University of Southampton

Faculty of Medicine

Human Health and Development

The Nutritional Care of Children and Young People with Chronic Kidney Disease

Matthew James Harmer

ORCID ID: 0000-0003-2977-0263

Thesis for the degree of Doctor of Philosophy

June 2021

University of Southampton
Faculty of Medicine
Human Health and Development
Doctor of Philosophy
Abstract

The Nutritional Care of Children and Young People with Chronic Kidney Disease
Matthew James Harmer

Chronic kidney disease (CKD) has a complex relationship with nutritional status. Children and young people with CKD have poorer nutritional status than their healthy counterparts. This may have an irreversible, negative, lifelong impact (1). This thesis explores how current clinical practice can characterise the clinical and nutritional status of children and young people with CKD, and proposes a relationship between disease severity, nutritional status, clinical outcomes, and disease activity.

A cross-sectional study reporting the growth data of children and young people attending a paediatric nephrology service found that short stature (height standard deviation score (SDS) <-2) and obesity (body-mass index SDS >2) were prevalent (10% and 12%, respectively), and variation not explained by disease severity alone.

A novel systematic framework approach to examining the literature was developed and when applied demonstrated the limitations of the published evidence on vitamin and mineral requirements in CKD to inform clinical decision making and management. What evidence that does exist is at high risk of bias. Application of this framework may be used to develop a more structured approach to management of micronutrients in CKD.

A cross-sectional study nutritionally characterising a cohort of 60 children and young people with CKD revealed variation of anthropometry, dietary intake, and blood concentrations of nutrients. Dietary intake assessment using current practice suggested that many children are at risk of nutritional inadequacy but was not supported by biochemical measures, which for the majority lay within the normal reference range. The exceptions were elevated concentrations of vitamins A and E.

Appetite was explored in this cohort through the development of a novel structured patient questionnaire that offers a broader and more complete characterisation of appetite. Using this approach revealed that self-reported appetite was poor in 25% of the cohort.

Poorer health-related quality of life (HRQoL), as assessed by a validated questionnaire (PedsQL) was reported in the cohort and was associated with poor nutritional status as marked by height SDS, and appetite. This association strengthens the importance of nutritional status as an important metric for patients.

A time-limited trial of a novel vitamin and mineral food for special medicinal purposes was poorly tolerated, but in those children who adhered to the intervention was associated with changes in blood concentrations of micronutrients, including selenium.

Finally, two putative markers of disease activity were explored. The distribution of red cell distribution width within the cohort was similar to that of a healthy population, and did not show any association with disease severity. RDW was negatively correlated with weight and mid-upper arm circumference, which may suggest a relationship between acute nutritional status and/or muscle mass. Urinary neutrophil gelatinase-associated lipocalin (uNGAL) was demonstrated to be elevated, and a predictor of kidney disease progression at 12 months compared to protein-to-creatinine ratio. An association with uNGAL and plasma selenium and vitamin B12 concentrations was observed that may mark a possible underlying mechanistic understanding of micronutrient-kidney disease interactions.

Herein, it is demonstrated that there is variability in nutritional status, appetite, health-related quality of life, and proposed markers of disease activity that are not entirely explained through variation in disease severity. As current approaches routinely applied in clinical practice may not adequately describe the nutritional state of children with CKD, there may be value in introducing a more structured approach to nutritional management of CKD.

"But I know all about love already. I know precious little about the kidneys" – Aldous Huxley, Antic Hay.

Contents

LIST OF FIGURES

LIST OF TABLES

UNIVERSITY OF Southampton

Academic Thesis: Declaration Of Authorship

I, ...Matthew James Harmer..... [please print name]

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

[title of thesis] .....The Nutritional Care of Children and Young People with Chronic Kidney Disease
.....

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

Signed: .....M Harmer.....

Date: .....11/10/2021.....

Acknowledgements

I would like to thank my supervisors: Dr Caroline Anderson, Professor Philip Calder, Dr Rodney Gilbert and Dr Steve Wootton for their guidance during completion of my PhD. In addition, the wider academic and clinical team for their contributions: Professor Alan Jackson, Dr Kesta Durkin, Dr Shuman Haq, Dr Arvind Nagra, and Southampton Children's Hospital.

Thank you to my friends and family for their support and patience throughout. Most importantly of all, thank you to the children and their families who I have the pleasure of caring for, who have given their time to participate in my research.

LIST OF ABBREVIATIONS

AE	Adverse Event
AUC	Area under the Curve
BMI	Body Mass Index
CI	Confidence Interval
CKD	Chronic Kidney Disease
cm	Centimetre
CVI	Content Validity Index
CYP	Children and young people
DRV	Dietary Reference Value
ECM	Extracellular Matrix
eGFR	estimated Glomerular Filtration Rate
ESRD	End-Stage Renal Disease
FSMP	Food for Special Medicinal Purposes
HCP	Healthcare Professional
HD	Haemodialysis
Ht	Height
HRQoL	Health-related Quality of Life
IL-6	Interleukin-6
IQR	Interquartile Range
IMP	Investigational Medicinal Product
Kcal	Kilocalorie
KDOQI	Kidney Disease Outcome Quality Initiative
Kg	Kilogram
l	Litre
LRNI	Lower Reference Nutrient Intake
m	Metre
MHRA	Medicines and Healthcare products Regulatory Agency
µg	Microgram
mmol	Millimole
mOsmol	Milliosmoles
MUAC	Mid-Upper Arm Circumference
ng	Nanogram
PD	Peritoneal Dialysis
pmol	picomole
PRING	Paediatric Renal Interest in Nutrition Group
RE	Retinol Equivalent
ROC	Receiver Operator Characteristic
RNI	Reference Nutrient Intake
RRT	Renal Replacement Therapy
SD	Standard Deviation
SDS	Standard Deviation Score
SOP	Standard Operating Procedure
TEMPeReD	Trace Element Malnutrition in Paediatric Renal Disease
WC	Waist Circumference
WHtR	Waist-to-Height Ratio
Wt	Weight
UK	United Kingdom
uNGAL	urinary Neutrophil Gelatinase-Associated Lipocalin
uPCR	urinary Protein-to-Creatinine Ratio
USA	United States of America
VKDP	Vitamin K-Dependent Protein
WHO	World Health Organisation

1. INTRODUCTION

There is a complex inter-relationship between chronic kidney disease (CKD) in children and young people and the extent to which their metabolic demands for energy and nutrients may be satisfied-their nutritional status. Nutritional status may be directly impaired by the disease process, through poor intake, or increased losses, or iatrogenically through treatment. At the same time, poor nutritional status will impact the disease process and growth. Although well recognised as an important factor in the clinical care of children and young people, there continues to be a limited evidence base for recommendations regarding their nutritional care. Children and young people with CKD, despite significant input from the multidisciplinary team (including highly specialist paediatric renal dietitians), are reported to have poorer nutritional status than their healthy counterparts, and this poor nutritional status may have an irreversible, negative, lifelong impact; including poorer growth outcomes (1).

Current guidance (2) aims to maintain optimal nutritional status, with a normal pattern of growth and body composition through regular expert assessment and individualised care.

It is incumbent upon healthcare professionals with the mantle of care of this vulnerable patient group to strive for improvement.

This thesis explores the clinical utility of the approaches currently used to characterise the nutritional status of children and young people with chronic kidney disease in a tertiary care setting in order to critically examine how this information may inform clinical management. This characterisation encompasses several elements:

1. growth of the child/young person;
2. dietary intake;
3. anthropometry;
4. biochemical status;
5. appetite;
6. an overall appreciation of the quality of life of the child;

Each of these elements separately and together may be used to determine the extent to which the metabolic demands for energy and nutrients are met. They may be explored in different ways each with varying rigor and differing inference and interpretation – with the most rigorous interrogation limited to specific research methodologies. They offer an ability to determine a nutritional assessment. It is only through examination of all these elements together with disease severity that a true representation of the individual child/young person can be summarised, and a nutritional diagnosis can be made; including the current nutritional status and risk of under-nourishment determined. Therefore, this thesis considers each of these elements, and their relationships to each other.

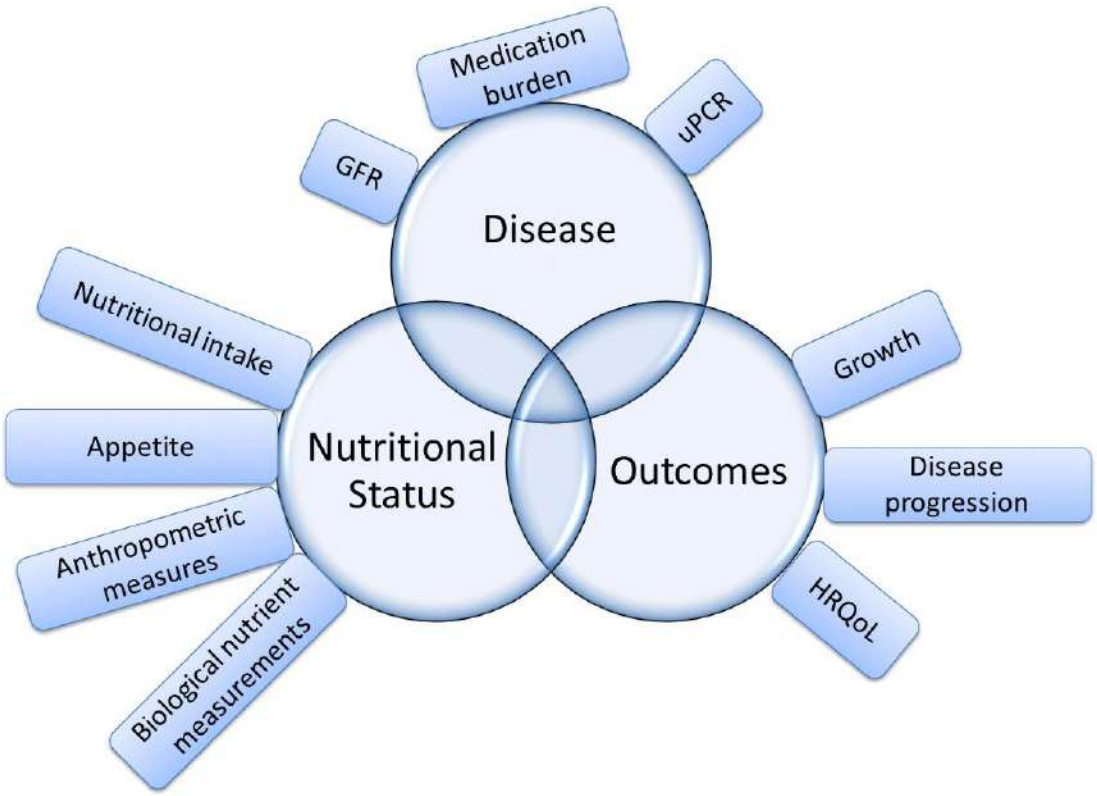


Figure 1. Conceptual framework for the relationship between disease, nutritional status and outcomes.

The proposed interaction between disease severity, nutritional status, and clinical outcomes in children and young people with chronic kidney disease. Each category may be captured in myriad methods each with its own advantages and limitations. Due to this, this thesis will use a number of measures for each category. Abbreviations: GFR – glomerular filtration rate; HRQoL – Health-related quality of life; uPCR – urinary protein-creatinine ratio.

This thesis proposes a relationship between disease severity, nutritional status, and clinical outcomes (see *Figure 1*). Children and young people with CKD may grow poorly. Whilst the degree to which growth is impaired is generally related to the severity of the disease (in that those with severe disease are shorter than those with normal renal function (3)), the extent to which the demand for energy and nutrients are met may also play an important part in determining the pattern of growth and progression of disease.

Poor intake, together with unusual losses and increased demands, may develop directly from the pathophysiology of the disease process, but may also develop indirectly as a result of clinical management. In clinical practice, those children and young people with the most overt clinical presentation of under-nourishment, usually those with the most severe or advanced disease are referred for nutritional intervention or are recruited into research studies.

Our approach to the nutritional management of children and young people with CKD is constrained by our lack of understanding of i) how the disease process, and its treatment, impacts on their requirements for energy and nutrients at different stages of the disease process and what they eat, ii) our ability to determine in routine clinical practice the extent to which the demand for energy and nutrients is satisfied, and iii) the extent to which

differences in nutritional state impact on growth, progression of disease and the quality of life of the child or young person.

Current nutritional management is dependent on identifying the child or young person at risk of undernourishment. This is largely dependent on obtaining a history, clinical examination and measures of height and weight, and subjective assessments of appetite and dietary intake based on recall. There are few biochemical measures relating to nutritional state that are used in routine practice, although some consideration may be given to iron status, vitamin D (calcium and phosphate), sodium and potassium.

Guidance on how best to identify those at nutritional risk and their management is limited. Current clinical practice recommendations regarding the nutritional care of children and young people with CKD have been composed by KDOQI (2). The guidance recommends review of energy and nutrient intake, regular height, weight and body-mass index assessment with the identification and treatment of nutritional inadequacies and metabolic derangement (such as metabolic acidosis). Specific nutritional management is limited to the assurance of adequate energy (defined as 100% estimated energy requirements); protein (defined as 100-140% dietary protein intake depending upon disease severity), and vitamin and mineral intake through diet with or without supplementation. Although serving as an initial starting point for clinical care, there is a lack of granularity in the recommendations of how these elements are assessed over and above estimation of dietary intake.

Taken together, improvements of the management of the nutritional aspects of CKD in children and young people requires a deeper and more complete characterisation of the nutritional needs of those with this condition. There is a particular need to study those with mild-moderate disease severity who have less overt signs and symptoms of nutritional deficits where we know so little.

The thesis additionally introduces the concept of disease activity as an additional actor within this relationship (see *Figure 2*). Current practice of determining the severity of disease is by measurement or estimation of the filtering capacity of the kidney; glomerular filtration rate (GFR). Although there are several methods to measure this, each with its own advantages and limitations, GFR remains only one aspect of renal function - the ability of the kidneys to filter, and only reflects the function of the kidney, not the underlying disease activity of CKD. As such, it tells us little about real-time changes in physiology due to the acquisition of complications associated with CKD or any medical intervention, or prognosis concerning progression of disease and deterioration of function. There is a common disease activity within the injured kidney that leads to the progressive fibrosis and loss of function, even once an initial insult has been removed.

There is a need to explore how this disease activity in children with CKD is related to physiological processes in order to better map the pathophysiology; including the interplay between nutrition and the disease processes.

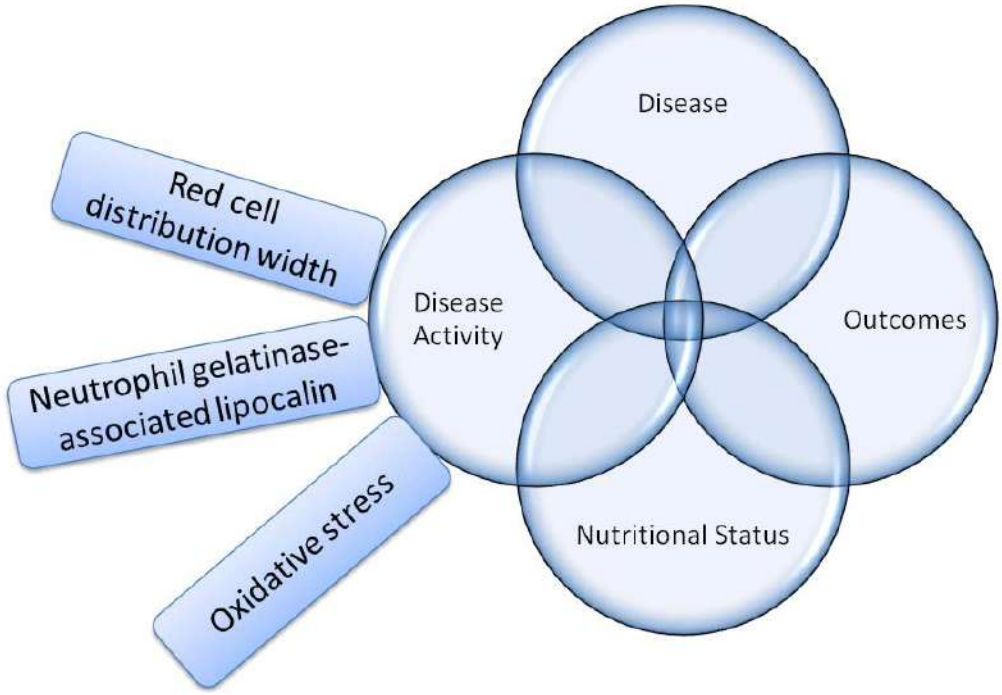


Figure 2. Conceptual framework for the relationship between disease, nutritional status, outcomes, and disease activity.

The proposed interaction between disease severity, nutritional status, clinical outcomes, and disease activity in children and young people with chronic kidney disease. Within the thesis, the concept of disease activity will be explored with two putative markers: red cell distribution width and neutrophil gelatinase-associated lipocalin.

This thesis takes a systematic approach to explore the structure and processes of the nutritional care of children and young people with CKD to explore the variation within these four concepts. To facilitate this, the thesis explores each of the above elements of nutritional status within a group of children and young people with CKD and reflect on the following research questions:

- What variation exists in nutritional status in those children and young people with CKD?
- What variation exists in the outcomes in those children and young people with CKD?
- What variation exists in the disease activity in those children and young people with CKD?
- How does the variation in these above concepts relate to disease severity; is there variation within these that are not fully explained by the variation in disease severity?
- What markers of nutritional status are associated with poor growth as defined by HtSDS<-2 and/or HtVelSDS<-2 in children and young people with CKD?
- What associations are there between well-being and nutritional status and children and young people with CKD?

1.1. CHRONIC KIDNEY DISEASE IN CHILDREN

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for 3 months or more, with implications for health (4). CKD is classified based on cause, glomerular filtration rate (GFR) category, and albuminuria category (4). It is a condition of irreversible kidney damage with progressive loss of kidney function that can progress to end-stage kidney disease at which point the roles of the kidney need to be as completely as possible replaced by medical intervention; including by dialysis.

The severity of CKD is graded depending upon the filtering function of the kidneys as assessed by glomerular filtration rate (GFR). Patients can subsequently be categorised into stages, the definitions of which are in *Table 1*, below, and apply throughout this text.

Table 1. KDIGO classification of CKD stage (4).

CKD Stage	GFR (ml/min/1.73m^2)	Description
1	≥90	Normal or high
2	60-89	Mildly decreased*
3a	45-59	Mildly-to-moderately decreased
3b	30-44	Moderately-to-severely decreased
4	15-29	Severely decreased
5	<15	Kidney failure
5D	In receipt of dialysis	In receipt of dialysis

*In the absence of kidney damage, neither GFR stage 1 or 2 fulfil the criteria of CKD. *compared to young adults.*

There are many causes of CKD. Congenital causes including congenital anomalies of the kidney and urinary tract (CAKUT) and hereditary nephropathies represent around two-thirds of children with CKD, and these tend to progress more slowly than acquired glomerulonephritides (5). Thus, those with the most severe disease (end-stage kidney disease, ESKD – defined as a GFR <15ml/min/1.73m^2, or in receipt of dialysis) have an over-representation of the faster-progressing glomerulonephritides.

Chronic kidney disease is becoming a worldwide epidemic with a prevalence 13·4% (10·6% in stages 3 to 5) (6). Within the paediatric population, the incidence and prevalence of CKD is increasing with a European incidence of stage 2-5 between 8-12 per million age-related population (pmarp) (5). Both the incidence and prevalence of those on renal replacement therapy (RRT) has doubled over a five-year period (7). In the UK, the prevalence of ESRD in those <16 years of age is 64.1pmarp (8). There is no UK data available of the number of children with non-ESRD CKD.

As in the adult population, in childhood CKD is associated with many complications and increased mortality (9); the leading causes of death being cardiovascular disease and infection (representing up to 40% and 50% of deaths, respectively) (5). The prevalence of complications of CKD including anaemia, hypertension, vitamin D deficiency, metabolic acidosis, hyperphosphataemia, hyperparathyroidism, malnutrition and mental health problems increase with disease severity (4).

Although progression of disease, with deterioration in kidney function is currently inevitable, the clinical course that an individual child makes is highly variable with varying rates of disease progression (10). The rates of progression to end-stage disease depends upon several identified risk factors, and likely many unknown (10).

The aetiology of the kidney injury affects progression of kidney disease, with those with glomerulonephridities progressing at a faster rate than those with congenital disorders (5). Low birth weight is associated with lower nephron number (11). Genetic variation may also increase susceptibility to and rate of progression of CKD as several genetic loci have been identified in the adult population (12). These risk factors are non-modifiable.

The most well-recognised modifiable risk factors are hypertension and proteinuria (5), but obesity is increasingly becoming more recognised in the paediatric population. Other variables, such as anaemia, and hypoalbuminaemia require prospective studies to confirm their effects seen in retrospective analyses in children but have increasing evidence in the adult population.

Although there are associations with markers of nutritional status, such as height with disease progression (13), the optimal nutritional assessment and management strategy for children and young people with varying severity of CKD is unknown.

1.1.1. Pathophysiology of CKD

Though the aetiologies, and therefore initial mechanism by which the kidney is injured, of CKD are highly heterogeneous, due to the close relationship of the different elements of the nephron and the shared vasculature, an injury in one part of the nephron is often an injury to all. There is a common pathophysiological pathway of the progression of CKD with glomerulosclerosis and tubulo-interstitial fibrosis (see *Figure 3*) (14). *Table 2* (below) outlines the potential damages and resultant consequences at different sites along the nephron. The exact mechanisms and pathways that by which kidney fibrosis is initiated and propagated is not fully understood, but is thought a combination of oxidative stress, inflammation, epithelial-to-mesenchymal transition (TEM), apoptosis, and extracellular matrix (ECM) deposition excess (15, 16). Following an initial injury, there is a common pathological process to the tubule-interstitial fibrosis and progression of disease. A decrease in the number of functioning nephrons leads to increased single-nephron GFR (snGFR) to compensate. The increased work of the nephron (including reabsorption within the proximal tubule) results in increased oxidative stress and activation of the renin-aldosterone-angiotensin system (RAAS). RAAS activation creates a positive feedback loop, increasing snGFR, but also increases expression of transforming growth factor-β which is a promoter of TEM and ECM deposition. Oxidative stress promotes inflammation that also drives fibrosis. The expansion of the ECM leads to capillary rarefication and local ischaemia resulting in further injury and oxidative stress (15, 16). The degree to which nutritional status; including availability of vitamins and minerals, impacts upon the development and progression of these pathological processes is unclear.

Table 2. Pathology observed in CKD in the different parts of the nephron (14).

	Damage	Consequences
Glomerulus	● Intra-capillary hypertension (e.g., in adaptation to increase GFR); ● Immunological injury; ● Metabolic injury (glucose, lipids, paraproteins); ● Genetic defects (e.g., Alport's syndrome; nail-patella syndrome).	● Diminished production of growth factors; ● Altered cell-matrix interactions; ● Expansion of mesangial matrix and basement membrane; ● Proliferation or loss of mesangial and endothelial cells; ● Changes in podocyte biology; ● Loss of podocytes; ● Altered permselectivity and proteinuria; ● Reduction of glomerular capillary area and blood flow.
Tubule	● Toxic or metabolic (glucose, lipids, filtered proteins, complement, cytokines); ● Ischaemia/hypoxia.	● Endoplasmic reticulum stress; ● Generation of ROS; ● Generation of mediators of inflammation; ● Altered generation of cytokines and growth factors; ● Change in ECM turnover; ● Generation of DAMP molecules; ● Activation of TLR's; ● Epithelial-mesenchymal transformation; ● Tubular epithelial apoptosis; ● Tubuloatrophy.
Peritubular Capillary	● Toxic or metabolic (glucose, lipids, filtered proteins, complement, cytokines); ● Immunological injury; ● Delivery of pro-inflammatory cytokines from glomeruli; ● Ischaemia/hypoxia secondary to: decreased post-glomerular blood flow or interstitial fibrosis; ● Loss of endothelial growth factors; ● Generation of pro-apoptotic factors by infiltrating leucocytes.	● Endothelial cell apoptosis; ● Capillary rarefication; ● Fibrosis
Interstitium	● Toxic or metabolic (glucose, lipids, filtered proteins, complement, cytokines); ● Immunological injury; ● Ischaemia/hypoxia secondary to capillary rarefication.	● Generation of mediators of inflammation; ● Generation of profibrotic cytokines; ● Activation of resident monocytes/dendritic cells; ● Infiltration and activation of inflammatory cells; ● Infiltration of bone marrow derived fibrocytes; ● Fibroblast activation and proliferation to myofibroblast; ● Altered matrix turnover and fibrosis.

Abbreviations: DAMP – Damage Associated Molecular Pattern; ECM – Extracellular Matrix; GFR – glomerular Filtration Rate; ROS – Reactive Oxygen Species; TLR – Toll-like receptors. Modified from: Schlondorff, 2008(14).

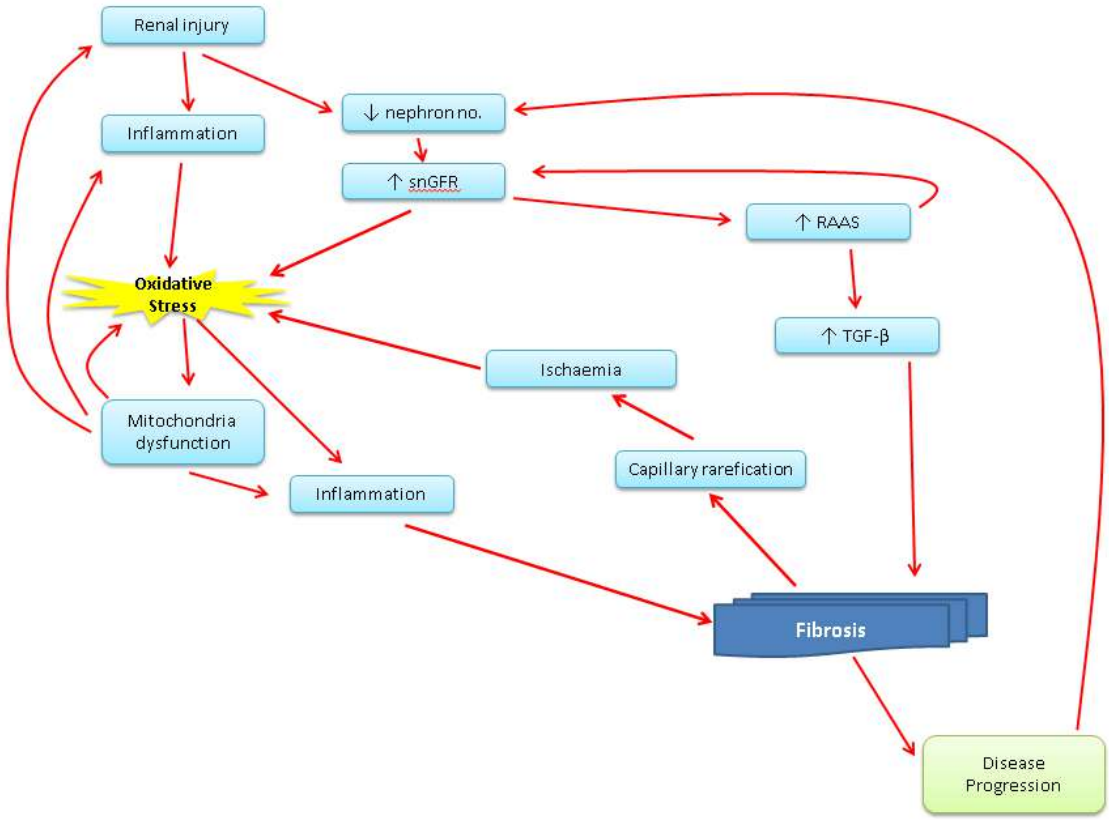


Figure 3. Pathophysiology of disease progression in CKD.

Following an initial injury, there is a common pathological process to the tubule-interstitial fibrosis and progression of disease. Following decreased nephron number, the increased work of the nephron results in increased oxidative stress and activation of the renin-aldosterone-angiotensin system (RAAS); creating a positive feedback loop. The expansion of the extracellular matrix leads to capillary rarefication and local ischaemia resulting in further injury and oxidative stress. Adapted from reviews (15, 16).

1.2. GROWTH IN CHILDREN WITH CKD

The assessment of growth is fundamental to paediatric care, as growth characterises childhood, and is the only timeframe in which this takes place. As such it is often the first assessment that a child or young person has on their attendance in the out-patient setting. Measured height in children and young people with CKD is lower than healthy peers (3, 17-21). A number of both potentially modifiable and unmodifiable factors have been associated with poor growth in CKD; including aetiology of disease, age of onset of disease, medical therapies (including steroid use), and suboptimal nutrition (22). Poor growth is important both as an outcome (as it relates to final adult height), and additionally is associated with increased mortality (23, 24).

Catch-up growth has been demonstrated in those in which some of the metabolic stressors and demands of CKD have been removed (i.e., in those that have undergone kidney transplantation, or with improved nutrition) during childhood (17, 25). Unfortunately, this catch-up growth is not universal, and final adult height remains lower than healthy peers in those that have undergone transplantation (3).

1.2.1. The usual pattern of growth

As depicted in *Figure 4*, linear growth (gain in length or height) of humans postnatally as characterised by height-against-time consists of three curves that represent the three stages of growth. These stages are infancy, childhood, and pubertal and, thus the model is known as the ICP model (26).

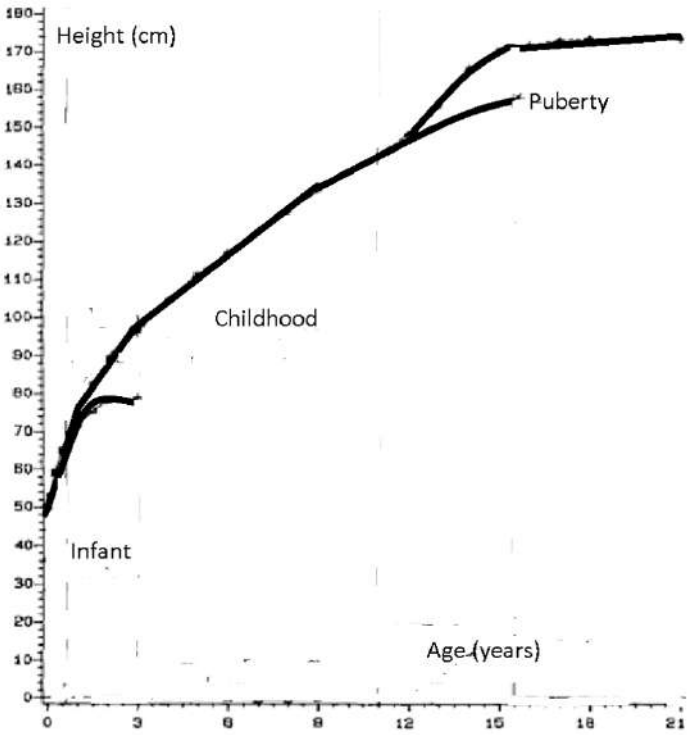


Figure 4. Graphical representation of the usual postnatal growth pattern of trajectory in humans (the 'ICP-model')) - from Kalberg et al, 1989 (26).

1.2.2. Foetal/prenatal

Prenatal growth is the most rapid phase of growth that starts with fertilisation of a single cell, and reaches, on average, 51cm length, 3.2kg term neonate (27). Embryogenesis occurs within the first trimester, and the second trimester brings the peak foetal length velocity of 2.5cm/week (28). Prenatal growth is mainly affected by placental function and its supply of blood and nutrients to the developing foetus, although genetics and hormonal function of the foetus also exert influence albeit to a lesser extent (29).

1.2.3. Infancy

In the first year of following birth, growth is rapid but there is a steady decline of growth velocity; halving over these 12-months (26). Nutrition is the most influential factor on growth in the infantile growth period (30). During this period of growth, extending up to about 3 years of age, intrinsic factors exert their influence and there is often catch-up and catch-down growth; finding a height centile that more closely reflects final adult height (29).

Schaefer et al (31), reported growth data from 321 pre-pubertal children with CKD from several European countries. These children had normal lengths at birth but dropped below the 3rd percentile during the first 15 months of life.

1.2.4. Childhood

Following the infantile growth period's deceleration, growth velocity remains relatively constant throughout the childhood growth period from the third year after birth at 5-6cm/year until the pubertal growth period begins (26). Within the childhood growth period, the effect of growth hormone (GH) becomes an increasingly important player, and it is at this age that abnormalities within the GH-IGF axis declare themselves as a result (29).

In Schaefer et al's aforementioned study (31), following the first year of life children with CKD had linear growth tracking parallel to the standard growth chart (mean height standard deviation score (SDS) = -2.37, SD± 1.6). Unfortunately, this study did not report delivery of nutritional management, but may suggest that nutritional management within the first year of life remains significantly important in children with CKD. It may be that during the childhood phase of growth, nutrition is less influential, although an alternative explanation is that healthcare professionals target growth along a centile line if above the 0.4^{th} percentile, and additional nutritional support may afford improved mean height scores.

1.2.5. Puberty

Puberty occurring at a skeletal age of 11 years (girls) and 13 years (boys) describes the transition from the pre-pubertal state, through the development of secondary sexual characteristics to the sexually mature adult with the arrival at final adult height upon fusion of the epiphyseal growth plates (29). The onset of puberty corresponds may be detected clinically with breast budding, or testicular enlargement, respectively and is initiated by the relaxation of the suppression of the hypothalamic-gonadotropin axis causing an increase in the sex hormones. In addition to increases in sex hormones characteristic of puberty, there are increases in GH, IGF-1 and insulin. During the pubertal growth period, there are significant sex-specific changes in body composition, and accounts for 20% of final adult height and 50% of adult peak bone mass. During puberty, girls have a higher rate of acquisition of fat mass compared to boys. Puberty is accompanied by a pubertal growth spurt that ends with oestrogen-mediated epiphyseal and metaphyseal fusion, and explains the sigmoidal curse of linear growth over time in the pubertal growth phase (29).

In paediatric CKD, delayed puberty (at least in those in receipt of dialysis) has variably been reported. In the review by Haffner and Zivicnjak (32), they report conflicting studies in which there may be up to 2.5years delay in puberty and its associated growth pattern with other studies finding no difference from healthy peers. This difference may be explained by the age of the studies, and possible advancements in care having a normalising influence on young people with CKD as it is the older studies that report delay (33). Once commenced, the steps of progression through the stages of puberty appear to be unaffected by CKD (32).

1.2.6. The assessment of growth in clinical practice

The assessment of growth in clinical practice is usually limited to linear growth - the measurement of height (or length in those children <2 years of age); the acquisition of mass through the measurement of weight; and, in infants, the measurement of head circumference. These measurements may then be compared to reference

standards for growth (27); and expressed as either placement on a centile or as a standard deviation score. These data may also be compared to calculation of mid-parental height (34), and form part of an individual's longitudinal data set where measurements over time are compared; including as height velocity.

The benefits of using such measures as a measure of growth are their relative ease in obtaining them, and the existence of a reference standard (27). Unfortunately, the measures tell researchers and clinicians little about the composition of the growing child, the obvious example being the acquisition of lean mass through bone and muscle deposition and that of adipose tissue are not discriminated between in these measures. The use of body composition measurement methodologies, such as bioelectrical impedance analysis and dual-energy x-ray absorptiometry are not recommended due to large margins of error and lack of validation in the paediatric CKD population (2). Techniques used for research purposes, such as cross-sectional imaging and isotope studies are limited by their availability, practicability, potential side-effects such as ionising radiation, and their validity in diseased groups (35).

Clinical practice guidance for the assessment of growth in childhood recommend the regular assessment of length/height, weight and, in those <3 years, head circumference. These measures are then compared to a reference standard (27). Crossing two major centiles on the growth chart is relatively common in infants and younger children, becoming uncommon once above the age of 3 years, and is used as indicator for possible investigation (36), although the sensitivity for the detection of pathology of this criterion may not be high enough to be used for screening (37). In the UK, referral for investigation is recommended for those children at 5 years who are below the 0.4^{th} centile for height (<-2.66 SD) (37), or if above 2 years and height centile is >2 below that of the calculated mid-parental height (38). Although the sensitivity and specificity are poor for the use in routine population-based screening (37), a fall in height SDS of more than 0.25 SD in a year (at least 3 measurements 6 months apart) has also been offered in The Netherlands (39).

There are no internationally agreed quality standards for the measurement of height and weight in children, but as clinicians and researchers, we must be able to have confidence in the measurements that are taken. It is essential to have agreed upon standard operative procedures (SOPs), assessment of competency and regular audit. Measurements such as height can be misleading in their apparent simplicity in obtaining, but different head positions during measurement, for example, can result in several centimetres difference of height (40). The World Health Organisation (WHO) has produced a training course in order to standardise measurements (41).

1.2.7. Disturbance in Growth

Causes of poor growth are myriad and include kidney disease. CKD should be considered in those presenting with their primary complaint as failure to meet growth targets as defined by standardised growth charts (27). This differs from the definition of "stunting" in which the child/young person has a height-for-age standard deviation score of ≤-2. In a population, it would be expected that approximately 2.5% of a cohort would fall into this category. The number of those that are not meeting their growth potential may be much greater in number as they may not lie below the "stunted" cut-off.

Poor growth has been associated with a number of short-term, long-term and inter-generational sequelae. These include: delay in meeting childhood developmental milestones (42), lower school achievement and adult income

(43), increased morbidity and mortality (43), and increased risk of birth complications (44), and small for gestational age offspring (44).

1.2.8. Growth and Nutrition

The adequate supply of energy and nutrients are essential for growth, and an inadequacy in one or more may result in detrimental effects on growth and development. Moreover, the limitation of a single nutrient if essential may completely limit the activity of growth. This was eloquently demonstrated in the landmark study by Golden and Golden (45), where limitation of a single nutrient was clearly demonstrated through supplementation of malnourished children with zinc and resulting resumption of growth with unchanged energy and protein supply. The extent of height deficit may be considered a reflection of the duration of under-nutrition (46). An extreme example is seen in those with severe malnutrition in the context of famine, but the full constellation of macro- and micronutrients are required for bodily function and growth, with vitamins and minerals acting with other molecules such as amino acids in complex pathways. Frank deficiencies act as clear examples of how severe restriction of supply of one player may have devastating systemic consequences (47). Under-supply not at the level to manifest as frank deficiency may have more subtle consequences within the growing and developing child/young person. They are potentially more vulnerable to unmet needs due to their requirement for growth that is not only unique to the pre-adult stage in the life-course, but time-limited with no further linear growth possible following growth plate fusion. It is therefore essential that adequate nutrition is supplied to the child/young person as there is only limited time for a trajectory of growth to be altered.

1.2.9. Catch-up Growth

Catch-up growth is where height velocity increases above the normal for age for at least one year after a period of growth inhibition, and may be complete or incomplete (48), with the child's anthropometry approaching the pre-insult growth trajectory (49). The pattern of tissue deposition during this catch-up growth is dependent upon the nutritional status (50), and may be as much as fifteen-times that of usual growth (51). If body composition has been altered through weight-loss (with loss of adipose tissue with relative lean mass sparing) an unbalancing of lean and fat mass has arisen. Prior to linear growth there must be a period of repletion (50). In many clinical scenarios, the process of return to the previous growth trajectory would also comprise this period of repletion before deposition of a balanced composition of tissues with usual ratio of lean and fat mass. During catch-up growth, the proportion of energy intake representing growth may be more than 50% (51), this compares to between 15-30% at birth and 5% from one year of age (52).

1.2.10. Growth in paediatric Chronic Kidney Disease

In CKD, it is well-recognised that growth may be detrimentally affected and there have been some efforts in characterising the growth of children with kidney disease, although hitherto, these data are mostly limited to those with the most severe kidney disease. Growth is important to children and young people with CKD and their parents (53), and in the post-transplant population, final adult height has been shown to be correlated with educational level, employment status; marital status; and independent living (54).

The North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) data from 1987-2010 (17) report that at the time of transplantation, mean height SDS of -1.75 SD, although with an improving trajectory (-1.23 SD in 2009).

In the UK, the most recent data from the renal registry (published in May 2019) reported that the median height SDS of those in receipt of dialysis was -1.9, and -1.1 in those with a functioning kidney transplant (3). Currently, the renal registry does not report on the growth data of those with less severe disease.

Data on the growth of children and young people with less severe CKD is limited. The multicentre North American CKiD study enrolled about 800 children with mild-to-moderate CKD (median GFR ≈ 50ml/min/1.73m^2) across 53 centres in the USA and Canada (18). These children had a median height SDS of -0.55 (IQR = -1.35 to 0.19), 12% of whom had a SDS ≤-1.88 (3rd percentile). Older reports of American children and young people reported a median height of those with a mean GFR of <75ml/min/1.73m^2 of 36.9% ≤ -1.88 SD (19).

Height SDS from Austrian children have also been reported both at referral to a paediatric nephrologist, and upon initiation of dialysis (20). At referral the 47 children (GFR = 24.7 ± 21.1) had a height SDS of -1.5. ± 2, compared to at dialysis initiation (GFR = 9.6 ± 3.3) height SDS was -1.3 ± 1.7.

In Turkey, pre-dialysis patients (CKD stage 1 to 4, n=20) have been reported to have a height SDS of -0.77, with 10% below -2 SD (21).

There are several both potentially modifiable and nonmodifiable factors that may influence growth in children with CKD (these are summarised in the *Table 3, below*).

Table 3. Variables known to contribute to poor growth in paediatric CKD (22).

Age of onset of disease;
Primary kidney disease;
Poor Nutrition* (increase calories; optimise diet);
Metabolic acidosis* (sodium bicarbonate supplementation);
Mineral bone disease of CKD* (phosphate-binders etc.);
Delayed puberty;
Urinary sodium loss* (sodium supplementation);
Urinary concentrating defect;
Steroid therapy* (steroid reduction as able);
Abnormal GH/IGF-1 axis* (recombinant GH therapy)
*potentially modifiable factors (treatment options)

Adapted from: Mahan et al, 2006 (22).

Poor growth in the paediatric CKD is associated with increased mortality (23), with those with height SDS <-2.5 with a two-fold risk of death compared to those with height SDS >-2.5 (23). Wong et al reported that for each 1 SD decrease in height SDS, children in receipt of dialysis or following transplantation had an increased risk of death of 14% (95% CI: 9%–34%) (24). The NAPRTCS data reported by Furth et al, also found an increased number of days in hospital and decreased school attendance in those with height SDS <-2.5 (23).

Medical management targeted to improve modifiable factors such as chronic anaemia, metabolic acidosis, and mineral-bone disease are important in the optimisation of the body for not only growth but disease progression and have internationally accepted guidance and recommendations (4).

Nutritional supplementation in malnourished children with CKD has been demonstrated to improve growth (25, 55, 56), although current medical and dietetic practice is not always successful, and response to this care is not

predictable. This may be due to the lack of understanding surrounding the causes of poor growth in children with CKD and the nutritional impact of the disease and its treatments.

Kari et al (25) report outcomes of 13 years of data for 81 children presenting under two years of age with severe CKD in whom early artificial feeding was commenced. As part of their routine clinical practice at this single institution, those with declining height velocity were offered feeding by nasogastric tube/gastrostomy (81% received). Although most children had a primary diagnosis of congenital abnormalities of the kidney and urinary tract, the cohort was variable and included several significant comorbidities that were likely to influence nutritional status. This approach has the advantage of reflecting real-life clinic experience, although may hide specific clinical phenotypes that may respond differently. Kari et al report that in those that did not undergo kidney transplantation height SDS improved from -2.34 at 6 months to -1.37 at 5 years of age, with BMI also increasing over this timeframe. The reported picture contrasts with the above-mentioned described work by Schaefer et al (31). Unfortunately, there is a lack of security that the supply of energy and nutrients are the main determinants of the difference between the two groups rather than other differences in care. Moreover, the optimal dietary prescription was not explored.

Parekh et al (55), in a study of 24 children with polyuric CKD (ten <10 ml/min1.73m^2; twelve 10-50 ml/min1.73m^2; and two >50ml/min1.73m^2) compared this group who were prescribed a sodium ±bicarbonate diluted milk formulation with a reference group. The study group was associated with greater energy and protein intake and higher height SDS including controlling for disease severity, aetiology of CKD and initial height SDS. Limitations of this, alongside the small sample size and the use of historic reference groups for comparison, include the inability to differentiate which elements this reported improvement in height SDS was associated with: energy intake, protein intake, sodium intake, bicarbonate intake, a combination of factors or some unknown element.

A Cochrane review of evidence (57), although limited, has demonstrated that those children with CKD and short stature increase height velocity with recombinant growth hormone (rhGH) administration, and that those who are pre-pubertal, and stage 3/4 CKD have a greater response than those who are post-pubertal or have more severe disease. Although there are no long duration trials that demonstrate an increase in final adult height in these children, cohort data suggests that in addition to increasing height velocity in the short term (first one to two years), final adult height may also be greater (58, 59). In addition to trials targeting this question directly, the role of nutritional status should also be included, as 'forcing' the system to growth through the exogenous administration of rhGH in the setting of an under-nourished body (including the availability of micronutrients, and key amino acids, for example), may have unintended consequences, in addition to recognised complications of rhGH treatment (60). In other groups of children with short stature, rhGH has demonstrated gains in height (sickle cell disease, and severe parasitic infection), but at the expense of the usual physiological pattern of tissue deposition (50).

Current recommendations are that children with stage 3–5 CKD or those on dialysis older than 6 months of age should be candidates for rhGH therapy if they have persistent growth failure (defined as a height below the 3rd percentile (SDS< -1.88) and a height velocity below the 25th percentile; SDS = -0.675) once other potentially treatable risk factors for growth failure have been adequately addressed and provided the child has growth

potential (60). In the aforementioned American CKiD study, 9% of the entire cohort and 23% of children with severe short stature (< 3rd percentile for height) were prescribed rhGH (18).

Catch-up growth has been demonstrated in those in which the demands of chronic kidney disease have been removed (i.e., in those that have undergone kidney transplantation) during childhood. Factors that influence the degree of catch-up growth include age (unlikely if transplantation is after 6 years old); graft function (how much of the ongoing physiological insult remains after transplantation), and the use of corticosteroids (which are well-known to suppress growth but used in immunosuppression regimens) (17). With regards to nutrition, as discussed above in the study by Kari et al, catch-up growth following nutritional intervention even in those with severe CKD is possible (25).

1.3. Chronic Kidney Disease and Nutritional Status

Nutritional assessment is "*the systematic process of collecting and interpreting information in order to make decisions about the nature and cause of nutrition related health issues that affect an individual*" (61). A commonly employed approach to nutritional assessment is the 'ABCDE' methodology (61). This uses an acronym to approach the assessment methodically.

Clinical assessment of the patient in nutritional assessment includes the examination of signs of malnutrition and signs of specific nutrient deficiencies, for example pallor in anaemia and Bitot's spots in vitamin A deficiency.

Dietary assessment is the process by which the diet is examined, and thus risk of nutritional inadequacy may be estimated. There are several methods that may be used each with their own advantages and disadvantages, discussed later.

Environmental and social factors that influence nutritional status; including financial stability and access to nutrient-rich foods, and cultural practices are reviewed in order to both identify risk and facilitate formulation of appropriate management plans.

Malnutrition has no universally accepted definition but is a term that is used to describe an imbalance in energy and/or nutrients that result in a change in body structure and function (62). Causes of malnutrition include inadequate dietary intake, decreased absorption, increased requirements, and increased losses which may be mediated through a diseased state (63). Malnutrition is associated with suboptimal function of many bodily systems; including skeletal muscle, cardiovascular, respiratory, and immunological (63).

There are many reasons why children and young people with CKD may have altered nutritional status and become malnourished. Firstly, intake of energy and nutrients is reduced for myriad reasons including diminished appetite, vomiting, and dietary restrictions. Secondly, demands for energy, protein and other nutrients may be altered. Thirdly, the medical interventions that are imposed upon the child or young person and complications such as infection may result in increased metabolic demands and nutrient losses. CKD is a pro-inflammatory state with elevated proinflammatory markers (64, 65). There is elevation in many pro-inflammatory cytokines, and lack of renal clearance of interleukin-6 (IL-6) (66). This inflammation has a significant impact upon nutritional status, with decreased appetite, metabolic dysfunction, contributing to alteration in body composition, with decreased muscle mass.

There have been several studies that have reported nutritional status of children with CKD and using these data reported malnutrition prevalence. Sozeri et al (21) reported 45% prevalence of malnutrition in a group of 42 Turkish children with CKD as assessed by the Subjective Global Assessment tool (67), including 20% in the pre-dialysis subgroup of 20. Apostolou et al (68) report the nutritional status of 30 children aged 1-16 years (17 with CKD stages 3/4 and 13 on peritoneal dialysis). They report malnutrition as assessed by the PeDISMART assessment tool to be 37%. This tool assesses four parameters 1) nutritional status as derived from weight SDS, 2) nutritional intake, 3) symptoms affecting intake and 4) overall disease impact. Score range for each parameter is zero to four with an adjustment of two points for children younger than 1 year, with a total score ranging from 0 to 18 (69). Apostolou et al also reported ABN scores in their group. ABN scores use a combination of anthropometric, bioelectrical impedance analysis, and intake parameters (70). In comparison, Edefonti et al's Italian cohort of 43 paediatric peritoneal dialysis patients malnutrition defined by ABN scores of 49% compared to 20% in Apostolou et al's mixed group (68). These studies demonstrate that using different definitions, result in different prevalence of malnutrition. Hitherto, these studies of nutritional characterisation have been focused on children and young people with the most severe disease; including those in receipt of dialysis.

1.3.1. Over-nutrition and Obesity in paediatric CKD

Obesity is becoming increasingly prevalent in CKD populations across the UK and Europe (18, 71). It has been reported to be an independent risk factor for development of kidney damage and end-stage kidney disease (72, 73).

Several proposed definitions of obesity exist, and to some extent these have been shifting with different standards by which an individual is compared. BMI thresholds of +1 SD and +2 SD (i.e. the 84.1st and 97.7th percentiles) have been recommended as overweight and obesity thresholds when using WHO growth charts as the standard or comparison (74).

Height-to-waist circumference ratio of >0.5 has been proposed as a cut-off for central obesity (75) with a recognition that central obesity confers a particular risk compared to increased weight-for-height *per se* (75).

1.4. Micronutrient Status in Children with CKD

Vitamins and minerals play essential roles within the body with inadequacy conferring morbidity and mortality. A significant proportion of children and young people from the general population are at risk from nutritional inadequacy with regards to vitamins and minerals (76). Those with CKD may be at increased risk with altered dietary intake (77). Moreover, the pathophysiological processes outlined above have vitamins and minerals playing significant roles, and their availability may have significant impact upon these processes.

Current guidance from the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) states that all children with CKD should have regular nutritional assessment and their micronutrient status reviewed regularly [1]. Oral micronutrient supplementation is recommended where dietary intakes appear insufficient so that the dietary intake with supplementation achieves the Reference Nutrient Intake (RNI) (or equivalent) (2). Little is known about the demands for, and losses of, micronutrients in children across differing pathophysiological states or treatment of CKD (2) so it is unclear whether an intake at or above the requirement of otherwise healthy individuals would be adequate in this patient group. The previous literature of biochemical

measures of micronutrient status in paediatric CKD is limited; it has been largely focused on those with the most severe disease and those in receipt of dialysis with often contradictory findings (2, 78-80).

1.5. Chronic Kidney Disease and Appetite

Appetite is the desire to eat food and is a complex entity in which both biological and psychological elements interact. Its control is not fully understood but involves a balance of various hormones and signalling pathways (81). In CKD, there are additional factors (such as metabolic toxins not cleared by poorly functioning kidneys, multiple medications, dialysis) that disrupt these processes (82).

During acute and chronic illness, appetite is perturbed (81), and in those with the most severe kidney disease, diminished appetite is a major cause for anorexia and malnutrition (82), and an important determinant of energy and nutrient intake (83).

It is recommended that appetite is assessed during clinical assessment of children with CKD (2), both as a marker of disease and as a means to identify those who are at risk from inadequate dietary intake of energy and nutrients.

In those with CKD, appetite has been shown to be associated with dietary energy and protein intake (83); to correlate with severity of kidney disease (84), hospital attendances (84), quality of life (84), and markers of nutritional status (85), although not associated with anthropometry in paediatric CKD patients (84). Decreased appetite is associated with decreased dietary intake of energy and protein (86, 87).

The pathogenesis of anorexia in CKD patients is multifactorial. Some of the changes in physiology that occur that may have a negative effect on appetite, including changes in taste; satiety; gastric irritation from medicines; alterations in the hormonal control of appetite, and inflammation with cytokine production (88). Those that receive dialysis have additional stressors that can have impact, including satiety during peritoneal dialysis, and nausea and poor appetite from the fluid shifts of haemodialysis. There is also the large area of the psychological impact of chronic illness and the intensive manipulation of children's lives (including diet) which can have significant impact on their relationship to food, with high prevalence of food aversion and picky eating, and depression is common (89, 90).

1.5.1. Assessing appetite

Often the clinical prompts that caregivers use for escalation of nutritional support are weight-loss or faltering growth. If the child has been identified as at risk from nutritional inadequacy then they may have dietary assessment from a healthcare professionals (HCP) such as a dietitian, who may be able to intervene before these cues have taken place, but often it is only when a child is failing to thrive that a dietitian is involved the child's care. If poor appetite is a marker of being at risk from nutritional inadequacy, then it has the benefit of occurring earlier. Earlier intervention with nutritional support may decrease the number of children that fail to meet their growth potential. Moreover, those children with CKD who have poor nutrition have more rapid progression of their disease (91).

Food record, diet recall or diet history are tools in the evaluation of disturbed appetite (2). There are additional biochemical and hormonal markers; such ghrelin concentrations that are accessible during research, but their role within clinical practice is limited by availability and cost (92). The development of cheap and easy methods that

HCP can use in clinical practice may be able to facilitate the characterisation of appetite and add to the nutritional assessment of children.

In *Table 4*, tools that have been developed for the clinical assessment of appetite are described. There is no currently available tool that has been developed and validated in the assessment of appetite in children with CKD. Due to the high prevalence of CKD in adults when compared to children, kidney-specific tools have only been developed in adult populations. Moreover, focus has been on those with end-stage kidney disease, rather than pre-dialysis, conservatively-managed (PDCM) CKD. For tools to be used in clinical practice, a balance must be struck between information gained and time taken for the tool to be completed.

Table 4. Previously developed tools for the assessment of appetite.

Tool (author, year)	Population	Notes
Appetite, Hunger and Sensory Perception (AHSP) questionnaire (de Jong, 1999 [22, 23])	Older people, The Netherlands (n=156)	29 items. Appetite associated with sensory changes and weight, but not in all sub-groups. Pros: Associated with nutritional state (MNA). Cons: 29-item tool; Older adults only; Not assessed against hormonal markers of appetite.
Appetite and Diet Assessment Tool, ADAT. (Borrowes et al, 2003 [24])	Adults receiving HD, USA (n=1846)	3 items. Correlated to serum creatinine; dietary intake of protein, carbohydrate, fat and energy. < 2% of group had "very poor" appetite. Appetite increased with time. Poor appetite/very poor appetite associated with lower protein and energy intake, and mortality. Pros: Quick to deliver; Large number of participants in multicentre study (n=1846); Longitudinal study; Associated with dietary intake. Cons: No relative portion size; Adults only; End-stage patients only; Not assessed against hormonal markers of appetite.
ADAQ (Lou et al, 2002 [25])	Adults receiving HD, Spain (n=44)	34 items. Appetite and Dietary Assessment Questionnaire. Correlation between the ADAQ score and protein-energy intake as assessed by diet-diary recall was highly significant, with a high sensitivity of predicting insufficient protein intake. Pros: Associated with protein intake. Cons: 34-item tool; No relative portion size; Adults only. End-stage patients only; Not assessed against hormonal markers of appetite.
SNAQ/CNAQ (Wilson et al, 2005 [26])	Older people in long-term care facilities, USA (n= 247)	An 8-item Council on Nutrition appetite questionnaire (CNAQ) and a 4-item derivative, the simplified nutritional appetite questionnaire (SNAQ) to identify elderly adults with anorexia and who are at risk from weight-loss. Both tools predicted involuntary weight-loss in excess of 5% of baseline body weight over the following 6 months. Pros: Quick to deliver; Associated with clinical outcomes. Cons: Older adults only; Not assessed against hormonal markers of appetite.

In clinical practice, appetite is assessed subjectively by direct questioning, (e.g., "How is your appetite?"), and through general dietary assessment. These methods are suboptimal as there are multiple confounding variables, making them not a direct surrogate for appetite. There is existing literature that finds them inaccurate; including in CKD cohorts (82). The lack of a formalised assessment tool means that a meaningful comparison between individuals and for an individual longitudinally is virtually impossible. The subjective nature of direct questioning may also result in a normalisation of poor appetite so that a child may perceive their appetite to be "good", but only relative to their previous feeling of appetite. There is no specific tool designed to evaluate

appetite in children with CKD. The ideal tool for this population would be a quick and easy to use, able to give a multi-faceted evaluation of a child's appetite, adding additional useful clinical information, and deliver a numerical score to enable tracking over time in a longitudinal fashion. Additional benefit may lie in the ability to predict clinical outcomes, possibly prior to anthropometric alterations manifest.

1.6. Chronic Kidney Disease and Health-Related Quality of Life

Health-related quality of life (HRQoL) is an individual's subjective perception of the impact of health status, including disease and treatment, on physical, psychological, and social functioning (93). Children with CKD, even those with disease on the milder end of the spectrum, have poorer HRQoL (90, 94-96). The physical and psychological impact of both the disease process itself and its management make CKD a particularly challenging condition with pervasive symptoms of nausea and lethargy, multiple clinic attendances and investigations, strict dietary modification, multiple medications (and their frequent adjustments), changes in physical health due to complications of CKD and psychological problems such as altered body-image, anxiety and depression.

HRQoL is not only determined by disease severity. In children with CKD, Gerson et al found that renal function did not correlate with HRQoL (95). Factors other than the disease itself contribute to HRQoL in those children with disease (97), and I would hypothesise that nutritional status impacts HRQoL. If such an association exists, then HRQoL assessment may offer a measure of how nutritional status impacts the child in a holistic, patient-centred way.

In cohorts of children with other disease states, poor nutritional status (98) and obesity (99) have been associated with poorer HRQoL, but this has not been explored in paediatric CKD. Micronutrient status has been associated with HRQoL (100), including selenium (101) with supplementation of selenium demonstrating improved scores (102-104), although there are contradictory findings (105).

Despite the recognition that HRQoL is important, there is limited data in this disease group with existing literature focusing upon end-stage disease and those following kidney transplantation. Of the studies that examine CKD stages 2 – 4 in the paediatric population (95, 96, 106-109), only one (108) examines nutritional status (evaluating the effect of short-stature).

1.7. Disease Activity in Chronic Kidney Disease

Although some aetiologies of CKD have a clear activity associated with kidney injury (systemic inflammatory conditions such as systemic lupus erythematosus, for example), there is a common disease activity within injured kidneys that leads to the progressive fibrosis and loss of function, even once an initial insult has been removed (see *Figure 3* depicting the pathophysiology of CKD).

There is a need to explore how this disease activity in children with CKD interacts with the known physiological processes to better map the pathophysiology; including the interplay between nutrition and the disease process. There is additional potential to use such markers clinically to both prognosticate and monitor response to therapeutic intervention, including nutritional manipulation.

CKD is well recognised as an inflammatory state (64), that worsens with increasing disease severity (110). Although this inflammation can be assessed through the measurement of circulating acute phase reactants and

cytokines, these markers are often non-specific; representing systemic inflammation. For example, C - reactive protein (CRP) which is elevated in a wide range of conditions, including acute infection.

1.7.1. Red cell distribution width

Red cell distribution width (RDW) has been purported to reflect inflammation in chronic disease states (111), and erythropoiesis is intimately linked with CKD. RDW has been associated with clinical outcomes (112-114).

RDW is a measure of the variability of erythrocyte volume and is calculated from the erythrocyte mean corpuscular volume (MCV) and the standard deviation of the erythrocyte population of the individual. Although calculated as part of the full blood count, it is not universally reported to the clinician. It is therefore accessible to the clinician through usual clinical care. RDW is influenced by the proportion of different sized erythrocytes in the population and may be increased (widened) with a larger proportion of smaller or larger erythrocytes. Examples of how RDW may be altered as given in *Figure 5*, below.

.

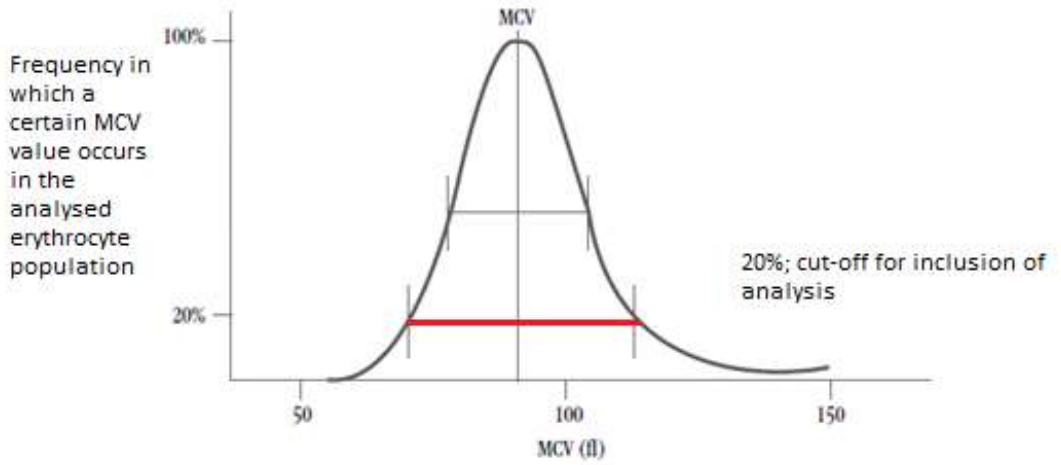


Figure 5. Red cell distribution width.

*Red cell distribution width (RDW-SD) is calculated as the distribution of erythrocytes at the 20% frequency level, that allow exclusion of outliers and is calculated with the formula: RDW = Standard Deviation*100/Mean Corpuscular Volume.*

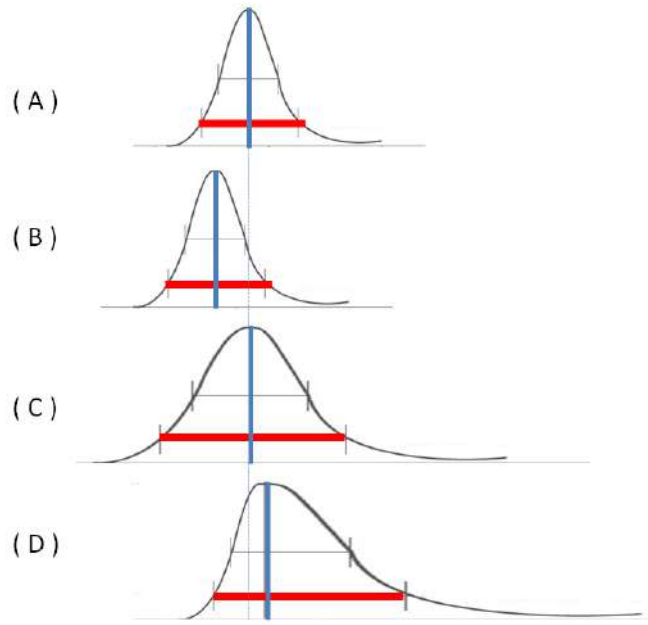


Figure 6. Example erythrocyte size frequency curves.

The figure demonstrates four proposed distribution curves. Subject (B) has a population of erythrocytes that are smaller than normal (A), but with the same variation in size (red cell distribution width (RDW), red lines). This curve may be seen in the context of iron-deficiency. Subject (C) has an increase in the variation in the size of erythrocytes (larger RDW), but with an unchanged mean corpuscular volume (MCV, blue lines). This curve may be seen in the context of a mixed iron, and vitamin B12 or folate deficiency. Subject (D) has increased MCV and RDW and represents an increase in the larger erythrocytes, for example Vitamin B12 or folate deficiency, or an increase in immature erythrocytes.

Many factors influence erythropoiesis and erythrocyte longevity and, therefore, potentially alter RDW; including the availability of nutrients such as iron, copper, glycine and B vitamins (see *Figures 6* and *7*, above and below). In CKD, there is systemic inflammation (66), and interruption of the usual erythropoietic signaling (115), as well as higher prevalence of nutritional inadequacies of iron, folate and vitamin B12 that increase the prevalence of anaemia (4).

Inflammation (as characterised by high-sensitivity CRP and erythrocyte sedimentation rate) has been associated with an increase in RDW (111). This may be due to alterations in the fragility of the erythrocyte membrane and / or effect on erythropoiesis.

Previous literature has demonstrated that increased RDW is associated with greater disease severity in chronic obstructive pulmonary disease (COPD) (112). A RDW >15.5% predicted outcomes following cardio-thoracic surgery (113). It has also been associated with higher mortality - with increased mortality associated with a RDW >14.2% in acute pancreatitis (114); >14.3% in COPD (112) and >18.6% critically unwell children in PICU (116). The generalisability of RDW suggests that it is a marker of a common mechanism, such as inflammation, although the exact processes by which RDW is associated with these disease states has not been elucidated.

In adult PDCM CKD (117), a RDW of >14.9% has been associated with increased mortality and a RDW >13.5% associated with decline in kidney function and initiation of dialysis (HR=1.47) (118). RDW has been

associated with endothelial dysfunction (as assessed by impaired flow-mediated dilatation) in adult CKD (119) independent of C - reactive protein (CRP) concentration. In paediatric kidney disease, RDW has been reported to predict the presence of nephritis and histopathological changes in Henoch-Schönlein Purpura (120), but no data in the paediatric PDCM CKD exists.

In search for a readily accessible marker of disease activity and / or nutritional status, RDW may reflect more than just disease severity but disease activity as a reflection of medium-term inflammation and its influence on the erythrocyte population. Other markers, such as CRP concentrations, increase in acute infection and may not be relied upon to reflect the CKD-associated inflammation, as minor, common infections elevate CRP beyond that observed in CKD-associated inflammation.

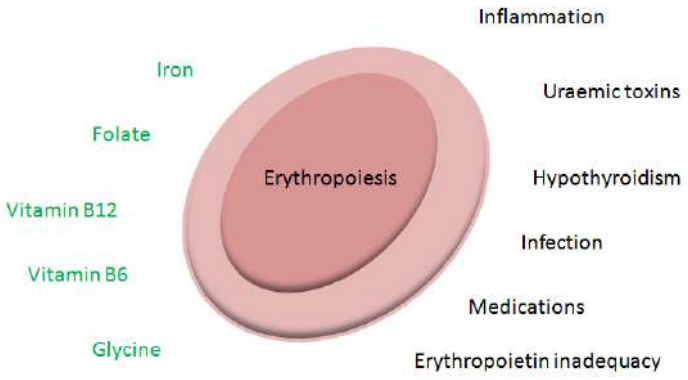


Figure 7. Factors in CKD that may influence erythropoiesis.

Anaemia is common in those with chronic kidney disease, and this is due to the many factors that may have a negative effect on erythropoiesis. In addition to limitation of erythropoietin in the failing kidney, inflammation and angiotensin-converting enzyme inhibitors may impair erythropoietin production. Inflammation also inhibits erythrocyte maturation and promotes erythrocyte cell death, and through the action of hepcidin, decreases availability of iron. Uraemic toxins and hypothyroidism decrease the life-span of the erythrocyte. Nutritional factors (green) include iron, folate and vitamin B12 availability, but also vitamin B6 which is essentially for amino acid metabolism, and the conditionally-essential amino acid glycine. Adapted from review articles (115, 121).

1.7.2. Neutrophil-gelatinase associated lipocalin

Neutrophil-gelatinase associated lipocalin (NGAL) is a biomarker that is showing promise for its utility in paediatric CKD. It is proposed that elevated NGAL is caused by the underlying inflammation present in CKD; being produced by kidney epithelial cells (122). Previous literature has demonstrated that NGAL concentrations in the CKD population are correlated with disease severity (123), and is able to predict disease progression (124).

NGAL is a 198-amino acid peptide with several known roles within the body. It acts as part of the innate immune system that binds to the siderophores that bacteria use to gain needed iron from the host organism, and through this mechanism, is bacteriostatic. NGAL has been demonstrated to be protective against the development of acute kidney injury (AKI), and has a role as a growth factor in kidney development and in

malignancy (125, 126). NGAL concentrations are low in healthy individuals (approximately 20ng/ml in blood and urine) and likely represent neutrophil expression (127-130) with rapid increase in expression following tissue injury (127).

NGAL has been shown to be elevated in kidney tubular damage in the setting of (CKD (131). The mechanism for higher NGAL in the CKD population is not clear. It has been suggested that increased kidney tubular production, increased glomerular loss, or decreased tubular reabsorption may be responsible. Mori and Nakao have proposed that elevated NGAL concentrations are more than a consequence of poor kidney clearance, but are caused by the underlying inflammation present in CKD; being produced by kidney epithelial cells (122). This is interesting as NGAL may, in this context, be a marker of disease activity rather than reflecting poorly functioning kidneys and reduced kidney clearance. Exogenous administration of NGAL in murine models of kidney injury confers a degree of protection (125), suggesting that NGAL is more than a marker of kidney injury, but may be part of the attempted repair mechanisms following injury.

Previous literature has demonstrated that NGAL concentrations in the CKD population are correlated with disease severity (123), and is able to predict disease progression (124); including being associated with increased risk of progressing to end-stage kidney disease (132).

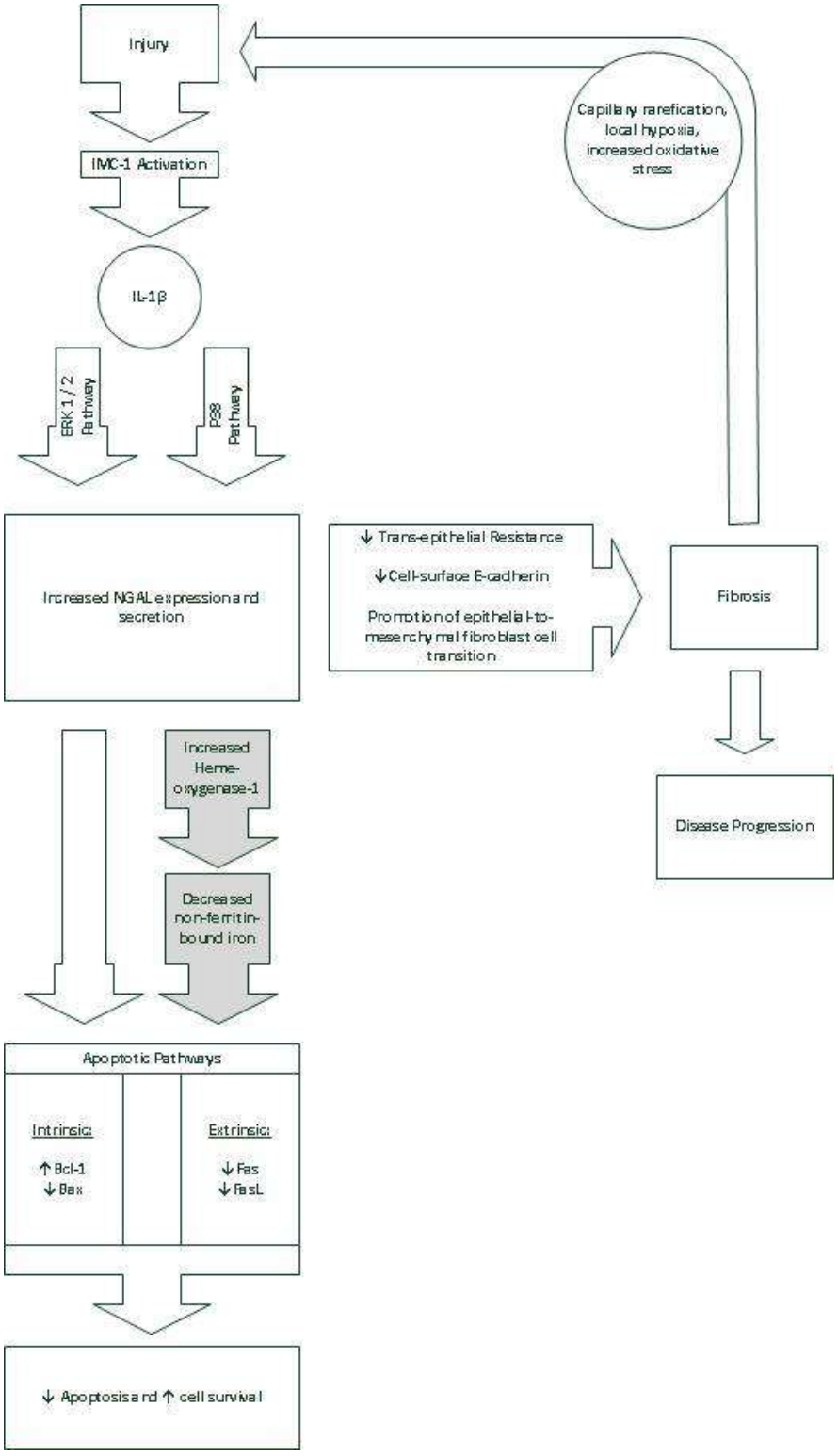


Figure 8. Proposed mechanisms of increased NGAL expression and its influence on apoptotic pathways.

Kidney injury induced inflammatory cascade, including increased expression of interleukin-1β (IL-1β), this via ERK1/2 and p38 pathways increase expression and secretion of NGAL. NGAL acts via heme-oxygenase-1 to modify both intrinsic and extrinsic apoptotic pathways to decrease apoptosis and increase cell survival. Abbreviations: FasL – Fas ligand; NGAL – neutrophil gelatinase-associated lipocalin.

NGAL may exist in 3 forms: a monomer; a homodimer; and a heterodimer with Matrix Metallopeptidase-9 (MMP9) (127). The binding of MMP9 to NGAL protects the enzyme from autolysis, preserving its activity (133). MMP9 as a matrix metallopeptidase, degrades extracellular matrix, allowing the infiltration of inflammatory cells. As reviewed by Cheng et al (134), MMP9 is associated with kidney fibrosis, which is the final common pathway of CKD. Its relationship with CKD is not linear with disease severity, with increased expression in the early stages of CKD (with collagen type-4 degradation, increased TGF-β expression and increased kidney fibrosis), but in more advanced CKD, MMP9 is lower. As the NGAL-MMP9 heterodimer, MMP9 seems to have an additional cell-signalling role (134).

NGAL promoter region has a binding site for NF-κβ, and its binding promotes cell survival and proliferation (135) and seems to act through the inhibition of both apoptosis signalling pathways; the intrinsic (Bcl-1 and Bax) and extrinsic (Fas and FasL) (136).

1.7.2.1. NGAL as a kidney protector

NGAL has been purported as a protective protein which decreases apoptosis in cells with higher NGAL expression. Tong et al demonstrated that over-expression of NGAL in human lung cancer cells reduced cytotoxic drug-induced cell death (137). In a model of ischaemia-reperfusion injury, Zang et al report that exogenous application of NGAL decreased apoptosis through the regulation of apoptosis factors (up-regulation of Bcl-1, and down-regulation of Bax and First apoptosis signal (Fas) and First apoptosis signal ligand (FasL)) (136), and agrees with Mishra et al's study showing decreased apoptosis in mice (125). Zang et al also report up-regulation of the NGAL receptors megalin and 24p3R in response to injury, and down-regulation with exogenous NGAL application.

This protection may be via heme oxygenase-1 (122), by reducing non-ferritin-bound iron. Devireddy et al (138) reported that the NGAL-siderophore complex without iron can chelate iron from cells, and through depriving these cells of iron, induce apoptosis. They hypothesise that NGAL may exert this effect as part of the termination of the immune response. Therefore, the action of NGAL may be cell-specific.

1.7.2.2. The influence on nutritional state

Review of the literature identified one study investigating association between nutritional status and NGAL in children with CKD (139); examining NGAL and iron status in dialysis patients. There is a suggestion from adult and in vitro literature that there is a relationship between some markers of nutritional status (iron status, vitamin D status, obesity, vitamin A pathways) and NGAL concentrations in patients with chronic kidney disease. The mechanistic relationship between these is not clear.

Iron

As previously described, NGAL binds to siderophores to sequester iron from the circulation, making it unavailable to pathogenic organisms that are reliant upon host iron. As such, NGAL is associated with iron metabolism. In adult CKD patients with anaemia, those with iron-deficiency (transferrin saturation, TSAT <20%) have been reported to have higher NGAL concentrations (140). A negative correlation was reported in a larger cohort of adult CKD patients (141), although a cut-off of TSAT <30% was used in this experiment, and analysis included those with and without anaemia, and also in a cohort of 40 children in receipt of dialysis (139).

Gunes et al (142) reported that children with iron-deficiency anaemia (IDA) had significantly higher creatinine-adjusted urinary NGAL, uNGAL and significant negative correlations between Hb, Hct%, RBC count, and creatinine-adjusted uNGAL. The authors postulate that this represents possible subclinical kidney injury in paediatric IDA patients whose kidney functions and serum electrolytes were normal. In paediatric dialysis patients, NGAL has been reported to have an inverse relationship with TSAT (139). Unfortunately, the potential causal relationship between NGAL and iron status has not been explored through the correction of an iron-deficiency anaemia and observation of NGAL concentrations thereafter fall.

Adiposity

In addition to neutrophils, epithelial cells and adipocytes express NGAL (143). Adipocyte numbers are thought likely static in adulthood, but may increase in childhood and adolescence under the influence of energy and nutrient intake (144). Number, active proliferation, and other factors may affect NGAL expression in adipocytes, but this has not been examined. Adiposity, therefore, may determine some degree of the baseline variability of NGAL expression. Moreover, NGAL changes in blood (and/or urinary) may be reflections of changes in adiposity or a changing adipocyte state.

Vitamin A

In a mouse model of kidney injury (145), tubular regeneration and proliferation has been shown to be mediated via vitamin A signaling pathways. Although the influence of vitamin A status in CKD has not been explored to date.

Vitamin C

Despite vitamin C being an important antioxidant, and that ischaemia-reperfusion injury is associated with an increase in oxidative stress, Sahraei et al were unable to demonstrate improvement of kidney tubule injury marker NGAL or graft function following kidney transplantation with administration of N-acetylcysteine ± vitamin C (146).

Vitamin D

In vivo and *in vitro* studies reported by Lucisano et al (147) demonstrated down-regulation of NGAL with administration of the 1, 25(OH)-vitamin D analogue paricalcitol in adult CKD patients. This was accompanied by a fall in inflammatory cytokines. The participants all had a low vitamin D status (<20ng/ml, with 70% <15ng/ml indicating deficiency). The association between vitamin D and NGAL may be down-regulation of inflammation, but the exact mechanisms have not been elucidated. In agreement, Zhang et al (148) demonstrated that in vitro, concentrations of vitamin D at 30ng/ml inhibited lipopolysaccharide-induced inflammation in human monocytes (IL-6 and TNF-α), but at concentrations of 15ng/ml suppression did not occur (148). In a randomized, placebo-controlled, double-blind study in adults with CKD stage 2 - 3 Alvarez et al (149) failed to demonstrate changes in NGAL and other inflammatory markers with supplementation (50,000 IU cholecalciferol weekly for 12 weeks followed by 50,000 IU alternate weeks for 40 weeks), although monocyte chemoattractant protein-1 concentrations were decreased at 12 weeks of supplementation (although not at 12 months).

1.7.2.3. What is normal uNGAL?

Previous studies have proposed normal reference ranges for uNGAL in children. Bennett et al (129) measured uNGAL in 368 healthy children, and reported 95th percentile for sex and age group (~45 children per group). The

age groups are arbitrary (3 - <5 years; 5 - <10 years; 10 - <15 years; and 15 - <18 years). There was a significant difference between sexes, being up to more than a factor of 10 greater in girls in the same age group (139.5 versus 10.9 ng/ml).

Rybi-Szuminśka et al (128) have also reported normal reference range correcting for urinary creatinine concentrations, measuring uNGAL in 172 children. Their proposed ranges (97.5th percentile) have been reported as broad age groups (0.2 – 5.9 years; 6 – 9.9 years; 10 – 13.9 years; 14 – 17.9 years), and for age in years. In contrast to Bennett et al's ranges, their proposed upper limit is graduated with a negative correlation with age. Rybi-Szuminśka et al's cohort did not have equal numbers across age-groups with a larger cluster in children aged 7 - 13 years, additionally the difference in sex reported by Bennett et al has not been considered, as no difference was found once uNGAL was adjusted for urinary creatinine.

Cangemi et al (130) reported uNGAL concentrations in a cohort of neonates (n=25) and older children (n=308). They report that neonates had higher uNGAL concentrations than older children, but without correlation with age otherwise. This agrees with Rybi-Szuminśka et al's cohort and may be explained by the tubular immaturity of the very young. Cangemi et al's uNGAL concentrations correlated with urinary creatinine, suggesting that correction of uNGAL with urinary creatinine should be performed. In concordance with creatinine-adjusted values reported by Rybi-Szuminśka et al and Nishida et al (150), Cangemi et al found no difference between the sexes in their cohort. The lack of creatinine-adjustment by Bennett et al, may explain the sex difference, but the increased likelihood of contamination of uNGAL from other sources, including the gastrointestinal tract in girls compared to boys, or hormonal difference may also account for differences.

1.8. Digitising Clinical Data

There is potential for technology and digital systems to be used to help to identify patients at risk by utilising data already gathered as part of usual clinical care. At present these data are used on an individual basis, and require the review, and evaluation of members of the clinical team. Clinicwide review may be accessed through retrospective reviews or as part of clinical audits, but is not used as part of usual care / service delivery.

It is important that medical professionals and researchers explore methods in which routinely collected clinical data may be used to understand their populations.

In addition, the development of clinical tools that may be conducted electronically before the clinical review may add important clinical information to the clinical team decision-making, and be time- and cost-saving.

1.9. Thesis Structure and Objectives

As described above, children and young people with CKD have outcomes (including growth) that are poorer than healthy peers. This continues to be the case despite the formulation of clinical practice guidelines; including the clear treatment goals for modifiable physiological disturbances such as metabolic acidosis, and anaemia (4). The variation of nutritional status in paediatric CKD has only partially been described previously. A greater exploration of this area is needed in order to guide future directions for research and essential for clinical care.

1.9.1. Aims and objectives

The aim of this thesis is to better understand the relationship between disease severity, nutritional status, and clinical outcomes, and thereby improve clinical care from the clinician's point of view.

To do this, the following objectives were undertaken:

1. To characterise the prevalence of poor growth and propose a way identify those at risk of under and over nutrition;
2. To explore and characterise vitamin and mineral status;
3. To explore and characterise nutritional intake and status;
4. To explore and standardise the assessment of appetite;
5. To explore and better understand health related quality of life;
6. To explore potential disease activity markers;
7. To explore relationships between each element, disease severity and clinical outcome.

This thesis takes a systematic approach to explore the variation of clinical outcomes, nutritional status, disease severity, and disease activity in children and young people with CKD. It will do this through exploration of the elements that are used in current clinical practice to characterise the nutritional status of children and young people with CKD and their relationships: growth; dietary intake; anthropometry; biochemical status; appetite; and disease severity. Additionally, HRQoL as a clinical outcome and disease activity and their relationship to nutritional status was explored. Disease activity characterization is through the measurement of putative markers of disease activity in CKD (red cell distribution width and neutrophil gelatinase-associated lipocalin). Throughout the thesis, there is a thread of how digital technology may be used in this exploration and potential to augment clinical care.

2. STUDY DESIGN AND GENERAL METHODOLOGY

2.1. OVERALL STRUCTURE OF THE THESIS

To address the overall aim of better understanding the relationship between disease severity, nutritional status, and clinical outcomes from the clinician view point, the thesis will explore each element systematically.

Firstly, a cross-sectional study reporting the growth data of a large cohort of children and young people attending a tertiary paediatric nephrology service is reported.

Secondly, a review of the evidence regarding vitamin and mineral status in children and young people with CKD is presented.

Thirdly, a cross-sectional study nutritionally characterising a small cohort of children and young people with CKD is presented. This consists of clinical characteristics, anthropometric measures, dietary intake data, and blood vitamin and mineral concentrations.

Fourthly, appetite is explored in this cohort of children with the development of an assessment questionnaire. This is then used to report the prevalence of anorexia within the characterised cohort.

Fifthly, HRQoL as described by a validated assessment tool (PedsQL^{TM}) is reported within the cohort and associations explored with markers of growth, nutritional status and disease severity.

Sixthly, an interventional study is reported in which a novel food for special medical purposes to supplement vitamins and minerals in the paediatric CKD population was given to a subgroup of CYP identified as being at risk from nutritional inadequacy from the above cross-sectional study.

Seventhly, results of two putative, potentially nutritionally-sensitive markers of disease activity within the cohort are described and associations with growth, nutritionals status explored.

Finally, the findings from these experiments are discussed in the thesis' final discussion.

2.2. ETHICAL APPROVALS

All experiments involving clinical subjects were approved by appropriate professional bodies.

3. GROWTH IN THE PAEDIATRIC NEPHROLOGY CLINIC

To explore the variation in growth in children and young people with CKD, a study describing basic anthropometric measures within a tertiary paediatric nephrology service was undertaken. This delivers a broad overview of the growth status of an entire service.

3.1. INTRODUCTION

Growth is one of the most important processes to consider in the care of children. The earliest description of normal growth recorded in the 6th century BC is by the Athenian statesman and poet Solon (151). Growth is not only a marker of illness and physiological stress upon the developing child / young person, but an important end-point in and of itself. Many diseased states, including kidney disease are associated with altered growth, although understanding the relationship between disease and growth is not straightforward.

In this chapter I will describe: the usual pattern of growth; how growth is assessed in clinical practice; disturbance of growth including within the context of chronic kidney disease; and present clinic-wide growth data at a single centre across the spectrum of kidney disease.

Although, as evidenced above, growth is important, data is not routinely collated and reviewed on a clinic-wide basis, and data nationally is limited to that of those receiving renal replacement therapy. In order to better understand the growth of the paediatric kidney population within a tertiary paediatric nephrology centre (Southampton Children's Hospital), a cross-sectional study collating routine anthropometric measures of the entire clinic population was undertaken.

The objectives of this study were to characterise the growth status of an entire paediatric nephrology clinic as determined by height and body mass index (BMI), and explore the relationship between these measures and kidney function - estimated glomerular filtration rate (eGFR).

3.2. METHODS:

All children (aged 0-17.99 years) who attended clinic appointments in 2015 with the paediatric nephrology service at Southampton Children's hospital were included in the study. Data on regional clinics (held at district general hospitals were not readily accessible and not included in the study). Children were identified using the child's hospital identification number and the clinic codes for paediatric nephrology available on the hospital's patient administration system, *CaMIS PACS+* (EMIS Health Group, Leeds, UK). A new kidney-specific database was developed using the hospital identification number as the child's identification number and the first measurements of the year for: age, gender, weight (kg), height (cm), body mass index (BMI, kg/m^2), plasma creatinine, date of clinic appointment, and clinic group code. Growth SDS (weight, height and BMI) were then generated, and eGFR using the modified Schwartz formula (152). Data quality checks and cleaning were also carried out manually using extreme values to check for entry errors (n=20).

3.2.1. Statistical Methods

Descriptive statistics; including mean and standard deviation scores (SDS) will be used to analyse the data; including breakdown by clinic group (group 1: general nephrology clinic; group 2: post-transplantation clinic; group 3: low-clearance clinic (those with eGFR<15ml/min/1.73m^2, or in receipt of dialysis)). The general nephrology clinic patients will then be stratified into degrees of kidney impairment as determined by eGFR

(calculated by modified Schwartz formula (152)), and classified as per the KDIGO CKD staging (4). All analysis was carried out using software by the Statistical Package for Social Sciences (SPSS version 22, SPSS Inc., Chicago, IL). A p-value of < 0.05 was used to indicate statistical significance.

3.3. RESULTS

A total of 819 children and young people were identified, with 754 (92%), 806 (98%), and 740 (90%) having a height, weight and BMI recorded, respectively. 56% (459) of these children had a plasma creatinine recorded at the same time as a height measurement, enabling kidney function to be estimated (eGFR).

These were then categorised as to attending one of three clinic categories: Group 1 – general nephrology clinic (n = 412); Group 2 – post-transplant clinic (n = 22); Group 3 – low clearance clinic (those with an eGFR <15ml/min/1.73m^2 or in receipt of dialysis, n = 25). Complete datasets were available for 401, 20, and 24 respectively (comprising 90%, 5%, and 5% of the total 445 analysed).

These 445 subsequently had calculation of height SDS, weight SDS, BMI SDS and eGFR and in attendance at the general nephrology clinic (non-dialysis, non-transplant, non-low clearance clinic).

As shown in *Figure 9*, the majority of children and young people's anthropometry lay within ±2SD for both height and BMI. Mean height SDS for the three groups were -0.19 (SD±1.34), -1.73 (SD±1.68) and -1.59 (SD±1.77) for groups 1, 2 and 3, respectively. 11% (84/754) of all height values recorded had a height SDS <-1.88, and 10% (75/754) <-2. Height SDS < -2 was much more prevalent in the low-clearance (group 3, 44%) and post-transplantation groups (group 2, 40%), with the general nephrology group (group 1) having 8% of children and young people below -2 SD. Mean BMI SDS for the three groups were 0.49 (SD±1.27), 1.34 (SD±1.14) and -0.31 (SD±1.52). The greatest number of children and young people with a BMI >2 SDS were in the transplant group (30%, group 2), followed by the general kidney group (12% group 1) then 4% in the low clearance group (group 3). There was a statistically significant difference between groups for height SDS, weight SDS, and BMI SDS as determined by one-way ANOVA (Height SDS: $F(2,751) = 22.817$, $p < 0.0005$; Weight SDS: $F(2, 803) = 20.076$. $p < 0.0005$; BMI SDS: $F(2, 14.908) = 9.126$, $p < 0.0005$). A Tukey *post hoc* test revealed that height SDS was significantly different between group 1 and 2 and between groups 1 and 3 ($p < 0.0005$), but not between groups 2 and 3 ($p = 0.943$). Weight SDS was significantly different between groups 1 and 3 ($p < 0.0005$), and between groups 2 and 3 ($p = 0.011$), but not groups 1 and 2 ($p = 0.424$). BMI SDS was significantly different between all group combinations (group 1 and 2: $p = 0.010$; group 1 and 3: $p = 0.007$; group 2 and 3: $p < 0.0005$).

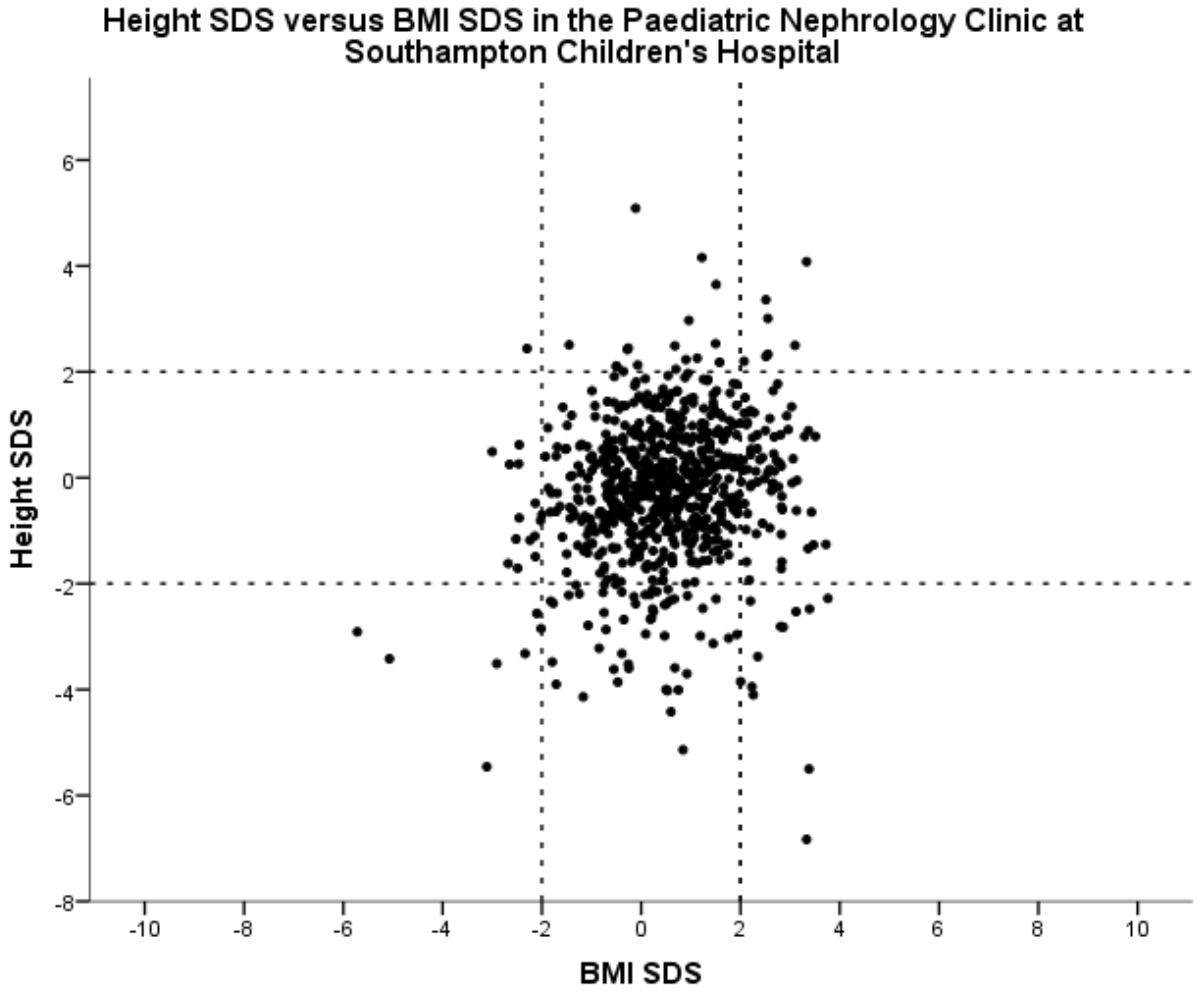


Figure 9. Height SDS versus BMI SDS for attendees at the paediatric nephrology clinic (n=445).

Graphical representation of Height SDS and BMI SDS for the nephrology clinic (n=740) reveals a picture that most children and young people lay within +2 and -2 SD for both measures. If outside that reference range, then more children and young people are short (HTSDS<-2) than tall (HtSDS>2) and heavy-for-height (BMISDS>2) rather than light-for-height (BMI<-2).

3.3.1. Kidney Function and Anthropometry

Growth data were compared to kidney function as determined by eGFR. The distribution of height SDS and BMI SDS for each KDIGO CKD staging group is displayed in *Figure 10*, below.

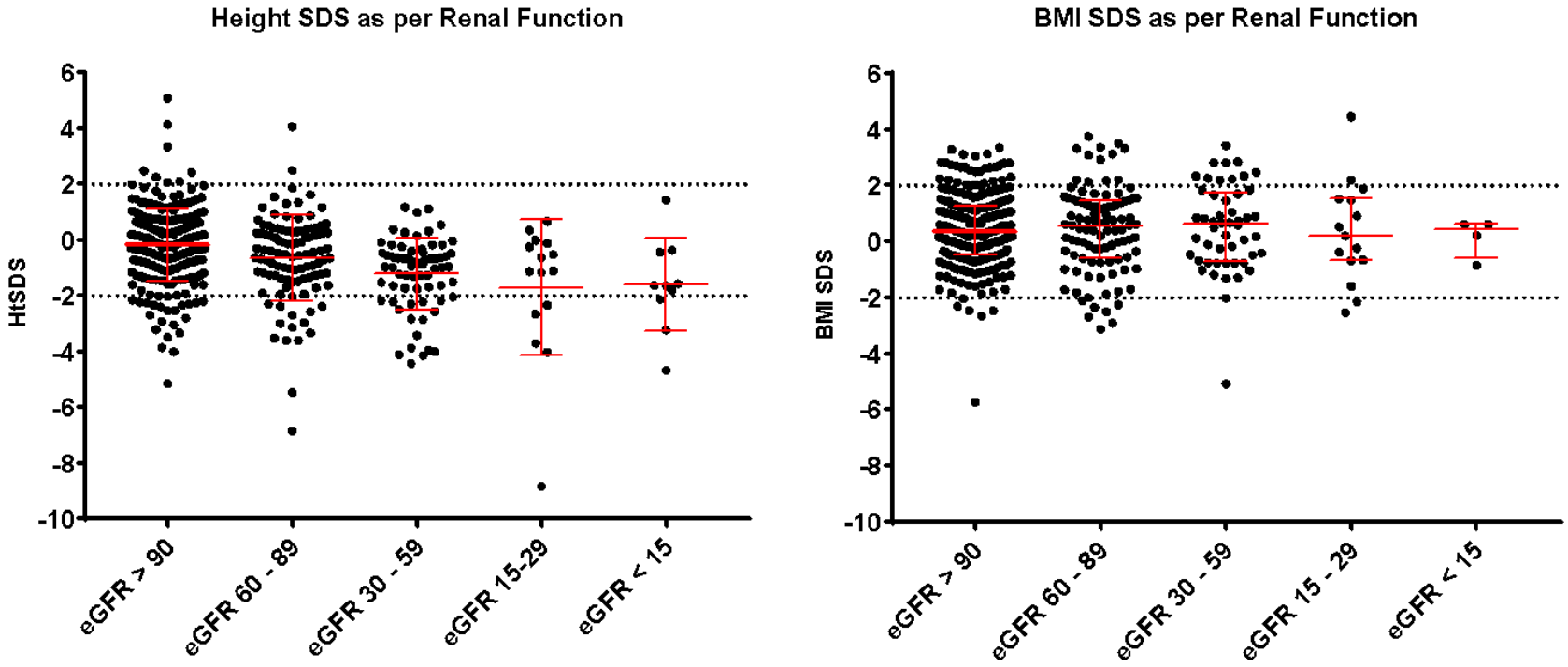


Figure 10. Height SDS and BMI SDS grouped as kidney function category (n=445).

The graphs depict height standard deviation scores (HtSDS) and body-mass index standard deviation scores (BMISDS) for different degrees of kidney impairment, as categorised by estimated glomerular filtration rate (eGFR). There is a general trend for those with more severe disease to have lower HtSDS, although there is a range of results with all eGFR categories having individuals with HtSDS<-2. A similar trend for BMISDS is not seen.

Association between eGFR and height SDS, weight SDS, and BMI SDS were sought by linear regression analysis. Analysis of all three groups together proved a statistically significant association between height SDS and eGFR (r^2=0.107, p=0.001), and was similar for weight SDS (r^2=0.094, p = 0.001). The same trend was shown for BMI SDS, but was not statistically significant (r^2=0.017, p = 0.059). Clinic groups were subsequently analysed separately.

For group 1 (general nephrology clinic), there was an association between height SDS and eGFR (r^2=0.034, p = 0.001), but not weight SDS (r^2 = 0.002, p = 0.357) or BMI SDS (r^2 = 0.004, p = 0.220).

In group 2 (post-transplantation), eGFR did not predict height SDS (r^2 = 0.010, p= 0.665), weight SDS (r^2 = 0.026, p = 0.499), or BMI SDS (r^2 = 0.015, p=0.610).

In group 3 (low clearance), eGFR did not predict height SDS (r^2 = 0.000, p = 0.920), weight SDS (r^2 = 0.070, p = 0.962), or BMI SDS (r^2 = 0.040, p = 0.352).

A multiple-linear regression model was constructed for each anthropometric measure SDS and eGFR, age, and sex for group 1. eGFR and age significantly added to the model to predict height SDS (r^2 = 0.058, p <0.0005) with increasing eGFR and age associated with greater height SDS. For weight SDS, only eGFR and age proved significant (r^2 = 0.019, p = 0.034), again with positive correlations. The model was unable to predict BMI SDS.

3.4. DISCUSSION

This is the first feasibility study to attempt to describe weight, height and BMI across all ages and stages of disease, and provide novel prevalence data for children with kidney disease in the UK, internationally and in Southampton Children's Hospital, using the routine collection of clinical data via hospital information technology systems.

As detailed above, data on growth in children with kidney disease is limited and mainly restricted to those with the most severe disease – those in receipt of dialysis and those that have received a kidney transplant. No growth information exists to describe the whole kidney population across the spectrum of disease.

These data show that 10% of the paediatric nephrology clinic have poor growth (HtSDS <-2); that obesity prevalence within the clinic are similar to that of the general population; and that kidney function may predict height SDS but not weight SDS or BMI SDS.

3.4.1. Stunting

Growth failure in paediatric CKD is associated with increased mortality (23, 24), with those with height SDS <-2.5 with a two-fold risk of death compared to those with height SDS >-2.5 (23), and increases with decreasing height SDS (24). From the total cohort, 11% of all height values recorded had a height SDS <-1.88, greater than the statistically likely 3%. The reasons for this are likely to include a percentage of children not fulfilling their growth potential due to poor kidney function, but also children and young people with other causes of short stature and medical therapy that has negative consequences on linear growth such as corticosteroids. Unfortunately, due to the lack of subject characterisation, these reasons cannot be determined.

3.4.2. Obesity

In this cohort of paediatric nephrology services attendees 12% of those in the general nephrology clinic (group 1) were obese (BMI SDS >2). This compares similarly to the general population, with rates of obesity in the UK population being 14% of children aged 2-15years (BMI >95th percentile) (153). In CKD, Wong et al reported a 'U'-shaped association between BMI and mortality, with low and high BMIs associated with an increased risk for death (24). Although traditionally nephrologists have been concerned about underweight patients, focus has now broadened to encompass both under- and overnutrition in relation to energy.

Obesity is becoming increasingly prevalent in CKD populations across the UK and Europe (71) and more prevalent than the canonical malnutrition state of low anthropometric scores and "protein-energy wasting" in both the conservatively-managed (18) and dialysis/post-transplant populations (71). It has been reported to be an independent risk factor for development of kidney damage and end-stage kidney disease (72, 73). The reasons behind obesity prevalence in this cohort are likely similar to those explored in the general population, with increased availability of nutrient-poor, energy-dense foods (76), coupled with levels of physical activity below recommended levels (child activity data from Sport England's Active Lives Children and Young People Survey states that only 18% of children and young people meet the recommended level of daily physical activity (154)). In those with CKD, it may be that dietary patterns and physical activity are similar to those in the general population. This is interesting as clinic attendees have access to healthcare professionals that others in the general population do not. It may be that such lifestyle aspects of children and young peoples' care are not addressed in the setting of the paediatric nephrology clinic, and the frequency of interactions is not great enough to enact lifestyle change and / or that healthcare professionals have inadequate training to recognise and intervene in such areas. Alternatively, those attending clinics are less able to perform adequate levels of physical activity due to ill-health and thus calorie requirements are less despite the abundance of calories available to children and young people in Western counties, like the UK.

3.4.3. Strengths of the study

Strengths of this study include the large number of children and young people in the cohort and that as a first step in examining the growth of the paediatric nephrology clinic, the lack of exclusion criteria allowed for a likely representative overview.

The use of electronic data has the potential to identify those children and young-people who are at risk and highlight them to healthcare professionals, such as dietitians to more formally assess them. For example, electronic systems could be programmed to email an alert to the clinical team if a child / young person's anthropometry lies outside of a given reference range (2SD, for example), and/or when there has been a change in the trajectory of growth (decrease in SDS of >0.25, for example). These examples of implementation of electronic data utilisation may increase the number of children / young people identified as at risk from growth failure, and enable identification sooner in their growth trajectory potentially allowing for earlier intervention and altering the course in a positive way.

This study may act as a template for the wider healthcare community that may be introduced into out-patient clinical settings to alert healthcare professionals to those with (or at risk of) growth failure.

3.4.4. Limitations

This study has several limitations, and care must be taken not to project these data as fully representative. Firstly, attendees at the paediatric nephrology service are diverse;with different stages of CKD from various aetiologies, to conditions in which kidney function may be additionally impaired, such as recurrent urinary tract infections.

Limitations also include the lack of longitudinal data. The study was a snapshot at a single point in time, and thus the identification of those at risk is only by absolute SDS and not a change over time. The study did not further characterise the cohort over and above their renal function where available. Some of the clinic attendees will have medical interventions already in place, or may have unusual but predictable growth trajectories (for example, those with a syndrome associated with short stature such as Turner and Down syndrome). Additionally, BMI SDS is just one method by which obesity is defined. Those with CKD have poorer lean mass (155), and thus, BMI may under-represent the percentage of attendees with increased adiposity (with relatively greater amount of adipose tissue to attain a given BMI). Fluid status in those with kidney conditions may also be altered, with the accumulation of interstitial water presented as oedema. Measures such as waist measurement cut-offs and body composition analysis may prove a better measure in this population.

Growth may be assessed in several different ways and height SDS is only one metric. Height velocity SDS and comparison to mid-parental height SDS may also be used, and may present different data. Unfortunately, these metrics were not captured in this study.

3.4.5. Future Directions

Future studies should include prospective, longitudinal data collection of growth data on children and young people with kidney disease with characterisation of medical and nutritional interventions. This would allow for a better understanding of growth trajectories of children and the influence of factors upon their course. Particular focus should be placed not only on the attainment of adequate final adult height, but also on the rising prevalence of obesity in the face of kidney disease, and the optimal way by which healthcare professionals can avoid their patients increasing cardiovascular and metabolic risk through its development.

3.4.6. Concluding Remarks

As evidenced by the above-described cross-sectional study, there is significant variation in the growth measures of children with kidney disease. It is important to understand the factors that influence these differences in order to enable healthcare providers to effect change to improve growth where possible.

In this chapter, growth has been explored in the context of paediatric CKD, reporting growth data as an overview of those attending a tertiary paediatric nephrology service in the UK and exploring kidney function (eGFR) as a contributing influence.

This study was unable to explore the nutritional influences on growth. Therefore, the possibility of micronutrient inadequacy in the paediatric CKD population is considered next, together with an exploration of the nutritional status in relation to growth and well-being of a child / young person with CKD. This is to more fully characterise children from a nutrition point of view to try and to identify potential risk factors / associations for poor growth.

3.4.7. Practice Points

- Applying a structured framework using IT to access growth data at the clinic level, information can be accessed and analysed to present patterns of growth. These data can be combined with clinical pathology data, such as serum creatinine concentrations to categorise patients.
- This level of analysis is limited here by the accessibility of patient characteristics and measures that are not usually captured electronically, easily or readily accessible outside of the individual patient level. The ability for different electronic systems to label, organise and share data generated in clinical practice would increase the ability to characterise patients; including identifying patterns in relation to measures of growth, such as height.
- All children and young people with CKD should have their height and weight measured and recorded electronically with SDS calculated at each healthcare encounter. Additional health data should be accessible to facilitate categorisation, and explore possible patterns of risk, such as in those with poorer growth.

4. REVIEW OF THE LITERATURE OF THE MICRONUTRIENT STATUS OF CHILDREN WITH CHRONIC KIDNEY DISEASE

Data informing clinical guidelines are limited with regards to vitamin and mineral status of children and young people with CKD. In order to explore the evidence-base in this area of nutritional status, the following chapter reports the results of a systematic-style review of literature. This will aim to demonstrate variation in the micronutrient status of children and young people with CKD and identify areas in which evidence is lacking.

Firstly, a framework to be used to methodically approach the literature is proposed. Secondly, two example nutrients are presented: Vitamin E and Selenium. These have been chosen due to their importance in the processes that protect the body against oxidative stress (as discussed earlier), and that anecdotally, blood concentrations of vitamin E are often elevated in CKD, and selenium dietary intake reported as low.

4.1. INTRODUCTION

Anecdote from clinical care suggests that there is a high prevalence of children at risk from nutritional inadequacy. To place this in the context of previous literature, a series of searches of the existing literature were performed.

4.1.1. Framework for the evaluation of the literature of micronutrient status

Although the supply of energy and nutrients to a person must clearly influence the structure and function of bodily systems and a person's resulting health, understanding the influence of individual nutrients in a living system is not easily determined. Firstly, the availability of individual nutrients is often linked to that of other nutrients. Secondly, disease states may add further complication with disruption of the usual homeostatic mechanisms. Kidney disease is such an example, with many nutrients not only needed for the healthy functioning of kidney tissue, but having handling of the nutrient and/or their metabolites by the kidney. A well-recognised example is vitamin A, in which the lack of the ability of the kidney to metabolise retinol-binding protein results in increased circulating concentrations, removal of retinol from hepatic stores and increased circulating retinol (156).

Nutrition research examining the micronutrient status of individuals is often reported as cohort studies of dietary intake and/or biological sample measures such as plasma zinc concentrations. These measurements in isolation are difficult to interpret and place into a clinical context for the practicing clinician. To facilitate the synthesis of the available literature in this area, a simple schema was developed in which to place reported results from the literature. Using such a framework, the literature may be placed into a logical context to understand where current literature exists and to what extent it is able to answer the questions necessary to make clinical decisions and to advise patients to alter intake through restriction, or supplementation, for example.

Figure 11 is a proposed schema that asks the question of whether a nutrient may be lacking in a target population. As with all experiments, interrogation of the literature should begin with an understanding of the physiology, and a reflection upon why in a given individual the nutrient is particularly important (step 1). In kidney disease, energy requirements may be increased, and as a result you may expect requirements of some B-vitamins necessary for energy metabolism to also be greater, for example. Step 2 asks whether from the existing literature there is evidence of altered status. Within this it is important to question the validity of the test being used. For example, does hair concentration of selenium truly reflect selenium status of an individual?

Unfortunately, most measures of nutriture have been validated in healthy individuals, and disease states may negatively influence this validity (serum zinc concentrations in the acute phase response, for example). Step 3 asks the evidence of the literature that altered status has a negative impact on the individual. Although it might be assumed that altered concentrations of a nutrient would disrupt structure and function of the system, there must be evidence that this is detrimental. Such negative impact is not necessarily absolute as altered availability of nutrients may be beneficial in particular circumstances. For example, the sequestration of iron for which the human has evolved specific mechanisms to limit its availability to pathogenic organisms. Step 4 asks for the evidence that intervention (for example, supplementation) improves status, and, step 5, that this intervention is associated with improved outcomes. Following these five steps, evidence-based guidance can be drawn up (step 6), and its implementation assessed (step 7). Often due to lack of available evidence but with the need for clinical practice guidance, the questions in this framework are not answered by the literature but clinical practice guidance formulated. Although such guidance may be useful for the healthcare professional, there must be an awareness of the potential lack of evidence behind such recommendations and an effort to fill in gaps in knowledge. It is only through a structured approach to evaluation of evidence that optimal nutritional care be determined for patients and for healthcare professionals to succeed in providing adequate nutritional care.

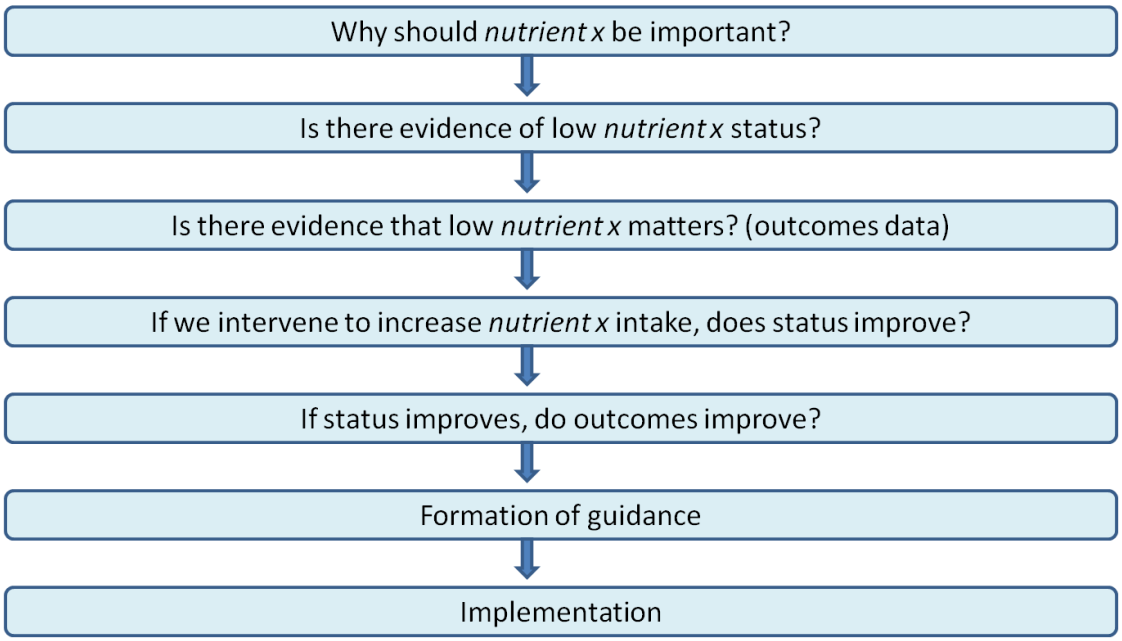


Figure 11. A stepwise approach to interrogating the literature of micronutrient status of children with CKD.

4.2. REVIEW OF THE VITAMIN E STATUS OF CHILDREN WITH CHRONIC KIDNEY DISEASE

4.2.1. Introduction

There are eight vitamers of vitamin E, which are classified into two groups: the tocopherols and the tocotrienols. Traditional thought is that only alpha-tocopherol is biologically active and there is no capacity for the body to convert other vitamers into this form. There is some suggestion that other vitamers may have function (157), but much of the existing literature and clinical guidance has focused solely on α-tocopherol; including the composition of dietary intake values.

Vitamin E is absorbed in the jejunum by passive diffusion following emulsification and micelle formation with bile-salts. Once in the enterocyte, tocopherols are incorporated into chylomicrons and transported to the liver, where alpha-tocopherol is transferred into very low-density lipoproteins (VLDLs), and then transported by the circulation to tissues and taken-up with lipoproteins. Elimination of vitamin E is predominantly through the faecal route, either not absorbed (30%) or in bile (158), with some additional loss through the skin (158). Alpha-tocopherol not bound to its transport protein is metabolised in the liver (to 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman, i.e. Alpha-CEHC. This metabolite is excreted in the urine (159).

Kidney interactions

Oxidative stress is greater in those with CKD than healthy peers and is associated with inflammation and uraemia found in this population. It is associated with risk of cardiovascular disease and mortality (160). There is evidence of increased oxidative stress even in mild CKD, and increases with degree of impairment and dialysis (161-164). Duration of dialysis (165) and the use of antioxidants within dialysis machines may also have an impact (166). Vitamin E is an antioxidant, acting to protect cellular damage via lipid peroxidation of unsaturated fatty acids within the phospholipid membrane by reactive species.Singlet molecular oxygen ($^1O_2$) is produced following lipid peroxidation, and reacts with lipid, protein and DNA, causing cellular damage. α -tocopherol 'quenches' this reactive molecule, and is additionally able to act as a proton-donor to reactive free-radical species, such as peroxyl radicals, neutralising them and ameliorating damage. α–tocopherol is superior to other vitamers in both its ability to inactivate $^1O_2$ (167), and donate hydrogen ions. Although carotenoids are better $^1O_2$ inactivators, plasma concentrations of vitamin E are much higher and therefore physiologically more significant (72). Following its oxidation when reacting with free-radicals, α-tocopherol may either interact with peroxyl radical to produce a metabolite or be reduced (and regenerated) via reducing agents including vitamin C and glutathione.

CKD is known to be a dyslipidaemic state with elevated triglycerides and triglyceride-rich lipoproteins (VLDLs, chylomicrons and chylomicron-remnants), and is associated with postprandial lipaemia (168). The mechanism of hyperlipidaemia in CKD is yet to be fully elucidated, but it has been observed that there is decreased lipoprotein lipase activity with both decreased gene expression and alteration of the inhibitor/activator ratio between apolipoprotein-C-III/apolipoprotein-CII, and insulin-resistance which increases hepatic synthesis of VLDL. Those receiving dialysis have additional potential mechanisms: In peritoneal dialysis (PD), the glucose load of dialysis fluid can increase insulin resistance, and in those receiving haemodialysis (HD), repeated heparinised dialysis sessions has been shown to decrease epithelial lipoprotein lipase concentrations. As vitamin E is transported bound to lipoproteins, blood concentrations increase with increasing lipid concentrations (169). Increased serum vitamin E concentrations in themselves may not imply truly elevated status because of their

displacement in high lipid states. In addition, there is evidence that there is increased oxidative stress in those with CKD, and therefore any given range of vitamin E may need to be higher for those with CKD due to extra 'demand' of the vitamin's role in the antioxidant pathways. It is unclear if the elevated serum vitamin E is due to displacement from hyperlipidaemia, and may cause vulnerability of cellular membranes to damage, despite apparent 'high' concentrations on blood analysis.

Interaction with other nutrients

High concentrations of vitamin E can reduce absorption of carotenoids (170), but the exact relationship and how this relates to dietary reference values, particularly in those with kidney disease has not been described.

It has been well-documented that high vitamin E concentrations have an antagonistic effect on vitamin K pathways, with documentation of increased bleeding tendency in animal experiments (171), decreased risk of thromboembolism (172) and decreased carboxylation of prothrombin and other markers of vitamin K status (173). Suggested mechanisms of this interaction include: shared enzymatic machinery needed for ω-oxidation, then β-oxidation; and role of vitamin E in promoting the xenobiotic pathways of excretion of vitamin, but the exact interaction(s) are yet to be elucidated.

Vitamin K may prove to be an important nutrient in those with CKD (174), but further limitation of vitamin-K dependent mechanisms by elevated vitamin E in those with CKD requires more exploration.

Current guidance

Due to its ability to alleviate oxidative stress in patients at risk of CVD, CKD and dialysis patients (aged < 9 years) should receive the daily recommended intake of vitamin E (2).

There is a need to review the literature in a structured way in order to understand the existing literature and identify areas that are lacking.

4.2.2. Methods

A systematic approach was undertaken with the search strategy outlined in *Appendix 11.2*. Articles were reviewed and a narrative review formed. Additionally, a meta-analysis for those children with pre-dialysis, conservatively-managed CKD was undertaken. Risk of bias was assessed through Newcastle-Ottawa Quality Assessment Scale (175).

4.2.3. Results

Numbers of articles identified with each search term are reported in *Appendix 11.2*. Articles were reviewed firstly by abstract and then full article as needed. As described in *Figure 12*, 43 articles were initially identified, with a total of 11 that were appropriate for inclusion in the review, and two suitable for the meta-analysis of pre-dialysis, conservatively-managed CKD patients. All articles were at high risk of bias (see *Appendix 11.2*).

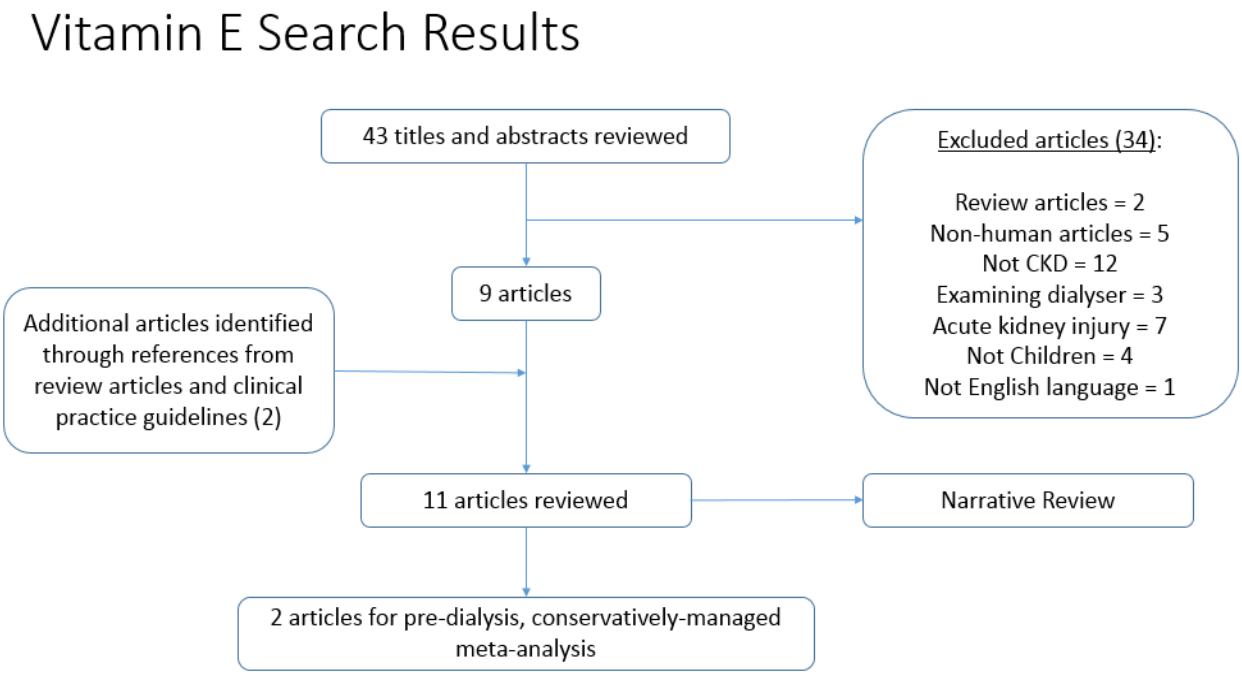


Figure 12. Diagram describing excluding articles, and those included in the review for the vitamin E status of children with CKD.

Narrative Review

A narrative review was undertaken due to the limited and disparate nature of the literature regarding vitamin status.

Dietary intake

One study in paediatric CKD patients reporting the dietary intake of vitamin E was identified, and found an adequate intake in those receiving PD (176). In this study, Coleman et al, report the dietary intake of seven children on PD as assessed by three-day dietary, and six out of seven reported intakes greater than recommended intakes. No studies were identified reporting dietary intakes in haemodialysis patients or pre-dialysis, conservatively-managed CKD patients.

Vitamin E status

Literature that exists generally examines the status of vitamin E in receipt dialysis; several of which report combined HD and PD cohorts.

Haemodialysis

In the adult CKD HD population, plasma vitamin E concentrations have been reported as low (177), normal(178), and high (179), and that a dialysis session does not affect concentrations (178, 180), with an absence of vitamin E in dialysis effluent (180). In paediatric populations, low (78), normal (79, 181), and high (80) plasma concentrations have also been reported.

Peritoneal dialysis

In the adult CKD PD population, plasma vitamin E concentrations have been reported low and high (182-184). In paediatric populations, low (78, 185), normal (79) (176), and high (186) concentrations have been reported.

Coleman and Watson (1992) reported adequate vitamin E intake (RDA, 1989) in seven paediatric PD patients (176).

Erythrocyte concentrations of vitamin E have also been measured. As erythrocytes are particularly prone to oxidative stress, it is not surprising that these concentrations have been reported as low in children (185).

Naseri et al (2015) reported their Iranian data of 43 dialysis patients (12 PD and 25 HD, mean age of 13.9 years). As an entire cohort, serum vitamin E concentrations as measured by HPLC were more likely to be low than normal or elevated with 72% low (as defined as <3, 6, and 5 µg/ml for children, teenagers and adults, respectively). 21% were within the normal reference range and 7% high (>9, 10 and 18ug/ml, respectively). For their HD patients (n=25), 84% had low concentrations, 12% normal, and 4% high. A similar picture was observed in their PD patients (67% low, and 33% normal). The authors reported mortality of their cohort over a four-year period of 25.6%. Those with low vitamin E concentrations did not have a higher incidence of death (p=0.175). This mortality is relatively high compared to UK data. The latest UK renal registry (2019) data (3) quotes 83 deaths in 1,575 (5.3%) children and young people starting RRT between 2003 and 2016, and four-year survival after initiation of RRT of between 87.6% (0-2 years of age) and 97.7% (8-12 years of age). As Naseri et al do not explore other measures of nutritional status (without basic anthropometric measures, for example), the reason for low vitamin E concentrations and higher mortality may be explained by a poor nutritional state, although severe malnutrition should have not been present with quoted serum albumin concentrations within the normal reference range. This study may not be representative of children and young people in other healthcare settings; including the UK.

Zwolińska et al (2006) (181) reported both plasma and erythrocyte concentrations of vitamin E in 21 haemodialysis patients, finding significantly lower plasma and erythrocyte concentrations compared to a control group (p<0.001). The author additionally measured dialysate concentrations of vitamin E, but unfortunately units for this measure are missing from the manuscript (0.123 SD±0.019). This same study report vitamin A concentrations that are lower than those of healthy controls. This finding is unusual in the context of CKD, in which elevated concentrations would be expected (80, 187), and casts doubt in the validity of these results. Reasons for this discrepancy may include measurement error in using HPLC which requires skilled analysis. A study of 16 laboratories found that only seven (44%) produced acceptable serum retinol results with as much as a 120% variation for measurements of retinol in sera (188).

Drukker et al (79) report the plasma vitamin E concentrations of children from Israel across the spectrum of treatment modalities (10 PDCM, 10 HD, 10 PD, and 10 with a functioning kidney transplant (with a mix of function – five good, five with a degree of graft impairment secondary to chronic rejection). Fasted serum vitamin E concentrations were within normal reference range for the author's laboratory (0.76 ±0.19mg/dl), and despite the presence of dyslipidemia (mostly hypertriglyceridemia) concentrations remained within the normal reference range after correction for total lipids (0.6-0.8mg/g).

Pre-dialysis, conservatively-managed CKD

In paediatric populations, a small number of studies have reported blood concentrations (79, 181, 189).

Iughetti et al (2008) (189) report the findings of a small cohort of children with PDCM CKD (n=9) from Italy. Authors found that lipoprotein composition, including that of tocopherols, were different in CKD compared to healthy controls. No difference was observed in vitamin E (α-tocopherol) concentrations (CKD: 22.17 SD±5.38, versus Controls: 14.45 SD±4.11µmol/l, p=0.492) – including after normalisation for plasma cholesterol concentrations (CKD: 5.05 SD±2.24 versus 4.58 SD±0.73nmol/mg, p=0.580). The mean GFR for the cohort was 41.1 ml/min/1.73m^2 (range: 9.6 to 75). Of note, mean concentrations of α-tocopherol in the CKD cohort was greater than the normal reference range used by clinical laboratories in the UK (10-21µmol/l).

In the article discussed above, Zwolińska et al (2006) (181) whilst examining markers of oxidative stress reported both plasma and erythrocyte concentrations of vitamin E in a paediatric cohort of 46 participants - grouped as moderate (serum creatinine <265.3µmol/l, n=32) and severe (serum creatinine ≥265.3µmol/l, n=14). The authors report significantly lower plasma and erythrocyte concentrations compared to a control group (n=27) (p<0.001). The authors additionally measured dialysate concentrations of vitamin E, but unfortunately units for this measure are missing from the manuscript (0.123 SD±0.019). This same study reports vitamin A concentrations that are lower than those of healthy controls. This finding is unusual in the context of CKD, in which elevated concentrations would be expected from both adult (187) and paediatric (80) data, and casts doubt on the validity of these results.

Meta-analysis of children with pre-dialysis, conservatively-managed CKD

Of the identified studies, two studies report PDCM and a healthy control group serum vitamin E concentrations. Zwolińska et al (2006) (181) report two groups, both plasma and erythrocyte concentrations. This gives a total of five concentrations in which to compare through a meta-analysis. To control for the differences in methodologies and samples, the standardised mean difference between the CKD group and the healthy controls along with confidence intervals were calculated. Results are displayed in *Figure 13*.

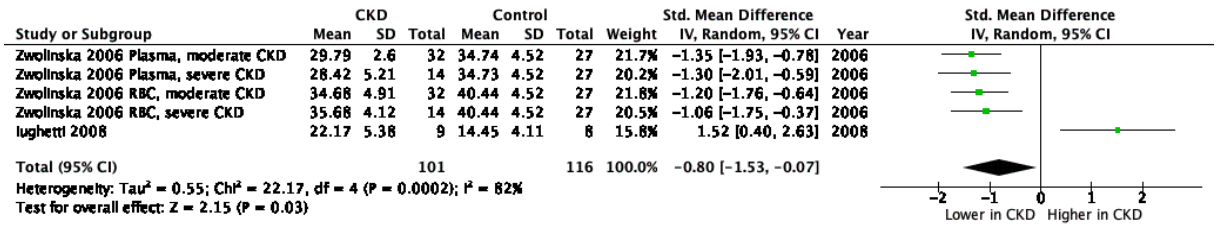


Figure 13. Meta-analysis of studies assessing the vitamin E status of children and young people with pre-dialysis, conservatively-managed CKD.

Supplementation studies

Supplementation of vitamin E has been offered as a potential intervention. There is murine model evidence that vitamin E supplementation may be beneficial for both kidney injury (190), and associated cardiovascular disease (191). In human subjects, interventional studies with supplementation of vitamin E show mixed results; showing

benefit (conservatively managed (192-194), PD(194), HD(194, 195)), and lack of benefit (conservatively managed (196), PD or HD (197)).

Meta-analysis of studies evaluating supplementation in different populations with vitamin E may have not shown benefit and may increase all-cause mortality (157), but there is thought that this may be due to supplementation with α–tocopherol alone, which decreases availability of the other vitamers, particularly tocotrienols, that may hold important roles that have not fully been elucidated (157).

Of the identified articles, four reported results of supplementation in the paediatric CKD population.

Tahzib et al, 1999 (198) report the findings of a small supplementation trial in children with focal-segmental glomerulosclerosis (FSGS) versus those with non-FSGS aetiology CKD (11 and 9 children, respectively). A significant improvement in proteinuria is reported in the FSGS group with no change in those of other aetiology following supplementation with 200 IU twice-daily for three months. The authors do not report the vitamin E status of the children, nor report measures of oxidative stress to better understand the mechanisms by which this supplementation has exerted its effect. Additionally, there is a lack of a FSGS-no supplementation control group. Despite its apparent positive effect on proteinuria, no change in eGFR was observed, although the length of the study was perhaps not long enough for this to be appreciable. The same authors describe their routine supplementation using vitamin E in those with FSGS since 1990 without side-effects based on animal studies and this clinical trial (199).

Modarressi et al, 2012 (200) report in a conference abstract the results of a vitamin E supplementation in paediatric HD cohort (n=15). In addition to subcutaneous erythropoietin 120 u/kg/week, oral folic acid 1 mg/day and iron 2 mg/kg/day, oral vitamin E 200 u/day for 3 months resulted in significantly higher concentrations of haemoglobin (Hb=11.4+/-1.7 vs. 10.1+/-1.9 g/dl and Hct=35.3 +/-5 vs. 31.3+/-6%, $P<0.05$). Although vitamin E status, and other factors that might contribute to haemoglobin differences (HD prescription, other nutritional parameters, aetiology of CKD etc.) are not reported and compared, the difference between the mean haemoglobin concentrations was 1.3g/dl, a roughly 10% difference that may translate into a clinically significant difference.

Farid et al, 2009 (201) reported that 21 of the 34 children studied (12 PDCM, 22 receiving HD) had evidence of elevated lipid peroxidation (elevated F2-isoprostane), and vitamin E supplementation (400 IU/day for 2 months) resulted in a significant decrease. Unfortunately, the authors do not report the vitamin E status of individuals. It may be variation in vitamin E status that explains the response/lack of response to this supplementation. These data are supported by the results from Cristol et al, 1997 (202) and Németh et al, 2000 (203). Cristol et al reported in that in a small cohort of adult HD patients (n=7), that vitamin E supplementation (500mg daily for 6 months) resulted in a significant increase in erythrocyte vitamin E and a reduction in rhEPO with stable haemoglobin concentrations. Németh et al (203) describe a supplementation trial in paediatric haemodialysis patients (n=10) and demonstrated a lack of the increase in oxidative stress induced by rhEPO administration with vitamin E supplementation, and moreover, a more rapid increase in haemoglobin compared to the same patients without supplementation. This may be due to increase in erythrocyte survival from decreased oxidative stress within the erythrocyte or by limiting endogenous erythropoietin expression suppression from oxidative stress–

mediated pathways (204). Similar to these other supplementation trials, vitamin E status of participants is not reported.

Larger studies are required with analysis of other potentially contributing / confounding factors; and perhaps analysis of lipid peroxidation that add evidence of the oxidative stress mechanism of anaemia and its potential improvement with vitamin E supplementation.

Summary of Narrative Review

Adopting the above framework for reviewing the available literature (*Figure 11*), the questions are thus answered:

1. ***Why should vitamin E be important?***

As discussed above there is a clear theoretical risk of altered status on vitamin E in those with CKD with potential risk of altered oxidative stress (and therefore requirements).

2. ***Is there evidence of altered vitamin E status in children with CKD?***

As outlined in the above narrative and systematic reviews, existing evidence is limited and is at high risk from bias. There is a lack of literature regarding dietary intake of vitamin E and biological markers of vitamin E status. The data that does exist is of small cohorts.

3. ***Is there evidence that altered vitamin E status in children with CKD matters?***

Although a theoretical risk may be hypothesised, there is no significant evidence that altered vitamin E status in children with CKD is associated with altered clinical outcomes. It may be that altered vitamin E status is associated with altered outcomes; with increased oxidative stress altering endothelial damage, cardiovascular disease risk, and mortality, but there is no literature available examining this question.

4. ***Does intervention change vitamin E status?***

A number of supplementation studies have been published in children (and adults) with CKD, although they do not examine vitamin E status (intake or biological measure). There is a suggestion that supplementation may improve haematological responsiveness to erythropoietin supplementation in dialysis patients, but studies are small, and at risk from bias.

5. ***Is this change associated with improved outcomes?***

Although not associated with a described change in vitamin E status, supplementation with vitamin E may result in a more rapid correction of anaemia. Due to the lack of status measurement it is unclear as to whether the supplementation in those individuals that showed improvement was due to a correction of low status to that within the normal, or that supra-normal delivery is associated with these outcomes. Larger studies that include assessment of nutriture are needed in order to begin to answer this question. Caution should be advised in supplementation without a clear understanding of the underlying mechanisms and interactions, as

supplementation trials for the prevention of cardiovascular disease (and cancer) have been associated with an increase in all-cause mortality (205).

4.2.4. Summary and conclusion

In this review the existing literature regarding vitamin E status in paediatric CKD has been explored, identifying a lack of studies to compose evidence-based guidance; including support of the current recommendation for clinical care.

Using a structured approach to examine the literature, a number of opportunities to better understand vitamin E in the context of paediatric CKD can be seen, and their exploitation may decrease unwanted variability in the clinical course of children, improve patient outcomes, and decrease healthcare costs.

Limited data exist for the vitamin E status of children with CKD, this is despite the theoretical influence of antioxidant nutrients, including vitamin E having influence over both progression of kidney disease and its complication, such as anaemia. Studies reporting vitamin E status have reported conflicting results with statuses lower than, the same as, and greater than the healthy population/controls. Supplementation trials that exist are small and have not reported vitamin E status at baseline. It has been suggested that FSGS may be a specific sub-group of CKD that may benefit from supplementation. True requirements of vitamin E for children and young people with CKD have not been determined, but theoretically may be greater due to the presence of increased oxidative stress. Small trials suggest that those in receipt of HD, and administration of erythropoietin (with their increased associated oxidative stress) may benefit from vitamin E supplementation with regards to anaemia and rhEPO dose, although larger studies are required. That being said, supplementation of vitamin E should be carefully studied as supplementation on the population level has been associated with an increase in all-cause mortality.

4.2.5. Recommendations clinical practice

Due to its ability to alleviate oxidative stress in patients at risk of cardiovascular disease, patients with CKD should receive the recommended daily intake of vitamin E. The role of additional supplementation above this, even in the pro-oxidative stress context of HD is yet to be proven.

Caution should be exercised with regards to vitamin E supplementation in paediatric CKD despite a theoretical benefit and some promising small study results. As there is a scarcity of robust evidence to support supplementation of vitamin E with evidence of increased mortality in supplementation trials in other populations – which cannot be currently explained.

4.2.6. Recommendations for future research

Much research directed towards understanding potentially altered vitamin E handling, and requirements in paediatric CKD, and the associated dyslipidemia is required. Perhaps first steps may be to explore the dietary intake of vitamin E and blood concentrations of vitamin E in children with CKD. The relationship with vitamin E status and hydrogen peroxide-induced haemolysis in children with CKD in addition to exploring how decreased kidney function alters alpha-CEHC concentration, and the influence of non-eliminated alpha-CEHC, would be beneficial.

As with all antioxidants, vitamin E may prove useful in rebalancing the redox scales unbalanced in those with CKD. More study is warranted in the role of supplementation of children with CKD, both using vitamin E and other antioxidants, including in combination (including selenium and niacin required for glutathione peroxidase activity, and vitamin C). This may be especially interesting in-view of the small studies reporting the potential benefit of vitamin E supplementation and haemoglobin.

As nutrition is the cornerstone for patient care, a concerted effort should be undertaken to increase knowledge about requirements of nutrients; including vitamin E to facilitate healthcare professionals supplying optimal nutrition to their charges.

4.3. REVIEW OF THE SELENIUM STATUS OF CHILDREN WITH CHRONIC KIDNEY DISEASE

4.3.1. Introduction

Selenium is a mineral that acts as a cofactor in several enzyme processes; there are around 25 selenoproteins. The action of which include: thyroid hormone metabolism; antioxidant defence; and immunological function (206). There are several glutathione peroxidase (GPx) enzymes, found in either the cytosol, mitochondria, or associated with the cellular membrane, and depending upon tissue type. The crucial role of GPx means that reduction in the function of selenoprotein results in cellular damage due to uncontrolled oxidative stress.

The selenium content of foodstuffs is highly variable depending upon the environment in which the food is raised. Selenium is randomly incorporated into sulphur-containing amino acids (cysteine and methionine) in place of sulphur; i.e., selenocysteine and selenomethionine. Selenium also exists independent of amino acids, as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). These forms are transported via a sodium-dependent transporter and passively absorbed, respectively, whilst amino acids have specific transporters.

In the bloodstream, selenium is transported to the liver bound to albumin or as free amino acids. Selenium is incorporated into selenoprotein P (50 - 80 %) in the liver for transportation, or as glutathione peroxidase-3 (GPx3) from the kidneys.

The Primary excretion pathway for selenium and mechanism by which homeostasis is maintained is via the urine as selenometabolites, the commonest of which is methyl-seleno-N-acetylgalactosamine (207).

Kidney interactions

There is some evidence in animal models that selenium may have a protective role against renal injury. Firstly, in rats, deficiency is associated with AKI (208),and supplementation correlated with improved serum creatinine, urea, and histopathological changes in cisplatin-induced Aksoy (209) and gentamicin-induced (210) AKI. When given in combination with erythropoietin in a murine model, selenium seemed to bestow some benefit in ischemia-reperfusion injury (211). Evidence in human subjects is lacking, although a randomized, controlled trial by Ghorbani et al in 2013 found that cancer patients who received cisplatin therapy had a decreased incidence of AKI when pre-treated with selenium (212).

Current Guidance

Patients should receive a daily dietary intake that meets the recommended intake, but routine supplementation is not recommended (2).

There is a need to review the literature in a structured way in order to understand the existing literature and identify areas that are lacking.

4.3.2. Methods

A systematic approach was undertaken with the search strategy outlined in *Appendix 11.2*. Articles were reviewed and a narrative review formed. Additionally, a meta-analysis for those children with pre-dialysis,

conservatively-managed CKD was undertaken. Risk of bias was assessed through Newcastle-Ottawa Quality Assessment Scale (175).

4.3.3. Results

Numbers of articles identified with each search term are reported in *Appendix 11.2*. Articles were reviewed firstly by abstract and then full article as needed. As described in *Figure 14*, 25 articles were initially identified, with a total of six that were appropriate for inclusion in the review, and two suitable for the meta-analysis of pre-dialysis, conservatively-managed CKD patients. All articles were at high risk of bias (see *Appendix 11.2*).

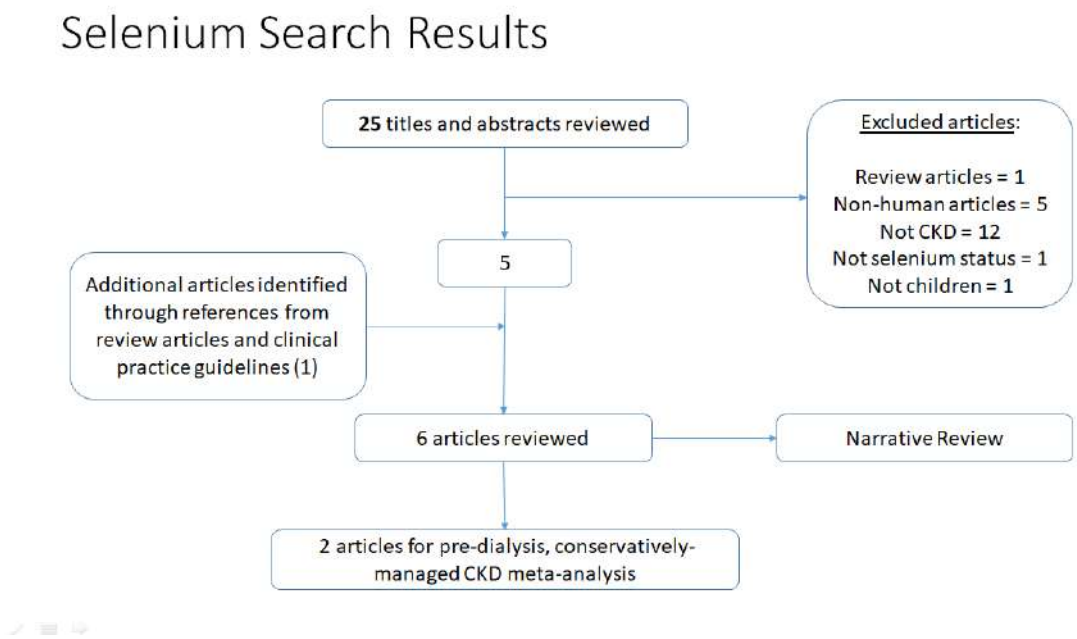


Figure 14. Diagram describing excluding articles, and those included in the review for the selenium status of children with CKD.

Narrative Review

A narrative review was undertaken due to the limited and disparate nature of the literature regarding vitamin status.

Dietary Intake

No studies reporting selenium dietary intake in children with CKD were identified on review of the literature. Ortac et al (213) attempted to quantify dietary selenium intake, but the return of dietary data from participants was poor enough that this element of their study was abandoned.

Selenium status

Selenium status has been assessed through serum concentration, hair and nail concentrations, as well erythrocyte levels and as activity of the selenoproteins, such as glutathione peroxidase (GPx).

Esmaeili and Rakhshanizadeh (2019) (214) report the trace element concentrations of a cohort of CKD patients from Iran into treatment modality (pre-dialysis, conservatively-managed CKD; HD; PD and healthy controls). Although serum selenium concentrations were lower in those in receipt of dialysis, the authors report no difference between selenium concentrations in conservatively-managed CKD (mean GFR = 8 ml/min/1.73m^2) compared to healthy controls. This study suggests that those on dialysis are at greater risk from nutritional inadequacy with lower selenium status, but the study does not directly explore the reasons for this. Moreover, the average concentration of the group with the lowest selenium concentration (CAPD, 100.44 µg/l) is within the normal reference range for our local hospital (1.28 µmol/l; 0.7-1.7 µmol/l), so the clinical significance of this statistical difference requires further exploration. The study does not explore the role of dietary intake, and although the authors postulate that the reported lower status in dialysis patients is secondary to dialysis losses, their study did not report dialysis effluent losses of selenium to justify their position.

Ortac et al (213) reported selenium status in 93 Turkish patients, including 32 patients with conservatively managed CKD (GFR=27ml/min/1.73m^2), 42 PD patients, 19 HD patients and 34 healthy children. Hair selenium concentrations and GPx activity were significantly lower in those with CKD compared to the control group. The authors report no correlation with disease severity (GFR) for either hair selenium concentrations or GPx activity, and a lack of agreement between hair selenium and GPx activity. Of note the healthy control group had a mean GFR of 84 ml/min/1.73m^2, which is not normal. The authors do not describe the selection process of the control group. Although an attempt was made to predict dietary intake, the authors report poor familial compliance with the study protocol in return of completed dietary data to characterise dietary selenium. Although the authors state that participants did not have contact with selenium containing shampoo products, they do not qualify how this was achieved. Moreover, hair and nail selenium concentrations are not felt to represent selenium status (215).

Joyce et al (2018) from the UK reported selenium concentrations in their dialysis cohort study of 47 children and young people (80). Their cohort consisted of 19 PD and 28 HD patients. 65% achieved normal ranges of selenium, with low concentrations reported in 28%, and high in 7%. As in the above studies, dietary intake and dialysis losses are not characterised to understand the variation in those children and young people whose concentrations lay outside of the normal reference range.

Zwolińska et al (2004) report an examination of oxidative stress in 46 Polish children and young people with pre-dialysis CKD. Concentrations of selenium both in plasma and erythrocyte were lower in those with CKD compared to healthy controls. On analysis between those with less or more severe disease (serum creatinine above or below 265.3µmol/l), no difference was reported. Moreover, the average plasma values for the more severe disease group was 0.87 µmol/l (SD± 0.12) within the range found in the general population. This study did not evaluate dietary selenium intake. The same group reports the selenium status (plasma and erythrocyte concentrations) in the dialysis patient cohort (CAPD=10, HD=21, control 27) (216). The study reported dialysis patients having lower selenium concentration compared with their control group, but with mean value within the normal reference range (0.74 µmol/l compared to 1.07 µmol/l). Change in plasma and erythrocyte selenium concentration before and shortly after starting HD were significantly different, suggesting possible losses, although the effluent was not analysed. This reported study has the same limitation to those described above, without dietary characterisation.

Sommerburg et al (2002) report GPx activity in a cohort of children on HD. The authors report GPx in this cohort as almost twice as high than their other analysed groups. The selenium status of the children are not reported.

As described above, in paediatric PDCM patients, lower biological samples have been reported (213, 217). GPx activity has also been found to be lower compared to healthy controls, but with contradictory results regarding correlation between GPx activity and renal function; and activity and selenium status (213, 217). Low results were also described in those receiving PD and HD despite only trace amounts of selenium in ultrafiltrates (216), but with contradicting studies (186). The above data is also limited by the lack of corresponding dietary intake analysis, as intake variability is likely to be the main determinant of status. In children and young people with blood concentrations lower than control groups, average concentrations are within the normal reference range.

The paediatric data are concordant with adult patients; with selenium being likely deficient in those receiving haemodialysis (218-220). In adults, whole blood and serum selenium has been reported to be correlated with degree of renal impairment (Yilmaz et al, 2015). CKD patients with low selenium have increased mortality, especially due to infection (221), but this serum selenium may be a reflection of nutritional status rather than causally-related; as serum selenium correlates with nutritional status in dialysis patients (222, 223). Some studies evaluating conservatively managed CKD patient have demonstrated that selenium supplementation increases selenium levels, reduces products of oxidative stress and increases plasma and erythrocyte GPx activity (208), but how (if at all) this converts into clinical outcomes is yet to be determined.

In conclusion, current guidelines for intake are based on general population and although there is no data on selenium ***intake*** of children with CKD, but the general population are at risk from not meeting needs so likely CKD children are too. There is a small body of evidence suggesting selenium ***status*** may lower in children with CKD, and this is concordant with literature from the adult CKD population. The mechanisms whereby this deficiency may result is unclear, and what implication this may have, or the effect of supplementation upon the health of children with CKD remains to be explored.

Meta-analysis of children with pre-dialysis, conservatively-managed CKD

Of the identified studies, two studies report PDCM and a healthy control group's plasma selenium concentrations. Zwolińska et al (2004) (217) report two groups, both plasma and erythrocyte concentrations. This gives a total of five concentrations in which to compare through a meta-analysis. To control for the differences in methodologies and samples, the standardised mean difference between the CKD group and the healthy controls along with confidence intervals were calculated. Results are displayed in *Figure 15*.

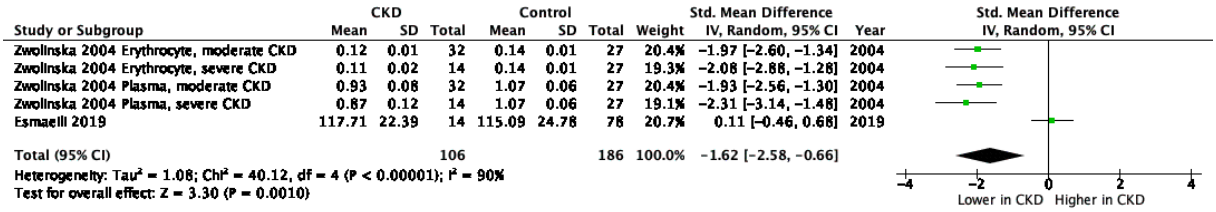


Figure 15. Meta-analysis of studies assessing the selenium status of children and young people with pre-dialysis, conservatively-managed CKD.

Supplementation studies

There are some animal studies that suggest that selenium supplementation may bring benefits for those with kidney disease. In chickens, selenium supplementation afforded protection against chromium IV-induced nephrotoxicity and oxidative damage (224). In mice, selenium supplementation had protective effects of sodium azide-induced oxidative stress (225). In diabetic kidney disease, a systematic review and meta-analysis of antioxidant supplementation was shown to have a positive effect of albuminuria, although no other markers of disease progression (226). There are no paediatric CKD supplementation studies available for review.

Summary of Narrative Review

Adopting the above framework for reviewing the available literature, the questions are thus answered:

1. ***Why should selenium be important?***

As discussed above there is a clear theoretical risk of altered status of selenium in those with CKD with potential altered risk of altered oxidative stress (and therefore requirements).

2. ***Is there evidence of altered selenium status in children with CKD?***

As outlined in the above review, evidence that exists is limited and is at high risk from bias. There is a lack of literature of dietary intake of selenium and biological markers of selenium status. The data that does exist is of small cohorts. Only one study was identified that reported dialysis losses, the clinical significance of this is difficult to assess.

3. ***Is there evidence that altered selenium status in children with CKD matters?***

Although a theoretical risk may be hypothesised, there is no significant evidence that altered selenium status in children with CKD is associated with altered clinical outcomes. It may be that altered selenium status is associated with altered outcomes; with increased oxidative stress altering endothelial damage, cardiovascular disease risk, and mortality, but there is no literature available examining this question.

4. ***Does intervention change selenium status?***

There were no identified selenium supplementation trials.

5. ***Is this change associated with improved outcomes?***

There were no identified selenium supplementation trials.

4.3.4. Summary and conclusion

In this review the existing literature regarding selenium status in paediatric CKD has been explored, identifying a lack of studies to compose evidence-based guidance; including support of the current recommendation for clinical care.

Using a structured approach to examine the literature, a number of opportunities to better understand selenium in the context of paediatric CKD can be seen, and their exploitation may decrease unwanted variability in the clinical course of children, improve patient outcomes, and decrease healthcare costs.

Limited data exist for the selenium status of children with CKD, this is despite the theoretical influence of antioxidant nutrients, including selenium having influence over both progression of kidney disease and its complications such as cardiovascular disease.

4.3.5. Recommendations clinical practice

Ensure adequate intake of selenium; and if deemed at risk of deficiency (or signs/symptoms) measurement of serum selenium as a marker of status could be made, although functional tests are not readily available in clinical care. Consideration should be made of supplementation for a trial period followed by reassessment; evaluating improvement in appetite, growth velocity, signs and symptoms of low status (lethargy, muscle-aches etc.), but caution should be exercised with regards to selenium supplementation due to the relatively small range between recommended intakes and upper tolerable limit.

4.3.6. Recommendations for future research

It may well be that glutathione peroxidase, primarily synthesized in the kidney and altered in relation to renal function. The conservation of selenium through the reabsorption of selenoproteins filter by the kidney is likely to be impaired by the dysfunctional kidney either through damaged mechanisms or through over saturation of the megalin-endocytotic process due to proteinuria seen in CKD. This may result in increased urinary selenium losses, and loss of control of the homeostatic process. Although functional assays are more likely to reflect body status of selenium, *GPx1* polymorphisms have been shown to influence both the enzyme's activity in response to selenium concentration (227) and oxidative stress response (228), and more research is needed to understand how this may alter its interpretation of selenium status.

Due to the increased oxidative stress that CKD represents, there may be increased demand for the defensive selenoproteins, how this increased oxidative stress changes the metrics by which nutriture is assessed or the requirement of the body in this state needs further exploration.

4.4. DISCUSSION

In this chapter, I have presented example systematic-style reviews assessing the available literature reporting vitamin E and selenium status of children and young people with CKD, focusing on PDCM CKD. To summarise, the existing literature is limited, conflicting, and at high risk of bias. The literature available is not strong enough to base clinical practice guidelines upon, and much research is needed in order to better understand those at risk from nutritional inadequacy.

The reviews are systematic-style reviews rather than full systematic reviews. This was due to limitations of time and resources for a second reviewer. Therefore, although methodical and reproducible literature searches were undertaken, there may be increased risk of bias.

In the subsequent chapter, I will report the findings of a cohort study which examines vitamin and mineral status in a cohort of children and young people with CKD and place these measures in the context of clinical and nutritional status; including that of growth.

4.4.1. Practice Points

- There is limited evidence to underpin the current recommended dietary intakes. Although the current recommended intakes can be used as a starting point for clinical care, a critical, individual approach should be taken to identify those that may have nutritional inadequacy of one or several vitamins and minerals. An understanding of the biological handling of these nutrients is important for this approach to be taken.

5. TRACE ELEMENT AND MALNUTRITION IN PAEDIATRIC RENAL DISEASE (TEMPERED) STUDY

Following a broad overview of the paediatric nephrology service, the subsequent chapter details a study to characterise in more depth a smaller cohort of CYP with CKD. This will aim to demonstrate variation in nutritional status in those children and young people with CKD and begin to explore relationships with variables; including disease severity.

5.1. INTRODUCTION

There is limited data regarding the nutritional status of children and young people with CKD. To explore the variation in nutritional status of children and young people with CKD across the spectrum of disease a cohort of children and young people are characterized through the described Trace Element Malnutrition in Paediatric Renal Disease (TEMPeReD) study.

5.2. STUDY DESIGN

An observation cross-sectional study was conducted to clinically and nutritionally characterise a cohort of children with CKD. In addition to baseline data, follow-up data was collected at six and twelve months.

Nutritional characterisation consists of: anthropometric measures, dietary assessment, blood concentrations of micronutrients, appetite assessment. Additionally, blood measurements of kidney function, urinary protein-to-creatinine ratio, and other routine clinical indices together with health-related quality of life questionnaire scores.

A thorough exploration of appetite and health-related quality of life are covered in subsequent chapters.

5.2.1. Inclusion Criteria

- Aged between 3 and 17.99 years;
- CKD (stages 2 to 5D and including post-transplantation);
- Under care of the paediatric nephrology team at Southampton Children's Hospital;
- Informed consent.

5.2.2. Exclusion Criteria

- Aged <3 years or ≥18 years;
- CKD stage 1;
- Lack of informed consent.

5.2.3. Data Collected at baseline visit

- Clinical details; including aetiology of CKD; duration of illness and medications;
- Anthropometric measures; including height, weight, mid-upper arm circumference and waist circumference;
- Bloods samples;
- Urine samples;
- Health-related quality of life assessment and appetite assessment questionnaires.

5.2.4. Ethical Approvals

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from all individuals included in the study, with informed consent obtained from care-givers and informed assent as appropriate.

5.3. METHODS

5.3.1. Recruitment

A total of 160 patients were identified through upcoming patient clinic attendance lists. All patients that were eligible were sent study information and a letter of invitation by post, with a subsequent face-to-face invitation on attendance at routine clinical appointment.

Children were recruited on a 'first come, first served' basis, until 60 children and young people were recruited. Seven families declined to participate. The sample size of 60 was chosen as a pragmatic number of the available families, with no use of power calculation to determine sample size. All data were collected upon attendance at routine clinic out-patient review.

5.3.2. Growth and Anthropometry

The following body measurements were obtained: height, weight, mid-upper arm circumference, waist circumference as per hospital and research facility standard operating procedures and performed by the clinical and research team that had been trained and assessed in their performance. These values were subsequently used to calculate body-mass index, waist-to-height ratio, and where appropriate standard deviation scores (SDS) with comparison to reference standards (27). Height velocity was calculated with comparison to height measurement in the clinic six-months prior to the baseline visit, and SDS calculated.

5.3.3. Estimation of Dietary Intake

Estimation of dietary intake of the children and young people is through 24-hour dietary recall, using a multiple-pass approach (an unstructured, uninterrupted listing of all foods and beverages consumed, followed by a structured approach to data collection including memory cues, ending in an unstructured question for any other foods recalled and included several additional memory cues). Food intake was coded and analyzed using Netwisp computer software (Tinuviel Software Ltd, UK), which provides comprehensive nutrient analyses using a large food database, to give estimated nutrient content and compared with dietary reference values.

5.3.4. Biological samples

Blood and urine samples were collected from participants at the time of usual blood sampling and if the participant was not anuric, respectively. Samples were analysed by the clinical science laboratory at University Hospital Southampton NHS Foundation Trust through their quality assurance framework and using their normal reference values. Additional aliquots of blood and urine were stored at -80'C to facilitate batch analysis, the details of which are described in the relevant chapters.

5.3.5. Follow-up data

Data (height, weight, and serum creatinine) were collected at 12 months following baseline assessment through usual clinical follow-up with no additional hospital attendance and collected as described above.

5.3.6. Data handling

A dedicated database was developed to collate routine clinical and research data. A working group of healthcare professionals with an interest in paediatric nephrology and nutrition comprising of an associate professor in nutrition, a paediatric kidney dietitian and a clinical research fellow in paediatric nephrology composed a first draft of the data points that the proposed database should encompass, and through an iterative process and refinement of a Microsoft Access Database was developed.

5.4. RESULTS

The TEMPeReD study is divided into four subsections that are now discussed separately, followed by a summary and discussion of the findings as a whole. Firstly, the cohort characteristics are described as an entire cohort and as a subgroup of pre-dialysis, conservatively-managed CKD children and young people. It is this subgroup that continues to be the focus of the subsequent analyses. Secondly, data regarding growth and anthropometry are reported and discussed. Thirdly, dietary intake data from the cohort including energy, micronutrient and dietary fibre intake. Fourthly, blood concentrations of micronutrients are explored. Finally, the results as a whole are summarised and discussed.

5.4.1. TEMPeReD Cohort characteristics

Sixty children with CKD were recruited; 46 of whom were conservatively managed, 10 had previously undergone kidney transplantation, and four were receiving dialysis (three haemodialysis, one peritoneal dialysis). Mean age 10.7 years (±4.0), with 40 (66.7%) boys and 20 (33.3%) girls.

Table 5. Patient characteristics for the entire TEMPeReD cohort (n=60).

Patient Characteristics	
Age (years)	Mean = 10.658 (SD±3.99, range = 3.72 to 17.78)
Gender	Male = 40 (66.7%), Female = 20 (33.3%)
eGFR (ml/min/1.73m^2)	Mean=52.68 (SD±24.54, range =5.10 to 116.00)
CKD stage	
Stage 2	22 (36.7%)
Stage 3a	9 (11.7%)
Stage 3b	18 (20%)
Stage 4	6 (10%)
Stage 5/5D/5Tx	5 (23.3%)
Renal Replacement Therapy	
Conservatively managed	55 (91.7%)
Peritoneal dialysis	1 (1.6%)
Haemodialysis	4 (6.7%)
Plasmapheresis	1 (1.6%)
Received a kidney transplant graft	10 (16.7%)
Time since diagnosis (months)	Mean = 88.68 (SD±53.44, range = 8 to 203)
Systolic blood pressure SDS	-0.50 (SD±0.85)
Diastolic blood pressure SDS	-0.52 (SD±0.94)
Aetiologies	
Hypoplasia/dysplasia	14 (23.3%)
Obstructive nephropathy	11 (18.3%)
Nephrotic syndrome	5 (8.3%)
Haemolytic uraemic syndrome	8 (13.3%)
Reflux nephropathy	7 (11.6%)
Ischaemic injury	5 (8.3%)
Idiopathic	4 (6.6%)
Post-infective glomerulonephritis	1 (1.6%)
Tubulointerstitial nephritis	1 (1.6%)
Drug-induced nephropathy	1 (1.6%)
Wilms' tumour	1 (1.6%)
Polycystic kidney disease	1 (1.6%)
Kidney venous thrombosis	1 (1.6%)

Demographic details of the entire cohort at baseline. Abbreviations: CKD – chronic kidney disease; eGFR – estimated glomerular filtration rate; ICR – interquartile range; SD – standard deviation; SDS – standard deviation score.

Table 6. Demographic details of the pre-dialysis, conservatively managed cohort.

True conservatively managed children (i.e., no dialysis and never received a kidney transplant)	
Total	46
Age (mean; years)	10.50 (SD±4.19)
Gender	Male = 28 (60.87%), Female = 18 (39.13%)
eGFR (mean; ml/min/1.73m^2)	57.35 (SD±23.95)
CKD stage	
2	21 (45.7%)
3a	7 (15.2%)
3b	12 (26.1%)
4	5 (10.9%)
5	1 (2.2%)
Diagnosis	
Dysplasia	11 (23.9%)
Obstructive Nephropathy	8 (17.4%)
HUS	8 (17.4%)
Reflux Nephropathy	7 (15.2%)
Ischaemic Injury (e.g., HIE)	3 (6.5%)
Idiopathic	2 (4.3%)
Nephrotic Syndrome	1 (2.2%)
Syndrome-Related	1 (2.2%)
Tubulo-interstitial Nephritis	1 (2.2%)
Wilms' Tumour	1 (2.2%)
Kidney Venous Thrombosis	1 (2.2%)
Polycystic Kidney Disease	1 (2.2%)
Number of medications	3.89 (SD±2.54)
Anti-hypertensive Use	26 (56.5%)
Dose of enalapril (median; mg/kg)	0.1838 (IQR±0.11)
Time since diagnosis (mean; months)	93.27 (SD±55.34)
Systolic blood pressure SDS	-0.52 (SD±0.84)
Diastolic blood pressure SDS	-0.52 (SD±0.50)

Demographic details of the conservatively managed subgroup only. Abbreviations: CKD – chronic kidney disease; eGFR – estimated glomerular filtration rate; ICR – interquartile range; SD – standard deviation; SDS – standard deviation score.

5.4.2. Growth and Anthropometry at Baseline

Within the cohort, most children lay within normal reference ranges for growth parameters (-2 to +2SD). There was a shift of the population to be shorter and heavier-for-height than the reference population, and a large variation in height velocity SDS.

5.4.2.1. Poor Growth

From the entire cohort (n=60), 17 (28%) had poor growth defined by HtSDS <-2, and 17 (28%) defined by HtVelSDS <-2. These were not the same children, as only six had both HtSDS and HtVelSDS below -2.

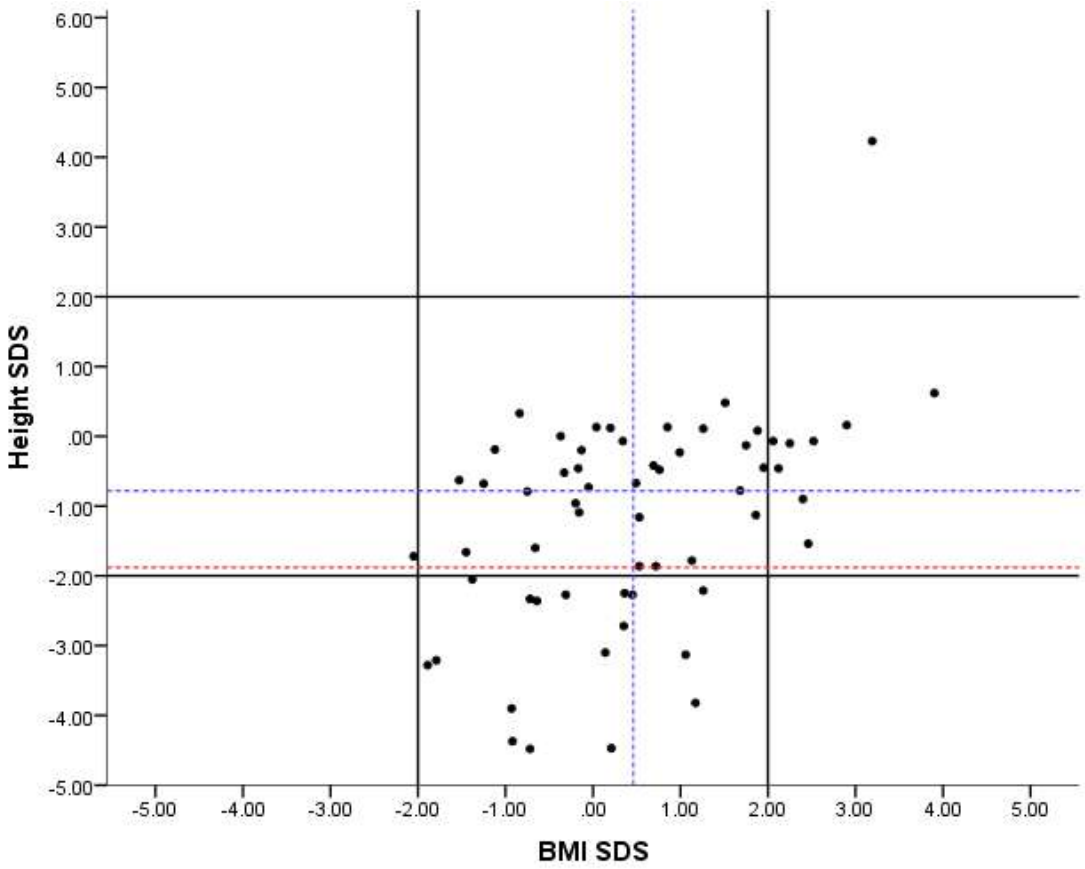


Figure 16. Height SDS and BMI SDS for the cohort (n=60).

Height SDS and BMI SDS are plotted with normal reference lines of -2 and +2 SD. Dotted blue lines represent the mean BMI SDS and median Height SDS. The red dotted line represents a height SDS of -1.88 that has been associated with an increase in morality. Although canonical descriptions of those with kidney disease describe "short and thin" individuals, these data can be broadly divided into three growth phenotypes: Normal height and weight-for-height (centre quadrant); short and light-for-height (bottom middle quadrant); and normal height and heavy-for-height (middle, right hand side quadrant). Abbreviations: BMI – body mass index; SDS – standardised deviation score.

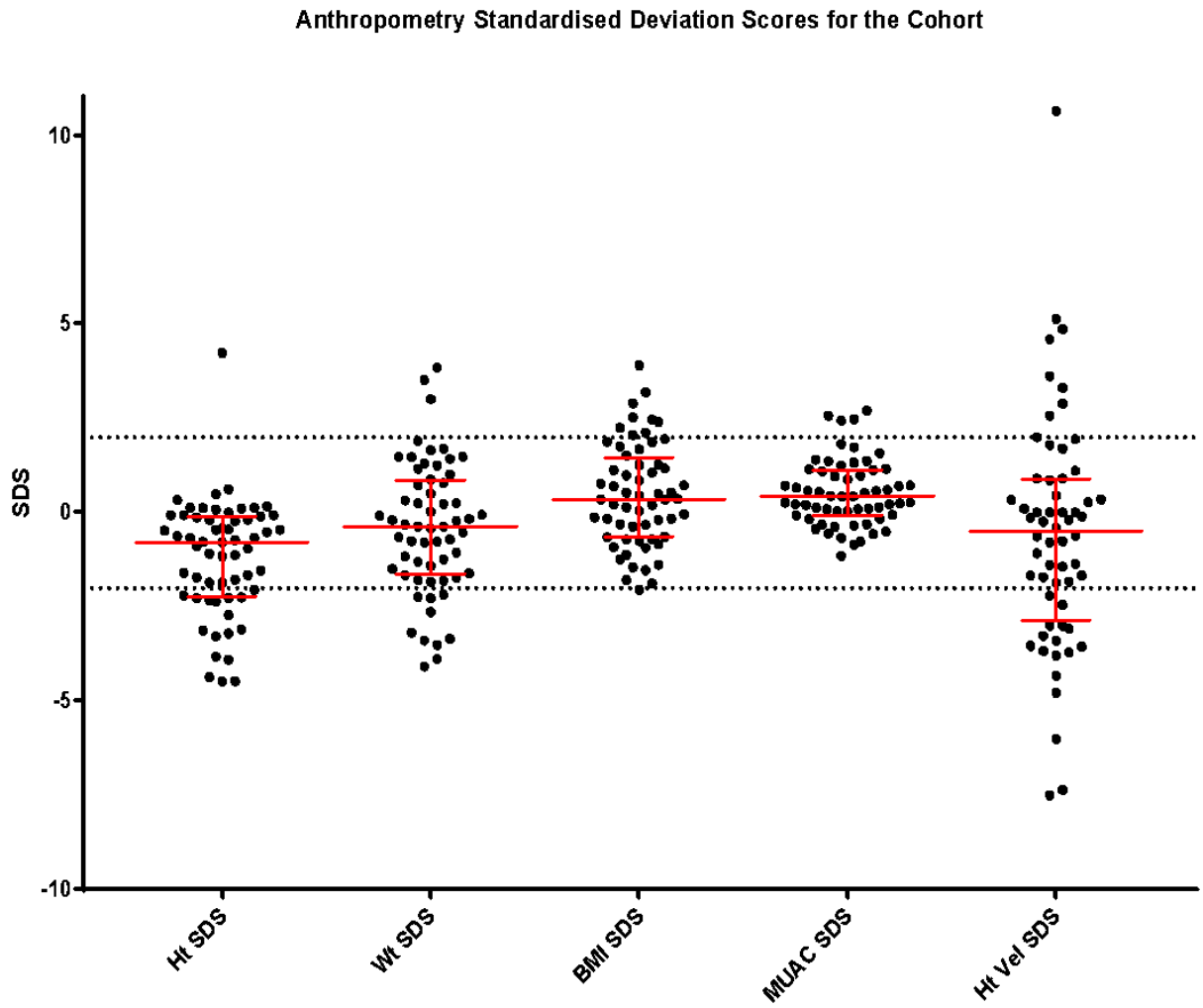


Figure 17. Baseline anthropometry SDS for the TEMPeReD cohort (n=60).

Standardised deviation scores for height, weight, body mass index, mid-upper arm circumference and height velocity are shown for the entire cohort. Median scores with interquartile range are shown in red. The black dotted lines represent the normal reference range of -2 to +2 SDS. Most children lay within the normal reference range for measurements, but compared to the reference standard, the cohort were shorter, with a large variation in height velocity. The variation in height velocity may represent a shifting of the normal growth curve to the right, with delayed pubertal growth spurt, and or catch-up growth following. Abbreviations: BMI – body mass index; Ht – height; Ht Vel – height velocity; MUAC – mid-upper arm circumference; SDS - standardised deviation score; Wt – weight.

5.4.3. Pre-dialysis, Conservatively-Managed (PDCM) Subgroup.

A similar picture of growth was reported in the PDCM subgroup (i.e., dialysis and transplant recipients excluded) to the entire cohort. Median height SDS was -0.65 (IQR=2.03, range = -4.48 to 4.23.). Mean values (with standard deviations) of weight SDS and BMI SDS for the cohort were -0.43 (±1.81), and 0.32 (±1.41), respectively. HtSDS, WtSDS, BMI SDS, MUAC SDS and Height velocity SDS are displayed in *Figure 18*.

5.4.3.1. Poor growth

From the PDCM group (n=46), 12 (26%) had poor growth defined by HtSDS <-2, and 17 (26%) defined by HtVelSDS <-2. These were not the same children, as only six had both HtSDS and HtVelSDS below -2. There was no significant difference between the sexes.

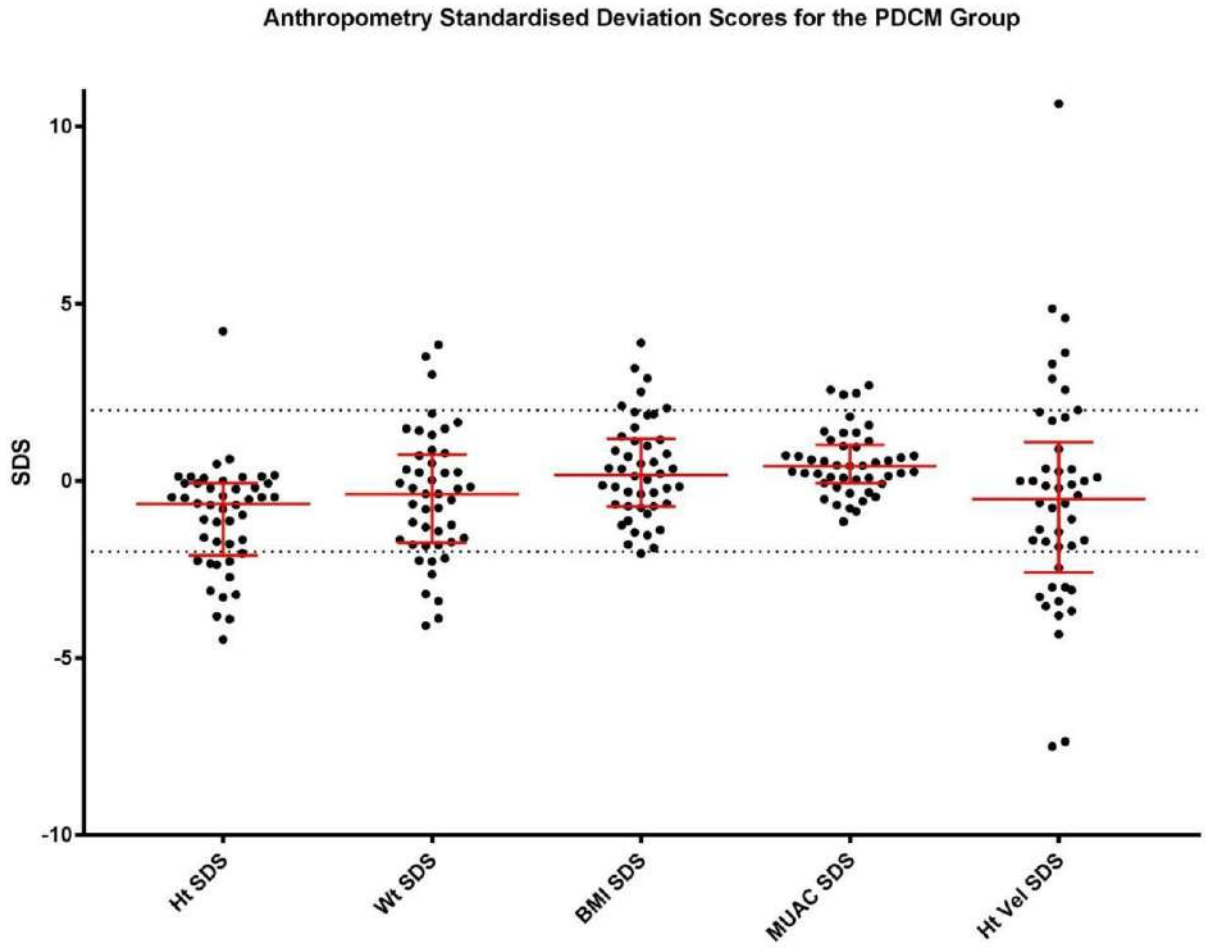


Figure 18. Baseline anthropometry SDS for the Pre-dialysis, conservatively-managed subgroup (n=46).

Standardised deviation scores for height, weight, body mass index, mid-upper arm circumference and height velocity are shown for the entire cohort. Median scores with interquartile range are shown in red. The black dotted lines represent the normal reference range of -2 to +2 SD. Most children lay within the normal reference range for measurements, but compared to the reference standard, the PDCM group were shorter, with a large variation in height velocity. The variation in height velocity may represent a shifting of the normal growth curve to the right, with delayed pubertal growth spurt, and or catch-up growth following. Abbreviations: BMI – body mass index; Ht – height; Ht Vel – height velocity; MUAC – mid-upper arm circumference; PDCM – Pre-dialysis, conservatively-managed; SDS - standardised deviation score; Wt – weight.

Factors that may influence the presence of poor growth were explored. There was no difference between those with HtSDS<-2 and those with HtSDS>-2 for: Age (Mann-Whitney U test (MWU) t=214.5, p=0.793); time since diagnosis (MWU t=205.5, p=0.970); number of medications (MWU t=267, p=0.111); eGFR (MWU t=227, p=0.565); degree of proteinuria (uPCR) (MWU t=153.5, p=0.964); dose of ACE-inhibitor (mg/kg) (MWU t=188.5, p=0.686); C-reactive protein (MWU t=196, p=0.422); or level of deprivation (IDACI score) (MWU t=190, p=0.849). PTH and serum albumin were significantly different (PTH: MWU t=91.5, p=0.038; serum albumin: MWU t=262, p=0.022), but this significance did not remain following Bonferroni correction (p=0.380 and p=0.220, respectively). Median PTH in stunted children was 2.7 pmol/l (IQR±1.5) and serum albumin was 42 g/l (IQR±3.75). Compared to those not stunted, PTH was 5.7 pmol/l (IQR±7.9) and serum albumin was 39.5 g/l (IQR±4.5).

HtSDS and BMISDS demonstrated a positive correlation (S.rho=0.568, p<0.0005). There is no clear pattern with regards to sex distribution or aetiology of CKD with regards to those that have poor growth (HtSDS<-2) or obese (BMISDS>2). Graphs demonstrating this are in *Appendix 11.3*.

5.4.3.2. Height Velocity

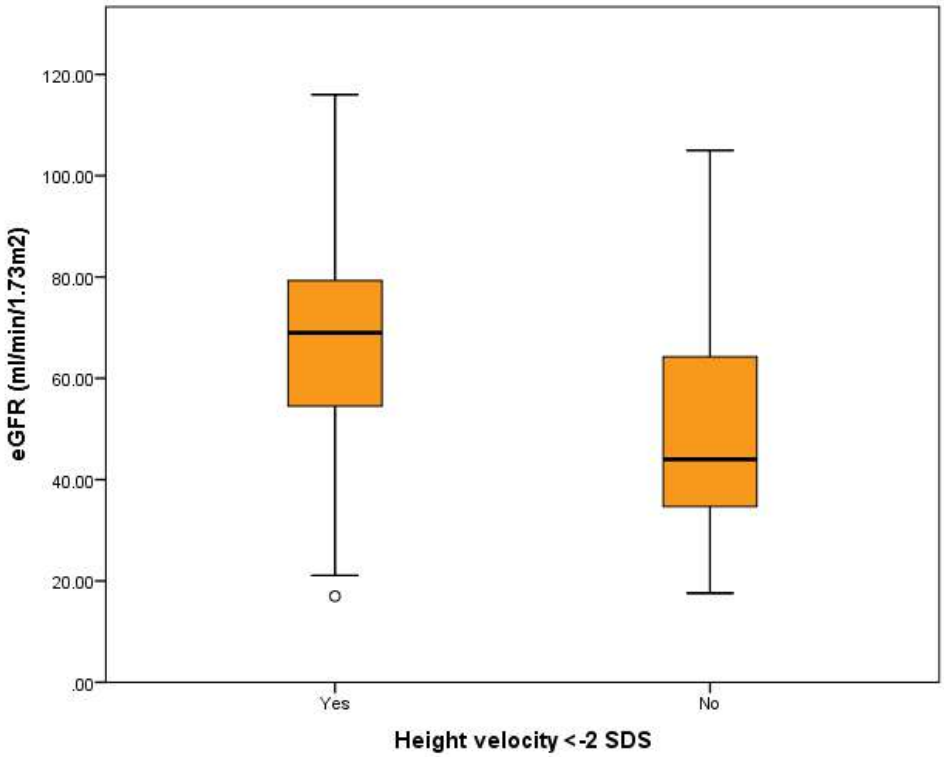
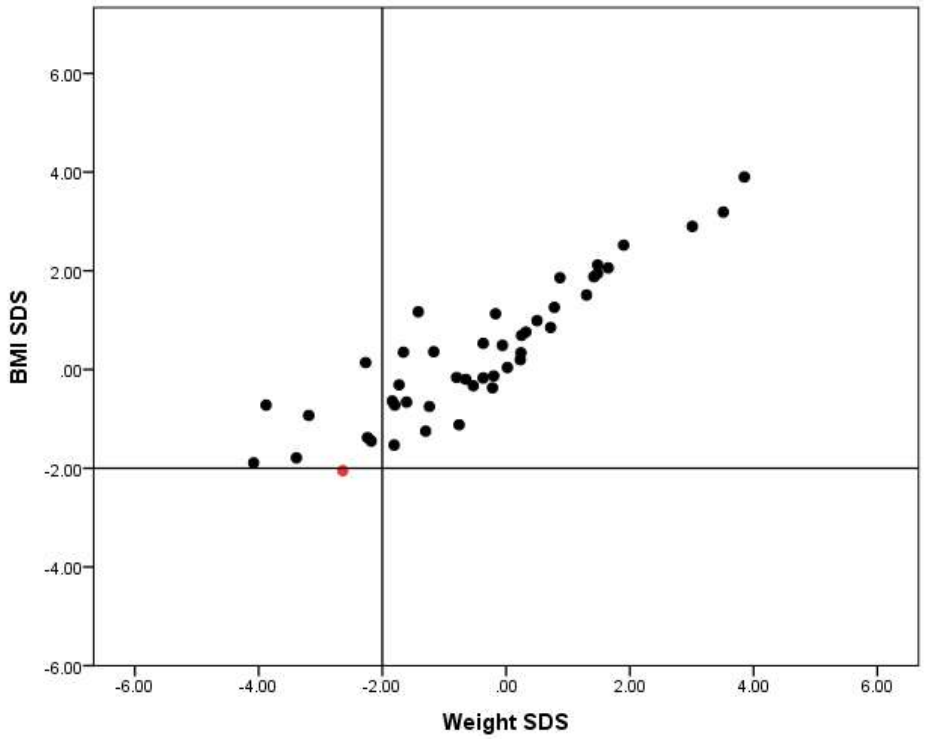


Figure 19. Poor growth as defined by height velocity versus kidney function in the pre-dialysis, conservatively-managed CKD subgroup (n=46).

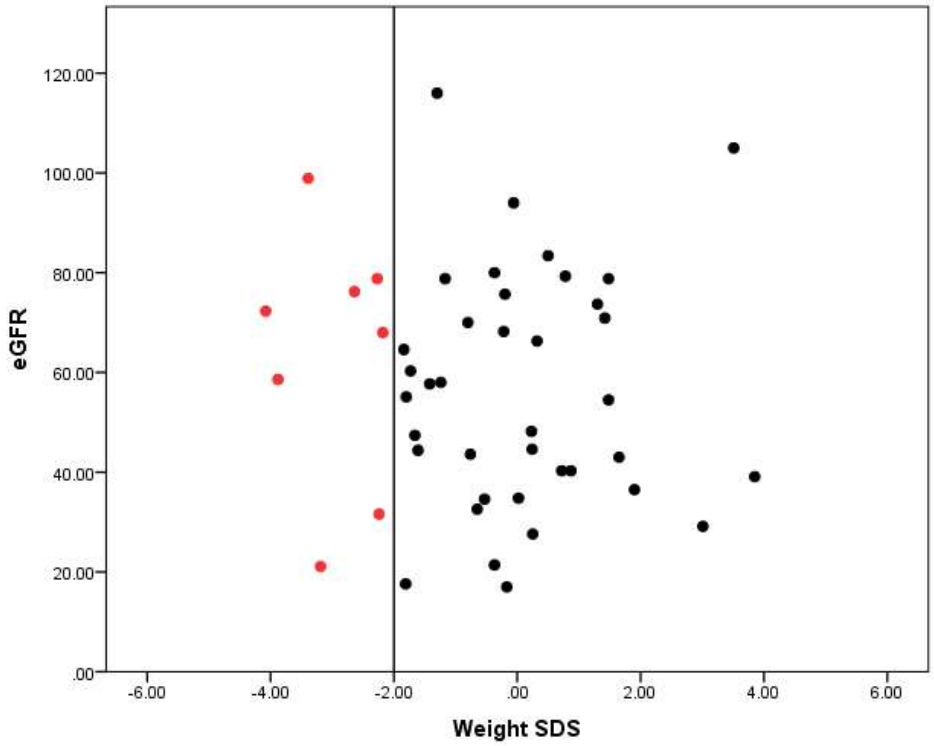
If grouped as either (i) HtVel SDS <-2, or (ii) HtVel SDS >-2, there was a significant difference in eGFR (t-test t(44=2.329, p=0.025) with those with HtVel SDS <-2 having higher eGFR (65.54 SD±24.59ml/min/1.73m^2 versus 49.84 SD±21.14 ml/min/1.73m^2). There was no difference between groups in degree of proteinuria, or medication burden. Those with HtVel SDS <-2 were younger (8.49 SD±3.61 versus 12.35 SD±3.87 years; t(44)=-3.495, p=0.001) and more recently diagnosed (73.68 SD±49.45 versus 109.17 SD±55.68 months; t(44)=-2.277, p=0.028).

5.4.3.3. Under-weight and Wasting

17% of the cohort are under-weight (WtSDS <-2), with one child wasted (BMI SDS <-2). There is no relationship with degree of kidney impairment (eGFR).



(1)



(2)

Figure 20. Weight SDS versus eGFR within the TEMPeReD cohort (n=60).

(1) Red circles represent those individuals with WtSDS <-2 and BMI SDS <-2. (2) Red circles represent those individuals with WtSDS <-2. There is no correlation between weight SDS and kidney function (eGFR).

5.4.3.4. Obesity

In addition to stunted children, the cohort also had a proportion of obese children. When comparing different definitions of obesity, the prevalence of obesity in the cohort was: BMI SDS >2 = 13%; WHtR >0.5 = 41%; WC >90th centile = 11%. Kappa-agreement values between the definitions were: BMI SDS >2/WHtR >0.5 κ=0.351; BMI SDS >2/WC >90th centile κ=0.484; WHtR >0.5/WC >90th centile κ=0.111. There was no difference in rates of BMI-defined or WHtR-defined obesity between girls and boys.

There was no difference in BMISDS depending upon CKD stage (One-way ANOVA F(5)=0.275, p=0.924), and no correlation between eGFR and BMISDS (Pearson correlation coefficient = -0.084, p=0.581).

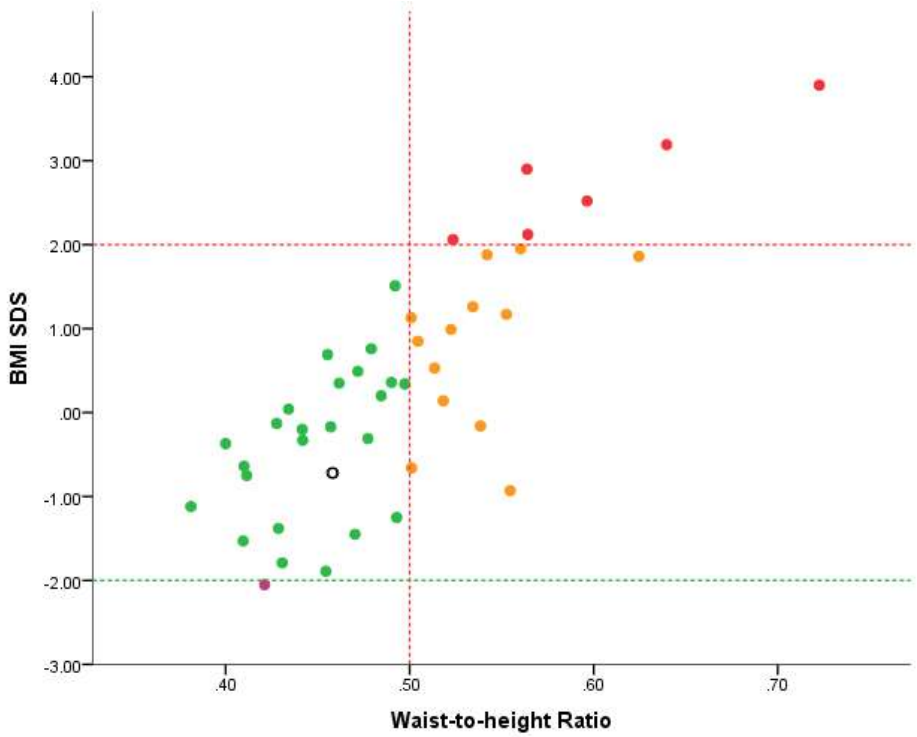


Figure 21. Obesity (BMI SDS) versus central obesity (waist-to-height ratio) (n=60).

13% of children and young people were obese (BMISDS>2), but with abdominal obesity (WHtR>0.5), 41% were defined as obese. There was a strong correlation between BMISDS and Waist-to-height ratio (Spearman's rho = 0.830, p<0.0005). Red circles represent those that are obese by both definitions; amber circles represent those with central obesity defined by waist-to-height ratio >0.5; green circles represent those that lay within the normal reference range for both measures; and the purple circle presents the individual that has a low weight-for-height (BMISDS <-2).

5.4.3.5. Follow-up data at 12 months

Data (height, weight, and serum creatinine) were collected at 12 months following baseline assessment through usual clinical follow-up.

A change in height SDS of 0.2 was *a priori* determined to likely represent a true deviation in SDS. Examining the entire cohort (n=60), 52 had height measurements available at 12 months and from these 32 (61.5%) showed no change in height SDS (±0.2 SDS difference), six (11.5%) a decrease in HtSDS of greater than 0.2 SDS, and 14 (26.9%) an increase in height SDS of greater than 0.2 SDS. In the PDCM subgroup (n=46), 42 had available

12 month measurements and of these no change, a decline and an increase in height SDS were seen in 27 (64.3%), five (11.9%), and 10 (23.8%), respectively.

Paired t-test analysis for HtSDS at baseline and 12-months were significantly different with a mean decline in height SDS for the entire cohort of 0.12 (SD±0.39, t(51) = -2.311, p = 0.025) and for the PDCM cohort of 0.13 (SD±0.42, t(41) = -2.025, p = 0.049).

When examining the subgroup of those with poor growth at baseline (HtSDS<-2), a greater proportion of children and young people increased their HtSDS (entire cohort those with height SDS <-2 (n=15): no change = 3 (20%), decrease = 1 (6.7%), increase = 11 (73.3%). PDCM subgroup with HtSDS <-2 (n=11): no change = 2 (18.2%), decrease = 0 (0%), increase = 9 (81.8%)). Paired t-test analysis did not reach statistical significance (t(14)=-1.623, p = 0.127. PDCM with HtSDS <-2 subgroup: t(10) = -1.600, p = 0.141).

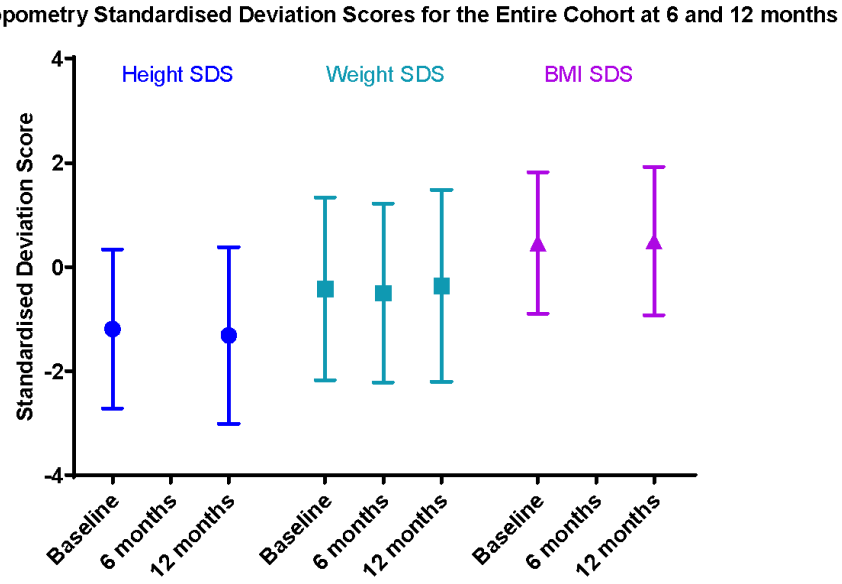


Figure 22. Mean standardised deviation scores for height, weight and body mass index for the TEMPeReD cohort at baseline, 6 months and 12 months follow-up (n=52).

Mean height SDS, weight SDS, and BMISDS at baseline and follow-up visits. At the average level, there was no observable difference.

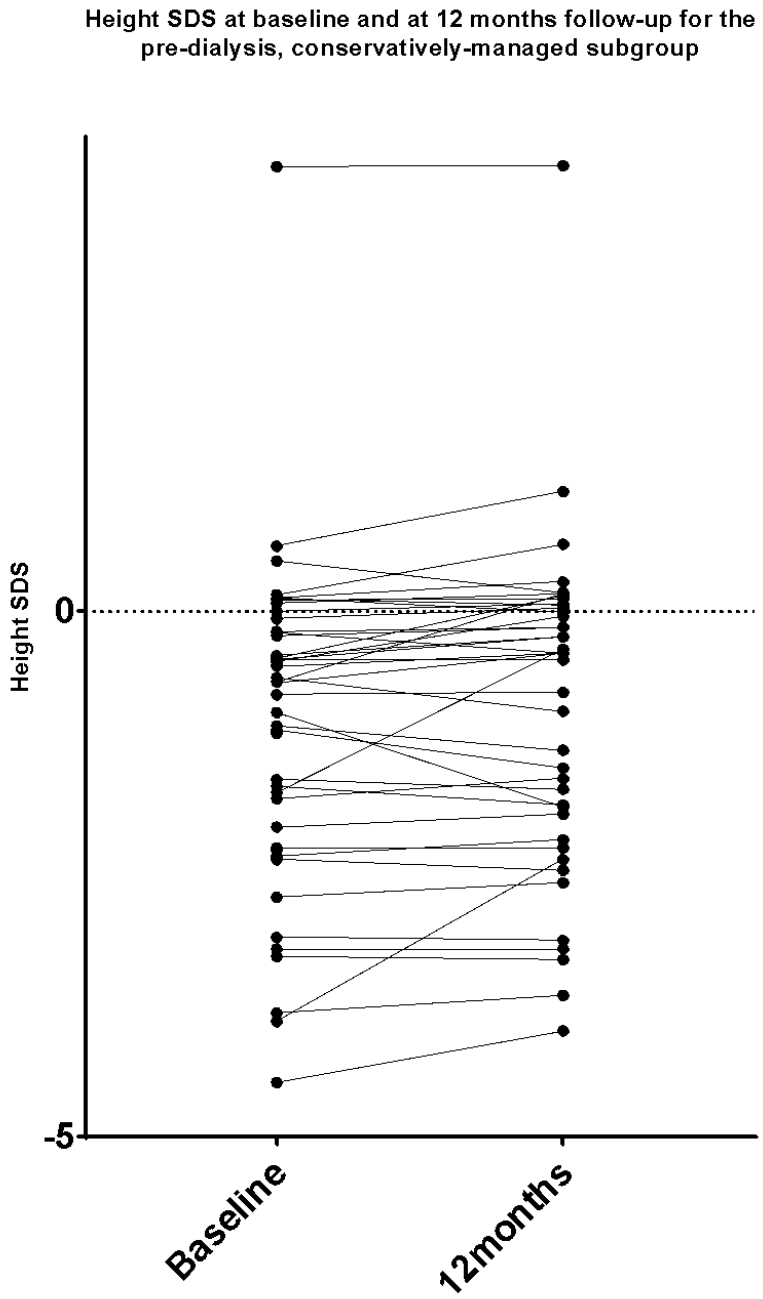


Figure 23. Height standard deviation score at baseline and 12 months for individual children and young people in the pre-dialysis, conservatively-managed subgroup of the TEMPeReD cohort (n=36).

Within the PDCM subgroup, no difference was seen between baseline and 12-month follow-up visits when taken as an average, some children and young people increased their HtSDS and some decreased.

At clinic appointments at 12 months following baseline visit, change in eGFR was calculated. The change in kidney function from baseline to 12 months for the PDCM subgroup was 0.39 ml/min/1.73m^2 (SD±9.12) with 36 from a maximum of 46 had both serum creatinine and height measured and therefore had data pairs for analysis available. As shown in *Figure 24*, there was no association between the change in kidney function (those with the most rapidly deteriorating kidney function) and change in HtSDS. This is confirmed with Cohen's Kappa agreement analysis of those with decline in eGFR and those with declined HtSDS of -0.094.

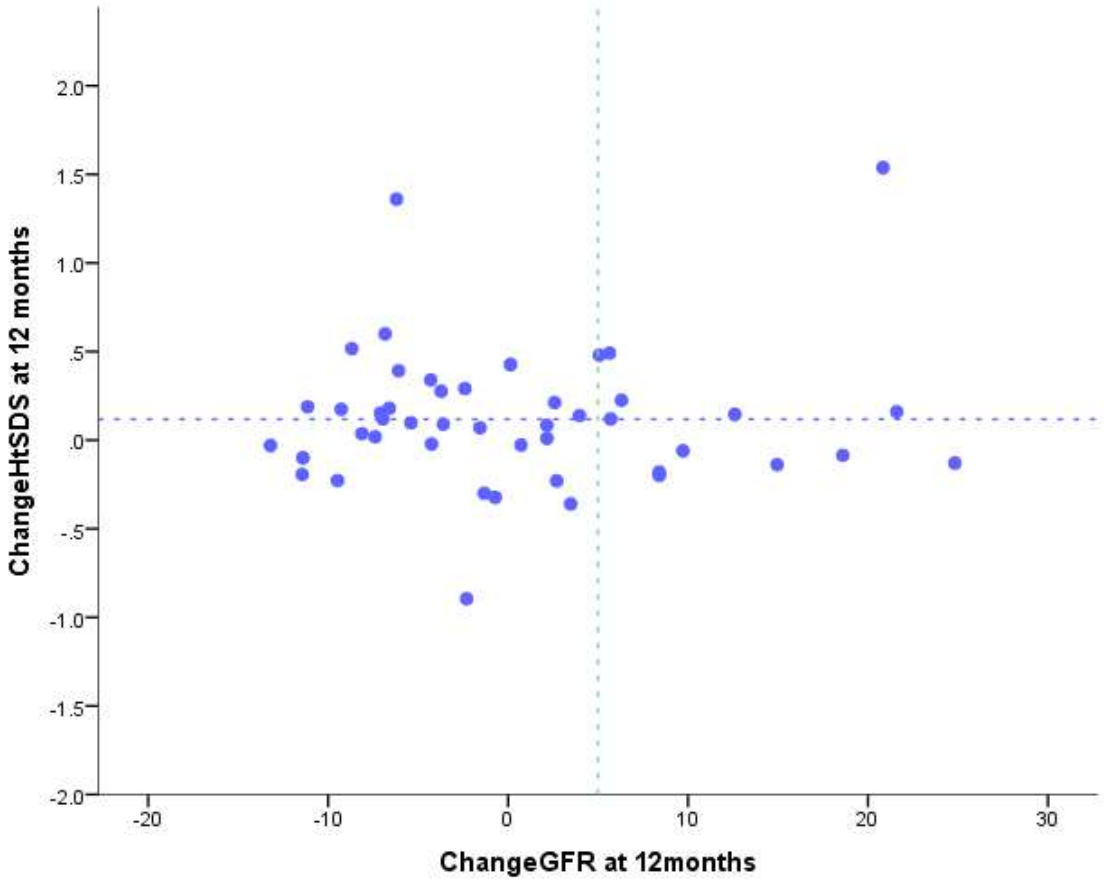


Figure 24. Change in height SDS and change in eGFR at 12 months for those in the pre-dialysis, conservatively-managed subgroup (n=36).

There is no agreement between those with a decreased HtSDS and those with decline in kidney function (Cohen's Kappa = -0.094).

5.4.4. Discussion

There are several measures that can be used to characterise growth. In the TEMPeReD cohort, a significant proportion of those were short (HtSDS<-2 = 28%), and the same proportion had linear growth (HtVelSDS<-2 = 28%), and 10% of the cohort had poor growth by both definitions. The proportions in these categories were not different in those with PDCM CKD, and disease severity (eGFR, CKD stage, number of medications) does not explain those with poor growth.

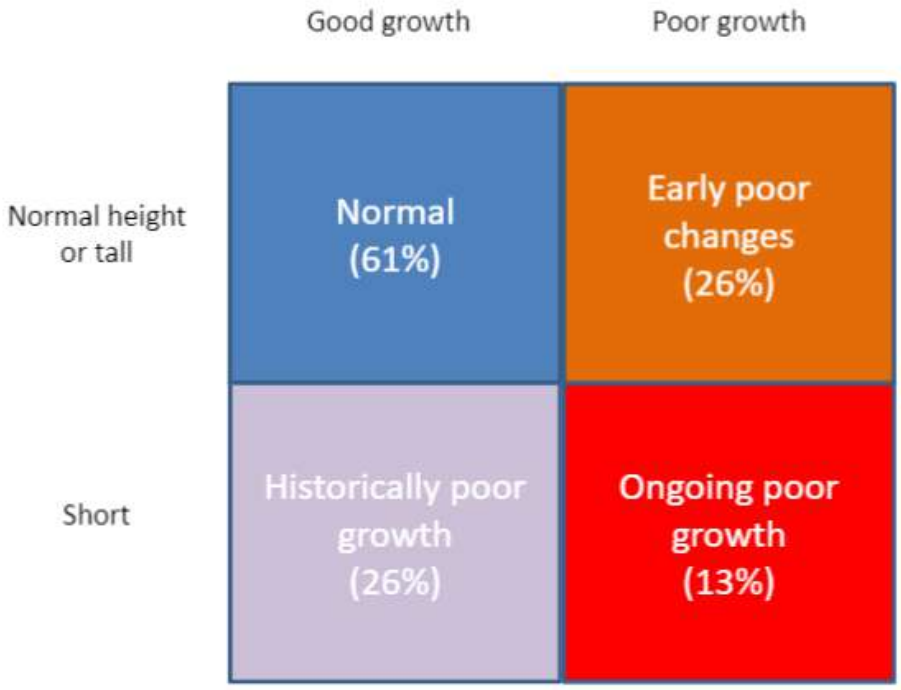


Figure 25. Growth determined by height SDS or height velocity SDS.

Blue square (61% of PDCM group) represents those with normal or high HtSDS and HtVelSDS. These children have normal (or tall) height and are gaining linear growth above normally or quickly (such as catch-up growth). The purple square (26% of PDCM group) represents those who are short, but are now either growing normally or having catch-up growth. The orange square (26% of PDCM group) represents those that are normal (or tall) height but whose linear growth is slow. This may be where an insult has occurred and the child has stopped growing, but this is acute enough that children are not short for their age yet. The child may have previously travelled along a high percentile prior to the insult. Those in the red square (13% of PDCM group) are both short and are not growing appropriately. This may represent those with a chronic, ongoing process that has either not responded to intervention or has been unrecognised.

In this cohort of pre-dialysis, conservatively-managed paediatric patients, growth patterns can be divided into three broad populations: (1) Those with height and BMI within the normal limits for a population; (2) those that are short in stature, but with a normal BMI; and (3) those with an elevated BMI, but with a normal height. The largest proportion of patients fall into the “normal height and BMI" category, and there were no children that fell

into the traditionally described anthropometric phenotype of "short and skinny" CKD patient, rather those falling out of the "normal" parameters are short (HtSDS<-2) or fat (BMISDS>2).

5.4.4.1. Stunting

A significant proportion of the cohort was stunted. Stunting is recognised as a problem worldwide, and a target variable in the World Health Organisation Global Nutrition Targets 2025 (To decrease the number of under-5's who are stunted by 40%).

The difference in albumin concentrations with higher concentration in those that are stunted may represent improved nutritional status due to recognition of growth problem, but without improvement in hHtSDS. PTH concentrations were lower in those who were stunted. Other markers of disease severity did not demonstrate differences, and this may be a statistical chance event. Alternatively, PTH concentrations may be a reflection of bone turnover, although as values lay within the normal reference range it is difficult to be convinced of clinical significance of the observed differences. As the effect of stunting is long-lived, with the impact of pre-first 1,000 days of life state having greatest impact, with less capacity for catch-up growth thereafter, it may be that in this cohort, there is limited ability to alter the trajectory of these stunted children who will remain stunted into adulthood.

There is a need to more deeply explore the reasons why the population has a high risk of stunting, as this is not fully explained by severity of disease, or length of disease duration.

5.4.4.2. Height velocity

Those with a lower HtVel (<-2 SDS) were younger and diagnosed more recently. This may reflect later diagnosis, and result in a potential delay in medical and dietetic management.

5.4.4.3. Under-weight

Although 17% of children were under-weight (WtSDS <-2), this was not observed when BMI was plotted. The lower WtSDS observed in these individuals is likely due to proportionately lower weight in shorter individuals.

5.4.4.4. Obesity

There is a significant proportion of the cohort that is obese (13%). This compares similarly to the general population, with rates of obesity in the UK population being 14% of children aged 2-15years (BMI >95th percentile)(153). World Health Organisation Global Nutrition Targets 2025 state that the number of children that are overweight should not increase.

Obesity is becoming increasingly prevalent in CKD populations across the UK and Europe (71) and more prevalent than the canonical malnutrition state of low anthropometric scores and "protein-energy wasting" in both the conservatively-managed (18) and dialysis/post-transplant populations (71). It has been reported to be an independent risk factor for development of kidney damage and end-stage kidney disease (72, 73). The reasons behind obesity levels in this cohort are likely similar to those explored in the general population, with increased availability of nutrient-poor, energy-dense foods, coupled with potential for different levels of physical activity. In those with CKD, it may be that dietary patterns are similar to those in the general population; especially in the

early (milder) stages of the disease where there is less dietetic in-put, and physical activity levels may be lower than healthy peers due to the effects of disease.

Alternative markers of obesity and cardiovascular risk include markers of central adiposity/obesity (for example, WHtR>0.5 and WC >90th centile). More children (35%) had abdominal obesity (WHtR>0.5) than BMI-defined obesity. The reason for this discrepancy may be BMI under-representing the degree of adiposity. In CKD, it is well-recognised that there is wasting of mean-mass ("protein-energy-wasting") thought secondary to the chronic inflammatory state that CKD represents. In this scenario, the accompanying loss of lean mass would mask an increase in fat mass, as BMI is only a measure of weight-for-height and does not represent body composition.

Similar prevalence of obesity as defined by BMI and WC>90th centile was observed, but not identifying the same individuals. This is likely due to the crude measure of obesity that BMI represents. The discrepancy between WC>90th centile and WHtR>0.5-defined obesity prevalence rates is likely due to the relatively high prevalence of stunting in the population. WHtR>0.5 should also be used to define obesity in this chronic disease population in order to account for skewed height measurements compared to a healthy population.

Looking at the pattern of development of obesity, the spectrum between healthy BMI and obesity. Children tend to develop abdominal (central) obesity prior to being formally classified as obese by BMI. This may be due to the tendency to lose linear growth (and be short), and then in an attempt to "make the child grow" be given additional calories. Unfortunately, the child needs more than just calories, and the resulting picture is one of relative short statures for weight (or high weight for height). There is some evidence that when children increase adiposity abdominal fat is increased (229).

Obesity is associated with increased risk of cardiovascular disease and mortality. In addition, CKD is a well-known risk factor for cardiovascular disease, with the leading causes of mortality in this population being cardiovascular disease (5), but abdominal fat is more pathogenic (230-232) .

Which is the best anthropometric measure to define or screen for obesity? These data show that prevalence of obesity varies depending upon definition used, likely due to body composition changes and prevalence of stunting. As central adiposity may pose the greatest risk to children in relation to their cardiovascular outcomes, a definition that is based on this parameter may be most appropriate. Additionally, a measure that is most sensitive could be used, with subsequent formal body composition analysis to truly determine adiposity, for example through skin-fold thickness measurement. The use of bioelectrical impedance analysis to determine body composition in this population is still a matter of debate, due to fluid and electrolyte shifts that occur in real disease, but all measures of adiposity are unreliable in this population due to fluid overload etc., and so a combination of methods is perhaps best pursued.

In current clinical practice there is no mechanism by which characteristics of the cohort or individual patients' phenotype can be readily accessed. No data exists on the numbers of children with CKD, as only those with the most severe disease; those in receipt of kidney replacement therapy are formally identified by the national registry. This lack of a mechanism to capture these data makes the evaluation of the disease group challenging and hinders the development of the clinical service to best serve the needs of the patients and their families.

5.4.4.5. Collection and collation of routine clinical data

During routine clinical practice, an immense amount of potentially valuable data is gathered about patients. Despite this, collated and organised data is largely unavailable to clinicians and researchers in any meaningful / accessible way. This results in the need to perform either prospective studies or retrospective reviews and audits of the patients' clinical records which is time-consuming and only gathered in response to specific questions, e.g., for the evaluation of clinical practice against a given standard. If data are collected prospectively, this offers the opportunity for almost instantaneous presentation of grouped data and outliers with comparison against a given standard, and moreover has the potential to be used in research and quality improvement to identify otherwise unseen associations, for example.

Data has the potential to be used nationally (and internationally) to compare healthcare systems. For example, in intensive care, databases are used to determine risk-adjusted outcomes using disease severity scores. Recording such data may seem daunting, but through electronic databases and 'buy in' from healthcare professionals such data can be gathered. The purpose of this project was to develop and trial a database to gather data for the use in clinical practice; including nutritional status and interventions. It will be used to prove usefulness to the clinical team of such a database through the provision of multiple clinical audits and the collection of data for research projects.

This project acts as an example of how the composition of such a database can be useful, and not only used in the delivery of this project, the database is continuing to be used for ongoing clinical data collection at Southampton Children's Hospital with adjustments and reiterations as directed by the clinical team.

Accessibility of the database to the paediatric kidney dietetic team was a recognised barrier to its implementation. Prior to more widespread incorporation into clinical care, for data protection, security and privacy reasons, the database was hosted on the hospital's shared drive that is password protected. As this system is a different process by which clinical information is obtained and stored, then users would need to access several systems simultaneously.

For greater utility, the database must be accessible to researchers. If there are the usually attending clinical team then specific ethics approvals may easily be sought, but for other research teams, such approvals may be less easily obtained with explicit informed consent. Recent developments in the UK renal registry (UKRR) and the national ethical government approval this affords, may also help in the future, proving clinical systems and the UKRR can link digitally.

The greatest barrier to implementation of the database was allocation of time by the practising dietitian. Dietitians felt that there was not enough time to complete the 'additional' administrative work that the database represented when they had had little time to complete other essential tasks. A local quality improvement project found that additional data entry led to more than a 3-fold increase in duplication documentation and time and reduced time on direct patient care.

5.4.4.6. Limitations

Limitations of the study include small cohort size and single centre design that may mean that the results are not generalisable. Error may have been introduced through measurement, as although all measurers were trained,

following the standardised operating procedure, measurements were not conducted by the same individual for each patient and on follow-up visit.

5.4.4.7. Concluding Remarks

A database facilitates collection of clinical and research data. Poor growth can be characterised in different ways and those using different definitions may identify different children and young people.

5.4.4.8. Practice Points

- The routine calculation of height velocity SDS may identify children and young people with poor growth who represent a separate, but not mutually exclusive group than those identified by HtSDS alone.
- Similarly, different anthropometric measures of obesity identify different groups of children and young people at risk from obesity. The incorporation of these readily accessible measures may be integrated into clinical practice to offer a more detailed characterisation in addition to height, weight and BMI.

5.5. DIETARY INTAKE OF THE TEMPEREDCOHORT

5.5.1. Introduction

Chronic kidney disease (CKD) is associated with altered dietary habits (233). Children and young people with CKD often have decreased calorie intake (234), although limited data exists for children with CKD, especially concerning micronutrients and dietary fibre. A greater understanding of the nutrients that may be more at risk from being inadequate in those with children and young people with CKD is beneficial, as it will aid healthcare professionals in supplying adequate nutrition to the growing child / young person.

In a first step to characterise nutritionally a cohort of children and young people with CKD, assessment of their dietary intake was undertaken as per usual clinical practice. The aim of the TEMPeReD study in its entirety was to characterise nutritionally the cohort in order to stratify the cohort for risk of nutritional inadequacy and subsequent growth impairment.

Poor nutrition and dietary intake can result in poorer growth (235), this can be a limitation of both energy and nutrients, but also may present itself in the context of a single limiting nutrient (236). A review of the literature regarding dietary intake of micronutrients in children and young people with CKD is presented in *Chapter 4*.

Dietary Assessment Methods

Dietary assessment is used in clinical practice to help nutritionally characterise an individual's diet, assess compliance with a dietary prescription such as a low potassium diet and review food-group choice and patterns.

Dietary assessment of an individual is the process of recording and analysing all food and drink that someone consumes, and then comparing it to a given standard (47). In the UK, dietary reference values such as EAR or RNI are used. For a completely accurate account, all the food and drink consumed should undergo chemical analysis for nutritional content, but as this is expensive and impractical clinically. Therefore, a prediction / estimation of intake is determined. There are several different methods and tools that have been developed for this purpose, but in clinic the multiple-pass 24-hour recall is commonly used – this is often a practice that is pragmatically possible within the clinical environment and constraints and does not place additional burden on the subject (and / or carer) in need to perform tasks prior to clinic attendance. Although commonly, 24-hour dietary recall is not an assessment of habitual dietary exposure, but a short-term 'snapshot'. The method may more accurately be used to compare to groups than an individual's exposure for which a large number of day's data would be needed for nutrients that have large day-to-day variation (237).

The reasons for dietary assessment of children with kidney disease has been explored in *Figure 26*. From a clinician's point of view, questions to be answered in the dietary assessment are:

1. Is the child at risk from nutritional inadequacy?
2. Is the child at risk for poor outcomes (e.g., poor growth, increased mortality)?
3. Is the child complying with dietetic prescription (e.g., low phosphate diet)?

Current clinical assessment of dietary assessment is based around the multiple-pass dietary recall, and following analysis, comparison against recognised dietary reference values (47).

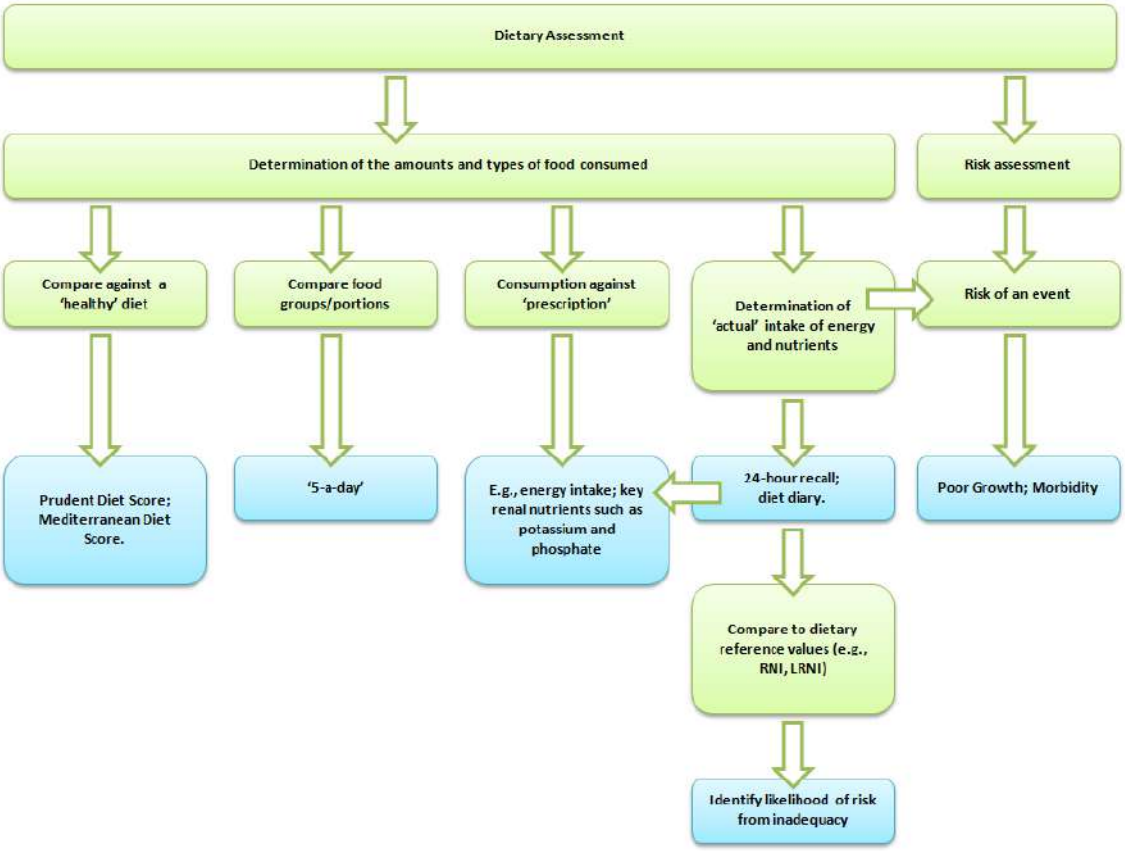


Figure 26. Schematic representation for potential information derived from dietary intake analysis.

There are many reasons why dietary analysis is needed. Depending upon the question(s) needed by professionals, then different dietary assessment tools may be more or less appropriate.

Dietary intake of energy, nutrients and dietary fibre

Recommended intake of energy, nutrient and dietary fibre have been set for healthy children and young people, with some more limited guidance for those with CKD (2, 47, 238). There are many reasons why those with CKD may not meet these recommendations, including that not all the general healthy population meet these recommended levels (76) and that possible effect of dietary restriction placed onto children and young people with CKD that limit availability of foods rich in dietary fibre. Despite these recognised potential influencing factors, literature regarding the dietary intake of children and young people with CKD is limited and has not been evaluated as per the current usual practice of a pragmatic single 24-hour recall, and whether this method demonstrates differences in those with adequate or poor growth.

There is a lack of data on the dietary intake of children and young people with CKD despite multiple factors that may increase the risk of not meeting their dietary requirements. Those data that exist are incomplete and not contemporary. The adequate / inadequate supply of nutrients in the diet is the cornerstone to nutritional status.

Aim:
The aim of this subchapter is to explore dietary intake of children and young people with CKD.

Objectives:

- To report contemporary dietary intake data for energy, nutrients, and dietary fibre of a cohort of children with pre-dialysis, conservatively-managed (PDCM) CKD using the current clinical methodology;
- Compare these dietary intake data with national UK data from the National diet and nutrition survey (NDNS) data (76);
- Explore whether the estimate intake as estimated by a single 24-hour recall identifies those children and young people that have low blood concentrations of the corresponding nutrient;
- Explore these data with regards to associated factors; including measures of adequacy of growth (height standardised deviation scores (Ht SDS) and height velocity standardised deviation score (Htvel SDS).

5.5.2. Methods

Participants were recruited to the TEMPeReD cross-sectional study as detailed in *Chapter 5.3*. Dietary assessment was performed by a single multiple-pass 24-hour recall. Following collection of dietary data, the food and drink were analysed using Netwisp computer software (Tinuviel Software, Ltd, UK) and nutrient composition compared to dietary reference values for sex and age, including Reference Nutrient Intake (RNI) or equivalent (47). Energy requirement was calculated using the Henry equation (239). Dietary fibre recommended intakes were taken from the Scientific Advisory Committee on Nutrition (SACN) 2015 "Carbohydrates and Health" report (238).

5.5.2.1. Statistical methods

Descriptive statistics; including mean and standard deviation scores (SDS) (or median and interquartile range, IQR depending upon distribution of variables) will be used to describe the data. Data were analysed as an entire cohort, and to decrease the risk of severely affected individuals skewing the data, as sub-groups: pre-dialysis, conservatively-managed (PDCM) CKD; those in receipt of dialysis; and those who have previously had a kidney transplant.

Comparison was made to both 100% of estimated requirement to control for sex and age and, as in usual clinical practice, 80% of RNI (or equivalent) to determine whether or not an individual is more at risk from inadequate intake. Therefore, the number of nutrients that in their dietary analysis had dietary intake of <80% RNI (or equivalent) was used to determine 'at risk'.

Data were compared to national dietary intake data from the NDNS (76), and between sub-groups. Correlations were explored between intake and other variables; including age.

Appetite was assessed by way of a Likert scale of subjective appetite assessment: very poor/poor/good/very good, and assigned a numerical value 1 to 4.

All analysis was carried out using software by the Statistical Package for Social Sciences (SPSS version 22, SPSS Inc., Chicago, IL). A p-value of < 0.05 was used to indicate statistical significance, but to decrease the risk of a type I error, where analysis of multiple variables occurs the p-value cut-off for significance will be adjusted by Bonferroni correction ($\alpha' = \alpha$ /test number) (240).

Comparison is made between nutrients that had at least one child with low intake (<80% RNI, or equivalent) and low blood concentrations by *Chi*-square analysis with the following interpretation for calculated *Phi* coefficients: -1.0 to -0.7 strong negative association; -0.7 to -0.3 weak negative association; -0.3 to +0.3 little or no association; +0.3 to +0.7 weak positive association; and +0.7 to +1.0 strong positive association.

5.5.3. Results

The dietary intake of the entire cohort (n=60) and subgroups (pre-dialysis conservatively managed only (n=46), post-transplantation only (n=10), and dialysis only (n=4)) with comparison to NDNS data are presented in *Appendix 11.4*.

Due to the relative size of the pre-dialysis, conservatively-managed subgroup (n=46) from the total of 60, subsequent analysis is focused on this more homogenous subgroup. Details of the pre-dialysis, conservatively-managed CKD patients (PDCM) cohort, including anthropometry and disease severity are earlier in the chapter (see *Table 6*).

5.5.3.1. Dietary Intake Compared to Dietary Reference Values

There is significant variation throughout the cohort in meeting recommended intakes. *Figure 27* shows mean and range of intake as expressed as a percentage of requirements. *Figure 28* shows the proportion of children and young people with intakes above the RNI (likely adequate intake), between the lower reference nutrient intake (LRNI) and RNI (at risk from inadequacy) and below the LRNI (likely inadequate intake). Mean intake of energy, potassium, magnesium, iron, iodine, selenium, zinc and vitamin K are all below recommended intake. Those nutrients which had the greatest number of children and young people with intake below the LRNI are therefore most at risk from not meeting their dietary intake are: Selenium (35%); Magnesium (35%); Iodine (30%); Zinc (30%); and potassium (24%).

5.5.3.2. Dietary Intake Compared to the current clinical standard of inadequacy (<80%RNI).

The percentage of children with dietary intake below 80% of RNI or equivalent is *depicted in Figure 29. The nutrients with the most children with dietary intake values less than 80% of RNI were vitamin K (76.1%), zinc (58.7%), iodine (56.5%).*

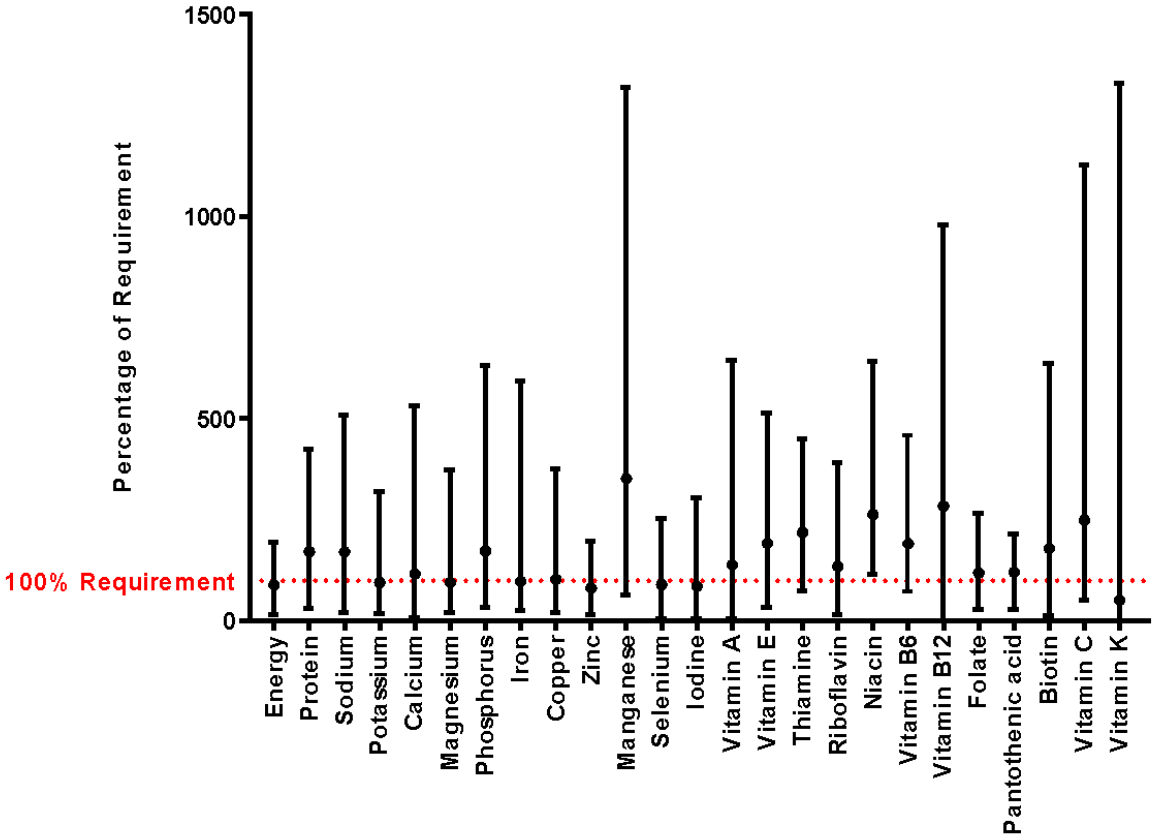


Figure 27. Mean and range of dietary intakes of the pre-dialysis, conservatively-managed CKD subgroup.

Mean and range of dietary intakes of the PDCM cohort expressed as percentage of requirement. The requirements are RNI values where they exist. Other requirements were estimated to be: Energy - estimated by the Henry equation (239); manganese - 16mcg/k/d; vitamin E – ratio of polyunsaturated fatty acids of 0.4; pantothenic acid – 3mcg/d; biotin – 10mcg/d; and vitamin K – 1mcg/k/d

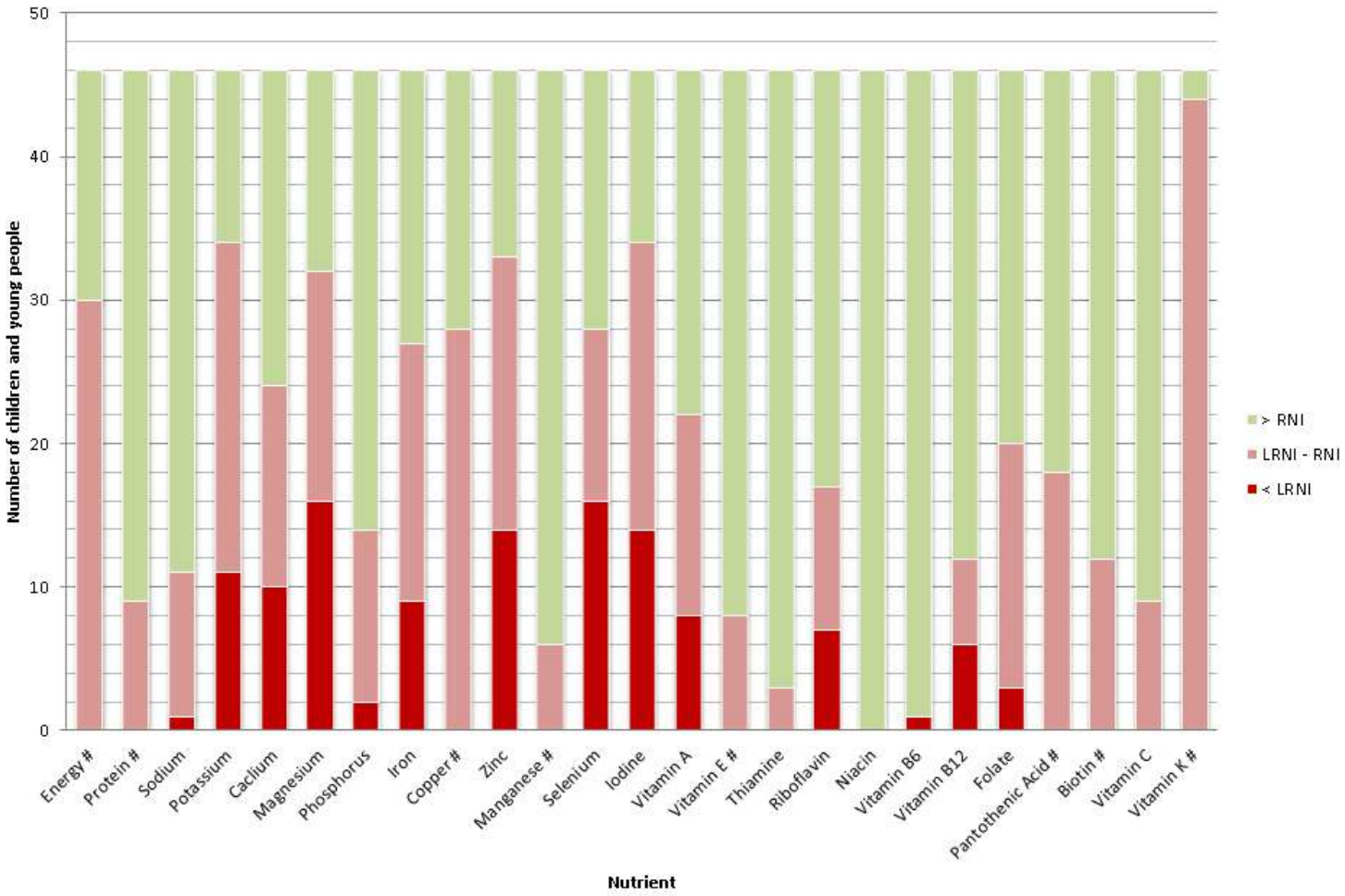


Figure 28. The number of children / young peoples with >RNI, <RNI and <LRNI within the pre-dialysis, conservatively-managed subgroup.

Where RNI value exist these were used, other requirements were estimated to be: Energy - estimated by the Henry equation (239); manganese - 16mcg/k/d; vitamin E – ratio of polyunsaturated fatty acids of 0.4; pantothenic acid – 3mcg/d; biotin – 10mcg/d; and vitamin K – 1mcg/k/d. # - No LRNI value available. Abbreviations: LRNI – lower reference nutrient intake, RNI – reference nutrient intake.

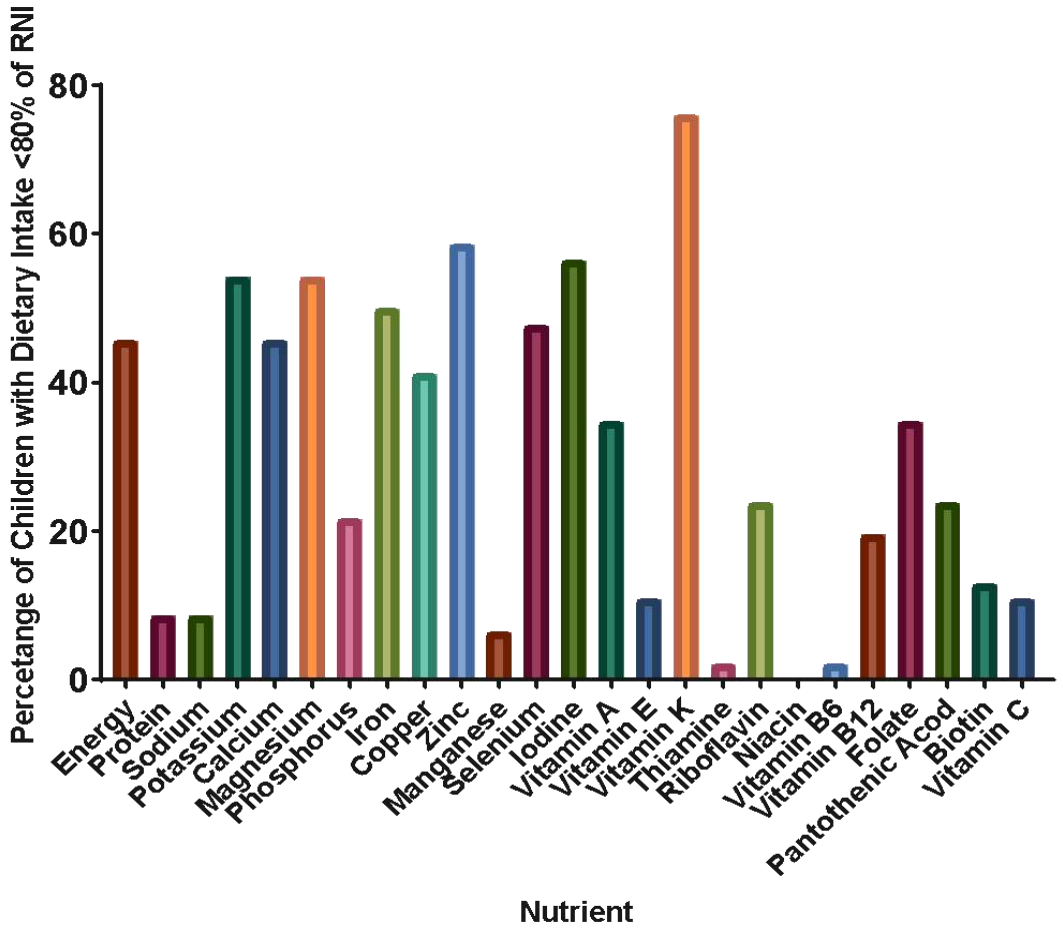


Figure 29. Percentage of children with dietary intake <80% RNI in the pre-dialysis, conservatively-managed subgroup.

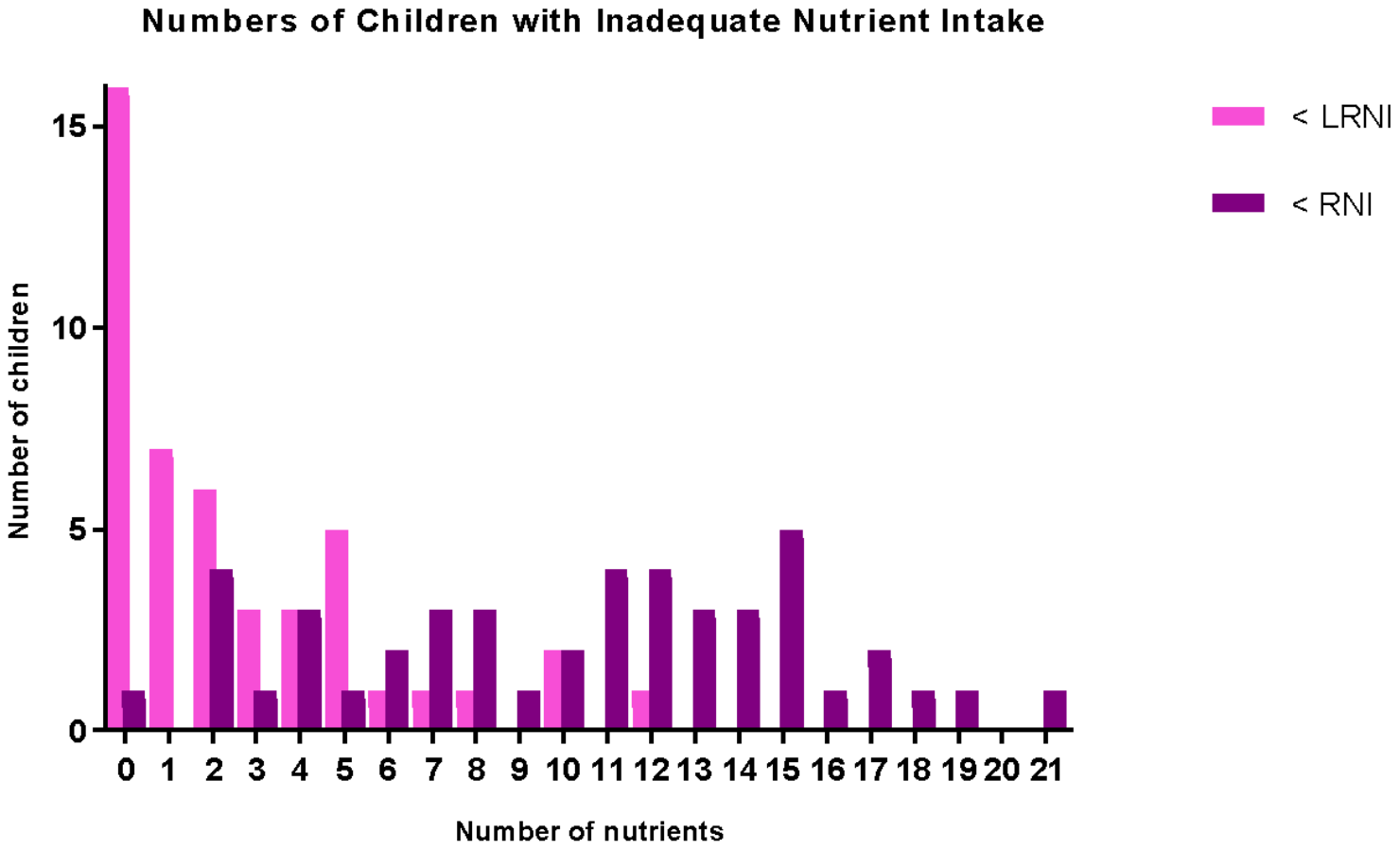


Figure 30. Distribution of the number of nutrients below recommended intakes in an individual child.

Abbreviations: LRNI – lower reference nutrient intake, RNI – reference nutrient intake.

5.5.3.3. Numbers of nutrients with intakes < LRNI

Most children had at least one nutrient's intake below LRNI (65.2%). The numbers of nutrients below LRNI for each child were: none - 34.8%; one - 15.2%; two - 13.0%; three - 6.5%; four – 6.5%; five - 10.9%; six - 2.2%; seven - 2.2%; eight - 2.2%; ten - 4.3%; twelve - 2.2%. The frequency of children with a given-number of nutrient intakes below LRNI and RNI are shown in *Figure 30*. For both sexes, older children were more likely to have inadequate intakes (see *Table 7*). Magnesium, selenium, zinc and iodine had the greatest proportion of children and young people with dietary intakes < LRNI (see *Figure 60* in *Appendix 11.4*).

5.5.3.4. Dietary Intake compared to national data

Comparison between NDNS data and the PDCM subgroup was grouped as per the NDNS data), in ages 4 to 10 years and 11to 18 years. Paired t-test analysis was performed to determine differences in mean intake between the PDCM cohort and NDNS data. In the 4 to 10 years age group, no differences were observed. In the 11 to 18 years age group (*table 8*), intake of energy, protein, magnesium, potassium, iodine, selenium, and zinc were all significantly lower in the PDCM subgroup, although only magnesium, potassium, selenium and zinc remained significant after Bonferroni correction for analysis of multiple variables.

Table 7. Number of nutrients with dietary intake < LRNI for different age and sex groups in the pre-dialysis, conservatively-managed subgroup (n=46).

	Males	*Females*
4 – 10 years	*1 ± 2*	*0 ± 0*
11 – 18 years	*3 ± 6.25*	*5 ± 5*

Table 8. Comparison between pre-dialysis conservatively-managed subgroup (n=46) and national data from the national diet and nutrition survey (ages 11 to 18 years).

	NDNS cohort		Pre-dialysis, Conservatively-managed TEMPeReD Cohort (n=21)				
Nutrient	NDNS mean	NDNS SD	Cons cohort mean	Cons cohort SD	T-value (df)	p-value	p-value (corrected)
Energy	1779	526	1507	577	T=(576) = 2.314	**0.021**	0.253
Protein	67.1	24.2	52.06	23.15	T=(576) = 2.799	**0.005**	0.064
Magnesium	215	75	160.57	59.26	T=(576) = 3.286	**0.001**	**0.013***
Potassium	2358	770	1795.67	675.03	T=(576) = 3.298	**0.001**	**0.012***
Iodine	126	87	75.76	675.03	T=(576) = 2.628	**0.009**	0.106
Selenium	42	20	26.67	19.48	T=(576) = 3.45	**0.001**	**0.007***
Zinc	7.5	2.8	5.53	2.50	T=(576) = 3.175	**0.002**	**0.019***

**- statistically significant. Only significant difference variables presented (full analysis table found in the Appendix 11.4).*

5.5.3.5. Does dietary intake data predict low status as assessed by blood nutrient concentrations?

Comparison of the nutrients that had at least one child with low intake (<80% RNI, or equivalent) and low blood concentrations is given in the table below (*Table 9*) with results of Chi-square analysis. Only vitamin C demonstrated a relationship between those identified as at risk of inadequacy of low intake (<80%RNI or equivalent) and low blood concentrations. Phi-coefficient was 0.422, representing a weak association, and this statistical significance was lost on Yates correction (p=0.269).

Table 9. Chi-squared analysis of those in the pre-dialysis, conservatively-managed CKD subgroup (n=46) comparing dietary intake <80% RNI with blood concentrations below the normal reference range.

Nutrient	Low intake (<80% RNI)	Low blood concentrations	Chi-square	P=value
Potassium	20	1	0.831	0.362
Phosphorus	8	1	3.756	0.053
Copper	16	1	1.506	0.220
Zinc	36	5	0.138	0.710
Manganese	2	2	0.062	0.803
Selenium	18	1	1.240	0.265
Vitamin A	13	1	2.049	0.152
Vitamin B12	9	1	0.298	0.585
Vitamin C	4	1	6.778	0.009*

* - *statistical significance, p<0.05.*

5.5.3.6. Does dietary intake data correlate with blood nutrient concentrations?

Correlations were explored between the dietary intake data of children and the blood concentration of the nutrient. Vitamin B6-PLP and folate demonstrated a correlation (S.rho = 0.325; p = 0.05, and S.rho = 0.474; p = 0.005, respectively). Although neither of these correlations would remain statistically significant if corrected for multivariable analysis (Full correlations are presented in *Appendix 11.4*).

5.5.3.7. Dietary intake and other patient characteristics.

There was no difference in eGFR, number of medications, HtSDS, WtSDS, BMI SDS, appetite in those with zinc, iodine, or iron intakes < 80% RNI compared to those with intakes >RNI.

Dietary intake was explored in relation to the age of the children and young people. Age was greater in those with intakes < 80% RNI (zinc: Mann Whitney U t=356, p=0.026; iodine: MWU t=381.5, p=0.007; iron: MWU t=370.5, p=0.02). In view of this, correlations were sought between age and dietary intake of nutrients. The strongest negative correlations were between age and: manganese (S.rho=-0.632); selenium (S.rho=-0.618);

vitamin B12 (S.rho=-0.515); vitamin K (S.rho=-0.479); folate (S.rho=-0.413). Full analysis details are found in *Appendix 11.4.*

Table 10. Percentage of pre-dialysis, conservatively-managed subgroup (n=46) with dietary intake < LRNI with comparison with national diet and nutrition survey data.

	Males 4-10 years	Males 11-18 years	Females 4-10 years	Females 11-18 years
Vitamin A	11% 7%	40% 14%	0% 12%	18% 18%
Riboflavin	11% 0%	20% 8%	14% 1%	18% 20%
Folate	6% 0%	20% 5%	0% 0%	0% 8%
Iron	11% 1%	30% 9%	0% 3%	36% 48%
Calcium	11% 1%	30% 12%	0% 1%	45% 19%
Magnesium	17% 0%	50% 27%	0% 3%	73% 48%
Potassium	0% 0%	50% 15%	0% 0%	45% 33%
Iodine	22% 5%	50% 16%	0% 7%	45% 26%
Selenium	13% 1%	50% 23%	0% 2%	82% 44%
Zinc	33% 4%	46% 17%	0% 13%	28% 22%

Comparison between the general population and the TEMPeReD cohort (PMCM subgroup) with regards to the percentage of children and young people with estimated intakes of less than the LRNI. The NDNS data (76) shown in red. In the majority, the PDCM CKD subgroup demonstrated a greater proportion at risk of nutritional inadequacy with intakes estimated to be below the LRNI.

5.5.3.8. Dietary Fibre

Dietary fibre was also estimated in the cohort. No child or young person met the requirement of dietary fibre (238). Mean intake as expressed as a percentage of requirement for dietary fibre was 31.2% for the entire TEMPeReD cohort and 34.6% in the PDCM subgroup. Details of dietary fibre intake for each age group (as per recommended intake) are displayed in *Table 11, below*.

There was no difference in dietary fibre intake as expressed as a percentage of requirement when comparing those with BMISDS >2 with those with BMISDS <2 (MWUT = 159, p = 0.215), and no correlations were found between dietary fibre intake (percentage of requirement) and BMISDS (S.rho = 0.156, p = 0.301), mid-upper arm circumference SDS (S.rho = 0.213, p = 0.156), waist-to-height circumference ratio (S.rho = 0.161, p = 0.286), or waist circumference SDS (S.rho = 0.250, p = 0.141).

Table 11. Dietary intake of fibre for the TEMPeReD cohort and the pre-dialysis, conservatively-managed subgroup (n=46) by age group.

		Fibre intake (g/day) Mean ± SD	Fibre intake (% of requirements) Mean ± SD	Number meeting requirements.
Entire cohort	All ages (n = 60)	6.7 ± 4.2	31.2 ± 19.9 %	0
	2-5 years (n = 7)	7.2 ± 3.8	47.9 ± 25.5 %	0
	5-11 years (n = 26)	5.7 ± 3.6	28.4 ± 18.2 %	0
	11-16 years (n =20)	8.1 ± 4.5	32.4 ± 18.2 %	0
	16-18 years (n = 7)	6.4 ± 5.5	21.3 ± 18.4 %	0
Pre-dialysis, conservatively-managed subgroup	All ages (n = 46)	7.5 ± 4.3	34.6 ± 20.0 %	0
	2-5 years (n = 7)	7.2 ±3.8	47.9 ± 25.5 %	0
	5-11 years (n = 18)	6.1 ±3.6	30.5 ± 17.8 %	0
	11-16 years (n = 16)	9.1 ± 4.5	36.5 ± 17.9 %	0
	16-18 years (n = 5)	7.4 ± 6.4	24.7 ± 21.3 %	0

Recommended dietary fibre intake: 2-5 years = 15g/day; 5-11 years = 20g/day; 11-16 years = 25 g/day; 16-18 years = 30g/day (238).

Dietary intake of fibre was also assessed for association with progression of disease. There was no association in the PDCM subgroup as a whole (S.rho = 0.158, p = 0.358), but on subgroup analysis for those that had a decline in their kidney function at 12 months (change in eGFR at 12months < 0ml/min/1.73m^2, n=19), a correlation between lower dietary fibre intake and greater decline in kidney function was seen: S.rho = 0.507, p= 0.027 - See *figure 31*, below).

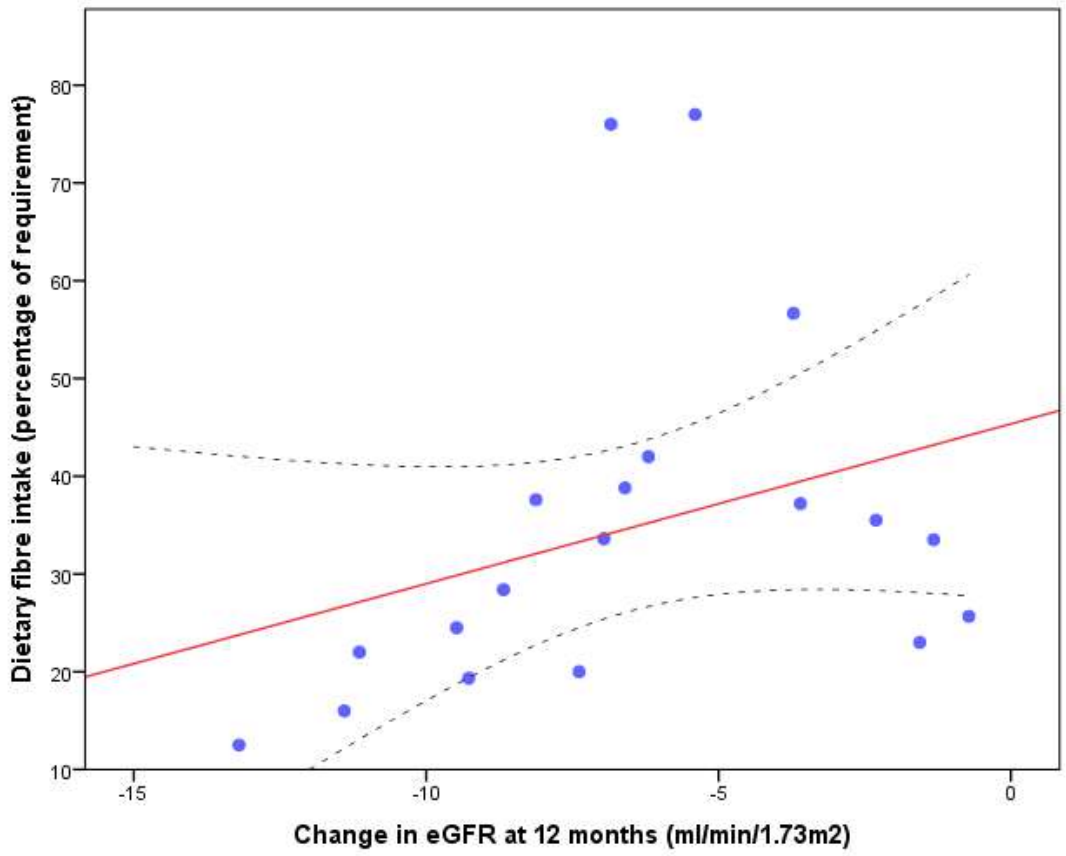


Figure 31. Association between dietary intake of fibre and decline in kidney function in the pre-dialysis, conservatively-managed CKD subgroup in those that had a decline in kidney function at 12 months.

On examination of those who had a decline in their kidney function at 12 months, there was a correlation between the degree of decline and their estimated dietary intake of dietary fibre with a correlation coefficient of 0.507.

5.5.3.9. Salt Intake

As shown in *Table 12*, intakes of the cohort were no different than from national data from the national diet and nutrition survey. Mean intakes of sodium were greater than the RNI for each age-group and greater than the recommended maximum intake for those aged 4 to 10 years. Despite average intakes of older children being lower than the recommended maximal, nearly half of children had intakes greater than this maximum, with proportionally a greater number of younger children exceeding, compared to older children (see *Table 13*).

The majority of children were taking anti-hypertensive agents (all enalapril), with 4 children taking multiple agents (other agents were amlodipine (4), and atenolol (1)). There was no difference in sodium intake in those receiving anti-hypertensives and those not (180 SD±113 %RNI versus 158 SD±83 %RNI, respectively). Dose of enalapril (mg/kg) did not correlate with sodium intake (Spearman's rho = 0.254, p=0.459). The number of children exceeding the maximum sodium intake was similar for those taking antihypertensives and those not (see *Table 13*). Dietary intake of sodium did not correlate with blood pressure SDS for those not prescribed anti-hypertensive agents.

There was no difference in sodium intake depending upon diagnosis (Kruskal-Wallis test t(12)=14.915, p=0.246), with no difference between dysplasia patients and all other diagnoses (Mann Whitney-U test t=129.00, p=0.105). The number of children exceeding the maximum sodium intake was similar for those with a diagnosis other than kidney dysplasia and the group as a whole (see *Table 13*).

Table 12. Dietary sodium intake of the pre-dialysis, conservatively-managed subgroup (n=46) versus national data.

	Mean and SD sodium intake of CONS cohort (mg/d)	NDNS (years 5-6) intake (national average intake) (mg/d)	One-sample t-test result: t-statistic (degrees of freedom) and p-value.
Aged 4 to 10 years			
Males	1864 ± 854 (n=18)	1660	T(17)=1.016, p = 0.324
Females	2106 ±1230 (n=7)	1538	T(6)=1.222, p = 0.268
Aged 11 to 18 years			
Males	2534 ± 1146 (n=10)	2225	T(9)=0.852, p = 0.416
Females	1557 ± 774 (n=11)	1860	T(10)=-1.297, p = 0.244

Table 13. Dietary sodium intake of the pre-dialysis, conservatively-managed subgroup (n=46) versus RNI and recommended maximum intake levels.

	RNI (mg/d)	Maximal Sodium intake (SACN, 2003) (mg/d)	Mean and SD sodium intake of PDCM subgroup (mg/d)	Number of children exceeding maximum sodium intake (%)	With a diagnosis other than dysplasia (n=35): Number of children exceeding maximum sodium intake (%)	On antihypertensives	No antihypertensives
4 to 6 years	700	1200	1810 ± 849 (n=11)	8/11 (73%)	6/8 (75%)	5/6 (83%)	3/5 (60%)
7 to 10 years	1200	2000	2028 ± 1047 (n=14)	6/14 (43%)	6/13 (46%)	3/7 (43%)	3/7 (43%)
11 to 18 years	1600	2400	2022 ± 1068 (n=21)	5/21 (24%)	4/14 (29%)	3/13 (23%)	2/8 (25%)
Total				19/46 (41%)	16/35 (46%)	11/26 (42%)	8/20 (40%)

5.5.3.10. Does dietary intake predict poor growth?

When comparing dietary intake of energy and nutrients of those with poor growth (SDS <-2) and normal growth (SDS >-2) using either height SDS or height velocity SDS criteria, no nutrient was significantly different between the two groups. Additionally, the number of nutrients with estimated intakes of < 80% requirements were no different (Height SDS: MWUT = 193, p = 0.782; height velocity SDS: MWUT = 197, p = 0.860), and those < LRNI were not different between the two groups (Height SDS: MWUT = 172, p = 0.412; height velocity SDS: MWUT = 210.5, p = 0.868).

Dietary fibre

On scatter-plot, there was no association between dietary intake of fibre and (percentage of requirement) and height velocity SDS, and no difference between those with poor growth (Height velocity SDS <-2) and those with normal growth (Height velocity SDS >-2); MWUT = 203.5, p = 0.990. Although not reaching statistical significance, height SDS demonstrated a trend towards lower values with lower dietary fibre intakes as expressed as percentage of requirement (S.rho = 0.287, p = 0.053). No difference in those with poor growth (HtSDS <-2) and normal growth (HtSDS >-2); MWUT = 135, p = 0.084.

5.5.4. Discussion

The aim of this subchapter is to report the dietary intake of a cohort of children and young people using usual clinical practice. These data presented are the most comprehensive list of nutrients analysed for dietary intake in children with pre-dialysis CKD, reporting a wide selection of energy, nutrients and fibre in a contemporary cohort, albeit a single centre and in a small cohort. In addition, for contemporary data to be reported (due to changing eating habits and food availability, for example) some nutrient intake data including that for vitamin K have not previously been reported.

The main findings are: the large variation in estimated intakes, the high proportion of children and young people with estimated intakes below recommendations, that older children and young people at greater risk of having intake less than recommended intakes, and that none of the children and young people examined met recommended dietary fibre. Moreover, dietary fibre intake was associated with decline in eGFR at 12 months.

5.5.4.1. Intake of nutrients

These data show a variation in intake not related to disease severity, and that in the methods used here, most children / young people had at least one nutrient below the LRNI (65.2%). As in the general population from the national diet and nutrition survey (NDNS) (76), age was a significant influencing factor in the likelihood of meeting recommended intakes or not with older children and young people less likely to be in receipt of adequate nutrients. For younger children (4 to 10 years), boys had more nutrients with intakes below the LRNI, but in older children and young people (11 to 18 years), girls were more likely to have more nutrients below this recommended level.

Compared to the general population, the PDCM subgroup had a higher proportion of children / young people below the LRNI for the majority of nutrients presented in the NDNS data; including 82% of girls aged 11 to 18 years have selenium intake less than the LRNI. Average intakes in those aged 11 to 18 years were lower for

magnesium, potassium, selenium and zinc; this is despite the general population having mean intakes lower than the RNI.

Although much focus is placed in the clinical context on younger children in which a more paternalistic approach to care is taken by both caregiver and healthcare professional, older children and young people appear at risk from inadequate dietary intake of many nutrients. In addition to adequate health and growth throughout childhood and adolescence, adequate nutrition is also needed to ready the bodies of young people for pregnancy and the *in utero* nutrition of their children with potential negative implications for subsequent generation of children if maternal nutrition is suboptimal.

5.5.4.2. Dietary Fibre

Current guidance recommends between 15g and 30g per day of dietary fibre (238). There is significant evidence for the benefit of a high fibre diet in the general population with large epidemiological data demonstrating a relative reduced risk of cardiovascular disease in those with higher intakes of dietary fibre (241-244).

In the data presented here, no child or young person had a dietary intake of fibre that met these recommendations, with average intake for the cohort of only ~ 35% of recommended intakes. There was a pattern that younger children had intakes that were closer to their recommendations (although still less than half of recommendation intakes) and older children and young people had intakes less than a quarter of their recommended intakes. This is in-keeping with both adult CKD patients not meeting requirements (245), and national UK data from the NDNS survey of the general population in which children and young people failed to meet recommended intakes. In the NDNS dataset, fibre (AOAC) intakes were 14g/d and 15.3g/d for age groups 4-11 and 11-18 years, respectively (76) which are greater than the intakes reported in this study, and so the paediatric CKD population may be particularly vulnerable to not meeting their fibre recommended intake. The reasons for this may include dietary restrictions placed on individuals, although these do not tend to be imposed until more severe disease in which potassium and phosphate are more likely to become problematic in terms of elevated serum concentrations.

With these data in mind, the next question to be asked is whether supplementation of dietary fibre is therefore warranted in the paediatric CKD population. Dietary fibre has been demonstrated to alter transit times, alter satiety, act as an energy source for the gut microbiota, and affect the innate immune system of the gut mucosa both directly and indirectly (246). Supplementation of CKD patients with fibre has been associated with an increase in colonic bacteria and a decrease in serum urea by 12% (247). NHANES data from the USA have associated lower markers of inflammation (CRP) and mortality with higher fibre intakes in the adult CKD population (245).

Some amino acid fermentation products are nephrotoxic – with most focus placed on indoxyl sulphate and *p*-cresyl sulphate and have been associated with kidney disease progression, cardiovascular health and morality in those with CKD (248, 249).

There was no association between dietary intake of fibre and markers of growth. Although not reaching statistical significance, height SDS demonstrated a trend towards lower values with lower dietary fibre intakes as

expressed as percentage of requirement (S.rho = 0.287, p = 0.053). In concordance with previous literature, in those children and young people who had a decrease in their kidney function at 12 months, those with lower estimated dietary fibre intakes had the greatest decline (S.rho = 0.507, p= 0.027). Although this is only a small subgroup, this may be as a result of increased absorption of nephrotoxic amino acid fermentation products that may be more prevalent in lower fibre diets.

In children with normal kidney function, intake of dietary fibre (whole grain) has been associated with a lower risk of overweight/obesity even once controlling for potential confounders such as fruit, vegetable and dairy intake, age, sex and physical activity levels (250). No such association was found in the data presented here. The reason for this may be due to the small number of children and young people analysed (a constant problem for those investigating rare diseases such as CKD in childhood), and / or the potential inadequacy of dietary intake estimation, or that an association in obesity in those with CKD and dietary intake of fibre is not associated. This later explanation may be due to the body composition changes seen in those with CKD, and that definitions of obesity may not reflect adiposity in the same way to the general population.

Interventions that might be appropriate include dietary advice and counselling from the paediatric dietetic team, and / or supplementation with fibre supplements. Unfortunately, there is limited evidence to support supplementation in the paediatric CKD population, with no systematic review.

5.5.4.3. Dietary Sodium Intake

Salt (sodium chloride) is needed for normal bodily function, but in more economically-developed countries (MEDCs); including the UK, salt intake is greater than is required and moreover is above recommended intake levels (251). Recommended intakes exist, at least in part because high salt-intakes are associated with poorer health outcomes; including the development of hypertension, and associated cardiovascular disease; including death from stroke (252, 253).

Mean sodium intake of the cohort was above the RNI for age, with significant variation demonstrated in wide standard deviation values. Younger children were more likely to exceed the maximal dietary sodium intake then older children.

Despite potential under-reporting bias of sodium intake in this population due to wanting report “the right thing”, and regular contact with health professionals with a consistent message of decreasing sodium intake for the benefit on kidney health and blood pressure control within an at-risk population, sodium intake in this pre-dialysis, conservatively-managed subgroup remains high. Despite this, the blood pressures of the cohort did not reflect hypertension. This may be because the treatment with antihypertensives either for previously recognised hypertension or the prescription of ACE-inhibitors for proteinuria that additionally, lower blood pressure. Alternatively, it may be that the cohort may have a large number of individuals that “salt waste’ due to dysfunctional tubular function, and that a higher salt intake in this population is beneficial for maintenance of normonatremia.

Although 24-hour urinary sodium excretion is the most accurate method by which to estimate sodium intake (254), this is often not practical, especially in the paediatric setting and costly. In adults, food frequency questionnaires have been developed (254), but none are currently available that have been validated for children

with CKD. For ongoing clinical review it may be that a food frequency questionnaire is accurate enough in its prediction of intake to allow for assessment of concordance with a low-salt dietary prescription.

Further family education, and dietary assessment of sodium intake in this population. As much of the sodium intake of the UK population arises from processed foods, an increased awareness of this must be instilled in the families of our population to empower them to make healthy food choices. From a population level, efforts to decrease the salt-content of foods available through public health strategies must be pursued.

The reason why this cohort has a large proportion of children receiving likely inadequate intake of nutrients is not clear, but as energy intake is also low for many of the children, one explanation is that the 24-hour recall methodology for predicting dietary intake is generally under-predicting. As the majority of children in the nephrology clinic would be growing, it may be presumed that the children are receiving the minimum energy requirement for this. Although the energy prediction methodology (239) may also be inaccurate.

There was no association found between the intake of specific nutrients and or the number of nutrients estimated to below recommended intakes in those with adequate or poor growth. This may be due to dietetic intervention in the alteration of dietary intake in those with disease having an impact either directly or indirectly through nutritional status change upon growth. Alternatively, a difference may not have been observed due to the small sample size of the study, or due to the limitations of a single 24-hour recall used to assess the diet.

The current dietary assessment of children with CKD

With the above discussion and interpretation in mind, one limitation of the data presented is that estimation of dietary intake was undertaken (as per usual clinical practice) by 24-hour multiple pass dietary recall. These data may be used to support the hypothesis that current clinical practice of administering a single 24-hour recall does not adequately identify those with micronutrient inadequacy as defined as blood micronutrient concentrations below the normal reference range. Although, blood biochemical measures of micronutrients, as discussed in *Chapter 4*, may not reflect true body status of the nutrient, the high prevalence of anthropometric measures within two standard deviations of the mean of the growth standard (27) casts doubt of the significance of the these data as the child and young person appears to be growing appropriately.

The concerns about using this method include:

1. A single 24-hour recall is unlikely to be adequate to ensure that the predicted micronutrient intake reflects true dietary intake, especially of nutrients that are infrequently consumed. This is especially the case in paediatrics, where day-to-day variability may be greater than in adults (255).
2. The standards (dietary reference values) upon which these data are then compared have been determined for a healthy population. In using these standards, there is an assumption that the requirements for children with kidney disease do not differ from that of healthy children for the majority of micro nutrient intakes. This is unlikely, due to probable alterations in losses and metabolic demand. For example, B-vitamin is a necessity in energy metabolism.

However, there is value in using 24-hour recalls over and above that of nutrient analysis - to start discussions on cooking, shopping, dietary patterns, lifestyle, and to gain insight into daily life in the context of food and diet. In

addition, in those with a nutritional prescription of food restriction (for example, potassium or phosphate restriction) then this may aid assessment of concordance with this prescription.

Current clinical guidance states that dietary intake be assessed regularly by a skilled registered kidney dietitian by means of a 3-day diet diary or three 24-hour recalls, although current practice is usually a pragmatic single 24-hour recall.

The reasons for dietitians for using a single 24-hour recall rather than 3 day food diary or three 24-hour recalls has not been explored. The thoughts, opinions and understanding of the assessment techniques warrant exploration within the paediatrics kidney dietetic workforce. Anecdotally, this is explained as a 3-day food diary being impractical. If practitioners feel that such tools do not adequately estimate true intake (as suggested here) then other methods of determining risk of dietary inadequacy must be sought. If issues are purely surrounding education of the recommendations of the tool needing three non-consecutive days' analysis and /or time-limitation in the clinical environment, then other measures should be explored to remedy these issues.

There is no tool that easily identifies concordance with prescriptive diets (e.g., phosphate restriction) with reliance upon 24-hour recall and diet diaries. Such tools, as discussed in the introduction, have limitations and take a significant amount of time to administer and analyse. With such insecurity of the methodology in estimating true intake, we must consider whether inflicting such a burden upon children and their families is in their best interests, consumes time and expense in the health services, and offers the clinical team falsely reassuring or worrying data upon which they base their decisions.

As depicted in *Figure 26*, there are several goals that may be achieved by dietary assessment. The concordance with dietary prescription is an important part of a paediatric kidney dietetic review. It may be reasonable to assess a diet for the content of a particular nutrient of interest (e.g., potassium) only rather than completing an entire food diary, the validity of which is questionable. Therefore, the development of nutrient-specific food frequency questionnaires could be developed based on dietary data from paediatric CKD cohorts to determine which foods explain most of the variability of nutrient intake.

5.5.4.4. Limitations

As discussed above, limitations of this analysis include the small sample size introducing the possibility of type 1 and type 2 errors, the flaws and limitations of 24-hour recall as a dietary assessment method, and reliance upon the dietary analysis package. The latter may be eliminated through the use of paired, weighed food analysis, but this has significant implications on cost, and would be impractical for assessment of *ad libitum* intakes in the community setting.

5.5.4.5. Future Directions

Clinical tools for estimating dietary intake of energy, nutrients and fibre that are easy to use for patients and healthcare professionals must be developed and trialled. In current clinical practice, much of the time that a dietitian spends with the child / young person and their family is occupied in this estimation. Ideally, this information should be available prior to the consultation and allow for greater time in understanding how a given dietary intake has been achieved and discussion around how modification / optimisation may be accomplished.

The development of the technology-based formats completed and analysed prior to consultation may facilitate this.

Food group analysis would be useful in identifying which food groups contribute to key nutrients, such as potassium and phosphate in children and young people with CKD.

Prospective studies of dietary fibre intake, the influence of intake on circulating nephrotoxic substances including amino acid fermentation products and clinically meaningful outcomes such as disease progression, cardiovascular complications, and mortality are needed. Additionally, the role of supplementation must be addressed. Increasing fibre intake may be relatively easily achieved through the prescription of supplements, but whether this is as beneficial as dietary modification, and optimal supplement and impact upon medication burden and quality of life must be explored. Perhaps the first steps in exploring the role of dietary fibre in paediatric CKD is a review of the current clinical practice amongst specialist dietitians in the UK, and review of the literature on the subject in the form of a systematic review and a Delphi consensus process of guidance.

The thoughts, opinions and understanding of the assessment techniques of dietary intake warrant exploration within the paediatrics kidney dietetic workforce. Current evidence suggests that multiple 24-hour recalls are required, and that several recalls are needed for some nutrients. This is discordant with the current practice. This piece of work may be suited to a quality improvement project within the dietetic community.

5.5.4.6. Concluding Remarks

This analysis is in agreement in previous literature that places children and young people with CKD at risk from nutritional inadequacy, but that this dietary intake risk has not directly translated into biochemical abnormalities or growth. There is variation in dietary intake, and this variation is not explained by disease severity alone.

5.5.4.7. Practice Points

- The current practice is adequate.
- Current practice is inadequate to characterise the dietary intake. This is suggested by high proportion with either stable or improved height SDS at 12-months (>88%) despite a large number deemed at risk from inadequate intake and the lack of concordance between low blood concentrations. Therefore, if dietary analysis is required then a full characterisation should be performed through multiple-day analysis.
- Despite methodological insecurity at the individual level, these data suggest that children and young people with CKD are at increased risk of inadequate intake of dietary fibre with no child or young person meeting recommended intakes. Although not routinely evaluated, dietary fibre should be incorporated into routine clinical care with consideration for nutritional counselling and possible supplementation.

5.6. Trace Element and Vitamin Concentrations in Paediatric CKD Patients

5.6.1. Introduction

There is a lack of literature assessing the micronutrient status of children with CKD. Although the prevalence of nutritional risk of inadequacy was high in those in the TEMPeReD cohort, the prevalence of abnormal blood concentrations in such a population is unknown. Therefore, the TEMPeReD cohort's blood concentrations of vitamins and minerals were evaluated.

Nutrition is a cornerstone of the clinical management of children with chronic kidney disease (CKD). Current guidance from KDOQI states that all children with CKD should have regular nutritional assessment and their micronutrient status reviewed regularly [1]. In practice, attention is usually restricted to biochemical review of vitamin D and iron status whilst the adequacy of other micronutrients is largely determined by dietetic review of recent reported intake. Oral micronutrient supplementation is recommended where dietary intakes appear insufficient so that the dietary intake achieves the Reference Nutrient Intake (RNI) (or equivalent) (2).

This approach is constrained by several issues. Firstly, little is known about the demands for and losses of micronutrients of these children across differing pathophysiological states or treatment [1]. Secondly, brief dietary assessment, such as 24 hour recall, is unlikely to adequately and securely reflect the intake of micronutrients due to innate daily variability in intake and the preponderance of missing values in the food composition dataset [2]. Thirdly, the appropriateness of judging adequacy of apparent dietary intake against the RNI in this population and whether supplementation to 100% of RNI adequately addresses the needs of these children. Finally, whilst addressing vitamin D and iron, access to biochemical measures of micronutrient status are not uniformly available in all centres, not complete and not applied systematically within usual routine care, especially in those conservatively managed.

The previous literature of biochemical measures of micronutrient status in paediatric CKD is limited and has been largely focused on those with the most severe disease and those in receipt of dialysis. The micronutrient status of conservatively managed children with pre-dialysis disease is uncertain with both the risk of increased losses and poor dietary intake, but also the potential risk of excess intake and toxicity arising from inappropriate supplementation.

The aim of this study was to describe blood concentrations of micronutrients and current supplementation in a cohort of children with pre-dialysis, conservatively-managed CKD receiving current routine clinical practice.

5.6.2. Methods

Children aged between 3 and 18 years with conservatively-managed CKD (stages 2 to 5, not receiving dialysis and never received a kidney transplant) under the care of a tertiary paediatric nephrology service were identified using electronic notes, and invited to participate in the study. Participants were recruited on a first come, first served basis and following acquiring informed consent, assessed at routine clinic appointments. All participants were receiving routine clinical care, including usual dietetic care. Clinical, anthropometric, and nutritional support data were prospectively collected including estimated glomerular filtration rate (eGFR), sex, CKD aetiology, height, weight, and waist circumference. Height velocity was calculated over the previous 6-month period. Age and gender specific Standard Deviation Scores (SDS) for height, weight, height velocity, body mass

index (BMI) and waist circumference-to-height ratios (WHtR) were calculated against RCPCH/WHO growth reference standards (256). Blood samples were obtained from children and sent for routine clinical testing by the clinical chemical pathology department. These included renal profile, full blood count, vitamin D and ferritin. Further biochemical analysis of micronutrient status was conducted using research assays by the Nutrition Laboratory of The Wessex Investigational Science Hub at the University Hospital Southampton including vitamin E (total tocopherols), vitamin A (total retinol) and vitamin B6 (pyridoxal-5-phosphate) by high performance liquid chromatography); vitamin C (by colorimetric assay); and serum copper and zinc and whole blood manganese by inductively-coupled mass spectrometry. Where elevated vitamin E concentrations were determined, the patient record was interrogated for cholesterol profile to correct vitamin E status for cholesterol status, and compared to reference data (257). Blood measurements are reported as continuous variables and also as 'below reference range', 'within reference range', or 'above reference range'. The normal reference ranges are available in *Appendix 11.1*.

Anthropometric measures were also categorised; including 'tall for age' (height SDS >2 SDS), and obese (BMI SDS >2, or WHtR >0.5).

Data were collected and collated using the aforementioned dedicated database and subsequently analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Descriptive statistical analysis including percentages of different CKD stages was performed. Values were expressed as mean and standard deviations (SD) or median and interquartile range (IQR) as appropriate following assessment of distribution by Shapiro-Wilk test. Statistical significance was defined as a p-value of less than 0.05.

Correlations were explored between variables, including between micronutrient concentrations and the receipt of nutritional support reporting Pearson's coefficient (P.coeff) or Spearman's rho (S.rho). Differences between mean values were explored using t-test or Mann-Whitney U-test (MWUT), as appropriate. When comparison was made with national population data, the data were divided as appropriate per age-groups given in the national data (76).

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from all individual participants included in the study, with informed consent obtained from care-givers and informed assent as appropriate.

5.6.3. Results

Forty-six children with pre-dialysis, conservatively-managed CKD were enrolled into the study. Details of the cohort are given earlier. Median height SDS was -0.65 (IQR±2.03). Mean values (with standard deviations) of weight SDS and BMI SDS for the cohort were -0.43 (±1.81), and 0.32 (±1.41), respectively. Eight children (17%) had weight-for-age SDS <-2 (malnourished by ICD-10 definition), three children (7%) had weight-for-age SDS >2. One child (2%) was under-weight for height (BMI SDS <-2); thirteen children (28%) were at risk of being overweight (>85th percentile), six children (13.0%) had BMI SDS >2. Twelve children (26%) were short-for-age (height SDS <-2), one child (2%) was tall-for-age (height SDS >2). Twenty children (44%) had a WHtR >0.5. Median height velocity SDS was -0.55 (IQR±2.5), with 28% having a height velocity SDS <-2.

There was no correlation with anthropometric measures and biochemical measures of micronutrient status. All of the children had at least one biochemical measure of micronutrient status that fell outside of the normal reference range, and the median number of abnormal blood results per child was 2.5 (IQR±1). The most common abnormal measurement was an elevated vitamin E concentration (95%), followed by an elevated vitamin A (74%), elevated manganese (23%), low zinc (12%), and low vitamin D (13%). There was a low prevalence of blood concentrations of micronutrients below the normal reference range: Vitamin A (2%); Vitamin D (13%); Manganese (6%); Selenium (2%); Zinc (12%). The prevalence of other micronutrient concentrations below the lower limits of the reference range were: Vitamin B6 (2%); Vitamin B12 (2%); Vitamin C (2%); Copper (2%). No patients had Vitamin E or Folate measures below the lower limit of the reference range. There was no discernible pattern of abnormal micronutrient concentrations grouping in particular individuals, and no differences between sexes.

Table 14. Average blood concentrations of the pre-dialysis, conservatively-managed subgroup (n=46).

Nutrient (units)	Average	SD / IQR	Range Min	Range Max	Number lower than reference range	Number higher than reference range
Haemoglobin (g/l)	129.14	16.92	86.00	160.00	9	0
Sodium ǂ (mmol/l)	137.00	2.00	134.00	141.00	0	0
Potassium ǂ (mmol/l)	4.2	0.63	3.40	5.50	1	4
Corrected Calcium (mmol/l)	2.42	0.10	2.21	2.62	0	2
Magnesium ǂ (mmol/l)	0.82	0.13	0.71	1.21	0	2
Inorganic Phosphate (mmol/l)	1.36	0.23	0.70	1.93	1	1
Ferritin ǂ (µg/l)	45.00	84.50	14.00	488.00	0	4
Copper (µmol/l)	19.42	4.78	9.70	31.60	1	3
Zinc ǂ (µmol/l)	13.55	3.13	9.50	22.80	5	0
Manganese ǂ (nmol/l)	152.00	69.00	61.00	494.00	2	8
Selenium (µmol/l)	1.04	0.20	0.47	1.46	1	0
Vitamin A (µmol/l)	2.38	0.84	0.80	4.50	1	31
Vitamin E ǂ (µmol/l)	27.90	7.63	20.80	60.50	0	40
Vitamin B12 ǂ (ng/l)	384.00	364.25	100.00	1072.00	1	-
Folate € (ng/ml)	10.45	-	4.30	>25.00	0	6
Vitamin C ǂ (µmol/l)	70.60	42.75	6.20	200.10	1	2
Vitamin B6 ǂ (nmol/l)	80.20	39.20	19.40	253.60	1	2
Vitamin D ǂ (nmol/l)	95.00	72.00	36.00	239.00	5	-

ǂ *-Non-parametric distribution, average expressed as median and interquartile range (IQR), otherwise mean and standard deviation (SD) presented. As folate is expressed as >25ng/ml for those above the reference range, SD cannot be calculated for folate. There is no upper reference range for vitamin B12, or vitamin D.*

€- Geometric mean reported.

Vitamin A (total retinol) and vitamin E (total tocopherols) were elevated in the majority of children. There was a low prevalence in low nutrient measurements in the cohort.

A similar pattern of blood measurement abnormalities were seen in those with mild (CKD stages 1 and 2) compared to those with moderate-severe disease (CKD stages 3 and 4). However, elevated vitamin A was more prevalent in those more severe disease and eGFR was found to inversely correlate with vitamin A (P.coeff=-0.649; p<0.0005). In contrast, biochemical measures of vitamin B12, selenium and manganese concentrations showed positive correlations with eGFR: vitamin B12 (S.rho=0.321, p=0.044); selenium (P.coeff=0.309; p=0.047) and manganese (S.rho=0.353, p=0.037).

Twenty-eight children (70%) received some form of nutritional supplementation in the form of a feed, sip-feed or supplement (details of the nutritional products for the cohort are given in *Table 3*.). The most frequently prescribed nutritional support were iron as ferrous sulphate or ferrous fumarate (37%) and vitamin D supplements as cholecalciferol (28%). Two families had chosen to administer their own choice of 'over-the-counter' vitamin preparations (one a multivitamin, and one a vitamin C supplement). Those in receipt of sip-feed supplementation were more likely to be short (HtSDS<-2) (chi-square = 8.803; p=0.003).

Table 15. The various nutritional treatment products used by the cohort (n=60) with the number (and percentage) of children.

Nutritional Product	Number of Children (%)
Feeds:	
Neocate®	1 (2%)
Kindergen®	1 (2.2%)
Sip feed:	
Standard energy feed (1kcal/ml) (e.g., Pediasure®, Frebini®)	5 (11%)
Polycal®/Maxijul®	4 (9%)
Prozero®	3 (7%)
Scandishake®	1 (2%)
High energy feed (1kcal/ml) (e.g., Fortini®).	1 (2%)
Calogen®	1 (2%)
Supplements:	
Iron supplements (e.g., ferrous sulphate, ferrous fumarate)	17 (37%)
Vitamin D supplements (Colecalciferol)	13 (28%)
Folic acid supplements	4 (9%)
Abidec®	2 (4%)
'Over-the-counter' vitamin C supplements	1 (2%)
Vitamin K supplements	1 (2%)
'Over-the-counter' standard multivitamin	1 (2%)

There was a range of support products prescribed to the cohort. The most common supplements prescribed were iron and vitamin D supplements. Two families self-prescribed 'over-the-counter' vitamin preparations.

Two children were prescribed feeds (Kindergen®, Neocate®) as a primary source of nutrition and had a greater number of nutrient concentrations outside of the normal reference range compared to others in the cohort (median = 4.5 [no IQR available as only two children] versus 2 (IQR±1); MWUT=81.50, p=0.031). Those in receipt of folic acid supplements had higher folate concentrations (median=25.00 IQR±7.60ng/ml versus 10.7 IQR±9.85ng/ml; MWUT=129.00, p=0.004).

Vitamin A

The majority of children had elevated vitamin A concentrations (74%) – see *Figure 32* and *Table 14*. Twelve (26%) of children were in receipt of nutritional support that contained vitamin A; 10 (22%) being in the form of a sip-feed/supplement rather than a feed. Prescription of nutritional support containing vitamin A; or sip-feed or supplement did not correspond to higher vitamin A concentrations (t(40)=-0.917, p=0.365; t(36.146)=1.014, p=0.317; and t(28.781)=0.578, p=0.568, respectively). Vitamin A concentrations did not correlate with albumin-corrected calcium concentrations (P.coeff=-0.051, p=0.747).

Serum vitamin A concentrations negatively correlated with Hb concentrations (P.coeff=-0.499, p=0.001), with higher vitamin A concentrations in those with a Hb <120g/l (3.12 SD±0.80 versus 2.18 SD±0.75, t(40)=-3.313, p=0.002). Sixteen (35%) children were receiving iron supplementation.

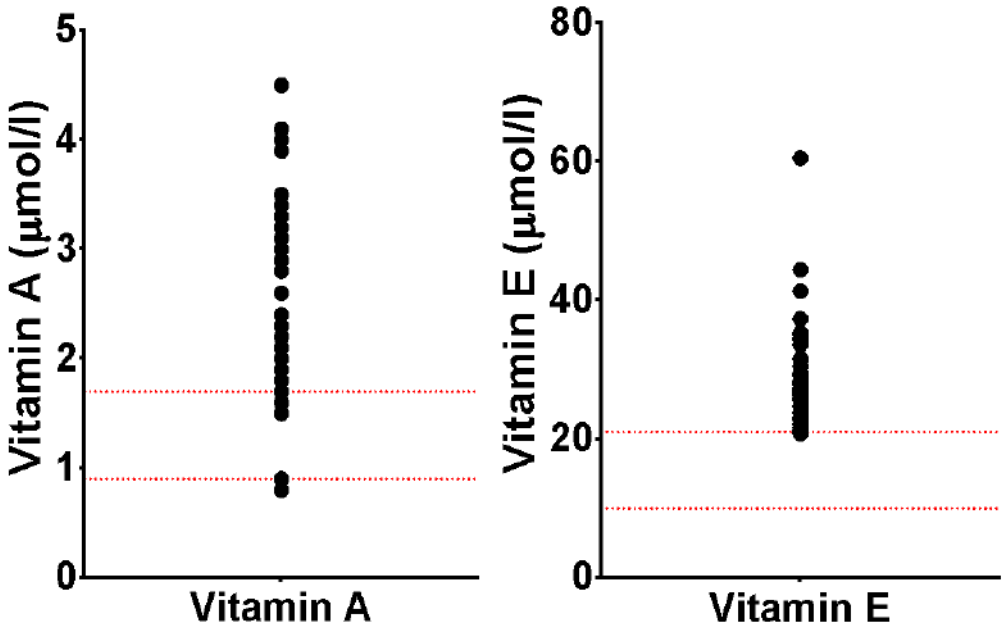


Figure 32. Distribution of blood concentrations of vitamins A and E in the pre-dialysis, conservatively-managed subgroup.

Reports the distribution of vitamin A (total retinol) and vitamin E (total tocopherols) in the cohort with the normal reference ranges indicated by the dotted lines. Both vitamins were elevated in the majority of children with a range of values up to 3-times the upper normal reference range. The clinical significance of this has yet to be determined in children with chronic kidney disease.

Vitamin E

As shown in *Table 14* and *Figure 32*, most children had elevated vitamin E concentrations (95%). There was no difference between those who were deemed at risk of obesity (BMI SDS >2, or WHtR>0.5) than those who were not (BMI: MWUT=27.00, p=0.714; WHtR: MWUT=122.50, p=0.124).

Of the 46 children, 25 children had (non-fasted) cholesterol profiles available (54.3%), with a mean total cholesterol of 4.56mmol/l (SD± 1.08). Twelve children (48%) had total cholesterol concentrations >4.40mmol/l (borderline), and six (24%) had high levels (>5.15mmol/l). Mean non-HDL cholesterol concentrations were 3.06mmol/l (SD± 1.14mmol/l; borderline/high (>3.18mmol/l) = 10 (40%); high (>3.73mmol/l) = 4 (16%). Total or non-HDL cholesterol concentrations did not correlate with vitamin E concentrations (S. rho=0.359, p=0.092; and S.rho=0.308, p=0.152, respectively). 48% had vitamin E to cholesterol ratio >90th percentile.

Folate

Those in receipt of folate supplements had significantly higher serum folate concentrations (median=25.00 IQR±7.60 versus 10.7 IQR±9.86; MWUT=129.00, p=0.004) and 3 out of 5 high values were in those taking supplements. No children were taking medication known to alter folate absorption or metabolism.

Manganese

23% of children had high whole blood manganese concentrations and 6% low. Those with a Hb <120g/l had higher whole blood manganese concentrations than those with higher Hb (median=128.00 IQR±66.00 versus 169.00 IQR±106.75; MWUT=62.5, p=0.038).There was no correlation with Hb or mean corpuscular volume (S.rho=0.097, p=0.578 and -0.305, p=0.075, respectively), and no difference in manganese concentrations in those in receipt of iron supplements or not (MWUT=143.5, p=0.907).

5.6.4. Discussion

This study reports biochemical markers of micronutrient status in a cohort of paediatric pre-dialysis, conservatively-managed patients receiving usual routine clinical and dietetic care at our centre. In contrast to that reported for children with CKD receiving dialysis, when compared to normal reference ranges this cohort of children had few overt biochemical signs of poor micronutrient status. However, biochemical markers of both vitamin A and vitamin E were consistently markedly elevated above the reference range even in those with less severe disease, and despite limited oral micronutrient supplementation. Caution is advised in the interpretation of these biochemical measures of micronutrient status as the measure taken is primarily from the transport pool (blood), not necessarily representing the functional pool, and may be affected by several other factors, including inflammation.

There is no agreed-upon formalised nutritional treatment protocol or structured framework for children with CKD and so there is likely to be marked variations in clinical practice, often within centres over time and between attending physicians or dietitians. This is reflected in the various nutritional products prescribed to the cohort, and variation seen between centres. Although personalised treatment plans are essential, a standardised approach to nutritional treatment should help decrease unwanted variability and enable characterisation of

nutritional management and status of this rare disease. Having poor kidney function was not a determinant of the prescription of nutritional support in this cohort, but being short-for-age was.

A high proportion of children had raised vitamin A (73.8%), with a strong negative correlation with kidney function. Joyce et al (80) recently reported micronutrient data in their paediatric dialysis population, showing a high prevalence of elevated vitamin A and higher average concentrations than this cohort.

Elevated concentrations are unlikely to be due to excessive intakes, increased absorption (already high in healthy children), or decreased losses (already low in healthy children). It is therefore likely to be due to alterations in metabolism (increased hepatic mobilisation and / or decrease uptake from peripheral tissues) due to the disease process and / or its treatment.

Chronic hypervitaminosis A can cause a number of signs and symptoms that are seen in the CKD population. These include anorexia, nausea, vomiting, and increased risk of bone fracture. The aetiology of these symptoms in those with CKD is multifactorial; including uraemia and mineral-bone disease of CKD, but it may be that a degree of elevated vitamin A also contributes. Moreover, vitamin A regulates bone turnover, with excessive vitamin A resulting in decreasing bone mineral density through inhibition of osteoblasts, and the stimulation of osteoclasts. Elevated vitamin A is associated with hypercalcaemia (258), and has been observed in both those adults receiving dialysis (187, 259, 260), and in paediatric dialysis patients where retinol-binding protein correlated with hypercalcaemia (261). The data presented here did not show an association with vitamin A and albumin-corrected calcium concentrations, although there are many factors that may mask a potential association, and retinol-binding protein was not measured.

Vitamin A excess acts, via the redistribution of iron and the inhibition of erythropoiesis, to cause anaemia. It is unknown to what extent elevated vitamin A contributes to the anaemia described in CKD. Our data demonstrate a correlation between Hb and vitamin A, although further larger studies are required to determine the influence of vitamin A concentrations on the presence of anaemia in this population, and any beneficial effect of lowering it (for example, through dietary manipulation) may have.

Although there is recognition that elevated vitamin A concentrations are common in CKD, there are limited therapeutic options. Current dietetic practice is to choose feeds and supplements that have the lowest vitamin A within them. Limitation of vitamin A intake (removal of vitamin A-containing supplements) in HD patients, has been shown to decrease serum vitamin A concentrations (260). There is currently no evidence that the elevated vitamin A concentrations, except at the extreme end of toxicity, has an impact upon outcome. Moreover, limiting vitamin A intake below requirements in an attempt to decrease blood retinol concentrations may have negative consequences on health.

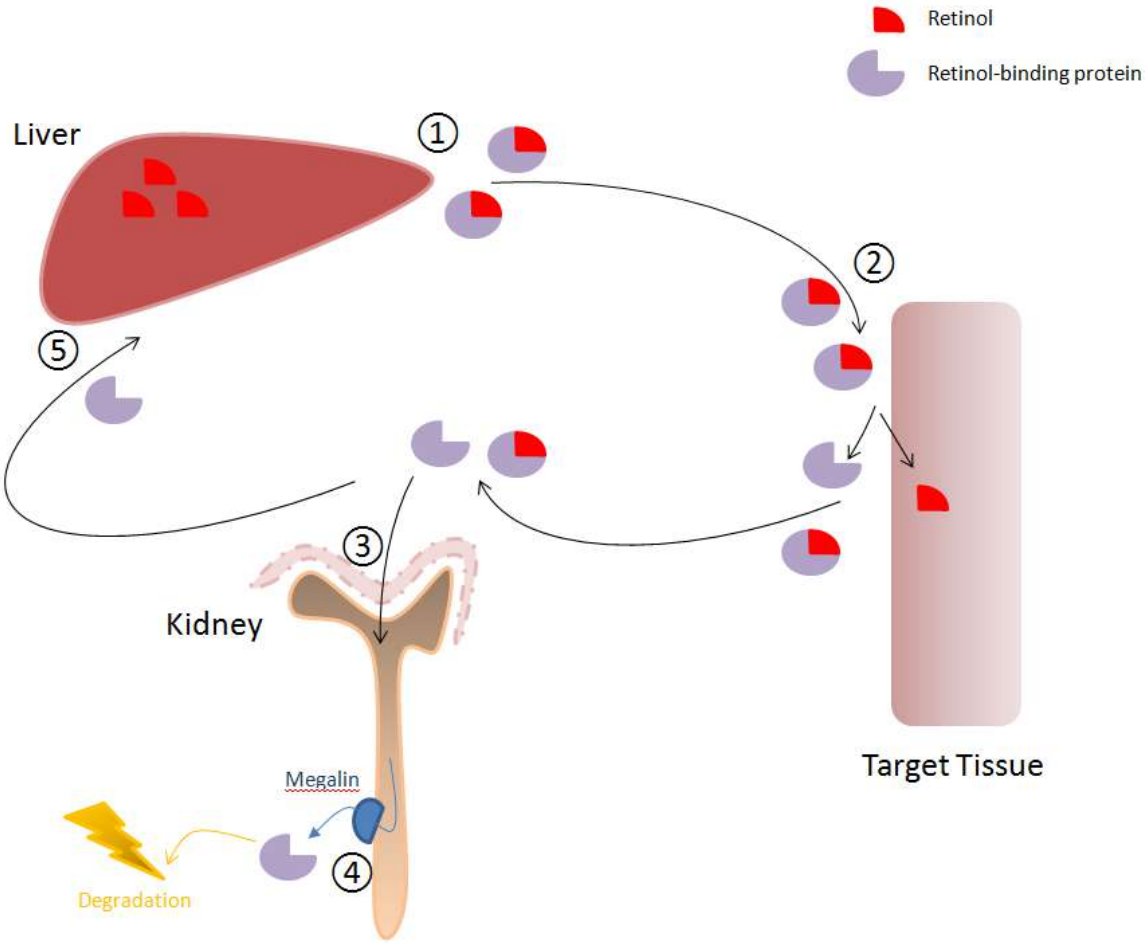


Figure 33. Proposed mechanism for the observed elevation in circulating vitamin A in CKD.

①Retinol is released into the circulating pool from the liver bound to retinol-binding protein. ②At the peripheral target tissue, retinol is cleaved from the binding protein at the target tissue, leaving Apo-Retinol-binding protein in the circulation. ③Within the kidney, Apo-Retinol-binding protein is freely filtered through the glomerulus. ④In the proximal tubule, the megalin, multi-ligand endocytotic receptor reabsorbs the retinol-binding protein, where it is degraded. With progressive nephron-loss, this degradation does not occur and apo-retinol-binding protein is exposed to the liver, promoting retinol secretion ⑤This results in loss of the usual homeostatic mechanism for circulating retinol, and elevated levels. Although those with proteinuria observed in CKD may exceed the threshold for the megalin receptor ⑤with lack of reabsorption within the proximal tubule, the additional urinary loss of retinol binding protein does is not great enough to match the lack of degradation due to nephron-loss in those with decreased glomerular filtration.

A high proportion of children had increased vitamin E concentrations (95.2%). Vitamin E has been previously reported as low (78, 185), normal (79, 176, 181, 189), and high (186). The data reported in this study suggest that vitamin E is elevated in CKD, agreeing with the recent study in which Joyce et al (80) reported micronutrient data in their paediatric dialysis population; showing a high prevalence of elevated vitamin E in paediatric dialysis patients, and higher average concentrations than this cohort; suggesting the possibility of a 'dose-response' relationship between disease severity and vitamin E, although in these data correlation was not statistically significant (p=0.059).

Similarly to the elevations in vitamin A, the increased concentrations observed are likely to be due to metabolic alteration, and as the main source of vitamin E is adipose tissue, it may be linked to the altered lipid-handling in CKD.

Vitamin E measurement is affected by fasting/non-fasting status, and may explain some of the inconsistency in the literature. Additionally, CKD is associated with dyslipidemia, and although only a subset of the cohort had cholesterol profiles available, the prevalence of dyslipidemia is similar to that reported in the CKiD cohort which report prevalence of 45% (262); not high enough attempt to explain the high vitamin E status found, especially as this cohort had better eGFR (57 versus 43ml/min/1.73m^2). The findings were not associated with markers of obesity. Not all the vitamin E concentrations were adjusted for cholesterol status, but in about half the subgroup where this was possible cholesterol-adjusted vitamin E concentrations were greater than the 90th percentile. Excess vitamin E is associated with coagulopathy and decreased platelet function (263), and trials of high-dose supplementation have demonstrated an increase in all-cause mortality (205, 264). Unfortunately, this study did not measure coagulation or leucocyte function in order to evaluate this possible negative impact. Future work should examine this. Vitamin E toxicity can also cause nausea, fatigue and muscle weakness, symptoms that are also seen in those with CKD.

5.6.4.1. Limitations

There are limitations to this study as these measures were measured at a single time point and compared against the reference ranges used in our centre and not followed prospectively over time. We were also restricted to the choice of measures of biochemical status and could only conduct those available to us through our pathology service. For example, biochemical measures of B vitamin status such as those that determine activation coefficients are not routinely available. This cross-sectional study was limited by the number of participants and the single-centre design so findings may differ in other centres subject to their practice.

5.6.4.2. Future Directions

Future research should include the measurement of functional status of micronutrients which may be able to capture true requirements of micronutrients.

5.6.4.3. Concluding Remarks

This is the first report of a cohort of children with pre-dialysis, conservatively-managed CKD in which a large panel of micronutrient concentrations have been reported in a formalised way. This is despite the availability of most of these tests through routine clinical pathology. There was no overt nutritional inadequacy as determined by these biochemical markers in this seemingly at risk population for which no structured nutritional therapy

framework exists. Although caution is advised due to the measures not being a direct measure of body status, but the transport pool that is subject to many other factors. These findings are likely to be generalisable throughout the UK. Future measurement of nutritional status should include these measures, but efforts should be made to find and utilise markers that truly reflect body status. The most notable finding was the high prevalence of high concentrations of vitamins A and E, for which potential detrimental effects and the mechanisms underlying the elevation warrant investigation.

5.6.4.4. Practice Points

- Children and young people with pre-dialysis, conservatively-managed CKD have few overt biochemical signs of poor micronutrient status, but have a high prevalence of elevated biochemical markers of vitamin A and vitamin E even in those with less severe disease. The impact of this is unknown. Caution is advised in the interpretation of biochemical measures of micronutrient status as the measure taken is primarily from the transport pool (blood), not necessarily representing the functional pool, and may be affected by several other factors, including inflammation.
- Clinical practice should endeavour to capture changes in status though a structured process such as annual reviews of status. In doing so, we can begin to characterise the nutritional profile of this rare population.

6. APPETITE ASSESSMENT IN CHILDREN WITH CHRONIC KIDNEY DISEASE

Appetite is important as both a marker of kidney disease, a marker of nutritional status, and a major determinant of dietary intake. As such, the next chapter describes an exploration of appetite in the TEMPeReD cohort. Firstly, an appetite assessment questionnaire was developed, and then used to consolidate information from a cohort of characterised children and young people with CKD. The herein-described study aims to demonstrate variability of appetite within this cohort and explore its relationship to measures of growth, nutritional status and disease severity.

6.1. INTRODUCTION

Appetite is the desire to eat food, and is a complex entity in which both biological and psychological elements interact. Its control is not fully understood, but involves a balance of various hormones and signalling pathways. In those with illness, such as chronic kidney disease (CKD), the changes in the milieu that alter appetite make the picture even more complex.

During acute and chronic illness, appetite is perturbed (81), and in those with the most severe kidney disease, diminished appetite is a major cause for anorexia and malnutrition (82), and an important determinant of energy and nutrient intake (83).

It is recommended that appetite is assessed during clinical assessment of children with CKD (2), both as a marker of disease and as a means to identify those who are at risk from inadequate dietary intake of energy and nutrients.

In clinical practice, appetite is assessed subjectively by direct questioning, (e.g., "How is your appetite?"), and through general dietary assessment. These methods are suboptimal as there are multiple confounding variables, making them not a direct surrogate for appetite. There is existing literature that finds them inaccurate; including in CKD cohorts (82). The lack of a formalised assessment tool means that a meaningful comparison between individuals and for an individual longitudinally is virtually impossible. Additionally, the subjective nature of direct questioning may result in a normalisation of poor appetite so that a child may perceive their appetite to be "good", but only relative to their previous feeling of appetite. There is no specific tool designed to evaluate appetite in children with CKD. The ideal tool for this population would be a quick and easy to use, able to give a multi-faceted evaluation of a child's appetite, adding additional useful clinical information, and deliver a numerical score to enable tracking over time in a longitudinal fashion. Additional benefit may lie in the ability to predict clinical outcomes, possibly prior to anthropometric alterations manifest.

Aim:

The aim of this chapter is to explore appetite in children and young people with CKD.

Objectives:

- Explore views and opinions about appetite assessment held by healthcare professionals working with children and young people with CKD;
- Review the current clinical practice of appetite assessment;
- Develop a questionnaire to explore appetite;

- Explore appetite using this newly developed questionnaire in a cohort of children and young people with CKD; including its relationship with growth.

Methods

A structured approach to the development of the ModSNAQ questionnaire was taken, to firstly explore health professionals' perceived need for an assessment tool followed by review of current clinical practice surrounding the assessment of appetite. A schema describing the structure of the approach in tool development and its subsequent assessment in a population of children with CKD is given in Figure *34*.

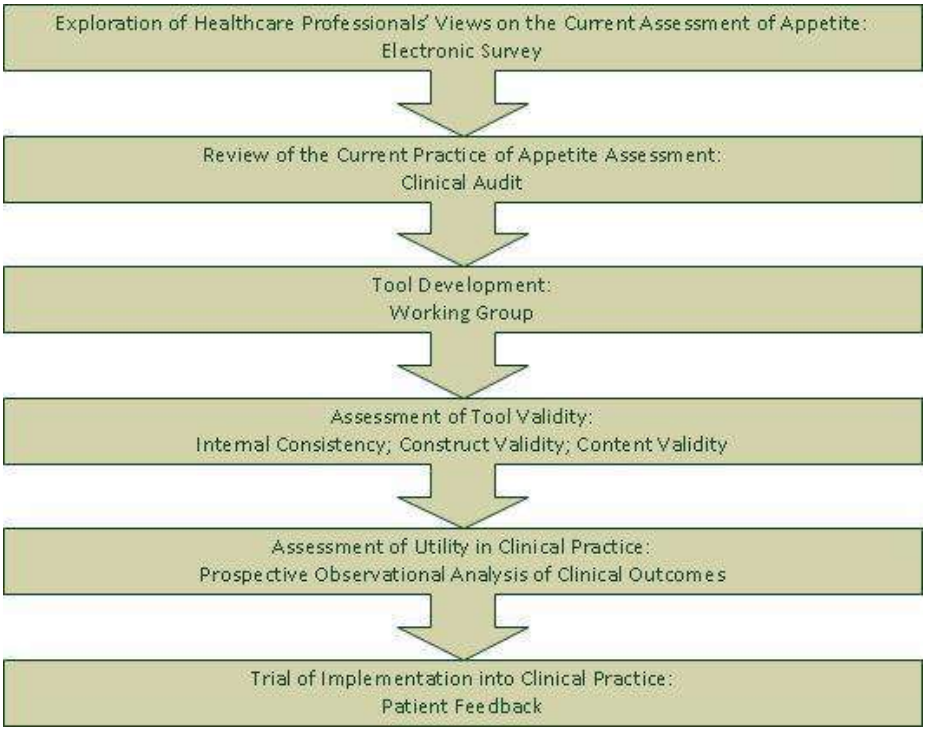


Figure 34. Schema describing the development and assessment of the ModSNAQ appetite assessment questionnaire.

6.1.1. Exploration of HCP's Views on the Current Assessment of Appetite

An electronic survey hosted online through "SurveyMonkey" (SurveyMonkey Inc, San Mateo, California, USA, www.surveymonkey.com) was composed and invitations delivered via email to HCP involved in the nutritional care of children with CKD at a UK teaching hospital.

6.1.2. Review of the Current Clinical Practice of Appetite Assessment

A sample of randomly selected 25 dietetic reviews conducted by paediatric kidney dietitians were reviewed and assessed for their evaluation of the patient's appetite. Clinical notes were interrogated for 100% compliance with: patient/care-givers' opinion of appetite; change in appetite; satiety; details of meal size and frequency.

6.1.3. Questionnaire Development

A working group of HCP with an interest in paediatric kidney nutrition was formed and consisted of: a paediatric nephrologist; two paediatric kidney dietitians; a professor of nutrition, an associate professor in nutrition, and a clinical research fellow in paediatric nephrology. The questionnaire was based upon the SNAQ questionnaire (265).

6.1.4. Assessment of Questionnaire Validity

Content validity was assessed by a group of HCP involved in the nutritional care of children with kidney problems, including local paediatric nephrologists, clinical nurse specialists, and paediatric kidney dietitians from throughout the UK. This was performed through an electronic survey conducted through anonymous email response. Internal consistency and utility in clinical practice was assessed in a cohort of children attending routine clinical appointments.

Children within the TEMPeReD cohort were recruited as detailed in *Chapter 5*, and consisted of children and young people aged 3-18 years identified through the upcoming patient clinic attendance list. All patients that were eligible were sent study information and a letter of invitation by post, and were recruited on a 'first come, first served' basis, until 60 children were recruited. Children (with parental help where appropriate) were asked to complete the questionnaire at routine clinic appointments, and clinical, anthropometric collected. The responses were then used to assess internal consistency and its changes with removal of questions; construct validity; to determine a cut-off for 'poor appetite'.

6.1.5. Assessment of Utility in Clinical Practice

Within the clinical cohort, height standard deviation scores (Ht SDS), weight standard deviation scores (WtSDS), and calculated body mass index (BMI) were assessed at baseline and at 6 months, and estimated glomerular filtration rate (eGFR), height standard deviation scores (HtSDS), weight standard deviation scores (WtSDS), and calculated body mass index SDS (BMISDS) were assessed at 12 months. Changes in SDS of >0.2 and eGFR >10ml/min/1.73m^2 are considered to be clinically significant, and prediction of occurrence of these outcomes by the ModSNAQ questionnaire was evaluated.

6.1.6. Statistical Methods

Data were analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America), and analysed as an entire cohort and as the PDCM group alone. Simple descriptive statistics, including calculation of percentages were used to present data from the electronic survey, the clinical audit, and patient feedback.

Content validity of the ModSNAQ questionnaire was assessed by an expert panel with calculation of congruency percentage, individual- Content Validity Index (CVI), and scale-CVI (266). Each question was scored by nine reviewers (paediatric nephrologists; paediatric kidney dietitians; nutrition researchers) on a Likert scale from 1 to 4 (1=not relevant, 2=somewhat relevant, 3=relevant, 4=very relevant). Scores of 3 or 4 were deemed as relevant, and those 1 or 2 not relevant. Internal consistency was assessed by calculation of Cronbach's alpha, and the value of each question was assessed through this re-calculation with questions removed from the ModSNAQ questionnaire. Construct validity was assessed through correlation of the ModSNAQ questionnaire with the

current best clinical practice of subjective self-assessment of appetite as "very poor/poor/good/very good", which was converted into a 4-point Likert scale.

Descriptive statistical analysis was performed including percentages of those receiving dialysis. In those variables that demonstrate normal distribution, mean and standard deviation (SD) were calculated, and in those that were not normally distributed, median and interquartile range (IQR) were calculated. Differences between groups were assessed by either t-test, Mann-Whitney U or Kruskal-Wallis (determined by distribution of data, and number of groups). Correlations between variables were assessed by Pearson's correlation or Spearman's rank order correlation tests according to distribution, and following identification of variables with significant correlations, linear regression and multiple linear regression were used to determine the contribution of effect of the variables on the variability seen in ModSNAQ scores. In order to assess the predictive power of the questionnaire, receiver operating characteristic curve (ROC) analysis was performed and area under the curve (AUC) calculated. *A priori* AUC value thresholds were determined to be: > 0.900 = excellent; 0.800 – 0.900 = good; 0.700 - 0.800 = fair; 0.600 – 0.700 = poor; and 0.500 - 0.600 = fail. Cut-off values of the ModSNAQ were determined by Youden's index, and optimal sensitivity and specificity given. Statistical significance was defined as a p-value <0.05.

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from all individuals included in the study, with informed consent obtained from care-givers and informed assent as appropriate.

6.2. RESULTS

6.2.1. Review of the Current Clinical Practice of Appetite Assessment: Clinical Audit

Within the clinical service, appetite assessment is inconsistent, even during paediatric kidney dietitian reviews. The percentages of those assessments that commented on particular aspects of appetite are shown in *Table 16*.

Table 16. Results of the clinical audit assessing documentation of appetite assessment in clinical practice (n=25).

Component of appetite	Percentage documented
Subjective assessment of appetite	52%
Change in appetite	32%
Comment on patient's satiety	0%
Documentation of the number of meals/day	80%
Documentation of the portion size of meals	76%
Comment on change in portion size of meals	20%

Clinical audit of the documentation of aspects of appetite assessment. Number of meals a day portion size were the most regularly recorded components. No assessment included details of patient satiety.

6.2.2. Exploration of HCP's views of the current assessment of appetite in children with CKD

An electronic survey was sent to clinicians, dietitians, and clinical nurse specialists within the paediatric nephrology team with six responses (75% response rate). 100% of responders felt that appetite was important in nephrology patients' clinical care, and that it should be assessed in clinical practice. 17% felt that appetite was

assessed at every clinic, with this being informally within the consultation (100%), and 67% documented in the patient health record. 100% felt that a more robust tool would be helpful in their clinical practice.

60 children with CKD were recruited; 46 of whom were PDCM; 10 had previously undergone kidney transplantation; and 4 were receiving dialysis (three haemodialysis, one peritoneal dialysis). Mean age 10.7 years (±4.0), with 40 (66.7%) boys and 20 (33.3%) girls.

6.2.3. Validation of Questionnaire Validity

Content validity Content validity was assessed by a panel HCP including the UK national paediatric kidney dietitian group. The average congruency percentage (percentage of questions deemed relevant to appetite assessment) was 94%. The Content Validity Index for each question (percentage of responders scoring ≥3 out of 4 – relevant/very relevant) was: Question 1 =78%; Question 2 =100%; Question 3 =100%; Question 4 =100%; Question 5 =89%; Question 7 =100%. Scale-CVI (the number of questions having all responders classify them as relevant / very relevant) was 83%.

Internal consistency as assessed by Cronbach's alpha coefficient in a cohort of 60 children attending the nephrology clinic, giving a value of 0.911, which demonstrates a high level of internal consistency. On evaluation of Cronbach's alpha if an item was deleted, all questions showed lower values if deleted (range of Cronbach's alpha = 0.883 to 0.908). Subgroup assessment revealed similar results (males only = 0.895; females only = 0.933; age <10years only = 0.940; age ≥10years only = 0.885).

6.2.4. Evaluation of the ModSNAQ Questionnaire in a clinical cohort

Median scores of the entire cohort and the PDCM group were 19.5 (IQR±7) and 20 (IQR±7.25) from a total of 24 (minimum of 6), respectively.

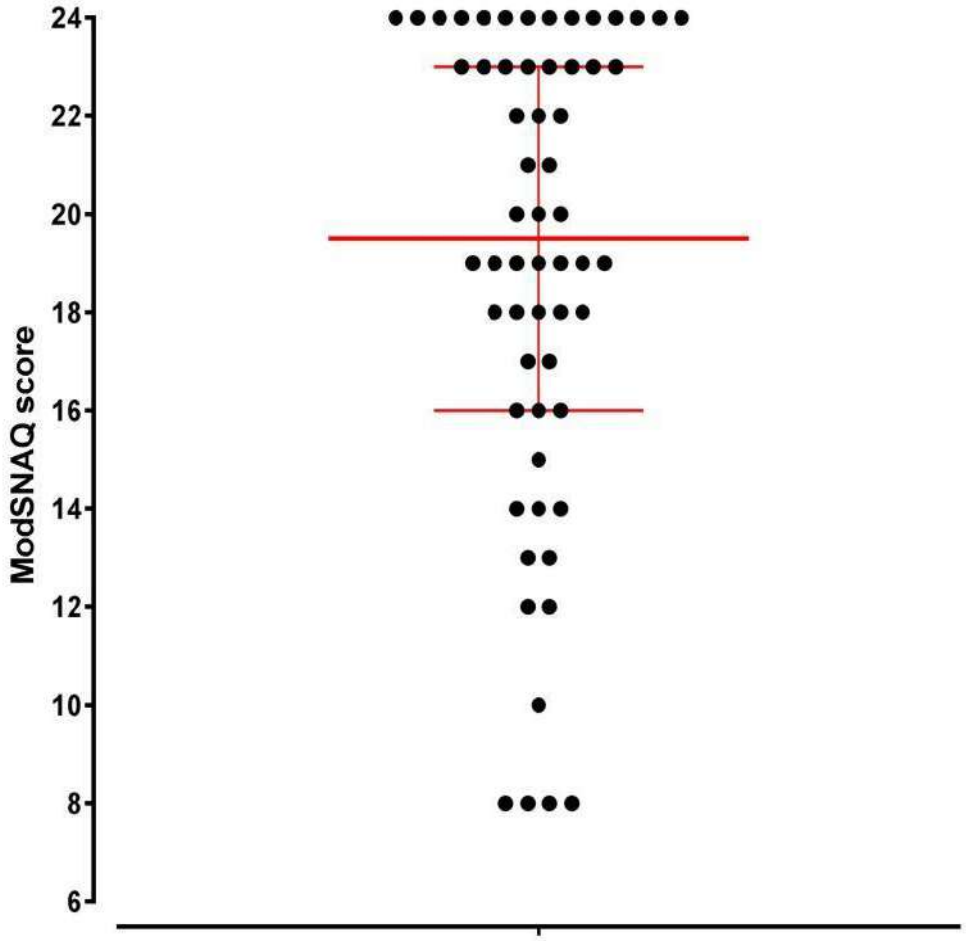


Figure 35. ModSNAQ questionnaire scores for the TEMPeReD cohort (n=60).

ModSNAQ appetite assessment tool scores for the entire cohort are shown. The minimum possible score is 6, and the maximum is 24. Median and interquartile range are shown in red. Many of the cohort scores were high, with 23% scoring maximally. No children scored a minimum score.

In the entire cohort, 14 out of 60 (23%) children rated their appetite as poor or very poor. This was similar to the PDCM group alone (11 out of 46 children, 24%). 4 (6.7%) and 3 (6.5%) in the respective groups rated their appetite as very poor.

ROC curve analysis to identify those children with Likert scale assessed poor/very poor appetite produced an AUC of 0.954. Youden's index analysis demonstrated that a score of 15.5 as a cut-off value (sensitivity = 95.75% and specificity = 21.4%).

Dietary intake of energy, protein, and sodium, potassium and phosphorus were evaluated in relation to ModSNAQ scores (*Table 17*). When analysing the entire cohort, total intake of protein, sodium, potassium and phosphorus correlated with ModSNAQ scores, with energy intake being close to statistical significance. These correlations were stronger in analysis of the PDCM group alone.

Table 17. Correlations between ModSNAQ questionnaire scores and dietary intake of energy and nutrients for the entire TEMPeReD cohort and pre-dialysis, conservatively-managed subgroup.

	Entire Cohort (n=60)	Pre-dialysis, conservatively-managed subgroup (n=46)
Energy (kcal/d)	S.rho = 0.250, p = 0.054	S.rho = 0.330, p = 0.025*
Energy (% requirement)	S.rho = 0.122, p = 0.355	S.rho = 0.156, p = 0.301
Protein (g/d)	S.rho = 0.326, p = 0.011*	S.rho = 0.382, p = 0.009*
Protein (% requirement)	S.rho = 0.253, p = 0.051	S.rho = 0.211, p = 0.158
Sodium (mg/d)	S.rho = 0.357, p = 0.005*	S.rho = 0.439, p = 0.002*
Sodium (% requirement)	S.rho = 0.302, p = 0.019*	S.rho = 0.339, p = 0.021*
Potassium (mg/d)	S.rho = 0.358, p = 0.005*	S.rho = 0.396, p = 0.006*
Potassium (% requirement)	S.rho = 0.247, p = 0.057	S.rho = 0.186, p = 0.216
Phosphorus (mg/d)	S.rho = 0.333, p = 0.009*	S.rho = 0.375, p = 0.010*
Phosphorus (% requirement)	S.rho = 0.231, p = 0.076	S.rho = 0.186, p = 0.218

*Correlations between ModSNAQ questionnaire scores and dietary intake of energy and nutrients as assessed by 24-hour dietary recall. * - signifies statistical significance (P<0.05). Abbreviations: kcal – kilocalorie; S.rho – Spearman's rho.*

The ability of ModSNAQ scores to predict meeting energy and protein intake was assessed by ROC curve analysis. As an entire cohort, AUC for prediction of meeting energy requirements was 0.714 (95%CI: 0.523 - 0.906), and for protein AUC was 0.487 (95%CI: 0.324 - 0.649). On analysis of the PDCM group alone, AUC for meeting energy and protein requirements were 0.529 (95%CI: 0.340 - 0.718) and 0.653 (95%CI: 0.434 - 0.872), respectively.

There was no correlation between ModSNAQ scores and eGFR (S.rho = 0.010, p = 0.937); or number of medications (S.rho = -0.042, p = 0.748). eGFR values were no different in those with poor appetite (ModSNAQ score <15.5) and those with good appetite (ModSNAQ score ≥15.5) (MWUT = 306.5, p = 0.986).

ModSNAQ scores correlated with HtSDS (S.rho = 0.263, p = 0.042), and more significantly with analysis of the PDCM group alone (S.rho = 0.324, p = 0.028). HtVelSDS did not demonstrate correlation (S.rho = 0.112, p = 0.394 and S.rho = 0.147, p = 0.330 for the entire cohort and the PDCM group, respectively).

Despite this correlation, those with poor growth (HtSDS <-2) did not have lower ModSNAQ scores in the cohort as a whole or in the PDCM group (MWUT = 261, p = 0.085 and MWUT = 146, p = 0.143, respectively).

From the entire cohort, 52 children had HtSDS at 12 months available for analysis. Change in HtSDS at 12 months did not correlate with ModSNAQ scores (S.rho = -0.019, p = 0.895), and ModSNAQ scores did not predict a decline in HtSDS of > 0.2 (AUC = 0.600 (95%CI: 0.381 - 0.819)). Comparing those with poor or good appetite (ModSNAQ <15.5 versus ≥15.5), those with poor appetite had an average increase in BMI SDS, rather than decrease (0.28 IQR±0.90 versus -0.2 IQR±0.52; MWUT = 262.5, p = 0.048). This remained the case when examining the PDCM group alone (0.28 IQR±0.90 versus -0.05 IQR±0.55; MWUT = 202.5, p = 0.040).

In the PDCM group, difference between those whose kidney function decline by > 10ml/min/1.73m2 at 12 months (n=3) and those with less of a decline (n=33) neared statistical significance with lower scores in those with greater decline in kidney function (Median = 22 (IQR 7) versus 8 (IQR n/a); MWUT = 16.5, p = 0.057).

6.2.5. Component Analysis of the ModSNAQ questionnaire

The individual component responses are displayed in *Figure 36*, below. Number of meals consumed per day (Questions 3 and 4) showed less variation in response than the other questions, with no children having fewer than two meals per day.

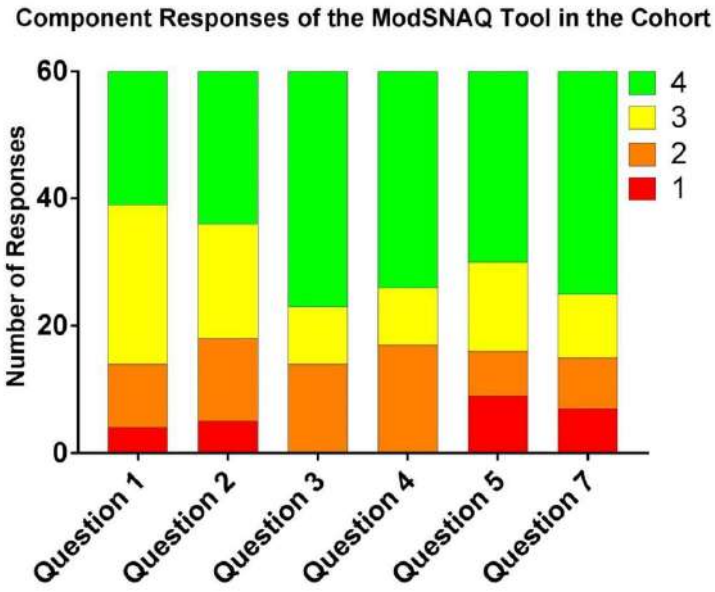


Figure 36. Number of responses for each component of the ModSNAQ questionnaire of the TEMPeReD cohort (n=60).

The Figure demonstrates the responses of the cohort for the components of the ModSNAQ tool. Each question scores from 1 (red) to 4 (green). Question 1 (A) subjective rating of appetite; Question 2 (B) Satiety; Question 3 (C) Usual meal number; Question 4 (D) Current meal number; Question 5 (E) Portion size; and Question 7 (F) Dietitian's opinion. Question 6 is not scored, and allows responders to comment on their appetite. Portion size and the dietitian's score (Questions 5 and 7) had more top and bottom scores, compared to self-rated appetite (question1) and satiety (question 2).

(A) (B)

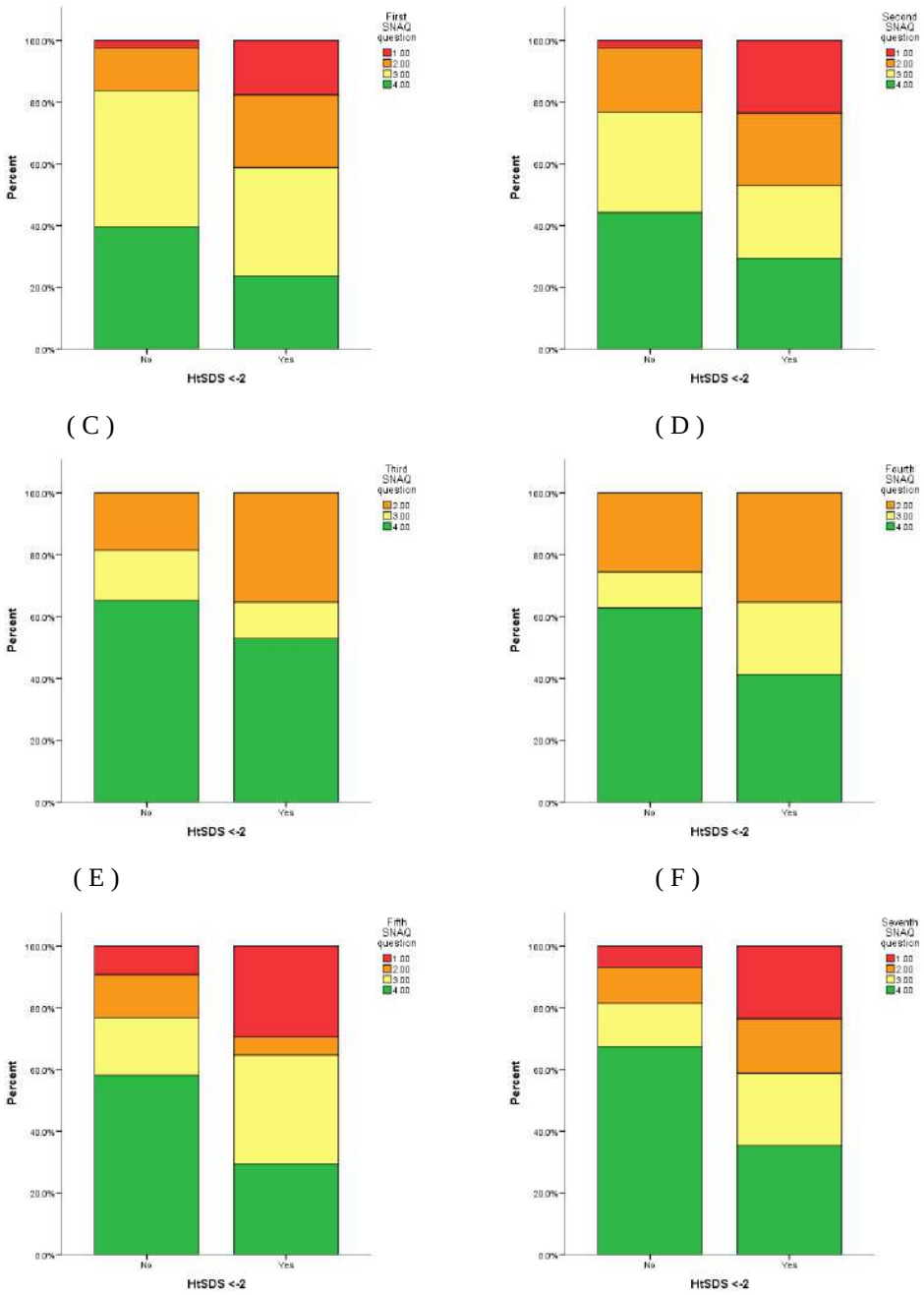


Figure 37. ModSNAQ scores depending upon growth status for the entire cohort (n=60).

Figures demonstrate the responses of the cohort for the components of the ModSNAQ questionnaire for those with poor growth (height standardised deviation score <-2) and those with normal growth (height standardised deviation score >-2). Each question scores from 1 (red) to 4 (green). Question 1 (A) subjective rating of appetite; Question 2 (B) Satiety; Question 3 (C) Usual meal number; Question 4 (D) Current meal number; Question 5 (E) Portion size; and Question 7 (F) Dietitian's opinion. Question 6 is not scored, and allows responders to comment on their appetite. There is a trend for those with poorer growth to respond to questions with a lower score for all questions. The questions asking about the number of meals consumed had no responders scoring 1 (red), reporting that all children had at least two meals a day. Abbreviations – HtSDS – height standardised deviation score.

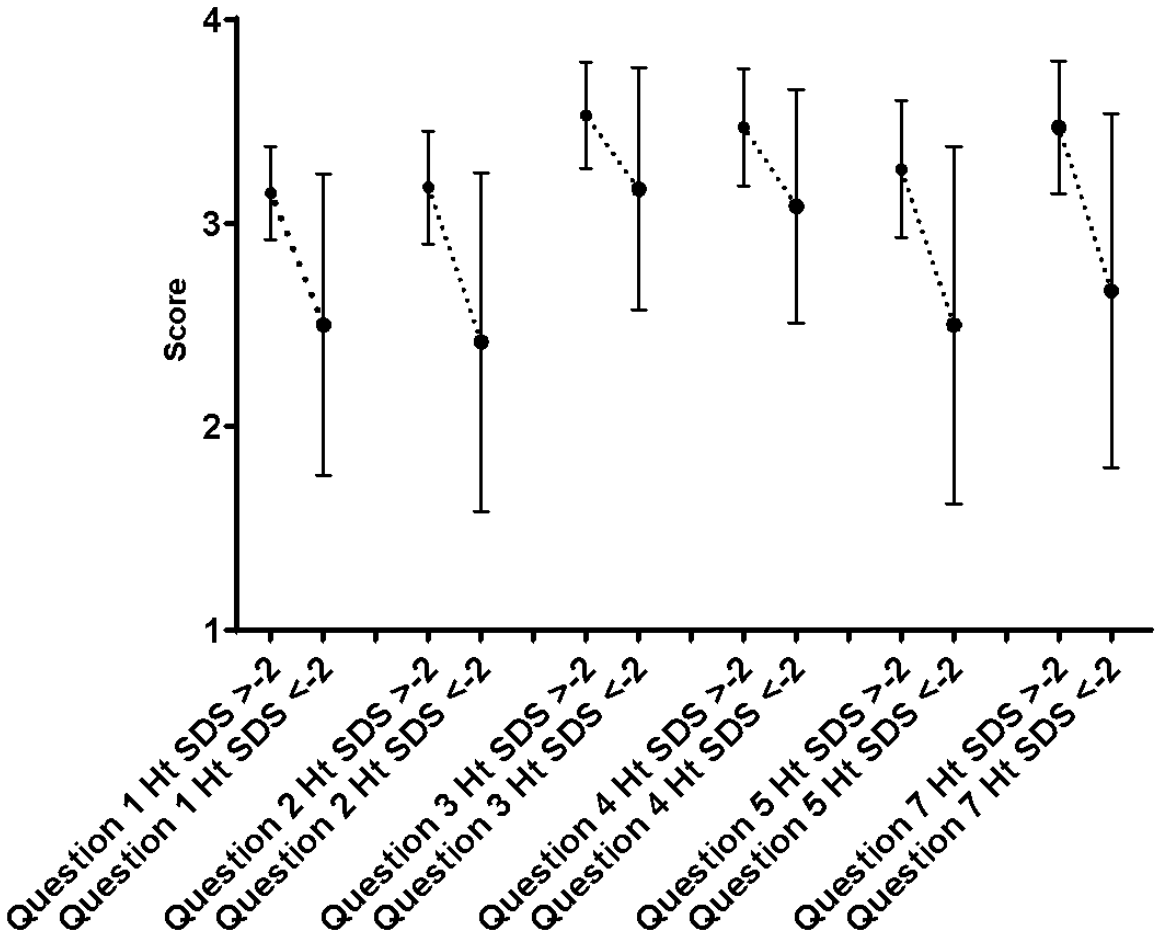


Figure 38. Component Analysis of the ModSNAQ questionnaire comparing those with HtSDS >-2 and those with HtSDS <-2 in the pre-dialysis, conservatively-managed subgroup (n=46).

Mean (and 95% confidence intervals) shown for each component question for the PDCM subgroup arranged in pairs of those with adequate growth (HtSDS >-2, n=43) and those with poor growth (HtSDS <-2, n=17).

6.3. DISCUSSION

A clinical need for a formalised appetite assessment was confirmed through both a survey of HCP's views, and through a retrospective clinical audit evaluating assessment of appetite. Following the questionnaire's development, the ModSNAQ was validated on a clinical cohort and then used to test the hypothesis that those children with poorer appetite would have poorer dietary intake, poorer growth, poorer nutritional status, and progress more rapidly in their kidney disease.

The results demonstrated a high prevalence of poor appetite that cannot be fully explained by disease severity alone. Appetite was associated with energy and nutrient intake. Poor appetite was associated with increased BMI, but not linear growth. Appetite scores neared significance for prediction of progression of kidney disease.

These data show, using a novel appetite assessment questionnaire, that the prevalence of poor / very poor appetite in this cohort is about 25%. This is higher than that previously reported. In the adult haemodialysis population in the HEMO study (83), 38% reported diminished appetite, 7% poor appetite. In the CKiD study of 879 children with PDCM CKD in the USA (84) "poor/very poor" appetite had a prevalence of 4% (1% very poor) as assessed on a 5-point Linkert scale (very good / good / fair / poor / very poor). Although the HEMO cohort is significantly different than that present here (adult patients undergoing haemodialysis in the USA), it would be expected that those with more severe disease, undergoing significant medical intervention known to influence appetite would have a higher prevalence of poor appetite than the group of PDCM patients. The CKiD cohort is similar to the cohort presented here (children with pre-dialysis CKD), with a similar average eGFR (51.7ml/min/1.73m^2). It may be that the increased prevalence of poor/very poor appetite in the cohort presented here is a 'truer' reflection. The ModSNAQ questionnaire has the additional benefit of capturing proxy measures of intake (portion size and meals per day) rather than a purely subjective rating of appetite.

A systematic review of the ability of appetite scoring systems to predict energy intake concluded that only half of studies demonstrated agreement (267). The ModSNAQ questionnaire scores did correlate with intake of energy and nutrients, although not when expressed as percentage of requirements. There may be a more influential dietary component on appetite as there seems to be a specific relationship between protein intake and appetite that does not exist for other macronutrients. Rats select protein-rich foods when exposed to protein-deficient diets, or during periods of additional protein requirements, such as pregnancy (268), and these findings are congruent with those of food choices and reduction in appetite in healthy humans (269). Although in this cohort, the strongest correlation with appetite was sodium intake.

Appetite as assessed by ModSNAQ score was not lower in those with poor growth (HtSDS<-2, or HtVelSDS<-2). In the CKiD study, those with worse appetite were found to be more likely to improve their anthropometric scores (84). In the data presented here, those with ModSNAQ scores <15.5 (i.e., poor appetite) had an average increase in BMI SDS at 12 months compared to those with good appetite who had an average decrease. A similar pattern in change in HtSDS was not observed. This may be interpreted as medical and dietetic intervention increasing BMI but this not translating into linear growth.

Averaged anthropometric measurements alone may not be ideal in the assessment of changes. The paediatric CKD population is composed of a mixed group, with an increasing prevalence of obesity (34% of the CKiD study had BMI > 85th percentile). Therefore, there are groups of children that may be trying to decrease their

BMI SDS over time, whilst others are trying to increase it due to being underweight. Future studies should be large enough for sub-group analysis of those of varying anthropometric categories. Although appetite is one factor in modifying these measurements, many others are also at play, including medical (e.g., growth hormone therapy) and dietetic intervention (e.g., supplemental feeds).

Although appetite may be seen as both a marker of disease and a nutritional risk factor, other modifying factors that may not be encompassed within the questionnaire. The development of the questionnaire aimed to introduce more of an objective assessment of appetite though the introduction of meal volume and number, there remains a significant subjective element to the responses. In the aforementioned HEMO study (83), it was found that appetite may improve with time. This may be due a resetting of biological systems once in a period of stability, and/or that in those with disturbed appetite that a 'new normal' is developed by which they now judge their appetite.

6.3.1. Limitations

There are several limitations to this study. Firstly, although a pragmatic approach was taken in the composition and size of the cohort used, a larger cohort from several centres, with longitudinal design would be of benefit. Validation of the ModSNAQ is needed against appetite as assessed through more objective measures. Dietary intake was assessed through 24-hour recall that has limitations including under-reporting due to poor recall and conformity bias.

6.3.2. Future Directions

The ModSNAQ questionnaire is now being trialled in routine clinical practice as part of the instigation of an annual review process for end-stage kidney disease patients that has been introduced. The questionnaire should be used as part of a larger, longitudinal study, or through collection of routine data as part of this annual review process. This would allow for ascertaining any predictive power that the questionnaire might have, and its potential utility in clinical decision-making.

The questionnaire, if modified to remove the dietitian's assessment, may be useful as a tool to identify those CYP at risk – requiring nutritional assessment. This needs to be explored further but has potential to 'triage' CYP into dietetic services if undertaken prior to hospital attendance for out-patient clinics, for example.
Appetite in the paediatric CKD population could be explored further through qualitative studies – an area that is also limited in the medical literature.

6.3.3. Concluding Remarks

There is a high prevalence of poor appetite that cannot be fully explained by disease severity alone. Poor appetite is associated with subsequent increased BMI, but not linear growth that may be attributable to currently employed interventions.

6.3.4. Practice Points

- A multidimensional, structured assessment of appetite captures appetite with greater granularity, and is quick and easily to integrate into clinical practice.
- Using this approach, the prevalence of poor appetite is greater than previously reported. This may have implications for risk of nutritional inadequacy for the paediatric CKD population.

- Appetite is not closely correlated to disease severity, and thus those with mild-moderate disease should also be assessed.
- The use of a scoring system may facilitate both longitudinal and between person comparisons.

7. HEALTH-RELATED QUALITY OF LIFE IN CHILDREN WITH CHRONIC KIDNEY DISEASE

So far, I have explored the relationship between measures of growth and objective measures of nutritional status. Health-related quality of life is a summative statement of an individual's well-being. In order to test the hypothesis of the influence of nutritional status on growth and well-being, the TEMPeReD cohort of children and young people will be evaluated using a well-recognised and validated assessment tool and comparisons made between those with poor growth and those with adequate growth.

7.1. INTRODUCTION

Health-related quality of life (HRQoL) is an individual's subjective perception of the impact of health status, including disease and treatment, on physical, psychological, and social functioning (93). Children with CKD, even those with disease on the milder end of the spectrum, have poorer HRQoL (90, 94-96).

Despite the recognition that HRQoL is important, there is limited data in this disease group with existing literature focusing upon end-stage disease and those following kidney transplantation. Of the studies that examine CKD stages 2 – 4 in the paediatric population (95, 96, 106-109), only one (108) examines nutritional status (evaluating the effect of short-stature). There is therefore a need to examine the effect of nutritional status; including obesity on HRQoL, and any association of HRQoL on future nutritional status.

The aims of this study are to report the HRQoL scores as assessed by the validated PedsQL^{TM} questionnaire and to explore the relationship of HRQoL scores to markers of nutritional status, particularly markers of growth. It will also examine concordance between the scores of the child and their parent/carer.

7.2. METHODS

A cross-sectional, observational study was performed to determine the HRQoL of a cohort of children with CKD and its relationship to markers of nutritional status. The TEMPeReD study is described in *chapter 5*.

7.2.1. Measures

As detailed previously, basic clinical and anthropometric data were collected; including height, weight, waist circumference, mid-upper arm circumference (MUAC) and standard deviation score (SDS), body mass index (BMI) and waist circumference-to-height ratio (WHtR) calculated. Appetite was assessed by a simple Likert scale by the child: Very poor-1, Poor-2, Good-3, Very good-4. Definitions of obesity were BMI SDS >2; MUAC SDS >2 or >25cm (270); and a WHtR >0.5 (75). Short stature was defined as a height SDS <-2. Blood for analysis of markers of the micronutrients copper, selenium, zinc and manganese were collected, and analysed through the clinical pathology department.

Further anthropometric and clinical data were collected via the electronic clinical record at 6 months and 12 months subsequent to initial assessment, and change in weight SDS, BMI SDS, height SDS and estimated glomerular filtration rate (eGFR) calculated.

The HRQoL was assessed using the PedsQL^{TM} tool (271). This is a series of questions posed to the child and the parent / caregiver that assess physical, emotional, social and schooling aspects of the child's life (*Appendix 11.6*).

The PedsQL™ tool was developed to measure HRQoL in children and has been validated for a number of chronic health conditions (272-274). It was highlighted in a systematic review of tools to assess HRQoL as one of the more thoroughly developed measures and has been validated for a wide age range of children (275). Although originally developed and validated for a USA population, it has been validated for a UK population of both healthy children and those with chronic conditions (asthma, diabetes, and inflammatory bowel disease) (276).

The PedsQL™(271) consists of two questionnaires; one for the child and one for a parent/care-giver. They ask a series of questions that assess four domains: physical, emotional, social and schooling aspects of the child's life. It is scored as individual domains and as a total score (all four domains), and is expressed as a percentage of maximum score. The tool is available in age-appropriate versions: toddler (2 – 4years), young child (5 – 7 years), older child (8 – 12 years) and teenager (13 – 18 years). There is no child (self-rater) questionnaire for those age 2-4years.

As a marker of deprivation, Income Deprivation Affecting Children Index (IDACI) scores were calculated using participants' postcodes. United Kingdom Department of Communities and Local Government data (2015) via the online platform that provides a selection of official statistics and data outputs on a variety of themes related to the UK Department of Housing, Communities and Local Government (277).

7.2.2. Statistical Analysis

Data were analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Statistical significance was defined as a p-value of less than 0.05. Descriptive statistical analysis was performed; including distribution of gender and age-group (as defined by the PedsQL™ questionnaire).

Independent t-tests were performed to compare: the questionnaire scores with both healthy control data from Varni et al's cohort (271). Concordance between self-reported and parent proxy questionnaire scores were compared using a paired t-test.

Correlation was explored between HRQoL scores and markers of nutritional status by examination of scatter-plot, and if appropriate, calculation of correlation coefficient values (Spearman's rho).

Data were firstly analysed as an entire cohort and then as treatment modality subgroup: pre-dialysis, conservatively-managed; and post-kidney transplantation.

7.2.3. Ethical Approval

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from parents/carers with parental responsibility. Informed assent was additionally obtained from those children/young people as participants in the study if age appropriate.

7.3. RESULTS

A total of 60 children and their parent-proxy were recruited as detailed in *Chapter 5*. The results were firstly analysed as an entire cohort and then anlaysed by treatment modality subgroup.

7.3.1. Entire Cohort

The composition of the TEMPeReD cohort is described in detail in previous chapters, and was composed of 60 children and young-people with chronic kidney disease. Both the child / young-person completed a questionnaire and a caregiver completed a corresponding questionnaire. If the child was below the age of five years (n=7), only a parent-proxy questionnaire was completed – as per the usual protocol for the questionnaire. All participants enrolled into the study completed the questionnaires, therefore, a total of 113 (60 parent-proxy and 53 child (self-rater) questionnaires were completed and analysed.

As presented in *Tables 18* and *19*, overall scores from the cohort were significantly lower than the healthy control data published by Varni et al (271) across all domains ($p<0.00001$). There were no significant differences found between scores depending upon sex as assessed by independent t-test (*Table 20*). Concordance between the child (self-rater) and parent-proxy scores were compared (*Table 21*), Scores for the emotional domain were significantly lower in the parent proxy compared to the child's own scoring ($p=0.005$). Following sub-group analysis by age-group, statistical significance only remained in those aged 5 – 7 years ($p=0.046$). The cohort was evaluated for difference in PedsQL^{TM} scores between different treatment subgroups (PDCM, dialysis, and post-transplantation). Total child (self-rater) and parent=proxy scores were not significantly different from one another as assessed by one-way ANOVA test (Child: $F(2, 49) = 0.862$, $p = 0.429$; parent-proxy: $F(2,57) = 0.459$, $p = 0.634$). There was also no difference between the groups on domain sub-group analysis.

Table 18. Comparison between child (self-rater) scores for the entire cohort (n=60) with healthy control reference data.

Children with CKD at Southampton Children's Hospital (self-rater)					Healthy Control Data (Varni et al)				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
64.01 (21.74)	65.99 (20.69)	68.29 (22.71)	58.51 (17.29)	65.03 (17.09)	86.86 (13.88) **P<0.00001**	78.21 (18.64) **P<0.00001**	84.04 (17.43) **P<0.00001**	79.92 (16.93) **P<0.00001**	82.87 (13.16) **P<0.00001**

*Mean PedsQL™ scores with breakdown by domain scores for the self-reported 'child' questionnaire with comparison to healthy control data from Varni et al (2003 (271)). Standard deviation in parentheses. Statistically significant (p<0.05) comparisons in **bold**. Children with CKD reported significantly lower scores across all domains than healthy controls.*

Table 19. Comparison between Parent-proxy scores for the entire cohort (n=60) with health control reference data.

Children with CKD at Southampton Children's Hospital (parent-proxy)					Healthy Control Data (Varni et al)				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
64.46 (24.88)	59.21 (21.28)	70.44 (21.01)	61.00 (18.88)	63.43 (17.45)	83.26 (19.98) **P<0.00001**	80.28 (16.99) **P<0.00001**	82.15 (20.08) **P<0.00001**	76.91 (20.16) **P<0.00001**	81.34 (15.92) **P<0.00001**

*Mean PedsQL™ scores with breakdown by domain scores for the parent-proxy questionnaire with comparison to healthy control data from Varni et al (2003) (271). Standard deviation in parentheses. Statistically significant (p<0.05) comparisons in **bold**. Parents of children with CKD reported significantly lower scores across all domains than healthy controls.*

Table 20. Statistical comparison between males and females across all domains for the entire cohort (n=60).

Domain	t-value (degrees of freedom)	p-value
Child (self-rater)		
Physical	t(26.069) = -0.807	0.427
Emotional	t(36.070) = -1.058	0.297
Social	t(32.409) =-0.499	0.621
School	t(29.191) = -0.945	0.353
Total	t(29.756) = -0.916	0.367
Parent-Proxy		
Physical	t(34.103) = -1.008	0.320
Emotional	t(38.049) = -0.629	0.533
Social	t(38.531) = -0.847	0.402
School	t(36.749) = -1.001	0.323
Total	t(38.703) = -1.257	0.216

Comparison of HRQoL was made between girls and boys, with no significant differences found when the entire cohort was analysed by independent t-test.

Table 21. Examination of concordance between child and parent for the entire cohort (n=60).

	Child (Self-rated) Scores					Parent Proxy Scores				
	Physical	**Emotional**	**Social**	**School**	**Total**	**Physical**	**Emotional**	**Social**	**School**	**Total**
2 - 4 years	x	x	x	x	x	65.24 (25.30)	63.75 (29.37)	76.25 (25.60)	74.02 (25.39)	67.59 (21.84)
5 – 7 years	55.58 (29.40)	66.88 (29.39)	62.29 (24.15)	56.25 (15.98)	62.81 (21.35)	52.12 (28.54) P=0.799	**51.25 (18.85) P=0.046**	72.19 (17.70) P=0.310	58.13 (21.87) P=0.786	57.48 (18.14) P=0.327
8 – 12 years	66.08 (20.47)	64.42 (17.79)	68.08 (22.09)	59.90 (18.03)	64.79 (16.77)	68.78 (23.56) P=0.531	57.12 (21.64) P=0.068	68.27 (21.26) P=0.848	58.65 (16.47) P=0.744	63.47 (16.24) P=0.518
13 – 18 years	64.78 (20.14)	67.85 (21.32)	71.25 (23.74)	57.50 (17.51)	66.36 (16.46)	61.98 (24.21) P=0.499	62.64 (17.91) P=0.276	70.22 (21.07) P=0.407	60.35 (17.59) P=0.873	63.46 (17.09) P=0.163
Total	64.01 (21.74)	65.99 (20.69)	68.29 (22.71)	58.51 (17.29)	65.03 (17.09)	64.46 (24.88) P=0.943	**59.21 (21.28) P=0.005**	70.44 (21.01) P=0.802	61.00 (18.88) P=0.870	63.43 (17.45) P=0.113

*Mean PedsQL™ scores for each age group and domain. Standard deviation in parentheses. Nb. there is no self-reported score for those aged 2-4years. P-values refer to differences between the self-reported and parent-proxy scores, with statistically significant (p<0.05) comparisons in **bold**. Scores for the emotional domain were significantly lower in the parent proxy compared to the child's own scoring (p=0.005). Following sub-group analysis by age-group, statistical significance only remained in those aged 5 – 7 years (p=0.046).*

7.3.1.1. Growth

Those with poor growth as defined by HtSDS and HtVel SDS were compared to those with adequate growth. The results are presented in *Tables 22* and *23*. There is a marked difference between the comparison depending upon which definition of poor growth used, with a 'across the board' lowering of scores when HtSDS is used, but with HtVel SDS is used, no difference is seen in child(self-rater) scores even on subgroup analysis. For the parent-proxy questionnaires, although for HtVel SDS the total score remains lower in those with HtVel SDS<-2, upon reviewing each domain for differences, only the emotional domain is significantly different.

Table 22. Comparison between those with poor growth (HtSDS<-2) and those with adequate growth for the entire cohort (n=60).

Domain	Poor growth (HtSDS<-2), n=17. Mean (±SD)	Adequate growth (HtSDS>-2), n=43. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	48.72 (±21.18)	70.81 (±18.50)	t(25.629) = -3.606	0.001*
Emotional	53.44 (±19.47)	71.53 (±18.90)	t(28.083) = -3.126	0.004*
Social	58.96 (±25.27)	72.43 (±20.51)	t(24.175) = -1.875	0.073
School	54.38 (±17.31)	60.34 (±17.20)	t(28.685) = -1.151	0.259
Total	53.19 (±16.65)	70.29 (±14.64)	t(25.773) = -3.546	0.002*
Parent-Proxy				
Physical	45.33 (±20.91)	72.03 (±22.28)	t(31.185) = -4.374	<0.0005*
Emotional	50.15 (±21.66)	62.79 (±20.27)	t(27.738) = -2.074	0.047*
Social	60.00 (±18.03)	74.57 (±20.85)	t(33.793) = -2.265	0.011*
School	54.64 (±16.01)	63.52 (±19.50)	t(35.598) = -1.816	0.078
Total	50.73 (±12.95)	68.45 (±16.52)	t(37.323) = -4.402	<0.0005*

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth by independent t-test analysis. Those with poor growth as defined as a HtSDS <-2 had lowers scores than those with adequate growth (HtSDS>-2).On analysis of each domain component, within the child (self-rater) questionnaire social and school domains were not significantly different, and within the parent-proxy questionnaire, the school domain was not significantly different.*

Table 23. Comparison between those with poor growth (HtVel<-2) and those with adequate growth for the entire cohort (n=60).

Domain	Poor growth (HtVel SDS<-2), n=17. Mean (±SD)	Adequate growth (HtVel SDS>-2), n=43. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	57.79 (±21.45)	66.31 (±21.68)	t(23.452) = -1.267	0.218
Emotional	60.71 (±18.59)	67.93 (±21.31)	t(26.454) = -1.192	0.244
Social	60.42 (±23.06)	71.18 (±22.18)	t(22.456) = -1.509	0.145
School	59.29 (±16.62)	58.22 (±17.73)	t(24.662) = 0.201	0.843
Total	61.13 (±17.53)	66.47 (±16.93)	t(22.536) = -0.983	0.336
Parent-Proxy				
Physical	55.88 (±20.96)	67.86 (±25.70)	t(35.856) = -1.865	0.070
Emotional	47.50 (±23.55)	63.84 (±18.64)	t(24.337) = -2.531	0.017*
Social	64.85 (±21.87)	72.65 (±20.50)	t(27.761) = -1.266	0.216
School	60.32 (±14.65)	61.27 (±20.46)	t(40.924) = -0.200	0.842
Total	56.84 (±14.07)	66.04 (±18.11)	t(37.664) = -2.094	0.043

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth by independent t-test analysis. Those with poor growth as defined as a Htvel SDS <-2 did not have lower scores in the child(self-rater) questionnaire. In the parent-proxy questionnaire, total scores were lower, but only the emotional component was significantly different.*

7.3.2. Post-transplantation Subgroup

Subgroup characteristics are described previously in *Chapter 5*. The PedsQL™ scores for the post-transplantation subgroup (n=10) are shown in *Table 24*. When compared to normative data (271), total scores for both the child (self-rater) and parent-proxy questionnaires were significantly lower than healthy control data. On component analysis, within the child (self-rater) questionnaire, the emotional and social components lost their significance.

Concordance between child (self-rater) and parent-proxy questionnaires was tested using paired t-test. Scores were similar across all domains except the Emotional domain in which children/young people scored themselves significantly higher than their caregivers (68.00 SD± 17.83 versus 48.50 SD± 11.80, t(9)=3.032, p = 0.014).

Table 24. Mean (standard deviation) PedsQL™ scores for the post-transplantation subgroup (n=10) compared to healthy controls.

Children with CKD at SCH (self-rater)					Healthy Control Data (Varni et al)				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
62.38 (19.50)	68.00 (17.83)	74.50 (19.64)	52.00 (19.03)	63.81 (15.21)	86.86 (13.88)*	78.21 (18.64)	84.04 (17.43)	79.92 (16.93)*	82.87 (13.16)*
Children with CKD at SCH (parent-proxy)					**Healthy Control Data (Varni et al)**				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
64.38 (16.94)	48.50 (11.80)	69.50 (16.24)	52.50 (15.70)	59.46 (9.51)	83.26 (19.98)*	80.28 (16.99)*	82.15 (20.08)*	76.91 (20.16)*	81.34 (15.92)*

**Significant difference for t-test p<0.05. Note that there is no self-rated score for those aged <5 years. Comparison is from healthy control data from Varni et al(271).*

Table 25. Mean (standard deviation) PedsQL™ scores for the post-transplantation subgroup (n=10) with comparison between child (self-rater) and parent-proxy scores.

Child (Self-rated) Scores					Parent Proxy Scores				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
62.38 (19.50)	68.00 (17.83)	74.50 (19.64)	52.00 (19.03)	63.81 (15.21)	64.38 (16.94)	48.50 (11.80)*	69.50 (16.24)	52.50 (15.70)	59.46 (9.51)

**- statistically significant, for t-test p<0.05. Note that there is no self-rated score for those aged <5 years.*

7.3.2.1. Growth

Those with poor growth as defined by HtSDS and HtVel SDS were compared to those with adequate growth. The results for the post-transplantation subgroup are presented in *Tables 26* and *27*. These show that for those with HtSDS <-2, only the physical component of the parent-proxy questionnaire was different compared to HtSDS >-2. For HtVel SDS-defined poor growth, only the emotional domain of the parent-proxy questionnaire was significantly different.

Table 26. Comparison between those with poor growth (HtSDS<-2) and those with adequate growth for the post-transplantation subgroup (n=10).

Domain	Poor growth (HtSDS<-2), n=4. Mean (±SD)	Adequate growth (HtSDS>-2), n=6. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	53.13 (±15.09)	68.54 (±20.83)	t(7.856) = -1.356	0.213
Emotional	70.00 (±15.81)	66.67 (±20.41)	t(7.681) = 0.290	0.779
Social	71.25 (±16.52)	76.67 (±22.73)	t(7.849) = -0.436	0.675
School	45.00 (±5.77)	56.67 (±23.80)	t(5.845) = -1.151	0.295
Total	58.97 (±4.56)	67.03 (±19.31)	t(5.804) = -0.983	0.365
Parent-Proxy				
Physical	48.44 (±5.41)	75.00 (±12.66)	t(7.214) = -4.554	0.002*
Emotional	53.75 (±11.81)	45.00 (±11.40)	t(6.401) = 1.163	0.286
Social	65.00 (±10.80)	72.50 (±19.43)	t(7.886) = -0.782	0.457
School	48.75 (±10.31)	55.00 (±18.97)	t(7.845) = -0.672	0.521
Total	53.26 (±7.48)	63.59 (±8.82)	t(7.353) = -1.989	0.085

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth by independent t-test analysis demonstrated no differences between the groups, except for within the physical component of the parent-proxy questionnaire.*

Table 27. Comparison between those with poor growth (HtVel SDS<-2) and those with adequate growth for the post-transplantation subgroup.

Domain	Poor growth (HtVel SDS<-2), n=3. Mean (±SD)	Adequate growth (HtVel SDS>-2), n=7. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	70.41 (±30.88)	58.93 (±14.37)	t(2.382) = 0.616	0.592
Emotional	60.00 (±21.79)	71.43 (±16.51)	t(3.044) = -0.814	0.475
Social	73.33 (±30.55)	75.00 (±16.33)	t(2.507) = -0.089	0.936
School	50.00 (±21.79)	52.86 (±19.55)	t(3.479) = -0.196	0.856
Total	63.77 (±24.79)	63.82 (±11.92)	t(2.408) = -0.003	0.998
Parent-Proxy				
Physical	69.79 (±14.09)	62.06 (±18.53)	t(5.122) = 0.721	0.503
Emotional	36.67 (±7.64)	53.57 (±9.45)	t(4.797) = -2.979	0.032*
Social	68.33 (±28.87)	70.00 (±10.80)	t(8.000) = -0.140	0.892
School	48.33 (±2.87)	54.28 (±18.80)	t(6.618)= -0.815	0.443
Total	57.61 (±3.92)	60.25 (±11.31)	t(7.959) = -0.545	0.600

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth by independent t-test analysis. Those with poor growth as defined as a HtVel SDS <-2 only demonstrated a difference in the emotional component of the parent-proxy questionnaire.*

7.3.2.2. Association with emotional component of the PedsQL™ questionnaire

On exploration for association between the emotional domain of the PedsQL (child, self-rater), correlations were found with time since diagnosis (S.rho = -0.683, p = 0.030); eGFR (S.rho = 0.738, p = 0.015); and number of anti-hypertensive medications (S.rho = -0.701, p 0.024). There was no correlation with total medication burden (S.rho = -0.581, p = 0.078), deprivation score (S.rho = 0.268, p = 0.486), or lower nutrient intake (number of nutrients below LRNI) (S.rho = -0.295, p = 0.407). Those with significant correlations were further explored using multiple-linear regression analysis. Only eGFR added significantly to the model, and described 67% of the variability in emotional domain score (r2 = 0.670, p = 0.004).

Multiple-linear regression analysis was performed to determine the domain that contributed greatest to the overall scores. A model was constructed for both the child (self-rater) and parent-proxy questionnaire scores, with all domains adding significantly to the model predicting the total score. In the child (self-rater) questionnaire, the domain that contributed greatest to the model was the physical domain with a β value of 0.323 (values for other domains: emotional = 0.223, social = 0.197, school = 0.242). For the parent-proxy scores, the physical domain again held the greatest influence on the total score with a β value of 0.348 (values for other domains: emotional = 0.217, social = 0.217, school = 0.217).

7.3.3. Pre-dialysis, conservatively-managed Subgroup

A total of 46 children with pre-dialysis, conservatively-managed CKD were recruited. Due to the removal of other variables, such as dialysis, and immunosuppressive drug regimens, additional analyses were undertaken to explore other variables' influence on HRQoL; including appetite. The cohort had a mean age of 10.50 ± 4.19 years. 18 (39.1%) were female. There were no significant differences between boys and girls; including age, time since diagnosis, eGFR, height SDS, weight SDS, BMI SDS, WHtR and appetite.

7.3.3.1. Anthropometry

Mean values (with standard deviations) of height SDS, weight SDS and BMI SDS for the cohort were -1.03 (±1.51), -0.43 (±1.81), and 0.32 (±1.41), respectively. The majority of children were within normal growth limits (±2SD). A greater number of children were obese as defined by WHtR than by WtSDS or BMISDS definitions. Eight children (17.4%) had weight SDS <-2 (malnourished by ICD-10 definition), three children (6.5%) had weight SDS >2. One child (2.2%) was under-weight for height (BMI SDS <-2), six children (13.0%) were overweight for height (BMI SDS >2). 12 children (26.1%) were short-for-age (height SDS <-2), one child (2.2%) was tall-for-age (height SDS >2). 20 children (43.5%) had a waist circumference-to-height ratio greater than 0.5.

7.3.3.2. HRQoL Scores

HRQoL scores are presented in *Table 28*. HRQoL scores were lower in all domains for both the self-rated and parent proxy components of the PedsQL™ tool than healthy control populations (Varni et al). There was good concordance between the self-rater and parent-proxy scores (t-test: t(37)=0.281, p=0.780). Details of subgroup analysis for different ages and PedsQL™ domains are available in *Table 29.*

Table 28. PedsQL™ scores for the pre-dialysis, conservatively-managed subgroup (n=46) compared to healthy controls.

Children with CKD at SCH (self-rater)					**Healthy Control Data (Varni et al)**				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
65.10 (±22.57)	34.38 (±24.65)	65.48 (±23.98)	58.88 (±16.91)	64.22 (±18.00)	86.86 (±13.88)*	78.21 (±18.64)*	84.04 (±17.43)*	79.92 (±16.93)*	82.87 (±13.16)*
Children with CKD at SCH (parent-proxy)					**Healthy Control Data (Varni et al)**				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
65.27 (±26.27)	61.47 (±22.53)	70.52 (±22.80)	63.48 (±19.68)	64.64 (±19.13)	83.26 (±19.98)*	80.28 (±16.99)*	82.15 (±20.08)*	76.91 (±20.16)*	81.34 (±15.92)*

**Significant difference for t-test p<0.0005. Standard deviation in parentheses. Note that there is no self-rated score for those aged <5 years.*

Table 29. Mean Child (Self-rater) versus Parent-proxy PedsQL™ Scores of the cohort of paediatric pre-dialysis, conservatively-managed CKD patients, and test for child-parent concordance using paired t-test analysis.

		Physical domain	Emotional domain	Social domain	School domain	Total score
2 - 4 years N=7	Parent-proxy	70.99 ± 27.88	61.43 ± 27.49	76.43 ±27.65	71.43 ± 26.29	68.76 ± 24.31
5 – 7 years N=8	Child(self-rater)	54.02 ± 31.13	61.88 ± 32.06	55.73 ± 30.41	53.75 ± 16.85	56.01 ± 24.18
	Parent-proxy	52.12 ± 28.54	47.50 ± 17.53	67.50 ± 24.35	55.00 ±23.30	55.02 ± 20.16
	Paired t-test Result	T(7) = 0.329, P = 0.752	T(7) = 2.308 P = 0.054	T(7) = -1.555, P = 0.164	T(7) =-0.166, P = 0.873	T(7) = 0.244, P = 0.814
8 – 12 years N=15	Child(self-rater)	70.24 ± 19.10	66.33 ± 17.16	67.33 ± 18.70	62.83 ±16.44	67.23 ± 14.92
	Parent-proxy	73.18 ±26.64	63.67 ± 24.31	68.33 ± 22.73	64.33 ±15.22	67.33 ± 18.37
	Paired t-test Result	T(12) = -0.467, P = 0.649	T(12) = 0.600, P = 0.560	T(12) = -0.457, P = 0.656	T(12) = -0.241, P = 0.814	T(12) =0.044, P = 0.966
13 – 18 years N=16	Child(self-rater)	70.24 ± 19.10	66.33 ± 17.16	67.33 ±18.70	62.83 ±16.44	67.23 ± 14.92
	Parent-proxy	73.18 ± 26.64	63.67 ± 24.31	68.33 ± 22.73	64.33 ± 15.22	67.33 ± 18.37
	Paired t-test Result	T(14) = 0.846, P = 0.412	T(14) =-0.108, P = 0.915	T(14) = 0-.343, P = 0.737	T(14) = -1079, P = 0.299	T(14) = 0.355, P=0.728
Total N=46	Child(self-rater)	65.10 ± 22.57	64.38 ± 21.65	65.48 ± 23.98	58.88 ± 16.91	64.22 ± 18.00
	Parent-proxy	65.63 ± 40.63†	61.47 ± 22.53	75.00 ± 35.00†	63.48 ± 19.68	64.64 ± 19.13
	Paired t-test Result	T(37) = 0.179, P = 0.859	T(37) = 1.249, P = 0.220	T(37) = -1.045, P = 0.303	T(37) = -1.056, P = 0.298	T(37) = 0.281, P = 0.780

Scores expressed as percentages with standard deviation in parentheses. † - median and interquartile range.. Nb. there is no self-reported score for those aged 2-4years. P-values refer to the difference between the self-reported and parent-proxy score for each age-group.

7.3.3.3. HRQoL and Kidney function

Although HRQoL scores correlated with kidney function (eGFR) for the child(self-rater) scores (Pearson's coefficient = -0.362, p=0.026), this was not the case for the parent-proxy scores (Pearson's coefficient = -0.133, p=0.377). Although there is correlation of self-rated PedsQL^{TM} scores, there were no difference in PedsQL^{TM} scores for either self-rater or parent-proxy components between CKD stages (self-rater: F=2.284, df=5, p=0.07; parent-proxy: F=1.117, df=5, p=0.367). Degree of proteinuria (uPCR) did not correlate with HRQoL scores; including individual domain scores (total score (child) Spearman's rho = 0.185, p=0.870; total score (parent-proxy) Spearman's rho = -0.05, p=0.755).

Table 30. Comparison of PedsQL^{TM} scores of those with height SDS <-2 and those >-2 in the pre-dialysis, conservatively-managed subgroup (n=46).

Domain	Poor growth (HtSDS<-2), n=12. Mean (±SD)	Adequate growth (HtSDS>-2), n=34. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	46.14 (±24.04)	72.82 (±16.99)	t(14.257) = -14.257	0.005*
Emotional	45.00 (±15.33)	72.27 (±18.79)	t(22.709) = -4.647	<0.0005*
Social	52.12 (±26.39)	70.93 (±21.08)	t(15.471) = -2.105	0.052
School	55.45 (±18.36)	60.28 (±16.44)	t(16.905) = -0.756	0.460
Total	49.20 (±18.32)	70.33 (±14.06)	t(15.040) = -3.435	0.004*
Parent-Proxy				
Physical	43.12 (±24.52)	73.08 (±23.03)	t(18.323) = -3.696	0.002*
Emotional	47.71 (±24.76)	66.32 (±19.86)	t(16.283) = -2.351	0.032*
Social	57.92 (±20.61)	74.97 (±22.11)	t(20.615) = -2.417	0.025*
School	57.40 (±17.74)	65.63 (±20.13)	t(21.777) = -1.332	0.197
Total	49.22 (±14.77)	70.07 (±17.60)	t(19.220) = -2.464	0.023*

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth by independent t-test analysis demonstrated significantly lower scores in those with poor growth (HtSDS<-2). On component analysis, within the child (self-rater) questionnaire, social and school components were not significantly different. Within the parent-proxy questionnaire, the only component that was not significantly different was school.*

7.3.3.4. HRQoL and Anthropometry (markers of growth and obesity)

Those who were stunted (Height SDS <-2) had lower HRQoL scores than those who were not for both the child (t(15.039) = -3.4356; p = 0.0037) and parent questionnaires (t(22.8722) = -4.10670; p = 0.0004). On component analysis, physical, and emotional components of the child (self-rater) and the physical, emotional, and social components of the parent-proxy were lower in those with HtSDS-defined poor growth (see *Table 30)*. When examining those with poor growth defined by HtVel SDS, there was no difference in scores; including upon component analysis (see *Table 31).*

Those with obesity (as defined by BMI SDS (>2), MUAC SDS (>2) or waist circumference-to-height ratio, WHtR (>0.5)) did not demonstrate different scores from non-obese patients (see *Tables 52-54 in Appendix 11.7*).

7.3.3.5. HRQoL and appetite

Children whose appetite was described as either "good" or "very good" had better scores than those with appetites described as "poor" or "very poor" (child – t(36) = 2.851; p=0.007 and parent - t(44) = 2.910; p=0.006).

7.3.3.6. HRQoL and micronutrient status

Correlations were explored between the plasma levels of copper, selenium, zinc, and whole blood manganese. None of these measures correlated with HRQoL scores (see *Table 32*).

Table 31. Comparison of PedsQL™ scores of those with height velocity SDS <-2 and those >-2 in the pre-dialysis, conservatively-managed subgroup (n=46).

Domain	Poor growth (HtVel SDS<-2), n=12. Mean (±SD)	Adequate growth (HtVel SDS>-2), n=34. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	57.04 (±18.93)	67.60 (±23.31)	t(16.275) = -1.379	0.187
Emotional	60.56 (±20.68)	65.56 (±22.15)	t(14.197) = -0.623	0.543
Social	53.15 (±20.81)	69.31 (±23.92)	t(15.180) = -1.962	0.068
School	61.67 (±16.96)	58.02 (±17.10)	t(13.467) = 0.563	0.583
Total	58.14 (±17.20)	66.12 (±18.11)	t(13.982) = -1.201	0.250
Parent-Proxy				
Physical	55.21 (±22.07)	68.81 (±27.57)	t(24.023) = -1.715	0.099
Emotional	49.79 (±26.17)	65.59 (±19.92)	t(15.739) = -1.906	0.075
Social	62.92 (±22.71)	73.21 (±22.54)	t(19.208) = -1.352	0.192
School	64.21 (±15.66)	63.22 (±21.13)	t(26.076) = 0.170	0.867
Total	57.51 (±16.72)	67.15 (±19.52)	t(22.397) = -1.641	0.115

**-statistically significant (P<0.05). Comparison between those with poor growth and those with adequate growth as defined by HtVel SDS<-2 by independent t-test analysis demonstrated no difference between groups.*

Table 32. Correlation analysis between PedsQL™ scores and blood micronutrient levels.

	Child (Self-rater) PedsQL™	Parent-proxy PedsQL™
Serum Copper	Spearman's rho = -0.105, p = 0.553.	Spearman's rho = -0.255, p = 0.104.
Serum Selenium	Spearman's rho = -0.183, p = 0.299.	Spearman's rho = 0.165, p = 0.295.
Serum Zinc	Spearman's rho = -0.324, p = 0.061.	Spearman's rho = -0.180, p = 0.254
Whole Blood Manganese	Spearman's rho = -0.260, p = 0.173	Spearman's rho = -0.060, p = 0.731.

7.3.3.7. Multiple-linear Regression Analysis

Variables that demonstrated correlation were used to perform multiple linear regression analysis to explore relationships between variables and total PedsQL™ scores. Additionally, as the major determinant of total score was the emotional component score, this was also examined.

Child (self-rater) HRQoL

On multiple linear regression for correlated variables (eGFR and HtSDS), these two variables statistically significantly predicted child self-assessed HRQoL (total score) $F(2,35)=12.436$, $p<0.0005$, $R^2=0.415$. Both HtSDS and eGFR added statistically significantly to the prediction of child-assessed HRQoL (HtSDS $p<0.0005$, eGFR $p=0.008$).

Parent-proxy HRQoL

On multiple linear regression for positively-correlated variables (HtSDS and IDACI), these two variables statistically significantly predicted parent-proxy HRQoL (total score) $F(2,42)=11.695$, $p<0.0005$, $R^2=0.358$. Both HtSDS and IDACI added statistically significantly to the prediction of child-assessed HRQoL (HtSDS $p=0.001$, IDACI $p=0.005$).

Emotional component determinants

Correlations with the emotional component of the child (self-rater) questionnaire were sought and those that demonstrated significant correlation (eGFR: s.rho = -0.351, p = 0.031; HtSDS: s.rho = 0.556, p<0.0005; MUAC SDS: s.rho = 0.415, p = 0.010; WtSDS: s.rho = 0.503, p = 0.001; BMI SDS: s.rho = 0.346, p = 0.033) were used to construct a multiple linear regression model. On analysis, eGFR and HtSDS added significantly to the model (p = 0.019 and p<0.0005, respectively) with an r^2 of 0.435 (p<0.0005). β values for eGFR and HtSDS were -0.311 and 7.985, respectively.

7.4. DISCUSSION

This is the first data of HRQoL as assessed by the PedsQL™ 4.0 in children with pre-dialysis, conservatively-managed CKD within the UK, and explores the association of nutritional status with HRQoL. The aim of this study was to report PedsQL™ scores in a UK population of children with CKD and explore the association of nutritional status on them, specifically to determine whether poor growth was associated with poorer HRQoL. I hypothesised that disease severity, obesity and short stature would demonstrate association, but of the nutritional status measures evaluated, poor appetite and short-stature demonstrated a strong association with lower HRQoL scores.

These data show that HRQoL scores was significantly lower in this population; including in the subgroup of pre-dialysis conservatively-managed CKD children compared to healthy control data, with mean score differences greater than the minimal clinically important difference (MCID) score of 4.4 (child self-assessed questionnaire) and 4.5 (parent-proxy questionnaire) (271). Existing literature is limited and has focused on those with the most severe disease - those with end-stage disease (eGFR <15ml/min/1.73m^2, in receipt of including dialysis, or who have undergone kidney transplantation). On the whole these report that HRQoL is lower in children with CKD compared to healthy children, but with some conflicting results (96, 106-108, 278-286). Such conflict may be explained by the heterogeneity of the tools used, but other factors may also be contributory.

There was no difference in scores according to age or gender in this cohort, although previous studies have shown lower scores in older children/young-people(286) and in girls (285, 286). Time since diagnosis did not correlate with HRQoL scores. This may be explained by the heterogeneity of the cohort, with a potential mix of those whose HRQoL worsens with time, and those that have altered "new normal" – a phenomenon described as "response shift" (287).

7.4.1. HRQoL Concordance between child (self-rater) and parent-proxy scores

There was significant correlation between child (self-rater) and parent-proxy scores. On evaluation of the entire cohort, the emotional domain scored significantly lower in parent-proxy scores compared to the self-rater scores. This difference only remains in the post-transplant subgroup and not the PDCM on subgroup analysis. When broken down by age subgroup, statistical significance only remained for the youngest children (aged 5-7 years). It might be expected that a lack of concordance between self-rater scores and parent–proxy scores may be lower in older children in which complexity of emotions and independence are greater. It may be that this discordance in the more intensively managed children and young people is observed due to the small number in the subgroup skewing these data, but children and young people may not have such insight into the emotional states discussed in the questionnaire (feeling scared, sad, or angry, and worry about the future). Alternatively, parents may be transposing their own feelings onto their child. Although these feelings are valid in themselves and may have implications for the patient, I did not explore the caregivers' quality of life or feelings regarding their child's health, emotional state or feeling/worries/concerns about the future of their child. Adult caregivers also have the benefit of a wider knowledge of other children (and themselves as a child) and therefore have given benchmarks by which to 'judge' their child in this respect. Studies involving a greater number of children are required to confirm or refute this finding and to explore, perhaps qualitatively, the differences between the emotional HRQoL component score differences. Discordance between self-reported and parent-proxy scores has been reported previously in those with dialysis and post-kidney transplantation (281, 288, 289) with other literature showing greater psycho-social score discordance in those approaching adulthood (108, 290).

A degree of disagreement is not surprising as the child's perception of quality of life will be different, at least in part due to parental expectations for their child based on their own life-experiences; experiences that the child would not necessarily draw comparison with. This potential lack of comparison on the child's part is one reason why HRQoL should be assessed by children and parents / caregivers in order not to over-score those that 'don't know any different'. Previous studies suggest that discordance between self-reported and parent-proxy questionnaires increases as the child's age increases (108). This is true not only for teenagers approaching adulthood, but demonstrated by Razzcuk et al, also for younger children (291).

7.4.2. HRQoL and disease severity

Child (self-rater) HRQoL scores correlated with eGFR, but parent-proxy scores did not, and no correlation was found between scores and degree of proteinuria. As the kidney impairment would have the greatest impact upon the individual, then other modifying factors on the caregiver may explain the lack of correlation observed in the parent-proxy scores. As proteinuria may be modified through medical intervention (such as the use of angiotensin II converting enzyme inhibitors), proteinuria is not a complete marker of disease severity.

Although eGFR did correlate with child (self-rater) HRQoL scores in our cohort, Gerson et al found that eGFR did not correlate with HRQoL (95). A lack of correlation may be explained by the fact that eGFR is not a true reflection of disease severity as it is too simplistic a marker. Furthermore, it does not take into consideration co-morbidities, social factors, or other contributors to HRQoL.

7.4.3. HRQoL and growth

As previously reported in other disease-groups (108), stunted children reported lower HRQoL scores than their non-stunted counterparts, and was the strongest influence on multiple regression analysis. The reasons for this may be two-fold. Firstly, linear growth is a summation of events, is affected by myriad factors and is a proxy measure for everything detrimental that has happened to the child. Secondly, short-stature may have psychological impact on the child, as both a visual reflection of a child's self-perceived health, and a noticeable difference between themselves and their peers. In keeping with the latter suggestion, the emotional component of the score was significantly influenced by height SDS independent of kidney function (eGFR).

On component analysis, the school domain was not different between those with adequate and those with poor growth (including on subgroup analysis). This may be due to practices employed by healthcare professionals and a family to try to limit impact upon school insofar as is practicable. Previous literature examining school attendance in children and young people with chronic health needs has reported that those with chronic ill health have twice as many days absent from school each year (292). That being said, absenteeism is not the main influencing factor on scholastic achievement with social factors, including deprivation contributing more significantly (292, 293).

7.4.4. HRQoL and obesity

Despite previous reports from other cohorts of children (294), these data do not demonstrate a lower HRQoL in those with markers of obesity (BMI SDS, waist circumference-to-height ratio). The reason for this may be a shift of the perceived normal body shape and "accepted" adiposity in children. Rates of obesity in the UK population is now 14% of children aged 2-15years (BMI >95th percentile)(153), and compare similarly to the percentage of those obese in this cohort (BMI >2 SDS). Therefore, despite children being obese, if their day-to-day functioning is not negatively impacted by this, then such children do not stand out from their peers, and so are perceived as "normal", or even healthy – Both obese children and their parents' ability to recognise themselves as too heavy is only 26% and ≤48%, respectively(153).

7.4.5. HRQoL and appetite

HRQoL scores were significantly higher in those with good appetite versus poor appetite. As appetite is a complex function of many factors in which general health and well-being impact upon, this is not surprising. In addition, eating is an important social setting – which much of family life is structured around. Alteration of feeding habits, including through eating less and a focus of the medicalisation of the normal social activity (advice to eat certain foods, avoid certain foods and to encourage oral intake in order to increase calorie intake, for example) may have an impact on how they view themselves compared to others, and hence their perceived HRQoL.

7.4.6. HRQoL and micronutrient status

Biomarkers of micronutrient status did not correlate with HRQoL scores. These measurements are prone to many factors, and have a high degree of buffering within the normal range; it is only at the extremes of nutrition that these values become abnormal. In addition, levels may be affected by other factors such as underlying inflammatory state. As discussed elsewhere, the markers by which we assess micronutrient status (blood concentrations) may not truly be reflective of bodily status, especially in the context of CKD.

7.4.7. Limitations

There are several limitations to this study. Firstly, the cohort, although larger than other reported cohorts, is only 46 children of children with pre-dialysis, conservatively-managed CKD, and ten following transplantation. Additionally, the study only measured HRQoL at a single time-point meaning that we were unable to examine changing scores with time (including during changes in nutritional status and treatment). Nutritional status is a complex, multi-faceted concept and this study only examined a selection of variables. Each of these measures has their own limitations (for example BMI not truly reflecting body composition). Not all variables that may have an impact on HRQoL such as markers of resilience and patient efficacy were explored.

7.4.8. Future directions

Further exploration of factors that could be influencing HRQoL, especially those that are modifiable, is needed with the aim to improve the HRQoL of our patients. It is not unreasonable to predict that the nutritional changes; including dietary restriction found in CKD and changes of body composition may have a detrimental effect upon HRQoL. Therefore, further studies analysing the impact would be valuable in order to optimise management strategies to improve HRQoL. A larger multi-centre cohort would allow for exploration of different aetiologies and the effect this may have on HRQoL in addition to minimising type 2 errors. Longitudinal studies of HRQoL in this disease-group are also needed. Although there is cross-sectional data comparing treatment modalities, it would be useful to know how an individual's HRQoL changes with time; with the changing disease severity, changing treatment modality, changing nutritional status, transitioning from paediatric to adult services. This would facilitate the exploration of the ways different management strategies, including nutritional intervention influence HRQoL. Finally, the emotional component of the HRQoL assessment presented here seemed most strikingly different from healthy peers, and work focused on exploring the emotional health; including its relationship to diet, nutrition and dietetic management should be undertaken. Such work could include a qualitative study exploring the perceptions and opinions of children, young-people and their caregivers with regards to the nutritional management of kidney disease, as this is an area lacking in the current literature.

The routine introduction of HRQoL as a measure in clinical care may allow for a more patient-focused approach to care. If the multi-disciplinary team is focused upon improving a patient-centred value, patients and their families may be more engaged in decision-making, and those decisions may be more focused on improving HRQoL rather than markers of disease, for example that may seem disconnected from day-to-day for families.

7.4.9. Concluding Remarks

HRQoL is lower in pre-dialysis, conservatively-managed paediatric CKD patients than in healthy control data. Examination of multiple nutritional variables revealed that nutritional status is associated with HRQoL. Other significant variables were eGFR for child (self-rater) scores and level of deprivation for parent-proxy scores.

HRQoL is a measure of an overall status, and the perception of an individual's own health status is an important factor that healthcare providers should be aiming to improve. As healthcare services become more patient-centred, the measures by which they are evaluated must include patient-centred measures and by assessing HRQoL using a formalised tool, healthcare processes can be evaluated. HRQoL tools also have the potential to be used as screening tools or to identify areas to be explored during out-patient consultation for a more holistic consultation. For these reasons, HRQoL assessment should be considered for introduction into routine clinical care for ongoing holistic care of children with chronic illnesses, including CKD, and to facilitate the acquisition of longitudinal data regarding the impact of changes in nutritional status and therapy.

7.4.10. Practice Points

- Assessment of the well-being of a child or young person can be readily performed by validated questionnaires in the out-patient setting.
- HRQoL is lower than healthy peers even in those with mild-moderate disease. Identifying early those with poor HRQoL may inform greater clinical care, and help identify those who need additional support. In addition longitudinal changes with disease progression and clinical care, including nutrition, could be tracked.

8. INTERVENTIONAL STUDY: EVALUATING A NOVEL FOOD FOR SPECIAL MEDICAL PURPOSES

The TEMPeReD cohort study (*Chapter 5*) reported many children and young people are at risk from not meeting dietary requirements of several vitamins and minerals. Additionally, the blood concentrations of vitamins and minerals measured in the TEMPeReD study did not reflect a similar picture as the dietary intake data, with most children and young people having blood concentrations within normal reference ranges (see *Chapter 5*). As discussed previously, the circulating pool of these vitamins and minerals may not be a true measure of status, especially in those with disease.

In the landmark study by Golden and Golden (45), the evidence of a limiting nutrient was clearly demonstrated through supplementation of malnourished children with zinc on the basis that a therapeutic response would provide the best evidence for inadequacy. In a similar fashion, a time-limited trial to examine the effects of supplementation of vitamins and minerals in a population determined to be at risk from nutritional inadequacy was undertaken and is presented in this chapter.

8.1. INTRODUCTION

Children and young people with kidney disease are at high risk of multiple nutritional deficiencies. Unfortunately, current nutritional practice involving the use of standard micronutrient supplements does not adequately meet the nutritional requirements for micronutrients and trace elements in all children and young people. The primary aim of this study is to explore acceptability and palatability of a kidney specific food for special medicinal purposes (FSMP) –a powdered blend of vitamin and trace elements specifically formulated for the dietary management of children and young people with kidney disease, and offers the opportunity to explore this study as a time-limited trial of such supplementation.

Healthy children are at risk from not meeting their dietary requirements of micronutrients. For example, >10% of children aged 4-10 years receive less than LRNI for vitamin A and zinc, and in children aged 11-18 years 22% and 35% receive less than LRNI for zinc and selenium, respectively (295). This highlights the potential of nutritional risk before any dietary restrictions are prescribed, and may potentially be further compounded by selective toddler food fads and teenage ‘junk’ diets.

The care of CYP with kidney disease involves scrupulous attention to detail in the child’s nutrition and then manipulation of nutrition, through placement of multiple dietary restrictions and the addition of supplemental nutrition. Dietary restriction, although potentially life-saving in its ability to control electrolyte derangement, for example, has unintended consequences, including the elimination of foodstuffs high in vitamins and minerals resulting in children being at increased risk of not meeting their dietary intake requirements.

Therefore, it is reasonable to predict that those children with CKD with a potentially more restrictive dietary pattern are at risk from nutritional inadequacy, by not meeting their recommended dietary intake of micronutrients. There is existing literature in support of this, (discussed previously) reporting lower dietary intakes of small paediatric CKD cohorts.

Current practice aims to use nutritional products designed specifically for children with kidney disease to meet the child’s possible nutritional deficits identified by reported dietary intakes. In the absence of specialist

products, standard formulations are used, with the kidney dietitian where possible choosing the lowest content option of the particular nutrient(s) of concern. This may result in excess of other nutrients. In practice, clinical guidelines recommend that for most micronutrients 100% of the RNI should be achieved for children with CKD (2), which may be facilitated by supplementation. Local audits and annual reviews highlight challenges in achieving this on a day-to-day basis.

At present there is no nutritionally complete FSMP specifically formulated for the dietary management of children with kidney disease. A selection of micronutrient supplements currently available in the UK are described in *Table 33*. As highlighted in the table, the majority of products do not supply a balanced supply of micronutrients, or supply a level adequate to meet RNI or equivalent. The micronutrient and trace element supplements have been developed for the requirements of the general (otherwise healthy) population; including those available 'over-the-counter', are developed for those without kidney disease. This is important to note as they contain vitamin A, the blood concentration of which has been shown to be elevated CKD. The preparations of supplements that have been developed for the kidney disease population consist of water-soluble vitamins only, and were originally designed for adults receiving dialysis. This reflects the evidence that dialysis increases water-soluble vitamin loss, that associated increased requirements may be difficult to meet through diet alone (contributed to by the dietary restriction of foodstuffs that are high in potassium and phosphate which tend to be present in water-soluble vitamin-rich foodstuffs), and the guidance to supplement with water-soluble vitamins in those in receipt of dialysis (2). Unfortunately, the evidence for this is largely expert opinion with extrapolation from adult studies. The formulation of such products is intended to ensure that the nutritional needs of the child are met, although the extent to which the combined diet and nutritional FSMP are sufficient to meet the demands of the child will vary.

Furthermore, on nutritional assessment it is anecdotally reported that nutrients such as selenium are less than RNI / LRNI.

In response to the recognition of this unmet clinical need the national Paediatric Renal Interest Nutrition Group (PRING) requested the development of a novel formulation of vitamins, minerals and trace elements to address the specific nutrient considerations in the dietary management of children with kidney disease. PRING approached industrial partners to assist. An industrial partner was identified (*Vitaflo*®) and collaboration between the national group undertaken to develop a FSMP to meet this need, based on the evidence available and expert opinion.

Due to limitations in dietary assessment techniques, it is not possible to determine with certainty the extent to which micronutrient requirements specific to children with kidney disease are or are not met. That is, the intake of infrequently taken micronutrients are unlikely to be adequately characterised by limited dietary intake tools such as 3-day 24-hour dietary recall. One pragmatic approach is to perform a time-limited trial of treatment directed at addressing those nutrients that are likely to be limiting, based on expert opinion. For this to be undertaken, predefined outcomes must be defined. The six questions (four primary and two secondary questions) to be asked to explore this FSMP, and their justifications, are discussed below:

Question one, is the formulation acceptable and palatable to the population?

It is important to determine acceptability and palatability, as even if formulations are effective, to determine if this patient group (children with kidney disease) would find the formulation unpalatable and refuse to take the product. If the latter is evident, any intended benefit will not occur and the product would not be used. In children with kidney disease, there are several reasons why such a formulation may not be palatable/acceptable. For example: like food, nutrients exist as complexes, often in small amounts compared to the volume of food; minerals have a metallic taste that some (especially children) may find unpalatable; and the addition of supplements with large number of solutes may cause osmotic effects within the gastrointestinal tract that cause side-effects such as delayed gastric emptying, and nausea. The latter are symptoms that children with CKD often report (88).

Question two, is the formulation safe to take?

Nutrient powders are formulated to ensure total nutrient intake is within the usual range of dietary intake. Any medical intervention, including nutritional support must assess the detrimental effects, and report Adverse Events (AE) throughout the study period.

Question three, if consumed to the recommended dose, does the formulation in addition to basal diet ensure that intake is greater than RNI (or equivalent) but below recommended upper intake levels?

The ability of the formulation to fulfil its primary aim should be assessed, despite being developed with the aim to ensure adequate (>RNI or equivalent) supply of nutrients to the patient. This is important because the dose is not individualised in the same way that a prescription medication could be.

Question four, if consumed to dose, does the formulation correct any apparent biochemical markers of micronutrient deficit?

It may be that at the dose provided (the primary aim is to ensure adequate intake, not to correct deficit) is insufficient to correct micronutrient deficiencies.

Question five, Does the addition of this formulation improve overall appetite?

Appetite is adversely affected in those with CKD (296) and its multifactorial causes have been described in *Chapter 6*. Appetite is also diminished in limitation of key micronutrients, such as zinc (297). Supplementation with micronutrients has improved appetite in other diseases (micronutrient supplementation for 6 months improved appetite in children with HIV, for example (298)). Decreased appetite in a setting of micronutrient limitation may be beneficial is some situations in which the ongoing consumptions of macronutrients in relatively large amounts represent a metabolic demand that is not advantageous to the body as it lacks the ability to appropriately handle them due to limitations of essential cofactors. Supplementation of nutrients in isolation has also been reported to increase mortality (vitamin E (264), zinc (299)). It is hypothesised that the supply of micronutrients in a balanced way would facilitate the body to efficiently utilise the supply of macronutrients; and be reflected in improved appetite.

Question six, Does the addition of this formulation improve quality of life?

Markers of health are varied, but not necessarily patient-centred. In the assessment of any intervention, the aim should be to improve the quality of life of the patient. A balance of positives that may result from treatment and negatives as perceived by the individual must be sought. One way to assess this is to use a tool to assess health-related quality of life (HRQoL)discussed in *Chapter 7*.

Therefore, the primary outcome of this study was the acceptability and palatability of a novel FSMP based on successful completion of the trial, and questionnaire responses. Secondary outcomes were changes in nutritional status (including blood biomarkers of micronutrient status), appetite and health-related quality of life scores (PedsQL^{TM}) following the trial.

Aim and Objectives of the Study

The aim of this study is to evaluate the acceptability and palatability of a newly-formulated FSMP for children with kidney disease. The study gives the additional opportunity to explore potential changes in nutritional status, appetite and HRQoL during such a trial, although the study is not powered for this analysis

Table 33. Currently available multivitamin preparations for children, and contribution of requirement for 10 year old boys.

	Dalavit	Wellkid multivitamin	Paediatric Dialyvit	Ketovite	Replavite and Hill-Vite	Dialyvite 800 with zinc 15	Nephrocap	Nephronex	Strovite Forte Syrup	Fruitivits	Trial FSMP.
Vitamin A (mcg RE)	1500 (300%)	200 (40%)	-	-	-	-	-	-	400 (80%)	500 (100%)	-
Thiamine (mg)	1 (143%)	0.7 (100%)	0.8 (125%)	3 (429%)	1.5 (214%)	1.5 (214%)	1.5 (214%)	1.5 (214%)	5 (714%)	1.2 (171%)	1.2 (171%)
Riboflavin (mg)	0.4 (40%)	0.8 (80%)	1 (100%)	3 (300%)	1.7 (170%)	1.7 (170%)	1.7 (170%)	1.7 (170%)	5.7 (570%)	1.4 (140%)	1.4 (140%)
Niacin (mg)	5 (42%)	8 (67%)	12 (100%)	9.9 (83%)	20 (167%)	20 (167%)	20 (167%)	20 (167%)	33.3 (278%)	15 (115%)	15 (115%)
Pantothenic acid (mg)	--	2 (67%)	6 (200%)	1.2 (40%)	10 (333%)	10 333%()	5 (167%)	10 333%()	8.3 (277%)	4 (133%)	4.7 (157%)
Vitamin B6 (mg)	0.5 (50%)	0.8 (80%)	2 (200%)	0.9 (90%)	10 (1000%)	10 (1000%)	10 (1000%)	10 (1000%)	6.7 (670%)	1.7 (170%)	1.7 (170%)
Folic acid mcg)	-	100 (67%)	1000 (667%)	750 (500%)	1000 (667%)	800 (533%)	1000 (667%)	1000¥ (667%)	333 (222%)	240 (160%)	400 (267%)
Biotin (mcg)	-	-	20 (100%)	510 (255%)	300 (150%)	300 (150%)	150 100%	300 (150%)	50 (100%)	112 (100%)	112 (100%)
Vitamin B12 (mcg)	-	2.5 (250%)	1 (100%)	-	6 (600%)	6 (600%)	6 (600%)	10 (600%)	6.7 (600%)	2.8 (280%)	2.8 (280%)
Ascorbic acid (mg)	50 (167%)	12 (40%)	10 (33%)	51 (170%)	100 (333%)	60 (200%)	100 (333%)	60 (200%)	100 (333%)	40 (133%)	60 (200%)
Vitamin D (mcg)	10 (100%)	10 (100%)	-	-	-	-	-	-	3.3 (33%)	15 (150%)	0.04 (0.4%)
Vitamin E (α-TE)	-	5 (100%)	6 (120%)	15 (300%)	-	-	-	-	6.7 (134%)	9.3 (186%)	6 (120%)
Vitamin K (mcg)	-	-	20 (79%)	1500 (9524%)	-	-	-	-	-	60 (381%)	30 (190%)
Iron mg)	-	5 (57%)	-	-	-	-	-	-	3.3 (38%)	10 (115%)	10 (115%)
Zinc (mg)	-	4 (57%)	8 (114%)	-	-	-	-	-	5 (71%)	10 (143%)	10 (143%)
Copper (mcg)	-	150 (21%)	800 (114%)	-	-	-	-	-	1000 (143%)	1000 (143%)	1000 (143%)
Iodine (mcg)	-	40 (36%)	-	-	-	-	-	-	-	169 (154%)	169 (154%)
Selenium (mcg)	-	-	-	-	-	-	-	-	-	41 (137%)	60 (200%)
Magnesium (mg)	-	-	-	-	-	-	-	-	-	201 (101%)	200 (100%)
Manganese (mg)	-	-	-	-	-	-	-	-	-	1.5 (298%)	1.5 (298%)
Chromium (mcg)	-	-	-	-	-	-	-	-	-	41 (1270%)	4 (127%)
Molybdenum (mcg)	-	-	-	-	-	-	-	-	-	68 (432%)	68 (432%)

Table 33. *Values for standard doses given (Dalavit = 0.6ml, Wellkid multivitamin liquid = 5ml, Paediatric Dialyvit = 1 tablet; Ketovite = 3 tablets; Replavite and Hill-Vite = 1 tablet; Nephrocap = 1 caplet; Nephronex = 1 caplet or 5ml if liquid preparation; Strovite Forte Syrup = 5ml; fruitivits = 6g; trial FSMP 6g). Abbrev.: RE – retinol equivalents. ¥ - 900 for liquid preparation. Highlighted in red are values < RNI or equivalent (percentage of RNI or equivalent in parentheses). Orange boxes highlight nutrients that exceed the upper recommended intake for a 10 year-old boy with CKD, i.e., no more than 100% RNI of vitamin A. Where needed, based on 50th centile weight (31.5kg) and intake of EAR for energy (1970kcal/d) and recommended intake for PUFA (i.e., 5mg/d vitamin E). Vitamin A has not been coded as 'low' due to concern of accumulation in CKD. As shown, the only supplement that offers an adequate micronutrient supplement without supplementation of vitamin A is the trial FSMP. Although vitamin A intake is lower than recommended levels, vitamin D is measured and supplemented independently as part of usual clinical care. Abbreviations: CKD – chronic kidney disease; FSMP – food for special medical purposes.*

8.2. METHODS

This interventional study was a phase 2a, first in-human, single-centre, open-label study. The novel FSMP was not considered an Investigational Medicinal Product (IMP), but a Food for Special Medicinal Purposes (FSMP) and therefore not subject to the same processes that an IMP would be. Details of what constitutes a FSMP are located in *Appendix 11.12*.

Patients were recruited as outlined in the cross-sectional observation study (TEMPeReD study) in *Chapter 5*. This was used as screening for inclusion for this interventional study.

A priori inclusion criteria for the interventional study were: recruitment for nutritional characterisation to the TEMPeReD cross-sectional study; in receipt of <80% RNI for more than 2 micronutrients on 24-hour recall dietary assessment (this is concordant with current dietetic practice of those deemed 'at risk of inadequacy'), or 2 micronutrients and have at least one micronutrient blood biomarker low; and consent to continue in the study for the interventional element.

These criteria were decided based on current clinical practice of decision-making as to whether to initiate nutritional support [Southampton Children's Hospital clinical guidelines]. 80% of RNI is used clinically as individuals tend to under-report dietary intake [Southampton Children's Hospital clinical guidelines]. The nutrients vitamin A, potassium and phosphate were not included as nutrients that determined eligibility as they are often restricted in the diet in those with CKD. Dietary intake of vitamin D or serum vitamin D status were also not included as vitamin D is supplemented and monitored independently with vitamin D being primarily supplied via exposure to sunlight (and/or supplementation) and not from the diet. Vitamin K intake was not used for inclusion due to its primary supply via the gastrointestinal microbiome and not through the diet.

Those that did not want to participate in the interventional study received usual nutritional care.

The target for enrolment into the interventional study was 15 participants. This was the number suggested by the Advisory Committee on Borderline Substances for the assessment of acceptability and palatability of FSMP products (300). Ethical approval was obtained for a maximum of 18 participants to be enrolled, to allow for drop-out through the study period.

The FSMP was formulated following discussions between an industrial partner *Vitaflo*® and the national group of specialist renal dietitians (PRING). This was prior to this study's development and took a number of years and formulation versions. PRING is a national working group of specialist paediatric kidney dietitians that have representation from all the paediatric nephrology centres in the UK. The FSMP formulation was determined based upon existing evidence and guidance (based largely on the KDOQI guidelines), and expert opinion through a modified Delphi approach of gathering expert opinion through a series of questions posed to the panel. The formulation was determined to be nutritionally complete, but lacking micronutrients that tend to accumulate in kidney disease, such as vitamin A, or regularly require dietary restriction, for example sodium, potassium and phosphate. The nutritional composition of the trial FSMP is shown in *Table 33* in the final column.

Dose of the FSMP

All children were given a standard dose of six grams (one sachet) to be mixed with water as per the manufacturer's guidance. This was the manufacturer and PRING consensus. No other changes to the diet were made, although there was no control over dietary intake of the cohort as free-living children no other nutritional interventions were made. The 6g of the FSMP was dissolved in 60ml water, and advised to be taken with food (as per manufacturer).

Outcomes Measures

Safety

Safety was assessed by physical examination, adverse events (AEs) – categorised by the Medical Dictionary for Regulatory Activities code, and laboratory tests. The severity of AEs was classified by the investigator as mild, moderate or severe. Laboratory tests included kidney function, and blood concentrations of vitamins and minerals.

Assessment of Acceptability and Palatability

Families were asked to complete a contemporaneous record and questionnaire regarding the trial FSMP that included details of the volume of the FSMP taken, any symptoms, and thoughts regarding palatability of the FSMP. The questionnaire was composed of tick-boxes, Likert scale questions and free text (see *Appendix 11.9*). This was based on a standard acceptability and palatability questionnaire proforma used for other studies. Free-text comments were assessed using a recognised framework for qualitative analysis (301) through their collation and grouped into themes in order to draw broader conclusions. Themes provide an accurate reflection of the content of the responders' feedback.

Assessment of Changes in Nutritional Assessment, Appetite and HRQoL

Nutritional assessment (anthropometry and biochemical markers of micronutrient) along with assessment of appetite through the ModSNAQ questionnaire (see *Chapter 7* and HRQoL (PedsQL^{TM}) were assessed at baseline (time of participation in the cross-sectional study) and repeated 90-days following commencement of taking the FSMP. This timeframe was agreed upon as a timeframe at which stores of any micronutrients would be replenished, represented an amount of time that measures of acceptability and palatability could be achieved – having placed the FMSP into routine daily life-, and that change in growth may begin to declare themselves.

Statistical Analysis

Data were analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Statistical significance was defined as a p-value <0.05. Descriptive statistical analysis was performed; including percentages of those receiving dialysis. Differences between mean values were assessed by paired t-test, and medians by Wilcoxon-signed Rank test.

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from all individuals included in the study, with informed consent obtained from care-givers and informed assent as appropriate.

8.3. RESULTS

47 children out of the 60 screened by the cross-sectional study were deemed eligible for invitation to participate in the interventional study on a first-come, first-served basis. All children that were approached to participate in the interventional study agreed to take part.

The interventional cohort consisted of 18 children (the maximum allowed from the ethical approvals for the study). There was a skew in boys compared to girls (88.9% versus 11.1%). The cohort were shorter, lighter, but heavier-for-their-height than healthy control data with median SDS being height = -1.34; weight = -0.48, and BMI = 0.49 with 27.8% having height <-1.88 SDS.

Children were at risk from not meeting their dietary requirements, with a mean of 9.94 nutrients being less than 80% of RNI or equivalent (range 3-19), and a mean of 4.56 nutrients being less than LRNI (range: 0 - 12).

Supply of Nutrients

As shown in *Figures 40* and *41*, analysis of the dietary intake of the group demonstrated a high prevalence of nutrient intake below 80% RNI and less than the LRNI. The addition of the FSMP to the intake of these participants greatly increased the intake of nutrients, and decreased the number of children identified as receiving intakes < 80% RNI (*Figure 42*). With supplementation with the FSMP, no child had intakes greater than recommended upper intakes. Although total (diet and FSMP) intake was for some nutrients many times the RNI or equivalent this was still within acceptable intakes. Noteworthy is Biotin where intake seems very high on review of the data. The intake has been expressed as a percentage of the lower end of the 'safe intake' (no RNI has been set) at 5mcg/d, but the stated range is from 5mcg/d to 200mcg/d (47), and if the higher value is used then the mean additional intake of biotin contributed by the FSMP is 56% of requirement.

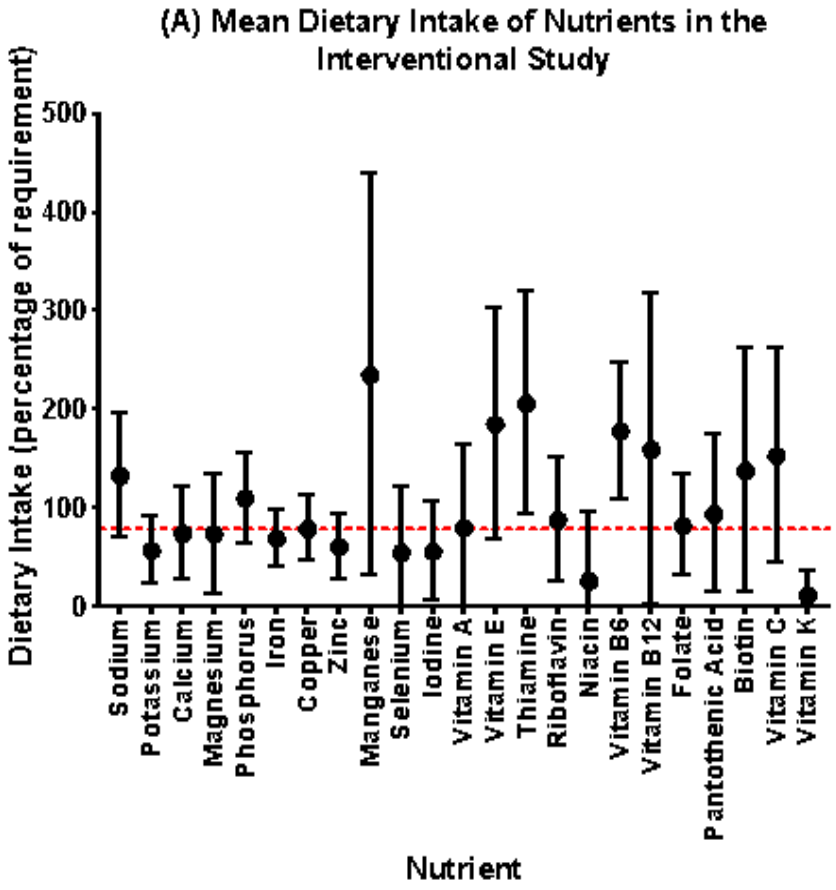


Figure 39. Dietary Intake of nutrients of the participants eligible to be enrolled into the interventional study – mean dietary intake of nutrients.

Mean and standard deviation of the dietary intake, with 80% RNI marked by the red dotted line. There are a significant number of children with dietary intake only below 80% RNI (the clinical cut-off for dietary intervention).

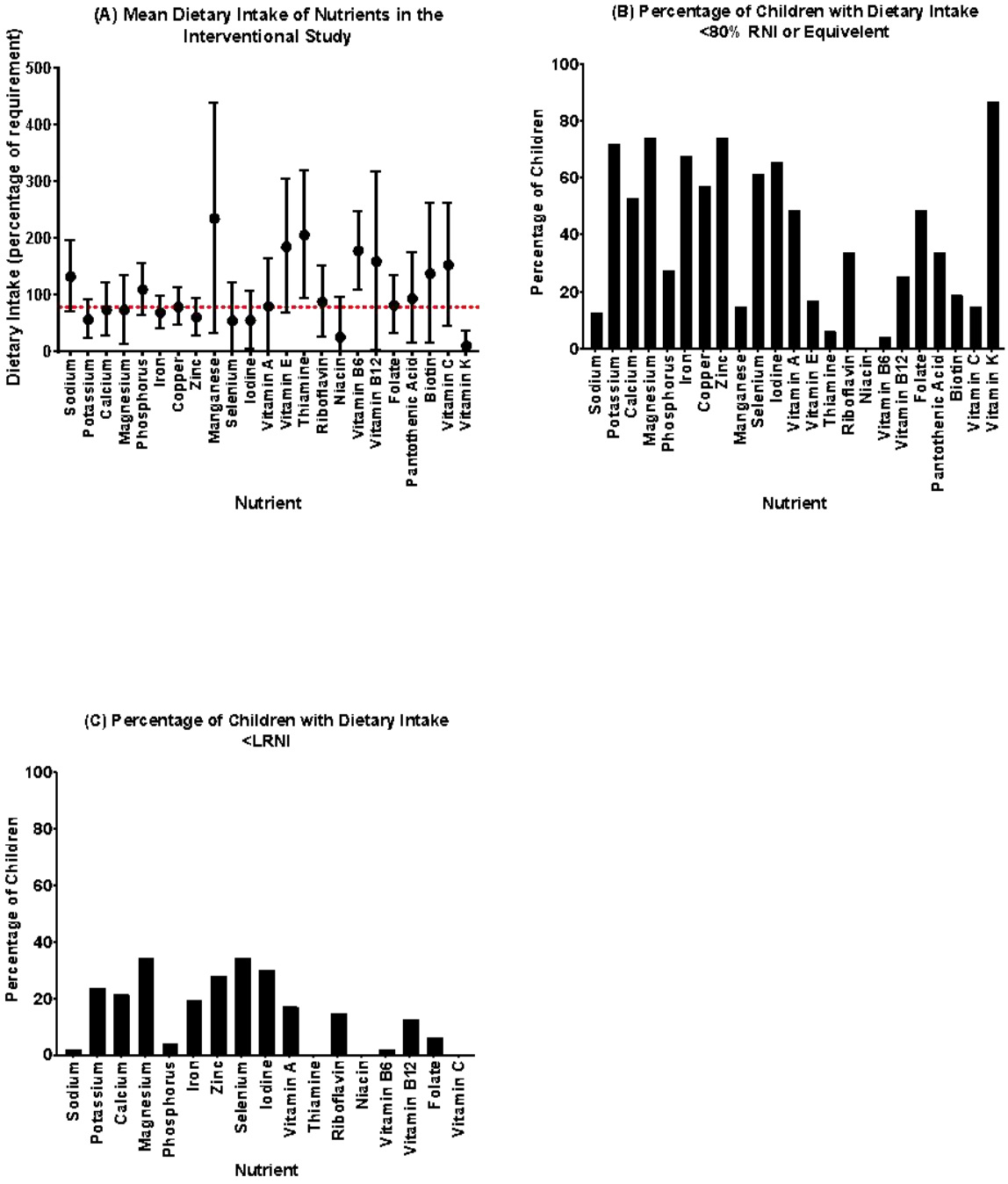


Figure 40. Dietary intake of nutrients of the participants eligible to be enrolled into the interventional study – percentage of children with dietary intake below 80% of RNI.

The percentage of children with inadequate intake (<80% RNI). There are a significant number of children with dietary intake below 80% RNI (the clinical cut-off for dietary intervention).

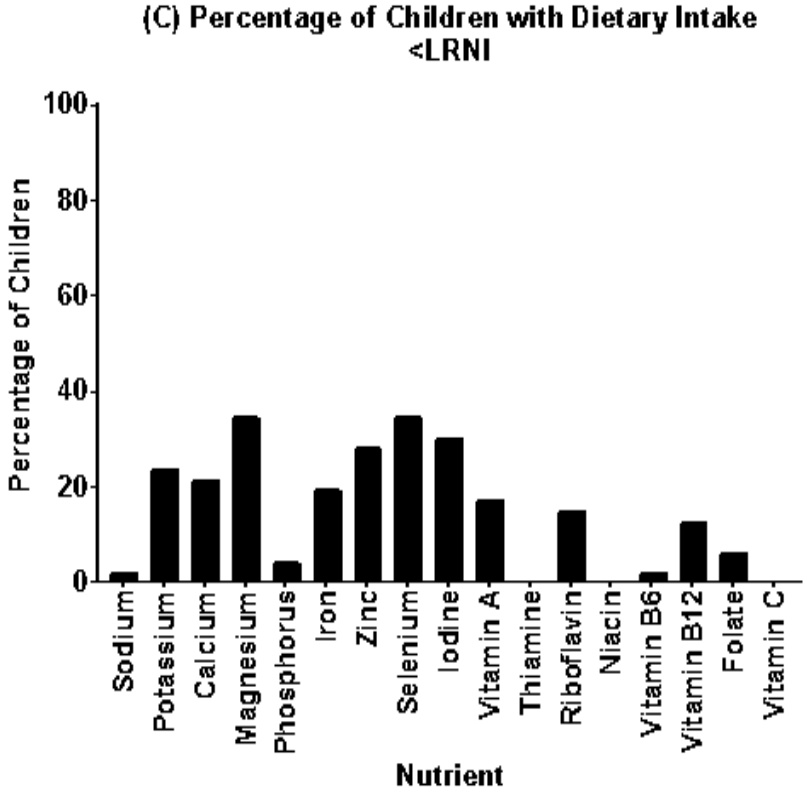


Figure 41. Dietary Intake of nutrients of the participants eligible to be enrolled into the interventional study – Percentage of children with intake < LRNI.

There are a number of children with dietary intake below the LRNI where individual inadequacy is likely. Six nutrients are not shown in graph (C) as there is no LRNI for these nutrients.

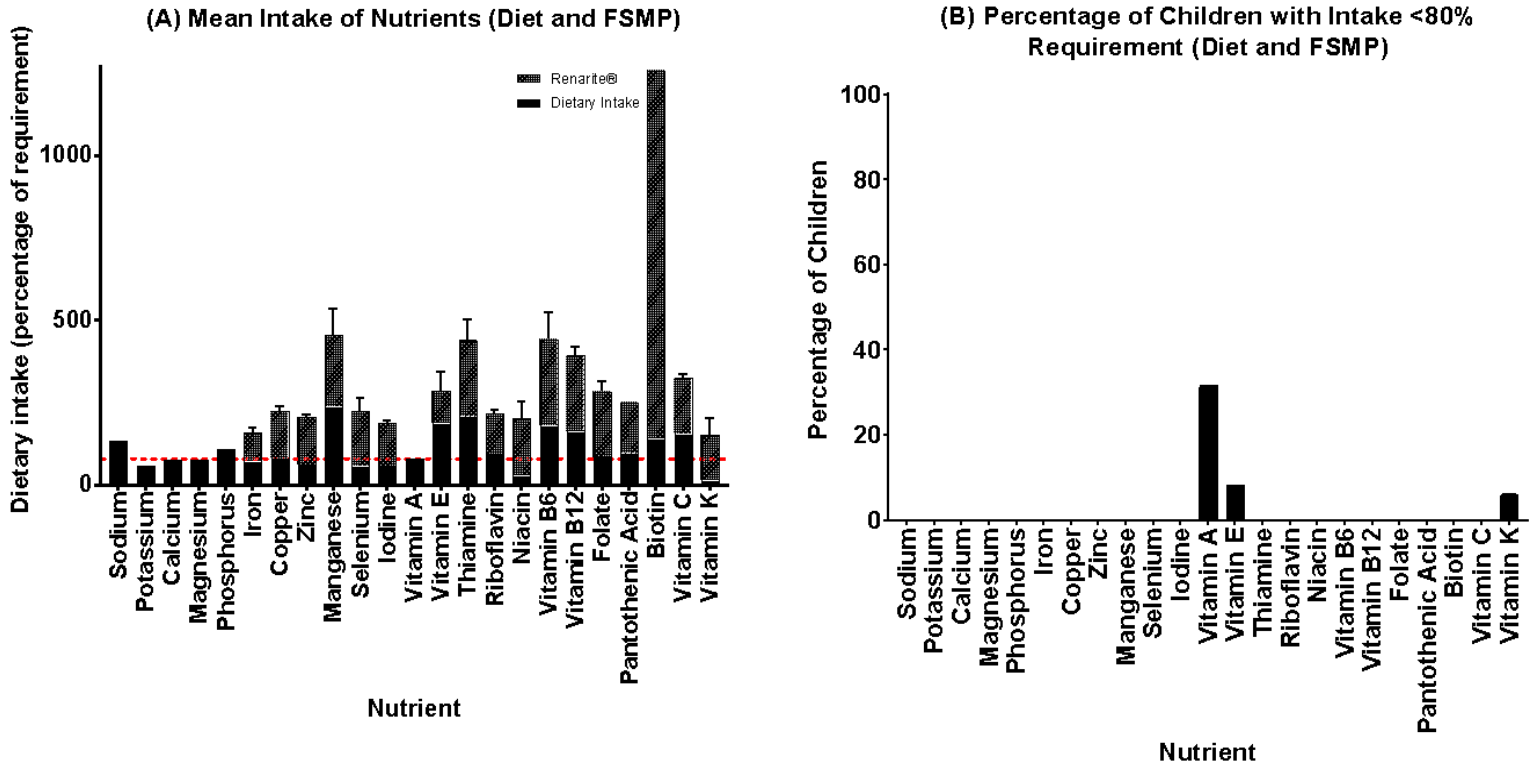


Figure 42. Intake (diet and trial FSMP) of nutrients of the participants eligible to be enrolled into the interventional study.

Graph (A) reports mean and SD intake (from diet and the FSMP), with 80% RNI marked by the red dotted line. Dietary intake is depicted as black bars and intake from the FSMP is represented by the hashed bars. Graph (B) reports the percentage of children with inadequate intake (<80% RNI). No children had intakes that were greater than the recommended upper intakes with supplementation.

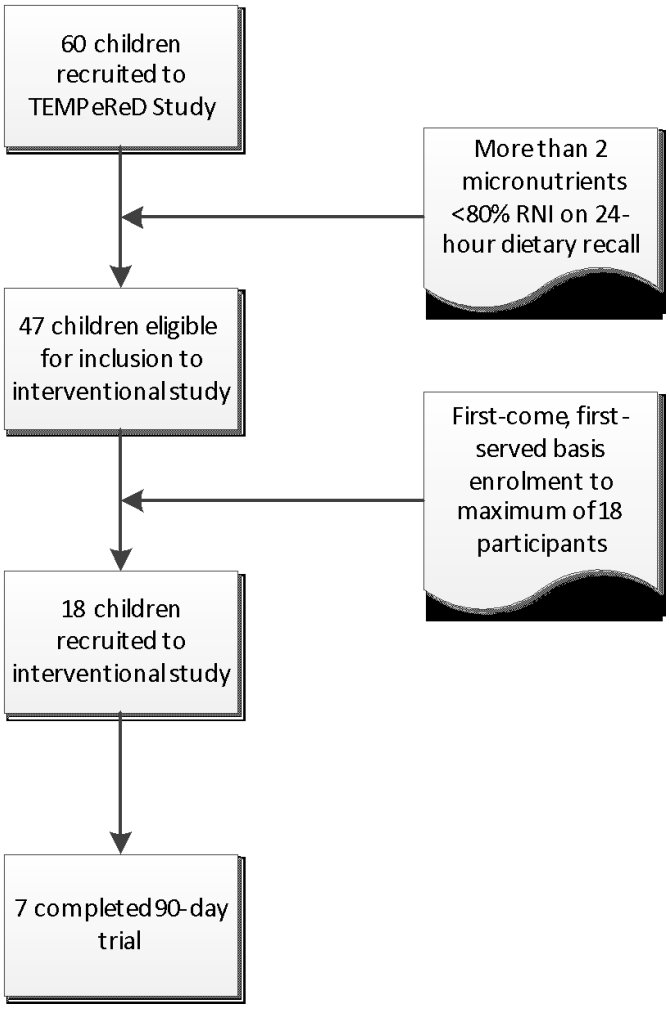


Figure 43. Consort diagram for the Interventional FSMP Study.

Sixty children were initially screened through the cross-sectional observation study, with 18 children recruited. There was a high loss of children from the study (61% loss) with only 7 children completing the study.

8.3.1. Reported Symptoms / Adverse Events During the Trial

A total of 14 adverse events (AE) were reported during the first week. After this period, no other AE were reported. Gastrointestinal symptoms were common during the study, and were cited as reasons for cessation of the FSMP. Families completed a symptom diary for the first week of the study. If reporting a symptom, responders were asked if such symptoms were unusual for the child; only unusual symptoms are presented in the table below. No non-gastrointestinal AEs were reported during the study period. All AEs were assessed as mild in severity. There were no Serious Adverse Events (SAE) reported throughout the study period.

Table 34. Reported Unusual Symptoms during the first week of taking the FMSP.

Day	1	2	3	4	5	6	7
Nausea/vomiting	1	2	1	0	0	1	0
Loose Stool	0	2	0	0	3	1	0
Abdominal pain	0	1	1	0	0	0	1

Reporting of gastrointestinal symptoms unusual for the child or young person showed that a number of children suffered these. Although such gastrointestinal symptoms were not uncommon in the group as a whole, caregivers felt that the FSMP may be a contributing factor.

8.3.2. Acceptability and Palatability Results

The reasons given by families for not continuing with taking the FSMP were grouped into themes. The first theme is that the 60ml minimal volume was considered a significant volume if a child was fluid restricted; one of the children was restricted to less than 200ml/day of free-fluids. These families felt that they needed to prioritise other fluids above the FSMP. Drinks were seen as something that children looked forward to in this situation, and felt that the FSMP did not taste 'nice enough' to be enjoyable; but considered a medication.

The second theme, which children who stopped the study over the first month reported, was a combination of unpalatable taste of the product with a rise of gastrointestinal symptoms including nausea, vomiting, abdominal cramps and loose stool. Some of these symptoms are known to occur in the cohort, but families felt that the FSMP may be exacerbating them, or for example that the poor taste precipitated nausea, or lowered the threshold for vomiting in the child.

The third theme was that upon completion of the first stage of the study, and completing the acceptability and palatability questionnaire at day 28, that families did not wish to continue for the full 90-day study. Reasons included poor taste, and the child refusing to take the FMSP.

The final theme was upon conclusion of the study at day 90. Of the remaining seven children, a single family wanted to continue taking the FSMP, and wanted to have a different formulation of the product.

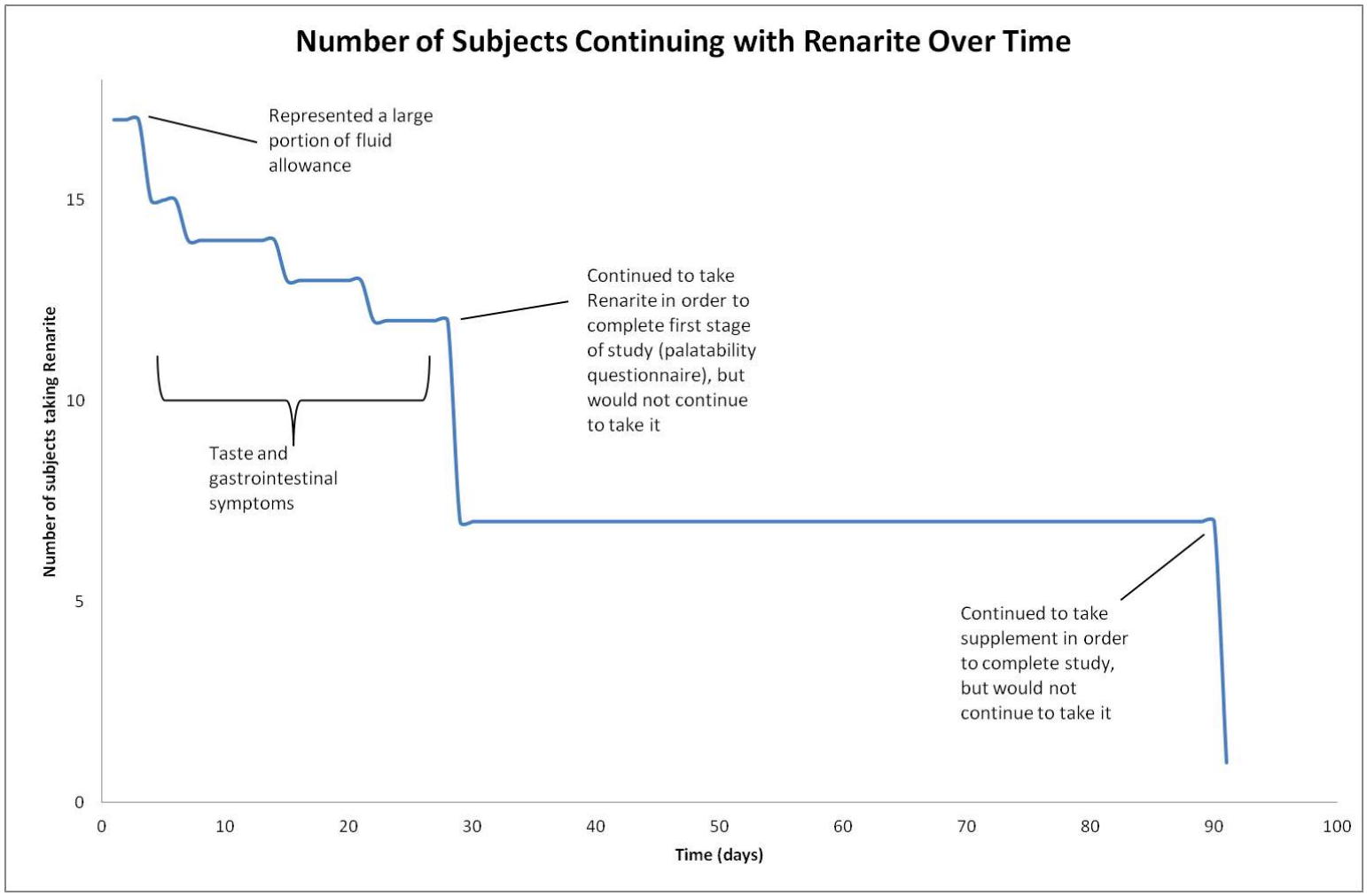


Figure 44. Kaplan-Meier plot of drop-out from the FSMP interventional study.

Themes of families' reasons for not continuing with the FSMP are annotated along the course of plot.

8.3.3. Themes from the Family Questionnaires Regarding the FSMP

A number of themes emerged from the comments offered by families completing the acceptability and palatability questionnaire. These are listed below, and although the majority of comments were negative or recommendations for changes, families emphasised the importance of nutrition and vitamin preparations. A table of the written comments grouped as 'positive comments', 'negative comments', 'reasons for not taking the product' and 'suggested improvements' is shown in *Appendix 11.13*.

Table 35. Themes of Comments from the Acceptability and Palatability Questionnaire.

● Peace of mind that a multivitamin can afford, especially in a child with a chronic disease, with nutritional problems, in whom 'over-the-counter' multivitamins are not suitable; ● *[The FSMP]* took too long to dissolve in the water, and sediment was often left at the bottom of the container; ● Poor taste; ● Preference for the formulation to be a tablet, rather than the effervescent powder.

8.3.3.1. Likert Scale Questions Results

Families completed an acceptability and palatability questionnaire at the end of the initial 28 day trial period (*Appendix 11.9*). A summary of the Likert scale responses are in the table below. Of the questions examining the product characteristics, only 'Taste' had a skew towards one arm of the scale; with 85.7% of responders answering "Really Dislike".

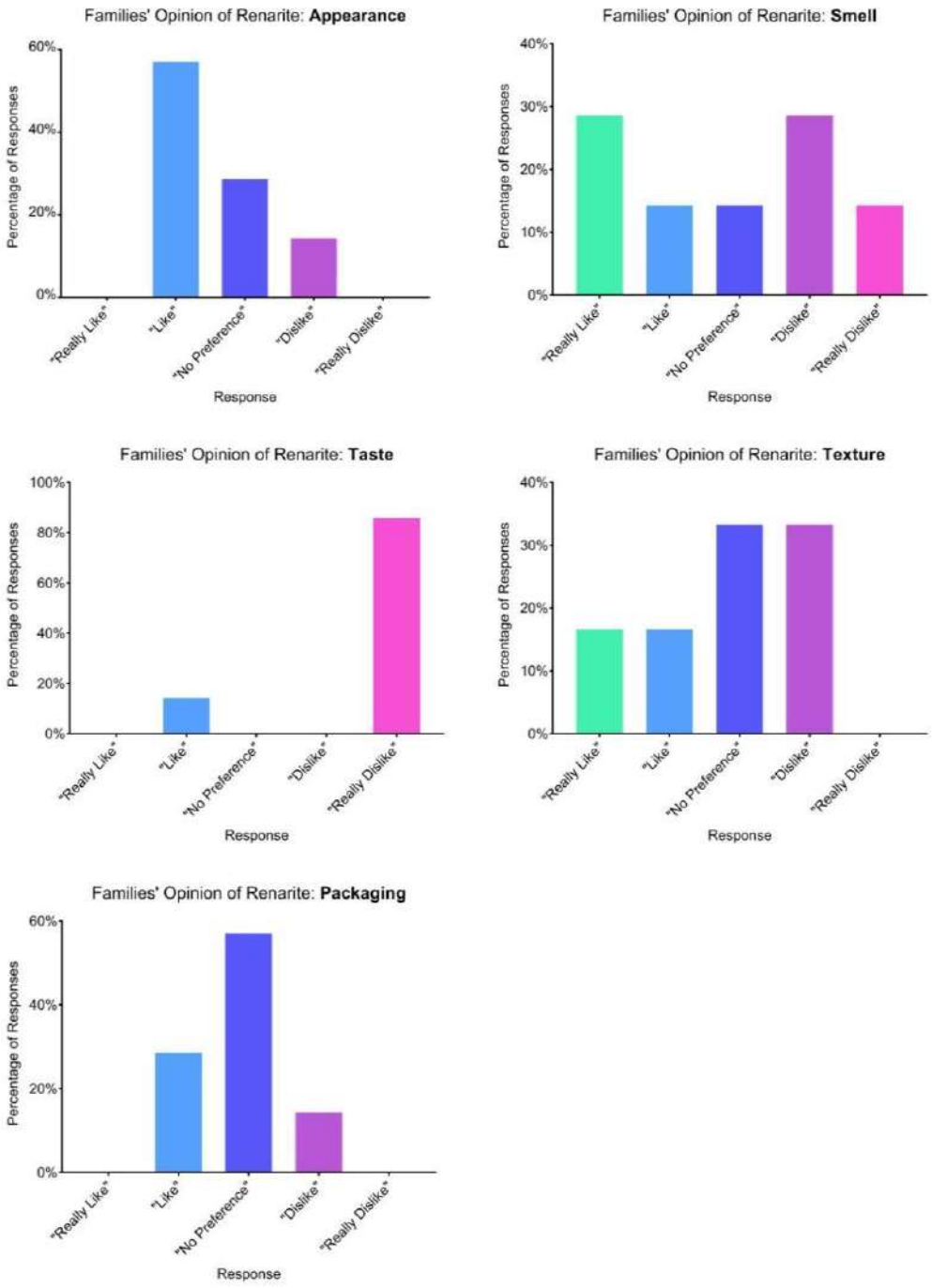


Figure 45. Summary of Likert scale responses to families' views of the FSMP characteristics from the day 28 acceptability and palatability questionnaires.

8.3.4. Assessment of Changes in Nutritional Assessment, Appetite and HRQoL

Variables measured at baseline and visit 2 (90 days later) are displayed in *Tables 36* and *39*, and *Figure 46*. There were no overt deficiencies detected on measurement of blood nutrient concentrations at baseline. Magnesium and copper concentrations were lower following the trial, and selenium concentrations higher. These

differences did not remain statistically significant following Bonferroni-correction. No other differences were found; including in appetite score.

Table 36. Comparison of Blood Nutrient Concentrations Pre-and Post-trial of FSMP (n=7).

Bloods Results mean (±SD) or median (±IQR) if non-parametric data			
	Baseline	**Visit 2**	**P-value**
Sodium (mmol/l)	137.29 (±2.29)	136.86 (±1.21)	0.448
Potassium (mmol/l)	4.17 (±0.72)	4.07 (±0.50)	0.606
cCalcium (mmol/l)	2.41 (±0.10)	2.37 (±0.08)	0.231
Magnesium (mmol/l)	0.90 (±0.14)	0.80 (±0.09)	0.031*
Phosphate (mmol/l) ǂ	1.29 (±0.77)	1.39 (±0.29)	0.063
Ferritin (ug/l) ǂ	55.00 (±154.00)	50.00 (±205.00)	0.398
Haemoglobin (g/l)	127.29 (±19.61)	120.29 (±28.55)	0.247
Vitamin B12 (ng/l) ǂ	554.00 (±202.00)	489.00 (±277.00)	0.612
Folate (ng/ml) Θ	17.50	25.00	-
Vitamin D (nmol/l) ǂ	76.00 (±74.00)	81.00 (±44.00)	0.612
Vitamin A (µmol/l) ǂ	2.20 (±1.90)	2.10 (±2.50)	0.172
Vitamin E (µmol/l)	28.59 (±4.45)	31.14 (±7.79)	0.215
Copper (µmol/l)	20.86 (±3.48)	17.29 (±3.41)	0.005*
Selenium (µmol/l)	1.00 (±0.21)	1.16 (±0.25)	0.014*
Zinc (µmol/l)	14.34 (±2.41)	14.37 (±2.19)	0.973
Vitamin C (µmol/l) ǂ	46.50 (±55.60)	47.70 (±41.50)	0.499
Manganese (nmol/l)	165.2 (±69.77)	209.80 (±102.44)	0.262
Vitamin B6 (nmol/l) ǂ	78.90 (±110.40)	142.25 (±197.45)	0.463

*Abbreviations: SD – standard deviation; IQR – interquartile range. *Statistical significance; p<0.05.* ǂ *- non-parametric, mean and IQR reported, and difference compared with Wilcoxon Signed Rank Test. Θ - Folate values once >25ng/ml are not expressed as integers; median reported.*

All participants that completed the trial period (n=7) had repeated blood measurements which demonstrated an increase in selenium concentrations for all individuals, although with variation in the increment. *Figure 46* below depicts selenium concentrations at baseline and then follow-up (visit 2, day 90).

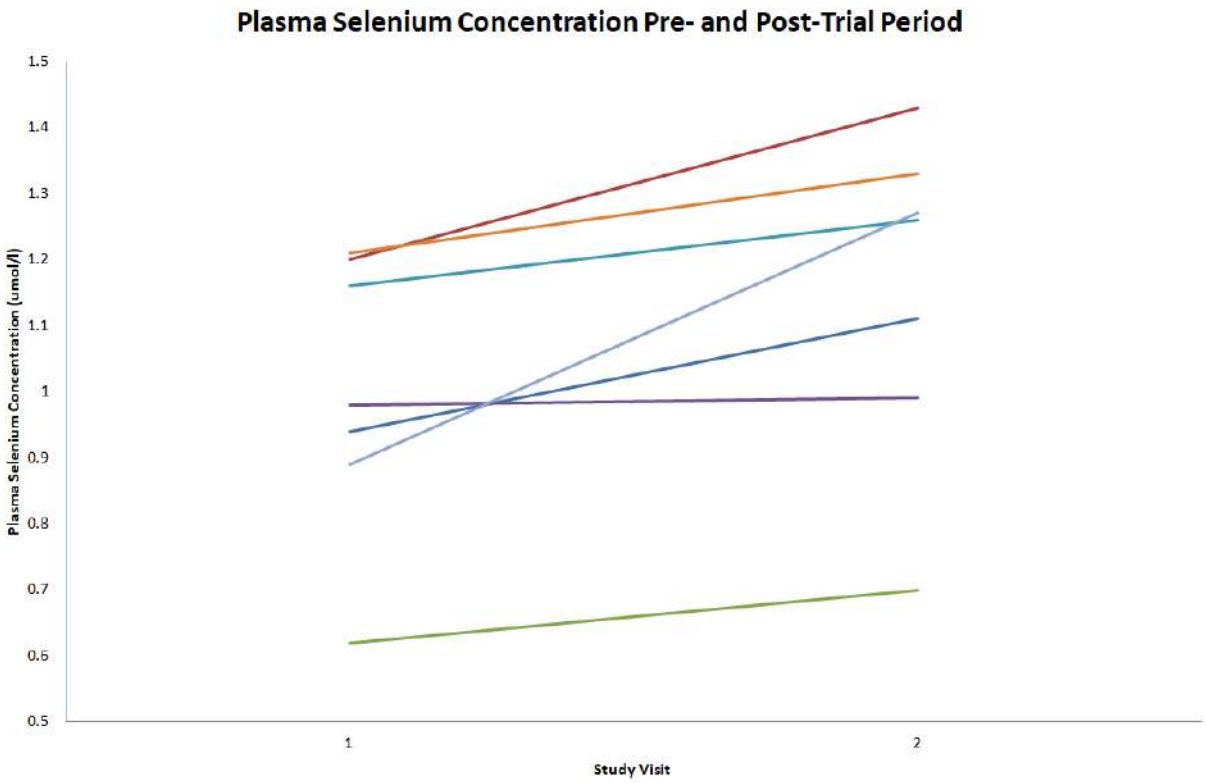


Figure 46. Pre- and Post-study Plasma Selenium Concentrations.

Plasma selenium concentrations increased for all participants, although the degree of change was variable and not seemingly related to the baseline (visit 1) selenium concentration. Mean concentration on visit 1 was 1 1µmol/l (SD±0.21) and at visit 2 was 1.16µmol/l (SD±0.25). The difference between the two time points was statistically significant (p=0.014).

Table 37. Comparison of Anthropometry, appetite, and HRQol Pre-and Post-trial of FSMP.

	Baseline mean(±SD)	**Visit 2** mean (±SD)	**P-value**
Anthropometry			
HtSDS	-2.14 (±1.23)	-2.08 (±1.29)	0.360
WtSDS	-1.32 (±1.37)	-1.37 (±1.29)	0.652
BMISDS	0.01 (±1.29)	-0.09 (±1.16)	0.546
MUAC (cm)	21.89 (±6.20)	21.97 (±4.69)	0.954
Appetite			
ModSNAQ Score	18.71 (±4.11)	20.00 (±5.06)	0.718
PedsQL™ Scores			
Child (self-rater):			
Physical Domain	62.06 (±20.12)	54.69 (±23.61)	0.922
Emotional Domain	70.00 (±27.16)	68.50 (±21.77)	0.842
Social Domain	64.17 (±28.53)	69.00 (±11.94)	0.667
School Domain	66.00 (±21.04)	56.00 (±16.73)	0.275
Total Child (self-rater) Score	65.57 (±22.95	63.61 (±13.90	0.795
Parent-Proxy:			
Physical Domain	60.00 (±25.33)	70.00 (±26.20)	0.008*
Emotional Domain	63.50 (±25.35)	59.00 (±14.32)	0.637
Social Domain	63.00 (±26.36)	60.63 (±24.69)	0.736
School Domain	65.00 (±8.66)	57.00 (±19.87)	0.347
Total Parent-Proxy Score	62.31 (±18.46)	63.48 (±17.81)	0.752

**Statistical significance; p<0.05. Abbreviations: BMISDS – body-mass index standard deviation score; HtSDS – height standard deviation score; WtSDS – weight standard deviation score.*

8.4. DISCUSSION

This chapter reports the findings of a small interventional trial examining the use of a novel FSMP as a balanced micronutrient supplement on a time-limited trial format. The main findings are that acceptability and palatability of the FSMP was poor with a high 'drop-out' rate and poor satisfaction; especially with regards to the taste profile.

8.4.1. Acceptability and Palatability of the FSMP

The evaluation of acceptability and palatability of medicinal formulations is essential. It can have a significant impact on the adherence to therapeutic regimens, and therefore clinical outcomes (302), and is highlighted in development guidance by the European Medicines Agency (EMA) (303).

Acceptability (defined as 'an overall ability of the patient and caregiver to use a medicinal product as intended') (304), and palatability (defined as, 'the overall appreciation of a (often oral) medicine by organoleptic properties such as vision (appearance), smell, taste, aftertaste and mouth feel (e.g. texture, cooling, heating, trigeminal response), and possibly also sound (auditory clues)') (304) are subjective and must therefore be assessed in such ways to allow users to describe their experiences, but for overall interpretation of the group qualitative evaluation is more difficult to interpret, therefore a mixed methods approach was used to evaluate the FSMP; including calculation of drop-out rate.

There was a significant drop-out of children in the study with 61% failing to continue the study longer than a month, and only one participant electing to continue using the supplement following cessation of the study when all were given the option. Taste was a big influence of acceptability of the product, being highlighted on both the free text element of the evaluation and in the post-trial questionnaire Likert scale rating of the FSMP's taste.

A preparation of powder that dissolved into a 'drink' was formulated due to the industrial partner's previous experience with similar products and quoted additional costs for different preparations to the manufacturer, and the suggestion that a liquid may be better tolerated in the paediatric population where swallowing tablet preparations can be challenging. In this cohort, participants suggested that a tablet preparation would be preferable. This might be due to the high pill burden placed on children with CKD, who are 'used to' taking tablets compared to other groups who have difficulty due to lack of need to take tablets (305).

Granule preparations of minerals (iron) and other medications have been shown to be better tolerated than liquid preparations in the paediatric population (306, 307), and so development of a granule preparation may be better tolerated. In agreement with suggested improvements made by the cohort, Mistry et al reviewed the acceptability of paediatric medications and found that when compared to liquid preparations, other formulations were preferred (308).

Development of a different formulation; including different taste profiles may make such a FSMP more acceptable and palatable. The addition of different flavourings/sweeteners may better disguise the minerals contained within.

Although based on a similar product (Fruitivits®, see *Table 33*), this product was evaluated in a cohort of children aged 4 - 8years on a ketogenic diet for control of epilepsy (and therefore on a restricted diet) (Details of this study: https://clinicaltrials.gov/ct2/show/NCT02229318). This population is very different from the cohort

evaluated in the study presented here, and the evaluation was only for 7 days, substituting an already prescribed micronutrient supplement. The lack of acceptability and tolerability here therefore offers a learning point in product development as to the differences in patient groups.

Children with CKD offer a unique challenge for FSMP as they have altered taste (309, 310), and increased prevalence of nausea and vomiting (1). Additionally, many already have a high medication burden, and the addition of other "medications"; including nutritional support, may make unacceptability more likely (311).

An alternative explanation for the poor acceptability of the FSMP is that the participants' taste is altered due to a physiological protective mechanism to avoid the ingestion of certain vitamins and minerals whilst in a state where their bodies are unable to cope with the metabolic and / or biochemical demands of its ingestion. Unfortunately, this study was unable to explore the possibility of this, but one way would be to offer different preparations of FMSP with different amounts of vitamins and minerals and examine the pathways by which each of the micronutrients are handled by the body.

8.4.2. Safety

There were a number of AE reported during the study period, these were solely gastrointestinal symptoms; including nausea and vomiting. In view of the poor taste profile of the product nausea, and vomiting would be more likely. As the AEs were only reported during the first week, this is likely due to drop-out from the study of those participants that suffered from AE, with only those that did not suffer AEs continuing. All the AEs were minor in severity. There were no SAEs, and this is expected for a FSMP and low-risk for such trials. In view of the low risk of the product, these AE/SAE data and the lack of derangement of biological markers measured, it is likely that the FSMP is safe.

In the development of nutritional products, one factor that may increase gastrointestinal side-effects is the osmolality of the product. Hypertonic formulations can result in fluid-shifts and resulting nausea, vomiting, bloating and diarrhoea. Most formula feeds have an osmolality of 250-800mOsmol/l. The osmolality of the FSMP once made up to 60ml is 180mOsmol/l, and therefore not hypertonic (defined as >300mOsm/l), although the osmolality would have been greater if the FMSP was made up in less water (possibly due to trying to limit the volume for fluid allowance or taste reasons), although this was not reported by participants.

8.4.3. Nutrient Intake

With the assumption that nutritional intake from diet was unchanged, then total intake of micronutrients contained with the FSMP was increased throughout the trial-period, and decreased the number of children at risk of inadequacy as assessed by total nutrient intake below 80% RNI or equivalent. The trial did not examine total intake throughout the trial period, but no other dietary intervention was made - families were not advised to change their diet in any way except for the addition of the FSMP; changes may have occurred both prompted by the nutritional assessment that was integral to the study, and due to the seasonality of foodstuffs. With a three-month period between start and finish of the trial, foods exposed to the families may have changed, influencing intake. Although the greatest impact on total intake of the nutrients is likely to be the addition of the FSMP.

8.4.4. Assessment of Changes in Nutritional Assessment, Appetite and HRQoL

Although the primary purpose of the study was to evaluate the acceptability and palatability of the FSMP, there was an opportunity to explore nutritional status changes in the cohort. This is especially important in the context of clinical assessment, monitoring and outcome markers. One reason why little difference was observed between the two time points is the size of the exposed cohort, and as stated *a priori*, the study was not powered to detect such changes. Observational studies are required to capture this daily information clinically.

8.4.5. Blood Micronutrient Concentrations

Plasma magnesium and copper were lower following the trial; selenium was higher, although none remained statistically significant following Bonferroni-correction for multiple comparisons (p=0.744; p=0.120; p=0.336, respectively). It is not surprising that supplementation in this population did not produce a significant change in biochemical values as their measurements mostly lay in the normal reference range at baseline. In those that completed the trial period of 90 days, little change was observed in the biochemical measures. Magnesium and copper levels were lower following the trial. This may be due to biological variation, but better nutritional control may result in lower levels due to limitation of some biological functions that have now become available. They may also be affected by the pro-inflammatory state, although no change in CRP, albumin or zinc was demonstrated. There may have also been competition between nutrients for absorption mechanisms, such as the divalent cation transport. High amounts of supplemental zinc have been demonstrated to decrease copper absorption, and is thought to be due to upregulation of the metal chelating protein metallothionein in intestinal cells. Metallothionein has a higher affinity for copper than zinc, copper is then 'locked' into these cells that are sloughed into the gastrointestinal lumen and lost in faeces, resulting in increased copper loss (312, 313).

Folate and selenium levels were higher following the trial, and these may be true results of improved nutrition. It is known that the selenium intake of the UK population is poor (76), and hence supplementation may improve this, as 47.8% of the cohort had a selenium intake of less than 80% of RNI upon baseline assessment.

8.4.6. Anthropometry

As demonstrated in the baseline anthropometric data of the entire cohort, most anthropometric measures lay within the normal reference range of -2 to +2 SDS, the exception being height SDS. There was no difference in anthropometry measurements between time points. This is not surprising due to: 1. growth being multifactorial and; 2. the supplementation period being relatively short; and 3. there was no change in blood markers of micronutrients. As markers of nutritional deficit, height SDS represents a longer-term nutritional status compared to weight SDS, and BMI SDS scores. As the children's BMI are not low, then it may be assumed that any acute nutritional deficit may have been corrected, and as such biochemical measures would more likely be normal also as a correction of the circulating pool of nutrients would occur sooner than in other nutritional status markers such as weight, and weight SDS would increase faster than height SDS.

8.4.7. Appetite

There was no difference in appetite scores between time points. With no alterations in blood biomarkers, this may be expected. In addition, the poor taste profile of the supplement may have a negative impact on the appetite of the child that masked any improvement due to micronutrient status improvement. Other studies evaluating micronutrient supplementation over similar timeframes in children with malnutrition in less economically

developed countries found similar lack of appetite increase (314). No children in the analysis had baseline zinc levels below the normal reference range, and this specific micronutrient-appetite association was not seen as zinc supplementation may only be beneficial to improve appetite in those with zinc deficiency (297, 315).

8.4.8. Health-Related Quality of Life (PedsQL™ Scores)

The only statistically different result was parent-proxy physical scores, and this did not remain after Bonferroni-adjustment of multiple comparisons (p=0.192). The parental opinion of their child's physical ability may have been scored higher following the trial in the awareness that the child was taking a supplement that they associated with "better health". HRQoL scores were not lower despite the addition of an additional "medication", although medication burden has been reported to negatively impact HRQoL (316). As previously stated, possible lack of observed difference between time-points may be due to sample size number.

8.4.9. The Novel FSMP

The determination of the nutrient content of FSMP was not clearly evidence-based, and developed through discussions between members of PRING. It was mostly based on the recommended intakes of healthy individuals, with additional expert knowledge such as the knowledge taken from dietary recalls and nutrient assessment as part of routine clinical practice. For example: selenium intake is often reported as low. The decision-making, and evidence that have led to the specific make-up of the product are not available, and in-view of the lack of available literature in micronutrient requirements was based largely on this expert opinion. This methodology has clear flaws with the inability to clearly justify the composition of the supplement, and a lack of a structured framework by which the composition was determined. Although offering a product for which there is a clear need, a greater evidence-base is needed in order to be sure that such supplementation is appropriate. Although there is no evidence that such supplementation is harmful, it does represent an additional medication-burden for families, and could represent a significant cost to healthcare systems if extended to all CKD patients. The feedback from families was that the burden that this represented was out-weighed by the security of supply of the micronutrients.

8.4.10. Limitations

There are many limitations to this study. The study is of a small cohort number of children who completed the study, increasing likelihood of type 2 error in identifying changes in measures. Additionally, although statistically correcting for multiple comparisons through applying a Bonferroni-correction may be helping in controlling for type 1 errors, it may risk losing identification of important changes that warrant further exploration although needed replication in large cohorts.

The primary outcome was the acceptability and palatability of the FSMP, and the study was not powered to find differences in the variables in micronutrient status, anthropometry, appetite, and HRQoL. Dietary intake was not assessed during the intervention period of the study. Therefore, any changes observed (or lack of changes) may be due to changes in dietary intake, not the supplementation. For example, families may have felt secure in the knowledge of their child receiving a nutritionally complete supplement, and placed less emphasis on the diversity of foods consumed or that the delivery of given micronutrients altered the tastes for other foodstuffs. Ideally, the trial of supplementation should be able to control for dietary intake. Without complete control of dietary intake (for example, as part of a prolonged residential stay within a research study), such evaluation of

diet is limited to the previously discussed dietary assessment methods and their own limitations – especially with regards to the accurate representation of infrequently taken nutrients. This highlights one of the many difficulties with nutrition research.

Markers of growth including height SDS may require a longer intervention time to begin to detect positive changes. Markers of vitamin and mineral status are not only influenced by the status of that nutrient, but many other factors, including inflammation. I propose that time-limited trials of supplementation are the most sensitive marker for inadequacy of a given nutrient, but require significant time, and assume adequacy of other nutrients which is unlikely in a malnourished individual – as suggested by these data in which children tended to have assessments indicating inadequate intake of multiple nutrients rather isolated inadequacies. Each nutrient has different handling within the body therefore time to repletion and depletion might be different for each nutrient and individual child, including different phenotypes of CKD.

However, the purpose of the FSMP was to facilitate the supply of micronutrients in a population who have a likely inadequate intake through diet alone, and the FSMP seems to offer this.

The *a priori* inclusion criterion of being in receipt of <80% RNI for more than 2 micronutrients on 24-hour recall dietary assessment has significant criticism. A single 24-hour recall alone is unlikely to truly reflect dietary intake; whilst this reflects current clinical practice, dietary intake of micronutrients, for example, is more sporadic and longer periods of assessment are needed to more accurately predict true intake. Although a single 24-hour recall is used in clinical practice, it is recommended to perform at least three 24-hour recalls (2), although even this is unlikely to predict true intake of most micronutrients especially for older children or 3-4 day diet diaries for younger children (317). The cut-off of 80% of RNI although justified by clinical practice guidelines (national PRING guidance) to take into account under-reporting and additional lenience so that over-identification of dietary inadequacy is less likely to occur. This value is not based upon clear evidence, but has been agreed upon as a 'pragmatic' method clinically. If this determination of dietary inadequacy is questionable, it is therefore possible that none of the children suffered from true dietary inadequacy.

8.4.11. Future Directions

There is a recognised need for a nutritionally complete micronutrient supplement that is suitable for children with kidney disease, but the currently tested micronutrient supplement was not acceptable to the population that it was intended for despite previous work performed by the industrial partner. It is advised that the profile of the supplement must be altered to be more acceptable and re-evaluated within a cohort of families with CKD. This could, in the first instance, be performed as a "focus-group" taste test as part of Patient and Public Involvement (PPI) work in study development. Following this, larger-scale testing on improvement of nutritional outcomes is needed in appropriately powered studies.

Further observational, long-term research is needed to determine the best way(s) to identify those at risk from micronutrient inadequacy during the clinical life-course of kidney disease. Previous literature in healthy individuals have stated that many days' dietary analysis are required to accurately predict micronutrient intake (317). Perhaps the development of a specifically-designed food frequency questionnaire would be a dietary

assessment method that may prove more accessible and accurate in identifying those at risk within a clinical setting.

8.4.12. Concluding Remarks

Although the formulation of this novel FSMP almost eliminated those children at risk from micronutrient inadequacy through the potential combined diet/supplement delivery of micronutrients, translation into clinical practice was not possible. This was largely due to the unacceptable nature of the FSMP described by the study population apparently due to poor taste profile and side-effects. Different formulations and preparations should be developed and trialed, as a gap in the ability to ensure micronutrient supply remains unfilled.

8.4.13. Practice Points

- A balanced, nutritionally complete micronutrient suitable for children and young people remains unavailable.
- Consideration should be taken to unpalatability of supplements and other nutritional interventions as potential protective mechanisms.
- Nutrient-nutrient interactions need to be considered. Increasing intake of one nutrient may decrease the absorption and resultant status of other nutrients.
- However, the need to make key nutrient adjustments to help delay the progression of disease, present further challenges here.
- Supplementation with folate and selenium increased blood concentrations. This may represent an improvement in status and increasing intake should be considered in those at risk.

9. IN SEARCH OF A MARKER OF DISEASE ACTIVITY

9.1. INTRODUCTION

As discussed above, the disturbed growth of children with CKD cannot be explained by nutritional inadequacy as characterised by the methods of assessment routinely employed in the clinical setting. Although traditionally stratified into clinical phenotypes by disease severity, a measure of disease activity may be more useful.

The pathophysiology underlying the disease process in CKD is discussed in *Chapter 1* and illustrated in *Figure 3*. There is a need to explore how disease activity in children with CKD interacts with the known physiological processes in order to better map the pathophysiology; including the interplay between nutrition and the disease process. There is additional potential to use such markers clinically to both prognosticate and monitor response to therapeutic intervention, including nutritional manipulation.

CKD is well recognised as an inflammatory state (64), that worsens with increasing disease severity (110). Although this inflammation can be assessed through the measurement of circulating acute phase reactants and cytokines, these markers are often non-specific; representing systemic inflammation. For example, C - reactive protein (CRP) which is elevated in a wide range of conditions, including acute infection.

This chapter will explore the possibility of a readily accessible, nutritionally sensitive marker of this disease activity through the application of markers to a cohort of paediatric pre-dialysis, conservatively-managed (PDCM) CKD patients. It is hypothesised that those with poor growth would have higher disease activity as assessed by these markers.

Red cell distribution width (RDW) has been purported to reflect inflammation in chronic disease states (111), and erythropoiesis is intimately linked with CKD. RDW has been associated with clinical outcomes (112-114).

Neutrophil-gelatinase associated lipocalin (NGAL) is a biomarker that is showing promise for its utility in paediatric CKD. It is proposed that elevated NGAL is caused by the underlying inflammation present in CKD; being produced by kidney epithelial cells (122). Previous literature has demonstrated that NGAL levels in the CKD population are correlated with disease severity (123), and is able to predict disease progression (124).

9.2. Red Cell Distribution Width

9.2.1. Introduction

The relevance and associations of RDW in current clinical practice have been discussed in *Chapter 1*. I hypothesize that RDW is a marker of medium-term inflammation and therefore disease activity in paediatric PDCM CKD, and that higher RDW is associated with markers of growth (height SDS, height velocity SDS), nutritional status, and progression of kidney disease. The aim of the study was to test these hypotheses.

Aim:

The aim of this subchapter is to explore the relationship between RDW and growth and nutritional status.

Objectives of the study are to:

- To report the RDW of a cohort of paediatric PDCM patients;
- To explore the association to RDW on the growth and nutritional status of children with PDCM CKD.
- To evaluate the predictive power of RDW with clinical outcomes of disease progression and nutritional status.

9.2.2. Methods

Children were recruited to the TEMPeReD study as described in *Chapter 5*. Although not routinely reported to the clinician, RDW is calculated as part of the full blood count of routine clinical care bloods. RDW was measured by fluorescence flow cytometry using Sysmex XN-9000 (Sysmex UK Ltd., UK) as per usual clinical care.

Although the TEMPeReD study included children and young people having received a kidney transplant (n=10) and an additional four in receipt of dialysis, these were excluded from this analysis due to the potential significant confounding of immunosuppressive drugs, and dialysis-driven inflammation.

Distribution of RDW within the pre-dialysis, conservatively-managed (PDCM) group was explored with descriptive statistics performed, including median and interquartile range. If RDW was above or below the normal reference range, the individual's other data was examined for a pathological cause, such as iron-deficiency.

There is no standardised, agreed-upon normal reference range of RDW, although quoted normal reference ranges are similar. For this study the normal reference range used was 11.3 – 14.8%, which is consistent local pathology references ranges.

In addition to RDW, other erythrocyte indices were reported and explored to identify those with a recognized cause of increased RDW, and numbers of children with anaemia (Hb <120g/l) reported.

Associations were sought between RDW and measures of disease severity, inflammation, and nutritional status were explored through correlations and comparison of tertiles.

Finally, follow-up data gathered at 12 months following baseline visit (serum creatinine, height and weight) were used to evaluate the predictive power of RDW for disease progression (change in eGFR), and change in anthropometric defined nutritional status (change in HtSDS, WtSDS, and BMI SDS).

9.2.3. Results

Forty samples from the 46 children in the PDCM group had RDW available for analysis. One child had elevated RDW (28.1%). This patient had a MCV=70.8fL (microcytosis) which was felt likely to represent iron-deficiency anaemia and was excluded from further analysis. With this outlier excluded, RDW data remained non-parametric, with a median RDW of 12.5% (IQR=0.70; range = 11.4 to 14.8%). RDW data were divided into tertiles, with cut-offs of 12.30%, and 12.73%.

There were no differences in MCV, RDW, ferritin, or CRP between those that were anaemic (Hb <120g/l, n=8) and those not (see *Table 38*, below).

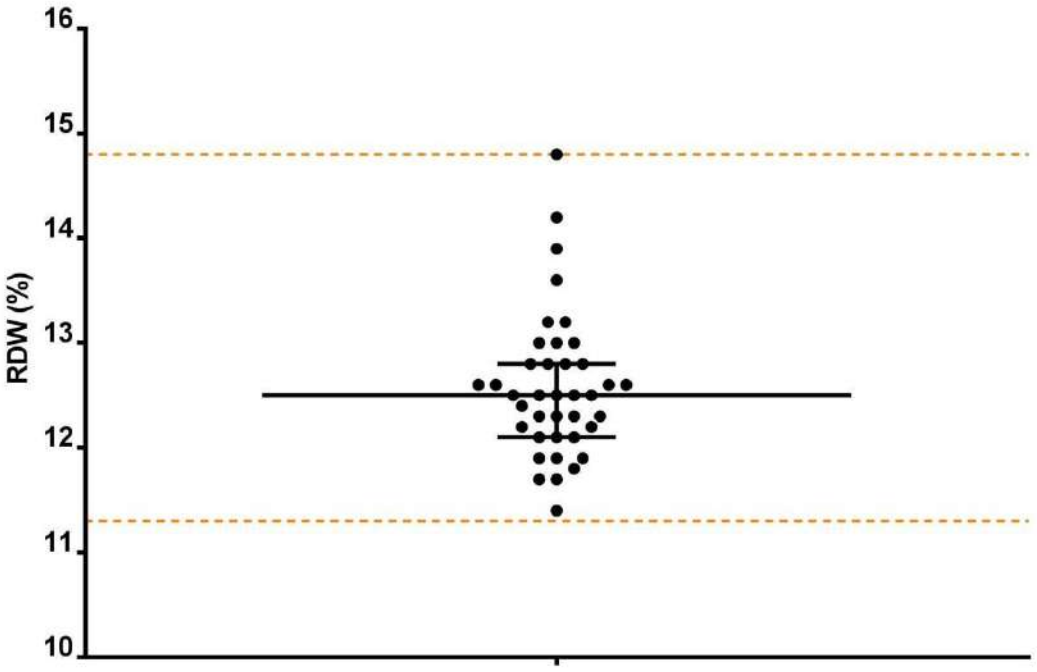


Figure 47. Red cell distribution in the PDCM group (n=39).

Distribution of red cell distribution width (RDW) within the Pre-dialysis, conservatively-managed (PDCM) group. Median and interquartile range are shown. The orange dotted lines represent the normal reference range.

Table 38. Red cell indices for the PDCM group, and anaemic/non-anaemic sub-groups.

Variable	PDCM group (n=39)	Anaemia (Hb <120g/l) (n=8)	Non-anaemia (Hb ≥120g/l) (n=31)	Difference between anaemia / non-anaemia; P=value
RDW (%) ‡	12.5 (±0.7)	12.4 (±1.15)	12.5 (±0.68)	0.798
MCV (fL) ‡	82.81 (±5.1)	82.50 (±6.90)	82.95 (±5.13)	0.645
Hb (g/l)	128.51 (± 17.00)	105.78 (±10.46)	135.33 (±12.33)	0.0001*
Ferritin (ug/l) ‡	55.00 (±88.5)	34.00 (±229.5)	56.00 (±75.0)	0.479
CRP (mg/l) ‡	1 (±2.5)	0 (±3)	1 (± 2.75)	1.000

*‡ - non-parametric data, median and interquartile range reported. * - statistically significant difference between anaemia and non-anaemic sub-groups. Abbreviations – CRP – c-reactive protein; Hb – haemoglobin; MCV – mean corpuscular volume; PDCM – pre-dialysis, conservatively-managed; RDW – red cell distribution width.*

9.2.3.1. Association with disease severity

There was no correlation between RDW and markers of disease severity: eGFR (S.rho = 0.018, p = 0.915); CKD stage (Kruskal-Wallis test=4.027, p=0.546) and medication burden (S.rho = 0.223, p = 0.172).

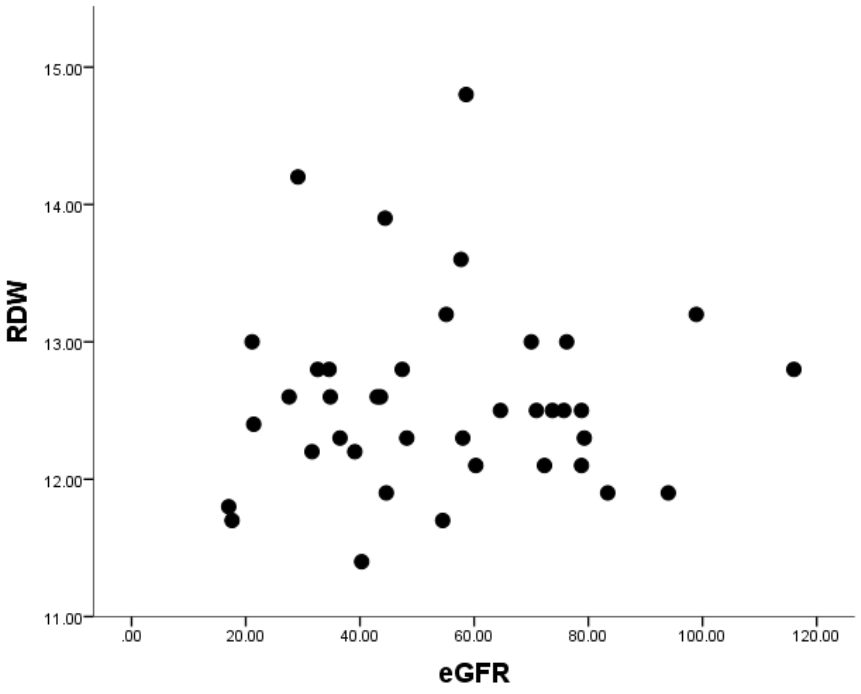


Figure 48. Graph of red cell distribution width versus eGFR in the PDCM group (n=39).

Red cell distribution width (RDW) was not correlated with kidney function (estimated glomerular filtration rate, eGFR) (Spearman's rho = 0.018, p = 0.915).

9.2.3.2. Association with C - reactive protein

CRP is a marker of inflammation. Although there was no difference in CRP concentrations between those in the lowest RDW tertile and those in the highest tertile (p = 0.211). There was a statistically significant correlation between RDW and CRP (S.rho = 0.375, p = 0.022). Three children had CRP concentrations ≥10mg/l. These may represent subclinical infection, and with their exclusion, correlation with RDW improved (S.rho = 0.454, p = 0.007).

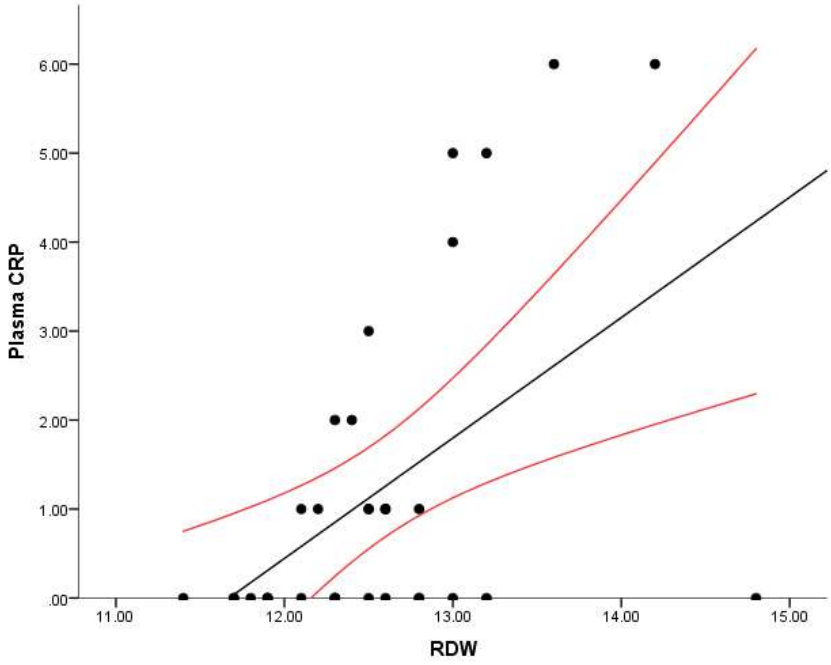


Figure 49. Plasma CRP versus RDW in PDCM group (CRP >10mg/l excluded).

There was a significant correlation between c-reactive protein (CRP, mg/l) and red cell distribution width (RDW, %) in the pre-dialysis, conservatively-managed group. Correlation was stronger following exclusion of those with CRP >10 mg/l, which may represent subclinical, acute infection rather than inflammation due to chronic kidney disease. Spearman's rho = 0.454, p = 0.007. Black line – line of best fit; red lines – 95% confidence intervals.

9.2.3.3. Association with markers of nutritional status

The data demonstrated correlation between RDW and WtSDS (S.rho = -0.344, p = 0.032), and MUAC (S.rho = -0.275, p = 0.023), but not HtSDS (S.rho = -0.260, p = 0.109), BMI SDS (S.rho = -0.275, p = 0.090), or WHtR (S.rho = -0.129, p = 0.432).

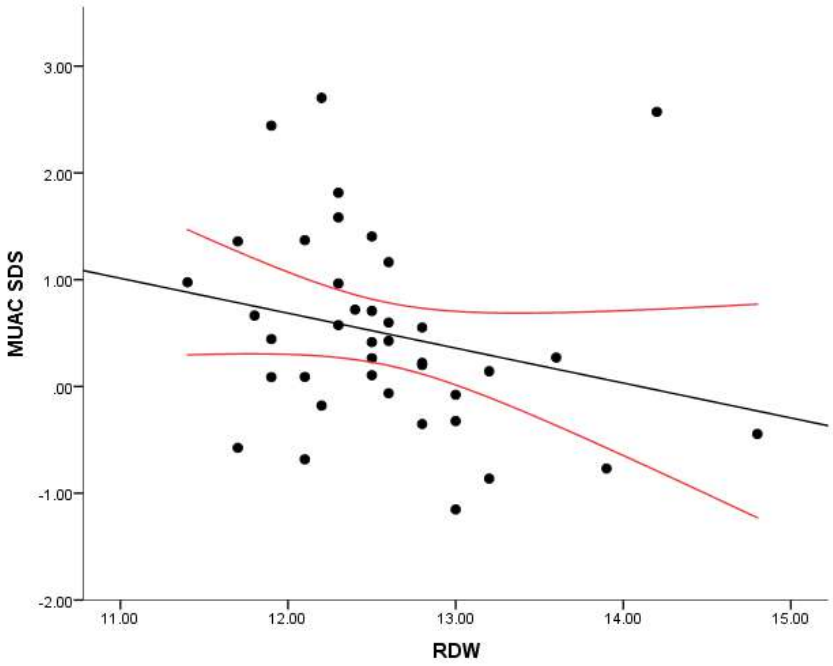


Figure 50. MUAC SDS versus RDW in PDCM group (n=39).

Mid upper arm circumference standardised deviation score (MUAC SDS) was negatively correlated with red cell distribution width (RDW, %) (Spearman's rho (S.rho) = -0.364, p = 0.023). Black line represents the line of best fit with red lines representing 95% confidence intervals. Although weight SDS was also negatively correlated (S.rho = -0.344, p = 0.032), Body mass index SDS and Waist-to-height-ratio was not (S.rho = -0.275, p = 0.090; and S.rho = -0.129, p =0.432, respectively). Although adiposity is associated with inflammation and inflammation is associated with increase in RDW, not all markers of adiposity are correlated with RDW. It may be that the inflammation, and RDW correspond with mid upper arm muscle mass, and increasing RDW is associated with muscle wasting and sarcopenia in the paediatric CKD. Abbreviations: RDW – red cell distribution width; MUAC – mid upper arm circumference; SDS – standardised deviation scores.

Although there was a trend for children in the highest RDW tertile to be shorter, have slower growth, and be lighter-for-height, these differences were not statistically significant. There were no differences in micronutrient concentrations between those in the lowest and those in the highest RDW tertiles (see *Table 40*, below).

Table 39. Differences between those lowest and highest RDW tertiles in the PDCM group (n=39).

Variable	Lowest RDW Tertile Mean (±SD)	Highest RDW Tertile Mean (±SD)	P-value
HtSDS	-0.65 (±1.7)	-1.72 (±2.70)	0.104
WtSDS	-0.14(±2.09)	-1.44 (±1.81)	0.126
BMISDS	0.64(±1.72	-0.30 (±1.40)	0.168
MUAC SDS	0.73(±1.09)	0.03 (±1.40)	0.130
Ht Vel SDS	-0.07(±3.02)	-1.67 (±1.00)	0.833
WHtR	0.50(±0.10	0.46 (±0.10)	0.211
Copper (µmol/l) ‡	17.58 (±3.73)	21.40 (±5.40)	0.056
Selenium (µmol/l)	1.05 (±0.20)	1.01 (±0.27)	0.641
Magnesium (mmol/l) ‡	0.83 (±0.14)	0.83 (±0.21)	0.880
Manganese (nmol/l)	149.09 (±45.80)	168.70 (±51.40)	0.367
Zinc (µmol/l) ‡	13.15 (±2.95)	13.10 (±3.48)	0.755
Vitamin B6 (nmol/l) ‡	80.20 (±42.00)	74.50 (70.55)	0.809
Folate (ng/ml) ‡	10.70 (±8.70)	17.50 (±15.25)	0.080
Vitamin B12 (ng/l) ‡	370.50 (±212.75)	446.00 (±705.00)	0.740
Vitamin C (µmol/l) ‡	76.85 (±60.00)	65.30 (±55.70)	0.449
Vitamin E (µmol/l) ‡	27.55 (±5.33)	29.05 (±8.00)	0.319
C-reactive protein (mg/l) ‡	0 (±1)	4 (±6)	0.211
Serum albumin (g/l) ‡	40.50 (±5.75)	38.50 (±4.50)	0.378

‡ - non-parametric data, median and interquartile range reported, and comparison made by Mann-Whitney U-test. Parametric data was compared using independent t-test. - statistically significant difference between the subgroups. There were no significant differences between the lowest and highest red cell distribution width tertiles in the PDCM group. There was a trend for those in the higher tertile to be shorter and lighter-for-height, with slower growth. Abbreviations: BMI SDS – body mass index standardized deviation score; eGFR – estimated glomerular filtration rate; HtSDS – height standardized deviation score; HtVelSDS – height velocity standardized deviation score; MUAC SDS – mid-upper arm circumference standardized deviation score; PDCM – pre-dialysis, conservatively-managed; RDW – red cell distribution width; SD – Standard deviation; uNGAL – urinary neutrophil gelatinase-associated lipocalin; uPCR – urinary protein-creatinine ratio; WHtR – Waist-for-height ratio.*

9.2.3.4. Prediction of clinical outcomes

Follow-up data at 12 months are described in *Chapter 5*. RDW did not show good predictive utility for progression of kidney disease (decrease in eGFR of >10ml/min/1.73m^2 at 12 months: 3 children from 32 available for analysis. AUC=0.224 (CI=0.024-0.424)), or changes in anthropometric SDS (Decline of HtSDS >0.5 at 12 months: 1/35 children. AUC = 0.691 (CI=0.528-0.854); Decline of WtSDS >0.5 at 12 months: 1/34 children. AUC = 0.227 (CI=0.083-0.371); Decline of BMISDS >0.5 at 12 months: 4/33 children. AUC = 0.297 (CI=0.091-0.504)).

9.2.4. Discussion

In this chapter, RDW for a group of paediatric PDCM CKD patients is reported, and its association with nutritional status and clinical outcomes at 12 months explored.

The distribution of RDW reported in this cohort is similar to that of a healthy population with maximum and minimum values within the limits of the normal reference range. RDW did not show an association with eGFR or other markers of disease severity. Although children with more severe disease are more likely to be anaemic for the reasons discussed above, clinical care is directed at correcting this with increased monitoring with increasing disease severity. With erythropoietin inadequacy corrected with exogenous erythropoietin, and correction of iron/folate, vitamin B12 deficiency that are actively investigated for in the presence of anaemia, variation in RDW may in be more influenced by systemic inflammation. Supporting this assertion is that CRP, a nonspecific marker of inflammation, was correlated with RDW. As highlighted in this group, the presence of acute infection (subclinical or otherwise) may cloud the association on the individual level and in small cohorts, such as this one. Exploration of inflammation as marked by other biomarkers, such as circulating cytokine levels or erythrocyte sedimentation rate (ESR) may strengthen the evidence of the link between systemic inflammation and RDW. It would be useful to evaluate markers of intra-kidney inflammation and their association with RDW in the context of CKD.

9.2.4.1. Association with markers of nutritional status

It was hypothesized that poorer growth would have higher RDW. Although there was a trend observed for this to be the case, this was not statistically significant. It may be that the sample size was not large enough to detect this difference, as there was large variation in height velocity SDS especially.

RDW was negatively correlated with WtSDS and MUAC SDS suggesting a possible relationship between acute nutritional state and RDW (lower WtSDS and MUAC SDS associated with greater variation in erythrocyte size), although the correlation with BMI SDS, that adjusts for height (a marker of chronic nutritional state), was not statistically significant. It may be that body composition changes account for this discrepancy. MUAC SDS may be more sensitive to changes in lean body mass, specifically muscle mass and the wasting that accompanies CKD (318). Body composition analysis, such as bioelectrical impedance analysis, may be able to explore this possible relationship in more depth. This hypothesis is supported by data from the National Health and Nutrition Examination Survey (NHANES) study in the USA which reported that elevated RDW was associated with sarcopenia (defined as appendicular skeletal muscle mass divided by weight below one SD of the mean of young adults) (319).

The lack of relationship between RDW and the presence of anaemia may be due to the aetiology of the anaemia. Anaemia of chronic disease can result in a normocytic or microcytic anaemia, and iron-deficiency (commonly observed in CKD) results in a microcytic anaemia.

Iron, folate, and vitamin B12 status all potentially influence RDW as inadequacy of iron results in a microcytosis, and inadequacies of folate and/or vitamin B12 cause a macrocytosis. If a mixed inadequacy is present both small and large erythrocytes are present, and the RDW increases. Formal irons studies were not performed in the cohort, but plasma folate and vitamin B12 concentrations were not correlated with RDW (Folate: S.rho = 0.221, p = 0.224; vitamin B12: S.rho=0.035, p = 0.835). This may be explained by the normal concentrations of the nutrients in the blood (no individuals were deficient) and that RDW lies within the normal reference range. Additionally, due to the method by which RDW is calculated, a decrease in MCV is more likely to result in an increase in RDW, but a macrocytosis does not. This is because increasing the denominator of the equation (see calculation in *Appendix 11.1*) may offset the increase in the width of the curve that RDW represents.

There was no difference in micronutrient blood concentrations and RDW, although plasma copper concentrations were approaching significantly different. Copper inadequacy is associated with anaemia which can be microcytic, normocytic, or macrocytic in morphology (320). Although copper intake is not assessed as part of the NDNS in the UK, there is a high prevalence of intake of other minerals (selenium and zinc) that are unlikely to meet requirements (< LRNI) (321).

Predictive power of RDW in the cohort

There are several explanations for why RDW did not show predictive power in this cohort. Firstly, RDW may not have this association in paediatric CKD patients. Secondly, the lack of significance may be due to the sample size and the short follow-up period. Hsieh et al's retrospective cohort study in >1000 adult CKD patients demonstrated a doubling of mortality in those with elevated RDW (>14.9%) covering a 7 year timescale (117). The health outcomes were also different; progression of kidney disease (decline in eGFR) and decline in growth trajectory not mortality (which is fortunately low in paediatric populations). It may be that RDW better reflects the factors that contribute to cardiovascular disease and infection. Hsieh et al's cohort differed in more ways than just age. Participants had more severe disease with a mean eGFR of 22.85 ml/min/1.73m^2 (SD±12.69) compared to this cohort's 54.65 ml/min/1.73m^2. Children are also different from adults in many other ways; including potential resilience of erythropoiesis to disease, that may explain the largely normal RDW measurements. An abnormally elevated RDW may have proved predictive, but none of this cohort had elevated levels. Larger studies are required encompassing a larger cohort, over a longer timeframe.

The advantages of using RDW as a measure of disease activity is that it is readily accessible and already measured routinely in clinical practice, although not always reported on the clinical facing results platform and immediately available to clinicians.

9.2.4.2. Limitations

Limitations of RDW include its wide number of influencing factors. Anaemia is common in CKD, and increased RDW in an individual may purely represent iron, folate and/or B12 inadequacy. The half-life of erythrocytes is approximately 120 days. Although this is lower in those with CKD (121), this means that for any change in

disease activity it is likely that the RDW would not be perceptively different for several weeks. As briefly referenced above, the calculation of RDW may be considered flawed, with asymmetrical influence of micro- and macrocytosis. Additionally, as the variation of 1 SD is used, cells that lie outside this window are not represented in the equation. As such, RDW is a 'blunt' cell population estimation, and does not characterize small subpopulation changes. It is likely that other clinical measurements/ phenotypic observations would serve equally well as markers at this level of erythrocyte population 'disruption'. A fundamental limitation of RDW as a marker of disease activity or as a nutritional risk marker is a lack of a clear mechanism by which RDW may be understood, over and above iron, folate and vitamin B12 inadequacies and the influence of disease severity on erythropoiesis (inadequate peritubular cell erythropoietin production, inflammation and uraemia effect on erythroblasts) and erythrocyte lifespan (inflammation, infection and uraemic toxins) (115, 121). Rather than a systemic marker, a kidney specific-marker is perhaps more likely to represent a marker of CKD disease activity. Additionally, at the individual level, many factors may influence RDW that would cloud clinical decision-making.

9.2.4.3. Future Directions

Future work may include evaluation of the association of clinical outcomes in paediatric CKD in a larger cohort over a longer timeframe; and exploration of the possible relationship between RDW and muscle mass (potentially assessed by cross-sectional imaging and/or anthropometric measures) and functionality such as handgrip strength.

9.2.4.4. Concluding Remarks

RDW is a readily accessible measure but has many influencing factors that make interpretation difficult. The potential association with muscle mass is an interesting suggestion that may be hypothesised from these data and could be the starting point of further body composition-inflammation research.

9.2.4.5. Practice Points

- Although correlations are observed between RDW and MUAC that may be a reflection of body composition, there is not enough evidence or understanding to support its use in clinical care.

9.3. URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (UNGAL)

9.3.1. Introduction

Neutrophil-gelatinase associated lipocalin (NGAL) is a biomarker that is showing promise for its utility in paediatric acute kidney injury (AKI). NGAL may be a nutritionally-sensitive, readily accessible marker that may reflect disease activity and/or nutritional status, as discussed in *Chapter 1*. There is a need to explore its relationship in paediatric CKD.

Aim:

The aim of this subchapter is to explore the relationship between uNGAL and growth and nutritional status in a cohort of children and young people with CKD.

Objectives of the study:

- Report the uNGAL concentrations of a cohort of paediatric PDCM patients;
- Report the number of elevated uNGAL concentrations in the cohort as defined by the previously defined normal references values by Bennett et al, and Rybi-Szuminśka et al; and assess the agreement of these methods;
- Test the hypothesis that those with poor growth have greater disease activity as assessed by uNGAL concentration.
- To evaluate the predictive power of uNGAL with clinical outcomes of disease progression and nutritional status.
- Test the hypothesis that those with higher uNGAL have lower micronutrient availability to the kidney.

9.3.2. Methods

Children with CKD were recruited to the TEMPeReD study as previously described. Following characterisation, including assessment of growth by height SDS and height velocity SDS, uNGAL was measured from samples collected at enrollment in the TEMPeReD study, the sample placed immediately on ice and then stored at -80˚C to allow for batch analysis. NGAL in urine was measured in duplicate by sandwich enzyme linked immunosorbent assay (ELISA), (Bioporto Diagnostics and supplied by Alpha Laboratories). Urines were diluted as required, from 1 in 50 to 1 in 5000 to achieve the final calculated reported results. The same kit lot was used throughout the analysis.

uNGAL concentrations were expressed as both absolute concentrations (ng/ml) and adjusted for urinary creatinine concentrations (ng/mg of creatinine). Values were compared to previously reported normal values (128, 129). Additionally, concentrations were stratified into tertiles and comparisons made between sub-groups.
Differences in uNGAL concentrations were sought with reference to anthropometry and measures of growth at baseline (height standard deviation score, Ht SDS; weight stand deviation score, Wt SDS; body mass index standard deviation score, BMI SDS; mid-upper arm circumference standard deviation score, MUAC SDS; height velocity standard deviation score, Htvel SDS; waist-height-ratio, WHtR) and change in Ht SDS at 6 months following initial visit. Differences in markers of disease activity (estimated glomerular filtration rate, eGFR; number of medications, and urinary protein-creatinine ratio, uPCR); inflammation (C-reactive protein, CRP; and serum albumin), and blood concentrations of micronutrients were also explored.

eGFR was calculated from the Schwartz formula (152). A deterioration of >10ml.min.1.73m^2 was *a priori* considered to likely represent a true deterioration in kidney function.

Those with CRP >20 mg/l were felt to have subclinical infection that may independently increase NGAL concentrations – and these subjects were excluded from analysis.

Statistical Analysis

Descriptive statistical analysis was performed; including distribution of gender and age-group. Differences between groups were explored using t-test, or Mann-Whitney U-test (MWUT) depending and correlations sought using Pearson or Spearman correlation tests dependent upon variable distributions. Data were analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Statistical significance was defined as a p-value of less than 0.05.

9.3.2.1. Ethical Approval

The study was approved by a Health Research Authority South East – Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from parents/carers with parental responsibility. Informed assent was additionally obtained from those children/young people as participants in the study if age appropriate.

9.3.3. Results

Forty-two children with PDCM CKD had uNGAL suitable for analysis. Two extreme outliers were excluded from subsequent analysis, with associated elevated CRP (>20mg/l) and felt to represent subclinical infection.

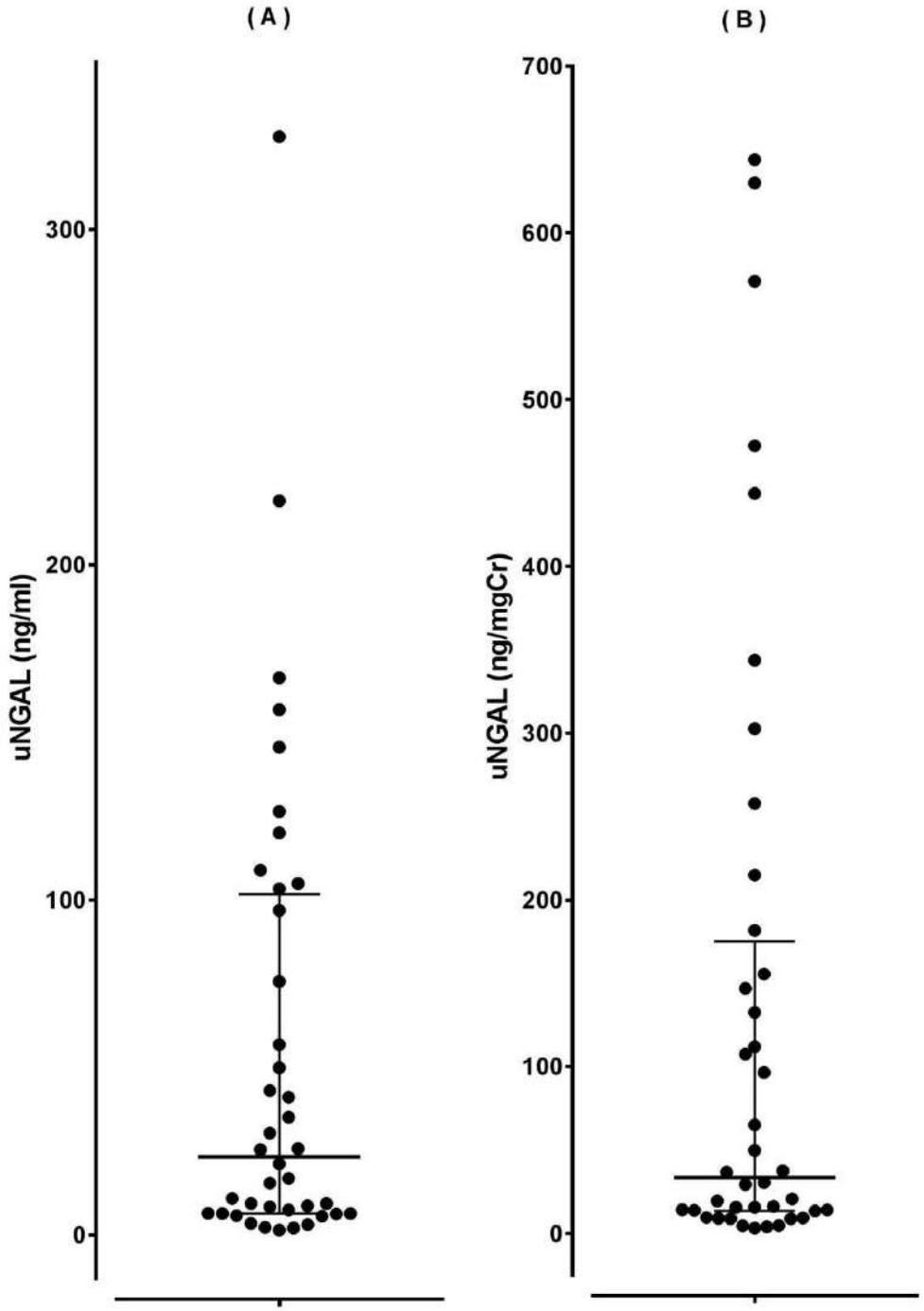


Figure 51. Distribution of uNGAL in the PDCM group (n=40).

uNGAL concentrations (A), and creatinine-adjusted uNGAL concentrations (B) for the Pre-dialysis, conservatively-managed group (PDCM). Median and interquartile range are displayed. The distribution of concentrations present as two groups: those with uNGAL concentrations below the 97.5th centile for age as defined by Rybi- Szuminśka et al (128), and distribution of varyingly elevated uNGAL concentrations. Abbreviations: Cr – creatinine; uNGAL – urinary Neutrophil gelatinase-associated lipocalin.

Elevated concentrations

Of 40 samples, 23 samples (57.5%) had concentrations of creatinine-adjusted uNGAL (ng/mlCr) greater than Rybi-Szumińska et al's 97.5th centile for age (128); 13 (32.5%) had uNGAL concentrations (ng/ml) greater than the Bennett et al's 95th percentile for age and sex (129). 13 (32.5%) had elevated uNGAL as defined by both criteria. These were the same children identified by Rybi-Szumińska et al's 97.5th centile for age alone, and Chi-square analysis for agreement between the two criteria demonstrated good agreement (χ^2=14.235, p<0.0005).

Difference between those with normal and elevated creatinine-adjusted uNGAL concentrations

Comparison was made between those with creatinine-adjusted uNGAL above and below the 97.5th percentile proposed by Rybi-Szumińska et al. Selected variables evaluating differences between the two groups are listed in *Table 40*, below. The PDCM group's uNGAL measurements were additionally stratified into tertiles: 33.3rd percentile = 15.2242ng/mgCr; 66.6th percentile = 118.9089ng/mgCr. Comparison was made between the lowest and highest tertiles (see *Table 41*, below).

Table 40. Differences between normal and elevated creatinine-adjusted uNGAL subgroups in the PDCM group (n=40).

Variable	Normal creatinine-adjusted uNGAL Mean (±SD)	Elevated creatinine-adjusted uNGAL Mean (±SD)	P=value
Age (years)	9.47 (±4.14)	12.31 (±3.32)	0.038*
Time from diagnosis (months)	90.33 (±55.71)	99.85 (±52.44)	0.609
eGFR (ml/min/1.73m^2)	64.91 (±22.85)	45.34 (± 27.71)	0.015*
uPCR (mg/mmol)‡	16.5 (±7.25)	56.0 (±100.0)	0.003*
Plasma Selenium (µmol/l)	1.15 (±0.15)	1.00 (±0.17)	0.016*
C-reactive protein (mg/l) ‡	0 (±1)	1 (±4)	0.159
Serum albumin (g/l) ‡	40.5 (±5.75)	40.0 (±6.5)	0.219

‡ - non-parametric data, median and interquartile range reported, and comparison made by Mann-Whitney U-test. Parametric data was compared using independent t-test. - statistically significant difference between the subgroups. Abbreviations: df – degrees of freedom; eGFR – estimated glomerular filtration rate; PDCM – pre-dialysis, conservatively-managed; SD – Standard deviation; uNGAL – urinary neutrophil gelatinase-associated lipocalin; uPCR – urinary protein-creatinine ratio.*

Table 41. Differences between lowest and highest tertiles for creatinine-adjusted uNGAL in the PDCM group (n=40).

Variable	Lowest creatinine-adjusted uNGAL Tertile Mean (±SD)	Highest creatinine-adjusted uNGAL Tertile Mean (±SD)	P-value
Age (years)	9.29 (±3.49)	10.58 (±3.96)	0.386
Sex (% female)	15.4	76.9	-
Time since diagnosis (months)	91.62 (±54.94)	90.69 (±51.44)	0.965
eGFR(ml/min/1.73m^2)	68.14 (±22.76)	44.73 (±22.88)	0.015*
uPCR (mg/mmol) ‡	17.5 (±21.5)	77.0 (±170.5)	0.002*
Number of medications	3.15 (±1.86)	5.15 (±2.41)	0.026*
C-reactive protein (mg/l) ‡	0 (±1)	2 (±5)	0.035*
Serum albumin (g/l) ‡	40 (±7)	38 (±4.5)	0.186
Height SDS ‡	-0.68 (±2.19)	-1.09 (±2.22)	0.960
Weight SDS	-0.59 (±1.75)	-0.29 (±2.18)	0.704
BMI SDS	0.19 (±1.51)	0.52 (±1.65)	0.602
MUAC SDS	0.35 (±0.85)	0.76 (±1.14)	0.317
Height velocity SDS	-1.31 (±2.95)	-1.60 (±2.91)	0.960
Waist-to-height ratio ‡	0.49 (±0.08)	0.50 (±0.09)	0.960
Plasma Vitamin A (µmol/l) ‡	2.00 (±0.60)	2.60 (±1.00)	0.026*
Plasma PLP (vitamin B6) (nmol/l) ‡	86.60 (±22.40)	78.80 (±38.75)	0.119
Plasma Folate (ng/ml)	12.90 (±14.40)	10.90 (±10.60)	0.478
Plasma Vitamin B12 (ng/l) ‡	514 (±358)	345 (±220)	0.035*
Plasma Vitamin C (µmol/l) ‡	79.80 (±27.7)	83.8 (±41.3)	0.494
Plasma vitamin D (nmol/l)	108.64 (±66.43)	102.08 (±48.87)	0.783
Plasma Vitamin E (µmol/l) ‡	29.3 (±9.60)	29.5 (±10.20)	0.459
Plasma Copper (µmol/l)‡	21.0 (±7.3)	19.9 (±3.7)	0.955
Whole Blood Manganese (nmol/l) ‡	157.50 (±60.25)	130.50 (±144.75)	0.633
Plasma Selenium (µmol/l)	1.17 (±0.16)	1.02 (±0.18)	0.049*
Plasma Zinc (µmol/l)	14.43 (±2.25)	12.85 (±2.18)	0.095

‡ - non-parametric data, median and interquartile range reported, and comparison made by Mann-Whitney U-test. Parametric data was compared using independent t-test. - statistically significant difference between the subgroups. Abbreviations: eGFR – estimated glomerular filtration rate; PDCM – pre-dialysis, conservatively-managed; PLP = pyridoxal-5-phosphate; SD – Standard deviation;SDS – standardized deviation score; uNGAL – urinary neutrophil gelatinase-associated lipocalin; uPCR – urinary protein-creatinine ratio.*

9.3.3.1. Association with disease severity

Creatinine-adjusted uNGAL was higher in those with more severe disease as defined by eGFR, uPCR, and number of medications (see *Table 22*, above), with significant linear correlations (eGFR: S.rho = -0.430, p = 0.006; uPCR: S.rho = 0.551, p < 0.0005; number of medications: S.rho = 0.378, p = 0.016).

9.3.3.2. Association with inflammation

CRP was significantly higher in those with higher creatinine-adjusted uNGAL but did not demonstrate a significant linear correlation (S.rho = 0.287, p = 0.089). Plasma albumin, which is suppressed in inflammation, was not associated with creatinine-adjusted uNGAL.

9.3.3.3. Association with anthropometry

Although there was a trend of children with higher uNGAL to be shorter, have slower growth velocity, and be heavier-for-height, none of the variables were significantly different. There was no difference between those with poor growth (HtSDS<-2) and those with adequate growth (HtSDS>-2) (MWU t=161; p=0.503).

9.3.3.4. Nutritional Status: Height Velocity at Baseline

Mean height velocity SDS at baseline was -0.68 (SD±2.85). The distribution of the HtVelSDS is depicted in *Figure 52*. The cohort was divided into three groups: HtVelSDS>2 (rapid height gain), 2≥HtVelSDS≥-2 (normal height gain), and HtVelSDS<-2 (poor height gain). There was no difference in creatinine-adjusted uNGAL between the three groups (Kruskal-Wallis test = 0.734, p = 0.693).

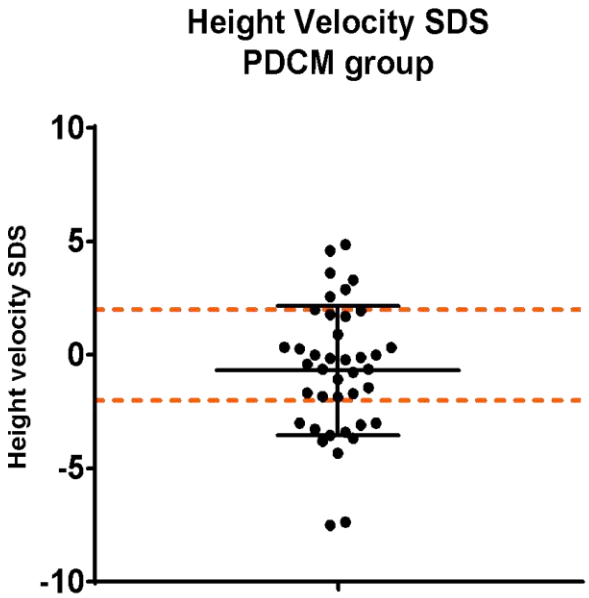


Figure 52. Height velocity SDS at baseline in the PDCM group (n=42).

Height velocity standardised deviation score(SDS) at baseline in the pre-dialysis, conservatively-managed CKD group. Most children had height velocities within -2 and +2 SDS (shown by the orange dotted lines), although a significant proportion had growth significantly lower. A small number had height velocities higher than + 2SDS. Mean and SD for the group are represented by the black line and error bars. There is significant variation in height velocity, some of this variation may be a result of delay of the pubertal growth spurt observed in some with chronic kidney disease, this may result in children both lower then if followed longitudinally higher than their healthy peers.

9.3.3.5. Association with micronutrient concentrations

Micronutrient concentrations were examined for association with higher creatinine-adjusted uNGAL (see *Table 41*). Vitamin A was higher in those with higher creatinine-adjusted uNGAL concentrations, and plasma selenium and vitamin B12 were lower. Vitamin A concentrations demonstrated a positive linear relationship to creatinine-adjusted uNGAL (S.rho = 0.403, p = 0.013) and plasma selenium a negative linear relationship (S.rho = -0.327, p = 0.048). Spearman's correlation test was not significant for vitamin B12 concentrations (S.rho = -0.293, p = 0.083). To explore the potential for plasma selenium to be suppressed by the presence of inflammation, there

was no correlation between CRP and plasma selenium concentrations (S.rho = -0.109, p = 0.527). Due to the higher representations of females in the higher upper tertile for creatinine-adjusted uNGAL, differences in plasma selenium concentrations and plasma vitamin B12 were explored, with no difference found between males and females (Plasma selenium (µmol/l): males = 1.07 SD± 0.19, females = 1.05 SD± 0.17; t(35) = 0.294, p = 0.771. Vitamin B12 (pmol/l): males = 518 SD± 256, females = 381 SD± 226; t(34) = 1.603 p = 0.118).

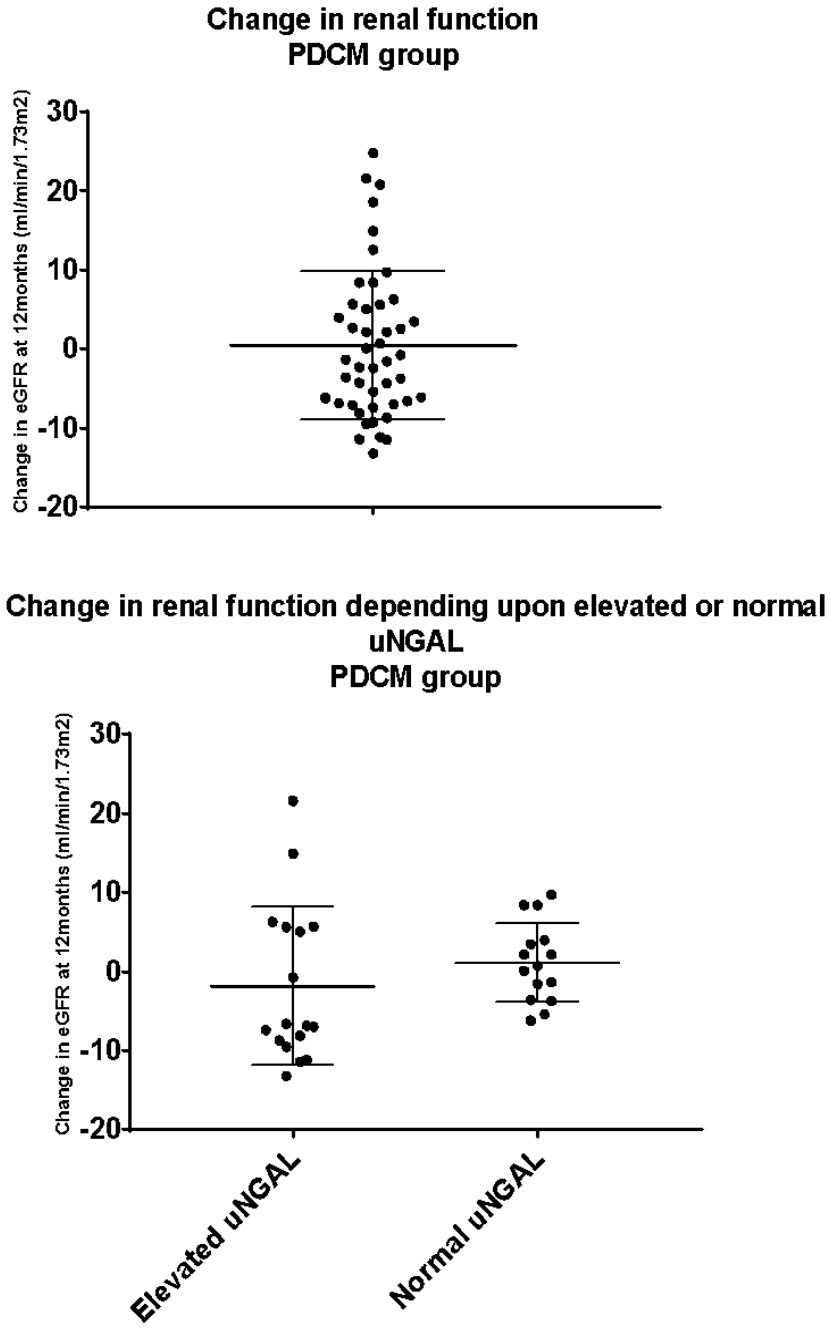


Figure 53. Change in kidney function at 12 months for the PDCM group, and subgroups by elevated (n=17) or normal uNGAL (n=15).

Change in kidney function at 12 months as determined by change in estimated glomerular filtration rate. There was a wide range of values, and no difference depending upon whether children had creatinine-adjusted uNGAL greater than the 97.5th centile for age (lower graph). Abbreviations: eGFR – estimated glomerular filtration rate; PDCM – pre-dialysis, conservatively-managed; uNGAL – neutrophil gelatinase-associated lipocalin.

Within the group, 32 had paired height and serum creatinine measurements at 12 months following baseline measurements available allowing for the calculation of eGFR at 12 months. There was a large variation in the change in eGFR at 12 months (mean change = -0.43 SD±8.11; range: -13.2 to +21.6 ml/min/1.73m^2), see *Figure 53*. There was no pattern of creatinine-adjusted uNGAL when categorised eGFR at 12 months as worse (eGFR >10ml/min/1.73m^2 decline at 12 months), unchanged (eGFR within ± 10ml/min/1.73m^2) and improvement in eGFR (eGFR >10ml/min/1.73m^2 increase at 12 months), see *Figure 54*, below.

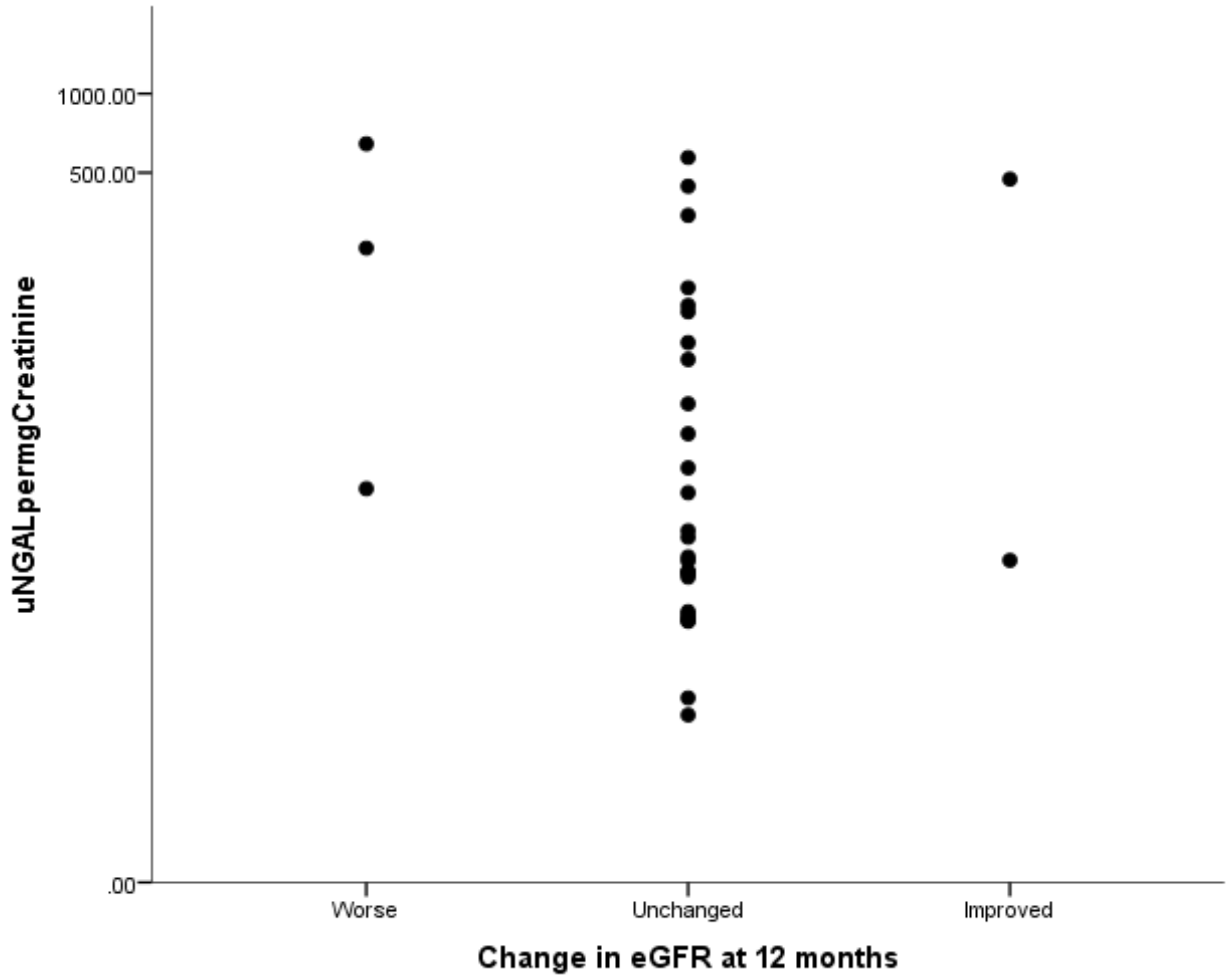


Figure 54. Urinary NGAL concentrations at baseline and change in eGFR at 12 months as categorised as worse, unchanged, or improved (n=32).

Three children (9.4%) had a deterioration of eGFR of >10 ml/min/1.73m^2 at 12 months. Using ROC curve analysis, the ability of creatinine-adjusted uNGAL to predict this deterioration was evaluated and compared to other variables associated with disease progression. The results of this analysis are displayed in *Figure 55*, below. Creatinine-adjusted uNGAL performed better than baseline eGFR, CRP and uPCR in the prediction of a decline in kidney function as assessed by estimated glomerular filtration rate at 12 months from baseline measurement with area under-the-curve (AUC) of 0.816 (95% CI: 0.596-1.000) compared to: Baseline eGFR = 0.425 (95% CI: 0.056-0.794); CRP = 0.453 (95% CI: 0.091-0.816); uPCR = 0.762 (95% CI: 0.468-1.000), although confidence intervals were large. Youden's index analysis demonstrated that cut-off values were:

Creatinine-adjusted uNGAL = 24.87 ng/mgCr (sensitivity = 66.7% and specificity = 83.3%); uPCR = 139 mg/mmol (sensitivity = 66.7% and specificity = 83.3%); and CRP = 2.5 mg/L (sensitivity = 33.3% and specificity = 83.3%)..

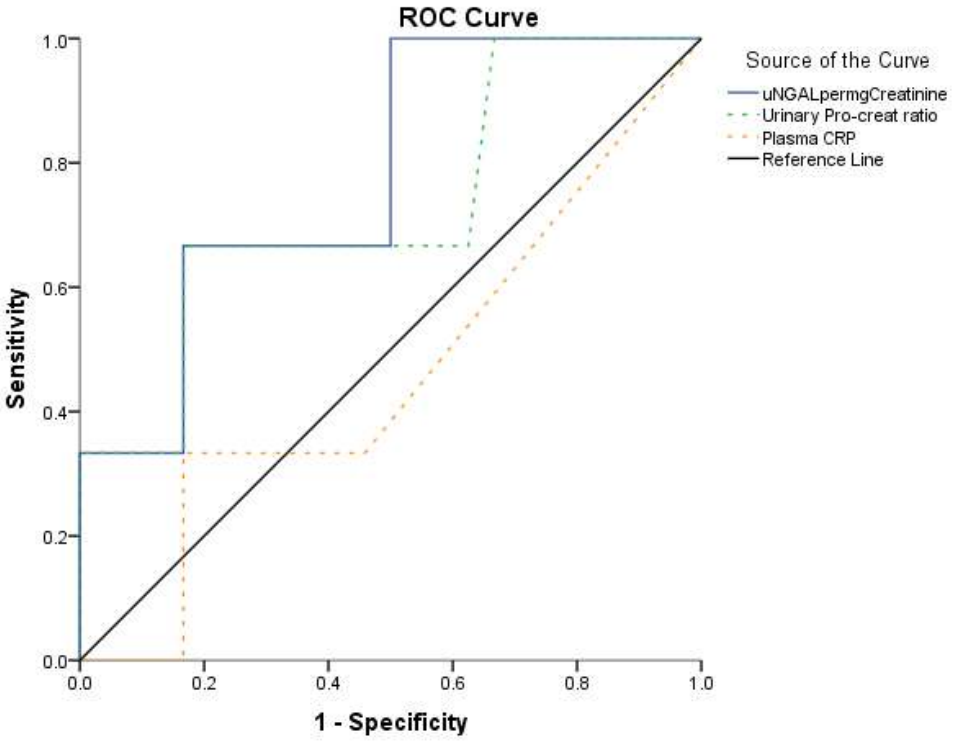


Figure 55. ROC curve analysis for the prediction of a deterioration of kidney function of more than 10ml/min/1.73m^2 in the PDCM group (n=32).

Creatinine-adjusted uNGAL performed better than c-reactive protein (CRP) and urinary protein-to-creatinine ratio (uPCR) in the prediction of a decline in kidney function as assessed by estimated glomerular filtration rate at 12 months from baseline measurement. Area under the curves (95% confidence intervals) for test variables were: creatinine-adjusted uNGAL= 0.816 (0.596-1.000); baseline eGFR = 0.425 (0.056-0.794); CRP = 0.453 (0.091-0.816); uPCR = 0.762 (0.468-1.000). Abbreviations: CRP – C-reactive protein; uNGAL – urinary neutrophil gelatinase-associated lipocalin.

9.3.3.7. Prediction of clinical outcomes: deterioration in growth

In addition to evaluating uNGAL in those with poor growth as assessed by HtSDS and HtVelSDS at baseline, follow-up height measurement at 12 months was also assessed. From the PDCM group, 35 had height measurements available for analysis at 12 months with paired baseline uNGAL. The median change in height SDS at 12 months was 0.08 (IQR±0.25). Prediction for a deterioration in HtSDS of >0.2 (n=5) was assessed using ROC curve analysis. Creatinine-adjusted uNGAL was unable to predict deterioration in growth at this level (AUC = 0.481; CI: 0.181 – 0.781). It was also unable to predict improvement in HtSDS of >0.2 SDS (AUC = 0.563, CI: 0.354 – 0.773).

9.3.4. Discussion

There is evidence to suggest that NGAL may play an important role in kidney injury and repair. In this study, uNGAL was found to be elevated in the PDCM group, and a better predictor of kidney disease progression at 12 months than other readily accessible markers that have been associated with disease progression (albeit with large confidence intervals). Although there was a trend of children with higher uNGAL to be shorter, have slower growth velocity, and be heavier-for-height, none of the variables were significantly different.

uNGAL is an attractive biomarker to explore clinical utility as it is readily accessible, and with promising application in the detection of acute kidney injury (322) may become increasingly available in clinical practice.

Limitations of using uNGAL as a biomarker of disease activity or of nutritional status include the large number of variables that have influence over uNGAL concentrations; including acute infection (323). Additionally, quoted normal reference ranges of uNGAL are based on small cohorts, especially so when these cohorts are divided into age-specific groups. Therefore, the reference standard to which uNGAL concentrations are compared needs more study to ensure its validity.

9.3.4.1. Elevated concentrations.

Those with elevated concentrations were older than those with normal levels. This is despite the using age-specific 97.5th percentile proposed by Rybi-Szuminśka et al as the cut-off for elevation. The explanation of this may be that the reference quoted by Rybi-Szuminśka et al does not truly reflect the normal range of individuals. Although, except for the very young, others have found little variation depending upon age. The length of disease did not appear to be the cause of this as time since diagnosis was not different between the groups. Alternative explanations include, older children with CKD may well have increased disease activity. During puberty, it is commonly observed that there is deterioration in kidney function (324), and older children are more likely to be pubertal. The pubertal status of the child may also increase uNGAL concentrations independent of disease activity and kidney function.

9.3.4.2. Comparing those with lower and those with higher creatinine-adjusted uNGAL

Being in the higher tertile was associated with being female and this is consistent with the previously reported data of healthy children and young people who report higher values in females compared to males (128, 129). Age and duration of disease (time since diagnosis) were not associated factors.

9.3.4.3. Disease severity

Those with higher creatinine-adjusted uNGAL (highest tertile) had more severe disease (eGFR, uPCR, and number of medications). This is in agreement with previous studies (123).

9.3.4.4. uNGAL and Nutritional Status: Markers of Adiposity and growth

Although a trend for high creatinine-adjusted uNGAL to be associated with shorter, heavier-for-height with poor growth, no statistical differences were found. It might be expected that those with the greatest disease activity would grow the poorest, and those with quiescent disease grow better, although poorer kidney function will also impact on growth.

Urinary NGAL has been reported to be higher in adult CKD (creatinine clearance >25ml/min/1.73m^2) patients who were overweight/obese compared to their normally-weighted counterparts (325). These patients had greater

protein and sodium chloride intake. Although the NGAL difference may be adiposity-related inflammation-derived, dietary patterns may lead to an increased likelihood of metabolic acidosis in CKD that has a negative impact on kidney function and disease progression.

Obesity has been associated with the progression of kidney disease (72, 73) and with systemic inflammation (326). Bennett et al (129) reported that in healthy children and young people NGAL concentrations did not correlate with Ht SDS, Wt SDS or BMI SDS, in-keeping in this cohort.

It is perhaps noteworthy that although not statistically significant, the relationship between MUAC and creatinine-adjusted uNGAL seems opposite to that of RDW and MUAC (*Chapter 9.2*), with a trend of increasing MUAC with higher creatinine-adjusted uNGAL. This might be explained by the uNGAL being more representative of kidney pathology than systemic inflammation. Body composition analysis is warranted to explore the relationship between loss of lean muscle, the presence of sarcopenia and putative markers of disease activity, including NGAL.

9.3.4.5. uNGAL and Nutritional Status: Micronutrient Status at Baseline

On examination of creatinine-adjusted uNGAL by tertile and comparing those in the highest versus the lowest, vitamin A concentrations were higher in those with higher creatinine-adjusted uNGAL, and selenium and vitamin B12 concentrations were lower. There was a linear relationship with vitamin A and, as vitamin A concentration increases in a dose-dependent manner with decreasing kidney function (261) (as demonstrated and discussed in *Chapter 5*), this relationship is likely due to collinearity. The difference in the percentage of females in the lowest and highest tertiles of creatinine-adjusted uNGAL can not explain this alone as there was no difference in selenium and vitamin B12 concentration between males and females, although data from the national diet and nutrition survey has reported that females tend to have lower dietary intake of selenium with almost twice as many girls aged 11 to 18 years receiving less than the lower reference nutrient intake (LRNI) for selenium, and more likely to have low serum vitamin B12 concentration compared to their male counterparts (321).

Selenium

Plasma selenium is often lowered in inflammatory states as part of the acute phase response (327), and as uNGAL increases with inflammation, their association may be in relation to inflammation rather than to each other. Although there was no linear correlation between plasma selenium and CRP in this analysis. CRP has limitations in the characterisation of inflammation, but there remains the possibility of an independent association between selenium status and disease activity.

As discussed in *Chapters 4* and 5, selenium has many roles within selenoproteins, including within glutathione peroxidase and control of oxidative stress. Although all except one child of the PDCM group had plasma selenium concentrations within normal reference range (mean = 1.04 SD± 0.2, range 0.47 to 1.46 µmol/l), there is debate regarding whether this range truly reflects optimal concentrations, as its derivation is based on the absence of frank disease in otherwise healthy individuals, and does not correspond to maximal activity of selenoproteins. In those with chronic disease, exposed to increased oxidative stress, demand for selenium may be increased still further. The association of decreased plasma selenium concentrations and increased disease activity may be the result of increased demand of selenium-dependent processes, or that the limitation of

selenium through inadequate dietary intake of selenium has a negative impact upon disease activity in those with CKD.

The UK are at increased risk of inadequate selenium intake compared to other populations, as the amount of selenium that foods contain is highly dependent upon the conditions in which it is raised; the selenium content of the soil. This varies greatly depending upon geographical region. Selenium content of the UK's wheat has decreased from the 1970's and is associated with sourcing of wheat from Europe rather than North America. Correlating with this, the nation's selenium intake has decreased (321).

Further research is needed to determine whether selenium requirements differ in children with CKD from that of the general population. Initial study should be focused on the activity of selenoproteins with varying plasma selenium concentrations, and intake of selenium. Whether supplementation with selenium would have disease modifying action warrants careful evaluation. In contrast to other vitamins and minerals, selenium has a relatively small therapeutic window prior to toxic effects may be observed (47). The increased mortality observed in those with supplemental vitamin E (205, 264) serves as an example of a need for greater understanding of the metabolism of nutrients prior to advocating increase intakes, although it may be reasonable, if subsequent data provides mechanisms suggestive of benefit, to develop studies that increase dietary intakes to similar to usual intakes in countries where selenium intake is higher, including in the Finland, where bio-fortification of selenium crops has resulted in increased population intake inline with the USA (328).

Vitamin B12

Serum vitamin B12 concentrations were lower in those with higher creatinine-adjusted uNGAL. The reason for this is unclear.

Girls had lower vitamin B12 concentrations compared to boys, although dietary intake for vitamin B12 in the TEMPeReD cohort was not reported as lower in girls (see *Chapter 5*). NDNS data show a higher prevalence of holotranscobalamin (the vitamin B12 form that can enter cells) below 32pmol/l (the concentrations associated with evidence of vitamin B12 deficiency) in girls compared to boys in those aged 11-18 years (321). One explanation could be that lower vitamin B12 status is associated with higher disease activity (greater uNGAL concentrations), and due to the lower vitamin B12 status in girls, disease activity is greater in girls compared to boys. With increased tubular protein loss signified by higher uNGAL, there may be increased B12 loss via the urine.

At physiological concentrations, protein-bound vitamin B12 filtered by the glomerulus exceeds the daily intake (329). Therefore, if there is an inability to reabsorb this vitamin, additional losses may result in increased risk of inadequacy. Indeed, children with Donnai-Barrow Syndrome caused by a mutation in the megalin-encoding gene *LRP2*, exhibit urinary low-molecular protein loss, and are at risk from vitamin B12 deficiency due to this low molecular weight protein loss. Moreover, there is evidence to suggest that it is not only the liver that has stores of vitamin B12, but that the kidney may act as a store too (329). If so, the ability of the kidney in the context of CKD of different aetiologies may play a part in altering vitamin B12 metabolism and status.

Previous literature has demonstrated an association of higher serum vitamin B12 concentrations in children with kidney impairment (186, 330, 331) – although these studies were in dialysis patients, and in receipt of

supplementation in two cohorts as part of routine care (186, 331). Without this supplementation, vitamin B12 status is likely to be low with increased loss through dialysis (332). In those with pre-dialysis, conservatively-managed CKD, B12 status has been reported as no different from healthy controls and within the normal reference range (333, 334). In this cohort, there was a positive correlation between serum vitamin B12 concentrations and eGFR (S.rho = 0.351, p = 0.036), i.e., those with more severe disease have a lower circulating vitamin B12 pool. This is contrary to previous literature (186, 330, 331) but may be due to the greater impact of dietary intake of vitamin B12 compared to adults with kidney impairment. Vitamin B12 metabolism in the context of CKD is complicated, and not fully understood.

In those without kidney disease, vitamin B12 concentrations are associated homocysteine concentrations due to their interaction in the methylation-demethylation pathways (see *Figure 56*, below) with low vitamin B12 concentrations being associated with elevated homocysteine concentrations (a risk factor for the development of cardiovascular disease (335)). In kidney impairment, homocysteine concentrations increase with decreasing kidney function independent of vitamin B12 status and total homocysteine concentrations have been reported in a meta-analysis as a risk factor for cardiovascular events and total mortality in patients with end-stage kidney disease not receiving vitamin supplementation or folic acid food fortification (336). Thus, this metabolism is disturbed in some way, although different studies have reported benefit, no benefit and harm with B-vitamin supplementation (337).

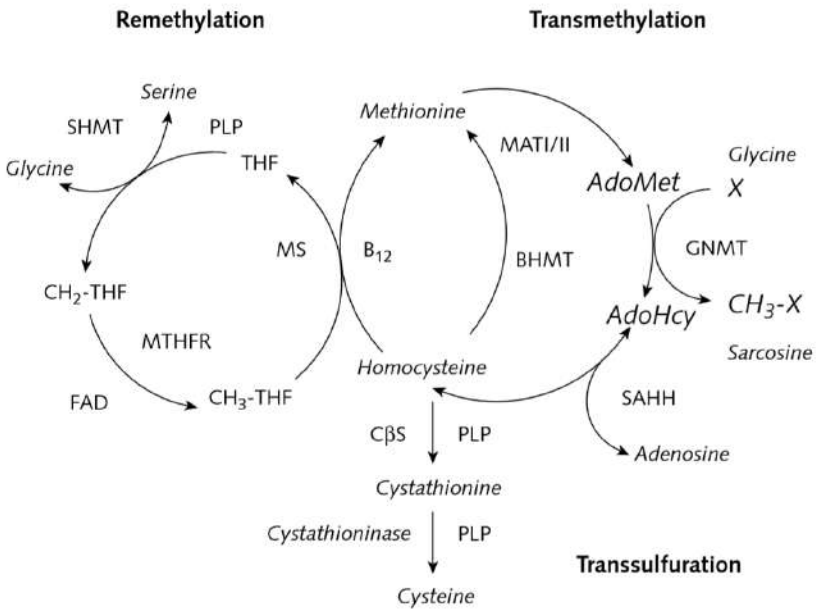


Figure 56. Methionine synthesis and transmethylation cycles and transsulfuration pathway.

Adapted from Shane, 2008 (338). Abbreviation: THF, tetrahydrofolate; FAD, flavin adenine dinucleotide; PLP, pyridoxal phosphate; B12, vitamin B12; AdoMet, adenosylmethionine; AdoHcy, adenosylhomocysteine; SHMT, serine hydroxymethyltransferase; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; MATI/II, methionine adenosyltransferase I and II; SAHH, AdoHcy hydrolase; CβS, cystathionine β-synthase; BHMT, betaine homocysteine methyltransmethylase; glycine NMT, N-methyl transferase).

Güneş et al (339) reported that in 12 otherwise healthy children with vitamin B12 deficiency, creatinine-adjusted uNGAL was higher than healthy controls. The authors suggest vitamin B12 deficiency causes kidney injury, as other markers of kidney injury were also elevated. An alternative explanation is that vitamin B12 availability has influence over NGAL concentrations either through modification of the disease process and / or expression of NGAL. Güneş et al's elevated creatinine-adjusted uNGAL concentrations were modest compared to the data presented here (19.5ng/mgCr, compared to 3.9ng/mgCr in the health controls), additionally, these children had an average haemoglobin below 120g/l, and therefore any kidney injury may be due to relative kidney hypoxia from anaemia rather than the direct effect of limitation of vitamin B12.

Unfortunately, this study did not measure homocysteine concentrations that may allow for deeper exploration of this area of metabolism. Future research should aim to understand mechanisms that may explain the possible interaction between these pathways, and kidney injury and repair.

9.3.4.6. uNGAL and Disease Progression

It was hypothesized that those with elevated creatinine-adjusted uNGAL would have faster deterioration in kidney function. In this PDCM group, uNGAL performed marginally better than the traditional marker of urinary protein-to-creatinine ratio in identifying those with a decline in eGFR >10ml/min/1.73m^2. The limitations of eGFR as a marker of disease progression may make a type 1 error more likely with significant variation. Additionally, follow-up of the study was only 12 months, and this might not be long enough to appreciate the deterioration in kidney function. The use of formal GFR (rather than estimated GFR) measurement in a larger cohort over a longer time period may be of benefit to confirm or refute findings from this cohort.

9.3.4.7. Limitations

There are unfortunately many factors that may increase uNGAL independent of kidney injury/repair that give uNGAL limitations. Poor growth was not explained by disease activity as assessed by uNGAL, although limitation of cohort size may be the reasons for this. It is also important to discuss that ROC curve analysis has a systemic bias to overestimate statistical significance in small sample sizes.

9.3.4.8. Future Directions

There is a suggestion that selenium availability may be important, and this warrants further investigation. More research is needed to understand how uNGAL may be used in clinical decision-making both as a marker of disease activity and nutritional status.

9.3.4.9. Concluding Remarks

uNGAL continues to show promise as a marker of disease activity and may represent a better predictor of kidney progression than current clinical used measurements.

9.3.4.10. Practice Points

- uNGAL may be superior to the urinary protein-to-creatinine ratio in predicting disease progression, but more research is needed to confirm these findings and understand the underlying mechanisms.

10. FINAL DISCUSSION

Within this thesis elements of nutritional status, well-being and disease activity of children and young people with CKD are explored.

- What variation in nutritional status exists in those children and young people with CKD?
- What variation exists in the outcomes in those children and young people with CKD?
- What variation exists in the disease activity in those children and young people with CKD?
- How does the variation in these above concepts relate to disease severity; is there variation within these that are not fully explained by the variation in disease severity?
- What are the markers of nutritional status associated with poor growth as defined by HtSDS<-2 and/or HtVelSDS<-2 in children and young people with CKD?
- What associations are there between well-being and nutritional status and children and young people with CKD?

The aim of this thesis was to explore the nutritional status of children and young people with chronic kidney disease (CKD), and the current clinical approach to nutrition within the UK. The hypothesis of this thesis was that those with children with CKD with poorer growth would have poorer health-related quality of life, nutritional status, and elevated levels of disease activity.

Poor nutritional status is associated with poorer outcomes; including increased mortality [4-7], and it makes sense that the availability of energy and nutrients have influence on the cellular processes that take place, many of which only occur with key nutrients, such as minerals. Despite this knowledge, it is unknown what the best nutritional management for children with CKD is.

This thesis takes the view of the paediatric nephrologist in the nutritional care of children with CKD. The ability of current practice of nutritional assessment to characterise children is evaluated, with identification of gaps and opportunities in the understanding of nutritional care. This led to the development, and trialing of measures that may improve such characterisation and aid clinical decision-making.

Firstly, a feasibility study describing anthropometry across all ages and stages of CKD was conducted and provided novel prevalence data in the UK, using the routine collection of clinical data via hospital information technology systems.

Secondly, a review of the literature of vitamin and mineral status in children and young people with CKD was conducted. Within which a structure with which to review the literature was developed and employed.

Thirdly, a traditional nutritional management approach was adopted through the assessment of risk of nutritional inadequacy by measuring dietary intake, anthropometry, and blood concentrations of vitamins and minerals in a cohort study.

Fourthly, holistic measures were explored using questionnaires in the assessment of appetite and HRQoL. A novel appetite assessment questionnaire was developed and used to explore appetite in the above-described cohort, and a validated questionnaire was used to explore HRQoL.

Fifthly, a time-limited trial of a novel food for special medical purposes was undertaken. Supplying a balanced vitamin and mineral supplement to a subgroup of children and young people at risk from not meeting their nutritional requirements.

Sixthly, the concept of disease activity is explored through the evaluation of putative markers of disease activity, i.e., RDW, uNGAL, and measures of mitochondrial bioenergetics.

The key messages from this thesis are:

- Methods of nutritional assessment currently employed in the clinical environment fail to fully capture the nutritional phenotype of children with CKD. More research is needed to develop markers of nutritional status, especially of specific vitamins and minerals.
- The evidence upon which clinical practice guidelines are based for the dietary requirements for vitamins and minerals for children and young people with CKD is limited and significant research is required before security in this area can be achieved.
- Prevalence of poor appetite in children and young-people with CKD may be greater than predicted, and that this potential under-recognition may be due to a hitherto narrow assessment of poor appetite.
- Well-being, as assessed by a well-research, validated questionnaire assessing health-related quality of life, is lower in those with CKD; even in those with mild-to-moderate disease. This measure of well-being has a relationship with markers of nutritional status and was lower in those with poor growth (HtSDS<-2).
- There was a trend for children and young people in the highest RDW tertile to be shorter, have slower growth, and be lighter-for-height, these differences were not statistically significant.
- Body composition changes may be associated with increased RDW, with lower WtSDS and MUAC SDS associated with greater variation in erythrocyte size.
- In pre-dialysis, conservatively-managed CKD, uNGAL is elevated and a better predictor of kidney disease progression at 12 months than other readily accessible markers. Although there was a trend of children with higher uNGAL to be shorter, have slower growth velocity, and be heavier-for-height, none of the variables were significantly different statistically.

The direction of causation and inter-relationships between these variables need further exploration and study.

10.1. Clinic wide assessment of growth

This feasibility study described weight, height and BMI across all ages and stages of disease, and provided novel prevalence data for children with kidney disease in the UK, internationally and in Southampton Children's Hospital, using the routine collection of clinical data via hospital information technology systems. Data on growth in children with kidney disease is limited and restricted on the whole to those with the most severe disease. The data presented in this chapter show short stature is prevalent (10% of paediatric nephrology clinics) with obesity levels similar to that of the general population. As is well described, this highlights that inadequacies of growth are common in paediatric nephrology. Some children with syndromic disease have lower growth potential, but others do not meet their potential for reasons of disease, its complication, and poor

nutrition. The benefit of covering the entire clinic allowed for a large number of children and young people to be included, but this study did not allow for detailed characterisation of the participants.

The use of electronic data has the potential to identify those children and young-people who are at risk and be highlighted to healthcare professionals. This study may act as a template for the wider healthcare community that may be introduced into outpatient clinical settings to alert healthcare professionals to those with (or at risk of) growth failure or obesity.

This methodology can also be used, to collect longitudinal data to monitor how the clinic as a whole and individuals change overtime. In the future, other clinical data could also be included to facilitate greater clinical information to be available, including other anthropometric data (such as markers of central adiposity) and nutritional interventions (Is the children/young person on a potassium or phosphate restriction?, for example).

10.2. Literature of the vitamin and mineral status of children and young people with CKD

The review of literature on vitamin and mineral status of children and young people with CKD demonstrated the lack of studies examining this important area of clinical care. The studies that exist are focused on those with the most severe disease, are of generally small size and no contemporaneous.

It is important to have clinical guidelines to decrease unwanted variation in clinical care. The guidance with this regard is based on very limited data, and on the whole (with notable exceptions of vitamin A, for example) states to ensure that individuals receive the recommended dietary intake as quoted for healthy individuals in receipt of otherwise adequate intake for energy and other nutrients. This is despite the knowledge that the kidney as an organ of homeostasis and metabolic influence may alter true requirements for an individual.

10.3. TEMPeReD

The TEMPeReD cross-sectional, observational study reported a high prevalence of children at risk from not meeting their nutritional needs through dietary intake. This increased risk was not borne out in the micronutrient blood concentrations in the same population, the vast majority of which were reassuringly normal despite the lack of a formalized, structured approach to nutritional support and supplementation. In a small cohort of these children at risk from nutritional inadequacy, a novel micronutrient supplement was trialed, which was poorly tolerated and did not have a measurable impact on nutritional status.

Elevated plasma vitamin A and vitamin E concentrations were almost universal, even in those with mild-to-moderate disease severity (PDCM). The impact of such elevations is not known.

Anthropometry of the cohort lay mostly within the normal distribution of the general population, but with a tendency for increased weight-for-height, MUAC and waist circumference that may represent adiposity. Despite this, a number of children had abnormalities of growth, and suggest that calorie limitation is not the only factor influencing growth.

10.3.1. Obesity and adiposity in paediatric CKD

Weight SDS, BMI SDS, waist-to-height ratio were all highly correlated – and this is to be expected. Importantly, central obesity has been reported to confer increased risk even in those that have not met the cut-off for obesity

as per BMI-based definition (75, 340). It is also important to propose tools and criteria that are readily accessible and easy to understand. More children were obese as defined by WHtR compared to BMI-criteria in the TEMPeReD cohort. As WHtR is not routinely used in clinical care as a definition of obesity, it may be that obesity (as defined by WHtR) may have a greater prevalence than previously reported – but that these children and young people are truly at increased risk compared to their WHtR <0.5 measured peers.

10.4. Nutritional assessment of children and young people with CKD

Following the exploration of nutritional assessment of children and young people through the TEMPeReD study and exploration of underlying literature, the security of the methods currently employed in the clinical setting is questionable. The current available tools do not offer the ability to make an adequate nutritional diagnosis. This includes limiting anthropometry to height and weight measurement without the ability to characterize body composition. Energy requirements can be accurately measured in the research setting, but in clinical practice even in the most difficult cases of poor growth, clinicians rely on estimated requirements. Dietitians use single 24-hour recall to estimate dietary intake and determine likelihood of nutritional adequacy/inadequacy despite this methodology unlikely to be predictive of actual intake. The reference standards for dietary intake which have been developed for the general population are applied to children and young people with possibly different requirements, but in whom little or known research has been performed to justify this. When assessing the vitamin and mineral status by biological measures, a simplistic approach is taken whereby the blood concentration of the nutrient is measured and compared to a normal reference range. This presupposes that this concentration reflects total body status in this disease population without research to underpin this assumption. The use of functional assays is more likely to represent the patient's needs. Additionally, treating those with high or low concentrations in the transport pool with either dietary advice or supplementation without evidence that this is beneficial to the patient, but risk unintended negative consequences to the patient. In summary, clinicians do not have the tools to adequately assess nutritional status but fool themselves that they do.

10.5. Appetite

In this thesis, a clinical need for a formalised appetite assessment was confirmed through surveys and clinical audit, and appetite assessing questionnaire developed and validated. On using the questionnaire to explore poor appetite in the TEMPeReD cohort, a high prevalence of poor appetite that cannot be fully explained by disease severity alone (25%). Appetite was associated with energy and nutrient intake. Poor appetite was associated with increased BMI, but not linear growth. This lack of association between poor growth and appetite may be due to medical and dietetic interventions employed by the clinical team that were not explored in this cohort, observational study.

10.6. HRQoL

This is the first data of HRQoL as assessed by the PedsQL^{TM} 4.0 in children with pre-dialysis, conservatively-managed CKD within the UK, and explores the association of nutritional status with HRQoL. These data show that HRQoL scores was significantly lower in this cohort; including in the subgroup of pre-dialysis conservatively-managed CKD children compared to healthy control data, with mean score differences greater than the minimal clinically important difference (MCID) score of 4.4 (child self-assessed questionnaire) and 4.5 (parent-proxy questionnaire) (271). The results suggest that, in agreement with existing literature mostly limited

to those with end-stage kidney disease, children and young people with mild-to-moderate CKD have lower HRQoL compared to their peers. The difference scores in the emotional domain of the questionnaire between the child/young person and their care-giver warrants further exploration. It highlights the need to evaluate both parties: the paediatric patient and their caregiver.

Kidney function per se, was not the only influencing factor upon HRQoL, and as previously reported in other disease-groups (108), stunted children reported lower HRQoL scores than their non-stunted counterparts, and was the strongest influence on multiple regression analysis. This highlights the importance of growth as a clinical outcome for healthcare professionals to focus on.

Despite this chapter demonstrating the relationship between well-being (as assessed by HRQoL) and poor growth, the nutritional influences between were not demonstrated.

Level of deprivation also, unsurprisingly, influences well-being and these data suggest the need to approach the child/young person with more than just physiological approach if healthcare professionals are to improve well-being. The emotional domain scores suggest the need for psycho-social support as part of the multidisciplinary team. Although many paediatric nephrology centres have clinical psychologists and social workers available, these are limited and as a result focused on those with the most pressing problems. These data make the case that professional mental health support may be needed for those with mild-moderate disease in addition to those with the most severe disease or overt psychological disruption due to the high prevalence of lower emotional domain scores.

HRQoL assessment should be considered for introduction into routine clinical care for ongoing holistic care of children with chronic illnesses, including CKD, and to facilitate the acquisition of longitudinal data regarding the impact of changes in nutritional status and therapy.

10.7. INTERVENTIONAL STUDY

Although the formulation of this novel food for special medical purposes almost eliminated those children at risk from micronutrient inadequacy through the potential combined diet/supplement delivery of vitamins and minerals, translation into clinical practice was not possible. Moreover, in the small number of children that completed the trial, only folate and selenium blood concentrations were higher at the end compared to baseline. This may be noteworthy, as the selenium intake is recognised to be lower in the UK compared to other countries, and the optimal plasma concentration has been the subject of some debate. There are reasons, as discussed early, that mean that requirements for selenium in CKD may be greater; including potential greater utilisation in those with higher levels of oxidative stress associated with both CKD and dialysis.

The high dropout rate was largely due to the unacceptable nature described by the study population apparently due to poor taste profile and side effects. Different formulations preparations should be developed and trialed, as a gap in the ability to ensure micronutrient supply remains unfilled.

10.8. MARKERS OF DISEASE ACTIVITY

Although disease severity is characterised in clinical practice (primarily through the measurement of GFR), there is no marker of disease activity. It was hypothesized that disease activity variation would be able to account for

the variation in nutritional status and disease progression observed in clinical practice that cannot readily or completely be explained by other markers that have been associated with these measures, and that those with poor growth would have the greatest disease activity.

RDW did not show an association with eGFR or other markers of disease severity, but was correlated with systemic inflammation (CRP). Although there was a trend observed between RDW and poorer growth, this was not statistically significant. It may be that the sample size was not large enough to detect this difference, as there was large variation in height velocity SDS.

RDW was negatively correlated with WtSDS and MUAC SDS suggesting a possible relationship between acute nutritional state and RDW. MUAC SDS may be more sensitive to changes in lean body mass, specifically muscle mass and the wasting that accompanies CKD (318). Body composition analysis, such as bioelectrical impedance analysis, may be able to explore this possible relationship in more depth.

Rather than a systemic marker (such as RDW), a kidney specific-marker was thought more likely to represent a marker of CKD disease activity, and therefore uNGAL was explored. uNGAL was found to be elevated in the PDCM group, and a better predictor of kidney disease progression at 12 months than other readily accessible markers that have been associated with disease progression (albeit with large confidence intervals). Although there was a trend of children with higher uNGAL to be shorter, have slower growth velocity, and be heavier-for-height, none of the variables were significantly different.

10.9. Digital technology in the National Health Service

One thread of the experiments described in the thesis is the potential for technology and digital systems to be used to help fulfil the NHS long-term plan (341). Using digital systems to identify those with anthropometric measures placing them at risk from growth failure or obesity, and the use of patient-led measures could increase efficiency in the system. Firstly, these tools could automatically highlight patients to be evaluated by paediatric kidney disease dietitians for nutritional assessment if not already earmarked to do so. Secondly, clinical data hitherto collected at consultation could be already collected and analysed prior to the patients' arrival in clinic, allowing increased time for discussion and motivational interviewing, for example. Digital options should be developed to improve patient experience and provide patient choice. In addition, technology may enable increased efficiency resulting in cost-savings both short-term with more patients evaluated by healthcare teams, and may allow for increased healthcare-patient-family interactions to allow for more oversight of concordance with nutritional prescription, for example.

In addition to growth which has been the focus of this thesis, obesity is a significant problem in the UK. It has been highlighted in the NHS Long-term plan as a priority to take action on childhood obesity within the next ten years (341). The use of digital technologies within the clinical environment both in paediatric nephrology and throughout the healthcare system should be targeted to highlight children at risk and facilitate intervention.

Example of digital technology methods to decrease prevalence of childhood obesity in the NHS:

- Child attends paediatric / primary care clinic appointment;
- Anthropometric data is collected and compared to growth chart standards;
- A child whose BMI >2 SDS or whose rate of change suggests rapid weight-for-height gain is identified by the digital technology platform and highlight to the clinical staff;
- Patient is highlighted to paediatric dietetic team and an appointment made;
- Dietary intake and physical activity data is collected from the family via online digital platform;
- These data are digitally analysed and report sent to dietitian;
- Face-to-face appointment with healthcare team to explore results / health prescription put in place / motivational interviewing;
- Ongoing mix of distance (digital) assessment and face-to-face follow-up to enable life-style change etc..

Figure 57. Potential example of how digital technology can result in new clinical pathways.

10.10. Challenges to the Multidisciplinary Team.

Providing adequate nutrition is an essential element of clinical care, and recognised in clinical practice documents for all NHS service users; specific paediatric service documents; and in international, national and local paediatric nephrology guidance. Despite this, there remains significant challenges to the delivery of optimal nutritional care to children and young people with CKD.

Challenges to providing optimal nutritional care include:

1. Acquisition of data; including accurate and regular recording growth data.
2. Interpretation of these data - Identification of those with or at risk from nutritional inadequacy. There is no formalized process by which those at risk are identified. Onus is placed upon individual clinicians to identify those in need of a nutritional assessment.
3. A lack of validated, practical tools by which to characterise the intake of children and young people with chronic kidney disease. Current methods are labour-intensive for both the family and healthcare professionals, and may not reflect true intakes especially for infrequently taken vitamins and minerals.
4. Establishing clear outcome goals for micronutrient requirements – current guidance of dietary intake values have been developed for otherwise healthy individuals not those with varying degrees of renal impairment or in receipt of renal replacement therapy;
5. Identifying and understanding the factors that may affect micronutrient requirements and intake in the setting of chronic kidney disease in individual patients;
6. Accurately measuring and interpreting markers of micronutrient status and recognizing the limitations of such measures (many biomarkers may not truly reflect status in the setting of CKD even if taken as a reflection of status in otherwise healthy individuals);
7. Translating and monitoring micronutrient requirements in clinical practice, in the dynamic state.

Although energy needs may be met through diet and supplementation, this does not necessarily result in adequate intake of micronutrients, without which usual metabolic processes are not possible. Achieving dietary

intake of these micronutrients can be challenging in otherwise healthy children (76) and is made even more challenging in the paradigm of chronic kidney disease as discussed above. As a result, diet and nutrition can be a source of frustration for patients, caregivers and healthcare professionals.

It is challenging to draw recommendations for micronutrients intakes and the current dietary reference values upon which guidance is based are derived from presumably healthy individuals not those with chronic kidney disease. There is no data available to derive different dietary reference values for paediatric CKD patients, although it is unlikely that requirements are the same. For example, increased metabolic demands are likely to increase the demands for B-vitamins involved in energy metabolism. True dietary intake requirements depend upon many factors including body stores, previous supplementation, nutritional status, gastrointestinal health and absorption, degree of renal metabolism, and losses through dialysis regimes, which are all dynamic.

A multitude of factors are taken into account in the clinical decision-making regarding estimation of an individual's micronutrient requirement and status. Some of these are explored in the figure below along with important outcomes to be monitored and drive the need for determination of such needs.

10.11. What impact has my PhD had?

- Development of annual review process within the clinical service to include assessment of appetite and health-related quality of life.
- Routine collection of nutritional data on clinical databases; including the nutritional interventions such as dietary restrictions.
- Greater emphasis on fibre intake assessment of children with CKD in the clinical service, with regular assessment as part of the usual clinical care. Development of work to explore the current UK paediatric dietetic management with regards to dietary fibre.
- Further research studies underway to qualitatively explore children and young people and their care-givers perceptions on the nutritional care of CKD.
- Reevaluation of a balanced vitamin and mineral supplement for paediatric CKD patients by an industrial partner – to bring to market a product that is more palatable.

10.12. Limitations

There are many limitations of the experiments used in this thesis. Firstly, given the observational nature of the TEMPeReD study, it could not establish that there was a cause-and-effect relationship between the variables. The interventional study was not designed to be powered to detect changes (with the primary aim to determine acceptability and palatability), but with a high drop-out rate had only a few participants complete the intervention.

There are many influencing factors on growth, and these change overtime. Moreover, HtSDS is a summative statement of factors (including nutritional status) and those factors may have changed despite the child / young person still being short in stature. Height velocity may be a better measure of this but continues to have limitations as a measure of growth in that it takes many months to achieve a measurement. A biological measure that represents the growth signaling that is specific and sensitive to changes to factors that influence growth is required and can be measured to determine the drive for growth.

Although only two participants were in receipt of growth hormone in the TEMPeReD cohort (one in the PDCM subgroup, and one in receipt of dialysis), these were not excluded from the analyses.

There remains opportunity to more greatly characterise each participant. The TEMPeReD study did not examine pubertal status, bone age or developmental status, and nor did it report biochemical measurements of associated hormones. From a nutritional point of view many vitamins and minerals have known enzymes that could have been measured to gain a better understanding of how the enzyme-activity may or may not be different in different nutritional states. Of particular interest may be selenoproteins as discussed above.

Within the thesis, kidney disease function was estimated using eGFR calculated, as per common clinical practice but the modified Schwartz formula (152), but has well-recognised limitations. The main limitation is its potential inaccuracy in the context of altered muscle mass from which creatinine is derived. The gold standard for measuring kidney disease function (GFR) is inulin clearance, but this is highly impractical to use for routine clinical care due to its intensity, invasiveness, and length of procedure, especially in children (342). There is ongoing research to investigate the clinical use of endogenous markers other than creatinine; including cystatin C. Cystatin C-based eGFR equations have been reported to be more reflective of true GFR in several adult clinical studies (343) and in children (344). It is not routinely used in clinical practice and was not available for the TEMPeReD study. Firstly, the cost of its measurement is much greater than that of creatinine (342), but additionally, not all studies in children have demonstrated significant superiority of cystatin C in children (345).

Within the thesis, when conducting multiple comparisons p-values with and without a Bonferroni-correction have been applied. Such correction may help in controlling for type 1 errors, but it may risk losing identification of important changes that warrant further exploration although needed replication in large cohorts. Logically, in paired analyses with the primary hypothesis tested (as in the interventional study changing selenium, magnesium, and copper status), then correction should not be needed. Bonferroni-correction is considered very conservative, but considered important to some statistician purests. In a similar fashion to Bonferroni-correction, Yates correction of *Chi*-squared comparisons may also be applied to avoid over-estimate for statistical significance in small sample sizes, but is also an conservative correction. In order to more roundly report the results, both uncorrected and corrected values are reported.

10.13. FUTURE WORK

10.13.1. Collection of routine clinical data

Routine clinical data collection is essential if the paediatric nephrology community are to make progress in improving care afforded to children with CKD. Firstly, acquisition of data related to growth and nutrition in the pre-dialysis, conservatively-managed population throughout the country (perhaps via the Renal Registry) would allow for an understanding of the current status of these children, and a beginning of understanding how these children are clinically managed. In an area of little evidence, large cohort studies such as this describe will be able to highlight clinical practice associated with better outcomes, and act to highlight areas of research interest in this area. This would have the added benefit of identifying groups or clinical units with outlying populations, and exploring these outliers may be able to direct optimal care. For example, if unit ‘A’ has growth data much better than other units, further analysis of the care given may point towards optimal care.

Locally, this routine collection of data would facilitate reflection upon local practice to increase the likelihood for following current clinical practice guidelines, for example.

Similar national data collection programmes have been introduced into epilepsy, asthma, diabetes, and in the neonatal and paediatric intensive care departments in addition to the UK Renal Registry (focused on end-stage disease). Locally, at Southampton Children's Hospital the research database developed for the TEMPeReD study will have ongoing refinement and development with subsequent deployment into routine clinical care.

10.13.2. Dietary intake data

The current clinical methodologies in acquiring dietary data are flawed in that the number of days diet analysed is limited and unlikely to represent true intake of vitamins and minerals in which foodstuffs that may be high in concentration are infrequently eaten. Methods for the clinical assessment of dietary intake need to be developed and validated. This may take the form of food frequency questionnaires. Alternatively, the development of biological markers of dietary intake and/or status could negate the need for diet histories for particular nutrient assessment – and could be focused on the concordance with nutritional prescription and counselling. For example, 24-hour urinary sodium excretion reflects sodium intake (254) – although this would not be practical for all the children within a clinic.

10.13.3. Vitamin and mineral status and requirements

Within the TEMPeReD study, elevated plasma vitamin A and vitamin E concentrations were almost universal, even in those with mild-to-moderate disease severity (PDCM). The impact of such elevations are not known, and requires further mechanistic study. Included in these experiments, researchers should examine for potential deleterious association of elevated vitamin E on vitamin K status, vitamin K-dependent proteins' activities. This may include vascular calcification and increase risk of cardiovascular disease and CKD progression; altered immunity; and effects on bone health and growth.

As discussed previously, the markers vitamin and mineral status have not been validated in children with CKD, and these must be revisited, with the exploration of biomarkers that truly reflect status.

Balance studies (or similar) to ascertain the true requirements for children with CKD should facilitate the composition of meaningful clinical practice guidelines. These should include the analysis of additional losses of vitamins and minerals in dialysis effluent and the effect of potentially altered requirements in those on immunosuppressive regimens and in-receipt regular broad-spectrum or urinary tract infection prophylactic antibiotics (potentially mediated through alter changes in the microbiome). For some vitamins and minerals (selenium, for example), more research is also required in healthy individuals in which requirements in the general population may truly be greater for optimal health, not just the avoidance of overt disease.

As part of these studies, further time-limited trials of supplementation in the paediatric CKD cohort would be useful in exploring the role of specific nutrient requirements. Some supplementation trials that may have promise include vitamins and minerals linked to the defence against oxidative stress as discussed earlier, and potentially B-vitamins and glycine with regards to CKD-related anaemia and erythropoietin dosing in both pre-dialysis, and dialysis patients.

There is a recognised need for a nutritionally complete vitamin and mineral supplement that is suitable for children and young people with kidney disease disease, but the supplement tested in this thesis was not acceptable to the population that it was intended for despite previous work performed by the industrial partner.

10.13.4. Obesity and adiposity

Future research is needed to evaluate the optimal definition of obesity in children and young people with CKD so that specific goals / targets may be created and tested to improve patient outcomes. In the first instance, collection of these data at the level of national registries and examining associations with outcomes over time may be the first step towards this. Additionally, evaluation of WHtR, for example, as related to body composition analysis (bioelectrical impedance analysis and cross-sectional imaging analysis) as a proxy measure for adiposity, in addition to other associated markers of cardiovascular risk such as lipid studies.

10.13.5. Appetite

The structured appetite assessment questionnaire developed within this thesis should be further tested in larger, multicentre cohorts of children and young people with CKD; including further validation studies. With removal of the seventh question, and subsequent validation the questionnaire could potentially be used as a screening tool for the wider nephrology clinic, and direct children and young people for further dietary and nutritional assessment.

10.13.6. Health-Related Quality of life

Further exploration of factors that could be influencing HRQoL, especially those that are modifiable, is needed with the aim to improve the HRQoL of our patients. It is not unreasonable to predict that the nutritional changes; including dietary restriction found in CKD and changes of body composition may have a detrimental effect upon HRQoL. Therefore, further studies analysing the impact would be valuable in order to optimise management strategies to improve HRQoL. A larger multi-centre cohort would allow for exploration of different aetiologies and the effect this may have on HRQoL in addition to minimising type 2 errors. Longitudinal studies of HRQoL in this disease-group are also needed. Although there is cross-sectional data comparing treatment modalities, it would be useful to know how an individual's HRQoL changes with time; with the changing disease severity, changing treatment modality, changing nutritional status, transitioning from paediatric to adult services. This would facilitate the exploration of the ways different management strategies, including nutritional intervention influence HRQoL. Finally, the emotional component of the HRQoL assessment presented here seemed most strikingly different from healthy peers, and work focused on exploring the emotional health; including its relationship to diet, nutrition and dietetic management should be undertaken. Such work could include a qualitative study exploring the perceptions and opinions of children, young-people as their caregivers with regards to the nutritional management of kidney disease, as this is an area lacking in the current literature.

The routine introduction of HRQoL as a measure in clinical care may allow for a more patient-focused approach to care. If the multidisciplinary team is focused upon improving a patient-centred value, patients and their families may be more engaged in decision-making, and those decisions may be more focused on improving HRQoL rather than markers of disease, for example that may seem disconnected from day-to-day for families.

10.13.7. Disease activity

Future work may include evaluation of the association of clinical outcomes and RDW in a larger cohort over a longer timeframe; and exploration of the possible relationship between RDW and muscle mass (and functionality such as handgrip strength).

In exploring the concept of disease activity, and the potential impacting role of nutrient availability and oxidative stress, I have considered that the mitochondria may be the area of cellular activity that may prove key. With altered supply of nutrients necessary for optimal function of the mitochondria interrupted, the usual process may not work to the same efficiency.

Those with CKD have dysfunctional mitochondria and dysfunctional mitochondria contribute to the oxidative stress that is present in the progressive kidney injury in CKD (346, 347). Measuring the functional capacity of mitochondria ex vivo has been performed; demonstrating altered mitochondrial function in many disease states.

Mitochondria produce reactive oxygen species during the production of ATP, and thus are a source of oxidative stress to the cellular machinery. Intra-mitochondrial redox imbalance may be one of the sources of reactive species – that are elevated in CKD. Moreover, mitochondria may be particularly vulnerable to any imbalance oxidative state as they are a site of reactive species generation.
Mitochondrial dysfunction may be the result of alteration is one of the following:

- Kreb's cycle efficiency;
- Respiratory chain efficiency;
- Mitochondrial membrane function;
- Control of oxidative stress and free radicals.
- Oxidative Stress in CKD

Even in the earlier, less severe stages of CKD (163) there are increased levels of markers of oxidative damage (e.g., markers of lipid peroxidation, decreased levels of the antioxidant protein glutathione (GSH) (163, 348). Moreover, the degree of imbalance worsens as disease severity increases and haemodialysis causes significant oxidative strain on the body over and above the CKD itself (163). The reasons why those with CKD have increased oxidative stress is not fully understood, but suggested mechanisms include deficiency in antioxidants and chronic inflammation (349). Additional mechanisms in those receiving haemodialysis are increased neutrophil activation (350) as well as the dialysis process itself (351). The increased oxidative stress causes damage to the cellular machinery; triggering further inflammation, apoptosis, and kidney fibrosis.

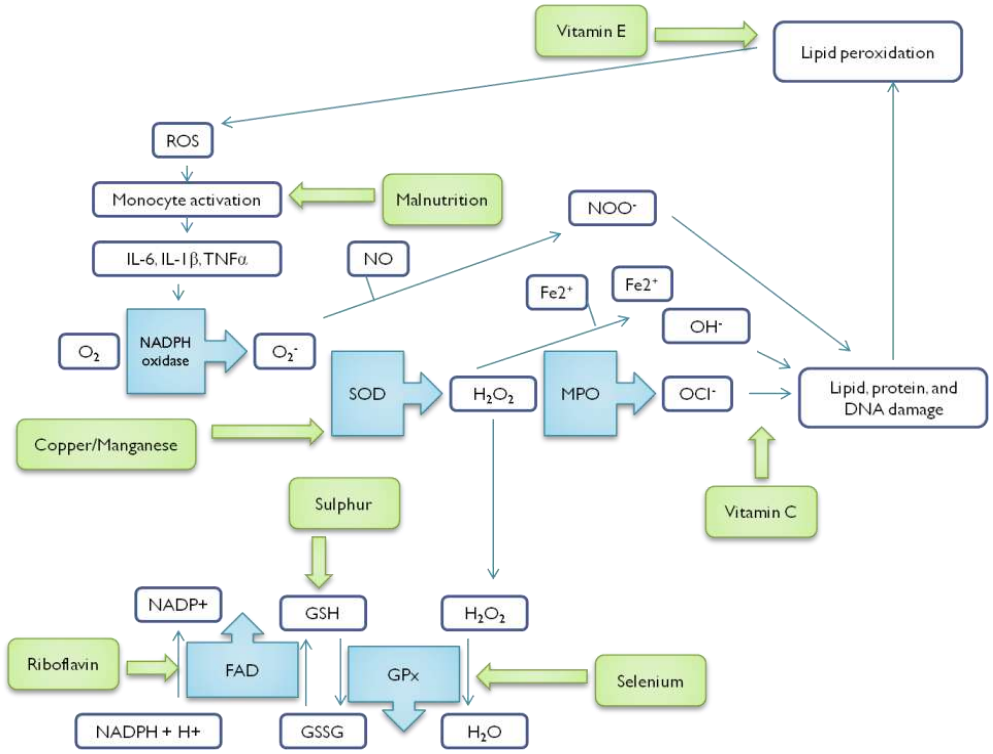


Figure 58. Generation of reactive species, glutathione's role in controlling redox state and some of the potential nutritional sensitive points.

In CKD, there is neutrophil activation, and increased inflammation with increased generation of superoxide (O_2^-), this may be converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), at which point it can be reduced to water (H_2O) through the action of glutathione peroxidase (GPx) with the oxidation of antioxidant protein glutathione. Superoxide may also react with nitric oxide (NO) to form peroxynitrite (NOO^-) and hydrogen peroxide may form hydroxyl radicals (OH^-) or be converted to hypochlorous acid (OCl^-). The reactive species interact with the cellular machinery causing cellular damage. If reacting with lipids, they cause lipid peroxidation, and the generation of more reactive species driving the oxidative stress forward. Some micronutrients, such as copper, selenium and riboflavin are required for enzyme function in these pathways. Vitamin C acts as an antioxidant within the body, reducing free radical such as hydroxyl radicals and superoxide, and vitamin E also acts to reduce free radicals, and due to its lipid soluble nature is important in protecting lipid membranes from peroxidation. Abbreviations: ROS – Reactive oxygen species; IL – Interleukin; TNF – Tumour necrosis factor; NADPH - Nicotinamide adenine dinucleotide phosphate; SOD – Superoxide dismutase; MPO – Myeloperoxidase; GSH – Glutathione; GSSH – Oxidised glutathione; GPx – Glutathione peroxidase; FAD – Flavin adenine dinucleotide.

Mitochondrial dysfunction has previously been explored in CKD, existing literature examining mitochondrial dysfunction in muscle samples of those with CKD have reported decreased activity of mitochondrial enzymes such as citrate synthase and hydroxyacyl-CoA dehydrogenase (352). In animal models, CKD-associated sarcopenia in a murine model was preceded by a decrease in mitochondria number. Exercise testing of adult HD patients has demonstrated impaired the rates of recovery of both phosphocreatine and intracellular pH in skeletal muscle (353). Moreover, the slower recovery of the phosphocreatine relative to blood flow as assessed by near infrared spectroscopy (354) supports mitochondrial dysfunction in skeletal muscle of CKD patients.

Previous studies by Granata (346) and Rao (347) have examined mitochondrial function in peripheral blood mononuclear cells; demonstrating increased levels of oxidative stress; mtDNA damage, and decreased mitochondrial activity.

In a five-sixths nephrectomy murine model of CKD, administration of mitoTempo (a superoxide dismutase mimetic) improved body weight, kidney function (serum creatinine), kidney fibrosis (histological appearance, TGF-β, fibronectin, connective tissue growth factor (CTGF), plasminogen activator inhibitor-1 (PAI-1) expression), pro-inflammatory cytokine mRNA expression (IL-6, TNF-α, MCP-1, and IL-1β), and oxidative stress (superoxide dismutase-2 (SOD2) activity, mitochondrial and serum malondialdehyde (MDA), and total SOD concentrations (355).

This research suggests that the observed oxidative stress in CKD is of mitochondria origin and potentially amenable to manipulation. Despite this, supplementation studies in human subjects are far from conclusive in their results.

The mitochondrial stress test examines the mitochondrial oxygen consumption with the addition of inhibitor and promoters to different elements of the electron transport chain (oligomycin – a mitochondrial ATP-synthase inhibitor; carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) –an ionophore that shuttles hydrogen ions across the mitochondrial membrane, uncoupling ATP synthesis from oxygen consumption; Antimycin A – a complex III inhibitor). Tissue substrates for this method include peripheral blood mononuclear cells, and muscle fibres obtained through biopsy.

Proposed research questions to be answered and proposed experiments to be undertaken:

- Does the mitochondrial function (as assessed by mitochondrial stress test) of circulating monocytes in children with CKD differ to that of healthy controls?

I would hypothesise that the findings discussed above would be reflected in this methodology of mitochondrial function assessment with diminished mitochondrial function in those with CKD compared to healthy peers. This question may be answered through the undertaking of a mitochondrial stress test on samples of peripheral blood mononuclear cells from a cohort of patients with CKD with comparison with a group of healthy controls. Selection of a group containing varying degrees of kidney impairment would allow for the determination of a correlation between severity of disease and degree of mitochondrial dysfunction.

- Are urine-derived cells a viable source of mitochondria that may be assessed for mitochondria dysfunction?

Firstly, a method of the collection of urine-derived cells with preparation for mitochondria function analysis must be developed. The viability of this method may be tested with a selection of healthy volunteers. If proved viable (adequate yield of viable cells) with normal mitochondrial function in these healthy controls, a group of urine-producing patients with CKD could be evaluated. Urine-derived cells may have the advantages of firstly not requiring venesection, and secondly may more accurately reflect intra-kidney mitochondrial function.

- Does the mitochondrial function of circulating monocytes reflect the function of mitochondria within the kidney in children with CKD?

Comparison between mitochondrial function in sample peripheral blood mononuclear cells, biopsy-derived kidney tissue, and urine-derived cells. Collection of biopsy tissue from human subjects may prove challenging in the first instance, and so a comparison test could initially be performed on animals. Excess tissue acquired for diagnostic purposes, could be used – although the more common indications of kidney biopsy (steroids resistant nephrotic syndrome, nephritis) are likely to influence mitochondrial function over-and-above degree of kidney impairment.

- If dysfunctional, what nutritional measures could be employed to improve the functioning of these mitochondria?

This could include the improving the availability of substrates and cofactors needed for the Krebs cycle; the electron transport chain (e.g., coenzyme Q10); mitochondrial membrane function/integrity; and the control of oxidative stress and free radicals (e.g., anti-oxidants vitamins). Experimental design for this may include interventional trials.

Although work to date has focused on a possible pharmacological 'silver bullet' in improving the function of the mitochondria, or the use of nutrients as antioxidants. Other variables are likely to affect the function. Importantly this included the availability of various nutrients, including those involved in the Krebs cycle (such as B-vitamins). Experimentation is first needed to explore how the availability of such nutrients to dysfunctional mitochondria may improve their function through, for example, increasing efficiency of the Krebs cycle, and respiratory chain.

The role of coenzyme Q10 in CKD has begun to be examined, but those with a primary co-enzyme Q10 deficiency present a nephrotic syndrome initially, and therefore it is questionable whether established CKD mitochondrial dysfunction may be 'cured' by such therapy. It may be that co-enzyme Q10, may represent an incremental improvement in mitochondrial function in this clinical scenario, and that other measures, acting through different mechanisms can also improve the mitochondrial function can summate to 'normalise' mitochondrial function and ameliorate the progression of disease.

A readily accessible biomarker that can be used clinically that reflects mitochondrial function may prove useful in understanding what is happening within the individual's kidney and be used to tailor therapy. If mitochondrial function as assessed by a given approach is the marker of disease activity and is demonstrated to be nutritionally sensitive, it is not clinically useful if the patient (especially a paediatric one) needs to undergo a kidney biopsy. As CKD is a systemic disease, it may be mitochondrial function in other, readily accessible, tissues correlated with kidney mitochondrial function. Monocyte mitochondrial function has been examined. Therefore, evaluation

of monocyte mitochondrial function may represent a first step in a clinical translational measure of mitochondria function in the paediatric population.

Previous research (348, 356) examining the response altering mitochondrial redox state suggests that pushing the redox scales too far may result in reductive stress with impairment of function. Kirkman et al's healthy controls had evidence of impaired vascular function when mitoTempo (a mitochondrial-specific superoxide scavenger) was administered (348). This might explain the increase in all-cause mortality observed in vitamin E supplementation trials – administration may be beneficial to some with a tendency to oxidative stress, but in those in redox balance, over-reduction may have had unintended consequential impaired function. If this is the case, individual assessment targeting may prove the correct course of action.

10.14. Summary and Concluding Remarks

This thesis explores the variation of clinical outcomes, nutritional status, disease severity, and disease activity in children and young people with CKD. Variation in nutritional status, HRQoL and proposed markers of disease activity are not solely-related to disease severity as determined by eGFR.

The work contained within this thesis adds to the scientific literature through:

- Its exploration of growth within a regional paediatric nephrology clinic using information technological systems;
- Collation and interrogation of the existing literature on the vitamin and mineral status of children and young people with pre-dialysis, conservatively-managed CKD;
- Reporting the dietary intake using current clinical methodologies, vitamin and mineral blood concentrations, anthropometry, and health-related quality of life of a cohort of children with CKD;
- Exploration of appetite using a novel appetite assessment questionnaire;
- Exploration of two putative markers of disease activity.

Additionally, the work highlights the importance of acceptability and palatability of vitamin and mineral supplements and has aided in the development of a commercially available product in the future.
Finally, I have discussed areas in which researchers should direct their attention in order to better understand nutrition in the context of paediatric CKD and for clinicians to supply the optimal care for their patients.

11. APPENDICES

11.1. ANTHROPOMETRIC AND CLINICAL METHODOLOGIES

Basic clinical and anthropometric data were collected; including height, weight, waist circumference, and BMI and waist circumference-to-height ratios calculated. Definition of stunting was taken as HtSDS <-2. Definitions of obesity were BMI SDS >2; MUAC SDS >2 or >25cm (270); and a WHtR >0.5 (75).

11.1.1. Assessment of deprivation

As a marker of deprivation, Income Deprivation Affecting Children Index (IDACI) scores were calculated using participants' postcodes. United Kingdom Department of Communities and Local Government data (2015) via the online platform that provides a selection of official statistics and data outputs on a variety of themes related to the department of Communities and Local Government (http://opendatacommunities.org).

11.1.2. Body Mass Index (BMI)

Body mass index (BMI) was calculated using the following equation.

$$BMI = \frac{Weight\ (kg)}{(Height\ (m)\ )^2}$$

11.1.3. Standard Deviation Score (SDS)

For comparison of anthropometric measurements of different children of different sexes and ages, standard deviation scores (SDS) were calculated using the formula below. The standard upon which the measurement is compared for height, weight, and BMI is the World Health Organisation (WHO) growth charts 1996 (27).

$$SDS = \frac{Measured\ Value - Value\ at\ 50th\ percentile\ for\ standard}{Standard\ deviation\ of\ the\ standard}$$

For the follow-up data, a change in height SDS of 0.2 was a priori determined to likely represent a true deviation in SDS.

11.1.4. Kidney Function

Kidney function was estimated using readily available serum creatinine measurement and height with subsequent estimated Glomerular Filtration Rate (eGFR) using the Schwartz formula (152).

$$eGFR\ (ml/min/1.73m^2) = \frac{40 \times Height\ (cm)}{Serum\ Creatinine\ (mmol/l)}$$

Kidney function is stratified depending upon GFR as per international agreed criteria (357), and is displayed below.

Table 42. KDIGO classification of CKD stage.

CKD Stage	GFR (ml/min/1.73m^2)	Description
1	≥90	Normal or high
2	60-89	Mildly decreased*
3a	45-59	Mildly-to-moderately decreased
3b	30-44	Moderately-to-severely decreased
4	15-29	Severely decreased
5	<15	Kidney failure
5D	In receipt of dialysis	In receipt of dialysis

In the absence of kidney damage, neither GFR stage1 or 2 fulfill the criteria of CKD. *compared to young adults.

11.1.5. Normal Reference Ranges

Table 43. Normal Reference Ranges for children and young people aged 4 to 18 years.

Nutrient	Normal Reference Range
Sodium	133-146 mmol/l
Potassium	3.5 – 5.0 mmol/l
Corrected Calcium	2.2-2.6 mmol/l
Magnesium	0.7-1.0 mmol/l
Inorganic Phosphate	0.9-1.8 mmol/l
Ferritin	10-170 µg/l
Copper	12-26 µmol/l
Zinc	11-24 µmol/l
Whole Blood Manganese	73-210 nmol/l
Selenium	0.7-1.7 µmol/l
Vitamin A	0.9-1.7 µmol/l
Vitamin E	10-21 µmol/l
Vitamin B12	>130 ng/l
Folate	2.9-20.6 ng/l
Vitamin C	11-114 µmol/l
Vitamin B6	30-144 nmol/l
Vitamin D	>50 nmol/l

11.1.6. Red cell distribution width (RDW)

$$RDW = \frac{Standard\ Deviation\ of\ Mean\ corpuscular\ volume}{Mean\ corpuscular\ volume\ (MCV)} \times 100$$

The normal reference range for RDW is 11.3 to 14.8%.

11.1.7. Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL)

NGAL in urine was measured in duplicate by sandwich enzyme linked immunosorbent assay (ELISA), (Bioporto Diagnostics and supplied by Alpha Laboratories). The normal value of uNGAL 95th percentile of healthy individuals has been proposed (129) and varies depending upon age and sex. To control for the variation,

and setting uNGAL concentrations greater than the 95th percentile as lying outside the normal range, the percentage of the 95th centile for uNGAL was calculated. This means that these calculated values greater than 100% are considered elevated.

$$uNGAL\ \%95th\ percentile = \frac{Measured\ [uNGAL]}{[uNGAL] representing\ the\ 95th\ percentile} \times 100$$

11.2. Additional Tables and Figures for Review of Literature of the Micronutrient Status of Children with Chronic Kidney Disease

11.2.1. Search Strategy for Vitamin E

#	Database	Search term	Results
1	Medline	(paediatric* OR pediatric* OR child OR children OR infan* OR neonat* OR newborn OR adolesc* OR teenag* OR (young* ADJ3 (people OR person OR adult*))).ti,ab	2008684
2	Medline	((Chronic OR Fail* OR injur* OR "end stage" OR "replacement therapy" OR transplant*) ADJ3 (kidney OR renal)).ti,ab	261842
3	Medline	(uraemi* OR uremi* OR ((urea ADJ2 blood) AND (elevat* OR high* OR excess*))).ti,ab	36335
4	Medline	(haemodialysis OR hemodialysis OR "peritoneal dialysis").ti,ab	88022
5	Medline	(2 OR 3 OR 4)	335849
6	Medline	(1 AND 5)	26080
7	Medline	("vitamin E" OR "α-tocopherol" OR "alpha-tocopherol" OR "tocopherol" OR "tocopherols" OR "tocotrienols").ti,ab	40595
8	Medline	(6 AND 7)	37
9	EMBASE	(paediatric* OR pediatric* OR child OR children OR infan* OR neonat* OR newborn OR adolesc* OR teenag* OR (young* ADJ3 (people OR person OR adult*))).ti,ab	2434380
10	EMBASE	((Chronic OR Fail* OR injur* OR "end stage" OR "replacement therapy" OR transplant*) ADJ3 (kidney OR renal)).ti,ab	374200
11	EMBASE	(uraemi* OR uremi* OR ((urea ADJ2 blood) AND (elevat* OR high* OR excess*))).ti,ab	43742
12	EMBASE	(haemodialysis OR hemodialysis OR "peritoneal dialysis").ti,ab	116698
13	EMBASE	(10 OR 11 OR 12)	464840
14	EMBASE	(9 AND 13)	40076
15	EMBASE	("vitamin E" OR "α-tocopherol" OR "alpha-tocopherol" OR "tocopherol" OR "tocopherols" OR "tocotrienols").ti,ab	46338
16	EMBASE	(14 AND 15)	48

11.2.2. Search Strategy for Selenium

#	Database	Search term	Results
1	Medline	(paediatric* OR pediatric* OR child OR children OR infan* OR neonat* OR newborn OR adolesc* OR teenag* OR (young* ADJ3 (people OR person OR adult*))).ti,ab	2008684
2	Medline	((Chronic OR Fail* OR injur* OR "end stage" OR "replacement therapy" OR transplant*) ADJ3 (kidney OR renal)).ti,ab	261842
3	Medline	(uraemi* OR uremi* OR ((urea ADJ2 blood) AND (elevat* OR high* OR excess*))).ti,ab	36335
4	Medline	(haemodialysis OR hemodialysis OR "peritoneal dialysis").ti,ab	88022
5	Medline	(2 OR 3 OR 4)	335849
6	Medline	(1 AND 5)	26080
7	Medline	(selenium OR selenoprotein OR "glutathione perioxidase" OR GPx).ti,ab	35458
9	EMBASE	(paediatric* OR pediatric* OR child OR children OR infan* OR neonat* OR newborn OR adolesc* OR teenag* OR (young* ADJ3 (people OR person OR adult*))).ti,ab	2434380
10	EMBASE	((Chronic OR Fail* OR injur* OR "end stage" OR "replacement therapy" OR transplant*) ADJ3 (kidney OR renal)).ti,ab	374200
11	EMBASE	(uraemi* OR uremi* OR ((urea ADJ2 blood) AND (elevat* OR high* OR excess*))).ti,ab	43742
12	EMBASE	(haemodialysis OR hemodialysis OR "peritoneal dialysis").ti,ab	116698
13	EMBASE	(10 OR 11 OR 12)	464840
14	EMBASE	(9 AND 13)	40076
15	EMBASE	(selenium OR selenoprotein OR "glutathione peroxidase" OR GPx).ti,ab	43212
16	Medline	(6 AND 7)	24
17	EMBASE	(14 AND 15)	32

11.2.3. Synthesis Tables for Vitamin E and Selenium Literature Reviews.

Table 44. Literature of Dietary Intake of Micronutrients in Paediatric Chronic Kidney Disease

Author	Year	Age (mean)	Age SD	Age (range)	Country	CKD [cons]	CKD [PD]	CKD [HD]	Controls	Findings / Comments:
Vitamin E										
Coleman and Watson	1992	6.1	-	0.3-12.6	UK	-	5	-	-	Dietary intake of seven children on PD as assessed by three-day dietary, and six out of seven reported intakes greater than recommended intakes.
Drukker et al	1988	10.9	4.5	3-19	Israel	10	10	-	10	All treatment groups within the normal reference range and no different from one another.
Farid et al	2009	14.2	2.1	6-16	Egypt	12	-	22	34	Vitamin E supplementation (400 IU/day for 2 months) resulted in a significant decrease in markers of lipid peroxidation. Vitamin E status not reported of individuals.
Iughetti et al	2008	1	4.6	-	Italy	-	-	8	-	No different from healthy controls.
Joyce et al	2018	9.4	6.6	-	UK	-	19	28	-	High prevalence of elevated vitamin E, and higher average concentrations; suggesting the possibility of a 'dose-response' relationship between disease severity and vitamin E, although in these data correlation was not statistically significant (p=0.059).
Modarressi et al	2012	-	-	-	-	-	-	15	-	Abstract at conference. In addition to subcutaneous erythropoietin, folic acid, and iron, oral vitamin E 200 u/day for 3 months resulted in significantly higher concentrations of haemoglobin (Hb=11.4+/-1.7 vs. 10.1+/-1.9 g/dl and Hct=35.3 +/-5 vs. 31.3+/-6%, P<0.05). Although vitamin E status, and other factors are not explored.
Naseri et al	2015	13.8	6.3	1.6-25	Iran	-	12	25	-	Serum vitamin E concentrations are more likely to be low than normal or elevated with 72% low (as defined as <3, 6, and 5 µg/ml for children, teenagers and adults, respectively). 21% were within the normal reference range and 7% high (>9, 10 and 18ug/ml, respectively). For their HD patients (n=25), 84% had low concentrations, 12% normal, and 4% high. A similar picture was observed in their PD patients (67% low, and 33% normal). High mortality rate. Those with low vitamin E concentrations did not have a higher incidence of death (p=0.175).
Nemeth et al	2000	15.2	3.2	7-17	Hungary	-	-	10	-	Supplementation trial HD patients and demonstrated a lack of the increase in oxidative stress induced by rhEPO and a more rapid increase in haemoglobin compared to the same patients without supplementation. Vitamin E status not reported.
Tahzib et al	1999	12.9	1.2	-	USA	11	0	0	9	Controls were children with non-FSGS renal disease. Supplementation trial. A significant improvement in proteinuria is reported in the FSGS group with no change in those of other aetiology following supplementation with 200 IU twice-daily for three months. The authors do not report the vitamin E status of the children. No change in eGFR was observed, although the length of the study was perhaps not long enough for this to be appreciable.
Zwolinska et al	2006	12.9	-	-	Poland	46	-	21	27	Conservatively-managed children report lower plasma and erythrocyte concentrations compared to a control group (p<0.001). The authors additionally measured dialysate concentrations of vitamin E, but unfortunately units for this measure are missing from the manuscript (0.123 SD±0.019). This same study report vitamin A concentrations that are lower than those of healthy controls – questions validity.
Zwolinska et al	2009	13.2	3.5	-	Poland	-	10	-	27	Vitamin E Loss of vitamin E in the ultrafiltrate.
Selenium										
Esmaeili et al.	2019	12.7	-	5-18	Iran	14	45	63	78	Serum selenium concentrations lower in dialysis patients. No difference in conservatively-managed CKD (mean GFR = 8 ml/min/1.73m2) compared to healthy controls. Average concentration of the group with the lowest selenium concentration (CAPD, 100.44 µg/l) is within the normal reference range, so the clinical significance of this statistical difference

										requires further exploration. The study does not explore the role of dietary intake.
Joyce et al, 2018.	2018	9.4	6.6	-	UK	-	19	28	-	65% achieved normal ranges of selenium, with low concentrations reported in 28%, and high in 7%. As in the above studies, dietary intake and dialysis losses are not characterised to understand the variation in those children and young people whose concentrations lay outside of the normal reference range.
Ortac et al,	2006	11.1	4.4	-	Turkey	32	42	19	34	Hair selenium concentrations and GPx activity were significantly lower in those with CKD compared to the control group. No correlation with disease severity (GFR) for either hair selenium concentrations or GPx activity, and a lack of agreement between hair selenium and GPx activity. Control group had a mean GFR of 84ml/min/1.73m2, which is not normal. The authors do not describe the selection process of the control group.
Sommerburg et al.	2002	-	-	-	-	-	-	-	-	Abstract only. GPx activity in a cohort of children on HD. The authors report GPx in this cohort as almost twice as high than their other analysed groups. The selenium status of the children are not reported.
Zwolinska et al.	2004	12.0	-	-	Poland	46	-	21	27	Concentrations of selenium both in plasma and erythrocyte were lower in those with CKD compared to healthy controls. Comparing less or more severe disease (serum creatinine above or below 265.3µmol/l), no difference was reported. Average plasma values for the more severe disease group was 0.87 µmol/l (SD± 0.12) within the range found in the general population. This study did not evaluate dietary selenium intake.
Zwolinska et al.	2006	13.6	-	-	Poland	-	21	10	27	Plasma and erythrocyte concentrations) in dialysis patients are lower compared to the control group, but with mean value within the normal reference range (0.74 µmol/l compared to 1.07 µmol/l). Change in plasma and erythrocyte selenium concentration before and shortly after starting HD were significantly different, suggesting possible losses, although the effluent was not analysed. No dietary characterisation.

Risk of Bias Assessment of Literature Reviewed
Newcastle-Ottawa Quality Assessment Scale Results

Study Details	Selection (maximum score = 4)	Comparability (maximum score = 2)	Exposure (maximum score = 3)	Total Score (maximum score = 9)	Score ≥ 7? 0=no, 1=yes.
Coleman and Watson, 1992. (176)	*	**	*	4	0
Drukker et al, 1988. (79)	**	*	***	6	0
Esmaeili et al, 2019. (214)	**	-	*	3	0
Farid et al, 2009. (201)	*	-	*	2	0
Iughetti et al, 2008. (189)	**	*	***	6	0
Joyce et al, 2018. (80)	*	*	**	4	0
Modarressi et al, 2012. (200)	-	-	*	1	0
Naseri et al, 2015. (78)	*	*	*	3	0
Nemeth et al, 2000. (203)	*	-	*	2	0
Ortac et al, 2006. (213)	**	-	*	3	0
Sommerburg et al, 2002. (358)	-	-	*	1	0
Tahzib et al, 1999. (198)	*	-	*	2	0
Zwolinska et al, 2004. *(217)*	**	-	***	5	0
Zwolinska et al, 2006. *(181)*	**	-	***	5	0
Zwolinska et al, 2006. (216)	**	-	***	5	0
Zwolinska et al, 2009.	**	-	***	5	0

11.3. ADDITIONAL TABLES AND FIGURES FOR THE GROWTH AND ANTHROPOMETRY OF THE TEMPERED COHORT.

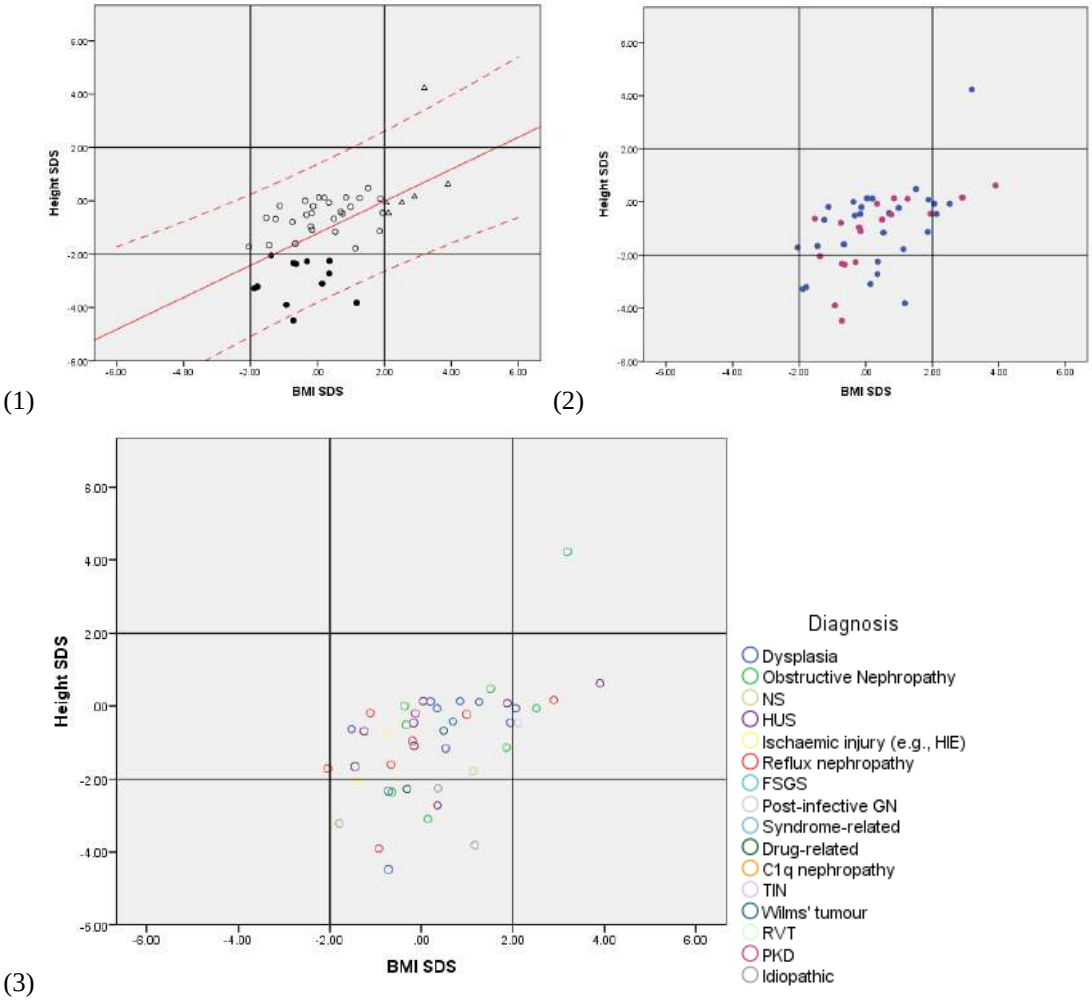


Figure 59. Height Standard Deviation Score Versus Body Mass Index Standard Deviation Score for the Pre-dialysis, Conservatively-Managed subgroup (n=46).

(1). Black circles represent those that are stunted (Height SDS <-2), and triangles those that are obese (BMI SDS >2). Line of best fit is shown with 95% confidence intervals (dotted red line). There was a correlation between the two measures (Spearman's rho=0.568, p<0.0005). (2). There is no clear pattern with regards to sex distribution (male= blue circles, females = pink circles). (3) There is no clear pattern with aetiology of CKD.

11.4. ADDITIONAL TABLES AND FIGURES OF THE DIETARY INTAKE OF THE TEMPEREDCOHORT.

Table 45. Absolute intake and percentage of recommended intake of a cohort of children within the pre-dialysis, conservatively-managed CKD subgroup (n=46).

Nutrient	Mean intake ± SD (‡-median intake ± IQR)	Mean dietary intake; % of recommended intake (± SD)
Energy (kcal/d)	1510 ± 593	89.6 (± 40.9)
Protein (g/d) ‡	45.7 ± 25.9	
Sodium (mg/d) ‡	1699 ± 1073	
Potassium (mg/d) ‡	1694 ± 939	95.4 (± 65.2)
Calcium (mg/d) ‡	586 ± 519	
Magnesium (mg/d) ‡	151 ± 85	95.1 (± 67.9)
Phosphorus (mg/d) ‡	844 ± 546	
Vitamin A (mcg/d) ‡	504 ± 436	
Vitamin E (mg/d)	5.1 ± 2.4	
Riboflavin (mg/d) ‡	1.29 ± 0.89	
Folate (mg/d)	173.74 ± 71.60	
Iron (mg/d) ‡	7.80 ± 4.45	97.5 (± 85.4)
Iodine (mg/d) ‡	82± 73.5	85.9 (± 65.3)
Selenium (mcg/d) ‡	27 ± 20.25	89.9 (± 62.2)
Zinc (mg/d) ‡	5.65 ± 3.08	81.1 (± 42.3)
Copper (mcg/d) ‡	665 ± 390	
Manganese (mg/d) ‡	1.45 ± 0.83	
Thiamine (mg/d) ‡	1.26 ± 0.93	
Niacin (mg/d) ‡	22.4 ± 11.65	
Vitamin B6 (mcg/d)‡	1.28 ± 0.93	
Vitamin C (mg/d) ‡	58 ± 51.0	
Vitamin B12 (mcg/d) ‡	2.1 ± 2.83	
Vitamin K (mcg/d) ‡	3 ± 7	51.3 (± 195)
Pantothenic acid (mg/d) ‡	3.4 ± 2.32	
Biotin (mcg/d) ‡	15.75 ± 12.44	

‡ - non-parametric distribution, median and interquartile range reported.

Table 46. Mean nutrient intake of the TEMPeReD cohort (n=60) versus 100%RNI.

Nutrient	Mean	Median	IQR	SD	Range min	Range high	P-value	Following Bonferroni correction.	Mean difference
Energy	88.7	80.5	49	43	16	246	***0.048****	1	-11.3
Protein	168.7	144	112	88	31	425	***<0.0005****	***0.0125***	68.7
Sodium	168.4	142.5	102	96	21	509	***<0.0005****	***0.0125***	68.4
Potassium	89.5	72	50	60	14	319	0.184	1	-10.5
Calcium	110.6	85.00	68	87	7	532	0.351	1	10.6
Magnesium	87.7	69.5	60	63	13	372	0.133	1	-12.3
Phosphorus	139.9	120	90	83	27	517	***<0.0005****	***0.0125***	39.9
Iron	91.2	75.5	53	77	24	592	0.381	1	-8.8
Copper	99.2	85	53	62	14	375	0.919	1	-0.82
Zinc	80.2	72	57	41	14	198	***<0.0005****	***0.0125***	-19.8
Manganese	318.4	260.5	277	228	40	1320	***<0.0005****	***0.0125***	218.4
Selenium	87.7	70.5	101	60	3	253	0.117	1	-12.3
Iodine	84.0	70.00	60	63	2	304	0.053	1	-16.1
Vitamin A	127.9	89.50	75	115	5	643	0.065	1	27.9
Vitamin E[$]	184.3	186	110	97	24	514	***<0.0005****	***0.0125***	84.3
Thiamine	205.5	206.5	104	81	73	450	***<0.0005****	***0.0125***	105.5
Riboflavin	127.2	114.5	94	80	14	391	***0.011****	0.275	27.2
Niacin	263.2	250	122	106	81	641	***<0.0005****	***0.0125***	163.2
Vitamin B6	179.8	165.0	70	71	51	458	***<0.0005****	***0.0125***	79.8
Panto' Acid[$]	264.2	111.5	57	237	0	980	***0.013****	0.325	18.9
Biotin[$]	110.1	157.5	-	60	15	266	***<0.0005****	***0.0125***	80.1
Vitamin B12	118.9	200	302	577	24	242	***<0.0005****	***0.0125***	164.2
Folate	180.1	100.5	73	115	11	638	0.198	1	10.1
Vitamin C	251.7	166	159	301	30	1849	***<0.0005****	***0.0125***	151.7
Vitamin K[$]	85.6	16.5	43	343	0	2657	0.745	1	-14.5

**Statistically significant difference. [$]As no defined RNI has been established, the following targets were used: Manganese target intake was taken to be 16mcg/k/day; Vitamin E target was taken to be 0.4 as a ratio to PUFA intake; Pantothenic Acid intake target was taken to be mg/day; Biotin intake target was taken 10mcg/day; Vitamin K intake target was taken to be 0.5mcg/k/day. One-sample t-test (Compared to 100%).*

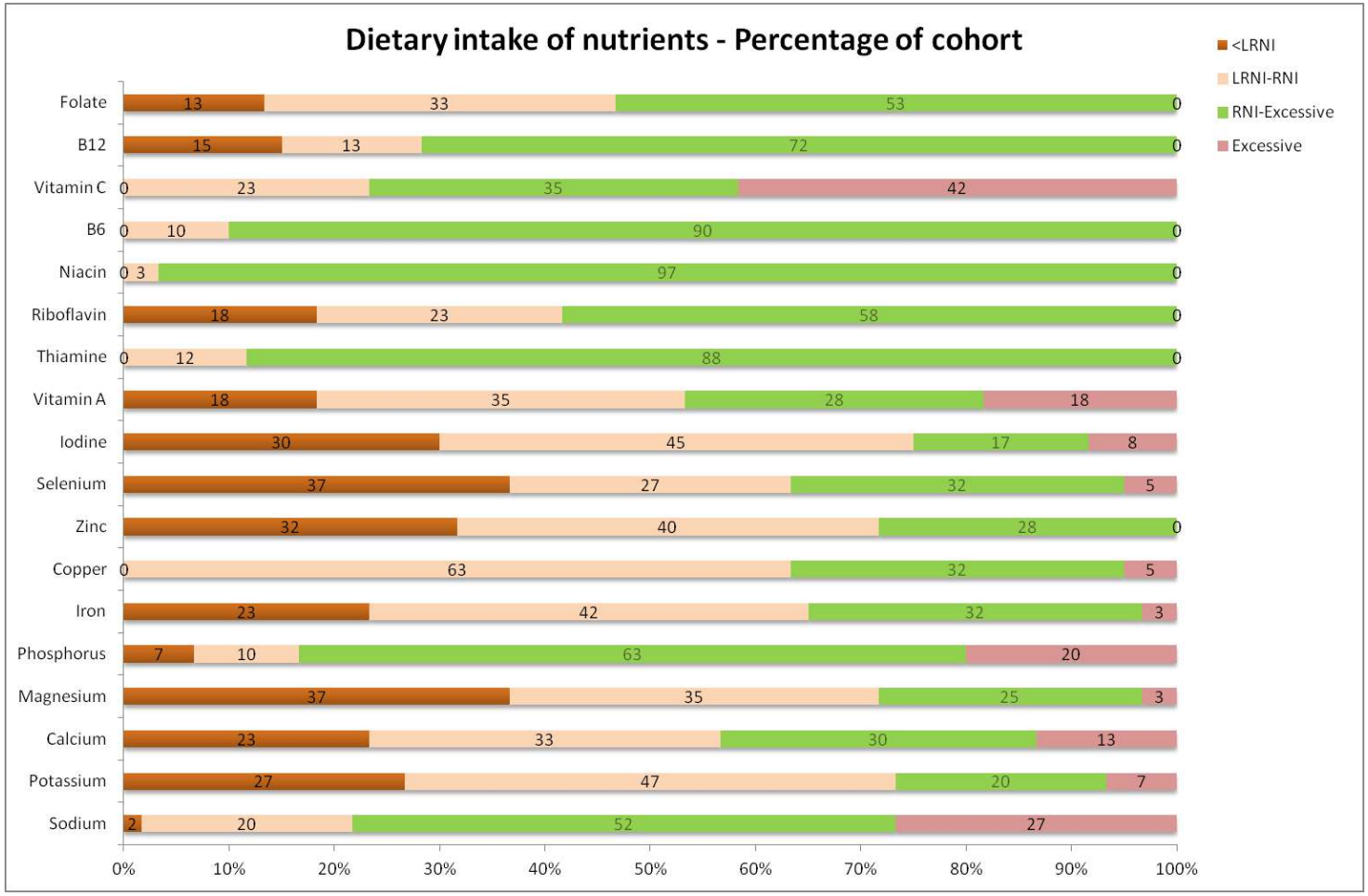


Figure 60. Percentages of the TEMPeReD cohort (n=60) with dietary intakes <LRNI, LRNI-RNI, RNI-excessive and >excessive levels.

N.b., Excessive defined as >200%RNI. Copper – no LRNI available for reference. Folate, B12, B6, Niacin, Riboflavin, thiamine – no excessive limit available for reference.

Table 47. Comparison of the TEMPeReD cohort (entire and pre-dialysis, conservatively-managed subgroup) with National Diet and Nutrition Survey data ages 4 to 10 years.

	NDNS cohort		Entire TEMPeReD Cohort (n=33)					Pre-dialysis, conservatively-managed TEMPeReD Cohort (n=25)				
Nutrient	NDNS mean	NDNS SD	Entire cohort mean	Entire cohort SD	T-value (df)	p-value	p-value (corrected)	Cons cohort mean	Cons cohort SD	T-value (df)	p-value	p-value (corrected)
Energy	1462	317	1429	588	t(526)= 0.5368	0.592	1.000	1527	643	T(518) = 0.736	0.462	1.000
Protein	54.1	13.2	49	23	t(526)= 2.028	***0.043****	0.516	51.2	23.6	T(518) = 1.16	0.245	1.000
Vitamin A	599	457	701	649	t(526)= 1.207	0.228	1.000	843.96	733.5	T(518) = 2.2	***0.028****	0.336
Riboflavin	1.45	0.53	1.23	0.81	t(526)= 2.224	***0.027****	0.324	1.31	0.85	T(518) = 1.07	0.285	1.000
Folate	183	63	154	71.4	t(526)= 2.533	***0.012****	0.144	173.09	72.15	T(518) = 1.08	0.282	1.000
Iron	8.2	2.6	8.61	8.15	t(526)= 0.7006	0.484	1.000	9.61	9.59	T(518) = 1.76	0.080	0.960
Calcium	781	270	707	515	t(526)= 1.42	0.156	1.000	745.48	556.72	T(518) = 0.289	0.773	1.000
Magnesium	188	49	159	80	t(526)= 3.105	***0.002****	***0.024****	179.3	82.78	T(518) = 1.15	0.250	1.000
Potassium	2115	520	1697	837	t(526)= 4.269	***0.000****	***<0.006****	1893.22	860.46	T(518) = 2.35	***0.019****	0.228
Iodine	129	64	104	72	t(526)= 2.114	***0.035****	0.420	108.09	72.17	T(518) = 1.42	0.155	1.000
Selenium	32	11	29	14	t(526)= 1.716	0.087	1.000	30.35	13.58	T(518) = 0.65	0.516	1.000
Zinc	6.1	1.8	10.5	26	t(526)= 3.685	***0.000****	***<0.006****	12.65	30.97	T(518) = 4.37	***0.000****	***<0.006****

Table 48. Comparison of the TEMPeReD cohort (post-transplant and receiving dialysis) with National Diet and Nutrition Survey data ages 4 to 10 years.

	NDNS cohort		Post-transplant TEMPeReD Cohort (n=5)					Dialysis TEMPeReD Cohort (n=3)				
Nutrient	NDNS mean	NDNS SD	Post-Tx cohort mean	Post-Tx cohort SD	T-value (df)	p-value	p-value-corrected	Dialysis Cohort mean	Dialysis cohort SD	T-value (df)	p-value	p-value (corrected)
Energy	1462	317	1184.20	266.54	T(498) = 1.952	0.051	0.612	1140.67	667.07	T(496) = 1.738	0.083	0.996
Protein	54.1	13.2	55.60	21.14	T(498) = 0.2513	0.802	1.000	22.87	16.31	T(496) = 4.081	***0.000****	***<0.006****
Vitamin A	599	457	341.60	75.92	T(498) = 1.258	0.209	1.000	378.67	189.43	T(496) = 0.8339	0.405	1.000
Riboflavin	1.45	0.53	1.18	0.82	T(498) = 1.11	0.267	1.000	0.48	0.33	T(496) = 3.164	***0.002****	***0.024****
Folate	183	63	132.80	43.69	T(498) = 1.777	0.076	0.912	65.00	42.00	T(496) = 3.238	***0.001****	***0.012****
Iron	8.2	2.6	6.96	1.90	T(498) = 1.063	0.288	1.000	5.03	1.42	T(496) = 2.108	***0.035****	0.420
Calcium	781	270	682.40	453.87	T(498) = 0.8066	0.420	1.000	270.67	162.50	T(496) = 3.268	***0.001****	***0.012****
Magnesium	188	49	130.60	48.85	T(498) = 2.606	***0.009****	1.000	68.00	36.76	T(496) = 4.233	***0.000****	***<0.006****
Potassium	2115	520	1536.2	506.26	T(498) = 2.477	***0.014****	0.168	647.00	518.82	T(496) = 4.875	***0.000****	***<0.006****
Iodine	129	64	118.00	84.34	T(498) = 0.3813	0.703	1.000	34.00	34.18	T(496) = 2.567	***0.011****	0.132
Selenium	32	11	29.00	15.00	T(498) = 0.6047	0.546	1.000	11.33	9.07	T(496) = 3.247	***0.001****	***0.012****
Zinc	6.1	1.8	6.70	2.03	T(498) = 0.7408	0.459	1.000	3.90	2.52	T(496) = 2.106	***0.036****	0.432

Table 49. Comparison of the TEMPeReD cohort (entire and pre-dialysis, conservatively-managed subgroup) with National Diet and Nutrition Survey data ages 11 to 18 years.

	NDNS cohort		Entire TEMPeReD Cohort (n=27)					Pre-dialysis, Conservatively-managed TEMPeReD Cohort (n=21)				
Nutrient	NDNS mean	NDNS SD	Entire cohort mean	Entire cohort SD	T-value (df)	P=value	p-value (corrected)	Cons cohort mean	Cons cohort SD	T-value (df)	p-value	p-value (corrected)
Energy	1779	526	1607.07	725.57	T(573) = 1.625	0.105	1.000	1507	577	T=(576) = 2.314	***0.021****	0.253
Protein	67.1	24.2	55.70	22.83	T(573) = 2.395	***0.017****	0.203	52.06	23.15	T=(576) = 2.799	***0.005****	0.064
Vitamin A	626	475	601.07	462.90	T(573) = 0.2665	0.790	1.000	570.05	480.32	T=(576) = 0.5295	0.597	1.000
Riboflavin	1.48	0.73	1.32	0.76	T(573) = 1.112	0.266	1.000	1.34	0.81	T=(576) = 0.859	0.391	1.000
Folate	208	91	177.52	85.90	T(573) = 1.703	0.089	1.000	179.38	73.83	T=(576) = 1.423	0.155	1.000
Iron	9.6	3.3	8.21	3.48	T(573) = 2.124	***0.034****	0.409	8.65	3.21	T=(576) = 1.296	0.196	1.000
Calcium	706	293	637.74	307.70	T(573) = 1.179	0.239	1.000	600.95	317.96	T=(576) = 1.607	0.109	1.000
Magnesium	215	75	166.00	64.84	T(573) = 3.333	***0.001****	***0.011****	160.57	59.26	T=(576) = 3.286	***0.001****	***0.013****
Potassium	2358	770	1896.15	793.76	T(573) = 3.038	***0.002****	***0.030****	1795.67	675.03	T=(576) = 3.298	***0.001****	***0.012****
Iodine	126	87	80.26	47.81	T(573) = 2.71	***0.007****	0.083	75.76	675.03	T=(576) = 2.628	***0.009****	0.106
Selenium	42	20	28.48	19.25	T(573) = 3.435	***0.001****	***0.008****	26.67	19.48	T=(576) = 3.45	***0.001****	***0.007****
Zinc	7.5	2.8	5.68	2.76	T(573) = 3.297	***0.001****	***0.012****	5.53	2.50	T=(576) = 3.175	***0.002****	***0.019****

Table 50. Comparison of the TEMPeReD cohort (post-transplant and receiving dialysis) with National Diet and Nutrition Survey data ages 11 to 18 years.

	NDNS cohort		Post-transplant TEMPeReD Cohort (n=5)					Dialysis TEMPeReD Cohort (n=2)				
Nutrient	NDNS mean	NDNS SD	Post-Tx cohort mean	Post-Tx cohort SD	T-value (df)	p-value	p-value-corrected	Dialysis Cohort mean	Dialysis cohort SD	T-value (df)	p-value	p-value (corrected)
Energy	1779	526	2157.80	1103.83	T(573) = 1.625	0.105	1.000	1162.50	306.18	T(548) = 1.66	0.098	0.606
Protein	67.1	24.2	66.68	19.28	T(573) = 2.395	***0.017****	0.204	67.60	13.72	T(548) = 0.03	0.977	1.000
Vitamin A	626	475	760.40	444.06	T(573) = 0.2665	0.790	1.000	321.50	190.21	T(548) = 0.91	0.366	1.000
Riboflavin	1.48	0.73	1.37	0.62	T(573) = 1.112	0.266	1.000	0.77	0.18	T(548) = 1.37	0.170	1.000
Folate	208	91	184.20	139.02	T(573) = 1.703	0.089	1.000	93.00	16.97	T(548) = 1.79	0.075	0.900
Iron	9.6	3.3	7.38	4.40	T(573) = 2.124	***0.034****	0.408	3.50	0.28	T(548) = 2.61	***0.009****	0.108
Calcium	706	293	839.60	194.61	T(573) = 1.179	0.239	1.000	460.00	83.44	T(548) = 1.19	0.236	1.000
Magnesium	215	75	196.60	89.81	T(573) =3.333	***0.001****	***0.012****	128.50	2.12	T(548) = 1.63	0.104	1.000
Potassium	2358	770	2433.20	1149.26	T(573) = 3.038	***0.002****	***0.024****	1576.50	361.33	T(548) = 1.43	0.152	1.000
Iodine	126	87	110.00	14.34	T(573) = 2.71	***0.007****	0.084	61.00	49.50	T(548) = 1.06	0.292	1.000
Selenium	42	20	36.00	20.53	T(573) = 3.435	***0.001****	***0.012****	26.50	3.54	T(548) = 1.10	0.274	1.000
Zinc	7.5	2.8	6.60	4.07	T(573) = 3.297	***0.001****	***0.012****	3.95	0.49	T(548) = 1.79	0.074	0.888

11.5. ADDITIONAL TABLES AND FIGURES OF THE BLOOD CONCENTRATIONS OF THE TEMPERED COHORT.

Table 51. Correlations between dietary intake and blood concentrations within the pre-dialysis, conservatively-managed subgroup (n=46).

Nutrient	Spearman's rho	p-value
Sodium	-0.056	0.724
Potassium	-0.011	0.947
Calcium / albumin-adjusted calcium	0.048	0.763
Magnesium	0.231	0.156
Phosphorus / inorganic phosphate	0.165	0.296
Iron / Ferritin	0.124	0.446
Copper	-0.005	0.976
Zinc	-0.189	0.231
Selenium	0.224	0.154
Manganese / whole blood manganese	0.180	0.301
vitamin A / total retinol	0.039	0.806
Vitamin E: PUFA ratio / total tocopherols	-0.004	0.978
Vitamin B6 / PLP	0.325	0.050*
Folate	0.474	0.005*
Vitamin B12	0.292	0.067
Vitamin C	0.163	0.329

*- *statistical significance, $p<0.05$.*

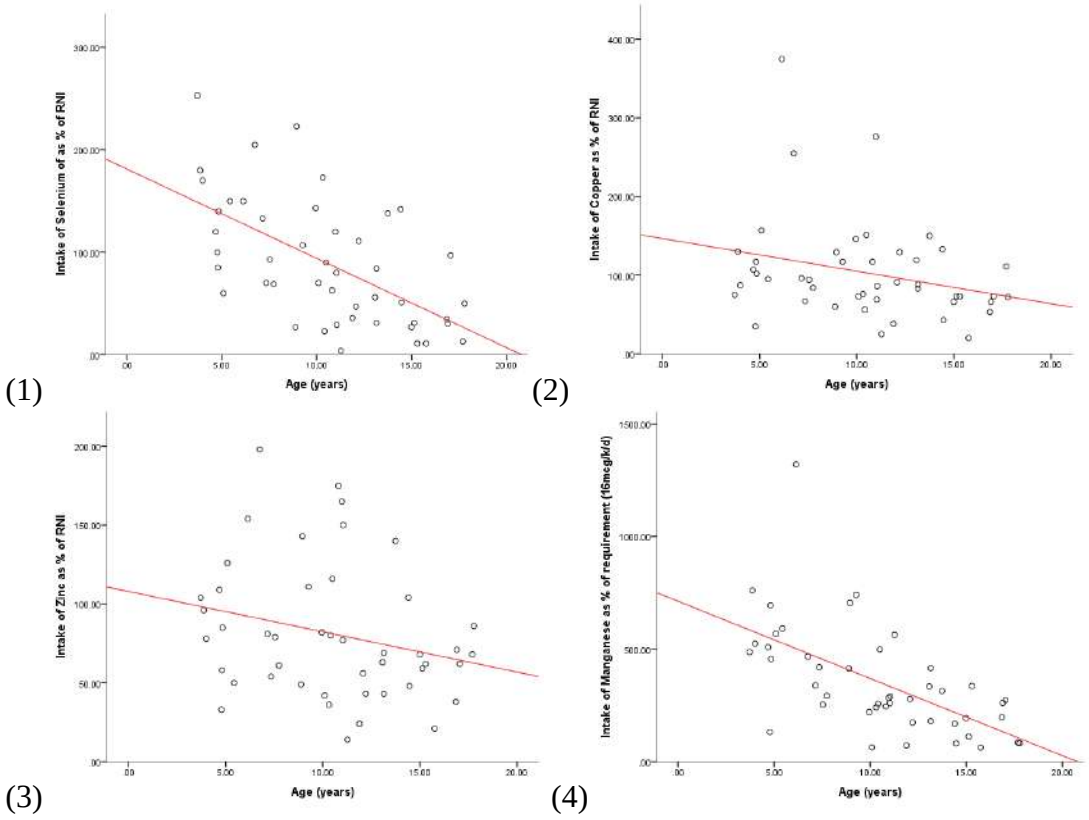
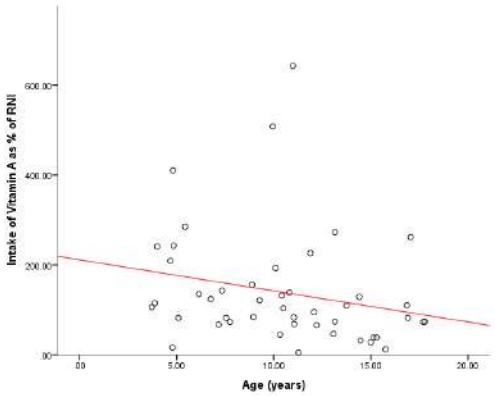


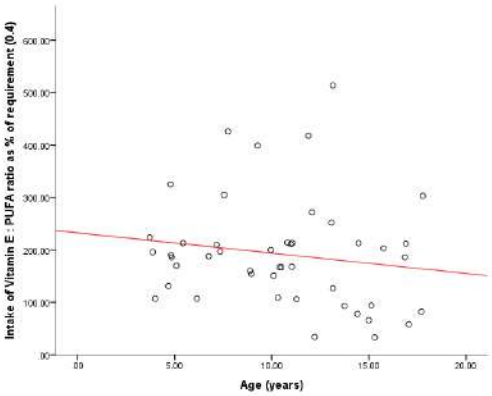
Figure 61. Intake of selenium, copper and manganese as a percentage of RNI compared to age in the pre-dialysis, conservatively-managed subgroup (n=46).

Intake of selenium (1), copper (2), zinc (3) and manganese (4) as percentage of RNI plotted against age in the pre-dialysis, conservatively-managed subgroup. Older children had lower intakes as expressed as a percentage of their requirements, with strongest correlation between age and manganese intake (selenium: S.rho=-0.618; copper: S.rho=-0.324; zinc: S.rho=-0.263; manganese: S.rho=-0.632).

(1)



(2)



(3)

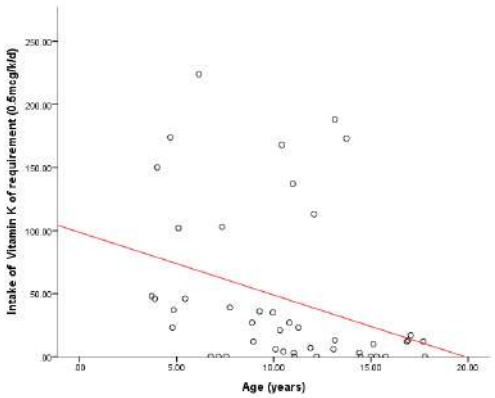
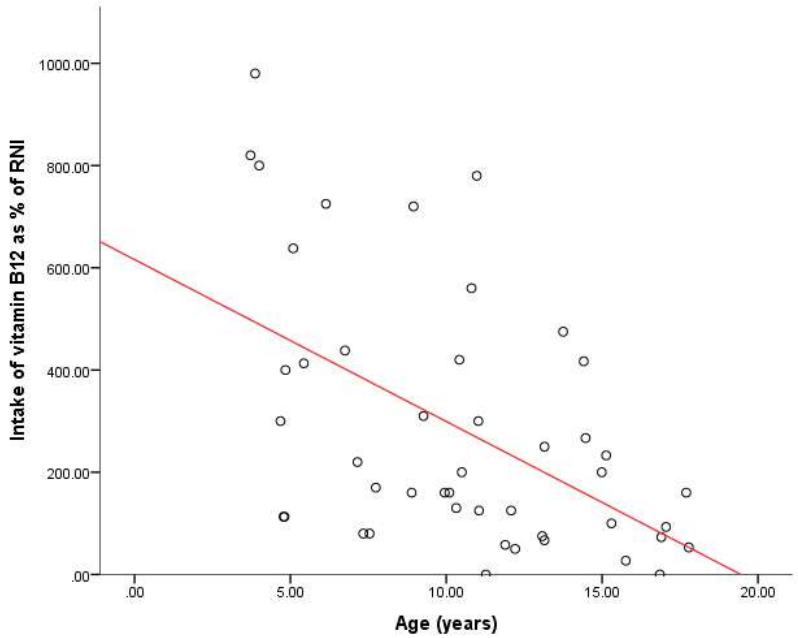


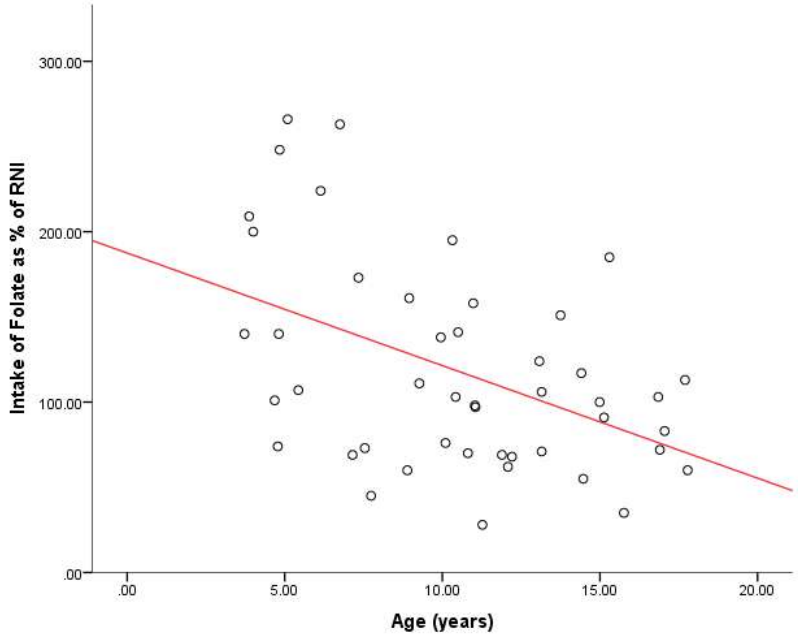
Figure 62. Intake of vitamin A (1) , vitamin E (2), and vitamin K (3) as percentage of requirements plotted against age in the pre-dialysis, conservatively-managed subgroup (n=46).

Outlier of vitamin K intake >2000% excluded from graph. Older children had lower intakes as expressed as a percentage of their requirements, but with less strong correlations than micro-minerals (vitamin A: rho=-0.364; vitamin E: rho=-0.210; vitamin K: rho=-0.479; vitamin K with outlier excluded: rho=-0.450).

Intake of fat-soluble vitamins were lower in older children, with vitamin A and vitamin K intake demonstrating good correlations with age (vitamin A: rho=-0.364, p=0.013; vitamin E: rho=-0.161, p=0.161; and vitamin K: -0.479, p=0.001).



(1)



(2)

Figure 63. Intake of vitamin B12 and folate as a percentage of RNI versus age (n=46).

On analysis of the water-soluble vitamins, only vitamin B12, and folate demonstrated correlation with age (thiamine: rho=0.175, p=0.243; riboflavin: rho=-0.227, p=0.130; niacin: rho=0.238, p=0.112; pantothenic acid: rho=0.113, p=0.455; vitamin B6: rho=0.168, p=0.264; biotin: rho=0.009, p=0.955; vitamin B12: rho=-0.515, p<0.0005; folate: rho=-0.413, p=0.004; vitamin C: rho=-0.180, p=0.231).

Intake of vitamin B12 (1), and folate (2) as percentage of RNI plotted against age in the pre-dialysis, conservatively-managed subgroup. Older children had lower intakes as expressed as a percentage of their requirements. This was not seen in other water-soluble vitamins (vitamin B12: rho=-0.515; folate: rho=-413).

ID#

PedsQL™

Paediatric Quality of Life Inventory
Version 4.0 - English (United Kingdom)

TEENAGER REPORT (ages 13-18)

INSTRUCTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you over the **PAST MONTH** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

*Over the **PAST MONTH**, how much of a **problem** has this been for you...*

ABOUT MY HEALTH AND ACTIVITIES *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than a couple of streets (about 100 metres)	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activities or exercise	0	1	2	3	4
4. It is hard for me to lift heavy things	0	1	2	3	4
5. It is hard for me to have a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I have aches and pains	0	1	2	3	4
8. I feel tired	0	1	2	3	4

ABOUT MY FEELINGS *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ON WITH OTHERS *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting on with other teenagers	0	1	2	3	4
2. Other teenagers do not want to be my friend	0	1	2	3	4
3. Other teenagers tease me	0	1	2	3	4
4. I cannot do things that other teenagers my age can do	0	1	2	3	4
5. It is hard to keep up with other teenagers my age	0	1	2	3	4

ABOUT SCHOOL / COLLEGE *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my school / college work	0	1	2	3	4
4. I miss school / college because of not feeling well	0	1	2	3	4
5. I miss school / college to go to the doctor or hospital	0	1	2	3	4

ID#
Date:

PedsQL™
Paediatric Quality of Life

Inventory

Version 4.0 English (United Kingdom)

PARENT REPORT for TEENAGERS (ages 13-18)

INSTRUCTIONS

On the following page is a list of things that might be a problem for **your teenager**.

Please tell us **how much of a problem** each one has been for **your teenager** during the **past ONE month** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

PedsQL 2

*In the past **ONE month**, how much of a **problem** has your teenager had with …*

PHYSICAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking 100 metres	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activities or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having aches or pains	0	1	2	3	4
8. Feeling tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting on with other teenagers	0	1	2	3	4
2. Other teenagers not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other teenagers	0	1	2	3	4
4. Not being able to do things that other teenagers his or her age can do	0	1	2	3	4
5. Keeping up with other teenagers	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

ID#

PedsQL™

Paediatric Quality of Life Inventory
Version 4.0 - English (United Kingdom)

CHILD REPORT (ages **8-12**)

INSTRUCTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you over the **PAST MONTH** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

There are no right or wrong answers.

*Over the **PAST MONTH**, how much of a **problem** has this been for you...*

ABOUT MY HEALTH AND ACTIVITIES *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than a couple of streets (about 100 metres)	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activities or exercise	0	1	2	3	4
4. It is hard for me to lift heavy things	0	1	2	3	4
5. It is hard for me to have a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I have aches and pains	0	1	2	3	4
8. I feel tired	0	1	2	3	4

ABOUT MY FEELINGS *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ON WITH OTHERS *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting on with other children	0	1	2	3	4
2. Other children do not want to be my friend	0	1	2	3	4
3. Other children tease me	0	1	2	3	4
4. I cannot do things that other children my age can do	0	1	2	3	4
5. It is hard to keep up when I play with other children	0	1	2	3	4

ABOUT SCHOOL *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my school work	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

ID#

Date:

PedsQL™
Paediatric Quality of Life

Inventory

Version 4.0 English (United Kingdom)

PARENT REPORT for CHILDREN (ages 8-12)

INSTRUCTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking 100 metres	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activities or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having aches or pains	0	1	2	3	4
8. Feeling tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting on with other children	0	1	2	3	4
2. Other children not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not being able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

ID#

TM

PedsQL

Paediatric Quality of Life

Inventory

Version 4.0 - English (United Kingdom)

YOUNG CHILD REPORT (ages 5-7)

Instructions for interviewer:

I am going to ask you some questions about things that might be a problem for some children. I want to know how much of a problem any of these things might be for you.

Show the child the template and point to the responses as you read.

If it is not at all a problem for you, point to the smiling face.

If it is sometimes a problem for you, point to the middle face.

If it is a problem for you a lot, point to the frowning face.

I will read each question. Point to the pictures to show me how much of a problem it is for you. Let's try a practice one first.

	Not at all	Sometimes	A lot
Is it hard for you to click your fingers?	☺	☐	☹

Ask the child to demonstrate by clicking his or her fingers to determine whether or not the question was answered correctly. Repeat the question if the child demonstrates a response that is different from his or her action.

PedsQL 2

Think about how you have been doing over the last few weeks. Please listen carefully to each sentence and tell me how much of a problem this is for you.

After reading the item, gesture to the template. If the child hesitates or does not seem to understand how to answer, read the response options while pointing at the faces.

PHYSICAL FUNCTIONING ***(problems with…)***	**Not at all**	**Sometimes**	**A lot**
1. Is it hard for you to walk?	0	2	4
2. Is it hard for you to run?	0	2	4
3. Is it hard for you to play sports or exercise?	0	2	4
4. Is it hard for you to lift big things?	0	2	4
5. Is it hard for you to have a bath or shower?	0	2	4
6. Is it hard for you to help in the home (like picking up your toys)?	0	2	4
7. Do you have aches and pains (***Where?***)	0	2	4
8. Do you ever feel too tired to play?	0	2	4

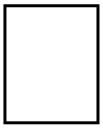
Remember, tell me how much of a problem this has been for you over the last few weeks.

EMOTIONAL FUNCTIONING ***(problems with…)***	**Not at all**	**Sometimes**	**A lot**
1. Do you feel scared?	0	2	4
2. Do you feel sad?	0	2	4
3. Do you feel angry?	0	2	4
4. Do you have trouble sleeping?	0	2	4
5. Do you worry about what will happen to you?	0	2	4

SOCIAL FUNCTIONING ***(problems with…)***	**Not at all**	**Sometimes**	**A lot**
1. Is it hard for you to get on with other children?	0	2	4
2. Do other children say they do not want to play with you?	0	2	4
3. Do other children tease you?	0	2	4
4. Can other children do things you cannot do?	0	2	4
5. Is it hard for you to keep up when you play with other children?	0	2	4

SCHOOL FUNCTIONING ***(problems with…)***	**Not at all**	**Sometimes**	**A lot**
1. Is it hard for you to pay attention in school?	0	2	4
2. Do you forget things?	0	2	4
3. Is it hard to keep up with schoolwork?	0	2	4
4. Do you miss school because of not feeling well?	0	2	4
5. Do you miss school because you have to go to the doctor or hospital?	0	2	4

How much of a problem is this for you?

Not at all	Sometimes	A lot
		

ID#

Date:

PedsQL™
Paediatric Quality of Life

Inventory

Version 4.0 English (United Kingdom)

PARENT REPORT for YOUNG CHILDREN (ages 5-7)

INSTRUCTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking 100 metres	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activities or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores, like picking up his or her toys	0	1	2	3	4
7. Having aches or pains	0	1	2	3	4
8. Feeling tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting on with other children	0	1	2	3	4
2. Other children not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not being able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with school activities	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

ID#

PedsQL
Paediatric Quality of Life

Inventory

Version 4.0 - English (UK)

PARENT REPORT for TODDLERS (ages 2-4)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **PAST MONTH** by circling:

0 if it is **never** a problem
if it is **almost never** a problem
if it is **sometimes** a problem
if it is **often** a problem
if it is **almost always** a problem

*In the **PAST MONTH**, how much of a **problem** has your child had with…*

PHYSICAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in active play and exercise	0	1	2	3	4
4. Lifting heavy things	0	1	2	3	4
5. Bathing	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having aches or pains	0	1	2	3	4
8. Feeling tired	0	1	2	3	4

EMOTIONAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Having trouble sleeping	0	1	2	3	4
5. Worrying	0	1	2	3	4

SOCIAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Playing with other children	0	1	2	3	4
2. Other children not wanting to play with him or her	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

Please complete this section if your child attends nursery or day care

NURSERY/DAY CARE FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Doing the same nursery/day care activities as peers	0	1	2	3	4
2. Missing nursery/day care because of not feeling well	0	1	2	3	4
3. Missing nursery/day care to go to the doctor or hospital	0	1	2	3	4

11.7. ADDITIONAL TABLES AND FIGURES FOR HRQOL CHAPTER.

Table 52. Comparison of PedsQL™ scores between those with obesity (BMISDS>2) and those without in the pre-dialysis, conservatively-managed subgroup.

Domain	Obese (BMI SDS >2), n=6. Mean (±SD)	Not obese BMI SDS <2, n=40. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	69.27 (±15.23)	64.31 (±23.80)	t(10.279) = 0.660	0.524
Emotional	72.50 (±22.75)	62.85 (±21.46)	t(6.778) = -0.981	0.369
Social	56.67 (±30.93)	67.14 (±22.68)	t(6.049) = -0.790	0.459
School	56.67 (±11.25)	59.30 (±17.88)	t(10.477) = -0.472	0.647
Total	67.75 (±15.87)	63.55 (±18.53)	t(7.801) =0.579	0.579
Parent-Proxy				
Physical	65.10 (±16.81)	65.10(±28.04)	t(9.825) = -0.023	0.982
Emotional	67.50 (±23.18)	60.56 (±22.59)	t(6.508) = 0.686	0.517
Social	70.83 (±26.72)	70.48 (±22.54)	t(6.115) = 0.031	0.976
School	61.88 (±13.91)	63.72 (±20.54)	t(8.683) = -0.282	0.785
Total	66.40 (±15.62)	64.37 (±19.76)	t(7.634) = 0.286	0.782

Table 53. Comparison of PedsQLTM scores between those with obesity (MUAC SDS>2) and those without in the pre-dialysis, conservatively-managed subgroup.

Domain	Obese (MUAC SDS >2), n=4. Mean (±SD)	Not obese (MUAC SDS <2), n=42. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	71.88 (±17.40)	64.52 (±23.07)	t(2.645) = 0.683	0.550
Emotional	78.33 (±16.07)	63.18 (±21.82)	t(2.678) = 1.518	0.237
Social	41.67 (±28.43)	67.52 (±22.89)	t(2.228) = -1.533	0.252
School	53.33 (±7.64)	59.36 (±17.46)	t(4.145) = -1.135	0.318
Total	69.20 (±14.02)	63.79 (±18.40)	t(2.631) = 0.625	0.582
Parent-Proxy				
Physical	71.87 (±19.42)	64.64 (±27.40)	t(4.233) = 0.683	0.530
Emotional	65.00 (±27.39)	61.13 (±22.38)	t(3.393) = 0.274	0.800
Social	70.00 (±30.28)	70.57 (±22.43)	t(3.321) = -0.037	0.973
School	58.65 (±17.09)	63.94 (±20.03)	t(3.832) = -0.583	0.593
Total	67.43 (±20.55)	64.37 (±19.24)	t(3.520) = 0.286	0.791

Table 54. Comparison of PedsQLTM scores between those with obesity (WHt>0.5) and those without in the pre-dialysis, conservatively-managed subgroup.

Domain	Obese (WHtR >0.5), n=19. Mean (±SD)	Not obese ((WHtR <0.5)), n=27. Mean (±SD)	t-value (degrees of freedom)	p-value
Child (self-rater)				
Physical	66.85 (±16.44)	63.96 (±26.10)	t(35.983) = 0.419	0.678
Emotional	68.00 (±22.66)	62.01 (±21.13)	t(28.536) = 0.818	0.420
Social	61.50 (±25.25)	68.08 (±23.32)	t(28.336) = -0.809	0.425
School	64.00 (±10.59)	55.54 (±19.51)	t(36.000) = 1.534	0.134
Total	66.62 (±14.58)	62.65 (±20.08)	t(35.494) = 0.707	0.484
Parent-Proxy				
Physical	64.75 (±21.82)	65.63 (±30.06)	t(43.934) = -0.115	0.909
Emotional	66.84 (±21.81)	57.69 (±22.65)	t(39.814) = 1.380	0.175
Social	73.16 (±23.41)	68.67 (±22.62)	t(38.051) = 0.650	0.520
School	65.88 (±16.90)	61.79 (±21.58)	t(43.422) = 0.720	0.475
Total	66.74 (±16.86)	63.15 (±20.77)	t(43.009) = 0.646	0.522

In the pre-dialysis, conservatively-managed subgroup, comparison of PedsQL^{TM} scores between those children / young people with obesity as defined by BMI SDS (>2SD); MUAC SDS (>2SD) or waist-to-height ratio (>0.5) did not demonstrate any differences; including on component analysis.

11.8. MODIFIED APPETITE ASSESSMENT QUESTIONNAIRE

Southampton Children's Hospital | University of Southampton | University Hospital Southampton NHS

Modified CNAQ-SNAQ Questionnaire:

Hospital Number: Date of appetite assessment:

1	Appetite is:	Very Poor	Poor	Good	Very Good		Scoring

2	When eating:	Patient feels full after eating only a few mouthfuls	Patient feels full after eating about half a meal	Patient feels full after eating most of the meal	Patient always completes meals and can manage a pudding		Scoring

3	My *normal* meal pattern is:	I eat 1 meal a day	I eat 2 meals a day	I eat 3 meals a day	I eat 3 meals a day plus snacks		Scoring

4	My *current* meal pattern is:	I eat 1 meal a day	I eat 2 meals a day	I eat 3 meals a day	I eat 3 meals a day plus snacks		Scoring

5	How do portion sizes compare to friends/siblings of similar age (+/- 2years)?	Less than half	About half	Slightly Less	Same or more		Scoring

	Things that put me off eating:	Food tastes different to what it used to	
		I feel too full	
		I don't have enough time	
		I worry about putting on weight	
		It makes me feel sick	
		Other.....	

7	Dietician's perception of patient's appetite:	Very Poor	Poor	Good	Very Good		Scoring

Modified from:
Wilson et al. "Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents" *Am J Clin Nutr* 2005;82:1074–81

11.9. CONSENT AND ASSENT FORMS

NIHR Southampton Centre for Biomedical Research
E Level, MP 113
University Hospital Southampton NHS Foundation Trust
Tremona Road
Southampton
SO16 6YD
caroline.anderson@uhs.nhs.uk
02381205330

CONSENT FORM

Participant Identification Number for this trial:

Title of Project: Trace Element Malnutrition in Paediatric Renal Disease (TEMPeReD)

Please initial box

1. I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I have been given a copy to keep. ☐
2. I understand participation of my child is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records. ☐
4. I understand that the information collected about my child will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
5. I agree to my child's General Practitioner being informed of my participation in the study. ☐
6. I agree that the samples will be kept by the research team. My child's blood and urine samples have been donated as gifts and therefore these anonymised samples may be used for ethically-approved future research. ☐
7. I agree to my child taking part in the above study. ☐

Name of Participant's Parent/guardian	Date	Signature
Name of Person taking consent	Date	Signature

ASSENT FORM

Participant Identification Number for this trial:

Title of Project: Trace Element Malnutrition in Paediatric Renal Disease (TEMPeReD)

Please initial box

1. I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I have been given a copy to keep. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐

5. I agree to my General Practitioner being informed of my participation in the study. ☐

6. I agree that the samples will be kept by the research team. My blood and urine samples have been donated as gifts and therefore these anonymised samples may be used for ethically-approved future research. ☐

7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

11.10. Blood Concentrations of Vitamins and Minerals for Different Treatment Modality Subgroups.

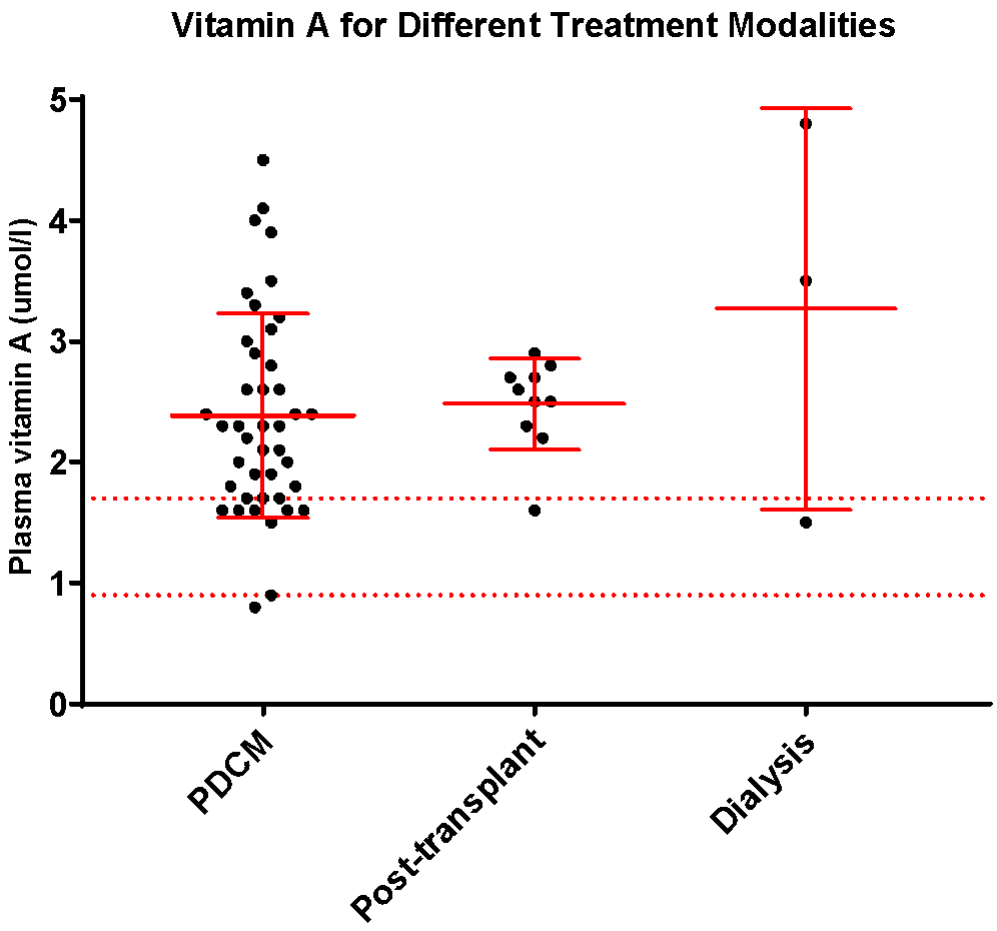


Figure 64. Plasma vitamin A concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities, with a significant proportion of children and young people having plasma vitamin A concentrations above the normal reference range.

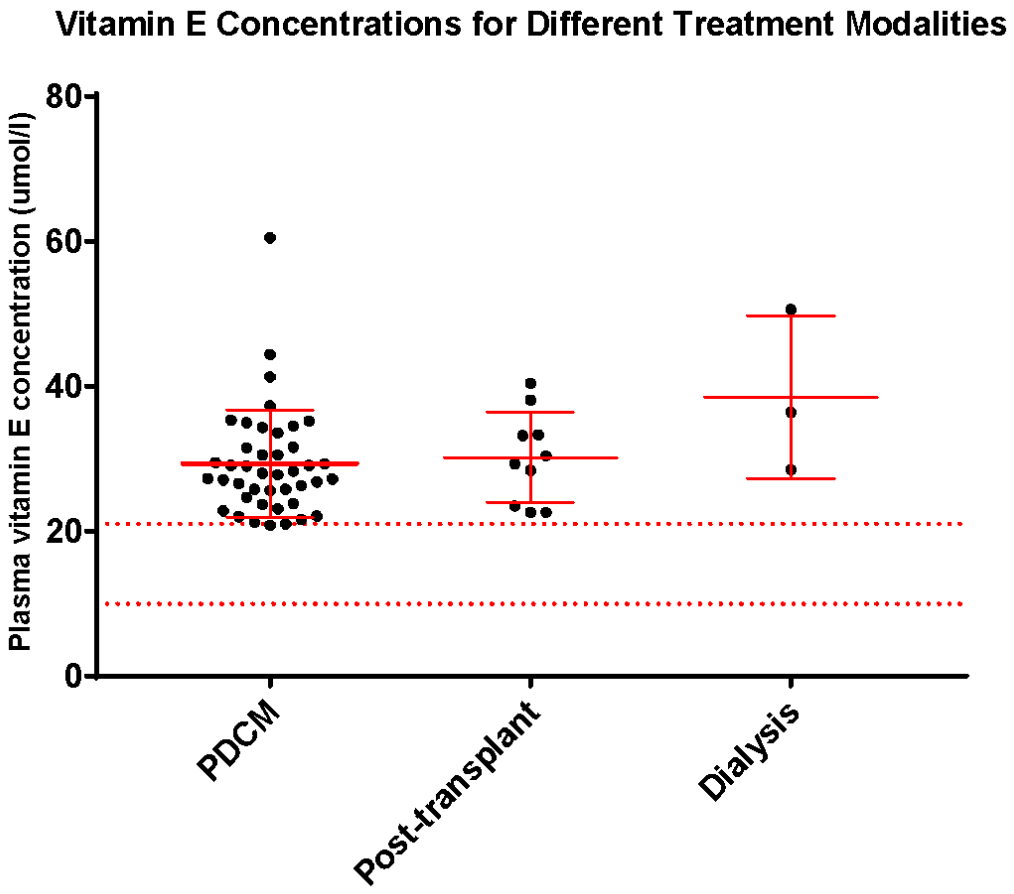


Figure 65. Plasma vitamin E concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities, with a significant proportion of children and young people having plasma vitamin E concentrations above the normal reference range; including all post-transplant and all dialysis patients above the reference range.

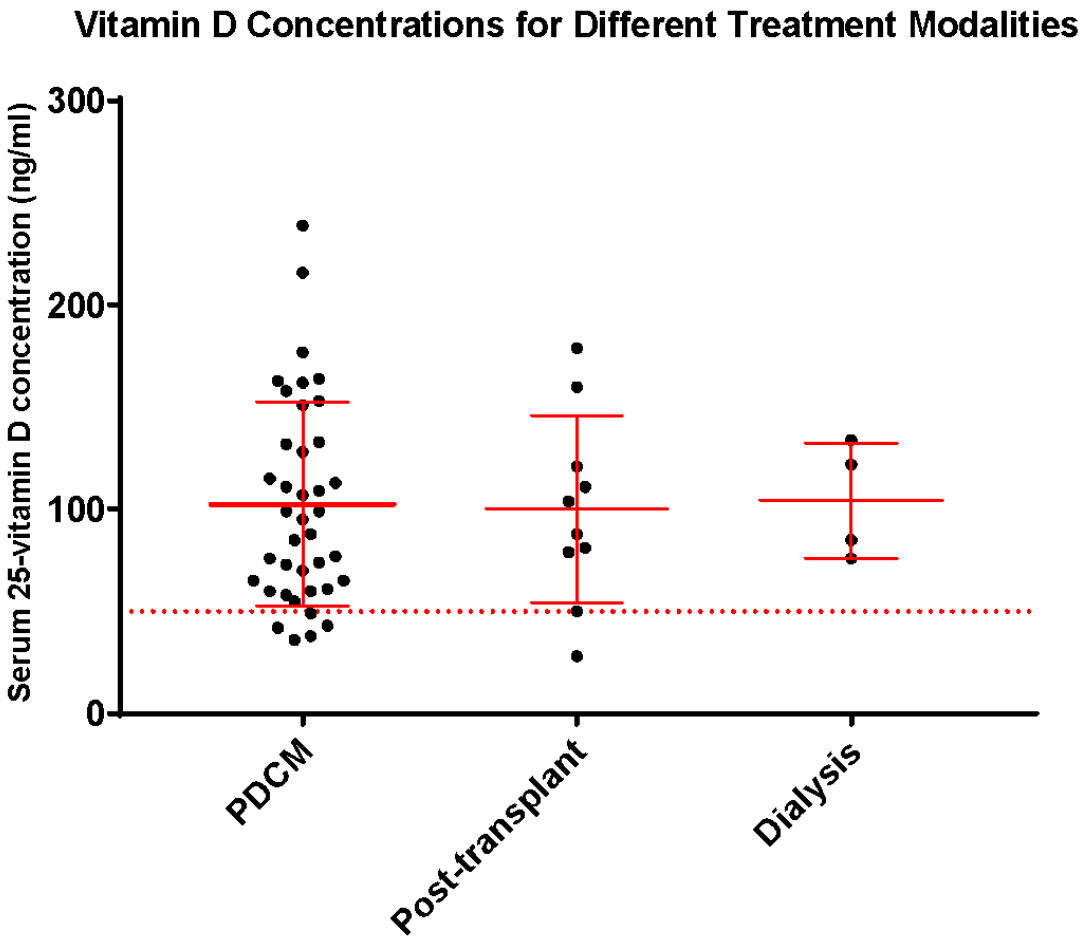


Figure 66. Serum 25-vitamin D concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted line represents the lower limit of the normal reference range for the laboratory (there is no upper limit). There were no significant differences between modalities for vitamin D concentrations.

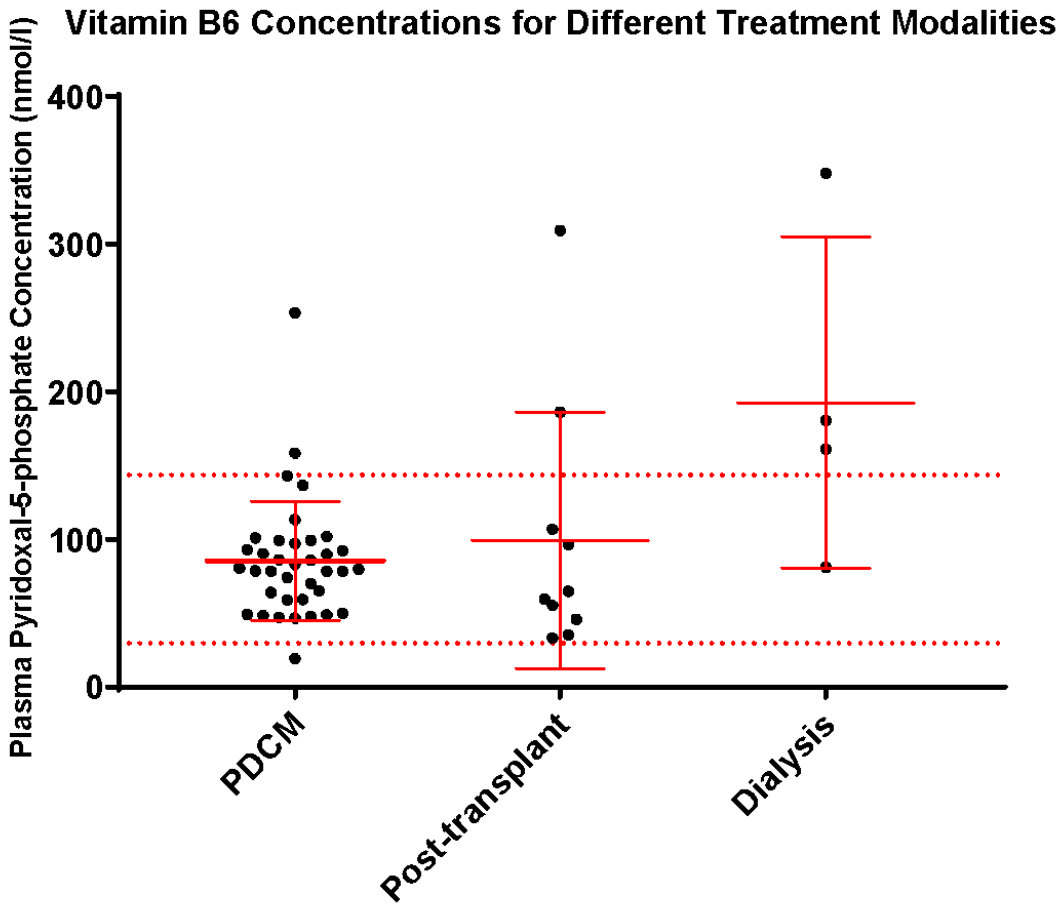


Figure 67. Plasma Pyridoxal 5-phosphate concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities for vitamin B6 (pyridoxal-5-phosphate) concentrations. Several children and young people had concentrations greater than the normal reference range <despite the absence of supplementation>. This may represent increased flux through the transport pool rather than true hypervitaminosis, and warrants further exploration to better understand potential altered metabolism of the vitamins in the context of chronic kidney disease, immunosuppressive regimens, and in dialysis.

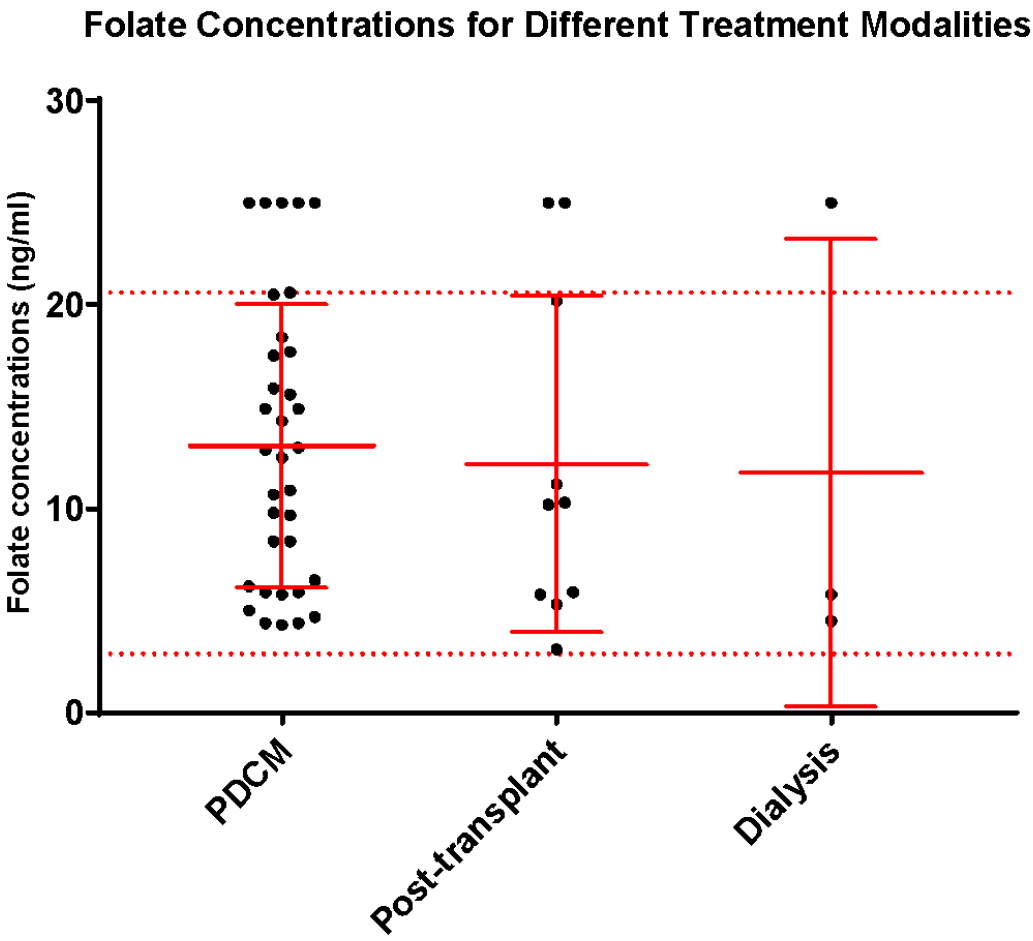


Figure 68. Plasma folate concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the lower limit of the normal reference range for the laboratory. There were no significant differences between modalities for folate concentrations.

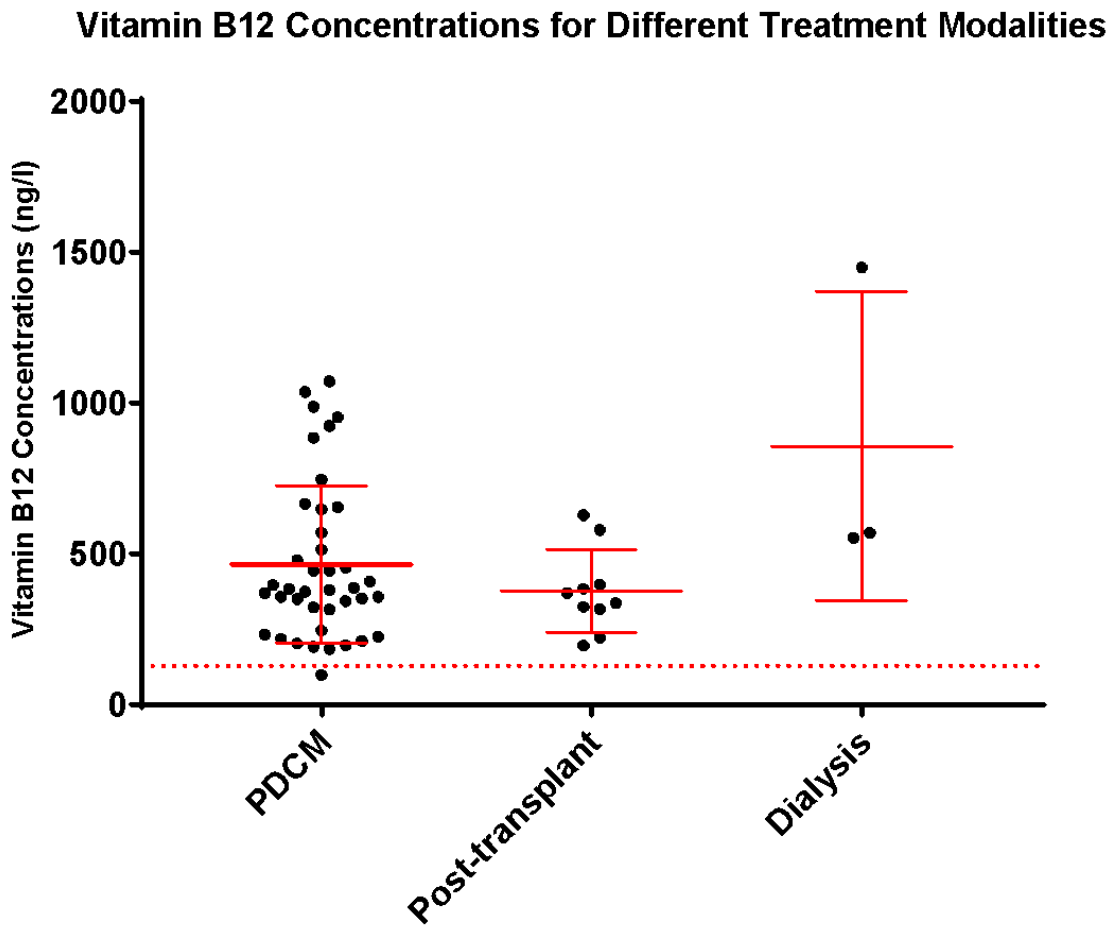


Figure 69. Vitamin B12 concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted line represents the lower limit of the normal reference range for the laboratory (there is no upper limit). There were no significant differences between modalities for vitamin B12 concentrations.

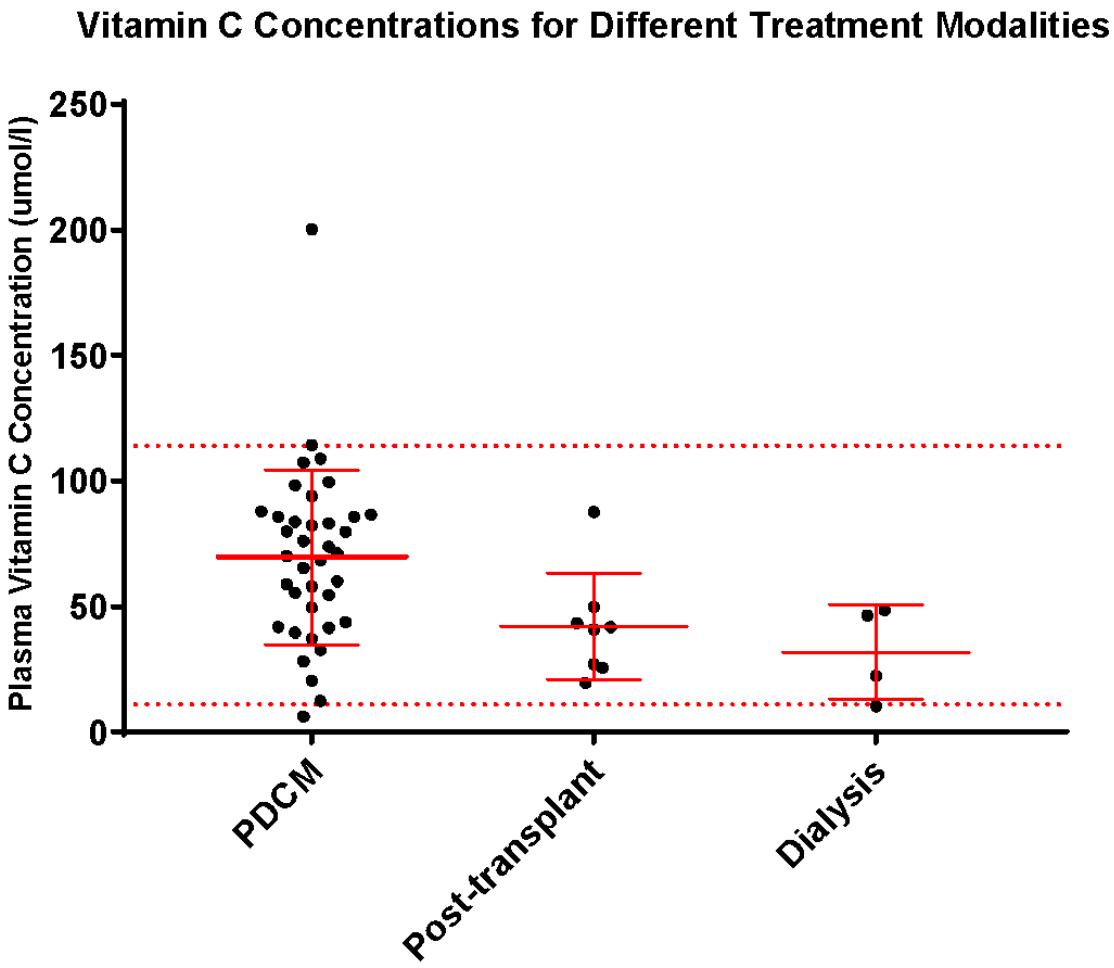


Figure 70. Plasma vitamin C concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities for vitamin C concentrations.

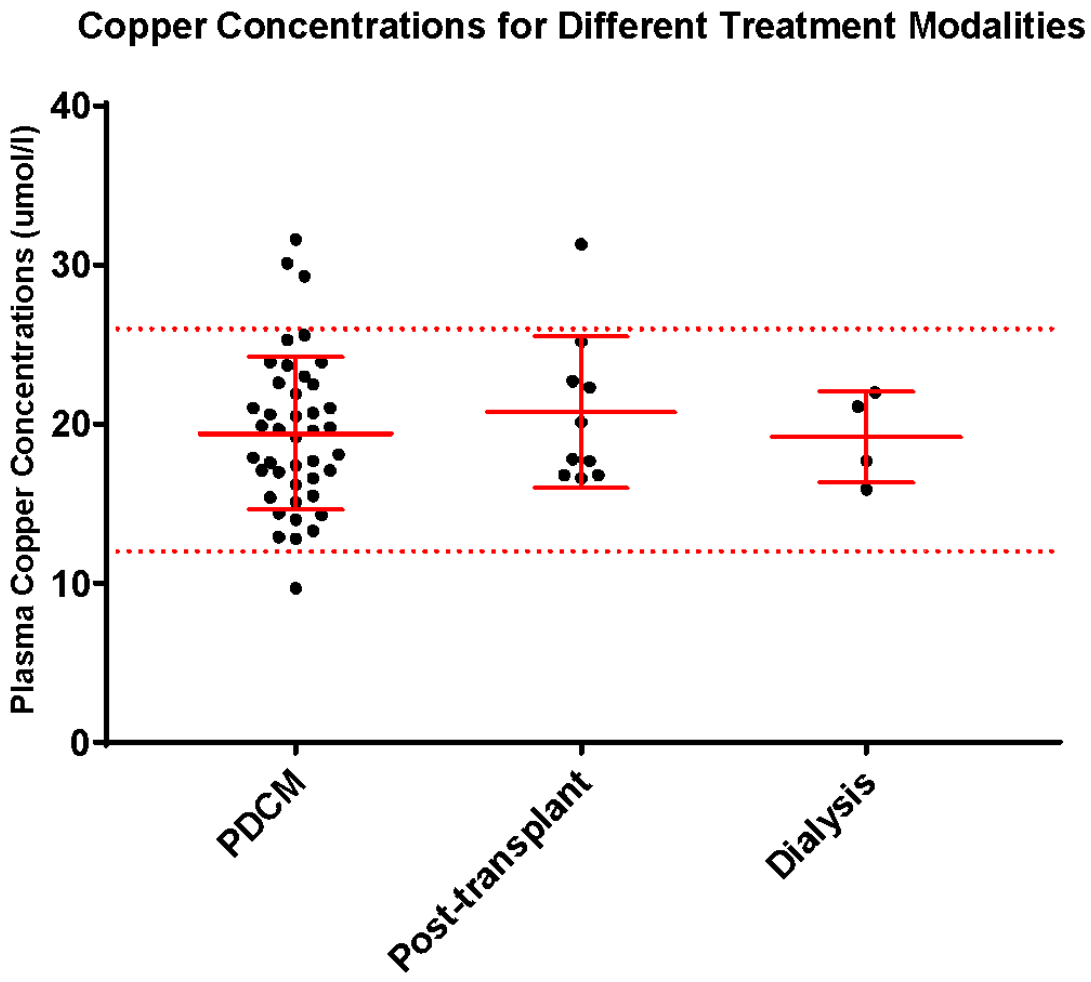


Figure 71. Plasma copper concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities for copper concentrations.

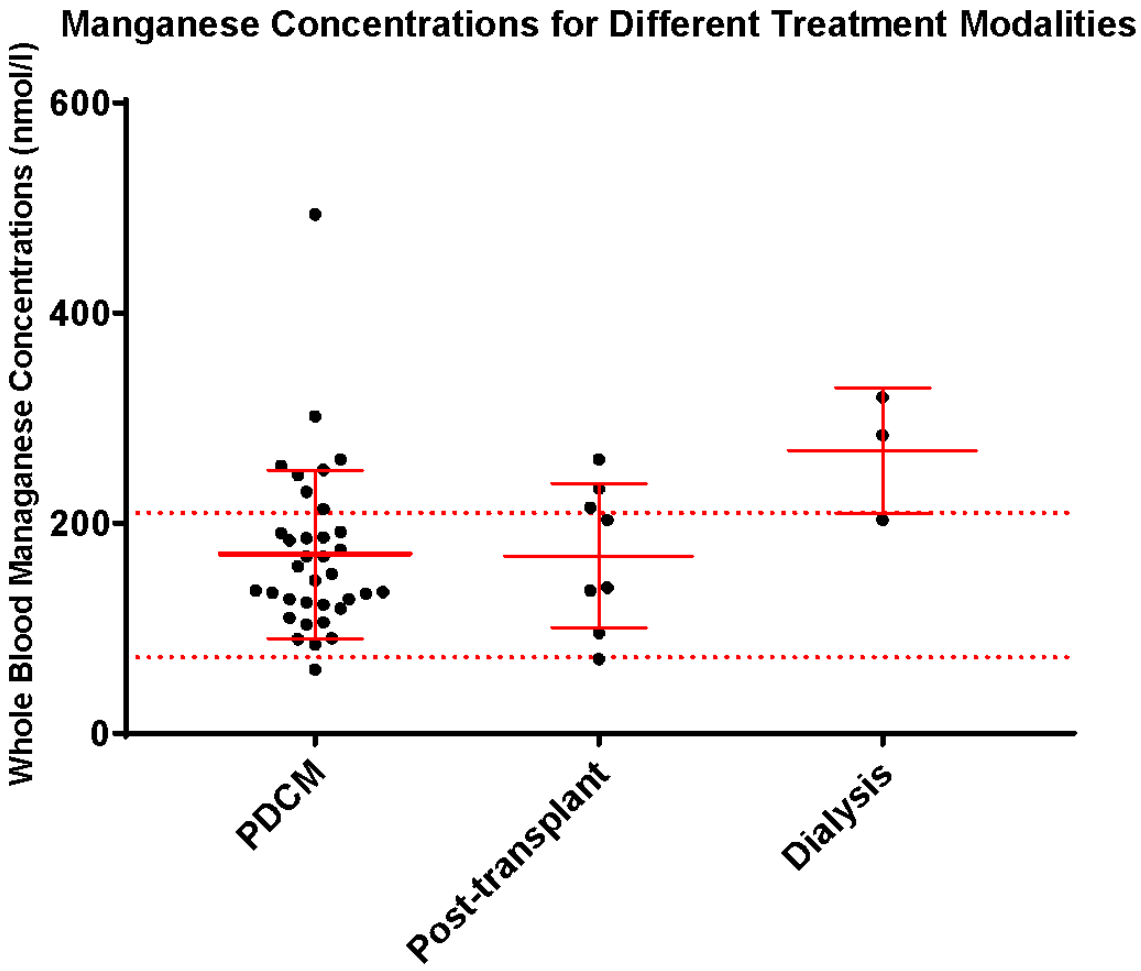


Figure 72. Whole blood manganese concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. The dialysis subgroup's concentrations were significantly higher than the pre-dialysis, conservatively-managed (PDCM) subgroup (Median concentrations: 284 versus 152 nmol/l; Mann-Whitney Test statistic = 11; p = 0.019). The reason for this could be related to the iron status of the subjects.

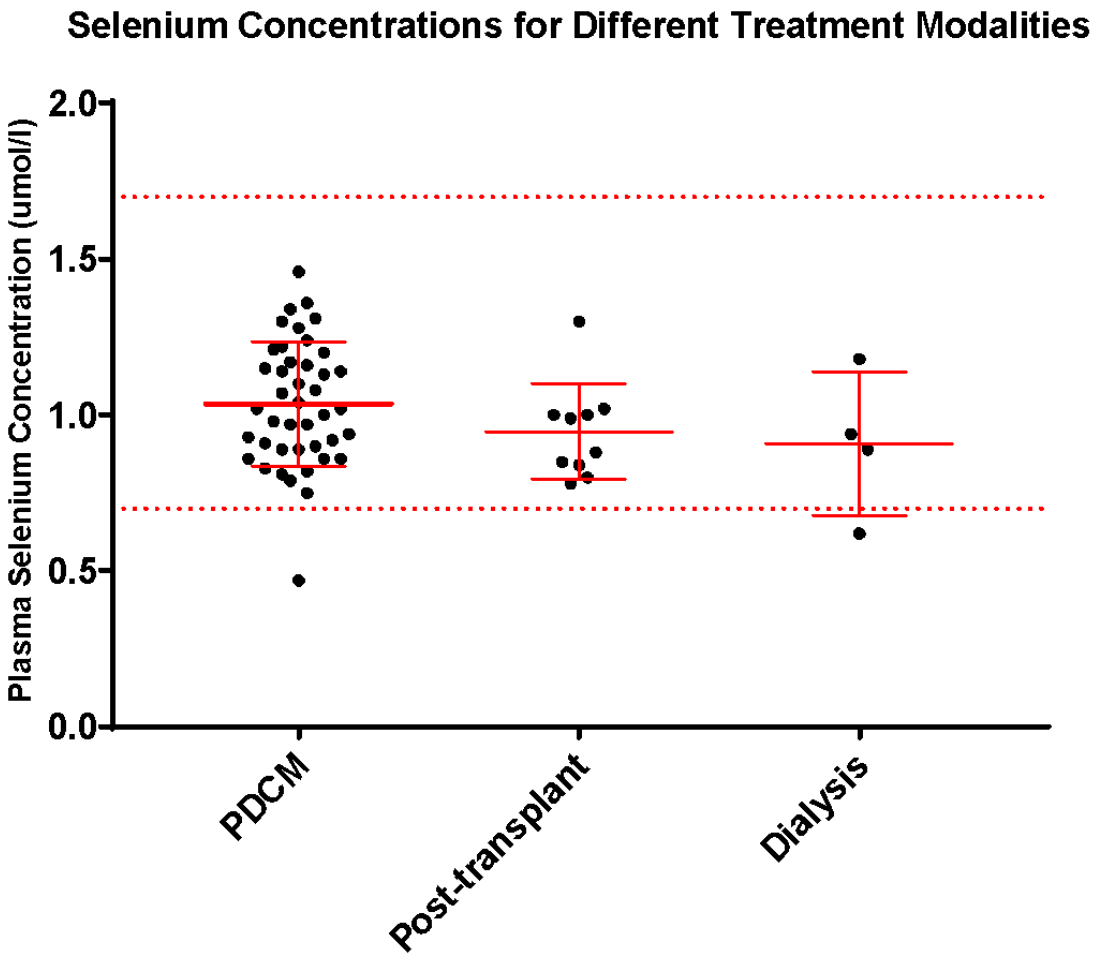


Figure 73. Plasma selenium concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities for plasma selenium concentrations.

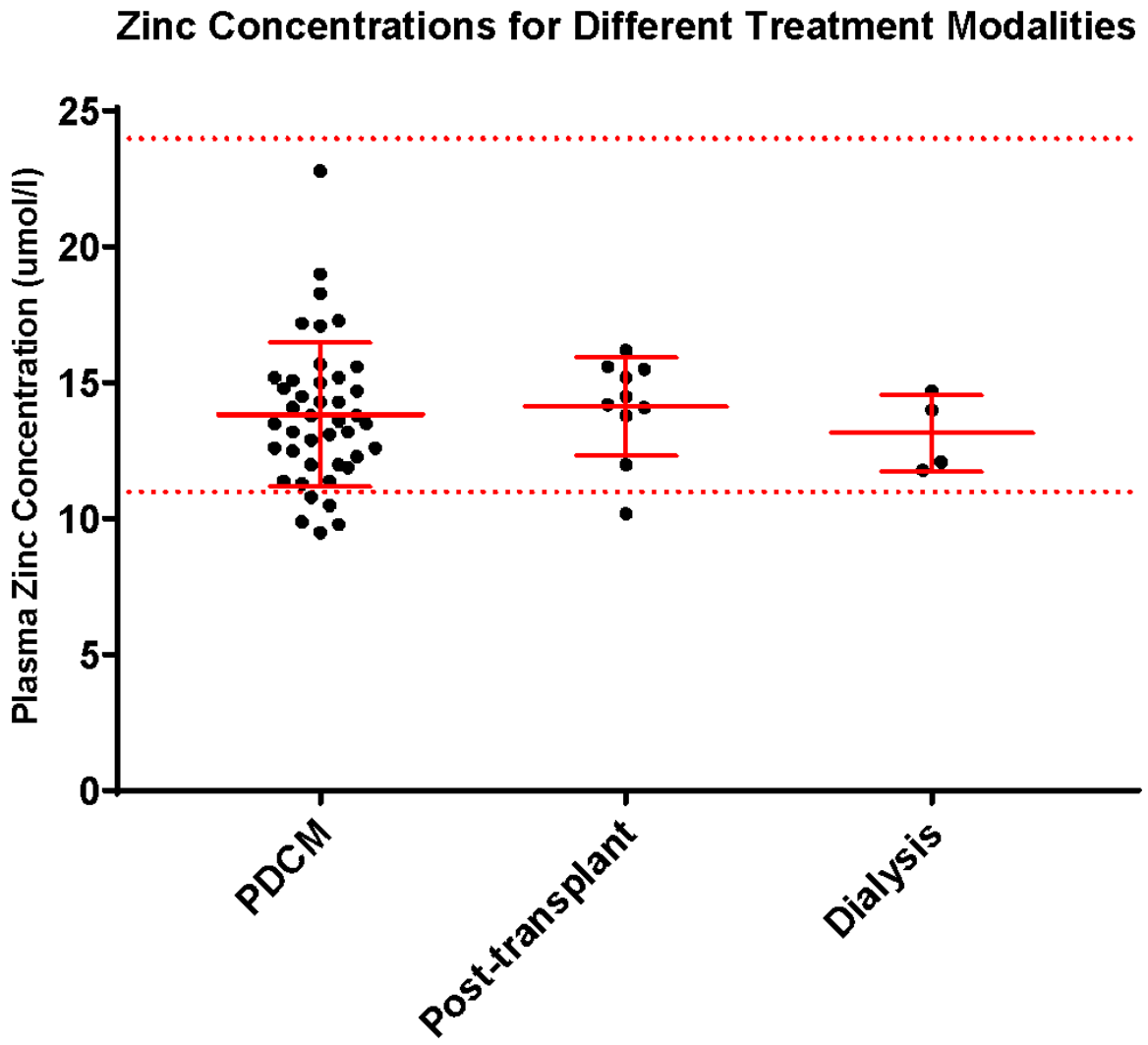


Figure 74. Plasma zinc concentrations in the TEMPeReD cohort divided into different treatment modalities.

PDCM = 46, Post-transplant = 10, dialysis = 3. Mean concentrations and standard deviations are depicted by error bars. Red dotted lines represent the normal reference range for the laboratory. There were no significant differences between modalities for plasma zinc concentrations.

11.11. What is a Food for Special Medicinal Purposes?

Legal interpretation from Loyens and Loeff (359).

"FSMP is specially processed or formulated food intended for the dietary management, under medical supervision, of patients (including children) who suffer from a disease, disorder or medical condition and whose dietary requirements cannot be met by modifying a normal diet only.

11.11.1. The definition includes different elements:

FSMP vs other products: *FSMP is food and not a medicinal product. The Notice specifies that the definition of FSMP has to be interpreted narrowly in order to distinguish it from foods other than FSMP such as food supplements or fortified foods which supplement the normal diet. In addition, a novel food ingredient that has been authorised to be used in FSMP will not automatically classify the final product as FSMP unless the product complies with the FSMP definition;*

'Specially processed' or 'specially formulated': *These concepts refer respectively to the manufacturing stage where substantial alteration of the initial product takes place (e.g. giving a specific consistency or viscosity to a product for the dietary management of dysphagia) and the development stage before the manufacturing (e.g. forecasting specific levels of energy and nutrients for products for patients with kidney disease). Products that are neither specially processed nor formulated should be excluded from the FSMP definition. This does not, however, preclude FSMP from containing ingredients of a 'natural composition';*

'Dietary management': *FSMP is intended to provide nutritional support to patients who suffer from a specific disease, disorder or medical condition which leads to either (a) 'limited, impaired or disturbed ability to take, digest, absorb, metabolise or excrete normal foods, nutrients or metabolites', or (b) 'other medically-determined nutrient requirements'. These conditions can be for instance the inability to digest or absorb sufficient foods/nutrients (e.g. short bowel syndrome) or the inability to excrete certain nutrients or their metabolites (e.g. phosphate and potassium for patients with kidney disease).*

When placing products on the market as FSMP, both FBOs and national authorities have to conduct their assessment on a case-by-case basis. Concretely, this implies that they have to assess how 'impossible, impractical, unsafe or nutritionally/clinically disadvantageous' it is for the targeted patients to satisfy their nutritional needs by exclusively modifying their normal diet. If their nutritional needs can be exclusively satisfied with foods other than FSMP, a product cannot be placed on the market as FSMP for the dietary management of those patients;

'Under medical supervision': *Because the consumers of FSMP are patients, their use must take place under medical supervision. In the context of the FSMP legislation 'patients' should be understood as 'people suffering from specific diagnosed diseases, disorders or medical conditions, who as a result of such disease, disorder or medical condition need to consume FSMP'. This means that products for consumers who do not suffer from any disease, disorder or medical condition or products which can be used without medical supervision for the dietary management of patients should not be marketed as FSMP. It is also important to note that although the role of healthcare professionals in recommending and supervising the use of FSMP is key. They have the full discretion to choose the most appropriate follow-up of their patients and may recommend the use of other products than FSMP. Therefore, the recommendation of a healthcare professional cannot be decisive to classify a product a FSMP because only a detailed case-by-case analysis by the FBO of the FSMP definition allows to classify a product as FSMP;*

'Modification of a normal diet': This concept refers to any adjustment to the diet by consuming foods other than FSMP. The aim of food supplements or fortified foods is to supplement or add nutrients or other substances to the normal diet. By supplementing or adding to the normal diet food supplements and fortified foods can be qualified as normal foods and apt to modify the normal diet. In addition, when analysing if the use of a specific product is safer or more practical than the exclusive use of food other than FSMP or if it has a clinical or nutritional advantage for the patient, FBOs and national authorities should take into account factors such as the severity of the disease, disorder or medical condition or the role of the specific product.

Although the definition includes different elements, the Notice clearly says that it must be interpreted in its entirety.

11.11.1.1. Categories of FSMP

FSMP legislation lists three categories in which FSMP can be classified:

Nutritionally complete food with a standard nutrient formulation used either orally or by enteral tube which contains all the necessary nutrients at appropriate levels and may be used as the only source of nutrition for patients, e.g. enteral formulas for gastroenterological conditions;

Nutritionally complete food with a nutrient-adapted formulation specific for a disease, disorder or medical condition which contains all the necessary nutrients at appropriate levels and may be used as the only source of nutrition for patients, e.g. MCT (Medium Chain Triglycerides) -containing formulas for malabsorption conditions;

Nutritionally incomplete food with a standard formulation or a nutrient-adapted formulation specific for a disease, disorder or medical condition which either does not contain all the necessary nutrients or the appropriate quantities to be used as the only source of nutrition for patients and is used in addition to normal foods, adapted diet or other FSMP, e.g. a protein substitute for metabolic conditions.

Conclusion

All in all, the Commission's Notice on FSMP attempts to give FBOs a sort of a check-list of required data they should compile in order to demonstrate that their product falls within the scope of FSMP legislation. FBOs should, however, carry out their assessment on a case-by-case basis. The necessary data should objectively demonstrate that 'the specific group of patients suffering from a disease, disorder or medical condition for which the product is intended have nutritional needs that are impossible, impractical or unsafe or nutritionally or clinically disadvantageous to satisfy through the exclusive consumption of food other than FSMP'. People for whom FSMP is intended should be easily distinguishable from those who are not in need of FSMP. When assessing the possibility to modify the normal diet through food other than FSMP, FBOs should refer to a typical person who suffers from the disease, disorder or medical condition for which the FSMP is considered. Finally, EFSA's scientific and technical guidance on FSMP can also be helpful for FBOs when deciding whether their product is correctly marketed as FSMP or not".

11.12. FAMILY FEEDBACK ABOUT THE NOVEL FSMP

Positive comments	Negative comments	Reasons for not taking	Suggested improvements
"Easy to drink"	"Took a while to dissolve"	Family away and forgot to take [FSMP] with them (5 days)	"Improve the taste"
"I really didn't like it to begin with but I've got used to it over time"	"Didn't like the taste"	"Refused to drink it"	"make into tablet form"
"I liked the bubbly sensation on my lips"	"Taste is quite bitter"	"Forgot"	"change flavour"
"It was quick to make"	"Some sediment at the bottom of the cup"		"x doesn't like it maybe tablet form maybe better"
"I felt like I had a lot more energy and didn't feel so ill"	"It tasted horrible by the end"		"Not as soluble as we'd have liked often left sediment in the bottom despite rigorous shaking"
"making of the drink was quick and easy"	"Quantity was too much"		"[They]'d prefer a tablet"
"I had piece of mind throughout that x was getting much needed vitamins etc"	"it's gross, too strong"		"Change to shape of the container so it's easier to mix [with a spoon]"
	"too strong and lumpy even when shake[n]"		"Different flavours, i.e., Blackcurrant. Not everyone likes orange"
	"did not like to taste, screamed throughout and didn't finish full amount"		"x found it a little better when made with ice-cold water"
	"he does not like the 'bits' that remain no matter how much I shake it"		
	"Tastes horrible"		
	"very strong tasting"		
	"Didn't really enjoy the taste"		
	"didn't take it because takes up my fluid [allowance] and doesn't taste nice"		

11.13. Full Analysis of Pre- and Post-Intervention with a Novel FSMP.

Table 55. Comparison of measurement at baseline and at visit 2 (90 day of trial of the FSMP).

	Baseline	Visit 2	
Anthropometry			
HtSDS	-2.14 (SD±1.23)	-2.08 (SD±1.29)	t(6)=-0.990; p=0.360
WtSDS	-1.32 (SD±1.37)	-1.37 (SD±1.29)	t(6)=0.474; p0.652
BMISDS	0.01 (SD±1.29)	-0.09 (SD±1.16)	t(6)=0.640;p=0.546
MUAC (cm)	21.89 (SD±6.20)	21.97 (SD±4.69)	t(6)=-0.061; p=0.954
Appetite			
ModSNAQ Score	18.71 (SD±4.11)	20.00 (SD±5.06)	t(5)=-0.382; p=0.718
Bloods Results			
Sodium (mmol/l)	137.29 (SD±2.29)	136.86 (SD±1.21)	t(6)=0.812; p=0.448
Potassium (mmol/l)	4.17 (SD±0.72)	4.07 (SD±0.50)	t(6)=0.544; p=0.606
cCalcium (mmol/l)	2.41 (SD±0.10)	2.37 (SD±0.08)	t(6)=1.332; p=0.231
Magnesium (mmol/l)	0.90 (SD±0.14)	0.80 (SD±0.09)	t(6)=2.802;p=0.031*
Phosphate (mmol/l) ǂ	1.29 (IQR±0.77)	1.39 (IQR±0.29)	Statistic=3.00; p=0.063
Ferritin (ug/l) ǂ	55.00 (IQR±154.00)	50.00 (IQR±205.00)	Statistic=19.00; p=0.398
Haemoglobin (g/l)	127.29 (SD±19.61)	120.29 (SD±28.55)	t(6)=1.283; p=0.247)
Vitamin B12 (ng/l) ǂ	554.00 (IQR±202.00)	489.00 (IQR±277.00)	Statistic=11.00; p=0.612
Folate (ng/ml) Θ	17.50	25.00	-
Vitamin D (nmol/l) ǂ	76.00 (IQR±74.00)	81.00 (IQR±44.00)	Statistic=17.00; p=0.612
Vitamin A (µmol/l) ǂ	2.20 (IQR±1.90)	2.10 (IQR±2.50)	Statistic=17.00; p=0.172
Vitamin E (µmol/l)	28.59 (SD±4.45)	31.14 (SD±7.79)	t(6)=-1.386; p=0.215
Copper (µmol/l)	20.86 (SD±3.48)	17.29 (SD±3.41)	t(6)=4.403; p=0.005*
Selenium (µmol/l)	1.00 (SD±0.21)	1.16 (SD±0.25)	t(6)=-3.414; p=0.014*
Zinc (µmol/l)	14.34 (SD±2.41	14.37 (SD±2.19)	t(6)=-0.035; p=0.973
Vitamin C (µmol/l) ǂ	46.50 (IQR±55.60)	47.70 (IQR±41.50)	Statistic=18.00; p=0.499
Manganese (nmol/l)	165.2 (SD±69.77)	209.80 (SD±102.44)	t(6)=-1.306; p=0.262
Vitamin B6 (nmol/l) ǂ	78.90 (IQR±110.40)	142.25 (IQR±197.45)	Statistic=14.00; p=0.463
PedsQL™ Scores			
Child (self-rater):			
Physical Domain	62.06 (SD±20.12)	54.69 (SD±23.61)	t(4)=0.104; p=0.922
Emotional Domain	70.00 (SD±27.16)	68.50 (SD±21.77)	t(4)=0.213; p=0.842
Social Domain	64.17 (SD±28.53)	69.00 (SD±11.94)	t(4)=-0.463; p=0.667
School Domain	66.00 (SD±21.04)	56.00 (SD±16.73)	t(4)=1.265; p=0.275
Total Child (self-rater) Score	65.57 (SD±22.95	63.61 (SD±13.90	t(4)=0.278; p=0.795
Parent-Proxy:			
Physical Domain	60.00 (SD±25.33)	70.00 (SD±26.20)	t(4)=-4.828; p=0.008*
Emotional Domain	63.50 (SD±25.35)	59.00 (SD±14.32)	t(4)=0.510; p=0.637
Social Domain	63.00 (SD±26.36)	60.63 (SD±24.69)	t(4)=0.361; p=736
School Domain	65.00 (SD±8.66)	57.00 (SD±19.87)	t(4)=1.064; p=0.347
Total Parent-Proxy Score	62.31 (SD±18.46)	63.48 (SD±17.81)	t(4)=-0.338; p=0.752

*Abbreviations: SD – standard deviation; IQR – interquartile range. *Statistical significance; p<0.05.* ǂ *- non-parametric, mean and IQR reported, and difference compared with Wilcoxon Signed Rank Test.* Θ *- Folate values once >25ng/ml are not expressed as integers; median reported.*

11.14. PUBLICATIONS IN PEER-REVIEWED JOURNALS

Harmer M, Wootton S, Gilbert R, Anderson C (2019) **Association of nutritional status and health-related quality of life in children with chronic kidney disease.** *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation* 28:1565-1573 (360).

Harmer M, Wootton S, Gilbert R, Anderson C (2019) **Vitamin B6 in Pediatric Renal Transplant Recipients.** *Journal of renal* nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 29:205-208 (361).

Quality of Life Research (2019) 28:1565–1573
https://doi.org/10.1007/s11136-019-02104-0

Association of nutritional status and health-related quality of life in children with chronic kidney disease

Matthew Harmer[1,2,4] · Stephen Wootton[2,4] · Rodney Gilbert[1,2] · Caroline Anderson[1,3,4]

Accepted: 8 January 2019 / Published online: 12 January 2019

Abstract

Purpose Health-related quality of life (HRQoL) is an important, patient-centred measure. Although nutritional status is altered in children with CKD, the impact of nutritional status on HRQoL in this population has not been explored. The aims of this study are to report the HRQoL scores as assessed by the validated PedsQL™ questionnaire and to explore the relationship of HRQoL scores to markers of nutritional status. It will also examine the concordance between the scores of the child and their parent/carer.

Methods A single-centre, cross-sectional, observational study was performed exploring the markers of nutritional status (anthropometry—including presence of obesity, micronutrient status and appetite) and HRQoL and assessed by the PedsQL™ questionnaire in children aged 3–18 years with pre-dialysis, conservatively managed CKD.

Results A total of 46 children were recruited, with a mean age of 10.5 years. HRQoL scores were lower than in healthy controls throughout all domains. Lower scores were associated with short stature and poor appetite. Markers of obesity or micronutrient status were not associated with HRQoL scores.

Discussion Nutritional status impacts upon HRQoL. Further study is needed to evaluate how changing nutritional status may affect HRQoL in children with CKD, and this may be used to facilitate the development of patient-centred treatment goals and plans.

Keywords Health-related quality of life · Children · Chronic kidney disease · Nutrition

Background

Chronic kidney disease (CKD) is an increasing problem in the UK and worldwide [1]. The disease and its management can have significant impacts upon the individual and their family, being associated with increased mortality and morbidity, and in children impaired growth [2].

Health-related quality of life (HRQoL) is an individual's subjective perception of the impact of health status, including disease and treatment, on physical, psychological and social functioning [3]. Children with CKD, even those with disease on the milder end of the spectrum, have poorer HRQoL [4–7]. The physical and psychological impact of both the disease process itself and its management make CKD a particularly challenging condition with pervasive symptoms of nausea and lethargy, multiple clinic attendances and investigations, strict dietary modification, multiple medications (and their frequent adjustments), changes in physical health due to complications of CKD and

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11136-019-02104-0) contains supplementary material, which is available to authorized users.

✉ Matthew Harmer
matthew.harmer@uhs.nhs.uk

[1] Southampton Children's Hospital, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton SO16 6YD, UK

[2] University of Southampton, University Road, Southampton SO17 1BJ, UK

[3] Department of Nutrition and Dietetics, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton SO16 6YD, UK

[4] NIHR Southampton Biomedical Research Centre–Nutrition, University Hospital Southampton NHS Foundation Trust, Southampton General Hospital, E-level, Tremona Road, Southampton SO16 6YD, UK

psychological problems such as altered body-image, anxiety and depression.

HRQoL is not only determined by disease severity. In children with CKD, Gerson et al found that renal function did not correlate with HRQoL [5]. Factors other than the disease itself contribute to HRQoL in those children with disease [8], and the authors hypothesise that nutritional status impacts HRQoL. If such an association exists, then HRQoL assessment may off a measure of how nutritional status impacts the child in a holistic, patient-centred way.

Malnutrition is common in CKD [9] and is associated with poorer outcomes [10]. It has been traditionally defined as poor nutritional status resulting from inadequate intake, but in CKD malnutrition is multifactorial in origin, with suppressed appetite, catabolism and chronic inflammation, altered dietary intake from dietetic therapy and nutrient losses through dialysis all contributing. In addition to under-nutrition, over-nutrition and obesity are becoming increasingly prevalent in CKD populations across the UK and Europe [11] and more prevalent than canonical malnutrition state of low anthropometric scores and lean tissue loss in both the conservatively managed [12] and dialysis/post-transplant populations [11]. Obesity has been reported to be an independent risk factor for development of renal damage and end-stage renal failure [13, 14].

In cohorts of children with other disease states, poor nutritional status [15] and obesity [16] have been associated with poorer HRQoL, but this has not been explored in paediatric CKD. Micronutrient status has been associated with HRQoL [17], including selenium [18] with supplementation of selenium demonstrating improved scores [19–21], although there are contradictory findings [22].

Despite the recognition that HRQoL is important, there are limited data in this disease group with existing literature focusing upon end-stage disease and those following renal transplantation. Of the studies that examine CKD stages 2–4 in the paediatric population [5, 6, 23–26], only one [25] examines nutritional status (evaluating the effect of short stature). There is therefore, a need to examine the effect of nutritional status, including obesity on HRQoL, and any association of HRQoL on future nutritional status.

Describing the subjective assessment of HRQoL of both the child and their caregiver is important as healthcare providers' focus is that of the child, but some children may be unable to describe their experiences, and caregivers make choices (including medical) dependent upon their assessment of the child's HRQoL. Discordance between self-reported and parent-proxy HRQoL scores has been reported previously in those with dialysis and post-renal transplantation [27–29], but not assessed in those with less severe disease.

The aims of this study are to report the HRQoL scores as assessed by the validated PedsQL™ questionnaire and to explore the relationship of HRQoL scores to markers of nutritional status. It will also examine concordance between the scores of the child and their parent/carer.

Methods

A cross-sectional, observational study was performed to determine the HRQoL of a cohort of children with pre-dialysis, conservatively managed CKD and its relationship to markers of nutritional status.

Participants

Children aged between 3 and 18 years with conservatively managed CKD (stages 2–5 as defined by Kidney Disease: Improving Global Outcomes (KDIGO) [30] [Pre-end-stage disease—glomerular filtration rate between 15 and 90 ml/min/1.73 m^2], not receiving dialysis and never received a renal transplant) under care of the paediatric nephrology team at Southampton Children's Hospital were identified using electronic notes, approached via a letter of invitation and subsequently assessed at routine clinic appointments. Both the child and a parent/caregiver were requested to independently complete the respective questionnaire.

Measures

Basic clinical and anthropometric data were collected, including height, weight, waist circumference, mid-upper arm circumference (MUAC) and standard deviation score (SDS), body mass index (BMI) and waist circumference-to-height ratio (WHtR). Appetite was assessed by a simple Likert scale by the child: Very poor-1, Poor-2, Good-3, Very good-4. Definitions of obesity were BMI SDS > 2; MUAC SDS > 2 or > 25 cm [31] and a WHtR > 0.5 [32]. Short stature was defined as a height SDS < − 2. Blood for analysis of markers of the micronutrients copper, selenium, zinc and manganese were collected and analysed through clinical pathology.

Further anthropometric and clinical data were collected via the electronic clinical record at 6 months and 12 months subsequent to initial assessment, and change in weight SDS, BMI SDS, height SDS and estimated glomerular filtration rate (eGFR) calculated.

The HRQoL was assessed using the PedsQL™ tool [33]. This is a series of questions posed to the child and the parent/caregiver that assess physical, emotional, social and schooling aspects of the child's life.

The PedsQL™ tool was developed to measure HRQoL in children and has been validated for a number of chronic health conditions [34–36]. It was highlighted in a systematic review of tools to assess HRQoL as one of the more

thoroughly developed measures and has been validated for a wide age range of children [37]. Although originally developed and validated for a USA population, it has been validated for a UK population of both healthy children and those with chronic conditions (asthma, diabetes and inflammatory bowel disease) [38].

The PedsQL™ [33] comprises two questionnaires: one for the child and one for a parent/caregiver. They ask a series of questions that assesses four domains: physical, emotional, social and schooling aspects of the child's life. It is scored as individual domains and as a total score (all four domains), and is expressed as a percentage of maximum score. The tool is available in age-appropriate versions: toddler (2–4 years), young child (5–7 years), older child (8–12 years) and teenager (13–18 years). There is no child (self-rater) questionnaire for those aged 2–4 years.

As a marker of deprivation, Income Deprivation Affecting Children Index (IDACI) scores were calculated using participants' postcodes. United Kingdom Department of Communities and Local Government data (2015) via the online platform provide a selection of official statistics and data outputs on a variety of themes related to the department of Communities and Local Government (http://opendatacommunities.org).

Statistical analysis

Data were analysed using SPSS version 20 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Statistical significance was defined a p value of less than 0.05. Descriptive statistical analysis was performed, including distribution of gender and age group (as defined by the PedsQL™ questionnaire).

Independent t-tests were performed to compare: the total questionnaire scores with both healthy control data from Varni et al's cohort [33]. Concordance between self-reported and parent-proxy questionnaire scores was compared using a paired t-test.

Correlation was explored between HRQoL scores and markers of nutritional status by examination of scatter-plot, and if appropriate, calculation of correlation coefficient values (Spearman's rho). Internal consistency between the domains of the questionnaires was tested using Cronbach's alpha.

Ethical approval

The study was approved by a Health Research Authority South East—Surrey Research Ethics Committee (REC Reference: 16/LO/0041). Informed consent was obtained from parents/carers with parental responsibility. Informed assent was additionally obtained from those children/young people as participants in the study if age appropriate.

Results

A total of 46 children with pre-dialysis, conservatively managed CKD were recruited with additional seven families refusing to take part in the study. These seven families did not represent a particular sub-group (a mix of degree of renal impairment levels, age and gender). The cohort had a mean age of 10.50 ± 4.19 years. Eighteen (39.1%) were female. The demographic and anthropometric details of the cohort are given in Table 1. There were no significant differences between boys and girls; including age, time since diagnosis, eGFR, height SDS, weight SDS, BMI SDS, WHtR and appetite.

Anthropometry

Mean values (with standard deviations) of height SDS, weight SDS and BMI SDS for the cohort were −1.03 (± 1.51), -0.43 (± 1.81) and 0.32 (± 1.41), respectively. The majority of children were within normal growth limits (± 2SD). A greater number of children were obese as defined by WHtR than by Wt SDS or BMI SDS definitions. Eight children (17.4%) had weight SDS < − 2 (malnourished by ICD-10 definition), three children (6.5%) had weight SDS > 2. One child (2.2%) was underweight for height (BMI SDS < − 2), six children (13.0%) were overweight for height (BMI SDS > 2). Twelve children (26.1%) were short-for-age (height SDS < − 2), one child (2.2%) was tall-for-age (height SDS > 2). Twenty children (43.5%) had a waist circumference-to-height ratio greater than 0.5.

Table 1 Demographic and anthropometric data of the cohort paediatric pre-dialysis, conservatively managed CKD

Variable	Averages
Age (years)	10.50 (SD ± 4.19)
Gender	Male = 28 (60.87%), Female = 18 (39.13%)
eGFR (ml/min/1.73 m^2)	57.35 (SD ± 23.95)
Number of medications	3.89 (SD ± 2.54)
Time since diagnosis (months)	93.27 (SD ± 55.34)
Height SDS[a]	0.65 (IQR = 2.03)
Weight SDS	−0.43 (SD ± 1.81)
BMI SDS	0.32 (SD ± 1.41)
MUAC SDS	0.52 (SD ± 0.92)
HtWR	0.49 (SD ± 0.07)

CKD chronic kidney disease, *eGFR* estimated glomerular filtration rate, *MUAC* mid-upper arm circumference, *SD* standard deviation, *SDS* standard deviation score, *WHtR* waist circumference-to-height ratio

[a]Median and interquartile range

HRQoL scores

All participants enrolled in the study fully completed the appropriate questionnaires. HRQoL scores are presented in Table 2. HRQoL scores were lower in all domains for both the self rated and parent proxy components of the PedsQL™ tool than healthy control populations (Varni et al). There was good concordance between the self-rater and parent-proxy scores (*t* test: $n = 38$, $t(37) = 0.281$, $p = 0.780$). Details of sub-group analysis for different ages and PedsQL™ domains are available in the *supplementary material*. There was a high level of internal consistency between domains of the questionnaires with a Cronbach's alpha of 0.810 (child self-rater) and 0.811 (parent-proxy).

HRQoL and renal function

Although HRQoL scores correlated with renal function (eGFR) for the child(self-rater) scores (Pearson's coefficient = −0.362, $p = 0.026$), this was not the case for the parent-proxy scores (Pearson's coefficient = −0.133, $p = 0.377$). Although there is correlation of self-rated PedsQL™ scores, there were no difference in PedsQLTM scores for either self-rater or parent-proxy components between CKD stages (self-rater: $F = 2.284$, df = 5, $p = 0.07$; parent-proxy: $F = 1.117$, df = 5, $p = 0.367$). Degree of proteinuria (urinary protein–creatinine ratio, uPCR) did not correlate with HRQoL scores, including individual domain scores (total score (child) Spearman's rho −0.185, p − 0.870; total score (parent-proxy) Spearman's rho = −0.05, $p = 0.755$).

HRQoL and anthropometry (markers of growth and obesity)

Those who were stunted (Height SDS < − 2) had lower HRQoL scores than those who were not for both the child ($t(15.0394) = -3.4356$; $p = 0.0037$) and parent questionnaires ($t(22.87220) = -4.10670$; $p = 0.0004$). Those with obesity (as defined by BMI SDS, MUAC and waist circumference-to-height ratio) did not demonstrate different scores from non obese patients. Children whose appetite was described as either "good" or "very good" had better scores than those with appetites described as "poor" or "very poor" (child—$t(36) - 2.851$; $p - 0.007$ and parent—$t(44) - 2.910$; $p = 0.006$).

HRQoL and micronutrient status

Correlations were explored between the plasma levels of copper, selenium, zinc and whole blood manganese. None of these measures correlated with HRQoL scores with p values between 0.061 and 0.731 (analysis available as Supplementary Material).

Multiple linear regression analysis

Variables that demonstrated correlation were used to perform multiple linear regression analysis.

Child (self-rater) HRQoL

On multiple linear regression for correlated variables (eGFR and Ht SDS), these two variables statistically significantly predicted child self-assessed HRQoL $F(2,35) = 12.436$, $p < 0.0005$, $R^2 = 0.415$. Both Ht SDS and eGFR added statistically and significantly to the prediction of child-assessed HRQoL (Ht SDS $p < 0.0005$, eGFR $p = 0.008$).

Parent-proxy HRQoL

On multiple linear regression for positively correlated variables (Ht SDS and IDACI), these two variables statistically significantly predicted parent-proxy HRQoL $F(2,42) = 11.695$, $p < 0.0005$, $R^2 = 0.358$. Both Ht SDS and IDACI added statistically significantly to the prediction

Table 2 PedsQLTM scores for the cohort compared to healthy controls

Children with CKD at SCH (self-rater)					Healthy control data (Vami et al)				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
65.10 (22.57)	34.38 (24.65)	65.48 (23.98)	58.88 (16.91)	64.22 (18.00)	86.85 (13.88)*	78.21 (18.64)*	84.04 (17.43)*	79.92 (16.93)*	82.87 (13.16)*
Children with CKD at SCH (parent-proxy)					Healthy control data (Vami et al)				
Physical	Emotional	Social	School	Total	Physical	Emotional	Social	School	Total
65.27 (26.27)	61.47 (22.53)	70.52 (22.80)	63.48 (19.68)	64.64 (19.13)	83.25 (19.98)*	80.28 (16.99)*	82.15 (20.08)*	76.91 (20.16)*	81.34 (15.92)*

Note that there is no self-rated score for those aged <5 years

*Significant difference for *t* test $p < 0.0005$

of child-assessed HRQoL (Ht SDS $p=0.001$, IDACI $p=0.005$).

Discussion

These are the first data of HRQoL as assessed by the PedsQL™4.0 in children with pre dialysis, conservatively managed CKD within the UK, and they explore the association of nutritional status with HRQoL. The aim of this study was to report PedsQL™ scores in a UK population of pre-dialysis, conservatively managed CKD and explore the association of nutritional status on them. We hypothesised that disease severity, obesity and short stature would demonstrate association, but of the nutritional status measures evaluated, poor appetite and short stature demonstrated a strong association with lower HRQoL scores.

These data show that HRQoL was significantly lower in this population of pre dialysis conservatively managed CKD children than healthy control data, with mean score differences greater than the minimal clinically important difference (MCID) score of 4.4 (child self-assessed questionnaire) and 4.5 (parent-proxy questionnaire) [33]. Existing literature is limited and has focused on those with the most severe disease—those with end-stage disease (eGFR < 15 ml/min/1,73 m^2, in receipt of including dialysis, or who have undergone renal transplantation). On the whole, these report that HRQoL is lower in children with CKD compared to healthy children, but with some conflicting results [6, 23–25, 28, 39–46]. Such conflict may be explained by the heterogeneity of the tools used, but other factors may also be contributory.

There was no difference in scores according to age or gender in this cohort, although previous studies have shown lower scores in older children/young people [46] and in girls [45, 46]. Time since diagnosis did not correlate with HRQoL scores. This may be explained by the heterogeneity of the cohort, with a potential mix of those whose HRQoL worsens with time, and those that have altered "new normal"—a phenomenon described as "response shift" [47].

HRQoL concordance between child (self-rater) and parent-proxy scores

There was significant correlation between child (self-rater) and parent-proxy scores, with no significant differences either in total scores or individual domains within any age groups. Discordance between self-reported and parent-proxy scores has been reported previously in those with dialysis and post-renal transplantation [27–29] with other literature showing greater psychosocial score discordance in those approaching adulthood [25, 48]. A degree of disagreement is not surprising as the child's perception of quality of life will be different, at least in part due to parental expectations for their child based on their own life experiences; experiences that the child would not necessarily draw comparison with. This potential lack of comparison on the child's part is one reason why HRQoL should be assessed by children and parents/caregivers in order not to over-score those that 'don't know any difference'. Previous studies that suggest that discordance between self-reported and parent-proxy questionnaires increases as the child's age increases [25]. This is true not only for teenagers approaching adulthood, but demonstrated by Razzcuk et al, also or younger children [49]. The reason for these data not reflecting differences may be because of the relatively small numbers within the cohort, although the difference between child (self-rater) and parent-proxy scores were close to statistical significance in the emotional domain ($p=0.054$).

HRQoL and disease severity

Child (self-rater) HRQoL scores correlated with eGFR, but parent-proxy score did not, and no correlation was found between scores and degree of proteinuria. As the renal impairment would have greatest impact upon the individual, then other modifying factors on the caregiver may explain the lack of correlation observed in the parent-proxy scores. As proteinuria may be modified through medical intervention (such as the use of angiotensin II-converting enzyme inhibitors), proteinuria is not a complete marker of disease severity.

Although eGFR did correlate with child (self-rater) HRQoL scores in our cohort, Gerson et al found that eGFR did not correlate with HRQoL [5]. A lack of correlation may be explained by the fact that eGFR is not a true reflection of disease severity as it is too simplistic a marker. Furthermore, it does not take into consideration co-morbidities, social factors or other contributors to HRQoL.

HRQoL and growth

As previously reported in other disease groups [25], stunted children reported lower HRQoL scores than their non-stunted counterparts, and it was the strongest influence on multiple regression analysis. The reasons for this may be twofold. Firstly, linear growth is a summation of events, is affected by myriad factors and is a proxy measure for everything detrimental that has happened to the child. Secondly, short stature may have psychological impact on the child, as both a visual reflection of a child's self-perceived health, and a noticeable difference between themselves and their peers.

HRQoL and obesity

Despite previous reports from other cohorts of children [50], these data do not demonstrate a lower HRQoL in those with markers of obesity (BMI SDS, waist circumference-to-height ratio). The reason for this may be a shift of the perceived normal body shape and "accepted" adiposity in children. Rates of obesity in the UK population are now 14% of children aged 2–15 years (BMI > 95th percentile) [51], and compare similarly to the percentage of those obese in this cohort (BMI > 2 SDS). Therefore, despite children being obese, if their day-to-day functioning is not negatively impacted by this, then such children do not stand out from their peers, and so are perceived as "normal", or even healthy—both obese children and their parents' ability to recognise themselves as too heavy is only 26% and ≤ 48%, respectively [51].

HRQoL and appetite

HRQoL scores were significantly higher in those with good appetite versus poor appetite. As appetite is a complex function of many factors in which general health and well-being impact upon, this is not surprising. In addition, eating is an important social setting—on which much of family life is structured around. Alteration of feeding habits, including through eating less and a focus of the medicalisation of the normal social activity (advice to eat certain foods, avoid certain foods and to encourage oral intake in order to increase calorie intake, for example), may have an impact on how they view themselves compared to others, and hence their perceived HRQoL.

HRQoL and micronutrient status

Biomarkers of micronutrient status did not correlate with HRQoL scores. These measurements are prone to many factors, and have a high degree of buffering within the normal range; it is only at the extremes of nutrition that these values become abnormal. In addition, levels may be affected by other factors such as underlying inflammatory state.

Limitations of study

There are several limitations to this study. Firstly, the cohort although larger than other reported cohorts is only 46 children. Additionally, the study only measured HRQoL at a single time-point meaning that we were unable to examine for changing scores with time (including during changes in nutritional status and treatment). Nutritional status is a complex, multi-faceted concept and this study only examined a selection of variables. Each of these measures has its own limitations (for example, BMI not truly reflecting body composition). Not all variables that may have an impact on HRQoL, such as markers of resilience and patient efficacy, were explored.

Future directions

Further exploration of factors that could be influencing HRQoL, especially those that are modifiable, is needed with the aim to improve the HRQoL of our patients. It is not unreasonable to predict that the nutritional changes, including dietary restriction found in CKD and changes of body composition, may have a detrimental effect upon HRQoL. Therefore, further studies analysing the impact would be valuable in order to optimise management strategies to improve HRQoL. A larger multi-centre cohort would allow for exploration of different aetiologies and the effect this may have on HRQoL in addition to minimising type 2 errors. Longitudinal studies of HRQoL in this disease group are also needed. Although there are cross-sectional data comparing treatment modalities, it would be useful to know how an individual's HRQoL changes with time, with the changing disease severity, changing treatment modality, changing nutritional status, transitioning from paediatric to adult services. This would facilitate the exploration of the ways of different management strategies, including nutritional intervention influence HRQoL.

Concluding remarks

HRQoL is lower in pre-dialysis, conservatively managed paediatric CKD patients than healthy control data. Examination of multiple nutritional variables revealed that nutritional status is associated with HRQoL. Other significant variables were eGFR for child (self-rater) scores and level of deprivation for parent-proxy scores.

HRQoL is a measure of an overall status, and the perception of an individual's own health status is an important factor that healthcare providers should be aiming to improve. As healthcare services become more patient-centred, the measures by which they are evaluated must include patient-centred measures and by assessing HRQoL using a formalised tool, healthcare processes can be evaluated. HRQoL tools also have the potential to be used as screening tools or to identify areas to be explored during outpatient consultation for a more holistic consultation. For these reasons, HRQoL assessment should be considered for introduction into routine clinical care for ongoing holistic care of children with chronic illnesses, including CKD, and to facilitate the acquisition of longitudinal data regarding the impact of changes in nutritional status and therapy.

Acknowledgements The authors would like to thank Professor Anne-Sophie Darlington for her guidance.

Author contributions All authors contributed to the conception and design of the study. MH and CA collected the data. MH analysed the data. All authors contributed to the interpretation of data; drafting and revision of the manuscript and approved the final version of the manuscript.

Funding This study was supported by the NIHR Southampton Clinical Research Facility. This report is an independent research by the National Institute for Health Research—Biomedical Research Unit Funding Scheme. Matthew J. Harmer is supported by a bequeathed fund for renal research. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.

Compliance with ethical standards

Conflict of interest No financial or non-financial conflict of interest is identified.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from parents/carers with parental responsibility. Informed assent was additionally obtained from those children/young people as participants in the study if age appropriate.

References

1. Harambat, J., van Stralen, K. J., Kim, J. J., & Tizard, E. J. (2012). Epidemiology of chronic kidney disease in children. *Pediatric Nephrology (Berlin, Germany), 27*, 363–373.
2. Greenbaum, L. A., Warady, B. A., & Furth, S. L. (2009). Current advances in chronic kidney disease in children: Growth, cardiovascular, and neurocognitive risk factors. *Seminars in Nephrology, 29*, 425–434.
3. Leidy, N. K., Revicki, D. A., & Geneste, B. (1999). Recommendations for evaluating the validity of quality of life claims for labeling and promotion. *Value in Health: The Journal of the International Society for Pharmacoeconomics and Outcomes Research, 2*, 113–127.
4. Rees, L. (2009). Long-term outcome after renal transplantation in childhood. *Pediatric Nephrology (Berlin, Germany), 24*, 475–484.
5. Gerson, A. C., Wentz, A., Abraham, A. G., Mendley, S. R., Hooper, S. R., Butler, R. W., Gipson, D. S., Lande, M. B., Shinnar, S., Moxey-Mims, M. M., Warady, B. A., & Furth, S. L. (2010). Health-related quality of life of children with mild to moderate chronic kidney disease. *Pediatrics, 125*, e349–e357.
6. McKenna, A. M., Keating, L. E., Vigneux, A., Stevens, S., Williams, A., & Geary, D. F. (2006). Quality of life in children with chronic kidney disease-patient and caregiver assessments. Nephrology, dialysis, transplantation: Official publication of the European Dialysis and Transplant Association -. *European Renal Association, 21*, 1899–1905.
7. Moreira, J. M., Bouissou Morais Soares, C. M., Teixeira, A. L., Simoes, E. S. A. C., & Kummer, A. M. (2015). Anxiety, depression, resilience and quality of life in children and adolescents with pre-dialysis chronic kidney disease. *Pediatric Nephrology (Berlin, Germany), 30*, 2153–2162.
8. Stone, M. B., Botto, L. D., Feldkamp, M. L., Smith, K. R., Roling, L., Yamashiro, D., & Alder, S. C. (2010). Improving quality of life of children with oral clefts: Perspectives of parents. *The Journal of Craniofacial Surgery, 21*, 1358–1364.
9. Fouque, D., Kalantar-Zadeh, K., Kopple, J., Cano, N., Chauveau, P., Cuppari, L., Franch, H., Guarnieri, G., Ikizler, T. A., Kaysen, G., Lindholm, B., Massy, Z., Mitch, W., Pineda, E., Stenvinkel, P., Trevino-Becerra, A., & Wanner, C. (2008). A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. *Kidney International, 73*, 391–398.
10. Worg, C. S., Gipson, D. S., Gillen, D. L., Emerson, S., Koepsell, T., Sherrard, D. J., Watkins, S. L., & Stehman-Breen, C. (2000). Anthropometric measures and risk of death in children with end-stage renal disease. *American Journal of Kidney Diseases: The Official Journal of the National Kidney Foundation, 36*, 811–819.
11. Bonthuis, M., van Stralen, K. J., Verrina, E., Groothoff, J. W., Alonso Melgar, A., Edefonti, A., Fischbach, M., Mendes, P., Molchanova, E. A., Paripovic, D., Peco-Antic, A., Printza, N., Rees, L., Rubik, J., Stefanidis, C. J., Sinha, M. D., Zagozdzon, I., Jager, K. J., & Schaefer, F. (2013). Underweight, overweight and obesity in paediatric dialysis and renal transplant patients. Nephrology, dialysis, transplantation: Official publication of the European Dialysis and Transplant Association. *European Renal Association, 28*(Suppl 4), iv195–iv204.
12. Rodig, N. M., McDermott, K. C., Schneider, M. F., Hotchkiss, H. M., Yadin, O., Seikaly, M. G., Furth, S. L., & Warady, B. A. (2014) Growth in children with chronic kidney disease: A report from the Chronic Kidney Disease in Children Study. *Pediatric Nephrology (Berlin, Germany), 29*, 1987–1995.
13. Hsu, C. Y., McCulloch, C. E., Iribarren, C., Darbinian, J., & Go, A. S. (2006). Body mass index and risk for end-stage renal disease. *Annals of Internal Medicine, 144*, 21–28.
14. Ribstein, J., du Cailar, G., & Mimran, A. (1995) Combined renal effects of overweight and hypertension. *Hypertension (Dallas, Tex: 1979), 26*, 610–615.
15. Brinksma, A., Sanderman, R., Roodbol, P. F., Sulkers, E., Burgerhof, J. G., de Bont, E. S., & Tissing, W. J. (2015). Malnutrition is associated with worse health-related quality of life in children with cancer. *Supportive Care in Cancer: Official Journal of the Multinational Association of Supportive Care in Cancer, 23*, 3043–3052.
16. Buttitta, M., Iliescu, C., Rousseau, A., & Guerrien, A. (2014). Quality of life in overweight and obese children and adolescents: A literature review. *Quality of Life Research: An International Journal Of Quality of Life Aspects of Treatment, Care and Rehabilitation, 23*, 1117–1139.
17. Duran Aguero, S., Gonzalez Canete, N., Pena D'Ardaillon, F., & Candia Johns, P. (2015). [Association of intake macro and micronutrients with life quality of life in elderly]. *Nutricion Hospitalaria, 31*, 2578–2582.
18. Gonzalez, S., Huerta, J. M., Fernandez, S., Patterson, A. M., & Lasheras, C. (2007). Life-quality indicators in elderly people are influenced by selenium status. *Aging Clinical and Experimental Research, 19*, 10–15.

19. Witte, K. K., Nikitin, N. P., Parker, A. C., von Haehling, S., Volk, H. D., Anker, S. D., Clark, A. L., & Cleland, J. G. (2005). The effect of micronutrient supplementation on quality-of-life and left ventricular function in elderly patients with chronic heart failure. *European Heart Journal, 26*, 2238–2244.
20. Oh, T. R., Kim, C. S., Bae, E. H., Ma, S. K., Han, S. H., Sung, S. A., Lee, K., Oh, K. H., Ahn, C., & Kim, S. W. (2017). Association between vitamin D deficiency and health-related quality of life in patients with chronic kidney disease from the KNOW CKD study. *PloS ONE, 12*, e0174282.
21. Johansson, P., Dahlstrom, O., Dahlstrom, U., & Alehagen, U. (2015). Improved health-related quality of life, and more days out of hospital with supplementation with selenium and coenzyme Q10 combined. results from a double blind, placebo-controlled prospective study. *The Journal of Nutrition, Health & Aging, 19*, 870–877.
22. Rayman, M., Thompson, A., Warren-Perry, M., Galassini, R., Catterick, J., Hall, E., Lawrence, D., & Bliss, J. (2006). Impact of selenium on mood and quality of life: A randomized, controlled trial. *Biological psychiatry, 59*, 147–154.
23. Reynolds, J. M., Garralda, M. E., Jameson, R. A., & Postlethwaite, R. J. (1988). How parents and families cope with chronic renal failure. *Archives of Disease in Childhood, 63*, 821–826.
24. Heath, J., Mackinlay, D., Watson, A. R., Harnes, A., Wirz, L., Scott, S., Klewchuk, E., Milford, D., & McHugh, K. (2011). Self-reported quality of life in children and young people with chronic kidney disease. *Pediatric Nephrology (Berlin, Germany), 26*, 767–773.
25. Al-Uzri, A., Matheson, M., Gipson, D. S., Mendley, S. R., Hooper, S. R., Yadin, O., Rozansky, D. J., Moxey-Mims, M., Furth, S. L., Warady, B. A., & Gerson, A. C. (2013). The impact of short stature on health-related quality of life in children with chronic kidney disease. *The Journal of Pediatrics, 163*, 736–741.e731.
26. Teixeira, C. G., Duarte Mdo, C., Prado, C. M., Albuquerque, E. C., & Andrade, L. B. (2014). Impact of chronic kidney disease on quality of life, lung function, and functional capacity. *Jornal de pediatria, 90*, 580–586.
27. Anthony, S. J., Hebert, D., Todd, L., Korus, M., Langlois, V., Pool, R., Robinson, L. A., Williams, A., & Pollock-BarZiv, S. M. (2010). Child and parental perspectives of multidimensional quality of life outcomes after kidney transplantation. *Pediatric Transplantation, 14*, 249–256.
28. Sundaram, S. S., Landgraf, J. M., Neighbors, K., Cohn, R. A., & Alonso, E. M. (2007). Adolescent health-related quality of life following liver and kidney transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons, 7*, 982–989.
29. Varni, J. W., Limbers, C. A., & Burwinkle, T. M. (2007). Impaired health-related quality of life in children and adolescents with chronic conditions: A comparative analysis of 10 disease clusters and 33 disease categories/severities utilizing the PedsQL 4.0 Generic Core Scales. *Health and Quality of Life Outcomes, 5*, 43.
30. Group, K. D. I. G. O. K. C. W. (2013). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney International, 3*, 1–150.
31. Chaput, J. P., Katzmarzyk, P. T., Barnes, J. D., Fogelholm, M., Hu, G., Kuriyan, R., Kurpad, A., Lambert, E. V., Maher, C., Maia, J., Matsudo, V., Olds, T., Onywera, V., Sarmiento, O. L., Standage, M., Tudor-Locke, C., Zhao, P., & Tremblay, M. S. (2016) Mid-upper arm circumference as a screening tool for identifying children with obesity: A 12-country study. *Pediatric Obesity*.
32. Ashwell, M., Lejeune, S., & McPherson, K. (1996). Ratio of waist circumference to height may be better indicator of need for weight management. *BMJ, 312*, 377.
33. Varni, J. W., Burwinkle, T. M., Seid, M., & Skarr, D. (2003). The PedsQL 4.0 as a pediatric population health measure: Feasibility, reliability, and validity. *Ambulatory Pediatrics: The Official Journal of the Ambulatory Pediatric Association, 3*, 329–341.
34. Varni, J. W., Burwinkle, T. M., Jacobs, J. R., Gottschalk, M., Kaufman, F., & Jones, K. L. (2003). The PedsQL in type 1 and type 2 diabetes: Reliability and validity of the pediatric quality of life inventory generic core scales and type 1 diabetes module. *Diabetes Care, 26*, 631–637.
35. Varni, J. W., Burwinkle, T. M., Katz, E. R., Meeske, K., & Dickinson, P. (2002). The PedsQL in pediatric cancer: Reliability and validity of the pediatric quality of life inventory generic core scales, multidimensional fatigue scale, and cancer module. *Cancer, 94*, 2090–2106.
36. Chan, K. S., Mangione-Smith, R., Burwinkle, T. M., Rosen, M., & Varni, J. W. (2005). The PedsQL: Reliability and validity of the short-form generic core scales and Asthma Module. *Medical Care, 43*, 256–265.
37. Eiser, C., & Morse, R. (2001). Quality-of-life measures in chronic diseases of childhood. *Health Technology Assessment (Winchester, England), 5*, 1–157.
38. Upton, P., Eiser, C., Cheung, I., Hutchings, H. A., Jenney, M., Maddocks, A., Russell, I. T., & Williams, J. G. (2005). Measurement properties of the UK-English version of the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales. *Health and Quality of Life Outcomes, 3*, 22.
39. Diseth, T. H., Tangeraas, T., Reinfjell, T., & Bjerre, A. (2011). Kidney transplantation in childhood: Mental health and quality of life of children and caregivers. *Pediatric Nephrology (Berlin, Germany), 26*, 1881–1892.
40. Qvist, E., Narhi, V., Apajasalo, M., Ronnholm, K., Jalanko, H., Almqvist, F., & Holmberg, C. (2004). Psychosocial adjustment and quality of life after renal transplantation in early childhood. *Pediatric Transplantation, 8*, 120–125.
41. Goldstein, S. L., Graham, N., Burwinkle, T., Warady, B., Farrah, R., & Varni, J. W. (2006). Health-related quality of life in pediatric patients with ESRD. *Pediatric Nephrology (Berlin, Germany), 21*, 846–850.
42. Eijsermans, R. M., Creemers, D. G., Helders, P. J., & Schroder, C. H. (2004). Motor performance, exercise tolerance, and health-related quality of life in children on dialysis. *Pediatric Nephrology (Berlin, Germany), 19*, 1262–1266.
43. Hamiwka, L. A., Cantell, M., Crawford, S., & Clark, C. G. (2009). Physical activity and health related quality of life in children following kidney transplantation. *Pediatric Transplantation, 13*, 861–867.
44. Falger, J., Landolt, M. A., Latal, B., Ruth, E. M., Neuhaus, T. J., & Laube, G. F. (2008). Outcome after renal transplantation. Part II: Quality of life and psychosocial adjustment. *Pediatric Nephrology (Berlin, Germany), 23*, 1347–1354.
45. Neul, S. K., Minard, C. G., Currier, H., & Goldstein, S. L. (2013). Health-related quality of life functioning over a 2-year period in children with end-stage renal disease. *Pediatric Nephrology (Berlin, Germany), 28*, 285–293.
46. Marciano, R. C., Bouissou Soares, C. M., Diniz, J. S. S., Lima, E. M., Silva, J. M. P., Canhestro, M. R., Gazzinelli, A., Melo, C. C. D., Dias, C. S., Correa, H., & Oliveira, E. A. (2011). *Behavioral disorders and low quality of life in children and adolescents with chronic kidney disease. Pediatric Nephrology, 26*, 281–290.
47. Sprangers, M. A., & Schwartz, C. E. (1999). Integrating response shift into health-related quality of life research:

RESEARCH BRIEF

Vitamin B6 in Pediatric Renal Transplant Recipients

Matthew Harmer, BSc(Hons), BM, MRCPCH,†‡ *Stephen Wootton, BSc(Hons), PhD,*†‡ *Rodney Gilbert, MA, Mmed(Paed), FRCPCH, DCH,*† *and Caroline Anderson, BSc(Hons), RD, PhD*§‡

Objectives: Our aim was to assess the vitamin B6 intake and biochemical status in a sample of children who have undergone renal transplantation.

Methods: A prospective observational study was performed in 10 pediatric renal transplant recipients to determine their vitamin B6 status through dietary assessment and serum Pyridoxal 5′-phosphate (PLP) measurement.

Results: Ten children (mean age of 11.9 years) had median serum PLP concentrations of 62.45 nmol/L (interquartile range ±83.40). Two children (20%) had values above the reference range, and none below. Mean vitamin B6 intake was 138.7% of reference nutrient intake (standard deviation ±35.2%). No children were in receipt of vitamin B6 supplementation.

Conclusion: There is no previous literature on vitamin B6 status in children who have undergone renal transplantation. In adult transplant recipients, elevated serum PLP concentrations have been described and ascribed to possible excessive intakes. In this sample, no children appeared biochemically deficient, but 20% had elevated concentrations. Dietary intakes were not excessive, and no children reported oral Vitamin B6 supplementation. Exploration of vitamin B6 metabolism in this population is required.

Introduction

VITAMIN B6, IN its active form pyridoxal 5′-phosphate (PLP), is essential as a coenzyme in the metabolism of amino acids and fatty acids and has additional roles in gene expression and immune function.[1] Pyridoxine is found in a variety of foods including meat, eggs, and vegetables.[2] Patients who have undergone renal transplantation are at risk for altered vitamin B6 status and metabolism because of altered dietary habits associated with dietary restraint and uremia, medication regimens that may affect metabolism, and continuing renal dysfunction.[3,4] Vitamin B6 may be of particular interest posttransplantation, given its role in the body's inflammatory and immune response.[5] Previous literature has shown that adult renal transplant recipients do not appear to have biochemical evidence of vitamin B6 deficiency, but a minority may have high serum PLP concentrations, which were believed to be associated with excessive dietary vitamin B6 intakes.[6] In the pediatric chronic kidney disease (CKD) population, dialysis has been associated with inadequate dietary vitamin B6 intake,[7,8] as well as normal,[9] high,[10] and low serum PLP concentrations.[11] In children with predialysis, conservatively managed CKD, poor dietary intakes have also been reported.[12] There is no available literature on intake or blood concentrations in children after renal transplantation.[13]

The aim of this study was to evaluate the vitamin B6 intake and status of a group of children who had received a renal transplant graft.

Methods

Children between 3 and 18 years of age who had received a renal transplant were identified via the electronic medical record and invited to participate in the study. Procedures were completed during usual clinic appointments to avoid unnecessary visits to the hospital. Data from the normal clinical record, including results from routine clinical blood results, were collected. These routine clinical blood tests included serum albumin, inorganic phosphate,

*Department of Paediatric Nephrology, Southampton Children's Hospital, University Hospital Southampton NHS Foundation Trust, Southampton, UK.
†Faculty of Medicine, University of Southampton, Southampton, UK.
§Department of Nutrition and Dietetics, University Hospital Southampton NHS Foundation Trust, Southampton, UK.
‡NIHR Southampton Biomedical Research Centre–Nutrition, University Hospital Southampton NHS Foundation Trust, Southampton General Hospital, E level, Southampton, UK.
Conflicts of Interest: No financial conflict of interest is identified.
Support: This study was supported by the NIHR. This report is independent research by the National Institute for Health Research Biomedical Research Unit Funding Scheme. M.H. is supported by a bequeathed fund for renal research. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.
Address correspondence to Dr Matthew Harmer, BSc(Hons), BM, MRCPCH, NIHR Southampton Biomedical Research Centre, Southampton Centre for Biomedical Research, Southampton General Hospital, MP 113, E-level, Tremona Road, Southampton SO16 6YD, UK. E-mail: matthew.harmer@uhs.nhs.uk

1051-2276/$36.00
https://doi.org/10.1053/j.jrn.2018.09.003

C-reactive protein (used as a surrogate marker for inflammation) and were analyzed through the clinical pathology department as per usual practice.

Dietary intake of the children was performed by an experienced registered dietitian (C.A.) by 24-hour dietary recall, using a multiple-pass approach (an unstructured, uninterrupted listing of all foods and beverages consumed, followed by a structured approach to data collection including memory cues, ending in an unstructured question for any other foods recalled and included several additional memory cues). Food intake was coded and analyzed using Netwisp computer software (Tinuviel Software Ltd, UK), which provides comprehensive nutrient analyses using a large food database, to give estimated nutrient content and compared with dietary reference values.[2] PLP was measured to assess vitamin B6 status, and concentrations were determined by high-performance liquid chromatography.[14] Folate and vitamin B12 status were also assessed as their status may have an impact on vitamin B6 status, sharing important biochemical pathways including the transsulfuration pathway. Folate and vitamin B12 concentrations were determined by measurement using a paramagnetic particle, chemiluminescent immunoassay on an Access Immunoassay System (Beckman Dxl 800).

Data were analyzed using SPSS version 20 for Windows (SPSS Inc., Chicago, IL). Statistical significance was defined a P value of less than 0.05. Descriptive statistical analysis was performed; including numbers of males and females. Values were expressed as mean and standard deviations (SDs) or median and interquartile range as appropriate after assessment of distribution by Shapiro–Wilk test. PLP concentrations were reported as "below reference range" (<30 nmol/L), "within reference range" (30-144 nmol/L), or "above reference range" (>144 nmol/L).[15] Relationships were explored through cross-tabulation, and correlations were explored between variables, including between PLP and estimated glomerular filtration rate (eGFR) reporting Pearson's coefficient or Spearman's rho. Serum albumin and phosphate, as important markers associated with disease progression, were also included in the analysis. Differences between mean values were explored using t-test or Mann–Whitney U-test, as appropriate.

Results

Ten children (8 males) with a mean age of 11.9 years, none of whom were receiving dialysis, were recruited to the study. All children consumed an oral diet; one of whom was on supplementary feed (Nutrini Energy Multi Fibre; Nutricia). No children were taking vitamin supplements. No children were in receipt of medications known to disrupt vitamin B6 metabolism, such as antibiotics or hydralazine for blood pressure control. Table 1 illustrates the group characteristics of participating patients.

Table 1. Group Characteristics

Variable	Mean (±Standard Deviation)
Age (y)	11.90 (±3.10)
eGFR (mL/min/1.73m^2)	48.06 (±11.59)
Inorganic phosphate (mmol/L)	1.35 (±0.14)
Albumin (g/L)	38.60 (±2.63)
Time since transplantation (mo)	36.43 (±21.11)
Etiology of Renal Failure	**Number of Children (%)**
Nephrotic syndrome	2 (20)
Focal segmental glomerular sclerosis	2 (20)
Dysplasia	2 (20)
Obstructive uropathy	2 (20)
Acute cortical necrosis	1 (10)
Unknown	1 (10)
Immunosuppressive Therapy	**Number of Children (%)**
Steroids	10 (100)
Calcineurin inhibitor (tacrolimus)	9 (90)
Azathioprine	2 (20)
Mofetil mycophenolate	3 (30)
mTOR inhibitor (sirolimus)	1 (10)

eGFR, estimated glomerular filtration rate; mTOR, mechanistic target of rapamycin.

PLP Concentrations

Median serum PLP concentrations were 62.5 nmol/L (interquartile range ±83.4; range = 33.4-309.5). Two children (20%) had values above the normal reference range (186.6 nmol/L and 309.5 nmol/l); no children had values below the normal reference range. No correlation between renal function (eGFR) and serum PLP concentrations were found (Spearman's rho = 0.418; $P = .229$). Those with the highest serum PLP concentrations were not those with the most impaired renal function (second and sixth highest GFR). On cross-tabulation, there was no pattern between those of elevated serum PLP and inorganic phosphate or albumin concentrations, underlying etiology of renal failure or immunosuppressive drug regimen.

Dietary Intake

Mean vitamin B6 intake was 1.3 μg/day (SD ± 0.6; range = 0.5-2.2 μg/day), and 138.7% of reference nutrient intake (SD ± 35.2; range = 77%-190%). No children had intakes below the lower reference nutrient intake. No children were in receipt of nutritional supplementation of vitamin B6. There was no association between those with elevated PLP concentrations and dietary intake (Mann–Whitney U-test T = 6.00; $P = .71$).

Other B-Vitamins

To explore the possibility of disturbance in shared pathways that may explain any altered vitamin B6 status, folate

and vitamin B12 status were also assessed. Mean plasma folate concentrations were 12.2 ng/mL (SD ± 8.2), and plasma vitamin B12 concentrations were 377.0 ng/L (SD ± 136.9). Of the 2 participants with elevated serum PLP concentrations, one had elevated folate (>25 ng/mL) with a vitamin B12 of 400 ng/L (rank order 8 of 10), the other had a folate of 3.1 ng/mL (rank order 1 of 10), and a vitamin B12 of 197 ng/L (rank order 1 or 10).

Discussion

Vitamin B6 status in renal transplant recipients has been seldom reported, and not previously in children. This is despite the recognition that vitamin B6 functions as an important cofactor in pathways that may be disturbed in renal disease, including amino acid and fatty acid metabolism, immune function, and oxidative stress.[5] This study reports vitamin B6 status in this population but also demonstrates that excessive dietary intake of vitamin B6 is unlikely to be the reason for high levels reported in this posttransplant population. Future study is warranted to examine the possible alteration in vitamin B6 metabolism that may be at play in this population.

Excessive intake of vitamin B6 has been associated with neuropathy with an upper recommended intake of children between 5 and 20 mg/day depending on age.[1] Although PLP is used as a marker for intake,[16] the validity of this has not been shown in the posttransplant population. There are many other factors that may affect PLP concentrations (see Fig. 1). There is no evidence that elevated PLP alone is associated with specific symptoms or outcomes but may represent altered flux through metabolic pathways rather than true hypervitaminosis.

There was no correlation with vitamin B6 status and renal function. This is in agreement with previous literature in adult patients.[6,17] There was also no correlation between PLP and C-reactive protein concentrations, and no pattern observed with folate and vitamin B12 levels. Previous literature has reported the association of low vitamin B6 and inflammatory markers.[18,19] The data reported here are in agreement with healthy adult data, suggesting that it may only be in those with vitamin B6 deficiency that such as association is observed.

This study reports serum PLP concentrations in a small group of pediatric renal transplant recipients that are at risk from vitamin B6 depletion because of both disease process and medical management.[4] This study did not demonstrate inadequacy but does report individuals with high concentrations. Jankowska's study of adult transplant recipients reported similar results but was unable to exclude excessive dietary intake as a contributing factor.[6] In the data reported here, elevated PLP was not associated with excessive intakes or supplementation and implies that other reasons for elevated concentrations must be sought. There are recognized limitations of the dietary analysis, but serum PLP concentrations above the normal reference range

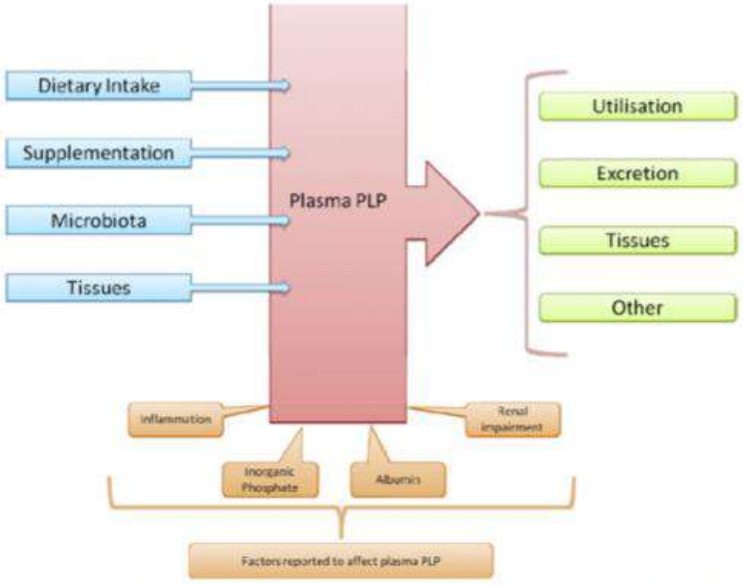


Figure 1. Schematic representation of influences over plasma pyridoxal-5-phosphate. PLP, pyridoxal-5-phoshate. PLP is metabolized, primarily to 4-pyridoxal phosphate, with excretion into the urine. As a transport pool, plasma PLP does not report flux through the system but may be altered by supply of the nutrient to the blood stream and its removal through metabolism and excretion. Several factors have been reported to modify plasma PLP[16] that may themselves altered in renal transplant recipients.

because of excessive intake are unlikely in the absence of supplementation.

Factors that influence the transport pool (plasma PLP) are shown in Figure 1. Colonic supply of B vitamins to the human host has not been evaluated. Magnúsdóttir et al demonstrated that a large number of species have the ability to synthesize vitamin B6.[20] If this is the cause of the hypervitaminosis B6 reported in these and other data, then it would be expected to find different composition of gut microbiota with a higher volume of those species that have vitamin B6 synthesis pathways (including *Bacteroides* spp.) and lesser proportion of those without those pathways (including *Clostridium difficile* and *Faecalibacterium* spp.). The gut microbiota is influenced by several factors, including recent antibiotic use and disease state. Those with CKD have a dysbiosis, with less diversity of species.[21] The effects of renal transplantation and immunosuppressive regimens on the gut microbiota need further study. The impact of vitamin B6 status on the risk of allograft rejection has not been characterized, and this warrants further investigation, as if immune function is depressed in those with lower or deficient concentrations of vitamin B6 as previously suggested,[6] then risk of acute rejection may be increased with its elevation, and increased vigilance needed.

Limitations

This study is limited by the very small sample size. Larger studies are needed exploring potential changes in metabolic pathway activity, the measurement of other nutrients, and potential role of the microbiome. In addition, the study did not explore if elevated PLP was associated with clinical outcomes; including risk of rejection.

Practical Application

Our data would indicate the need for caution in using supplements containing vitamin B6 in children postrenal transplantation. It is unclear whether serum PLP adequately marks vitamin B6 status in these circumstances. Therefore, the cost of laboratory measurement may not be justified although may be considered if signs and symptoms of hypervitaminosis B6 (neuropathy, photosensitivity, nausea, and heartburn) present. The role of vitamin B6 and immune function should be examined more closely.

Acknowledgments

The study team would like to thank the families and staff that continue to be generous in their time to help in conducting research at Southampton Children's Hospital.

Authors' contributions: All authors contributed to the conception and design of the study; M.H. and C.A. collected the data. M.H. analyzed the data. All authors contributed to the interpretation of data, drafting and revision of the article, and approved the final version of the article.

References

1. EFSA. Dietary reference values for vitamin B6. *EFSA J.* 2016;14:e04485.
2. Department of Health. Dietary reference values for food energy and nutrients for the United Kingdom. In: *Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy.* London, UK: Her Majesty's Stationary Office (HMSO); 2005.
3. Mydlik M, Derzsiova K, Zemberova E. Metabolism of vitamin B6 and its requirement in chronic renal failure. *Kidney Int.* 1997;62:S56-S59.
4. Lacour B, Parry C, Drueke T, et al. Pyridoxal 5'-phosphate deficiency in uremic undialyzed, hemodialyzed, and non-uremic kidney transplant patients. *Clin Chim Acta.* 1983;127:205-215.
5. Mooney S, Leuendorf JE, Hendrickson C, Hellmann H. Vitamin B6: a long known compound of surprising complexity. *Molecules.* 2009;14:329-351.
6. Jankowska M, Marszall M, Debska-Slizien A, et al. Vitamin B6 and the immunity in kidney transplant recipients. *J Ren Nutr.* 2013;23:57-64.
7. Kriley M, Warady BA. Vitamin status of pediatric patients receiving long-term peritoneal dialysis. *Am J Clin Nutr.* 1991;53:1476-1479.
8. Pereira AM, Hamani N, Nogueira PC, Carvalhaes JT. Oral vitamin intake in children receiving long-term dialysis. *J Ren Nutr.* 2000;10:24-29.
9. Warady BA, Kriley M, Alon U, Hellerstein S. Vitamin status of infants receiving long-term peritoneal dialysis. *Pediatr Nephrol.* 1994;8:354-356.
10. Don T, Friedlander S, Wong W. Dietary intakes and biochemical status of B vitamins in a group of children receiving dialysis. *J Ren Nutr.* 2010;20:23-28.
11. Stockberger RA, Parrott KA, Alexander SR, Miller LT, Leklem JE, Jenkins RD. Vitamin B-6 status of children undergoing continuous ambulatory peritoneal dialysis. *Nutr Res.* 1987;7:1021-1030.
12. Foreman JW, Abitbol CL, Trachtman H, et al. Nutritional intake in children with renal insufficiency: a report of the growth failure in children with renal diseases study. *J Am Coll Nutr.* 1996;15:579-585.
13. KDOQI. KDOQI clinical practice guideline for nutrition in children with CKD: 2008 update. Executive summary. *Am J Kidney Dis.* 2009;53(3 Suppl 2):S11-S104.
14. Talwar D, Quasim T, McMillan DC, Kinsella J, Williamson C, O'Reilly DS. Optimisation and validation of a sensitive high-performance liquid chromatography assay for routine measurement of pyridoxal 5-phosphate in human plasma and red cells using pre-column semicarbazide derivatisation. *J Chromatogr.* 2003;792:333-343.
15. Litchford M, ed. *Laboratory Assessment of Nutritional Status: Bridging Theory and Practice.* Greensboro, NC: CASE Software & Books; 2011.
16. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. *Annu Rev Nutr.* 2015;35:33-70.
17. Busch M, Gobert A, Franke S, et al. Vitamin B6 metabolism in chronic kidney disease–relation to transsulfuration, advanced glycation and cardiovascular disease. *Nephron Clin Pract.* 2010;114:c38-c46.
18. Meydani SN, Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD, Gershoff SN. Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. *Am J Clin Nutr.* 1991;53:1275-1280.
19. Friso S, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. *Circulation.* 2001;103:2788-2791.
20. Magnusdottir S, Ravcheev D, de Crecy-Lagard V, Thiele I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front Genet.* 2015;6:148.
21. Xu K-Y, Xia G-H, Lu J-Q, et al. Impaired renal function and dysbiosis of gut microbiota contribute to increased trimethylamine-N-oxide in chronic kidney disease patients. *Sci Reps.* 2017;7:1445.

11.15. POSTER PRESENTATIONS

Nutritional Status and Health-Related Quality of Life in Children with Chronic Kidney Disease (19th International Congress on Renal Nutrition and Metabolism, Genoa, Italy, Jun 2018).

A Novel Appetite Assessment Tool for the Use in Children with CKD (19th International Congress on Renal Nutrition and Metabolism, Genoa, Italy, Jun 2018).

Nutritional Status and Health-Related Quality of Life in Children with Chronic Kidney Disease

M. Harmer[1,2,3,4], R.D. Gilbert[1,3,4], S. Wootton[1,4], C.E. Anderson[1,3,4]

[1]Nutrition and Dietetics, [2]Child Health, [3]The Southampton Biomedical Research Centre (Nutrition), [4]University Hospital Southampton NHS Foundation Trust and the University of Southampton, United Kingdom

INTRODUCTION

Health-related quality of life (HRQoL) is an individual's subjective perception of the impact of health status, including disease and treatment, on physical, psychological, and social functioning [1]. HRQoL is lower in those with renal disease. In cohorts of children with other disease states, poor nutritional status and obesity have been associated with poorer HRQoL, but this has not been explored in paediatric Chronic Kidney Disease (CKD). There is therefore, a need to examine the effect of nutritional status, including obesity on HRQoL. The aim of this study is to report the HRQoL scores as assessed by the validated PedsQL™ questionnaire and to explore the relationship of HRQoL scores to markers of nutritional status.

METHODS

A cross-sectional, observational study was performed to determine the HRQoL of a cohort of children with pre-dialysis, conservatively-managed CKD and its relationship to markers of nutritional status.

Children between 3 and 18 years with conservatively-managed CKD (stages 2 to 5, not receiving dialysis and never received a renal transplant) were recruited. Both the child and a parent/care-giver were requested to independently complete the respective questionnaire.

Clinical and anthropometric data were collected; including eGFR, height, weight, waist circumference, and BMI and waist circumference-to-height ratios (WHtR) calculated. Appetite was assessed by a simple Likert scale by the child. Definitions of obesity were BMI SDS >2) MUAC SDS >2 or >25cm) and a WHtR >0.5. Short stature was defined as a height SDS <-2. Blood for analysis of markers of the micronutrients copper, selenium, zinc and manganese were collected, and analysed through the clinical pathology. Level of deprivation was estimated using IDACI (Income Deprivation Affecting Children Index).

The HRQoL was assessed using the PedsQL™ tool [2]. The PedsQL™ is comprised of two questionnaires: one for the child and one for a parent/care-giver. They ask a series of questions that assesses four domains: physical, emotional, social and schooling aspects of the child's life. It is scored as individual domains and as a total score (all four domains), and is expressed as a percentage of maximum score. The tool is available in age-appropriate versions: toddler (2 – 4 years), young child (5 – 7 years), older child (8 – 12 years) and teenager (13 – 18 years).

Figure 1. Example PedsQL™ questionnaire for a 5-7 year old

RESULTS

46 children and parent pairs were recruited to the study. Details of the cohort are given in *table 1*. HRQoL scores were lower in all domains in the cohort compared to healthy control data (*table 2*).

Those who were stunted (Height SDS <-2) had lower HRQoL scores than those who were not for both the child ($t(15.0394) = -3.4356$; $p = 0.0037$) and parent questionnaires ($t(22.87220) = -4.10670$; $p = 0.0004$). Those with obesity (as defined by BMI SDS, MUAC and waist circumference-to-height ratio) did not demonstrate different scores from non-obese patients. Children whose appetite was described as either "good" or "very good" had better scores than those with appetites described as "poor" or "very poor" (child – $t(36) = 2.851$; $p=0.007$ and parent - $t(44) = 2.910$; $p=0.006$) (*see figure 2*).

Correlations were explored between the plasma levels of copper, selenium, zinc, and whole blood manganese. None of these measures correlated with HRQoL scores .

Variables that demonstrated correlation were used to perform multiple linear regression analysis.

Child (self-rater) HRQoL

On multiple linear regression for correlated variables (eGFR and Ht SDS), these two variables statistically significantly predicted child self-assessed HRQoL $F(2,35)=12.436$, $p<0.0005$, $R^2=0.415$. Both Ht SDS and eGFR added statistically significantly to the prediction of child-assessed HRQoL (Ht SDS $p<0.0005$, eGFR $p=0.008$).

Parent-proxy HRQoL

On multiple linear regression for positively-correlated variables (Ht SDS and IDACI), these two variables statistically significantly predicted parent-proxy HRQoL $F(2,42)=11.695$, $p<0.0005$, $R^2=0.358$. Both Ht SDS and IDACI added statistically significantly to the prediction of child-assessed HRQoL (Ht SDS $p=0.001$, IDACI $p=0.005$)

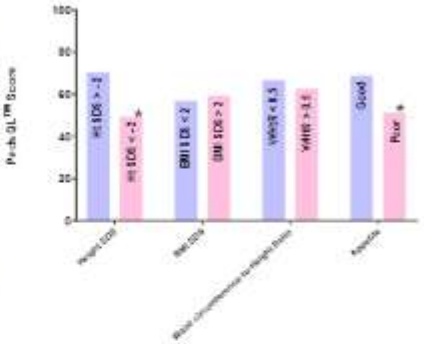


*Figure 2. Average HRQoL scores in those with different nutritional status. *Statistically significant difference between groups.*

DISCUSSION

Stunted children reported lower HRQoL scores than their non-stunted counterparts. Despite previous reports from other cohorts of children, these data do not demonstrate a lower HRQoL in those with markers of obesity (BMI SDS, waist circumference-to-height ratio). The reason for this may be a shift of the perceived normal body shape and "accepted" adiposity in children. HRQoL scores were significantly higher in those with good appetite versus poor appetite. As appetite is a complex function of many factors in which general health and well-being impact upon, this is not surprising. Biomarkers of micronutrient status did not correlate with HRQoL scores. These measurements are prone to many factors, and have a high degree of buffering within the normal range; it is only at the extremes of nutrition that these values become abnormal. In addition, levels may be affected by other factors such as underlying inflammatory state.

There are several limitations to this study; including number of participants, and no longitudinal measures. Nutritional status is a complex, multi-faceted concept and this study only examined a selection of variables. Each of these measures has their own limitations (for example BMI not truly reflecting body composition). Not all variables that may have an impact on HRQoL such as markers of resilience and patient efficacy were explored.

HRQoL is a measure of an overall status, and the perception of an individual's own health status is an important factor that healthcare providers should be aiming to improve. As healthcare services become more patient-centred, the measures by which they are evaluated must include patient-centred measures and by assessing HRQoL using a formalised tool, healthcare processes can be evaluated. HRQoL tools also have the potential to be used as screening tools or to identify areas to be explored during out-patient consultation for a more holistic consultation. For these reasons, HRQoL assessment should be considered for introduction into routine clinical care for ongoing holistic care of children with chronic illnesses, including CKD, and to facilitate the acquisition of longitudinal data regarding the impact of changes in nutritional status and therapy.

REFERENCES

1. Leidy NK, Revicki DA, Geoghegan B (1999) Recommendations for evaluating the validity of quality of life claims for labeling and promotion. Value in Health : the journal of the International Society for Pharmacoeconomics and Outcomes Research 2: 113-127.
2. Varni JW, Burwinkle TM, Seid M, Skarr D (2003) The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. Ambulatory pediatrics : the official journal of the Ambulatory Pediatric Association 3: 329-341.

A Novel Appetite Assessment Tool for the Use in Children with CKD

M. Harmer[1,2,3,4], R.D. Gilbert[2,3,4], S Wootton[3,4], C.E. Anderson[1,3,4]
[1]Nutrition and Dietetics, [2]Child Health, [3]The Southampton Biomedical Research Centre (Nutrition), [4]University Hospital Southampton NHS Foundation Trust and the University of Southampton, United Kingdom

INTRODUCTION

Self-reported appetite has been shown to correlate with severity of renal disease, hospital attendances, and quality of life [1], but is not assessed in a formalised fashion in clinical care. The lack of a formalised assessment tool means that a meaningful comparison between individuals and for an individual longitudinally is virtually impossible. Additionally, the subjective nature of direct questioning may result in a normalisation of poor appetite so that a child may perceive their appetite to be "good", but only relative to their previous feeling of appetite. Children with chronic kidney disease (CKD) are known to have altered appetite and changes in appetite and portend changes in clinical status, including broader nutritional status and deterioration of renal function. Herein we present the development of a new tool to assess the appetite of children with CKD as part of routine clinical care.

THE TOOL DEVELPOMENT

Exploration of healthcare professional views on the current assessment of appetite

An electronic survey was sent to clinicians, dietitians, and clinical nurse specialists within the local paediatric nephrology team with 75% response rate. 100% of responders felt that appetite was important in nephrology patients' clinical care, and that it should be assessed in clinical practice. 17% felt that appetite was assessed at every clinic, with this being informally within the consultation (100%), and 67% documented in the patient health record. 100% felt that a more robust tool would be helpful in their clinical practice.

Review of current practice Re: appetite assessment

Within the clinical service, appetite assessment is inconsistent, even for paediatric renal dietitian reviews. A sample of randomly selected 25 dietetic assessments were evaluated.

Subjective assessment of appetite = 52%
Change in appetite = 32%
Comment on patient's satiety = 0%
Documentation of number of meals/day = 80%
Documentation of the portion size = 76%
Comment on change in portion size = 20%

Tool development

A working-group of healthcare professionals with an interest in nutrition developed a questionnaire (modSNAQ). The questionnaire was based upon the SNAQ questionnaire [2] and modified by the working group. Face validity was assessed by the expert clinical team comprising of paediatric nephrology consultants, specialist paediatric renal dietitians, and professor in nutrition. Following this, further testing with lay members of the public was performed for readability, feasibility, layout and clarity.

Tool validity assessment

Internal consistency
Internal consistency reliability as assessed by Cronbach's alpha co-efficient in a cohort of 60 children attending the nephrology clinic, giving a value of 0.911, which demonstrates a high level of internal consistency. On evaluation of Cronbach's alpha if an item was deleted, all questions showed lower values if deleted (range of Cronbach's alpha = 0.883 to 0.909).

Subgroup assessment revealed similar results (males only = 0.895; females only = 0.933; age <10years only = 0.940; age ≥10years only = 0.885).

Tool validity assessment

Construct validity
60 children ranked their appetite on a Likert scale 1-4 (very poor/poor/good/very good) in addition to completing the modSNAQ.

ROC analysis to identify those children with Likert scale assessed appetite score of very poor or poor = 0.954 (Youden's index analysis demonstrated that a score of 15.5 as a cut-off value, with sensitivity of 95.7% and specificity of 21.4%).

Tool validity assessment

Content validity
Content validity was assessed by a panel HCP including the UK national paediatric renal dietitian group. The average congruency percentage (percentage of questions deemed relevant to appetite assessment) was 94%.

The Content Validity Index for each question (percentage of responders scoring ≥3 out of 4 – relevant/very relevant) was: Qu1 =75%; Qu2 =100%; Qu3 =100%; Qu4 =100%; Qu5 =69%; Qu7 =100%.

Scale-CVI (the number of questions having all responders classify them as relevant / very relevant) was 83%.

Utility in predicting clinical outcomes

In a cohort of 60 children with CKD, modSNAQ scores were measured alongside growth measurements at baseline and 6 and 12 months following appetite assessment.

ROC analysis was used to determine the power of the modSNAQ scores at baseline to predict decrease in weight SDS or BMI SDS (+0.5 SDS) at 6 months, and 12 months and decrease in height SDS and eGFR at 12 months. The modSNAQ tool showed predictive power for a decrease in weight SDS of > 0.5 SDS at 6 months with an area under the curve of 0.709 (95% CI = 0.444 - 0.975).

Trial of implementation into clinical practice

An initial trial of 12 patients in the low clearance clinic of the nephrology centre, assessing the acceptability of the modSNAQ revealed that 91.7% found it easy or very easy to complete.

100% were happy to use the tool in the future.
In one patient a problem was identified as a result from using the tool in clinic.

Future Directions

The modSNAQ tool is now being trialled in a larger paediatric CKD cohort as part of routine clinical care in order to formally assess appetite in a structured manner longitudinally.

Future research will focus on confirming predictive power of the tool, and it's potential utility to aid clinical decision-making.

REFERENCES

1. Ayestaran FW, Schneider MF, Kaskel FJ, Srivaths PR, Seo-Mayer PW, Moxey-Mims M, Furth SL, Warady BA, Greenbaum LA (2016) Perceived appetite and clinical outcomes in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany) 31:1121-1127
2. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, Diebold MR, Morley JE (2005) Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. The American journal of clinical nutrition 82:1074-1081

11.17. GLOSSARY OF TERMS

Adverse event (AE) − an unwanted clinical symptom, sign or disease, or an abnormal laboratory finding, that is related in time to, but is not necessarily caused by, the administration of an IMP to a subject in a clinical trial.

Advisory Committee on Borderline Substances (ACBS) – the UK committee responsible for advising approved prescribers about the prescribing of certain foodstuffs and toiletries.

Algorithm – procedure for making a series of choices among alternative decisions to reach an outcome.

Appetite – the desire to eat food.

Audit − a systematic and independent review of trial-related activities and documents, to find out if the activities were carried out, and if the data were recorded, analysed, and accurately reported, according to the protocol, SOP, GCP, GMP and regulatory requirements.

Bioavailability − a measure of how well a medicine is absorbed by the body.

Biomarker − a laboratory or clinical measure of the body's response to an event that might indicate that, for example an IMP is working. When a biomarker can replace a clinical endpoint it is called a surrogate endpoint.

Case report form (CRF) − a printed, optical, or electronic document designed to record all of the information that is required by the clinical trial protocol, and is to be reported to the sponsor, for each trial subject.

Chief investigator − leads a group of principal investigators.

Chronic Kidney Disease - abnormalities of kidney structure or function, present for 3 months or more, with implications for health.

Compliance − meeting the relevant requirements for a trial-related function.

Cytokine − small proteins produced by cells, mainly white blood cells, in response to an immune stimulus. They mediate and regulate immunity and inflammation.

Data Protection Act − legislation to give people the right of control of personal information that is held about them.

Declaration of Helsinki − guidelines of the World Medical Association that protect the rights, safety and well-being of subjects who take part in clinical trials, and are revised every four years – Directive 2001/20/EC is based on the 1996 version.

Disease Progression – a measurable decrease in kidney function.

Documentation − the process of creating records, in a written, magnetic, optical or other form, that describes the methods and conduct of the study, factors affecting it, and the action taken. Records include the protocol and any amendments, copies of submissions and approvals from the MHRA and REC, curricula vitae, information and consent forms, monitor's reports, audit certificates, relevant letters, reference ranges, raw data, completed CRF and the final study report.

Dose − the amount of an IMP given to the trial subject on one or more occasions (single- or multiple-dose). A dose may be one or more tablets, capsules, injections or other form of the IMP.

End-stage Kidney Disease (ESRD) – glomerular filtration rate of < 15ml/min/1.73m^2.

Efficacy − whether an IMP is effective.

Exclusion criteria − reasons for excluding a subject from a trial, such as taking another medicine, having an illness or having out-of-range laboratory results.

Fibrosis – the thickening and scarring of connective tissue, usually as a result of injury.

Food for Special Medicinal Purposes (FSMP) – a specially processed or formulated food intended for the dietary management, under medical supervision, of patients (including children) who suffer from a disease, disorder or medical condition and whose dietary requirements cannot be met by modifying a normal diet only.

General Medical Council (GMC) − registers and regulates UK physicians.

Good clinical practice (GCP) − an international ethical and scientific quality standard for designing, conducting, recording, monitoring and reporting studies that involve human subjects. GCP ensures that the rights, safety and well-being of the trial subjects are protected, and that the trial data are credible and accurate.

Health-related quality of life (HRQoL) - an individual's subjective perception of the impact of health status, including disease and treatment, on physical, psychological, and social functioning.

Human Tissue Act – legislation to regulate the removal, storage and use of human organs and tissues.

Inclusion criteria − conditions that must be met if a subject is to join a trial.

Informed consent − a process by which subjects voluntarily confirm their willingness to take part in a trial after having been fully informed about it. Informed consent is documented by means of a written, signed and dated consent form.

Investigational medicinal product (IMP) − a potential new medicine, a placebo or a comparator. Includes a marketed product when used or assembled in a way different from the approved form, or when used for an unapproved indication or to gain further information about an approved use.

Investigator − a researcher who carries out a clinical trial. A principal investigator leads a team of researchers. A chief investigator leads a group of principal investigators. In some units, the chief investigator and the principal investigator may be the same person.

in vitro – outside the body, such as in a test tube (the opposite of in vivo).

in vivo – in the living body.

Ligand – a molecule that binds to a protein or receptor.

Likert Scale – An agreement scale used to measure respondents' agreement with a variety of statements, and enable complex opinion to be quantified and compared.

Lower Reference Nutrient Intake (LRNI) – An amount of the nutrient that is enough for only the few people in a group who have low needs.

Medicines and Healthcare products Regulatory Agency (MHRA) − a body required by law to assess the safety, quality and efficacy of medicinal products and devices, and to enforce GCP, GMP and GLP.

Metabolism – the chemical process that occur to maintain life within a living organism.

Nephron – microscopic structural and functional unit of the kidney.

Osmolality – the concentration of a solution, expressed in terms of the amount of osmotically active solute per litre of the final formula solution.

Paediatric Renal Interest in Nutrition Group (PRING) – a national working group of specialist paediatric kidney dietitians that have representation from all the paediatric nephrology centres in the UK

Phase 1 − trials of an IMP in subjects, either healthy subjects or patients, who will not benefit from the IMP.

Phase 2 − early trials of an IMP in subjects with the target disease who are expected to benefit from the IMP.

Phase 3 − late trials of an IMP in many subjects with the target disease who are expected to benefit from the IMP.

Phase 4 − post-marketing trials of a medicine to compare it with other treatments.

Placebo − a preparation that looks and may taste like the IMP that is being tested but contains no active substance (a dummy medicine).

Pre-clinical studies − studies in laboratory animals in vivo or in tissues, cells, components of cells or biological fluids of laboratory animals or humans in vitro before the start of Phase 1 trials. Also called non-clinical studies.

Pre-dialysis, conservatively-managed CKD – the presence of chronic kidney disease, but with kidney great enough not to require renal replacement therapy.

Principal investigator − leads a team of investigators (researchers).

Reference Nutrient Intake (RNI) – An amount of the nutrient that is enough, or more than enough, for about 97% of people in a group. If average intake of a group is at RNI, then the risk of deficiency in the group is very small.

Research ethics committee (REC) − an independent group of medical and scientific professionals and members of the public, with no financial interests or affiliations with the sponsor or researchers, who give an opinion on the ethics of a trial.

Risk – potential for harm.

Serious adverse event (SAE) or serious adverse drug reaction (serious ADR) − any untoward medical event that at any dose of a medicinal product: results in death; is life-threatening; requires a stay in hospital or prolongs an existing stay in hospital; results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect.

Suspected unexpected serious adverse (drug) reaction (SUSAR) − a serious adverse event considered by the investigator or sponsor to be possibly or probably related to the IMP under test and for which the nature and/or severity differs from the information in the investigator's brochure.

Young Person (YP) – A person aged between 11 and 25 years.

12. REFERENCES

1. Rees L, Jones H. Nutritional management and growth in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2013;28(4):527-36.
2. KDOQI. KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 update. Executive summary. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2009;53(3 Suppl 2):S11-104.
3. Registry UR. UK Renal Registry 21st Annual Report - data to 31/12/2017. Available from https://wwwrenalregorg/publications-reports/. 2019.
4. Group KDIGOKCW. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney international. 2013;3(Suppl):1-150.
5. Harambat J, van Stralen KJ, Kim JJ, Tizard EJ. Epidemiology of chronic kidney disease in children. Pediatric nephrology (Berlin, Germany). 2012;27(3):363-73.
6. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. PloS one. 2016;11(7):e0158765.
7. Kerr M, Bray B, Medcalf J, O'Donoghue DJ, Matthews B. Estimating the financial cost of chronic kidney disease to the NHS in England. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27 Suppl 3:iii73-80.
8. Registry UR. 20th Annual Report of the Renal Association. Nephron. 2018;139(suppl1).
9. Mitsnefes MM. Cardiovascular morbidity and mortality in children with chronic kidney disease in North America: lessons from the USRDS and NAPRTCS databases. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2005;25 Suppl 3:S120-2.
10. Staples AO, Greenbaum LA, Smith JM, Gipson DS, Filler G, Warady BA, et al. Association between clinical risk factors and progression of chronic kidney disease in children. Clinical journal of the American Society of Nephrology : CJASN. 2010;5(12):2172-9.
11. Keijzer-Veen MG, Schrevel M, Finken MJ, Dekker FW, Nauta J, Hille ET, et al. Microalbuminuria and lower glomerular filtration rate at young adult age in subjects born very premature and after intrauterine growth retardation. Journal of the American Society of Nephrology : JASN. 2005;16(9):2762-8.
12. Kottgen A. Genome-wide association studies in nephrology research. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2010;56(4):743-58.
13. Seikaly MG, Salhab N, Gipson D, Yiu V, Stablein D. Stature in children with chronic kidney disease: analysis of NAPRTCS database. Pediatric nephrology (Berlin, Germany). 2006;21(6):793-9.
14. Schlondorff DO. Overview of factors contributing to the pathophysiology of progressive renal disease. Kidney international. 2008;74(7):860-6.
15. Nogueira A, Pires MJ, Oliveira PA. Pathophysiological Mechanisms of Renal Fibrosis: A Review of Animal Models and Therapeutic Strategies. In Vivo. 2017;31(1):1-22.
16. Gajjala PR, Sanati M, Jankowski J. Cellular and Molecular Mechanisms of Chronic Kidney Disease with Diabetes Mellitus and Cardiovascular Diseases as Its Comorbidities. Frontiers in Immunology. 2015;6(340).
17. Smith JM, Martz K, Blydt-Hansen TD. Pediatric kidney transplant practice patterns and outcome benchmarks, 1987–2010: A report of the North American Pediatric Renal Trials and Collaborative Studies. Pediatric transplantation. 2013;17(2):149-57.
18. Rodig NM, McDermott KC, Schneider MF, Hotchkiss HM, Yadin O, Seikaly MG, et al. Growth in children with chronic kidney disease: a report from the Chronic Kidney Disease in Children Study. Pediatric nephrology (Berlin, Germany). 2014;29(10):1987-95.
19. Seikaly MG, Salhab N, Gipson D, Yiu V, Stablein D. Stature in children with chronic kidney disease: analysis of NAPRTCS database. Pediatric Nephrology. 2006;21(6):793.

20. Boehm M, Riesenhuber A, Winkelmayer WC, Arbeiter K, Mueller T, Aufricht C. Early erythropoietin therapy is associated with improved growth in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2007;22(8):1189-93.
21. Sozeri B, Mir S, Kara OD, Dincel N. Growth impairment and nutritional status in children with chronic kidney disease. Iran J Pediatr. 2011;21(3):271-7.
22. Mahan JD, Warady BA. Assessment and treatment of short stature in pediatric patients with chronic kidney disease: a consensus statement. Pediatric nephrology (Berlin, Germany). 2006;21(7):917-30.
23. Furth SL, Stablein D, Fine RN, Powe NR, Fivush BA. Adverse clinical outcomes associated with short stature at dialysis initiation: a report of the North American Pediatric Renal Transplant Cooperative Study. Pediatrics. 2002;109(5):909-13.
24. Wong CS, Gipson DS, Gillen DL, Emerson S, Koepsell T, Sherrard DJ, et al. Anthropometric measures and risk of death in children with end-stage renal disease. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2000;36(4):811-9.
25. Kari JA, Gonzalez C, Ledermann SE, Shaw V, Rees L. Outcome and growth of infants with severe chronic renal failure. Kidney international. 2000;57(4):1681-7.
26. Karlberg J. On the construction of the infancy-childhood-puberty growth standard. Acta paediatrica Scandinavica Supplement. 1989;356:26-37.
27. WHO Child Growth Standards based on length/height, weight and age. Acta paediatrica (Oslo, Norway : 1992) Supplement. 2006;450:76-85.
28. van Uitert EM, Exalto N, Burton GJ, Willemsen SP, Koning AH, Eilers PH, et al. Human embryonic growth trajectories and associations with fetal growth and birthweight. Human reproduction (Oxford, England). 2013;28(7):1753-61.
29. Wei C, Gregory JW. Physiology of normal growth. Paediatrics and Child Health. 2009;19(5):236-40.
30. Tse WY, Hindmarsh PC, Brook CG. The infancy-childhood-puberty model of growth: clinical aspects. Acta paediatrica Scandinavica Supplement. 1989;356:38-43; discussion 4-5.
31. Schaefer F, Wingen AM, Hennicke M, Rigden S, Mehls O. Growth charts for prepubertal children with chronic renal failure due to congenital renal disorders. European Study Group for Nutritional Treatment of Chronic Renal Failure in Childhood. Pediatric nephrology (Berlin, Germany). 1996;10(3):288-93.
32. Haffner D, Zivicnjak M. Pubertal development in children with chronic kidney disease. Pediatric Nephrology. 2017;32(6):949-64.
33. Schaefer F, Seidel C, Binding A, Gasser T, Largo RH, Prader A, et al. Pubertal growth in chronic renal failure. Pediatric research. 1990;28(1):5-10.
34. Ranke MB. Towards a Consensus on the Definition of Idiopathic Short Stature. Hormone Research in Paediatrics. 1996;45(suppl 2)(Suppl. 2):64-6.
35. Wuhl E, Fusch C, Scharer K, Mehls O, Schaefer F. Assessment of total body water in paediatric patients on dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1996;11(1):75-80.
36. Bithoney WG, Dubowitz H, Egan H. Failure to thrive/growth deficiency. Pediatrics in review. 1992;13(12):453-60.
37. Hall DM. Growth monitoring. Archives of disease in childhood. 2000;82(1):10-5.
38. Excellence. TNIfHaC. Faltering Growth: recognition and managnement of faltering growth in children. NICE guideline NG75. 2017.
39. de Muinck Keizer-Schrama SM. [Consensus 'diagnosis of short stature in children.' National Organization for Quality Assurance in Hospitals]. Nederlands tijdschrift voor geneeskunde. 1998;142(46):2519-25.
40. de Onis M, Onyango AW, Van den Broeck J, Chumlea WC, Martorell R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. Food and nutrition bulletin. 2004;25(1 Suppl):S27-36.
41. Organisation WH. Child Growth Standards - Training course nad other tools http://www.who.int/childgrowth/training/en

[
42. Sudfeld CR, McCoy DC, Danaei G, Fink G, Ezzati M, Andrews KG, et al. Linear growth and child development in low- and middle-income countries: a meta-analysis. Pediatrics. 2015;135(5):e1266-75.
43. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition: consequences for adult health and human capital. Lancet (London, England). 2008;371(9609):340-57.
44. Addo OY, Stein AD, Fall CH, Gigante DP, Guntupalli AM, Horta BL, et al. Parental childhood growth and offspring birthweight: pooled analyses from four birth cohorts in low and middle income countries. American journal of human biology : the official journal of the Human Biology Council. 2015;27(1):99-105.
45. Golden MHN, Golden BE. Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition. The American journal of clinical nutrition. 1981;34(5):900-8.
46. Waterlow JC. Classification and definition of protein-calorie malnutrition. Br Med J. 1972;3(5826):566-9.
47. Department-of-Health. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. 2005.
48. Wit JM, Boersma B. Catch-up growth: definition, mechanisms, and models. Journal of pediatric endocrinology & metabolism : JPEM. 2002;15 Suppl 5:1229-41.
49. Prader A, Tanner JM, von HG. Catch-up growth following illness or starvation. An example of developmental canalization in man. The Journal of pediatrics. 1963;62:646-59.
50. Jackson aW. The energy requirments for growth and catch-up growth. In: Group IDeC, editor. Activity, energy expenditure and energy requirements in infants and children1989.
51. Ashworth. Regulation of weight and height during recovery from severe malnutrition. In: Chavez B, Basta, editor. Proc 9th Int Congr Nutr Vol 2: Karger, Bsel, Switzerland; 1975. p. 280-5.
52. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. World Health Organization technical report series. 1985;724:1-206.
53. Reynolds JM, Wood AJ, Eminson DM, Postlethwaite RJ. Short stature and chronic renal failure: what concerns children and parents? Archives of disease in childhood. 1995;73(1):36-42.
54. Broyer M, Le Bihan C, Charbit M, Guest G, Tete MJ, Gagnadoux MF, et al. Long-term social outcome of children after kidney transplantation. Transplantation. 2004;77(7):1033-7.
55. Parekh RS, Flynn JT, Smoyer WE, Milne JL, Kershaw DB, Bunchman TE, et al. Improved growth in young children with severe chronic renal insufficiency who use specified nutritional therapy. Journal of the American Society of Nephrology : JASN. 2001;12(11):2418-26.
56. Wingen AM, Mehls O. Nutrition in children with preterminal chronic renal failure. Myth or important therapeutic aid? Pediatric nephrology (Berlin, Germany). 2002;17(2):111-20.
57. Hodson EM, Willis NS, Craig JC. Growth hormone for children with chronic kidney disease. The Cochrane database of systematic reviews. 2012(2):Cd003264.
58. Haffner D, Schaefer F, Nissel R, Wuhl E, Tonshoff B, Mehls O. Effect of growth hormone treatment on the adult height of children with chronic renal failure. German Study Group for Growth Hormone Treatment in Chronic Renal Failure. The New England journal of medicine. 2000;343(13):923-30.
59. Rees L. Growth hormone therapy in children with CKD after more than two decades of practice. Pediatric nephrology (Berlin, Germany). 2016;31(9):1421-35.
60. Drube J, Wan M, Bonthuis M, Wühl E, Bacchetta J, Santos F, et al. Clinical practice recommendations for growth hormone treatment in children with chronic kidney disease. Nature Reviews Nephrology. 2019.
61. Association TBD. Model and Process for Nutrition and Dietetic

Practice. 2019.

62. BAPEN-Quality-Group. A toolkit for clinical commissioning groups and providers in England: malnutrition matters meeting quality standards in nutritional care. wwwbapenorguk. 2012.
63. Saunders J, Smith T. Malnutrition: causes and consequences. Clin Med (Lond). 2010;10(6):624-7.
64. Sylvestre LC, Fonseca KP, Stinghen AE, Pereira AM, Meneses RP, Pecoits-Filho R. The malnutrition and inflammation axis in pediatric patients with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2007;22(6):864-73.
65. Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney international. 2004;65(3):1009-16.
66. Garibotto G, Sofia A, Balbi M, Procopio V, Villaggio B, Tarroni A, et al. Kidney and splanchnic handling of interleukin-6 in humans. Cytokine. 2007;37(1):51-4.
67. Kalantar-Zadeh K, Kleiner M, Dunne E, Lee GH, Luft FC. A modified quantitative subjective global assessment of nutrition for dialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1999;14(7):1732-8.
68. Apostolou A, Printza N, Karagiozoglou-Lampoudi T, Dotis J, Papachristou F. Nutrition assessment of children with advanced stages of chronic kidney disease-A single center study. Hippokratia. 2014;18(3):212-6.
69. Karagiozoglou-Lampoudi T, Daskalou E, Lampoudis D, Apostolou A, Agakidis C. Computer-based malnutrition risk calculation may enhance the ability to identify pediatric patients at malnutrition-related risk for unfavorable outcome. JPEN J Parenter Enteral Nutr. 2015;39(4):418-25.
70. Edefonti A, Mastrangelo A, Paglialonga F. Assessment and monitoring of nutrition status in pediatric peritoneal dialysis patients. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29 Suppl 2:S176-9.
71. Bonthuis M, van Stralen KJ, Verrina E, Groothoff JW, Alonso Melgar A, Edefonti A, et al. Underweight, overweight and obesity in paediatric dialysis and renal transplant patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2013;28 Suppl 4:iv195-iv204.
72. Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. Annals of internal medicine. 2006;144(1):21-8.
73. Ribstein J, du Cailar G, Mimran A. Combined renal effects of overweight and hypertension. Hypertension (Dallas, Tex : 1979). 1995;26(4):610-5.
74. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bulletin of the World Health Organization. 2007;85(9):660-7.
75. Ashwell M, Lejeune S, McPherson K. Ratio of waist circumference to height may be better indicator of need for weight management. BMJ (Clinical research ed). 1996;312(7027):377.
76. Food_Standards_Agency_and_Public_Health_England. NDNS: Results from years 7 and 8 (combined). 2018.
77. Foreman JW, Abitbol CL, Trachtman H, Garin EH, Feld LG, Strife CF, et al. Nutritional intake in children with renal insufficiency: a report of the growth failure in children with renal diseases study. Journal of the American College of Nutrition. 1996;15(6):579-85.
78. Naseri M, Shahri HM, Horri M, Rasoli Z, Salemian F, Jahanshahi S, et al. Antioxidant vitamins status in children and young adults undergoing dialysis: A single center study. Indian journal of nephrology. 2015;25(4):206-12.
79. Drukker A, Itai T, Stankiewicz H, Goldstein R. Plasma vitamin E levels in uremic children and adolescents. Child nephrology and urology. 1988;9(4):208-10.
80. Joyce T, Court Brown F, Wallace D, Reid CJD, Sinha MD. Trace element and vitamin concentrations in paediatric dialysis patients. Pediatric nephrology (Berlin, Germany). 2018;33(1):159-65.
81. Yu JH, Kim M-S. Molecular Mechanisms of Appetite Regulation. Diabetes & Metabolism Journal. 2012;36(6):391-8.

82. Kalantar-Zadeh K, Block G, McAllister CJ, Humphreys MH, Kopple JD. Appetite and inflammation, nutrition, anemia, and clinical outcome in hemodialysis patients. The American journal of clinical nutrition. 2004;80(2):299-307.
83. Burrowes JD, Larive B, Chertow GM, Cockram DB, Dwyer JT, Greene T, et al. Self-reported appetite, hospitalization and death in haemodialysis patients: findings from the Hemodialysis (HEMO) Study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2005;20(12):2765-74.
84. Ayestaran FW, Schneider MF, Kaskel FJ, Srivaths PR, Seo-Mayer PW, Moxey-Mims M, et al. Perceived appetite and clinical outcomes in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2016;31(7):1121-7.
85. Gama-Axelsson T, Lindholm B, Barany P, Heimburger O, Stenvinkel P, Qureshi AR. Self-rated appetite as a predictor of mortality in patients with stage 5 chronic kidney disease. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2013;23(2):106-13.
86. Young V, Balaam S, Orazio L, Bates A, Badve SV, Johnson DW, et al. APPETITE PREDICTS INTAKE AND NUTRITIONAL STATUS IN PATIENTS RECEIVING PERITONEAL DIALYSIS. Journal of renal care. 2016;42(2):123-31.
87. van der Meij BS, Wijnhoven HAH, Lee JS, Houston DK, Hue T, Harris TB, et al. Poor Appetite and Dietary Intake in Community-Dwelling Older Adults. Journal of the American Geriatrics Society. 2017;65(10):2190-7.
88. Aguilera A, Codoceo R, Bajo MA, Iglesias P, Diéz JJ, Barril G, et al. Eating Behavior Disorders in Uremia: A Question of Balance in Appetite Regulation. Seminars in Dialysis. 2004;17(1):44-52.
89. Kogon AJ, Vander Stoep A, Weiss NS, Smith J, Flynn JT, McCauley E. Depression and its associated factors in pediatric chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2013;28(9):1855-61.
90. Moreira JM, Bouissou Morais Soares CM, Teixeira AL, Simoes ESAC, Kummer AM. Anxiety, depression, resilience and quality of life in children and adolescents with pre-dialysis chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2015;30(12):2153-62.
91. Zha Y, Qian Q. Protein Nutrition and Malnutrition in CKD and ESRD. Nutrients. 2017;9(3).
92. Arbeiter AK, Büscher R, Petersenn S, Hauffa BP, Mann K, Hoyer PF. Ghrelin and other appetite-regulating hormones in paediatric patients with chronic renal failure during dialysis and following kidney transplantation. Nephrology Dialysis Transplantation. 2009;24(2):643-6.
93. Leidy NK, Revicki DA, Geneste B. Recommendations for evaluating the validity of quality of life claims for labeling and promotion. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research. 1999;2(2):113-27.
94. Rees L. Long-term outcome after renal transplantation in childhood. Pediatric nephrology (Berlin, Germany). 2009;24(3):475-84.
95. Gerson AC, Wentz A, Abraham AG, Mendley SR, Hooper SR, Butler RW, et al. Health-related quality of life of children with mild to moderate chronic kidney disease. Pediatrics. 2010;125(2):e349-57.
96. McKenna AM, Keating LE, Vigneux A, Stevens S, Williams A, Geary DF. Quality of life in children with chronic kidney disease-patient and caregiver assessments. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(7):1899-905.
97. Stone MB, Botto LD, Feldkamp ML, Smith KR, Roling L, Yamashiro D, et al. Improving quality of life of children with oral clefts: perspectives of parents. The Journal of craniofacial surgery. 2010;21(5):1358-64.
98. Brinksma A, Sanderman R, Roodbol PF, Sulkers E, Burgerhof JG, de Bont ES, et al. Malnutrition is associated with worse health-related quality of life in children with cancer. Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer. 2015;23(10):3043-52.
99. Buttitta M, Iliescu C, Rousseau A, Guerrien A. Quality of life in overweight and obese children and adolescents: a literature review. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2014;23(4):1117-39.

100. Duran Aguero S, Gonzalez Canete N, Pena D'Ardaillon F, Candia Johns P. [Association of intake macro and micronutrients with life quality of life in elderly]. Nutricion hospitalaria. 2015;31(6):2578-82.
101. Gonzalez S, Huerta JM, Fernandez S, Patterson AM, Lasheras C. Life-quality indicators in elderly people are influenced by selenium status. Aging clinical and experimental research. 2007;19(1):10-5.
102. Witte KK, Nikitin NP, Parker AC, von Haehling S, Volk HD, Anker SD, et al. The effect of micronutrient supplementation on quality-of-life and left ventricular function in elderly patients with chronic heart failure. European heart journal. 2005;26(21):2238-44.
103. Oh TR, Kim CS, Bae EH, Ma SK, Han SH, Sung SA, et al. Association between vitamin D deficiency and health-related quality of life in patients with chronic kidney disease from the KNOW-CKD study. PloS one. 2017;12(4):e0174282.
104. Johansson P, Dahlstrom O, Dahlstrom U, Alehagen U. Improved Health-Related Quality of Life, and More Days out of Hospital with Supplementation with Selenium and Coenzyme Q10 Combined. Results from a Double Blind, Placebo-Controlled Prospective Study. The journal of nutrition, health & aging. 2015;19(9):870-7.
105. Rayman M, Thompson A, Warren-Perry M, Galassini R, Catterick J, Hall E, et al. Impact of selenium on mood and quality of life: a randomized, controlled trial. Biological psychiatry. 2006;59(2):147-54.
106. Reynolds JM, Garralda ME, Jameson RA, Postlethwaite RJ. How parents and families cope with chronic renal failure. Archives of disease in childhood. 1988;63(7):821-6.
107. Heath J, Mackinlay D, Watson AR, Hames A, Wirz L, Scott S, et al. Self-reported quality of life in children and young people with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2011;26(5):767-73.
108. Al-Uzri A, Matheson M, Gipson DS, Mendley SR, Hooper SR, Yadin O, et al. The impact of short stature on health-related quality of life in children with chronic kidney disease. The Journal of pediatrics. 2013;163(3):736-41.e1.
109. Teixeira CG, Duarte Mdo C, Prado CM, Albuquerque EC, Andrade LB. Impact of chronic kidney disease on quality of life, lung function, and functional capacity. Jornal de pediatria. 2014;90(6):580-6.
110. Aveles PR, Criminacio CR, Goncalves S, Bignelli AT, Claro LM, Siqueira SS, et al. Association between biomarkers of carbonyl stress with increased systemic inflammatory response in different stages of chronic kidney disease and after renal transplantation. Nephron Clinical practice. 2010;116(4):c294-9.
111. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Archives of pathology & laboratory medicine. 2009;133(4):628-32.
112. Tertemiz KC, Ozgen Alpaydin A, Sevinc C, Ellidokuz H, Acara AC, Cimrin A. Could "red cell distribution width" predict COPD severity? Revista portuguesa de pneumologia. 2016;22(4):196-201.
113. Collas VM, Paelinck BP, Rodrigus IE, Vrints CJ, Van Craenenbroeck EM, Bosmans JM. Red cell distribution width improves the prediction of prognosis after transcatheter aortic valve implantation. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. 2016;49(2):471-7.
114. Yao J, Lv G. Association between red cell distribution width and acute pancreatitis: a cross-sectional study. BMJ open. 2014;4(8).
115. Tsagalis G. Renal anemia: a nephrologist's view. Hippokratia. 2011;15(Suppl 1):39-43.
116. Sachdev A, Simalti A, Kumar A, Gupta N, Gupta D, Chugh P. Outcome Prediction Value of Red Cell Distribution Width in Critically-ill Children. Indian pediatrics. 2018;55(5):414-6.
117. Hsieh YP, Chang CC, Kor CT, Yang Y, Wen YK, Chiu PF. The Predictive Role of Red Cell Distribution Width in Mortality among Chronic Kidney Disease Patients. PloS one. 2016;11(12):e0162025.
118. Yonemoto S, Hamano T, Fujii N, Shimada K, Yamaguchi S, Matsumoto A, et al. Red cell distribution width and renal outcome in patients with non-dialysis-dependent chronic kidney disease. PloS one. 2018;13(6):e0198825.

119. Solak Y, Yilmaz MI, Saglam M, Caglar K, Verim S, Unal HU, et al. Red cell distribution width is independently related to endothelial dysfunction in patients with chronic kidney disease. The American journal of the medical sciences. 2014;347(2):118-24.
120. Xu H, Li W, Mao J-h, Pan Y-x. Association between red blood cell distribution width and Henoch–Schonlein purpura nephritis. Medicine. 2017;96(23):e7091.
121. Babitt JL, Lin HY. Mechanisms of Anemia in CKD. Journal of the American Society of Nephrology : JASN. 2012;23(10):1631-4.
122. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney international. 2007;71(10):967-70.
123. Basturk T, Sari O, Koc Y, Eren N, Isleem M, Kara E, et al. Prognostic significance of NGAL in early stage chronic kidney disease. Minerva urologica e nefrologica = The Italian journal of urology and nephrology. 2016.
124. Patel ML, Sachan R, Verma A, Kamal R, Gupta KK. Neutrophil gelatinase-associated lipocalin as a biomarker of disease progression in patients with chronic kidney disease. Indian journal of nephrology. 2016;26(2):125-30.
125. Mishra J, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. Journal of the American Society of Nephrology : JASN. 2004;15(12):3073-82.
126. Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, et al. An iron delivery pathway mediated by a lipocalin. Molecular cell. 2002;10(5):1045-56.
127. Virzi GM, Clementi A, de Cal M, Cruz DN, Ronco C. Genomics and biological activity of neutrophil gelatinase-associated lipocalin in several clinical settings. Blood purification. 2013;35(1-3):139-43.
128. Rybi-Szuminska A, Wasilewska A, Litwin M, Kulaga Z, Szuminski M. Paediatric normative data for urine NGAL/creatinine ratio. Acta paediatrica (Oslo, Norway : 1992). 2013;102(6):e269-72.
129. Bennett MR, Nehus E, Haffner C, Ma Q, Devarajan P. Pediatric reference ranges for acute kidney injury biomarkers. Pediatric nephrology (Berlin, Germany). 2015;30(4):677-85.
130. Cangemi G, Storti S, Cantinotti M, Fortunato A, Emdin M, Bruschettini M, et al. Reference values for urinary neutrophil gelatinase-associated lipocalin (NGAL) in pediatric age measured with a fully automated chemiluminescent platform. Clinical chemistry and laboratory medicine. 2013;51(5):1101-5.
131. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clinical journal of the American Society of Nephrology : CJASN. 2009;4(2):337-44.
132. Alderson HV, Ritchie JP, Pagano S, Middleton RJ, Pruijm M, Vuilleumier N, et al. The Associations of Blood Kidney Injury Molecule-1 and Neutrophil Gelatinase-Associated Lipocalin with Progression from CKD to ESRD. Clinical journal of the American Society of Nephrology : CJASN. 2016;11(12):2141-9.
133. Yan L, Borregaard N, Kjeldsen L, Moses MA. The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL. The Journal of biological chemistry. 2001;276(40):37258-65.
134. Cheng Z, Limbu MH, Wang Z, Liu J, Liu L, Zhang X, et al. MMP-2 and 9 in Chronic Kidney Disease. International Journal of Molecular Sciences. 2017;18(4):776.
135. Haussler U, von Wichert G, Schmid RM, Keller F, Schneider G. Epidermal growth factor activates nuclear factor-kappaB in human proximal tubule cells. American journal of physiology Renal physiology. 2005;289(4):F808-15.
136. Zang X, Zheng F, Hong HJ, Jiang Y, Song Y, Xia Y. Neutrophil gelatinase-associated lipocalin protects renal tubular epithelial cells in hypoxia-reperfusion by reducing apoptosis. International urology and nephrology. 2014;46(8):1673-9.
137. Tong Z, Wu X, Ovcharenko D, Zhu J, Chen CS, Kehrer JP. Neutrophil gelatinase-associated lipocalin as a survival factor. The Biochemical journal. 2005;391(Pt 2):441-8.

138. Devireddy LR, Teodoro JG, Richard FA, Green MR. Induction of Apoptosis by a Secreted Lipocalin That is Transcriptionally Regulated by IL-3 Deprivation. Science (New York, NY). 2001;293(5531):829-34.
139. Yazdani M, Merrikhi A, Beni ZN, Baradaran A, Soleimani N, Musazade H. Association between neutrophil geletinase-associated lipocalin and iron deficiency anemia in children on chronic dialysis. Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences. 2014;19(7):624-8.
140. Xiang D, Wang X, Liu P, Pan Y, Zhang Q, Chi X, et al. Increased NGAL level associated with iron store in chronic kidney disease with anemia. 2018;18(4):563-8.
141. Kim IY, Kim JH, Lee DW, Lee SB, Rhee H, Song SH, et al. Plasma neutrophil gelatinase-associated lipocalin is associated with iron status in anemic patients with pre-dialysis chronic kidney disease. Clinical and experimental nephrology. 2018;22(1):28-34.
142. Gunes A, Ece A, Aktar F, Tan I, Soker M, Karabel D, et al. Urinary Kidney Injury Molecules in Children with Iron-Deficiency Anemia. Medical science monitor : international medical journal of experimental and clinical research. 2015;21:4023-9.
143. Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. Clinical journal of the American Society of Nephrology : CJASN. 2010;5(12):2229-35.
144. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature. 2008;453:783.
145. Nakamura J, Sato Y, Kitai Y, Wajima S, Yamamoto S, Oguchi A, et al. Myofibroblasts acquire retinoic acid-producing ability during fibroblast-to-myofibroblast transition following kidney injury. Kidney international. 2019;95(3):526-39.
146. Sahraei Z, Salamzadeh J, Nafar M. Effect of N-acetyl cysteine and vitamin C on kidney allograft function biomarkers interleukin-18 and neutrophil gelatinase-associated lipocalin. Iranian journal of kidney diseases. 2015;9(1):56-62.
147. Lucisano S, Arena A, Stassi G, Iannello D, Montalto G, Romeo A, et al. Role of Paricalcitol in Modulating the Immune Response in Patients with Renal Disease. International journal of endocrinology. 2015;2015:765364-.
148. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. Journal of immunology (Baltimore, Md : 1950). 2012;188(5):2127-35.
149. Alvarez JA, Zughaier SM, Law J, Hao L, Wasse H, Ziegler TR, et al. Effects of high-dose cholecalciferol on serum markers of inflammation and immunity in patients with early chronic kidney disease. European journal of clinical nutrition. 2013;67(3):264-9.
150. Nishida M, Kawakatsu H, Okumura Y, Hamaoka K. Serum and urinary neutrophil gelatinase-associated lipocalin levels in children with chronic renal diseases. Pediatrics international : official journal of the Japan Pediatric Society. 2010;52(4):563-8.
151. Tanner J. A history of the study of human growth. London: Cambridge university Press; 1981.
152. Schwartz GJ, Haycock GB, Edelmann CM, Jr., Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics. 1976;58(2):259-63.
153. Conolly. Health Survey for England 2015: Children's BMI, overweight and obesity. Health and Social Care Information Centre. 2016.
154. England. S. Active lives children and young people survey academic year 2017/18. December 2018.
155. Pereira RA, Cordeiro AC, Avesani CM, Carrero JJ, Lindholm B, Amparo FC, et al. Sarcopenia in chronic kidney disease on conservative therapy: prevalence and association with mortality. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2015;30(10):1718-25.
156. Vannucchi MT, Vannucchi H, Humphreys M. Serum levels of vitamin A and retinol binding protein in chronic renal patients treated by continuous ambulatorial peritoneal dialysis. International journal for vitamin and nutrition research Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung Journal international de vitaminologie et de nutrition. 1992;62(2):107-12.

157. Gee PT. Unleashing the untold and misunderstood observations on vitamin E. Genes & nutrition. 2011;6(1):5-16.
158. Wu JH, Croft KD. Vitamin E metabolism. Molecular aspects of medicine. 2007;28(5-6):437-52.
159. Schultz M, Leist M, Petrzika M, Gassmann B, Brigelius-Flohe R. Novel urinary metabolite of alpha-tocopherol, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? The American journal of clinical nutrition. 1995;62(6 Suppl):1527s-34s.
160. Bayes B, Pastor MC, Bonal J, Foraster A, Romero R. Oxidative stress, inflammation and cardiovascular mortality in haemodialysis--role of seniority and intravenous ferrotherapy: analysis at 4 years of follow-up. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2006;21(4):984-90.
161. Ceballos-Picot I, Witko-Sarsat V, Merad-Boudia M, Nguyen AT, Thevenin M, Jaudon MC, et al. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. Free radical biology & medicine. 1996;21(6):845-53.
162. Ross EA, Koo LC, Moberly JB. Low whole blood and erythrocyte levels of glutathione in hemodialysis and peritoneal dialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1997;30(4):489-94.
163. Poulianiti KP, Kaltsatou A, Mitrou GI. Systemic Redox Imbalance in Chronic Kidney Disease: A Systematic Review. 2016;2016:8598253.
164. Zargari M, Sedighi O. Influence of Hemodialysis on Lipid Peroxidation, Enzymatic and Non-Enzymatic Antioxidant Capacity in Chronic Renal Failure Patients. Nephro-urology monthly. 2015;7(4):e28526.
165. Koca T, Berber A, Koca HB, Demir TA, Koken T. Effects of hemodialysis period on levels of blood trace elements and oxidative stress. Clinical and experimental nephrology. 2010;14(5):463-8.
166. Yang SK, Xiao L, Xu B, Xu XX, Liu FY, Sun L. Effects of vitamin E-coated dialyzer on oxidative stress and inflammation status in hemodialysis patients: a systematic review and meta-analysis. Renal failure. 2014;36(5):722-31.
167. Kaiser S, Di Mascio P, Murphy ME, Sies H. Physical and chemical scavenging of singlet molecular oxygen by tocopherols. Archives of biochemistry and biophysics. 1990;277(1):101-8.
168. Saland JM, Satlin LM, Zalsos-Johnson J, Cremers S, Ginsberg HN. Impaired postprandial lipemic response in chronic kidney disease. Kidney international. 2016;90(1):172-80.
169. Traber MG, Leonard SW, Bobe G, Fu X, Saltzman E, Grusak MA, et al. alpha-Tocopherol disappearance rates from plasma depend on lipid concentrations: studies using deuterium-labeled collard greens in younger and older adults. The American journal of clinical nutrition. 2015;101(4):752-9.
170. Hageman SH, She L, Furr HC, Clark RM. Excess vitamin E decreases canthaxanthin absorption in the rat. Lipids. 1999;34(6):627-31.
171. Wheldon GH, Bhatt A, Keller P, Hummler H. d,1-alpha-Tocopheryl acetate (vitamin E): a long term toxicity and carcinogenicity study in rats. International journal for vitamin and nutrition research Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung Journal international de vitaminologie et de nutrition. 1983;53(3):287-96.
172. Glynn RJ, Ridker PM, Goldhaber SZ, Zee RY, Buring JE. Effects of random allocation to vitamin E supplementation on the occurrence of venous thromboembolism: report from the Women's Health Study. Circulation. 2007;116(13):1497-503.
173. Booth SL, Golly I, Sacheck JM, Roubenoff R, Dallal GE, Hamada K, et al. Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. The American journal of clinical nutrition. 2004;80(1):143-8.
174. Cozzolino M, Mangano M, Galassi A, Ciceri P, Messa P, Nigwekar S. Vitamin K in Chronic Kidney Disease. Nutrients. 2019;11(1):168.
175. Zeng X, Zhang Y, Kwong JS, Zhang C, Li S, Sun F, et al. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. J Evid Based Med. 2015;8(1):2-10.
176. Coleman JE, Watson AR. Micronutrient supplementation in children on continuous cycling peritoneal dialysis (CCPD). Advances in peritoneal dialysis Conference on Peritoneal Dialysis. 1992;8:396-401.

177. de Cavanagh EM, Ferder L, Carrasquedo F, Scrivo D, Wassermann A, Fraga CG, et al. Higher levels of antioxidant defenses in enalapril-treated versus non-enalapril-treated hemodialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1999;34(3):445-55.
178. De Bevere VO, Nelis HJ, De Leenheer AP, Lambert WE, De Paepe M, Ringoir S. Vitamin E levels in hemodialysis patients. Jama. 1982;247(17):2371.
179. Stein G, Sperschneider H, Koppe S. Vitamin levels in chronic renal failure and need for supplementation. Blood purification. 1985;3(1-3):52-62.
180. Hultqvist M, Hegbrant J, Nilsson-Thorell C, Lindholm T, Nilsson P, Linden T, et al. Plasma concentrations of vitamin C, vitamin E and/or malondialdehyde as markers of oxygen free radical production during hemodialysis. Clinical nephrology. 1997;47(1):37-46.
181. Zwolinska D, Grzeszczak W, Szczepanska M, Kilis-Pstrusinska K, Szprynger K. Vitamins A, E and C as non-enzymatic antioxidants and their relation to lipid peroxidation in children with chronic renal failure. Nephron Clinical practice. 2006;103(1):c12-8.
182. Nikolakakis N, Kounali D, Tornaritis M, Anastassou A, Papadakis E, Kassotakis G, et al. Adipose tissue fatty acid composition, serum lipids, and serum alpha-tocopherol in continuous ambulatory peritoneal dialysis patients living on the island of Crete. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 1999;19(2):154-9.
183. Blumberg A, Hanck A, Sander G. Vitamin nutrition in patients on continuous ambulatory peritoneal dialysis (CAPD). Clinical nephrology. 1983;20(5):244-50.
184. Bonnefont-Rousselot D, Jaudon MC, Issad B, Cacoub P, Congy F, Jardel C, et al. Antioxidant status of elderly chronic renal patients treated by continuous ambulatory peritoneal dialysis. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1997;12(7):1399-405.
185. Zwolinska D, Grzeszczak W, Szczepanska M, Makulska I, Kilis-Pstrusinska K, Szprynger K. Oxidative stress in children on peritoneal dialysis. Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis. 2009;29(2):171-7.
186. Joyce B, Sinha, Reid. Evaluation of nutritional bloods in paediatric dialysis patients (Abstracts - 48th ESPN Meeting, Brussels, September 2015). Pediatric Nephrology. 2015;30(9):1638.
187. Farrington K, Miller P, Varghese Z, Baillod RA, Moorhead JF. Vitamin A toxicity and hypercalcaemia in chronic renal failure. British medical journal (Clinical research ed). 1981;282(6281):1999-2002.
188. Duewer DL, Kline MC, Sharpless KE, Thomas JB, Gary KT, Sowell AL. Micronutrients Measurement Quality Assurance Program: Helping Participants Use Interlaboratory Comparison Exercise Results To Improve Their Long-Term Measurement Performance. Analytical Chemistry. 1999;71(9):1870-8.
189. Iughetti L, Perugini C, Predieri B, Madeo S, Bellomo G, Bernasconi S, et al. Low-density lipoprotein oxidizability in children with chronic renal failure. Pediatrics international : official journal of the Japan Pediatric Society. 2008;50(4):447-53.
190. Tasanarong A, Kongkham S, Duangchana S, Thitiarchakul S, Eiam-Ong S. Vitamin E ameliorates renal fibrosis by inhibition of TGF-beta/Smad2/3 signaling pathway in UUO mice. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2011;94 Suppl 7:S1-9.
191. Shing CM, Fassett RG, Peake JM, Coombes JS. Effect of tocopherol on atherosclerosis, vascular function, and inflammation in apolipoprotein E knockout mice with subtotal nephrectomy. Cardiovascular therapeutics. 2014;32(6):270-5.
192. Saran R, Novak JE, Desai A, Abdulhayoglu E, Warren JS, Bustami R, et al. Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2003;18(11):2415-20.
193. Yukawa S, Mune M, Otani H. [Vitamin E disturbances in chronic renal failure]. Nihon rinsho Japanese journal of clinical medicine. 1999;57(10):2366-70.

194. Islam KN, O'Byrne D, Devaraj S, Palmer B, Grundy SM, Jialal I. Alpha-tocopherol supplementation decreases the oxidative susceptibility of LDL in renal failure patients on dialysis therapy. Atherosclerosis. 2000;150(1):217-24.
195. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet (London, England). 2000;356(9237):1213-8.
196. Ramos LF, Kane J, McMonagle E, Le P, Wu P, Shintani A, et al. Effects of combination tocopherols and alpha lipoic acid therapy on oxidative stress and inflammatory biomarkers in chronic kidney disease. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2011;21(3):211-8.
197. Himmelfarb J, Ikizler TA, Ellis C, Wu P, Shintani A, Dalal S, et al. Provision of antioxidant therapy in hemodialysis (PATH): a randomized clinical trial. Journal of the American Society of Nephrology : JASN. 2014;25(3):623-33.
198. Tahzib M, Frank R, Gauthier B, Valderrama E, Trachtman H. Vitamin E treatment of focal segmental glomerulosclerosis: results of an open-label study. Pediatric Nephrology. 1999;13(8):649-52.
199. Chan JC. Focal segmental glomerulosclerosis: a single center study of over two decades. World J Pediatr. 2007;3(4):260-4.
200. Modarresi A. 1462 The Effect of Oral Vitamin E on Renal Anemia in Chronic Hemodialysed Children. Archives of disease in childhood. 2012;97(Suppl 2):A415-A.
201. Farida Farid GM, Hala El-Khawas, Eman El-Hadidi, Tamer Sameeh. Plasma F2-Isoprostane: A Biomarker of Lipid Peroxidation: Correlation with Cerebral Haemodynamics in Children with Chronic Renal Failure Egypt J Neurol Psychiat Neurosurg 2009;46(1):235-46.
202. Cristol JP, Bosc JY, Badiou S, Leblanc M, Lorrho R, Descomps B, et al. Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1997;12(11):2312-7.
203. Nemeth I, Turi S, Haszon I, Bereczki C. Vitamin E alleviates the oxidative stress of erythropoietin in uremic children on hemodialysis. Pediatric nephrology (Berlin, Germany). 2000;14(1):13-7.
204. Oshima K, Ikeda Y, Horinouchi Y, Watanabe H, Hamano H, Kihira Y, et al. Iron suppresses erythropoietin expression via oxidative stress-dependent hypoxia-inducible factor-2 alpha inactivation. Laboratory investigation; a journal of technical methods and pathology. 2017.
205. Miller ER, 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Annals of internal medicine. 2005;142(1):37-46.
206. British_Nutrition_Foundation. Selenium and Health. 2001.
207. Francesconi KA, Pannier F. Selenium metabolites in urine: a critical overview of past work and current status. Clinical chemistry. 2004;50(12):2240-53.
208. Iglesias P, Selgas R, Romero S, Diez JJ. Selenium and kidney disease. Journal of nephrology. 2013;26(2):266-72.
209. Aksoy A, Karaoglu A, Akpolat N, Naziroglu M, Ozturk T, Karagoz ZK. Protective Role of Selenium and High Dose Vitamin E against Cisplatin - Induced Nephrotoxicty in Rats. Asian Pacific journal of cancer prevention : APJCP. 2015;16(16):6877-82.
210. Randjelovic P, Veljkovic S, Stojiljkovic N, Velickovic L, Sokolovic D, Stoiljkovic M, et al. Protective effect of selenium on gentamicin-induced oxidative stress and nephrotoxicity in rats. Drug and chemical toxicology. 2012;35(2):141-8.
211. Liu L, Liu C, Hou L, Lv J, Wu F, Yang X, et al. Protection against ischemia/reperfusioninduced renal injury by cotreatment with erythropoietin and sodium selenite. Molecular medicine reports. 2015;12(6):7933-40.
212. Ghorbani A, Omidvar B, Parsi A. Protective effect of selenium on cisplatin induced nephrotoxicity: A double-blind controlled randomized clinical trial. Journal of nephropathology. 2013;2(2):129-34.

213. Ortac E, Ozkaya O, Saraymen R, Yildiz N, Bedir A, Buyan N, et al. Low hair selenium and plasma glutathione peroxidase in children with chronic renal failure. Pediatric nephrology (Berlin, Germany). 2006;21(11):1739-45.
214. Esmaeili M, Rakhshanizadeh F. Serum Trace Elements in Children with End-Stage Renal Disease. Journal of Renal Nutrition. 2019;29(1):48-54.
215. Combs GF, Jr. Biomarkers of selenium status. Nutrients. 2015;7(4):2209-36.
216. Zwołińska D, Grzeszczak W, Szczepańska M, Kiliś-Pstrusińska K, Szprynger K. Lipid peroxidation and antioxidant enzymes in children on maintenance dialysis. Pediatric Nephrology. 2006;21(5):705-10.
217. Zwolinska D, Grzeszczak W, Kilis-Pstrusinska K, Szprynger K, Szczepanska M. Lipid peroxidation and antioxidant enzymes in children with chronic renal failure. Pediatric nephrology (Berlin, Germany). 2004;19(8):888-92.
218. Tonelli M, Wiebe N, Hemmelgarn B, Klarenbach S, Field C, Manns B, et al. Trace elements in hemodialysis patients: a systematic review and meta-analysis. BMC Medicine. 2009;7:25-.
219. Prodanchuk M, Makarov O, Pisarev E, Sheiman B, Kulyzkiy M. Disturbances of trace element metabolism in ESRD patients receiving hemodialysis and hemodiafiltration. Central European journal of urology. 2014;66(4):472-6.
220. Gomez de Ona C, Martinez-Morillo E, Gago Gonzalez E, Vidau Arguelles P, Fernandez Merayo C, Alvarez Menendez FV. Variation of trace element concentrations in patients undergoing hemodialysis in the north of Spain. Scandinavian journal of clinical and laboratory investigation. 2016;76(6):492-9.
221. Fujishima Y, Ohsawa M, Itai K, Kato K, Tanno K, Turin TC, et al. Serum selenium levels are inversely associated with death risk among hemodialysis patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(10):3331-8.
222. Saint-Georges MD, Bonnefont DJ, Bourely BA, Jaudon MC, Cereze P, Chaumeil P, et al. Correction of selenium deficiency in hemodialyzed patients. Kidney international Supplement. 1989;27:S274-7.
223. Dworkin B, Weseley S, Rosenthal WS, Schwartz EM, Weiss L. Diminished blood selenium levels in renal failure patients on dialysis: correlations with nutritional status. The American journal of the medical sciences. 1987;293(1):6-12.
224. Wan H, Zhu Y, Chen P, Wang Y, Hao P, Cheng Z, et al. Effect of various selenium doses on chromium(IV)-induced nephrotoxicity in a male chicken model. Chemosphere. 2017;174:306-14.
225. Hamza RZ, Al-Harbi MS, El-Shenawy NS. Ameliorative effect of vitamin E and selenium against oxidative stress induced by sodium azide in liver, kidney, testis and heart of male mice. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2017;91:602-10.
226. Bolignano D, Cernaro V, Gembillo G, Baggetta R, Buemi M, D'Arrigo G. Antioxidant agents for delaying diabetic kidney disease progression: A systematic review and meta-analysis. PloS one. 2017;12(6):e0178699.
227. Jablonska E, Gromadzinska J, Reszka E, Wasowicz W, Sobala W, Szeszenia-Dabrowska N, et al. Association between GPx1 Pro198Leu polymorphism, GPx1 activity and plasma selenium concentration in humans. European journal of nutrition. 2009;48(6):383-6.
228. Jablonska E, Gromadzinska J, Peplonska B, Fendler W, Reszka E, Krol MB, et al. Lipid peroxidation and glutathione peroxidase activity relationship in breast cancer depends on functional polymorphism of GPX1. BMC cancer. 2015;15:657.
229. Moreno LA, Fleta J, Mur L, Sarria A, Bueno M. Fat distribution in obese and nonobese children and adolescents. Journal of pediatric gastroenterology and nutrition. 1998;27(2):176-80.
230. Goran MI, Gower BA. Relation between visceral fat and disease risk in children and adolescents. The American journal of clinical nutrition. 1999;70(1 Part 2):149s-56s.
231. Maffeis C, Pietrobelli A, Grezzani A, Provera S, Tato L. Waist circumference and cardiovascular risk factors in prepubertal children. Obesity research. 2001;9(3):179-87.
232. Bassali R, Waller JL, Gower B, Allison J, Davis CL. Utility of waist circumference percentile for risk evaluation in obese children. International journal of pediatric obesity : IJPO : an official journal of the International Association for the Study of Obesity. 2010;5(1):97-101.

233. Hui WF, Betoko A, Savant JD, Abraham AG, Greenbaum LA, Warady B, et al. Assessment of dietary intake of children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2017;32(3):485-94.
234. Norman LJ, Coleman JE, Macdonald IA, Tomsett AM, Watson AR. Nutrition and growth in relation to severity of renal disease in children. Pediatric nephrology (Berlin, Germany). 2000;15(3-4):259-65.
235. Hizli S, Abaci A, Buyukgebiz B, Buyukgebiz A. Nutritional stunting. Pediatric endocrinology reviews : PER. 2007;4(3):186-95.
236. van Stuijvenberg ME, Nel J, Schoeman SE, Lombard CJ, du Plessis LM, Dhansay MA. Low intake of calcium and vitamin D, but not zinc, iron or vitamin A, is associated with stunting in 2- to 5-year-old children. Nutrition (Burbank, Los Angeles County, Calif). 2015;31(6):841-6.
237. Rutishauser IH. Dietary intake measurements. Public health nutrition. 2005;8(7a):1100-7.
238. Nutrition SACo. Carbohydrates and Health. London: The Stationary Office; 2015.
239. Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. Public health nutrition. 2005;8(7a):1133-52.
240. Shi Q, Pavey ES, Carter RE. Bonferroni-based correction factor for multiple, correlated endpoints. Pharmaceutical statistics. 2012;11(4):300-9.
241. Liu S, Buring JE, Sesso HD, Rimm EB, Willett WC, Manson JE. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. Journal of the American College of Cardiology. 2002;39(1):49-56.
242. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, Fruit, and Cereal Fiber Intake and Risk of Coronary Heart Disease Among Men. Jama. 1996;275(6):447-51.
243. Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, et al. Long-term Intake of Dietary Fiber and Decreased Risk of Coronary Heart Disease Among Women. Jama. 1999;281(21):1998-2004.
244. Pietinen P, Rimm EB, Korhonen P, Hartman AM, Willett WC, Albanes D, et al. Intake of dietary fiber and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Circulation. 1996;94(11):2720-7.
245. Krishnamurthy VM, Wei G, Baird BC, Murtaugh M, Chonchol MB, Raphael KL, et al. High dietary fiber intake is associated with decreased inflammation and all-cause mortality in patients with chronic kidney disease. Kidney international. 2012;81(3):300-6.
246. Brownlee IA. The physiological roles of dietary fibre. Food Hydrocolloids. 2011;25(2):238-50.
247. Bliss DZ, Stein TP, Schleifer CR, Settle RG. Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. The American journal of clinical nutrition. 1996;63(3):392-8.
248. Lin CJ, Liu HL, Pan CF, Chuang CK, Jayakumar T, Wang TJ, et al. Indoxyl sulfate predicts cardiovascular disease and renal function deterioration in advanced chronic kidney disease. Archives of medical research. 2012;43(6):451-6.
249. Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, et al. Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(4):1183-91.
250. Choumenkovitch SF, McKeown NM, Tovar A, Hyatt RR, Kraak VI, Hastings AV, et al. Whole grain consumption is inversely associated with BMI Z-score in rural school-aged children. Public health nutrition. 2013;16(2):212-8.
251. Nutrition SACo. Salt and Health. 2003.
252. He FJ, MacGregor GA. Reducing population salt intake worldwide: from evidence to implementation. Progress in cardiovascular diseases. 2010;52(5):363-82.
253. Zhao D, Qi Y, Zheng Z, Wang Y, Zhang XY, Li HJ, et al. Dietary factors associated with hypertension. Nature reviews Cardiology. 2011;8(8):456-65.

254. McLean RM, Farmer VL, Nettleton A, Cameron CM, Cook NR, Campbell NRC. Assessment of dietary sodium intake using a food frequency questionnaire and 24-hour urinary sodium excretion: a systematic literature review. 2017;19(12):1214-30.
255. Livingstone MB, Robson PJ. Measurement of dietary intake in children. The Proceedings of the Nutrition Society. 2000;59(2):279-93.
256. Cole TJ, Williams AF, Wright CM. Revised birth centiles for weight, length and head circumference in the UK-WHO growth charts. Annals of human biology. 2011;38(1):7-11.
257. Winklhofer-Roob BM, van't Hof MA, Shmerling DH. Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences. Clinical chemistry. 1997;43(1):146-53.
258. Bhalla K, Ennis DM, Ennis ED. Hypercalcemia caused by iatrogenic hypervitaminosis A. Journal of the American Dietetic Association. 2005;105(1):119-21.
259. Fishbane S, Frei GL, Finger M, Dressler R, Silbiger S. Hypervitaminosis A in two hemodialysis patients. American journal of kidney diseases : the official journal of the National Kidney Foundation. 1995;25(2):346-9.
260. Praga M, Diaz Rubio P, Morales JM, Canizares F, Ruilope LM, Gutierrez-Millet V, et al. Implications of hypervitaminosis A on the calcium-phosphate metabolism and on blood lipids in hemodialysis. American journal of nephrology. 1987;7(4):281-6.
261. Manickavasagar B, McArdle AJ, Yadav P, Shaw V, Dixon M, Blomhoff R, et al. Hypervitaminosis A is prevalent in children with CKD and contributes to hypercalcemia. Pediatric nephrology (Berlin, Germany). 2015;30(2):317-25.
262. Saland JM, Pierce CB, Mitsnefes MM, Flynn JT, Goebel J, Kupferman JC, et al. Dyslipidemia in Children with Chronic Kidney Disease: A Report of the Chronic Kidney Disease in Children (CKiD) Study. Kidney international. 2010;78(11):1154-63.
263. Kim JE, Han M, Hanl KS, Kim HK. Vitamin E inhibition on platelet procoagulant activity: involvement of aminophospholipid translocase activity. Thrombosis research. 2011;127(5):435-42.
264. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. Jama. 2007;297(8):842-57.
265. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, et al. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. The American journal of clinical nutrition. 2005;82(5):1074-81.
266. Polit DF, Beck CT. The content validity index: are you sure you know what's being reported? Critique and recommendations. Research in nursing & health. 2006;29(5):489-97.
267. Holt GM, Owen LJ, Till S, Cheng Y, Grant VA, Harden CJ, et al. Systematic Literature Review Shows That Appetite Rating Does Not Predict Energy Intake. Critical reviews in food science and nutrition. 2016:0.
268. Deutsch JA, Moore BO, Heinrichs SC. Unlearned specific appetite for protein. Physiology & behavior. 1989;46(4):619-24.
269. Griffioen-Roose S, Mars M, Siebelink E, Finlayson G, Tome D, de Graaf C. Protein status elicits compensatory changes in food intake and food preferences. The American journal of clinical nutrition. 2012;95(1):32-8.
270. Chaput JP, Katzmarzyk PT, Barnes JD, Fogelholm M, Hu G, Kuriyan R, et al. Mid-upper arm circumference as a screening tool for identifying children with obesity: a 12-country study. Pediatric obesity. 2016.
271. Varni JW, Burwinkle TM, Seid M, Skarr D. The PedsQL 4.0 as a pediatric population health measure: feasibility, reliability, and validity. Ambulatory pediatrics : the official journal of the Ambulatory Pediatric Association. 2003;3(6):329-41.
272. Varni JW, Burwinkle TM, Jacobs JR, Gottschalk M, Kaufman F, Jones KL. The PedsQL in type 1 and type 2 diabetes: reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales and type 1 Diabetes Module. Diabetes care. 2003;26(3):631-7.

273. Varni JW, Burwinkle TM, Katz ER, Meeske K, Dickinson P. The PedsQL in pediatric cancer: reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. Cancer. 2002;94(7):2090-106.
274. Chan KS, Mangione-Smith R, Burwinkle TM, Rosen M, Varni JW. The PedsQL: reliability and validity of the short-form generic core scales and Asthma Module. Medical care. 2005;43(3):256-65.
275. Eiser C, Morse R. Quality-of-life measures in chronic diseases of childhood. Health technology assessment (Winchester, England). 2001;5(4):1-157.
276. Upton P, Eiser C, Cheung I, Hutchings HA, Jenney M, Maddocks A, et al. Measurement properties of the UK-English version of the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales. Health and quality of life outcomes. 2005;3:22.
277. UK Ministry of Housing CaLG. http://opendatacommunities.org [
278. Diseth TH, Tangeraas T, Reinfjell T, Bjerre A. Kidney transplantation in childhood: mental health and quality of life of children and caregivers. Pediatric nephrology (Berlin, Germany). 2011;26(10):1881-92.
279. Qvist E, Narhi V, Apajasalo M, Ronnholm K, Jalanko H, Almqvist F, et al. Psychosocial adjustment and quality of life after renal transplantation in early childhood. Pediatric transplantation. 2004;8(2):120-5.
280. Goldstein SL, Graham N, Burwinkle T, Warady B, Farrah R, Varni JW. Health-related quality of life in pediatric patients with ESRD. Pediatric nephrology (Berlin, Germany). 2006;21(6):846-50.
281. Sundaram SS, Landgraf JM, Neighbors K, Cohn RA, Alonso EM. Adolescent health-related quality of life following liver and kidney transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2007;7(4):982-9.
282. Eijsermans RM, Creemers DG, Helders PJ, Schroder CH. Motor performance, exercise tolerance, and health-related quality of life in children on dialysis. Pediatric nephrology (Berlin, Germany). 2004;19(11):1262-6.
283. Hamiwka LA, Cantell M, Crawford S, Clark CG. Physical activity and health related quality of life in children following kidney transplantation. Pediatric transplantation. 2009;13(7):861-7.
284. Falger J, Landolt MA, Latal B, Ruth EM, Neuhaus TJ, Laube GF. Outcome after renal transplantation. Part II: quality of life and psychosocial adjustment. Pediatric nephrology (Berlin, Germany). 2008;23(8):1347-54.
285. Neul SK, Minard CG, Currier H, Goldstein SL. Health-related quality of life functioning over a 2-year period in children with end-stage renal disease. Pediatric nephrology (Berlin, Germany). 2013;28(2):285-93.
286. Marciano RC, Bouissou Soares CM, Diniz JSS, Lima EM, Silva JMP, Canhestro MR, et al. Behavioral disorders and low quality of life in children and adolescents with chronic kidney disease. Pediatric Nephrology. 2011;26(2):281-90.
287. Sprangers MA, Schwartz CE. Integrating response shift into health-related quality of life research: a theoretical model. Social science & medicine (1982). 1999;48(11):1507-15.
288. Anthony SJ, Hebert D, Todd L, Korus M, Langlois V, Pool R, et al. Child and parental perspectives of multidimensional quality of life outcomes after kidney transplantation. Pediatric transplantation. 2010;14(2):249-56.
289. Varni JW, Limbers CA, Burwinkle TM. Impaired health-related quality of life in children and adolescents with chronic conditions: a comparative analysis of 10 disease clusters and 33 disease categories/severities utilizing the PedsQL 4.0 Generic Core Scales. Health and quality of life outcomes. 2007;5:43.
290. Lopes M, Ferraro A, Koch VH. Health-related quality of life of children and adolescents with CKD stages 4-5 and their caregivers. Pediatric nephrology (Berlin, Germany). 2014;29(7):1239-47.
291. Razzouk BI, Hord JD, Hockenberry M, Hinds PS, Feusner J, Williams D, et al. Double-blind, placebo-controlled study of quality of life, hematologic end points, and safety of weekly epoetin alfa in children with cancer receiving myelosuppressive chemotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(22):3583-9.

292. Fowler MG, Johnson MP, Atkinson SS. School achievement and absence in children with chronic health conditions. The Journal of pediatrics. 1985;106(4):683-7.
293. Mackner LM, Bickmeier RM, Crandall WV. Academic achievement, attendance, and school-related quality of life in pediatric inflammatory bowel disease. Journal of developmental and behavioral pediatrics : JDBP. 2012;33(2):106-11.
294. Schwimmer JB, Burwinkle TM, Varni JW. Health-related quality of life of severely obese children and adolescents. Jama. 2003;289(14):1813-9.
295. Food_Standards_Agency_and_Public_Health_England. National Diet and Nutrition Survey: results from Years 5 and 6 (combined. 2016.
296. Huang B, Zhou Z, Xu H, Wang H, Liu B, Cui Y, et al. Diminished appetite predicts mortality of Chinese peritoneal dialysis patients. Biological research for nursing. 2014;16(3):241-9.
297. Krebs NF, Hambidge KM, Walravens PA. Increased food intake of young children receiving a zinc supplement. American journal of diseases of children (1960). 1984;138(3):270-3.
298. Mda S, van Raaij JM, Macintyre UE, de Villiers FP, Kok FJ. Improved appetite after multi-micronutrient supplementation for six months in HIV-infected South African children. Appetite. 2010;54(1):150-5.
299. Doherty CP, Sarkar MAK, Shakur MS, Ling SC, Elton RA, Cutting WA. Zinc and rehabilitation from severe protein-energy malnutrition: Higher- dose regimens are associated with increased mortality. American Journal of Clinical Nutrition. 1998;68(3):742-8.
300. ACBS.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/603695/Appendix5.pdf [Accessed 11/07/2018] [
301. Braun V, Clarke V. Using thematic analysis in psychology. Qualitative Research in Psychology. 2006;3(2):77-101.
302. Salunke S, Hempenstall J, Kendall R, Roger B, Mroz C, Nunn T, et al. European Paediatric Formulation Initiative's (EuPFI) 2nd conference commentary--Formulating better medicines for children. International journal of pharmaceutics. 2011;419(1-2):235-9.
303. van Riet-Nales DA, Wang S, Saint-Raymond A, Robert J-L. The EMA quality guideline on the pharmaceutical development of medicines for paediatric use. International journal of pharmaceutics. 2012;435(2):132-4.
304. Kozarewicz P. Regulatory perspectives on acceptability testing of dosage forms in children. International journal of pharmaceutics. 2014;469(2):245-8.
305. Liu F, Ranmal S, Batchelor HK, Orlu-Gul M, Ernest TB, Thomas IW, et al. Patient-centred pharmaceutical design to improve acceptability of medicines: similarities and differences in paediatric and geriatric populations. Drugs. 2014;74(16):1871-89.
306. Zlotkin S, Antwi KY, Schauer C, Yeung G. Use of microencapsulated iron(II) fumarate sprinkles to prevent recurrence of anaemia in infants and young children at high risk. Bulletin of the World Health Organization. 2003;81(2):108-15.
307. Verrotti A, Nanni G, Agostinelli S, Alleva ET, Aloisi P, Franzoni E, et al. Effects of the abrupt switch from solution to modified-release granule formulation of valproate. Acta neurologica Scandinavica. 2012;125(3):e14-8.
308. Punam M, Hannah B. Evidence of acceptability of oral paediatric medicines: a review. Journal of Pharmacy and Pharmacology. 2017;69(4):361-76.
309. Armstrong JE, Laing DG, Wilkes FJ, Kainer G. Smell and taste function in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2010;25(8):1497-504.
310. Correa M, Laing DG, Hutchinson I, Jinks AL, Armstrong JE, Kainer G. Reduced taste function and taste papillae density in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2015;30(11):2003-10.
311. Blydt-Hansen TD, Pierce CB, Cai Y, Samsonov D, Massengill S, Moxey-Mims M, et al. Medication Treatment Complexity and Adherence in Children with CKD. Clinical journal of the American Society of Nephrology : CJASN. 2014;9(2):247-54.
312. Broun ER, Greist A, Tricot G, Hoffman R. Excessive zinc ingestion. A reversible cause of sideroblastic anemia and bone marrow depression. Jama. 1990;264(11):1441-3.

313. Oestreicher P, Cousins RJ. Copper and zinc absorption in the rat: mechanism of mutual antagonism. The Journal of nutrition. 1985;115(2):159-66.
314. Dossa RAM, Ategbo E-AD, Van Raaij JMA, de Graaf C, Hautvast JGAJ. Multivitamin-Multimineral and Iron Supplementation Did Not Improve Appetite of Young Stunted and Anemic Beninese Children. The Journal of nutrition. 2001;131(11):2874-9.
315. Chao HC, Chang YJ, Huang WL. Cut-off Serum Zinc Concentration Affecting the Appetite, Growth, and Nutrition Status of Undernourished Children Supplemented With Zinc. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2018.
316. Chiu YW, Teitelbaum I, Misra M, de Leon EM, Adzize T, Mehrotra R. Pill burden, adherence, hyperphosphatemia, and quality of life in maintenance dialysis patients. Clinical journal of the American Society of Nephrology : CJASN. 2009;4(6):1089-96.
317. Pereira RA, Araujo MC, Lopes TdS, Yokoo EM. How many 24-hour recalls or food records are required to estimate usual energy and nutrient intake? Cadernos de Saúde Pública. 2010;26:2101-11.
318. Mak RH, Ikizler AT, Kovesdy CP, Raj DS, Stenvinkel P, Kalantar-Zadeh K. Wasting in chronic kidney disease. Journal of Cachexia, Sarcopenia and Muscle. 2011;2(1):9-25.
319. Kim J, Im J-S, Choi CH, Park CH, Lee JI, Son KH, et al. The Association between Red Blood Cell Distribution Width and Sarcopenia in U.S. Adults. Scientific reports. 2018;8(1):11484.
320. Halfdanarson TR, Kumar N, Li C-Y, Phyliky RL, Hogan WJ. Hematological manifestations of copper deficiency: a retrospective review. European Journal of Haematology. 2008;80(6):523-31.
321. NDNS years 1-4 combined https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012 (accessed 20 Nov 2017).
322. Zhang A, Cai Y, Wang PF, Qu JN, Luo ZC, Chen XD, et al. Diagnosis and prognosis of neutrophil gelatinase-associated lipocalin for acute kidney injury with sepsis: a systematic review and meta-analysis. Critical care (London, England). 2016;20:41.
323. Singer E, Marko L, Paragas N, Barasch J, Dragun D, Muller DN, et al. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta physiologica (Oxford, England). 2013;207(4):663-72.
324. Ardissino G, Testa S, Daccò V, Paglialonga F, Viganò S, Felice-Civitillo C, et al. Puberty is associated with increased deterioration of renal function in patients with CKD: data from the ItalKid Project. Archives of disease in childhood. 2012;97(10):885-8.
325. Eguchi K, Izumi Y. Insufficiency of urinary acid excretion of overweight or obese patients with chronic kidney disease and its involvement with renal tubular injury. 2018.
326. Stepien M, Stepien A, Wlazel RN, Paradowski M, Banach M, Rysz J. Obesity indices and inflammatory markers in obese non-diabetic normo- and hypertensive patients: a comparative pilot study. Lipids in health and disease. 2014;13:29.
327. Maehira F, Luyo GA, Miyagi I, Oshiro M, Yamane N, Kuba M, et al. Alterations of serum selenium concentrations in the acute phase of pathological conditions. Clinica chimica acta; international journal of clinical chemistry. 2002;316(1-2):137-46.
328. Eurola MH. Proceedings: Twenty Years of Selenium Fortification. Agrifood Research Reports. 2005;69.
329. Birn H. The kidney in vitamin B12 and folate homeostasis: characterization of receptors for tubular uptake of vitamins and carrier proteins. American journal of physiology Renal physiology. 2006;291(1):F22-36.
330. Don T, Friedlander S, Wong W. Dietary intakes and biochemical status of B vitamins in a group of children receiving dialysis. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2010;20(1):23-8.
331. Kriley M, Warady BA. Vitamin status of pediatric patients receiving long-term peritoneal dialysis. The American journal of clinical nutrition. 1991;53(6):1476-9.
332. Saifan C, Samarneh M, Shtaynberg N, Nasr R, El-Charabaty E, El-Sayegh S. Treatment of confirmed B12 deficiency in hemodialysis patients improves Epogen(R) requirements. International journal of nephrology and renovascular disease. 2013;6:89-93.

333. Litwin M, Abuauba M, Wawer ZT, Grenda R, Kuryt T, Pietraszek E. Folate, vitamin B12, and sulfur amino acid levels in patients with renal failure. Pediatric nephrology (Berlin, Germany). 2001;16(2):127-32.
334. Kang HG, Lee BS, Hahn H, Lee JH, Ha IS, Cheong HI, et al. Reduction of plasma homocysteine by folic acid in children with chronic renal failure. Pediatric nephrology (Berlin, Germany). 2002;17(7):511-4.
335. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J. 2015;14:6-.
336. Heinz J, Kropf S, Luley C, Dierkes J. Homocysteine as a Risk Factor for Cardiovascular Disease in Patients Treated by Dialysis: A Meta–analysis. American Journal of Kidney Diseases. 2009;54(3):478-89.
337. Wang AY-M. Does Vitamin B₁₂ Delay CKD Progression? American Journal of Kidney Diseases.
338. Shane B. Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food and nutrition bulletin. 2008;29(2 Suppl):S5-16; discussion S7-9.
339. Gunes A, Aktar F, Tan I, Soker M, Uluca U, Balik H, et al. Urinary levels of early kidney injury molecules in children with vitamin B12 deficiency. Archivos argentinos de pediatria. 2016;114(5):453-7.
340. Khoury M, Manlhiot C, McCrindle BW. Role of the waist/height ratio in the cardiometabolic risk assessment of children classified by body mass index. J Am Coll Cardiol. 2013;62(8):742-51.
341. NHS-England. The NHS Long Term Plan. 2019.
342. Pottel H. Measuring and estimating glomerular filtration rate in children. Pediatric nephrology (Berlin, Germany). 2017;32(2):249-63.
343. Vinge E, Lindergard B, Nilsson-Ehle P, Grubb A. Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults. Scandinavian journal of clinical and laboratory investigation. 1999;59(8):587-92.
344. Zappitelli M, Parvex P, Joseph L, Paradis G, Grey V, Lau S, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2006;48(2):221-30.
345. Conkar S, Mir S, Karaslan FN, Hakverdi G. Comparing different estimated glomerular filtration rate equations in assessing glomerular function in children based on creatinine and cystatin C. Journal of Clinical Laboratory Analysis. 2018;32(6):e22413.
346. Granata S, Zaza G, Simone S, Villani G, Latorre D, Pontrelli P, et al. Mitochondrial dysregulation and oxidative stress in patients with chronic kidney disease. BMC genomics. 2009;10:388.
347. Rao M, Li L, Demello C, Guo D, Jaber BL, Pereira BJ, et al. Mitochondrial DNA injury and mortality in hemodialysis patients. Journal of the American Society of Nephrology : JASN. 2009;20(1):189-96.
348. Kirkman DL, Muth BJ, Ramick MG, Townsend RR, Edwards DG. Role of mitochondria-derived reactive oxygen species in microvascular dysfunction in chronic kidney disease. American journal of physiology Renal physiology. 2018;314(3):F423-F9.
349. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. Free radical biology & medicine. 2000;28(10):1456-62.
350. Grooteman MP, Bos JC, van Houte AJ, van Limbeek J, Schoorl M, Nube MJ. Mechanisms of intra-dialyser granulocyte activation: a sequential dialyser elution study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1997;12(3):492-9.
351. Canaud B, Cristol J, Morena M, Leray-Moragues H, Bosc J, Vaussenat F. Imbalance of oxidants and antioxidants in haemodialysis patients. Blood purification. 1999;17(2-3):99-106.
352. Conjard A, Ferrier B, Martin M, Caillette A, Carrier H, Baverel G. Effects of chronic renal failure on enzymes of energy metabolism in individual human muscle fibers. Journal of the American Society of Nephrology : JASN. 1995;6(1):68-74.
353. Durozard D, Pimmel P, Baretto S, Caillette A, Labeeuw M, Baverel G, et al. 31P NMR spectroscopy investigation of muscle metabolism in hemodialysis patients. Kidney international. 1993;43(4):885-92.

354. Kemp GJ, Crowe AV, Anijeet HK, Gong QY, Bimson WE, Frostick SP, et al. Abnormal mitochondrial function and muscle wasting, but normal contractile efficiency, in haemodialysed patients studied non-invasively in vivo. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2004;19(6):1520-7.
355. Liu Y, Wang Y, Ding W, Wang Y. Mito-TEMPO Alleviates Renal Fibrosis by Reducing Inflammation, Mitochondrial Dysfunction, and Endoplasmic Reticulum Stress. Oxidative medicine and cellular longevity. 2018;2018:5828120-.
356. Mentor S, Fisher D. Aggressive Antioxidant Reductive Stress Impairs Brain Endothelial Cell Angiogenesis and Blood Brain Barrier Function. Current neurovascular research. 2017;14(1):71-81.
357. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. Pediatrics. 2003;111(6 Pt 1):1416-21.
358. Sommerburg O, Grune T, Ehrich JH, Siems WG. Adaptation of glutathion-peroxidase activity to oxidative stress occurs in children but not in adult patients with end-stage renal failure undergoing hemodialysis. Clinical nephrology. 2002;58 Suppl 1:S31-6.
359. Loeff La. https://www.loyensloeff.com/en-us/news-events/news/borderline-products-new-guidance-on-the-classification-of-food-for-special-medical-purposes-1 [Accessed 11/07/2018] [
360. Harmer M, Wootton S, Gilbert R, Anderson C. Association of nutritional status and health-related quality of life in children with chronic kidney disease. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2019;28(6):1565-73.
361. Harmer M, Wootton S, Gilbert R, Anderson C. Vitamin B6 in Pediatric Renal Transplant Recipients. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2019;29(3):205-8.