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Growth and Body composition in children with Inflammatory Bowel Disease

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ABSTRACT

FACULTY OF MEDICINE
HUMAN DEVELOPMENT AND HEALTH

DOCTOR OF PHYLOSOPHY

GROWTH AND BODY COMPOSITION IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE

BY MONA KESHTKARAN

Crohn`s Disease (CD) and Ulcerative Colitis (UC), two types of Inflammatory Bowel Disease (IBD), are chronic, relapsing inflammatory conditions of the gastro-intestinal tract. Approximately 25% of cases are diagnosed in childhood and adolescence; affected children suffer from symptoms such as abdominal pain, bloody diarrhoea, fatigue, and poor nutritional state. Poor growth, in terms of both height and weight, precedes diagnosis and further weight may be lost with successive inflammatory exacerbations. Changes in height and weight are used as a marker of both disease severity and response to treatment, but the associated changes in body composition with changes in weight are poorly characterised and understood, and rarely assessed in routine clinical care. Being able to determine the nature and size of any deficits in lean and fat mass may provide a better understanding of the disease process, whilst gains in height and lean tissue, relative to increases in fat mass, could also be used to mark the effectiveness of clinical management and improvement in nutritional state. Exclusive enteral nutrition is now being used in preference to anti-inflammatory therapy in children with IBD, but there are increasing concerns that the focus on weight gain as children move into remission with only modest gains in height reflects an inappropriate mix of tissue deposition with greater gains in fat than lean.

The central hypothesis of this thesis is that children with IBD present at diagnosis with a lean deficit, greater that that which can be simply attributed to their lack of height, and that conventional therapy, including exclusive enteral nutrition, may not adequately correct the nutritional state and deficit of lean tissue. In order to test this hypothesis, the work described in this thesis is presented in three parts. Firstly, a cross-sectional study of a convenient sample of children with CD and UC drawn from the regional IBD outpatient clinic to explore the extent and nature of the differences in height, weight and BMI expressed as SD scores, together with

simple measures of body composition using anthropometry. This initial study confirmed that whilst as a group, both CD and UC children exhibit only modest deficits in height, weight and BMI there was marked variance across the group with more pronounced deficits in some children. Lower Upper Arm Muscle Area SD scores and higher Triceps skinfold thickness SD scores would support the proposition of a general lean deficit and fat excess, even in children with BMI range within ± 2SD.

The second part explored different approaches to assessing body composition by i) determining the concurrent and face validity of different bioelectrical impedance devices using deuterium dilution space as a reference method and ii) the potential of using SIFT-MS to conduct real-time near-patient measures of deuterium abundance on breath vapour was examined in comparison to measures of deuterium abundance in saliva and urine assessed by both SIFT-MS and IRMS. These studies demonstrated important differences in lean mass were evident between devices. Deuterium abundance in saliva and urine by SIFT-MS was directly comparable to that by IRMS although higher levels of D_2O administration were required for optimal analytical performance; greater imprecision was evident in determining deuterium abundance in breath.

The third part described detailed measures of body composition (anthropometry, DXA, deuterium abundance in saliva by IRMS, and BIA) in a prospective inception cohort of eleven children with CD studied at diagnosis, and followed for the first year of treatment from active disease into remission using exclusive enteral nutrition. Lean deficits identified using both DXA and Upper Arm Muscle Area was evident at diagnosis greater than that which could be attributed to shortness. Treatment was associated with gains in height and weight, but in contrast to previous reports where corticosteroids were only used to induce remission, gains in lean mass over the first year of treatment using exclusive enteral nutrition were observed which were greater than that which could be attributed to an increase in height that reflect at least a partial correction of the lean deficit.

Contents

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Declaration

I, Mona Keshtkaran, declare that the thesis entitled 'Growth and body composition in children with inflammatory bowel disease' and the work in it are both my own and have been generated by me as the result of my original research.

I confirm that:

- this work was done wholly while in candidature for a research degree at the University of Southampton.

- none of this work has been submitted for a degree or other qualification at any institution.

- where I have consulted the published work of others, this is clearly attributed and the source given.

- I have acknowledged the help of my supervisors (Dr Stephen Wootton & Prof Marinos Elia) and the Paediatric IBD Research team led by Dr Robert Mark Beattie and colleagues within the Southampton NIHR Biomedical Research Unit (Nutrition, Diet & Lifestyle) in the conduct of this thesis.

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Main abbreviation used in the text:

A*	Abundance
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMI	Body Mass Index
BMI SDS	Body Mass Index z-score
CD	Crohn`s Disease
CRP	C Reactive Protein
D2O	Deuterium oxide
DDS	Deuterium Dilution Space
DXA	Dual energy X-ray absorptiometry
E*	Enrichment
EEN	Exclusive Enteral Nutrition
ESR	Erythrocyte Sedimentation Ratio
FFM	Fat Free Mass
FFMI	Fat Free Mass Index
FM	Fat Mass
FMI	Fat Mass Index
GI	Gastro- Intestinal
HSE	Health Survey England
Ht	Height
Ht SDS	Height z-score
IAEA	International Atomic Energy Agency
IBD	Inflammatory Bowel Disease
IL	Interleukin
Imp	Impedance
NHANES	National Health and Nutrition Examination Surveys
NOD	Nucleotide Oligomerisation Domain
PCDAI	Paediatric Crohn` s Disease Activity Index
PN	Parenteral Nutrition
PUCAI	Paediatric Ulcerative Colitis Activity Index
SCBR	Southampton Research Facility for Biomedical Research
SDS	Standard Deviation Score
SOP	Statement of Purpose
SOP	Statement od Purpose
TBW	Total Body Water
TNF	Tumour Necrosis Factor
TSF	Triceps Skinfold thickness

TSF SDS	Triceps Skinfold thickness z-score
UC	Ulcerative Colitis
UMA	Upper Arm Muscle Area
UMA SDS	Upper Arm Muscle Area z-score
VD	Volume of Distribution
WHO	World Health Organization
Wt	Weight
Wt SDS	Weight z-score
WTCRF	Welcome Trust Clinical Research Facility

Chapter 1 Introduction

1.1 Background to research

Inflammatory Bowel Disease (IBD) is a chronic relapsing inflammatory condition of the gastro-intestinal tract. Reduced food intake, nutrient malabsorption, enhanced intestinal losses and, altered metabolism often result in impairment of nutritional status. The development of the disease may lead to faltering growth and tissue acquisition which frequently precedes disease diagnosis.

Treatment, either by steroids or exclusive enteral nutrition, can control the inflammation. The goal is to remain in remission and prevent relapse. The extent to which inflammation is supressed and nutritional support provided would be expected to determine the subsequent pattern of growth and tissue accretion.

Studies have shown that children with IBD frequently have deficits in height and/or weight compared to other children of the same age. The standard markers of growth (height and weight for age) do not provide a picture of the composition of tissue. If tissue is deposited disproportionally, the majority of the weight gain in these children may be due to gain in fat compartment of the body rather than the balanced pattern of tissue accretion. This would imply that the balance of energy and nutrients does not adequately meet the needs of the child and may lead to poor growth, worsening inflammatory state and increased risk of relapse. Large proportions of fat and small percentages of lean can be detrimental, as depleted lean mass is associated with poor skeletal health, increased susceptibility to infection, morbidity and overall, poor-quality of life and increased fat mass is accompanied by complications of obesity.

Treatment of IBD over time can partially improve the deficits in weight and height, the correction is frequently incomplete as it fails to make up the deficits associated with the disease prior to diagnosis. In addition, the majority of diseases influence body composition in one way or another, and measurements are required both to characterise these effects and to assess the efficacy of treatment programs.

Few studies have been conducted on body composition in children with IBD and so the extent to which the excess body fat and depleted lean mass affect children with IBD is largely unknown, which makes it difficult to assess their nutritional status. In addition, much of our knowledge in this area has been acquired using research

methods that are not suitable for use in routine clinical settings. Further research is required on changes that occur in terms of body composition in children with IBD. A greater understanding of these changes may make it possible to use body composition measurements as means of evaluating growth and, therefore, assessing the efficacy of different interventions in these children. In addition, there is a need to determine the utility of approaches that can be applied in clinical settings as part of the nutritional assessment of the patient.

The central hypothesis of this thesis is that children with IBD present at diagnosis with a lean deficit, greater than which can be attributed to their shortness, and that conventional therapies, including exclusive enteral nutrition, may not adequately correct the nutritional state and deficits in lean tissue. The purpose of this work is to first assess whether in addition to any differences in growth assessed by weight and height measurements, there are differences in body composition (excess fat mass, and depleted lean mass) compared to a reference and general population. In addition, if such disturbances exist, this work aims to examine whether they can be corrected by current treatment.

In order to test this hypothesis, the work described in this thesis is presented in three parts. Firstly a cross-sectional study of convenient sample of children with CD and UC drawn from the regional IBD outpatient clinic was designed to investigate if in addition to deficits in height, weight and BMI, expressed as SD scores, lean deficits and fat excess measured by upper arm muscle area SD scores and triceps skinfold thickness SD scores was present in these children.

The second part of the thesis explored different approaches of measuring body composition. Firstly, the concordance of different bioelectrical impedance analysers was tested in both adults and children. In addition, the potential of using SIFT-MS to perform real-time, near –patient measures of deuterium abundance in on breath vapour was examined in comparison to measures of deuterium abundance in saliva and urine assessed by both SIFT-MS and IRMS. The third part of the study, focused on body composition measurements with a variety of devices (anthropometry, DXA, deuterium abundance in saliva by IRMS, and BIA) in a prospective inception cohort of eleven children with Crohn`s disease.

1.2 Thesis Outline

This thesis is divided into chapters beginning with an introduction to the field of research. The next section (chapter 2) reviews the current literature, concentrating on risk factors related to IBD, particularly growth and body composition abnormalities, highlighting limitations of existing knowledge. Chapter 3 focuses on investigating whether growth and body composition abnormalities exist in children with IBD using a cross-sectional study. Methods used during research and validation work to justify the use of the methods are presented in chapters 4 and 5. Chapter 6 focuses on a longitudinal study that was designed to investigate whether growth and body composition abnormalities persist in children with CD after treatment. Finally, Chapter 7 presents a general discussion. Additional information is shown in the appendices (Chapter 8).

Chapter 2 Review of the current literature

2.0.0 Introduction.

The purpose of this chapter is to introduce the area of research presented in the thesis, and to identify both what is known and what areas of uncertainty remains that require further examination. The review of the literature starts with an overview of the aetiology, epidemiology and aetiology of paediatric Inflammatory Bowel Disease (2.1). The following section (2.2) reviews clinical aspects and diagnosis of IBD, treatment and consequences related to IBD. The next section (2.3) describes normal growth in general and, in terms of body composition before reviewing (2.4) previous studies of growth and body composition in children with IBD. The review ends (2.5) with and overall summary, aims of the present research and hypothesis. A search using PubMed and Ovid was performed using words that are listed in the appendix to find relevant articles on inflammatory bowel disease, treatment of inflammatory bowel disease, growth and body composition in children with inflammatory bowel disease and, growth and body composition in normal children.

2.1.1. Inflammatory Bowel Disease.

IBD is characterised by chronic inflammation of the intestinal tract with variable periods of remission and exacerbation. Crohn`s disease (CD) and ulcerative colitis (UC) are collectively known as Inflammatory Bowel Disease (IBD) , are idiopathic, lifelong, inflammatory, relapsing, destructive conditions of the Gastro Intestinal (GI) tract [1], [2] with rising incidence [3]. CD and UC can be diagnosed at any age, however 25% of IBD presents in childhood, often in adolescence [4]. IBD causes significant gastrointestinal symptoms, including diarrhoea, abdominal pain, bleeding, anaemia, and weight loss together with more subtle symptoms of lethargy and decreased appetite. Although the incidence of IBD in childhood is high (25% of IBD diagnoses present in childhood), much of paediatric practice is adapted from adult studies and evidence, which puts forth the need for evidence based guidelines in children.

2.1.2 Epidemiology and, UK incidence.

IBD can occur at any age, with the peak age range of diagnosis in the second and third decades of life. In childhood, rates of IBD increase from the first year of life, with the highest rates in teenage years [5]. A recent systematic review of the epidemiological studies suggests that the incidence of paediatric IBD seems to be increasing for uncertain reasons and this increase has been demonstrated in both western regions such as Canada, France, and northern Europe and in former eastern European countries i.e. Croatia, and Hungary [6]. Another study conducted by *Henderson et al* [7]compared the incidence of IBD between 2003-2008 to 1990-1995. This study confirmed an increase in the incidence of IBD in Scotland as a total of 260 patients were diagnosed with IBD between 1990-1995 compared to 436 children in the period of 2003-2008. Whether these trends reflect a significant change toward a younger age of CD development, or simply better recognition of disease and an increasing CD at all ages is controversial. It has been argued that this may be due to earlier diagnosis of the disease [4]. Due to high and increasing incidence of IBD in children and adolescence more attention should be allocated to children with IBD.

Crohn`s disease and Ulcerative colitis both occur at the highest incidence in United Kingdom, North America and Europe. The only prospective national survey of IBD in children aged <16 years in the UK between June 1998 and July 1999 showed the incidence to be 5.2 per 100,000 individuals per year (60% CD, 28% UC and 12% IC) [8] . The incidence of IBD has increased over the last 30-40 years [4], temporal trends in incidence rates of CD in different areas can be observed in Figure 2-1. Whether this is due to improvement in diagnostic assessments, lifestyle and diet, or environmental exposure is unclear.

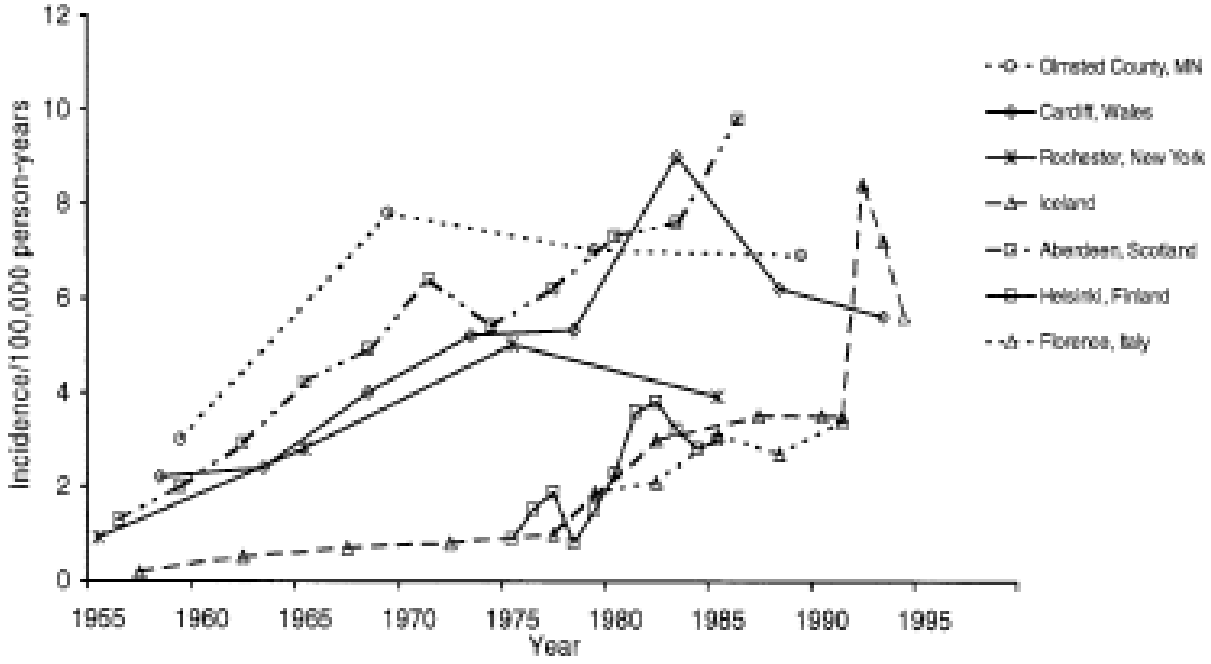

Figure 2-1 Temporal trends in incidence rates of CD in(Olmsted county Minnesota; Cardiff, Wales; Rochester New York; Iceland; Aberdeen; Scotland; Helsinki, Finland; and Florence, Italy. This figure is from reference [9].

2.1.3 Aetiology.

The aetiology of IBD is not understood well but is assumed to be multi-factorial. IBD is most likely to be caused by a complex interplay between genetic and environmental factors. It has been suggested that IBD results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host [10], [11]. This hypothesis is supported by the observation that almost all experimental murine models of mucosal inflammation depend upon the existence of microbiota. In simple terms, inflammation will not occur if mice are raised in a germfree environment [12]. The pathogenesis of the disease includes a dysregulation of early mucosal immune response to commensal microorganisms in genetically susceptible individuals under the influence of environment and immunologic factors [13]. The gut provides a vital barrier that consists of a thin epithelial layer linked by tight junctions. This surface is constantly exposed to various food components, commensal microbiota, and antigens. IBD is assumed to be an excessive cell-

mediated response to antigens of the normal bacteria microbiota that results in an impairment of the barrier function of the gut [14].

Dysfunctional immune host response to normal luminal components:

The normal relationship between commensal bacteria and the host is symbiotic [15]. It is assumed that the exposure to commensal bacteria down-regulates the inflammatory genes and restrains activation of NF-KB pathways, thus inhibiting the inflammatory immune response of the gut to the food antigens and microbiota. In IBD this tolerance is decreased, exposure to microbiota stimulates an inflammatory response by the cells lining the mucosa, resulting in a chronic and damaging immune response. In UC patients, triggers are possibly epithelial antigens or functionally altered aerobes; In CD, the antigens are thought to be anaerobic bacteria. Cell mediated arm of the adaptive immune response occurring in IBD may subsequently follow one of the two pathways. In CD patients an excessive T helper 1(T_H1) exists and UC is associated with excessive $T_H2$production. T helper cells are mediators of inflammation which produce various patterns of cytokines resulting in mucosal damage [16].

Altered microbiota:

Bacteria in the gastrointestinal provide key nutrients and prevent colonization by pathogens [17]. In adults, the `normal` microbiota has been shown to have both bacteria that prevent and stimulate the development of inflammation, under specific circumstances [18]. Changes in the microbiota may lead to production of different antigens that will stimulate inflammatory response. Dysregulation may also occur as a result of alteration in bacteria function and composition [16]. Differences in intestinal flora have been reported between patients with IBD and controls. In patients with IBD, depletion and reduced diversity of the mucosa-associated bacteroidetes have been observed [19]. Diet may affect microbiota in the gut, and subsequently lead to dysregulated immune response. An animal study conducted by *Lam et al* demonstrated that High fat diet altered the microbiota profile in the gut [20].

Defective barrier function:

The gastrointestinal tract is a complex interface between the external environment and the immune system, establishing a dynamic barrier that enables the absorption of dietary nutrients and the exclusion of potentially detrimental compounds from the intestinal lumen. IBD is associated with increased permeability of the epithelial lining of the gut leading to continuous stimulation of the mucosal immune system [21], [22]. There is evidence from animal models of IBD than deregulated permeability is a primary defect in individuals with IBD that may precede the development of the disease [16], [23]. *Lam et al* have demonstrated that high saturated fat diets increased gut permeability and inflammation [20].

Genetics:

Recent research demonstrates that heritable factors influence IBD; family and twin studies and the discovery of many susceptibility genes support the concept that IBD is highly heritable [24], [25]. It has been reported that 6%-32% of patients with Crohn`s Disease have a relative that suffers from CD [26]. The discovery that CD is associated with mutations of NOD2/CARD15 gene(Nucleotide Binding Oligomerisation Domain 2) , whose product is a bacteria- sensing cytoplasmic protein , suggests that IBD patients may have genetically altered immune system to recognize the gut flora in a normal manner [27]. These mutations cause a decrease in the ability of NOD2 to identify antigens and active cytokines, and subsequently permit antigenic material in the cell without stimulating an immune response.

2.2.1 Clinical Aspects and diagnosis.

Crohn`s disease:

Crohn`s disease (CD) affects the ileum and large bowel in more than 90% of patients, but it can affect any part of the GI tract from anus to mouth [28]. Symptoms of CD are heterogeneous and depend on the location, behaviour, and severity of disease. Common symptoms include diarrhoea for more than 6 weeks, abdominal pain and weight loss. Systematic symptoms of malaise, anorexia, or fever are also common [28].

No single assessment is sufficient for CD diagnosis [12]. The diagnosis is made using clinical history, physical examination, in conjunction with results of endoscopic, radiological, laboratory, and histological studies. In addition, haematological tests, such as complete blood count, CRP, ESR, and faecal calprotectin measurement are performed [13]. Since no single clinical or laboratory parameter consistently mirrors activity of intestinal inflammation in Crohn`s disease, assessment of disease activity in the clinical trial setting is achieved using Paediatric Crohn`s Disease Activity Index (PCDAI).

The PCDAI offers a score drawn from 11 variables (3 historical, 5 physical examination, and 3 laboratory) which ranges from 0 to 100, with higher scores demonstrating greater disease activity. The index takes in to account commonly obtained laboratory measurements of haematocrit, albumin, and erythrocyte sedimentation rate. In addition measurements of both height and weight are included in determining PCDAI and could contribute to a maximum of 20 points to the total [29].

The clinical course is usually chronic with periods of clinical activity and remission. It has been reported that 1 year after diagnosis, 10-30% of patients with CD have a relapse or exacerbation of the disease, 15-25% have low disease activity, and 50-65% experience remission. Long term follow up (10-15 years) demonstrated that most patients (up to 73%) had a chronic intermittent course of the disease, 10% experienced remission, while 20% showed a chronic course with continuous activity [12]. It can be concluded from this evidence that it is difficult to maintain remission in long-term. Therefore there is a need for new management goals and strategies that are capable of inducing remission and maintaining it in long-term.

Ulcerative colitis:

UC affects the mucosal layer of the large bowel, and it involves the rectum and may extend proximally to part or even to the entire colon [30]. Symptoms of UC depend upon the extent and severity of the disease [12]. Common symptoms are bloody diarrhoea, rectal bleeding, rectal urgency, abdominal pain during bowel movements, and fatigue. Systemic symptoms of anorexia or fever are consequences of a severe attack [13].

The diagnosis of UC relies upon a combination of medical history, endoscopic findings, and histological features on multiple colonic biopsies and negative stool tests for infectious agents [31]. Active disease can be associated with increased acute-phase reactants, C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), and a decrease in haemoglobin. In addition faecal calprotectin levels correlate well with histologic inflammation and can be used to predict relapse. In severely ill patients serum albumin level will decrease rather rapidly. In children with ulcerative colitis, the Paediatric Ulcerative Colitis Activity Index (PUCAI) is used to measure disease activity [32].

2.2.2 Clinical manifestations of IBD.

Inflammatory Bowel Disease is a chronic disease, with a potential to develop varied complications (stricture and fistulae in CD, dysplasia and carcinoma particularly in UC) [4]. In addition to gastrointestinal and extra intestinal symptoms of IBD, children with CD and UC will often experience one or more nutritional complications of their disease including growth failure, delayed puberty, osteoporosis, anaemia, and micronutrient deficiencies. This report focuses on this nutrition related consequences of IBD. Figure 2-2 summarizes the aetiology of malnutrition in children with IBD and is produced from reference [33].

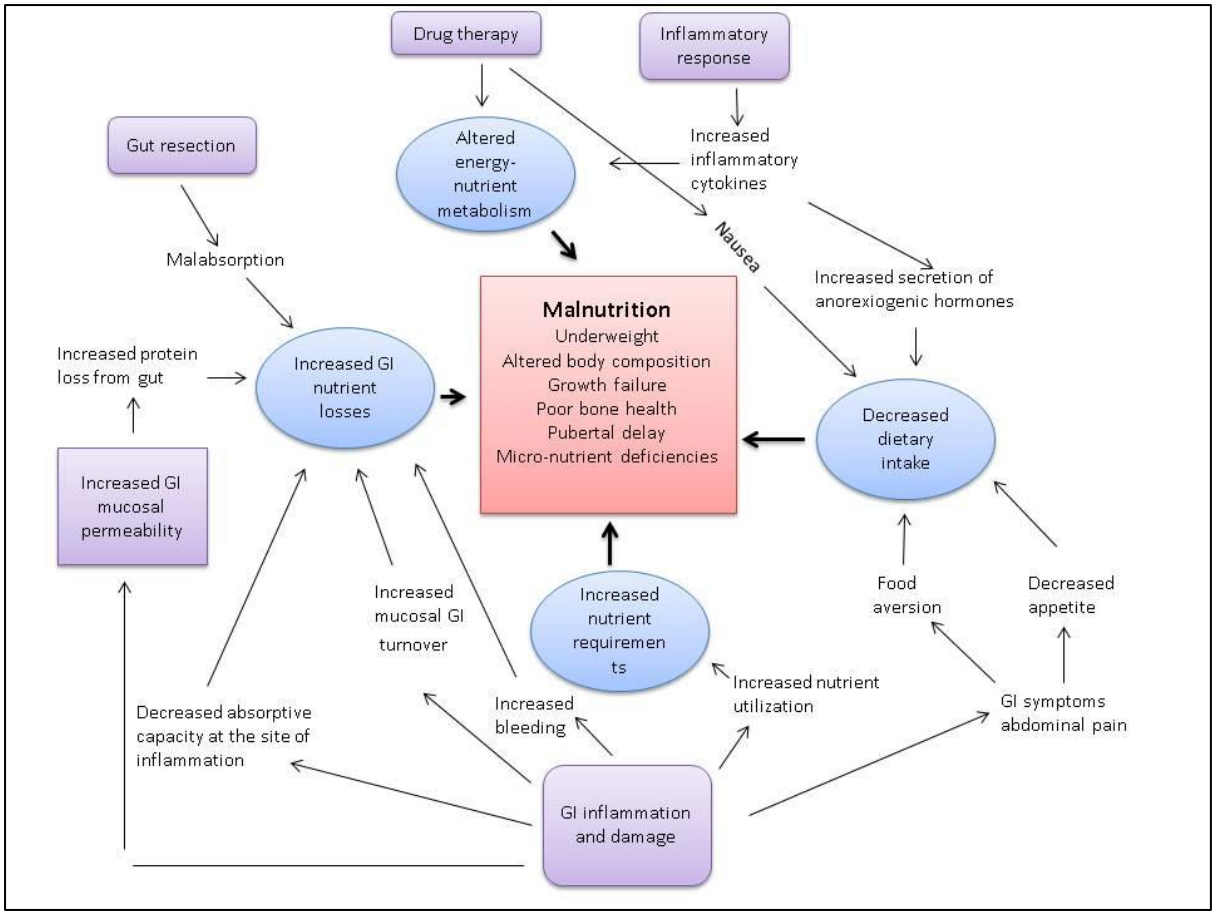

Figure 2-2: The aetiology of malnutrition in children with IBD, this figure has been produced from reference [33].

2.2.3 Nutritional status of children with IBD.

There are several factors that contribute to deterioration of the nutritional status in IBD patients, including decreased dietary intake, increased losses, malabsorption, drug-nutrient interactions, and altered energy/nutrient metabolism. Figure 2-2 summarizes the factors that lead to poor nutritional status in IBD.

Many patients with IBD have poor nutritional status at the time of diagnosis. Most patients present with weight loss and every relapse may be followed by further weight loss [34], [35]. Poor linear growth may also be evident at the initial assessment. A cohort study by *Kugathasan et al* showed that 102 of 456 children

(22%) of children with CD and 14 of 156 children (9%) of UC children in the North American cohort had a low BMI (<5th centile [35]. Recent data from adult and paediatric studies suggest that fewer patients present with underweight compared to previous studies and, indeed, a large proportion of patients, particularly UC patients, are overweight or obese [35]. *Long* et al [36] reported the prevalence of obesity in a cross-sectional study during 2007-2009 to be 20% in children with CD and 30% in UC children. Many pro-inflammatory markers associated with obesity and adipose tissue (leptin, resistin and ghrelin) have also been found to be linked with IBD. Therefore the increase in the level of these markers may mark or contribute to disease activity [37] . These findings highlight that nutritional problems do not only present in the form of weight loss, therefore there is a need to pay more attention to nutrition-related complications in the management of the condition, including the effect of disease on body composition changes in children with IBD.

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2.2.4. Growth failure

Growth is often impaired in children with IBD, and frequently precedes disease diagnosis [38]. Growth failure can be characterised by delayed skeletal maturation, faltering linear growth, and delayed onset of puberty [33]. The aetiology of growth impairment in children with IBD is multifactorial and includes poor nutritional state, corticosteroid use, disturbances of the growth hormone/insulin-like growth factor axis, and inflammation are all implicated. A summary of these factors are shown in Figure 2-3. Growth in children with IBD will be discussed in detail in section 2.5.8.

Growth retardation at diagnosis has been reported in 23-88% of children with CD and may precede gastrointestinal manifestations of the disease [39]. A table that summarizes different reported prevalence of growth deficits in children with IBD can be found in Appendix 1. Growth impairments have been found less common in CD than UC; however, growth impairment occurs in both groups [38]. About 30%-40% of children continue to have severe linear growth retardation during the disease course and several studies have shown that final height is affected in CD patients [40]. The variability in reported prevalence of growth failure in CD

children may be explained by differences in the definition of growth impairment, the population under study, and the disease phenotype (colon as opposed to small bowel) [41].

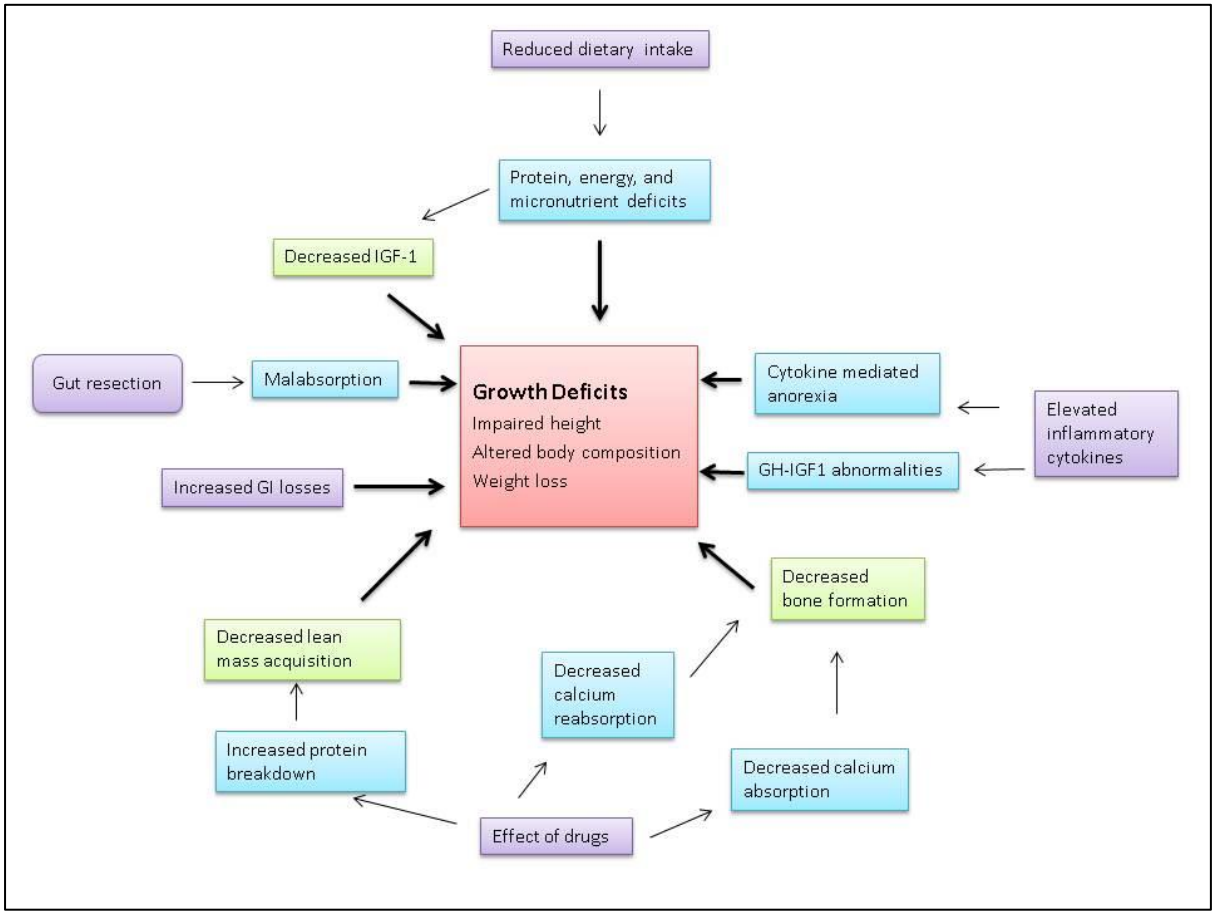

Figure 2-3 Aetiology of growth deficits in paediatric IBD.

2.2.5 Micronutrient deficiencies

Decreased dietary intake, malabsorption, and increased enteric losses, chronic inflammation have all been postulated to result in micronutrient deficiencies. Circulating concentrations of Zinc, Selenium, Copper, and vitamins A, E, C have all been reported at decreased in IBD patients compared to healthy controls and reference ranges [42]. Iron deficiency is also frequent is IBD patients which may lead to anaemia [43]. Anaemia is discussed in more detail in the following section.

2.2.6 Anaemia

Anaemia is a frequent complication of IBD. The prevalence of anaemia has been reported to be 40%-70% and anaemia affects quality of life and ability to work [44], [43] but is frequently under-diagnosed and treated [43]. The broad prevalence range in paediatric studies may be due to different methods used to describe anaemia including various cut-offs for haematocrit and haemoglobin values [45]. *Wiskin et al* demonstrated that 90% of children with Crohn's and 95% of children with Ulcerative Colitis (UC) were iron deficient at diagnosis. At follow up two years later 70% of children with Crohn's and 65% of children with UC were iron deficient [45]. Two types of anaemia are mostly present in IBD patients, iron deficient anaemia and the anaemia of chronic disease associated with chronic inflammation. It is widely believed that Iron-deficiency anaemia occurs as a result of GI bleeding, reduced dietary intake, and impairment of Iron absorption particularly when disease is located at major sites of iron absorption [46]. The clinical significance of anaemia in children with IBD and the extent to which correcting anaemia is associated with improvements in disease activity or growth has not been adequately determined [33]. Some studies suggest that the anorexia associated with iron deficiency may contribute to growth retardation [47]. Reports of the effects of Iron supplementation on linear growth of anaemic children have shown mixed results [48], [49], [50]. There is a need for more studies to examine the aetiology of anaemia and its relationship with growth failure or altered body composition both in normal children and children with IBD, and whether iron supplementation improves growth in children with IBD.

2.2.7 Poor bone health:

The pathophysiology of bone disease in IBD is complex [51]. Various factors may contribute to this condition including, chronic inflammation, malnutrition, decreased physical activity, and steroid therapy. The effect of these factors may be immediate, leading to fragility fractures during childhood or long term consequences due to sub-optimal bone accrual [52]. Poor bone health will lead to compromised linear growth, low bone mineral density, increased risk of fractures and lean mass deficits [51] .

The majority of adult bone mass is accumulated by the age of 18-20 years in boys and at the age of 16 years in girls. The average age of IBD diagnosis in children is 12 years with the majority of children diagnosed between 6-17 years, at the time when bone mass is acquired at the fastest rate [53]. Although longitudinal studies of bone mass accrual in children with IBD are limited, there is some evidence from short term studies that bone mass accrual is hampered in children with IBD [54]. *Sylvester et al* [54] observed that total body BMD expressed as Bone Mass Density SD Scores (mean +/- SD) was -0.78 +/- 1.02 for Crohn's disease (CD, n = 58), -0.46 +/- 1.14 for ulcerative colitis (UC, n = 18), and -0.17 +/- 0.95 for control (CL, n = 49) ($P < 0.01$, CD versus CL). After two years of follow up, BMD did not change significantly in IBD patients.

Longitudinal studies of bone mass accrual in healthy children have demonstrated that bone mass density is positively related to linear growth [55]. Cross-sectional studies have also demonstrated that there is a close relationship between height SD Scores and bone mass density [56], [57]. Height deficits frequently exist in children with IBD at diagnosis [58]. At diagnosis, biomarkers of bone formation and resorption are reduced to approximately 30-50% of normal in children with CD, and growth deficits are associated with bone metabolic activity [54].

Low bone mineral density is also considered as a potential risk in children with IBD and so, bone mineralization is an important aspect in the care of these children. Several studies have reported poor bone health in paediatric and adult patients with IBD [59], [56]. Low bone mineralization in IBD can be a result of low Vitamin D intake, the effect of pro-inflammatory cytokines on bone formation, corticosteroid exposure, delayed pubertal maturation and low physical activity [60].

One of the less appreciated consequences of poor bone health in children with IBD is a deficit in the accretion of lean mass. Equally, lean mass is significantly important for skeletal health possibly through the piezo electrical forces applied on bone during muscular contraction. Several cross-sectional and longitudinal studies have concluded that there is a relationship between lean mass deficits and bone mass or structure deficits [52], [61].

To conclude, IBD comprises primarily two disorders: CD and UC that are chronic, relapsing, inflammatory conditions of the GI tract with a potential to develop several

clinical manifestations. Under-nutrition, growth deficits, altered body composition, micronutrient deficiencies, anaemia, and poor bone health are common. The aetiology of the various local and systemic clinical manifestations are multi-factorial, and it is essential to be able to characterise and understand the factors that contribute to malnutrition in children with IBD such as poor dietary intake, drug therapy, inflammation, increased losses.

2.2.8 Treatment of IBD.

Guidelines currently in use for management of IBD have been published by the British Society of Paediatric Gastroenterology Herpetology and Nutrition (BSPGHAN) [62]. The IBD working group of BSPGHAN performed a comprehensive literature search of treatment modalities in paediatric IBD intervention studies using electronic databases (Medline, Pub med, Cochrane and Ovid). Evidence was graded using the Scottish Intercollegiate Guidelines Network 'SIGN' [63]. Unfortunately, there is very limited information on nutritional assessment and monitoring growth in children with IBD in the mentioned guidelines.

The primary goal of treatment for IBD is the management of inflammation and induction of remission, and subsequently maintaining remission and prevention of relapse. In recent years general well-being of patients with IBD has become an important outcome in the management of the disease [64]. In children, normal growth and pubertal development are additional goals of treatment. Impairment of longitudinal growth is a sensitive marker of persistent inflammatory activity, therefore it is often used as an indicator of successful treatment or sustained remission [65].

In children with CD, PCDAI which includes evaluation of longitudinal growth, is used as the indices of the patient`s overall response to treatment. In UC children, PUCDAI is used to measure disease activity and response to treatment [29], [32].

CD Treatment:

Exclusive Enteral Nutrition (EEN) or corticosteroids can be used as first line treatment to induce initial remission according to BSPGHAN guidelines. Recently increased understanding of the biological mechanisms of IBD has enabled the use

of a growing number of biological agents in the treatment of IBD. Many more biological agents have been studied in CD than in UC [66].

Conventional treatments:

Conventional treatments include amino salicylates and corticosteroids and the early introduction of immunosuppressive (such as azathioprine or 6-mercamtopurine) is encouraged as maintenance treatment. The efficacy of corticosteroids in the induction of clinical remission through the down-regualtion of proinflammatory cytokines has been well described [67]. However, in paediatric practice they are often used as a second-line therapy due to their side effects. Corticosteroids have adverse effect on growth, but their side effects also include obesity, and susceptibility to infection [68].

Nutritional therapy

Children presenting at many European paediatric IBD centres with new onset CD are treated with exclusive enteral nutrition, most commonly with a polymeric formula. Nutritional therapy has proven to be effective in inducing and maintaining remission in CD while promoting linear growth [65], [69]. Exclusive enteral nutrition (EEN) is a distinct therapy; it is an enteral formula, either elemental or polymeric, which is given exclusively (instead of normal diet) usually for six weeks [62]. EEN and corticosteroids have similar rates of disease remission; However, EEN has beneficial effects on nutritional status and growth [70].

Soo et al [71] retrospectively studied newly diagnosed paediatric patients with CD. 36 patients received EEN and 69 patients received corticosteroids. Remission was achieved 88.9% in the EEN group versus 91.3% in the corticosteroids group after a period of 3 months. As indicated, the two groups did not differ significantly in terms of induction of remission. However, weight SD score increased significantly after 12 months in the EEN group compared to corticosteroid group.

The theory behind the mechanism of action of EEN is multi-factorial. It has been demonstrated that EEN has anti inflammatory effects on the intestinal epithelial cells and may act to diminish inflammation through this process [72]. It is also proposed that EEN modulates the intestinal microbiota, suggesting inflammation may also be reduced by changes in the intestinal microbiota [73]. Furthermore, *Guzy et al* demonstrated that EEN reconstitutes epithelial cells, increases the

epithelial barrier function, decreases T cell activation and, decrease the release of pro-inflammatory cytokines [74].

EEN has been shown to be effective in increasing weight, but less effective in promoting linear growth [75], [76], [77] , [78]. For example, *Beattie et al* found that EEN resulted in increased weight SDS at 8 and 16 weeks with increased levels of insulin insulin-like growth factor (IGF)-1 in a cohort of 14 children [75]. A retrospective case-note review in newly diagnosed paediatric CD patients conducted by Cameron et al [78] demonstrated weight SDS improved from -1.3± 1.5 at baseline to -0.4 ± 0.9 following 8 weeks of EEN. In the same study BMI SDS increased significantly during follow up(6-24 months) , however, height SDS did not increase significantly from baseline (-0.6 ± 1.1) to the last follow up session after 24 months(-0.8 ± 1). . The extent to which it is possible to promote linear growth, weight gain and the accretion of lean tissue is discussed later (Section 2.4.8).

A recent, small randomized, controlled trial comparing steroids with EEN in the treatment of active paediatric CD demonstrated significantly better mucosal healing with EEN (75%) compared to steroids (33%) [77]. In a controlled trial of exclusive elemental diet versus high-dose steroids in 33 children with active CD, linear growth (assessed from height velocity over 6 months) was significantly greater in children receiving EEN than steroids alone [70]

Biologicals:

Monoclonal antibodies (commonly referred to as 'biologicals') are drugs that interfere with inflammatory response. The administration of biologicals is a relatively new method of treatment that could potentially be very successful concept in treatment of IBD. The first such product, infliximab, is available for treating CD and acts by inhibiting the functional activity of TNF*α*. Following treatment, *Anand et al* histologically assessed colonic biopsies and found an extensive reduction in measureable TNF*α*. Treatment was also associated with a reduction of CRP which is generally increased during inflammation [79]. Some initial data on the beneficial effects of biologicals in improving growth in paediatric IBD have been reported. *Hyhams et al* found significant improvements in height SD scores were observed in paediatric patients with CD that were treated with Infliximab [80]. *Walters et al* assessed the growth patterns of 32 children treated with Infliximab, these children showed improved height velocity, and increases in height centile [81]. *Malik et al* confirmed the beneficial effects of treatment with infliximab in children with CD,

significant improvements in BMI were detected after 1 year of treatmen [82]. *Moeeni et al* believe that the effects of biological in children with IBD may be due to improved inflammation with mucosal healing or due to direct effects upon growth hormone and IGF-1 signalling [83].

In conclusion, the main objective of treatment is to induce remission and prevention of relapse but when treating children, ameliorating or correcting any growth impairment is clearly important. The choice of therapy depends on the balance between short-term efficacy and long-term suitability. EEN is usually used as the first line management of children with CD, as it both induces remission and prevention of relapse and appears to promote weight and height in children which is not always seen with steroid treatment alone. Recently, biologicals have been used in treatment of IBD, particularly CD. Biologicals have been shown to improve growth in children with CD and their effects may be explained by mucosal healing or direct effects on IGF-1 and growth hormone signalling [83].

UC Treatment:

The treatment of paediatric UC is improving with improved understanding of the genetic and immunological basis of the disease [84]. At diagnosis the initial treatment for UC depends on disease extend and severity. Corticosteroids still remain the first-line therapy for clinical management of UC patients. 5-Aminosalcylic Acid (5-ASA) at high doses may induce remission, but in children it is more usually used in combination with corticosteroids to induce remission [85]. Corticosteroids induce remission due to their effect on reducing production of proinflammatory cytokines [66]. Recent guidance proposes that the use of corticosteroids should be limited, given their lack of efficacy as maintenance medications and significant side-effects [84].

2.3.1 Normal growth

In order to identify any perturbations of growth, it is necessary to firstly have a sense of what pattern of height and weight gain might be considered as appropriate. Only with this understanding and reference, is it possible to determine how growth may deviate from normality in order to be considered unusual or inappropriate. This demands a sense of typical or usual growth, as well as identifying what might be considered the limits of usual growth or variance.

A summary of normal growth and normal growth in terms of body composition is provided here to help a greater recognition of the nature of the growth deficits associated with the disease process and treatment.

Growth and development go hand in hand. The most rapid phase of longitudinal growth is in the mid-trimester foetus. Thereafter, rapid growth in infancy slows down through early childhood before a doubling of growth rate at puberty, followed by virtual cessation of growth when the epiphyses of the long bones fuse in the mid-teen years [86]. Growth is rapid in the first few months of life where increases in length, head circumference and weight may be used to mark linear growth.

It is generally agreed that there are at least three distinct endocrine phases of linear growth - infancy, childhood, and puberty - each regulated by different growth promoting systems [87]. The main influences on growth in foetal and early postnatal life are nutritional rather than endocrine (growth hormone does not play a great role in growth in infancy possibly because of immature growth hormone specific receptors) with hormonal factors becoming progressively more important from infancy onwards [86]. Growth during adolescence is related to both growth hormone and sex steroids, testosterone and oestrogen, and the adolescent`s growth spurt is the product of the two superimposed phases of childhood and puberty [87].

Prader [87] uses the following parameters to define the timing of the adolescent growth spurt. The starting point is the age of minimal pre-pubertal height velocity (AMHV), maximal pubertal growth is reached at the age of peak height velocity (APHV); and the spurt ends when the adult stature is but as this age cannot be precisely identified, and the age when 99% of predicted adult height (99AH) is completed was used. AMHV occurs at 9.9 years in girls and at 11.6 years in boys; (99AH) is reached at 15.2 years in girls and at 16.8 years in boys. At the same time as skeletal maturity, sexual maturation occurs in both genders. In girls, pubic hair and breast development begin between AMHV and reach maturity about one year before 99AH and menarche occurs always after APHV. In boys, testicular growth begins just after AMHV and ends late in the descending part of the growth spurt. Public hair and penile growth begin between AMHV and APHV. Penile growth is purely androgen dependant and ends shortly after APHV, whereas testicular growth is a much more complicated process depending on the gonadotropins and testosterone and takes much longer than penile growth.

Variations in the timing of puberty are wide [86]. Early pubertal development is defined as before the age of 8 years in girls and 9 years in boys. Delayed puberty is common in boys (a tendency often inherited from the father), but occurs less often in girls (where again the mother may have passed menarche late). A boy, who shows no signs of puberty at 14 years of age, or a girl at 13 years, warrants specialist assessment. A 16-year old boy with few or no signs of puberty may suffer psychological stress on this account, and may also be at lifelong risk of low bone density and other effects of late onset of androgen surge. Delayed puberty is a frequent finding in adolescents with chronic disease due to several mechanisms, including malnutrition and inflammation [88].

2.3.2 Monitoring and assessment of growth

Standardized charts are available for graphically recording height, weight and height velocity such that an individuals child`s growth can be compared to normative values. In the United Kingdom, WHO Growth Standards are used from birth to 4 years of age. From 4 y onwards the new charts that combine UK90 and WHO data and provide clear instructions on how to measure, plot and interpret the chart. An individual child's growth measurement can be represented as a percentile or as a standard deviation score, which are explained in detail in the following sections.

2.3.3 Centiles and standard deviations.

Standard Deviation Scores (SDS)

Standard Deviation Scores (SD scores, SDS or *Z* –score) are used to offer an age and gender independent characterisation against which weight and height measurements can be compared and has immediate advantages over expressing relative growth in terms of centiles. Firstly, SD scores are calculated based on the distribution of the reference population (both the mean and the standard deviation [SD]) thus, they reflect the reference distribution (for reference population the mean SDS is zero). Secondly, as standardized measures, SD scores are comparable across age, sex and measure (as a measure of "dimensionless quantity"). It is possible to provide conventional summary statistical terms to describe the central tendency and variance within a group of children continuous as a continuous variable. Thirdly, SD scores can be used to describe growth beyond the usual range of centile values.

The major limitation of *Z* -scores is that they are not straightforward to explain to the public [89].

Centiles:

A centile refers to the position of an individual within a given reference distribution). Centiles are possibly easier to understand and use in practice, both by health professionals and the public. A limitation of using centiles is that the same interval of centile values corresponds to different ranges in absolute values for different measurements. For instance, increments from 85th to 90th centile correspond to different ranges in subscapular and in triceps skinfold thickness. Even within the distribution of one measurement, the same increments at different percentile levels could correspond to different changes in both SD scores and absolute measures. In addition, it does not allow for quantifying the change in percentile values near the extremes of the reference distribution (e.g., people in the uppermost 1st centile can have very different absolute values). For these reasons, centiles are not recommended to be used to assess change in status over time, while change in SD score is a better measure. SD scores are more useful in research while percentiles are easier for use in clinical settings and by the public. SD scores and centiles can be converted to each other, but the commonly used cut points of each are not at exactly comparable levels. For example, SD scores of 2 and -2 correspond to the 97.7th and 2.3rd percentiles, while the 85th and 5th percentiles correspond to SD scores of 1.04 and -1.65, respectively [89].

2.3.4 Definition of Impaired growth

Growth impairment has often been defined as an obtained height lower than the 3rd or 5th centile or <1.6 or <2.0 SD scores or with the crossing of two centile bands or SD over time. These definitions do not consider the children who have grow poorly but still obtain a height above defined lower limits. For this reason, growth velocity is the most sensitive way to identify growth disturbances at any age but in practice, requires multiple measures over time. Delayed growth usually leads to delayed puberty when this developmental stage has arrived. It can be defined as a difference from the expected stage of maturation [90].

2.3.5 BMI and it`s limitations in interpreting body composition.

BMI is wildly used in clinical settings as a marker of growth and nutritional status. This measure was first introduced by mathematician Lambert Adolphe Jacques Quetelet and has sometimes been referred to as the Quetelet index [91]. It is simple to use in clinical settings, however, there are limitations regarding the use of BMI as it is not a sufficient tool to describe differences in body composition. Although there is evidence that adiposity (as fat mass) increases with BMI and so in general, can be thought of as marking fatness, it is a more predictor of fatness at the individual level. Lean mass also increases with increasing BMI, so a high BMI usually reflects both high fat and lean mass.

The total mass of the body can be simply subdivided into two parts – the mass of lean tissue or Fat Free Mass (FFM) and fat mass (FM).

Fat Mass Index (FMI) and Fat Free Mass Index (FFMI) are two indices that were first introduced by *Vanitallie et al* to overcome limitations of using only BMI to describe nutritional status [92]. The two suggested indices are calculated as follows:

$$FFMI = \frac{FFM(kg)}{ht(m)2}$$

$$FMI = \frac{FM(kg)}{ht(m)2}$$

The potential advantage is that only one component of body weight, i.e. FFM or FM, is related to the height squared.

The use of BMI to assess the body fat in children assumes that BMI represents adiposity independent of age, race, sex, and individual differences, meaning that all subjects with a similar BMI have the same degree of fatness, regardless of their age, sex, race, and individual differences. In a study by *Daniels et al* [93] BMI (kg/m^2) was calculated from height and weight measurements and compared against fat mass and percent body fat determined using dual-energy x-ray absorptiometry. Sexual maturation was evaluated by physical assessment. They demonstrated that BMI is not an equivalent measure of the percent body fat for each race-sex group. When BMI is used as a measure of body fatness in a research or clinical setting, it may be important to consider the maturation stage, race, gender.

Freedman et al [94] demonstrated that despite similar BMI levels of boys and girls, FMI was higher in girls than boys. There were also differences across race/ethnicity groups where BMI was shown to be dependent on race and gender so that different

Fat Mass Index (FMI) and Fat Free Mass Index (FFMI) in children of similar BMI values. They also concluded that the accuracy of BMI as an index of adiposity in children differs according to the degree of fatness. BMI can better predict body fatness among relatively heavy children (BMI for age ≥ 85th centile) but not among lighter children. *Maynard et al* found that the average incremental changes in BMI over single-year periods could mask differences in the changes in body composition changes in children, depending on race and gender [95]. *Demerath et al* [96] demonstrated that BMI was dependent on age and gender. Although BMI was similar in boys and girls, FMI and FFMI differed significantly between genders, particularly in later adolescence. Age differences also existed in the relationship between BMI and body composition as well. Older children generally had higher FFMI for a given BMI percentile than younger children, and older girls also had higher FMI than younger girls for a given BMI.

From these studies it can be concluded that BMI is not an accurate or precise measure of body composition, and should be interpreted by caution. Although BMI is relatively easy to use in clinical settings, it has limitations. Therefore, there is a need to develop simple bedside measurements of body composition that can assess body composition more accurately.

2.3.6 Why is final height of great importance?

Children with short stature are more likely to report themselves as a victim of bullying than their taller peers, or achieve significantly lower test scores. In a study of 2848 children aged 5–12 years, short boys who were achieving poorly in school were more likely to have to repeat a grade as they progressed through primary school than their normal height peers [97]. Because IQ was similar, it was concluded that societal perception, biased against the smaller boys, may have hampered their progression through school.

Adults with short stature are more likely to have difficulties in education, employment and relationships than those of normal height [98]. Short stature may also be associated with lower health status. It is associated with significantly higher percentage of body fat, particularly in women [99]. Women of short stature are at greater risk for obstetric complications because of a smaller pelvis. Small women

are at greater risk of delivering an infant with low birth weight, contributing to the intergenerational cycle of malnutrition, as infants of low birth weight or retarded intrauterine growth tend be smaller as adults [100]. For the mentioned reasons, height gain should be monitored regularly in childhood, and causes for any deficits should be identified rapidly. Subsequently, required measures should be taken in to account to eliminate the cause of the deficits if possible, and stimulate catch up growth.

2.3.7 The role of nutrition on growth and body composition

As children grow, they become taller (linear growth) and heavier (weight). However, the rate of height gain slows from birth through infancy and early childhood, remains relatively constant over 5-9 years before peaking and falling again through puberty. The rate of gain in height in comparison to the rate of weight gain is not always the same such that weight relative to height (expressed as the BMI) initially rises over the first year of life and then falls between 2y and 5-6y before rising again through to adulthood. This lack of concordance between rates of height gain and weight gain results in a characteristic pattern of change in BMI as children grow. The change in rate of weight gain reflects changes in the accretion of fat and lean. There is an absolute increase in the amount of both lean and fat as children become taller. *Wells* [101]has highlighted the inadequacy of expressing the changes in body composition in terms of percentage fat or lean in children as they grow and the need to normalize both FM and FFM independently for height. When normalized for height, it is clear that the rate of accretion of lean and fat differs in proportion to the rate of height gain such that the Fat Mass index (FMI: Fat Mass in kg/height in m^2) and Fat Free Mass Index (FFMI: Fat Free Mass in kg/height in m^2) change during growth. These changes follow a characteristic pattern over time and appear to be gender specific.

Hattori produced a chart in order to graphically present body constitution as a quantitative measure [102]. FFMI, and FMI were placed, one component on each axis. Wells [101] has used a Hattori chart to plot FFMI against FMI (and also lines to indicate BMI and %fat) in order to illustrate the changes in the relative proportions

of FM and FFM, Figure 2-4.

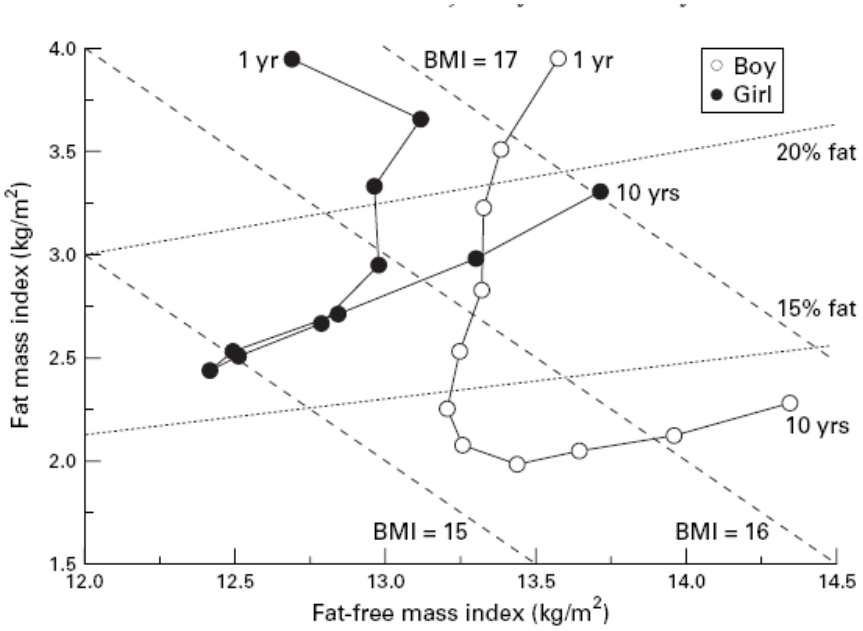

Figure 2-4: This chart illustrates the changes in the relative proportions of FM and FFM is reproduced from reference [101].

Whilst the rate of lean tissue gain is proportionate to the rate of gain in height over the first 6 months of life, fat tissue is gained faster than that expected with gaining height. From 6 to 12 months, lean is gained at a faster rate than height while fat gain is less than height gain The patterns are similar in both boys and girls, but the initial gain in lean relative to gain in height over the first month of life is greater. Whether this is caused as a result of gain in skeletal muscles or viscera is unclear, and there is still lack of evidence of how children grow in terms of body composition in general populations.

As the rate of gain in height, lean and fat changes over time, the relative fatness also changes (as percentage fat). Both genders show a U-shaped curve for BMI between 1 and 10 years although fat percentage increases only in girls again in the second half of childhood. This is illustrated in Figure 2-5 which shows how FMI and FFMI change during growth, from infancy to 10 y of age.

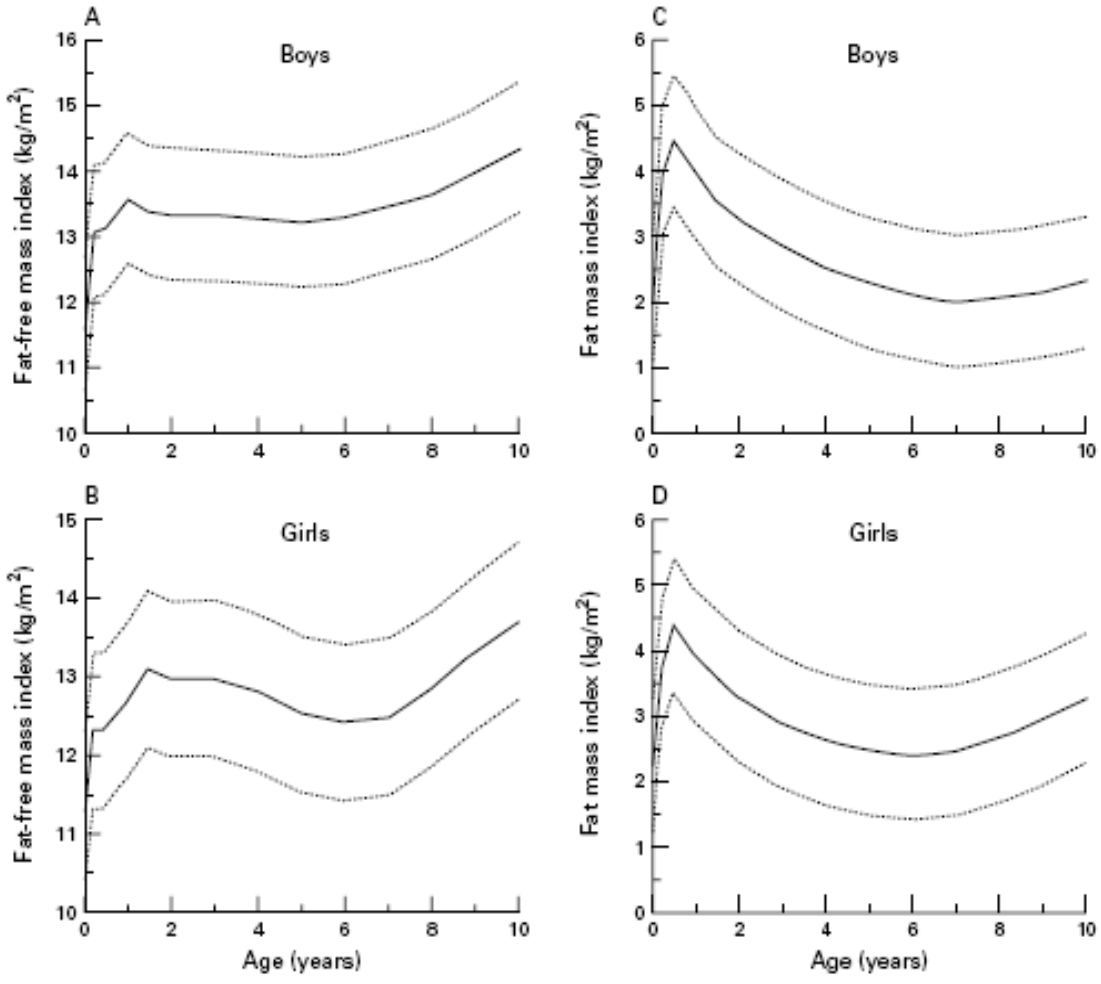

Figure 2-5: This figure shows show FMI and FFMI change from infancy to 10 years and is reproduced from reference [103] .

Boys show a relatively constant relationship between height and FFM between 1 and 6 y, with the FFMI (FFM/height$^{2)}$ increasing with age thereafter. The decrease in male BMI between 1 and 6 y is almost entirely due to a relative decrease in fatness, whereas the increase in BMI between 6 and 10 y is due to increasing FFMI with the percentage fat remaining relatively unchanged during this period. In contrast, girls show a decrease in both FMI (FM/ height^2) and FFMI between 1 and 5 y. The decrease in FMI is less marked than in the boy, and between 5 and 10y she shows an increase in both components.

Maynard et al [104] also describe the relative changes in body composition in older children and found that mean values for BMI and FFMI generally increased with age in both genders. Girls tended to have greater mean BMI than boys between 11y-16y. In boys, the mean FMI increased slightly with age but boys had lower FMI than that of girls at all ages. The pattern of mean FFMI was very different from that observed for FMI. FFMI increased progressively with age in both genders until the age of 14 y. After this age, there were only slight small increases in the mean FFMI for girls, whilst the mean FFMI for boys continued to increase significantly until 17y.

In boys, the increase in the BMI between the ages of 9-12y was due to increase in FMI and FFMI. Between 8 and 9 years and 12-17y, the increase in BMI was exclusively due to increases in FFMI, whilst FMI decreased. Between 17 and 18 years of age, both FMI and FFMI underlie the increase in BMI.

The annual changes in FMI and FFMI, and BMI for male individuals showed that between the ages of 9-12y, the increase in the BMI was due to increase in FM/height^2 and FFM/ height^2. Between 8 and 9 years and 12-17y, mean annual changes of BMI were exclusively due to increase in FFM/ height^2, whilst FM/ height^2 were decreasing at these ages. Between 17 and 18 years of age both FM/height^2 and FFM/height^2 caused the in increase in BMI.

The data for girls demonstrates that increases in FM/ height^2 and FFM/ height^2 contribute to the increase in BMI from 8-9y. Most of the increase in BMI from 10 to 14 years was due to the increase in FFM/ height^2. As girls reached adulthood,

between the ages of 16 and 18, BMI changes were almost exclusively driven by increases in FM/height^2, however, slight increases in FM/ height^2 were also observed.

Few studies have examined the relationship between FFM and BMI in healthy children [95], [105]. Most recent studies have focused on the relationship of fat mass and BMI [106], [107], [108]. As reported by *Demerath et al* [96], BMI increases are principally driven by increases in FFM in growing in children and particularly in adolescent boys. Therefore there is a need to further investigate the pattern of tissue accretion and changes occurring in FFM and FFM components of weight as in healthy growing children and adolescents in order to set a reference for FFMI and FMI in healthy children. New reference data are required that represent healthy contemporary children throughout the period of growth, based on measured values rather than predicted values. Such data are required to describe the broad changes in fat and lean and to describe the changing properties of FFM with age. Having a reference data for FFMI and FMI enables us to further investigate the effects of different diseases on childhood body composition.

2.4.1 Growth in children with IBD.

Growth abnormalities frequently exist in children with IBD, and often precede disease diagnosis. The pathophysiology of growth disturbance in children with IBD is multifactorial - see Figure 2-3. Inadequate energy intake leading to chronic malnutrition has long been implicated as the primary cause for growth failure in children with IBD. Energy deficit may be due to a combination of factors including symptoms of esophagitis and gastritis, cytokine-mediated anorexia, and fear of worsening gastrointestinal symptoms. Stool losses, including protein-losing enteropathy and steatorrhea, occur secondary to mucosal damage and may also account for nutrient deficiency. Consumption of corticosteroids can result in growth failure as well [33].

Decreased linear growth may be one of the earliest signs of CD in pediatrics. It has been indicated that there is a direct correlation between the duration of impaired linear growth before the diagnosis and treatment of CD and the development of severe growth retardation [38]. Therefore, early diagnosis and the introduction of remission are crucial to reduce the degree of growth retardation. Improvements in growth can be used as a useful marker of the efficacy of treatment of children with

IBD. Hence, growth in children with IBD should be closely monitored following diagnosis and treatment.

2.4.2 Energy deficit

Signs of energy under-nutrition are present in many patients with IBD at diagnosis [8]. Several factors may lead to an energy deficit in these children, including decreased dietary intake and cytokine mediated anorexia. Inflammation of the gastrointestinal tract and the related symptoms of pain, nausea and diarrhea, as seen in CD, often lead to a loss of appetite and reduced food intake. As a result, children often experience early satiety and decrease their oral intake to avoid these symptoms, on both conscious and subconscious level [109]. In addition cytokine mediated anorexia may result in loss of appetite and reduced dietary intakes in these children [110].

In pediatric CD patients, energy intake was reported lower than healthy controls and the national recommendations [111], particularly during the active phase of the disease [109]. Children with active CD consumed on average 1757 kJ less than their siblings matched for their height and weight. In addition, for 21% of patients the energy intake was lower than estimated energy requirements compared to 10% of controls [111].

2.4.3 Altered energy and nutrient metabolism

Altered energy-nutrient mechanism is another possible cause that may result in faltering growth in children with IBD. Higher rates of energy expenditure, as basal metabolic rate per unit FFM, are commonly believed to contribute to the energy deficit. Some, but not all studies, have reported higher BMR/FFM in CD patients compared to UC patients and healthy controls [112], [113]. *Azcue et al* [114] found that FFM was significantly depleted in absolute terms in patients with Crohn's but that the Resting Energy Expenditure (REE) was not different from the controls whether expressed as calories per kg of body weight or per kg of lean body mass. The reason why this may happen is unclear, inflammation and inflammatory cytokines may play a role [112]. Failure to correctly adjust energy expenditure for differences in FFM mass may give rise to an apparent hyper-metabolism as the intercept for the slope of the relationship between basal metabolism and FFM is usually positive. *Wiskin et al* [115] demonstrated that there is a weak negative

association between REE per kg FFM and PCDAI. Using the linear regression model authors showed that there was a statistically significant relationship between PCDAI and REE. For each point increase in PCDAI there was a decrease in REE of 3.2 kcal.

2.4.4 Malabsoption and increased Gastrointestinal nutrient losses

It is reasonable to predict that absorption of specific nutrients would be impaired when the disease is located at the site of specific nutrient absorption or if this area has been resected [116]. Iron malabsorption may occur in CD patients as a result of excessive production of hepcidin, a hepatic peptide mediating the absorption of Iron at the level of erythrocyte [117]. In addition to malabsorption in IBD, loss of nutrients may exist as a consequence of intestinal mucosal damage, and through protein enteropathy due to increased permeability of gut. Studies using whole gut lavage have shown that disease activity was paralleled with gastrointestinal protein loss [118].

2.4.5The effect of drugs on nutritional status

Corticosteroids have been shown to inhibit linear growth and delay puberty in children. However, children may differ in their susceptibility to corticosteroid induced growth inhibition. Corticosteroids may lead to a reduction in bone mass and osteopenia via several different mechanisms, including direct effects on bone formation and bone resorption and indirect effects via actions intestinal calcium absorption, renal tubular calcium reabsorption [119]. Several other drugs used in IBD treatment can directly affect nutritional intake or alter absorption, metabolism and excretion of nutrients. An example is the interaction of methotrexate with folate metabolism which leads to nausea and reduced food intake [33].

In addition rates of protein breakdown may affect the capability to acquire and maintaining lean tissue and bone, and so linear growth. Altered rates of protein metabolism have been described in children with active CD and the severity of inflammation correlates well with the rate of protein breakdown. For example, *Steiner et al* demonstrated in their study that, the use of corticosteroids lead to significant alteration in protein metabolism within 2 weeks of consumption in children with newly diagnosed CD. The rates of whole body protein breakdown, protein oxidation, and, protein loss were elevated significantly in these patients [120]. In conclusion , use of steroids have adversely affect lean tissue and bone

acquisition, deficiencies in bone mineral density, and the opportunity to attain their full potential adult height

2.4.6 Role of inflammatory cytokines on growth

Pro-inflammatory cytokines are produced by numerous cell types and have a variety of actions including, T-cell activation, induction of acute phase proteins and inflammatory chemical pathways. Evidence supports that imbalance of pro-inflammatory cytokines exist in patients with IBD [121]. There is evidence in children with inflammatory conditions abnormalities in the Growth hormone- Insulin like growth factor (GH-IGF) occur, this may be a result of GH deficiency, GH resistance, and decreased IGF-1 levels. In addition there is also evidence that inflammatory cytokines are associated with reduced levels of insulin like growth factor binding protein (IGFBP-3). Reduced levels of IGFBP-3 result in increased clearance of circulating free IGF-1. Inflammatory cytokines may also induce anorexia, which may subsequently lead to growth failure [110].

2.4.7 Management and prevention of growth impairment in children with IBD

Early recognition of impaired growth is of great importance in children with IBD. In caring for children with IBD, it is of value to attain sense of growth prior to diagnosis. Pre-illness height measurements will help in distinguishing the impact of chronic intestinal inflammation; however, one of the problems in management of children with IBD is that due to initial general symptoms of the disease, it is not usually diagnosed rapidly. Part of evaluating the response to therapy in children with IBD is assessment of normal growth, and examining whether catch-up growth to pre-illness centiles is being achieved. A properly calibrated and reliable stadiometer is required for accurate and precise measurements of height. Height velocity must be calculated subsequently [41].

One of the complications in assessing growth in response to therapy is the relatively long interval of time required to determine a meaningful height velocity. Published normal standards for height velocity during childhood are based on height measurements over a period of 6 or 12 months [122]. When growth velocity is calculated over short period of time, small errors in individual measurements

appear significant, and the normal variation in growth is overlooked. The recommendation is that height velocity should be calculated over intervals no shorter than 6 months [123].

The first-line management of growth failure in IBD is control of active disease and inflammation. When choosing the treatment in children with IBD, treating to facilitate growth is of great importance. Avoidance of long-term corticosteroid therapy, more frequent use of enteral nutrition as an alternate primary therapy in CD should be considered. The goal of all these strategies is to improve growth prior to puberty in these children. New biologic therapies have opened new avenues in pediatric IBD management as they have been demonstrated to improve growth in children with IBD and other chronic diseases [81].

2.4.8 Growth in children with IBD

Linear growth in IBD

Impairment of linear growth is common in children with IBD especially in CD. A longitudinal study from Sweden conducted by *Hildebrand et al* showed that in children with CD, the mean height SDS declined slowly during the 5 years preceding diagnosis and remained significantly decreased through the 4th year after diagnosis. After diagnosis, the children with CD grew with a normal velocity but no significant catch-up growth occurred and the mean weight for height SDS decreased during the 5 years before diagnosis. Children with UC had height SDS above the reference mean from 5 years to 1 year before the diagnosis. The score then decreased during both the year before and the year after diagnosis and weight for height decreased during two years before diagnosis [124].

These findings demonstrate that at the time of diagnosis, weight for height was more affected than height, and weight started to deviate earlier than height both in children with UC and those with CD. These findings suggest that the main cause of growth failure in IBD may be malnutrition. However, *Kanof et al* [38]found that height velocity decreased before or simultaneously with the weight change in children with CD. The authors postulated from these findings that energy intake is not the primary reason for linear growth deficits in IBD children.

Growth deficits are more prevalent in patients with CD compared to UC patients. In a prospective study which defined growth failure as a height SD score of -1.64, the reported prevalence of growth failure in children with UC was 9%, compared to 38% in children with CD [125]. In the study conducted by *Hildebrand et al*, the reported prevalence of growth failure, defined as a height velocity SD score of <2.0 during at least one period between age 3 and the end of puberty, was 34% in UC patients compared to 65% in CD patients [124].

The prevalence of short stature has been shown to range 13-88% depending on what criteria are used to define the growth deficit [126], [38] whilst in UC patients it ranges 3-21 % [126], [127]. These figures support the commonly accepted principle that the prevalence of growth deficits are higher in CD than in UC .Only one of the studies focused on Height Velocity which is a more sensitive marker of poor growth [38]. This study shows that 88% of patients with CD have a height velocity below the 3rd centile prior to diagnosis. The two studies conducted by *Griffiths et al* (22) and *Hilderband et al* [124]found that at diagnosis the prevalence of short stature varies 13-21% in CD patients but in contrast, only 3% in UC patients identified short stature. We can conclude from these figures that the growth impairment in children with IBD, particularly CD patients occurs prior to gastrointestinal symptoms.

It is unclear whether current treatments of paediatric IBD can improve growth outcomes in these children. Salma Malik et al [128] studied case notes of 142 patients with CD retrospectively. The mean Height SD score at diagnosis was −0.5 (−3.3 to 2.6) which was lower than the mean mid-parental height SD score of 0.2 (−2.0 to 1.4). The mean height SD score did not change significantly from −0.0 (−0.9 to 2.0) to −0.0 (−0.8 to 1.0) (p=0.45) between year 1 and year 2 after diagnosis. No significant changes were noted between year 2 and year 3 following diagnosis (−0.0 (−0.8to 1.0) to 0.0 (−0.9 to 0.7) (p=0.07)). The authors concluded that therapy was associated with a reduction in growth failure but this was not associated with sufficient catch-up growth to cause an improvement in overall height SDS.

Weight in children with IBD

Most children with CD have a history of weight loss or have failed to gain weights prior to diagnosis. For example, *Sawczenko et al* [126] found that at diagnosis the mean weight SD score was -1.06 and that 27% of children with CD had a weight

below the 3rd centile(which is about the equivalent of -2 SDS). The weight loss in UC was less marked, with a mean SD score of -0.32 and 9% below the 3rd centile. Similarly, Gri*ffith and Hugot* reported that 80% of 386 Canadian children diagnosed with CD had a history of weight loss over 10 years [129]. The reports in the literature tends to focus on the prevalence of weight loss at presentation as it is assumed that weight will normalise with treatment; therefore, there is a need for more longitudinal studies to examine weight gain in response to treatment. For example, maintenance of appropriate weight gain also continues to be an important issue following diagnosis. In a cohort of 41 children with CD, the 18 children with active disease had low mean SD scores for weight, height, and BMI than the 23 children in remission [109].

In a study conducted by *Sentongo et al* [39] , height SD score (Ht SDS), weight SD Score (Wt SDS) and Upper arm muscle area SD score (UMA SDS) were measured as markers of growth in children with CD and controls. Boys with CD had significantly lower Height SD score, Weight SD scores and UMA SD scores compared with control subjects. On the other hand, significant differences were not observed between the females with CD and control subjects.

Pfefferkorn et al [58] analysed growth outcomes in 176 CD patients younger than 16 years old at diagnosis who were studied for 2 years children and found that growth abnormalities persist despite current therapies. Their results established that the distribution of Height SDS remained similar during the 2 year observation period and that the growth delay persists in many children with CD following diagnosis, despite improved disease activity.

Burnham and co-workers [130] studied 104 patients with Crohn`s disease and 233 healthy controls aged 4-25 and found that subjects with CD had significantly lower height-for-age and weight-for-age than healthy controls. However, one drawback of this study is that controls were significantly younger than subjects with CD, which puts forward the need for a study which cases and controls are matched for age.

More recently, *Lee et al* [131] undertook a prospective assessment of height in 295 children with CD and UC who had been diagnosed between 2002 and 2008 in Boston, USA. 22% of these children (90% of which had CD) had linear growth impairment (height for age SD score of -1.64 or less) documented on one or more measurements following diagnosis. The final mean adult height SD score in this

cohort was -0.39. The main predictors of mean adult height in this study were found to be lower parenteral height and the patients' lowest height SD score.

In a recent British study, *Sawczenco et al* examined the final height of 23 individuals who had been diagnosed CD prior to turning 16. This group had a final adult height of -0.29 SDS which is very similar to that observed in North America. The mean final height of these patients was 2.4 cm less than the parental or target height [132].

In summary, the studies on growth in children with IBD confirm that growth deficits exist in both CD and UC children. In addition these studies convey that the prevalence of growth deficits and the severity of growth are more in CD children. Most studies focus on growth deficits at presentation. Little is known about how children grow during different phases of the disease processes, or how it differs by type of therapy or nutritional state. Therefore, the effect of different stages of the disease (e.g. remission and relapse) on height, weight and BMI is still not known. A longitudinal study, following children with IBD from diagnosis during their continuing care is needed. Such studies would make it possible to first monitor growth patterns of IBD children during different stages of the disease, second compare growth patterns of UC and CD children, third evaluate different therapies used in clinical settings. These findings will help us establish appropriate growth management strategies and therapies for these children in clinical settings.

2.4.9 Body composition in children with IBD

There are several factors that may result in differences between the body composition of IBD patients and healthy individual. The elevated levels of pro-inflammatory cytokines may alter energy metabolism, protein turnover. In addition the use of corticosteroids increases body fat with catabolic effects on lean mass. Moreover, physical activity which plays a major role in the normal development, growth and normal tissue accretion has been reported lower in pediatric patients with CD compared to healthy controls [133].

Recent studies show that alterations in body composition may be associated with clinical outcomes; In studies in a mixed hospital population and those following major vascular surgery, a low FFMI has been shown to be associated with a longer hospital stay [134], [135]. It has also been reported that it has been in patients with

chronic obstructive pulmonary disease that a low FFMI is a stronger predictor for mortality than is a low BM [136]. In a study by *Venrooij et al* [137] low FFMI was associated with the occurrence of respiratory tract infections. The direction of causality remains unclear as to whether a low FFMI simply marks more active/aggressive disease reflects a worse or whether for any level of disease, a low FFMI is associated causally with a worse outcome. One possible explanation for the association found between a low FFMI and the occurrence of adverse outcome in general could be that a low FFMI represents insufficient nutritional reserve. In other words, less metabolically active cell mass results in a longer recovery period after surgery, because patients do not have an adequate response to operative stress and are unable to handle complications well [138], [139].

2.4.10. Review of methods of measuring body composition uses in this thesis

Assessment of human body composition is a central component in the assessment of nutritional status. There is increasing recognition of the need to measure body composition in children. First, the rising prevalence of obesity in children is concerning and has increased the request for measuring FM in children and younger adults [140]. Second, body composition can be used as a marker of nutritional status and well-being, and monitoring changes in body composition of patients indicates whether nutritional state is declining or improving. Third, measurement of body composition is essential for optimum clinical care during hospitalization because, the size of the FFM is an important index of energy and fluid requirements. In addition, childhood growth deficits may not be identified using only height and weight measurements; therefore body composition measurements are required in the assessment and treatment of childhood with growth disorders.

There are different methods of measuring body composition which are discussed below, field methods of measuring body composition and reference methods. Reference methods are generally more expensive, inconvenient for the subjects, and are not applicable in routine clinical settings.

First two reference methods of measuring body composition (deuterium oxide dilution technique and dual energy X-ray absorptiometry), and subsequently field methods (Bioelectrical impedance analysis, and skinfold thickness) are discussed.

Deuterium oxide dilution

Hydrometry is the measurement of body water. Water is most abundant constituent of the body, typically comprising over 60% of the body weight and approximately 73% of the Fat Free Mass; however these percentages may differ significantly with age, level of body fatness, and health status [91]. Water is the most abundant component of the body and is predominantly associated with FFM; therefore, the measurement of TBW, as well as the distribution of water extracellular (ECW) and intracellular (ICW), is essential to the assessment of body composition. TBW is typically estimated via measurement of dilution of isotope tracers. The concentration of either hydrogen or oxygen isotopes in biological fluids after equilibration are measured and used to estimate TBW. Deuterium as deuterium oxide (D_2O) and ^{18}O as ($H_2{}^{18}O$) are tracers labeled with stable isotopes and can be used to assess body water. Deuterium oxide is more widely used in research purposes due to its availability.

Hydrometry is based on the dilution principle. This principle states that the volume of the solvent (body water) is equal to the amount of the compound (isotopic water) added to the solvent divided by the concentration of the compound in that solvent. Five basic assumptions are applied to the tracers and isotope dilution technique for estimating TBW.

1) The trace is distributed only in body water [141]

This assumption is not true as the tracers enter other pools within the body. This is known as non-aqueous exchange.
This assumption is violated slightly as, tracers exchange to a small degree with non-aqueous molecules. Deuterium exchanges with exchangeable hydrogen atoms in body protein, it is also sequestered in to fat and protein as these are synthesized, therefore the dilution is assumed to be is 4.1% higher than TBW [141].

2) The tracer is distributed evenly throughout all water compartments.

This is true for water in the body, but not for the water leaving the body as water vapor, which is subject to isotopic fractionation. The effect of increased insensible

water losses, which contain less deuterium than body water, is to concentrate the deuterium oxide left behind, which leads to an underestimation of TBW and therefore an overestimation of body fat. It is important to avoid physical activity during the equilibration period to avoid increasing the rate of breathing and transdermal evaporation [141].

3) Tracer's equilibration is achieved relatively rapidly.
This assumption is true in healthy participants (equilibration is usually reached after 2-5 hours), but water turn over is slower in the elderly, pregnant women and patients with expanded extracellular water volume (such as malnourished children with edema), and during systematic shock, therefore a longer equilibration time should be allowed in these participants [142].

4) Neither the tracer nor body water is metabolized during the equilibration time.

This is probably not true, but precautions should be taken to minimize losses. Body water is a dynamic system with a variety of inputs (drink, food and metabolic water) and outputs (urine, feces, sweat, breath etc). When TBW is measured using the equilibration technique participants should be asked to avoid physical activity during the equilibration period, and therefore minimize insensible water loss [141].

5) The constant hydration of FFM is 73.2%.

There is a wide range in inter-individual variation of FFM. *Siri* estimated that the biological variability in the water component of the FFM to be 2% [143].

Two fluid samples (blood, saliva, urine, or breath) are collected. The first sample provides a baseline measure and is taken just before the administration of a tracer. The second sample provides a measure of the concentration of the tracer taken after a sufficient amount of time for the tracer to equilibrate with all water spaces. Although TBW can be measured using non-radioactive stable isotopes, this procedure is rarely undertaken in clinical practice due to lengthy duration of the procedure. In addition equations derived in previous decades are not necessarily valid for contemporary infants and children (59).

Measuring total body water using deuterium oxide is an expensive and time-consuming procedure. It can not be used routinely in clinical practices. Therefore there is a need to validate simple bedside methods such as BIA with deuterium

oxide dilution technique.

Dual energy X-ray absorptiometry (DXA)

The basic principle underlying DXA technology is that the attenuation of X-rays with high and low photon energies is measurable and is dependent on the thickness, density, and chemical composition of the underlying tissue. The attenuation, or weakening, of x-ray energies through fat, lean tissue and bone varies due to differences in the densities and chemical composition of these tissues. The limitations of DXA come partly from the assumptions this method is based on. First, DXA assumes the same amount of fat over bone as over neighboring bone-free tissue. Indeed, soft tissue determination may not be as accurate in the arms, legs, and thorax as in bone-free regions of interests because only few bone-free pixels are available for direct fat and lean mass calculations [144].

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is a rapid, non-invasive, and relatively inexpensive method for assessing body composition in clinical settings. With this method, low-level electrical current is passed through the subject and the impedance (Z), or opposition to the flow of the current is measures by BIA. The Total Body Water (TBW) can be estimated from the impedance measurement, as the electrolytes in body water are conductors of electrical current. When the volume of TBW is large, the current flows more easily through the body. The resistance to the current flow is greater in individuals with large amounts of body fat, as adipose tissue is poor conductor of electrical current due to it`s relatively small water content.

BIA is applicable in routine clinical settings, as it does not require a high degree of technician skill, and it is comfortable for patients [145].

Understanding the BIA Results

There are several measurements of importance that are reported in the BIA results. Here are the conventional definitions of these measurements.

Impedance: The resistance of a component at a given frequency which comprises of two parts, resistance and reactance and so, Impedance = Resistance + Reactance
Resistance: Resistance is a measure of opposition to current flow in a circuit, which is constant for all frequencies.

Reactance: Reactance is the part of impedance that is due to components within the circuit acting as capacitors or inductors. The reactance imposed by capacitors is called the capacitive reactance which is inversely proportional to the signal frequency and the capacitance. The latter is defined as the ability of a capacitor to store electrical charge. On the other hand, the reactance caused by the presence of inductors in a circuit is called the inductive reactance and is proportional to the frequency and the inductance. Here, the latter is the natural property of the component which is the ability of an inductor to store magnetic energy.
In the human body capacitance arises from cell membranes, and the Resistance from extra and intra-cellular fluid (BIA).Impedance is the term to describe the combination of the two.
The relationship between capacitance and resistance is interesting because it reflects different electrical properties of tissues that are affected in various ways by disease, nutritional status and hydration status [146]. When resistance and reactance are plotted against each other phase angle is used to predict clinical outcome Figure 2-6.

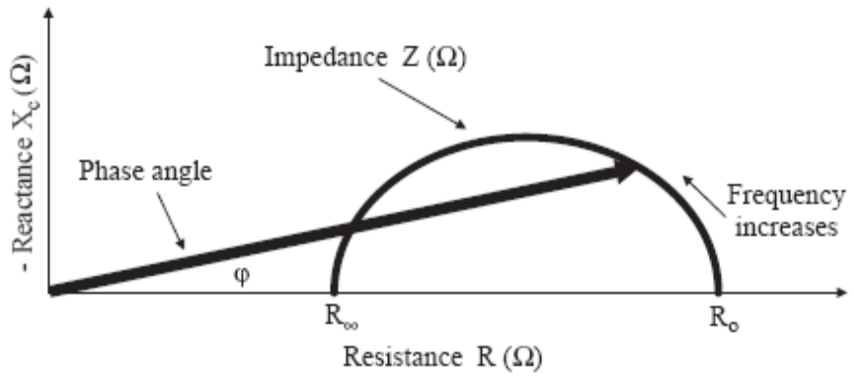

Figure 2-6**:** The relationship between capacitance and resistance, this figure has been extracted from reference [147] .

Phase Angle: is a measurement of body's overall health. Phase angle is based on total body resistance and reactance and is independent of height, weight and body fat [147]. Lower phase angles appear to be consistent with either cell death or a

breakdown of the cell membrane while higher phase angles suggest large quantities of intact cell membranes [147] Phase angle has been found to be a prognostic marker in several clinical conditions such as HIV infection, liver cirrhosis, chronic obstructive pulmonary disease, sepsis, lung cancer [148].

Skinfold thickness:

Skinfolds are direct measures of the thickness of subcutaneous adipose tissue. Skinfold thickness has been widely used in clinical settings as it is relatively easy to measure, and inexpensive [91]. Technicians taking skinfold measurement should be trained to develop their skills in taking measurements in order to obtain the most accurate results.

2.4.11 Review of the studies on body composition with IBD

Relatively few studies have been conducted on body composition in children with IBD and so it is difficult to discern wwhether EEN is associated with lean tissue gains is less clear.

Royall et al [76] investigated the effect of using total enteral nutrition as a primary treatment in 30 children with CD and showed that proportionate gains in body protein, body fat, and body water were achieved following EEN. Total body protein was assessed by neutron activation, fat was assessed by dual energy x-ray absorptiometry, and water was assessed by bioelectric impedance analysis. Weight increased by 1.9 ± 0.3 kg ($P<0.005$), and the composition of the weight gained was comparable to that observed in normal tissue (65% water, 18% fat and 18% protein).

The effect of EEN on bone density was investigated by *Werkstetter et al* [149]. 10 newly diagnosed paediatric patients with CD were examined by Peripheral Quantitative Computed Tomography (pQCT) at diagnosis and after 12 and 52 weeks following an 8-weeks treatment with EEN. The authors reported that trabecular bone density and muscle cross-sectional area increased significantly after 12 weeks (P value= 0.006 and 0.00 respectively). No further changes were observed between 12 weeks and 52 weeks following the start of EEN.

Azcue et al studied 24 children with CD (mean age of 13.1y) with at least moderately active disease (PCDAI >150) on various mixed treatments and has probably conducted the most detailed study of body composition to date. Anthropometric measurements, BIA, TBK by ^{40}K, TBW by $H_2{}^{18}O$, and ECW by Bromide Space measurements were conducted. The conclusion from this study was that lean body mass was significantly depleted in absolute terms in patients with CD compared with controls, but not as a percentage of body weight. However, it was not clear whether these children lost lean mass over the period of their disease or did not gain appropriate lean body mass from the beginning. It is also unclear whether this lean deficit is a result of less skeletal muscle mass or viscera. In addition, there is lack of agreement in the results of different methods of measuring body composition which highlights the need for validating field measurements with standard methodologies for measuring body composition in children [112].

Thayu et al [149] studied lean mass and fat mass by DXA in 78 newly diagnosed children with Crohn`s disease aged 5-21 years, and 669 healthy controls. 10% of children with Crohn`s disease and 37% of the control group were reported as of black race. Lean mass was reported adjusted for height and expressed as a Standard Deviation Score (LM-Ht SDS) was significantly lower in nonblack ($P < 0.001$) and black ($P < 0.01$) females with CD compared with controls. Within the males, LM-Ht SDS were substantially lower in the nonblack subjects with CD compared with controls ($P < 0.001$) and slightly greater in the three black subjects with CD compared with controls ($P = 0.07$). Children with CD exhibited LM-Ht SDS significantly lower in females compared with males ($P < 0.05$). Lean Mass and Fat Mass adjusted for height and expressed as a Standard Deviation Score (FM-Ht SDS) in subjects with CD were not associated with PCDAI, site of disease, duration of symptoms.

A longitudinal study was conducted by *Thayu et al* on children with CD [151]. Whole body lean mass and fat mass were assessed using DXA in 78 CD subjects and 669 healthy controls, ages 5-21 years. Gender specific SD scores for Lean Mass (LM-Ht) and Fat Mass (FM-Ht) adjusted for height was derived using linear regression models in controls. This study confirmed that CD was associated with significantly lower height and BMI for age. Within CD subjects, FM-Ht and LM-Ht SD scores were significantly lower in females compared with males (FM-Ht SDS: _0.66 $\pm$ 0.83 vs.

_0.08±0.95, p<0.01; LM-Ht SDS: _1.12 ±1.12 vs. _0.57 ± 0.99, p < 0.05). In females, CD was associated with significantly lower LM-Ht SDS (p _ 0.001) and FM-Ht SDS (p _ 0.001), adjusted for age, race and Tanner stage, and compared with controls. Lean and fat were significantly greater in older females with CD; 47% of adolescent females had LM-Ht SDS <-2 SDS. In non-black males, CD was also associated with lower LM-Ht SDS (p <0.02); FM-Ht SDS deficits were not significant. *Burnham et al* assessed lean mass using DXA scans (Hologic QDR 2000 bone densitometer) in 104 CD patients and 233 healthy controls. The results of this study showed that individuals with CD had significantly lower height, weight , BMI-for age and lean mass-for-height SD scores than healthy controls(all P<0.0001). Lean mass-for-height was subsequently adjusted for age and race, and still was significantly decreased in subjects with CD. One of the drawbacks of this study was that controls were significantly younger and less mature than CD patients [130].

Burnham et al published a subsequent article based on the same dataset as the previous study mentioned. Assessment of lean mass as a sex-specific SD scores relative to height also showed significant deficits in Crohn`s disease patients. The lean mass–for height SD scores were significantly lower in male subjects (-0.58 ±0.87) and female subjects (-0.65 ± 1.03) with Crohn`s disease compared to controls (0.00 ± 1.00), (P -value: 0.0002) in males and (P –value: 0.0004) in females. When the SD scores were calculated relative to age, the deficits were greater:(-1.16±1.19) in males and (-0.98 ± 0.97) in females (both P value: 0.0001); lean mass–for age z-scores were significantly lower than lean mass–for height SD scores in the subjects with CD (P value :0.0001).

Bechtold et al measured muscle cross-sectional area (CSA), which is a reasonable surrogate for total muscle mass, using peripheral quantitative computed tomography(pQCT) in 143 newly diagnosed patients with IBD(45 UC, 98 CD). The authors found out that, patients had a significantly reduced muscle CSA for age and height compared to age and sex matched healthy controls [151].

In a study conducted by *Sylvester et al* [61] 42 newly diagnosed children with IBD were followed for two years. Body composition was measured at baseline, 1 and, two years later after diagnosis for CD patients and at diagnosis for unaffected controls. The results indicated that, FFM SD scores were significantly lower than those of unaffected children at diagnosis (P<0.001) and did not increase significantly during the course of the study [61].

Varllie et al [152] studied 11 children with Crohn`s disease before and after resection surgery and compared them against healthy controls. Fat Free Mass was measured by the sum of skinfold thickness method, and was reported to be lower in children with Crohn`s disease compared to healthy controls, and did not change before and after surgery.

Boot et al [56] studied 55 patients (34 boys and 21 girls, age range 4–18); 22 children had Crohn's disease and 33 Ulcerative Colitis, body composition was measured using DXA. The mean SDS of lean tissue mass was −1.04 (SD 1.41), of bone mineral content −1.05 (1.27), of fat mass −0.64 (1.02), and of percentage body fat −0.38 (1.11); all were significantly lower than normal (all $p<0.001$ except for percentage body fat $p<0.02$).

Mauras et al [153] studied 10 children (6 males, 4 females), with IBD. All had been diagnosed at least 1 year previously. Nine had Crohn's disease and one had a form of non-specific IBD. Body composition was assessed at baseline and was repeated after four months. Patients received daily injections of recombinant human growth hormone (rhGH) after baseline assessment. The main aim of the study was to measure protein and carbohydrate turnover, however, fat mass and fat free mass were estimated by DXA. The results demonstrated a significant increase in fat free mass (baseline visit: 25.1 6± 2.0 kg; 4 months: 28.1± 2.3; P-value .001). While the total fat mass did not change significantly (baseline visit: 9.3 6 ± 1.2 kg; 4 months: 8.6 ± 1.3; P-value: NS), since the patients were growing, when the data are expressed as a percentage of the total weight, there was a significant decrease in percent fat mass as measured by DEXA scanning after rhGH therapy (baseline visit: 26.0% ± 2.2%; 4 months: 22.5% ± 2.2%; P-value 0.001).

The remaining studies have been largely conducted in adults, many of whom would have developed the condition in childhood or early adolescence whilst they were still growing such that any structural deficit accrued in childhood would result in a lack of stature and lean mass in adulthood. *Jahnsen et al* [154]studied adults (21-75y) with CD (n =60) and UC (n =60) who were mostly in remission phase were monitored and concluded that patients with CD were significantly lighter, shorter, and had less lean body mass compared to UC patients and healthy controls. In contrast, *Sentong et al* [39]reported no significant differences between adult CD patients and controls in terms of FFM and FM. *Valentini et al* [155]and Rocha and

co-workers [156]also concluded that as children with IBD grow up, they turn in to adults who have lower height, weight, and lean mass.

Most measurements of body composition have been made using DXA scans. One of the drawbacks of this method is that it assumes the mount of fat over bone is the same as that in the adjacent soft-tissue background. In regions such as thorax, arm and head, the percentage of bone free pixels is much lower and may be insufficient for accurate soft-tissue and hence bone determinations to be made. Another limitation of DXA is that, during whole body scans, measurements are made over a very wide range of tissue dept. Depths can vary from about 1 to 30 cm. At high tissue depths (> 20-25 cm), the amount of fat and bone mineral are over estimated [157].

In the previous studies described above, body composition was measured using devices that were expensive, time -consuming and not applicable in routine clinical settings. *Werkstetter et al* [133], measured body composition of children with IBD using Bioelectrical Impedance analysis (BIA) with a multi-channel device at 5, 50, and 100 kHz(Nutriguard M). The phase angle α was applied as an index for quality of lean mass as it is associated with cell size, and integrity of the cell membrane, and on the other hand on its pure resistive behaviour mainly dependent on tissue hydration (hydration factor for FFM is approximately 73.3%). SubsequentlySD scores were calculated based on the reference data from *Bosy- Westphal et al* [158] The mentioned reference data was gathered from data from 10,127 girls (11.5 ±3.9 years, 6–17 years), 6110 boys (9.5 ± 3.2 years, 6–17 years). A single tetrapolar BIA measurement of resistance (R) and reactance (Xc) was taken at a fixed frequency of 50 kHz using (BIA 2000-S, Data Input, Frankfurt, Germany). This study [133] found that, patients with IBD had significantly lower SD scores for phase angle − 0.64 (− 0.95 to 0.34), height -0.54 (-0.84 to -0.24), weight -0.75 (-1.04 to -0.46), and BMI -56(-0.83 to -0.28) than controls(Phase angle SDS 0.09 (− 0.16 to 0.35), height SDS 0.18 (-0.12 to 0.47),weight SDS -0.35(-0.31 to 0.23), and BMI SDS-0.15(-0.39 to 0.10), P value< 0.015.

As stated before, *Gerasimidis et al* explored growth and body composition changes in 17 children with active CD treated exclusively with a polymeric feed for 6-8 weeks [159]. The main focus of this study was changes in micronutrient levels following treatment with EEN. Body composition was measured using Bioelectrical Impedance Analysis and converted to SD scores. The authors reported that weight SD scores (-

1.3), BMI SD scores (-2.2) and Lean index SD scores (the exact number of lean index SD scores were not reported) improved significantly following 30 days of EEN use and were reported -0.5 and -1.0 respectively, P value< 0.001.

Taken together, the majority of published studies are case-control studies. Whilst it appears that children with IBD have less lean in general, such studies cannot clarify whether these children fail to gain the appropriate lean mass in the first place, or acquire lean mass and then lose it during their course of the disease. There is a need to investigate the changes in the body composition compartments during the active phase of the disease and also remission phase to find out the changes in their body composition in detail. The most useful study would be a prospective longitudinal study which would follow children from first presentation at diagnosis throughout the disease course. Measurements could be conducted at different stages of the disease to better characterize the pattern of tissue accretion or loss.

Further research is required to clarify the factors that contribute to growth abnormalities in children with IBD and the factors that should be taken in to account in order to improve growth in these children. A greater understanding of the changes that occur in terms of body composition in children with IBD may make it possible to use body composition measurements as means of evaluating growth and therefore evaluating different interventions in these children. Improving the knowledge in body composition measurement methods and growth evaluation may also help lead to early diagnosis of the disease.

In conclusion, childhood IBD is associated with malnutrition, malabsorption, delayed puberty, decreased physical activity, elevated inflammatory cytokines. Each of these factors may influence growth and alter body composition. In children, altered body composition can be used as a marker of poor nutritional status and overall health. The majority of studies on growth are cross-sectional studies that have focused on height and weight deficits at the time of diagnosis, such studies have demonstrated that height and weight deficits re present in children with IBD. There is a lack of evidence from longitudinal studies following growth and body composition in children with IBD, as it has been assumed that growth impairment and altered body composition are corrected

2.5.1 Summary of literature and aims of research

The review of the literature has primarily attempted to provide a background of Inflammatory Bowel Disease (IBD), describing both aetiology and clinical manifestations. It has attempted to provide an overview of growth and body composition in healthy children before discussing the effect of IBD on growth and body composition. The review demonstrated that the pathophysiology of IBD is multi-factorial and still not well-understood; a greater appreciation of the aetiology may lead to improving treatment methods and reducing consequences of the disease. In addition the review has highlighted that whilst 25% of IBD occurs before the age of 18, adequate attention has not been allocated to the effect of growth and body composition differences of this population. Growth and body composition measurements can be used as surrogates of the efficacy of different treatments; therefore, it is essential to monitor growth and body composition closely after diagnosis.

Crohn`s disease (CD) and Ulcerative colitis (UC) are collectively known as Inflammatory Bowel Disease (IBD). 25% of IBD happens in children, many children have poor nutritional status at presentation of the disease, which may worsen during the clinical course. Growth impairment is a very common consequence of IBD which may precede the clinical symptoms. Inadequate dietary intake, increased losses, malabsorption, altered energy demands and the effect of treatment drugs may all contribute to faltering growth. The initial goal of treatment is to control inflammation, induce remission with minimal adverse effects resulting in enabling normal growth and development. Exclusive Enteral Nutrition (EEN) has been proven to be drug of choice in children with IBD, as it is as effective as steroids in inducing remission, particularly in CD, and improves growth and nutritional status in these children

A review of the paediatric growth studies in IBD conveyed that growth deficits have been reported instantly in children with IBD at diagnosis and after treatment. However more recently, it has been shown down following treatment weight gain is achieved in children but linear growth has not been shown to improve remarkably.

Our knowledge of body composition in paediatric IBD is very limited, particularly body composition changes during the course of the disease and treatment. Few studies have been conducted on body composition in children with IBD and so the extent to which the excess body fat and depleted lean mass affect children with IBD is mainly unclear, which makes it challenging to evaluate their nutritional status. There is not enough evidence of how current treatments effect growth and body composition. There is a need for more longitudinal studies to examine how body composition changes during the course of IBD and in response to treatment. In addition, much of our knowledge in this area has been attained using research methods that are not suitable for use in routine clinical settings. Further research is required on changes that exist in terms of body composition in children with IBD. A greater understanding of these changes may make it possible to use body composition measurements as means of evaluating growth and, therefore, assessing the efficacy of different interventions in these children.

In the majority of studies, body composition has been measured using reference methods. Reference methods of measuring body composition are both time-consuming and expensive. They are not used routinely in clinical settings. One of the methods that is simple to perform and is assumed to generate valid and reliable results is Bioelectrical Impedance Analysis (BIA). There is a need to validate the results generated by BIA devices against the reference methods of body composition in order to be able to use BIA as a valid bedside method of body composition in clinical settings and in research purposes.

Chapter 3 Cross-sectional study of children with IBD.

3.1.1 Introduction:

The cross-sectional study was conducted to evaluate the prevalence of growth and body composition deficits and any association with disease activity. The aim of this study was twofold: Firstly to investigate whether growth impairment exists in children with IBD I and whether they differ between CD and UC. Secondly whether it was possible to identify differences in lean mass and fat mass using simple anthropometric techniques and provide initial scoping data to inform the design of the subsequent longitudinal study.

3.1.2 Study Design:

Patients were recruited and studied from children attending the Wessex paediatric gastroenterology service. This service provides tertiary paediatric gastroenterology care for a population of about 650,000 from 10 district general hospitals spread over a large geographical area including the Channel Islands and the Isle of Wight.

All children with confirmed histologically of IBD according to international criteria [160]and treated following published guidelines [161], [162] aged between 5 and 18 years were eligible to participate. This included children with any level of disease activity, on any or no treatment and with disease at any location in the gastro-intestinal tract. Recruitment occurred in two ways. Firstly, patients attending the hospital for a routine visit (outpatient appointment or endoscopy) were identified from clinic and endoscopy lists. They were then contacted by telephone prior to their appointment. An information pack was sent to those patients that were keen to participate. Secondly, children at routine hospital visits were given written information and an opportunity to discuss the research project. Following a follow up phone call they were recruited to take part in the research at their next routine hospital visit. Consent was obtained at the point of the study entry on the morning of their routine hospital visit. Measurements were made in the morning following an overnight fast.

Clinical exams of patients were conducted by Dr Tony Wiskin. Height, weight measurements and bioelectrical impedance analysis were performed by the author. However the Bioelectrical Impedance Data was used by Dr Tony Wiskin in his thesis and are not presented here.

Ethical approval was granted from the Isle of Wight, Portsmouth and South-East Hampshire, local research ethics committee (Study number 09/H0501/70).

3.1.3 Disease activity:

Disease activity was scored using the Pediatric Crohn`s Disease Activity Index (PCDAI) [163] for children with CD and the Pediatric Ulcerative Colitis Activity Index (PUCAI) for those with UC [32] both use a combination of symptoms, growth and serological data to produce a disease activity score.

3.1.4 Study subjects:

63 patients (38M; 25F) with CD aged 6.0 to 17.8y (mean 13.7 y) in both active disease and remission and, and 31 UC patients (14M; 17 F) aged 5.90 to 17.79 years (mean 12.86 years); in both active disease (n 26) and in remission (n 5) attending routine clinics were studied on one occasion in the morning following an overnight fast. The characteristics of patients recruited in the study are summarized in Table 3-1.

Table 3-1 General characteristics of UC and CD patients.

	IBD+	CD+	UC+	P value*
Number Of patients	94	63	31	
Age (mean ± SD) (years)**	13.44 ± 3.01	13.72 ± 2.81	12.86 ± 3.35	0.196
Female/Male	52/42	25/28	17/14	0.498
Active disease/remission	29/65	24/39	5/26	0.030

*Chi squared test for comparison between CD and UC
**The age range was 6.01-17.75 for CD and 5.9-17.74 for UC
+IBD = inflammatory bowel disease, CD= Crohn's disease, UC = Ulcerative Colitis

Although the age and sex distribution did not differ between CD and UC, the proportion of subjects with active disease was significantly higher in CD (38) than UC (16%).

3.1.5 Anthropometric measurements

Height was measured with the head in the Frankfort plane using a validated stadiometer nearest 0.1 cm(Leicester Height Measure) and weight was measured using (a digital scale to the nearest 0.1 KG)in light clothing after voiding; both measures were performed on a single occasion at Southampton General Hospital Welcome Trust Clinical Research. These measurements were made according to the related Statement of Purposes (SOP) available in Southampton Centre for Biomedical Research (SCBR) (see Appendix).
Height and weight measurements were then converted to Standard Deviation Scores (SDS) against UK 1990 Growth Charts (LMS growth programme; Harlow Healthcare, South Shields UK; www.healthforallchildren.co.uk).

3.1.6 Body composition measurements

Mid upper arm circumference was measured on the non-dominant side of the body according to the existing SOP available in SCBR (see appendix 2). Skinfold thickness measurements at triceps, subscapular, biceps and suprailiac were taken in triplicate from the non-dominant side of the body using a skinfold calliper (Holtain Ltd. Crymych, UK) following the method of *Tanner and Whitehouse* [164] from the SOP available in SCBR, see appendix 2. In all but one child, triceps skinfold (TSF) and mid-arm circumference (MUC) were also measured and used to determine Upper Arm Muscle Area (UMA); both TSF and UMA were then expressed as SDS according to *Anthropometric standards for the assessment of growth and nutritional status developed by Frisancho* [165].

3.1.7 Reference datasets:

The British 1990 reference data set was used to calculate height, weight and BMI SD scores; this data set was collected in the 1980s. The UK1990 charts used existing UK data from 17 different British sources and were constructed using Cole's LMS

method, which allowed for a choice of centiles; and the conversion of measurements to standard deviation scores.

Health Survey for England data sets for 2009 and 2010 were available from the repository held at Essex University. It provided recent anthropometric information from the general population in contrast to the previously determined datasets for the UK1990 reference data on growth and provided an alternate, more contemporaneous statement of usual heights and weight of children in the UK. They were used to calculate height, weight and BMI SD scores using LMS growth program; Harlow Healthcare, South Shields UK; www.healthforallchildren.co.uk. Since the age of this population was reported according to their last birthday, it was assumed that the average age was the midpoint between consecutive years (e.g. an age of 6.5 years was assigned to children who were aged 6 years at the time of their last birthday).

Standards for upper arm anthropometry, developed by *Frisancho* [165], were used to calculate UMA SDS and TSF SDS. The anthropometric standards are based on the combined samples of the first and the second National Health Survey Examination Surveys of the U.S.A. conducted during 1971-1975, and 1976-80. The methods to measure upper arm circumference and triceps skinfold thickness are described by *Frisancho* who also provided a formula for calculating mid upper muscle area:

$$Upper\ Arm\ Circumfrence = C$$

$$Upper\ Arm\ Muscle\ Area\ (UMA) = [C - (Ts \times \pi)]^2/(4 \times \pi\ )$$

From this data SD scores were calculated using the charts provided by the same author.

3.1.8 Statistics:

Descriptive statistics were used to characterize the population studied. Independent t tests were used to examine differences between CD and UC. Paired t tests were used to compare differences in the SD scores of the IBD subjects obtained using the UK 1990 reference data and those obtained using the HSE reference (population) data. The analyses were undertaken using SPSS 18 (Chicago, USA). Regression analysis (with and without adjustments for height) was used to

establish relationships between disease activity measured by PCDAI and PUCDAI and Fat Free Mass (FFM) measured by UMA SDS. The results are presented as mean and SD.

3.2.0 Results:

3.2.1 Assessment of growth:

The distribution of SD scores for height, weight, and BMI in IBD are shown in Figure 3-1 . This figure shows a wide scatter in the results with a suggestion that weight has the largest discrepancy with the UK1990 reference data. When the IBD patients were divided in CD (Figure 3-1) and UC (Figure 3-3) the height SDS and to a lesser extent weight and BMI SDS tended to be lower in CD than UC. This is confirmed by examination of the z-scores for height, weight, and BMI in Table 3-2. The greatest discrepancy was for height (-0.54 VS 0.46), however significant difference was also noted for weight (-0.56 VS 0.15). The overall height, weight and BMI SD scores of children with IBD as UC and CD boys and girls is summarized in Table 3-2 and shown in Figure 3-2 and Figure 3-3. Height SD scores were plotted against BMI SD scores to explore the inter-relationships between BMI and height and better characterise the children those who were short, those who were underweight and those who were both short and under-weight demonstrate the distribution of

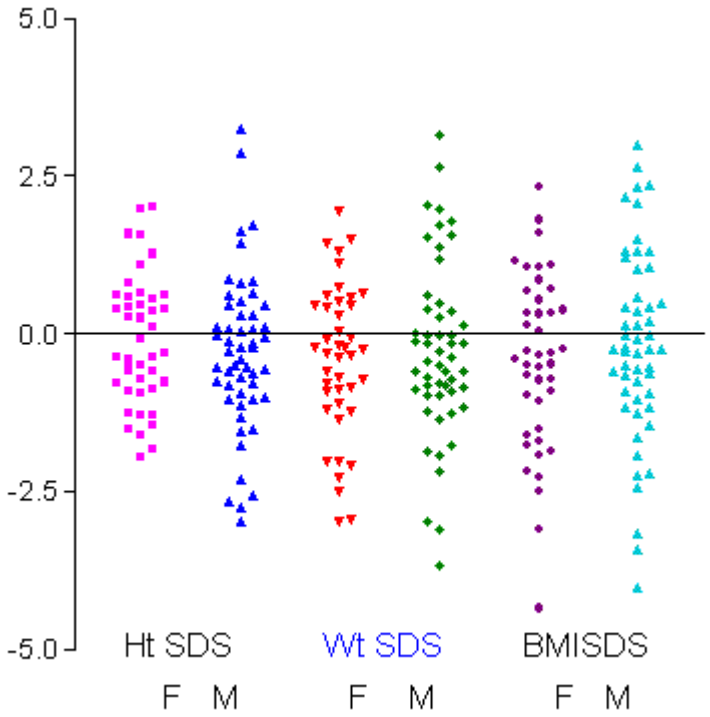

Figure 3-1: The distribution of height, weight, and BMI SDS in IBD children

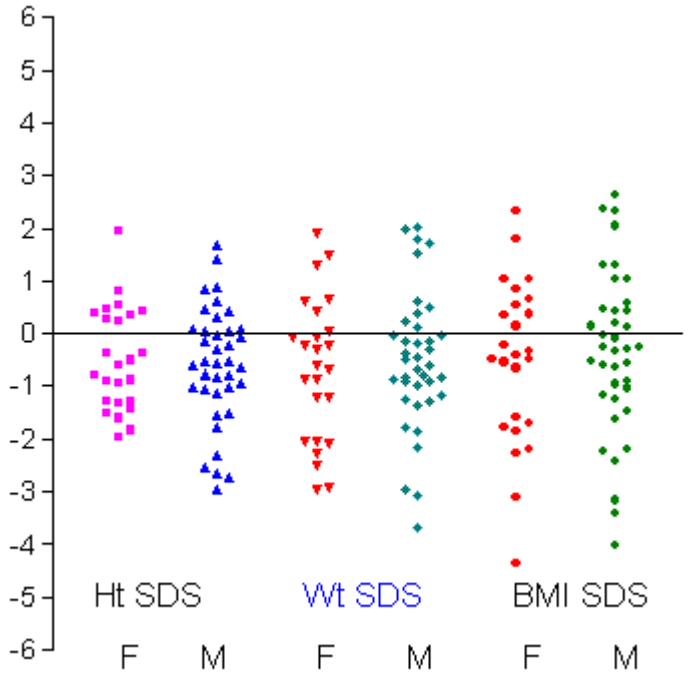

Figure 3-2: The distribution of height, weight and BMI SDS in CD children.

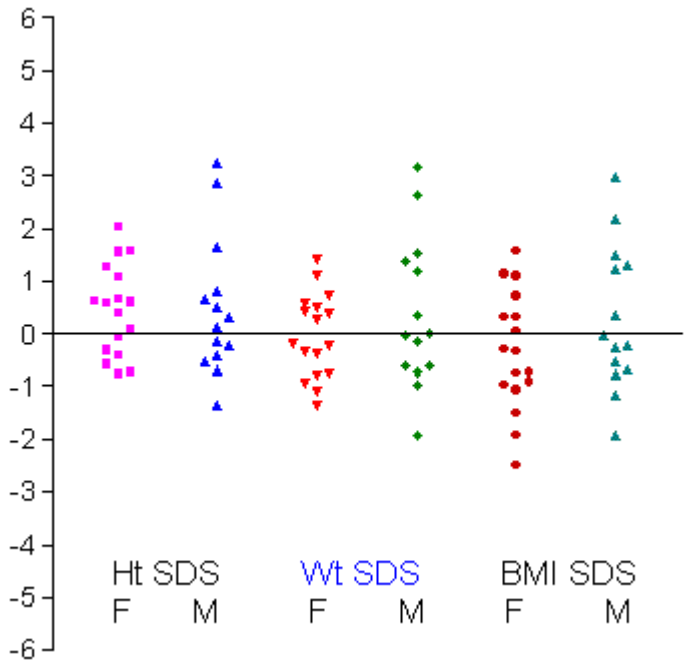

Figure 3-3. The distribution of height, weight and BMI SDS in UC children.

Table 3-2 Height, weight and BMI SD scores of children with IBD over all and divided in to UC and CD compared to the British 1990 reference data.

	Range	Mean	P value*
IBD			
Age (years)	5.9-17.75	13.44 ± 3.01	
Height SDS	-2.97-3.25	-0.21 ± 1.15	0.080
Weight SDS	-3.66-3.16	-0.32 ± 1.30	0.016
BMI SDS	-4.35-2.98	-0.29 ± 1.46	0.055
CD			
Age	6.02-17.75	13.72 ± 2.81	
Height SDS	-2.97-1.96	-0.54 ± 1.05	<0.00
Weight SDS	-3.66-2.04	-0.56 ± 1.32	0.004
BMI SDS	-4.35-2.63	-0.40 ± 1.54	0.040
UC			
Age	5.9-17.74	12.86 ± 3.35	
Height SDS	-1.34-3.25	0.46 ± 1.06	0.021
Weight SDS	-1.92-3.16	0.15 ± 1.12	0.456
BMI SDS	-2.48-2.98	-0.05 ± 1.25	0.802

*P value compared to the 1990 reference data

Table 3-3 demonstrates the differences between the two groups in terms of their height, weight and BMI SD scores. The most striking difference between the groups

is the discrepancy in height (-1.00 SD score; P <0.001) followed by weight (-0.71 SD score; P = 0.011). The BMI SD score did not differ significantly between CD and UC (-0.35 SD score).

Table 3-3 Comparison between UC and CD children in terms of height, weight, and BMI SDS.

	CD	UC	Mean difference (CD-UC)	P value
Age	13.72 ± 2.81	12.86 ± 3.35	0.85	0.196
Height SDS	-0.54 ± 1.05	0.46 ± 1.06	-1.00	< 0.001
Weight SDS	-0.56 ± 1.32	0.15 ± 1.12	-0.71	0.011
BMI SDS	-0.40 ± 1.54	-0.05 ± 1.25	-0.35	0.275

Further insights into the differences in growth discrepancies between CD and UC can be obtained by plotting height SD scores and BMI SD scores in Figure 3-4 and Figure 3-5. The plot for CD patients shows that 8 of the children are distributed in area A of the figure, which represents height within ± 2sd and BMI <-2sd, children in this category are classified as wasted according to World Health Organization (WHO) guidelines [166]. A smaller proportion of children (3M) are in area C which represents BMI within ± 2sd and height < -2 SDS, and are referred to as stunted by WHO guidelines. Even a smaller proportion is in area B (2M), which represents both stunting and wasting. 5 children (4M, 1F) are located in Area D, meaning that they are obese according to WHO guidelines (BMI SDS>2 and height SDS within ± 2 SDS).

In contrast, in UC patients there is only one child that is wasted (area A), two are obese and the rest are within the ± 2SD for both BMI and height SDS. Children with CD are more likely to be stunted, wasted and stunted plus wasted (13/63; 21%) when compared to UC (1/31; 3%) (P=0.026, Chi squared test).

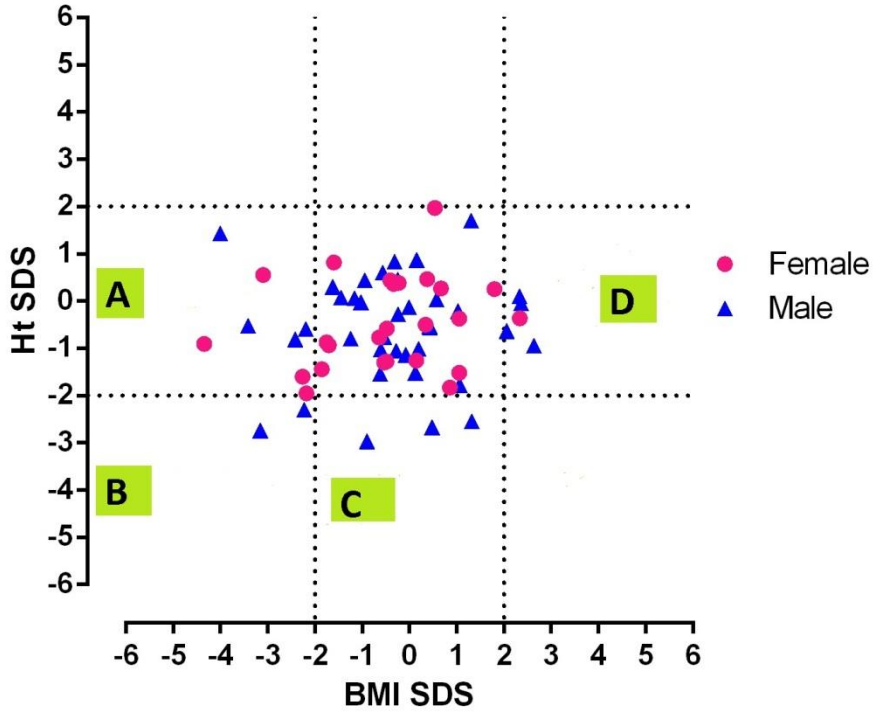

Figure 3-4 Cross plot of height SDS and BMI SDS in CD patients.

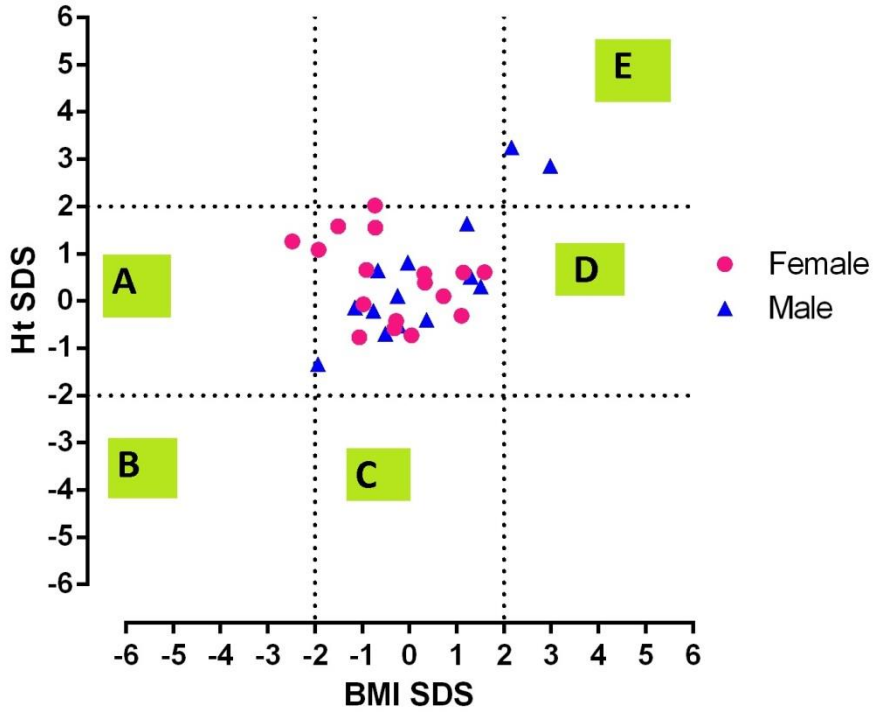

Figure 3-5 Cross plot of height SDS and BMI SDS in UC patients.

3.2.2 Comparison of growth in IBD with a current reference population

Table 3-4 shows that children participating in the 2009 and 2010 health Surveys for England (HSE) (the same years as the cross sectional IBD study) had a height SD score close to zero, whilst the BMI and weight SD scores were significantly greater than zero. The distribution of height weight and BMI at ages between 6 and 18 years divided by gender (the age range of the children in our IBD study) is shown in Figure 3-6 to Figure 3-11. The points on the graph display data from HSE data (the relevant centile is mentioned on the figure) on the British 1990 growth charts. The distribution of height was comparable to that of the 1990 UK growth charts, since the values for the 9th, 50th and 91st centiles at different ages approximated to those of the 1990 UK growth charts. In contrast, the median values of the HSE population (50th centile) for weight and BMI were shifted upwards (indicating that children had become heavier). This was associated with a large upward shift of the 91st centile and little change in the position of the 9th centile.

This means that the differences in height between IBD children and the current population is reasonably comparable to that observed with the UK 1990 growth data. However, the discrepancies in weight and BMI are more pronounced when compared to the current population of children than with the children who contributed to the UK 1990 growth charts.

Table 3-4 Comparson between the British 1990 growth refrence data and HSE.

	HSE	P value
Height Z-score	-0.002 ± 1.109	0.779
Weight Z-score	0.322 ± 1.175	0.000
BMI Z-score	0.427 ± 1.232	0.000

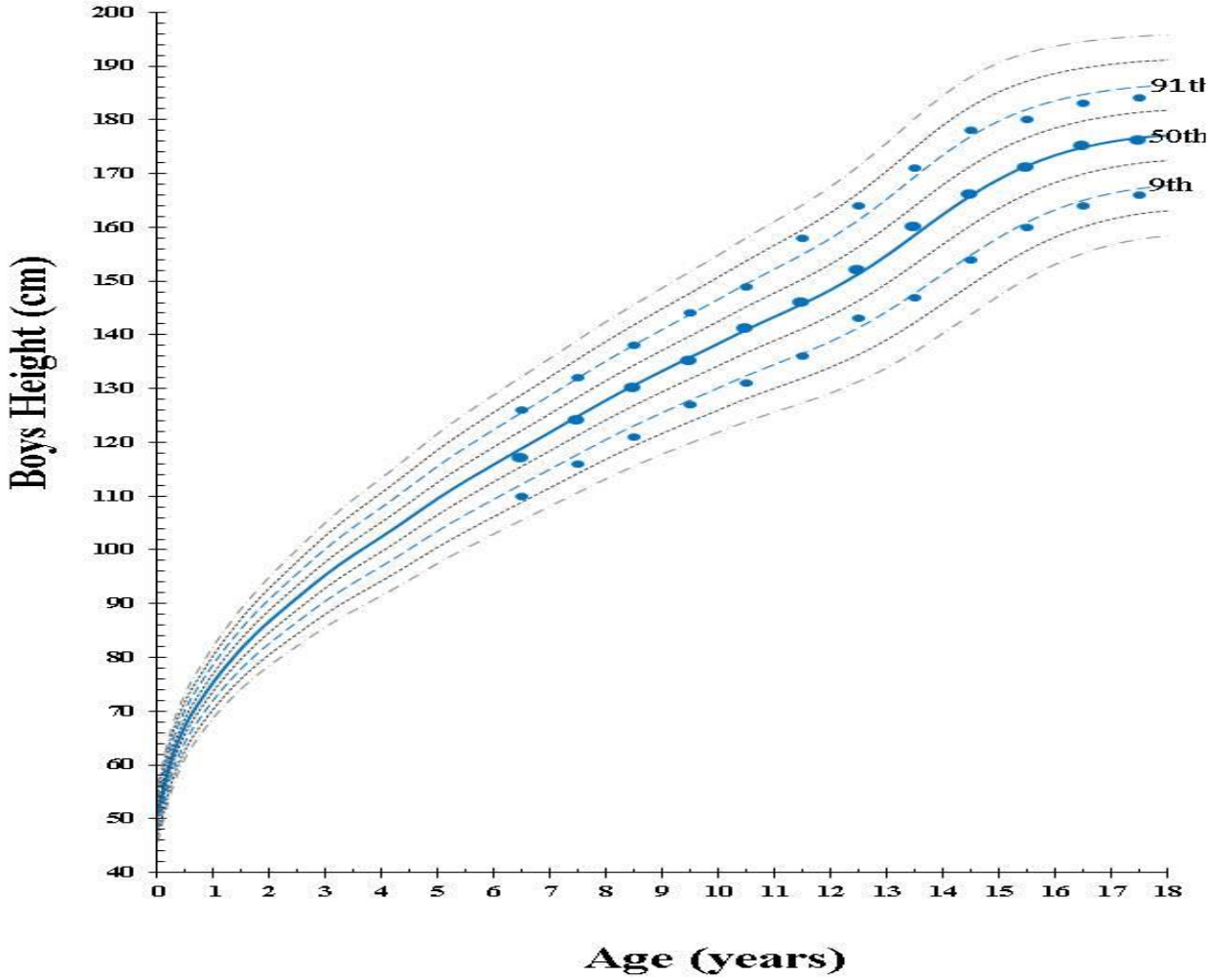

Figure 3-6 Comparison of height from British 1990 reference data and HSE population in boys (amalgamation of results obtained from the HSE 2007, 2008, 2009 and 2010; N =). The dots are the results of the boys from the HSE overlayed on the 1990 centile curves.

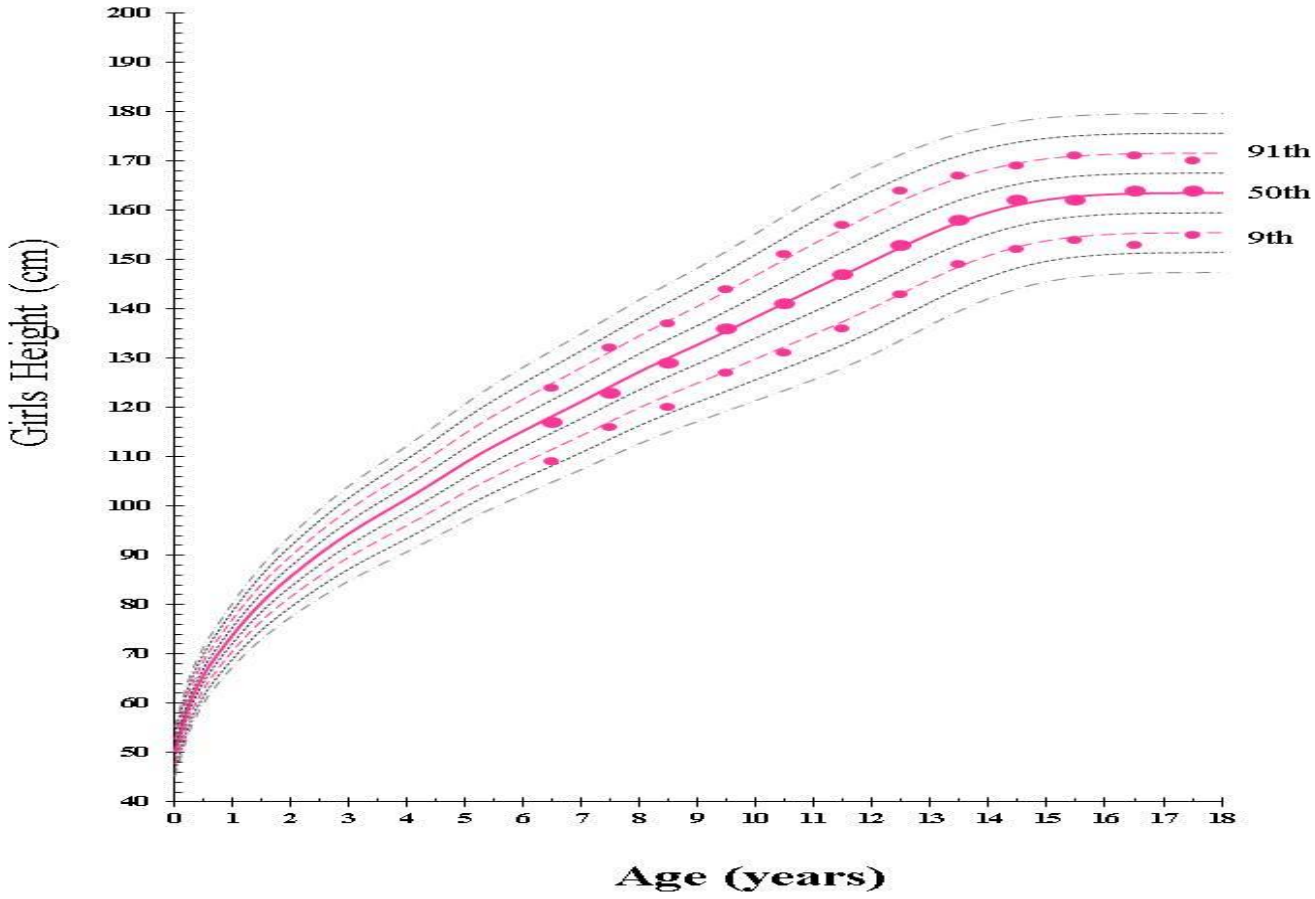

Figure 3-7 Comparison of height from British 1990 reference data and HSE population in girls.

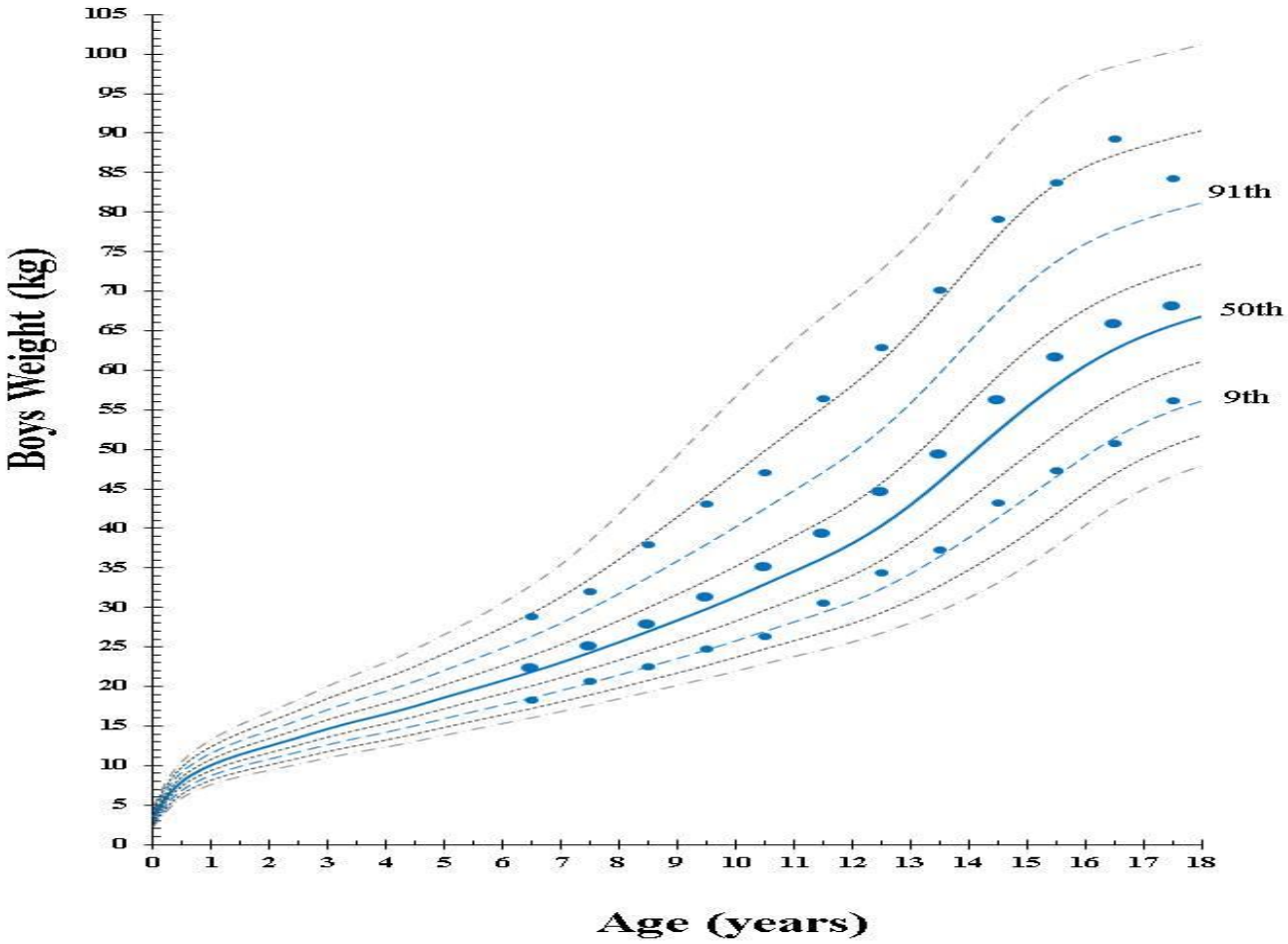

Figure 3-8 Comparison of weight from British 1990 reference data and HSE population in boys.

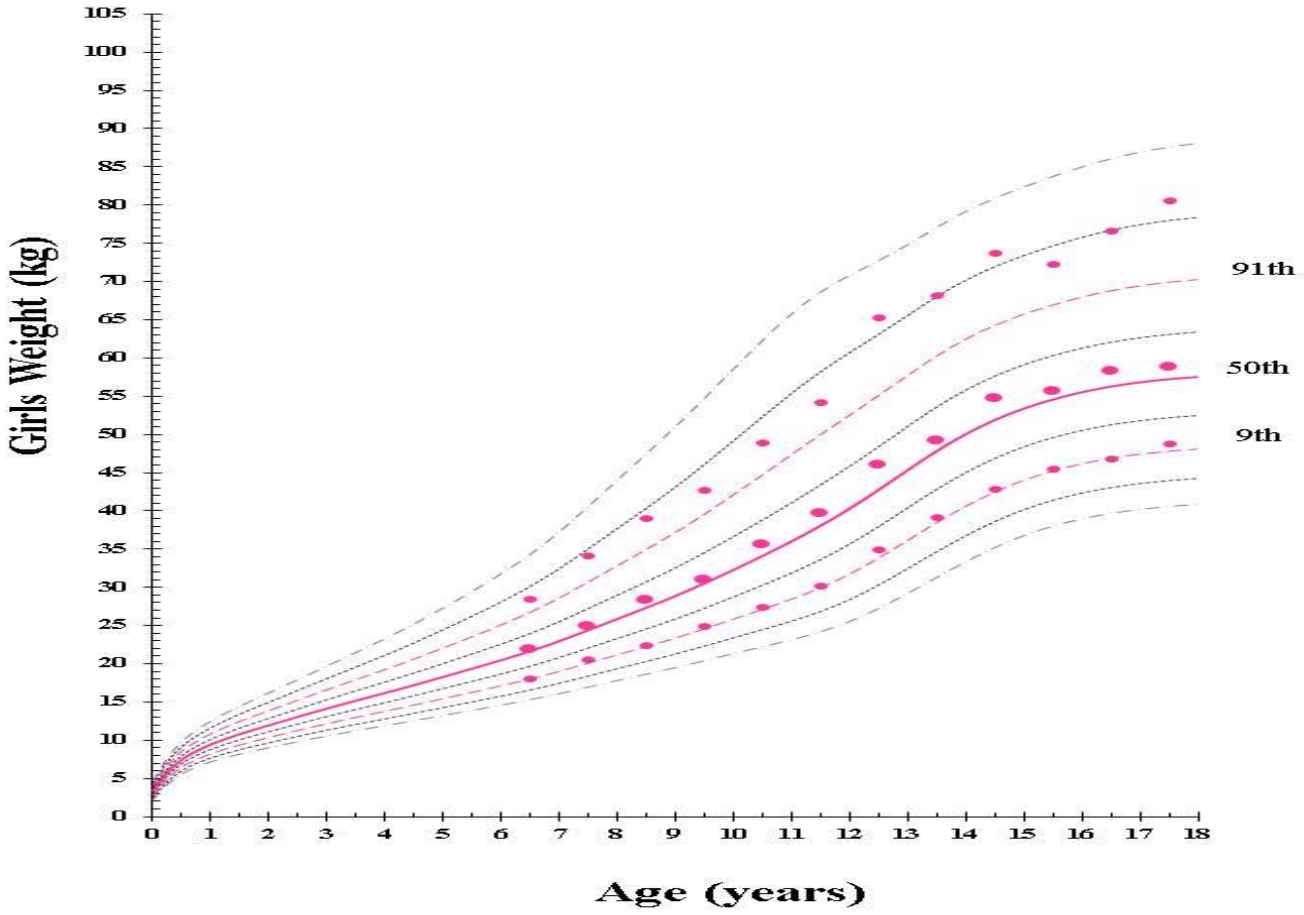

Figure 3-9 Comparison of weight from British 1990 reference data and HSE population in girls

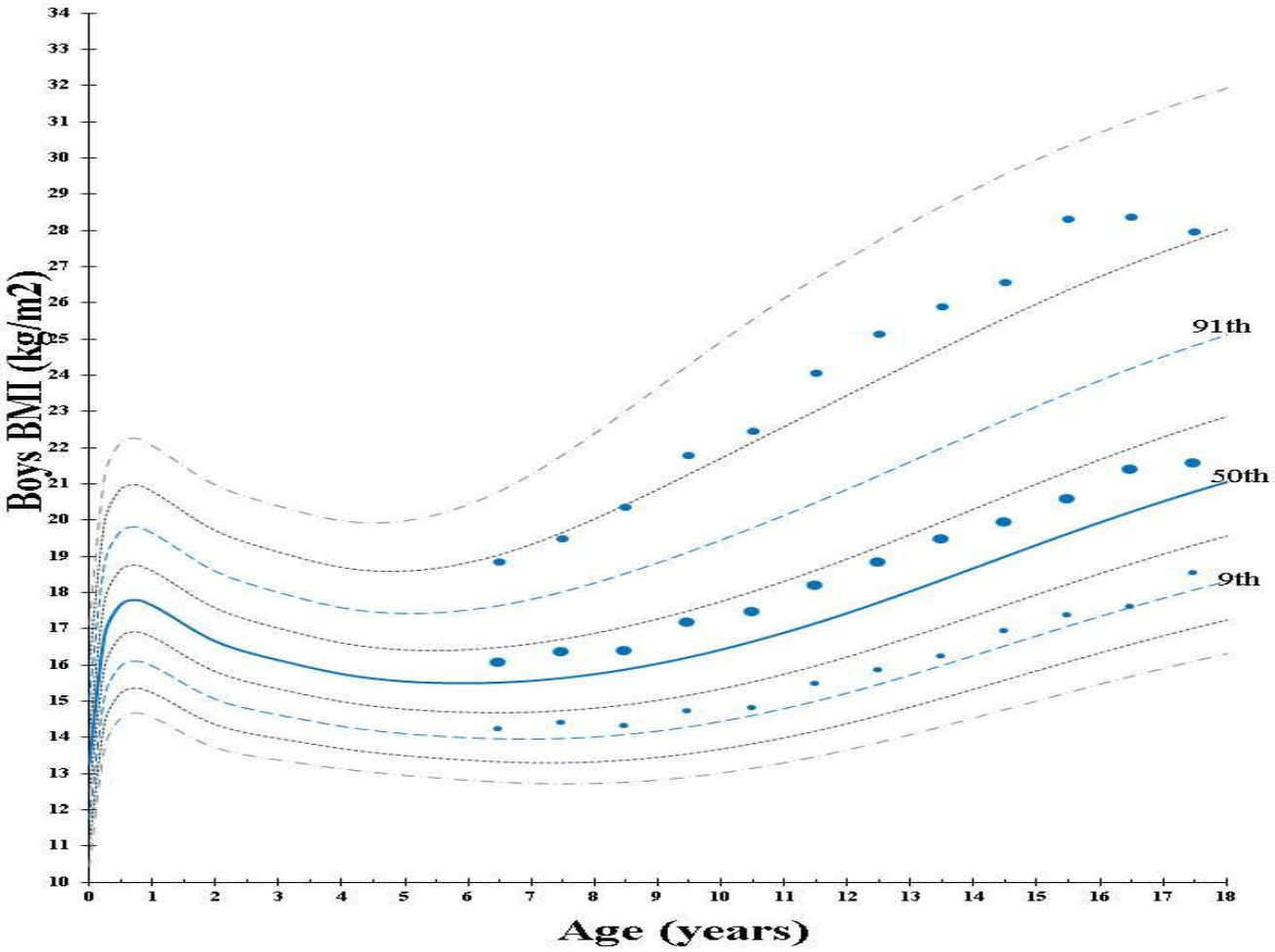

Figure 3-10 Comparison of BMI from British 1990 reference data and HSE population in boys.

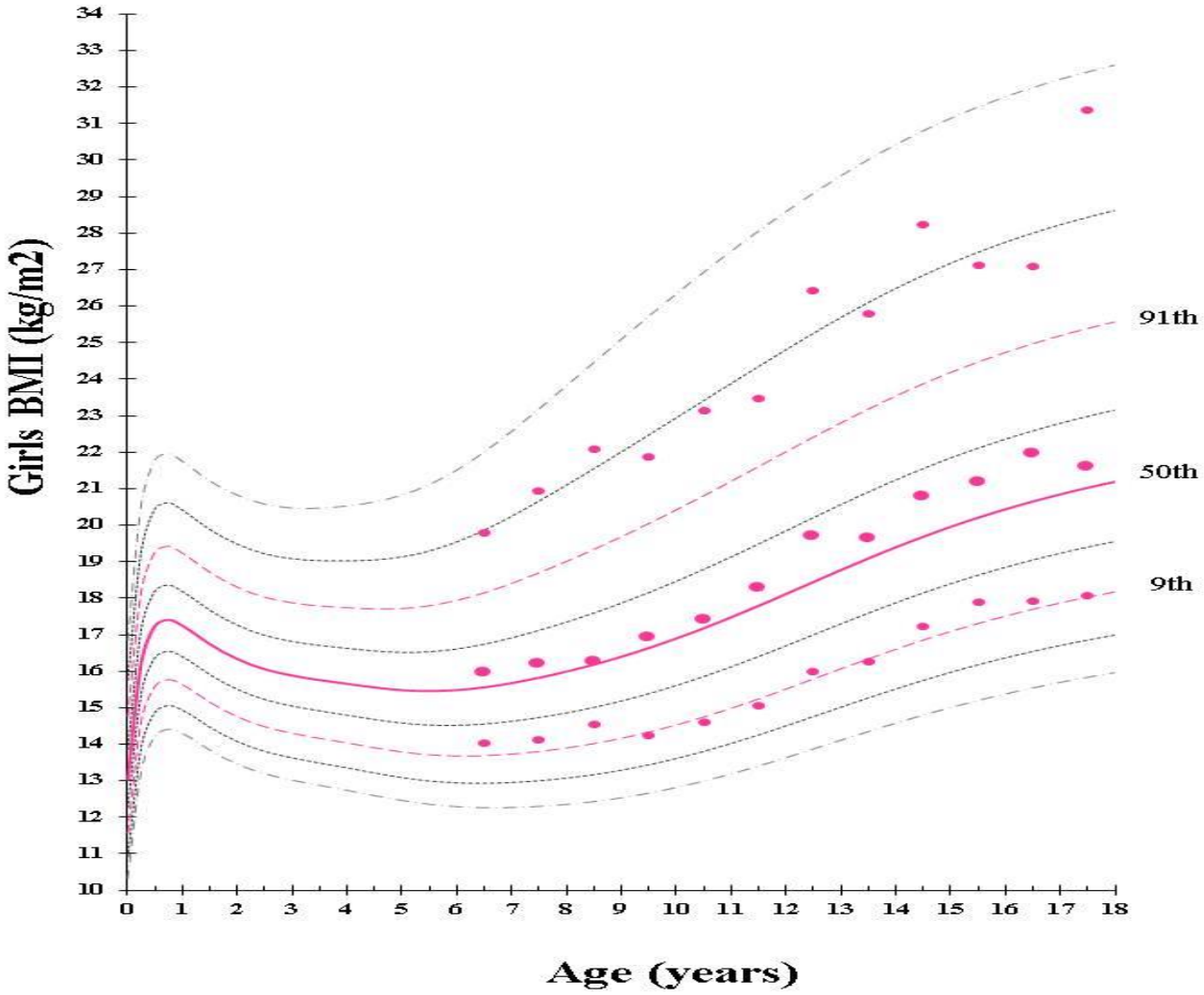

Figure 3-11 Comparison of BMI from British 1990 reference data and HSE population in girls

As previously mentioned, weight and BMI SD scores for Health Survey for England have increased compared to 1990 British growth reference data; therefore, comparing children in the cross-sectional study to this reference may overlook some growth deficits. In the next stage, height, weight and BMI SD scores of IBD children recruited in the cross-sectional study were compared against the data from Health Survey for England. The mean differences and standard deviations are summarized in Table 3-5. Subsequently IBD patients were divided into CD and UC groups and compared against the reference data of health survey for England, these comparisons are summarized in Table 3-6, and Table 3-7.

Table 3-5 Comparison of height, weight, and BMI SDS of IBD children with HSE.

	IBD	HSE	Mean Difference	P value
Height SDS	-0.21 ± 1.15	-0.002 ± 1.109	-0.20	0.069
Weight SDS	-0.32 ± 1.30	0.322 ± 1.175	-0.65	<0.001
BMI SDS	-0.29 ± 1.46	0.427 ± 1.232	-0.72	<0.001

Table 3-6 Comparison of height, weight, and BMI SDS of CD children with HSE.

	CD	HSE	Mean Difference	P value
Height SDS	-0.54 ± 1.05	-0.002 ± 1.109	-0.541	<0.001
Weight SDS	-0.56 ± 1.32	0.322 ± 1.175	-0.887	<0.001
BMI SDS	-0.40 ± 1.54	0.427 ± 1.232	-0.835	<0.001

Table 3-7 Comparison of height, weight, and BMI SDS of UC children with HSE.

	UC	HSE	Mean Differences	P value
Height SDS	0.46 ± 1.06	-0.002 ± 1.109	0.467	0.010
Weight SDS	0.15 ± 1.12	0.322 ± 1.175	-0.168	0.424
BMI SDS	-0.05 ± 1.25	0.427 ± 1.232	-0.484	0.029

3.2.3 Assessment of body composition

UMA SDS and TSF SDS were used as proxies for measuring body composition in children with IBD. UMA SDS was obtained in 93 IBD patients (63 CD, 30 UC) and TSF SDS was collected in 93 IBD patients (62 CD, 31 UC). The distribution of UMA SDS and TSF SDS in IBD patients is demonstrated in Figure 3-12. Subsequently Figure 3-13 and Figure 3-14 show the distribution of UMA SDS and TSF SDS separately in CD and UC children.

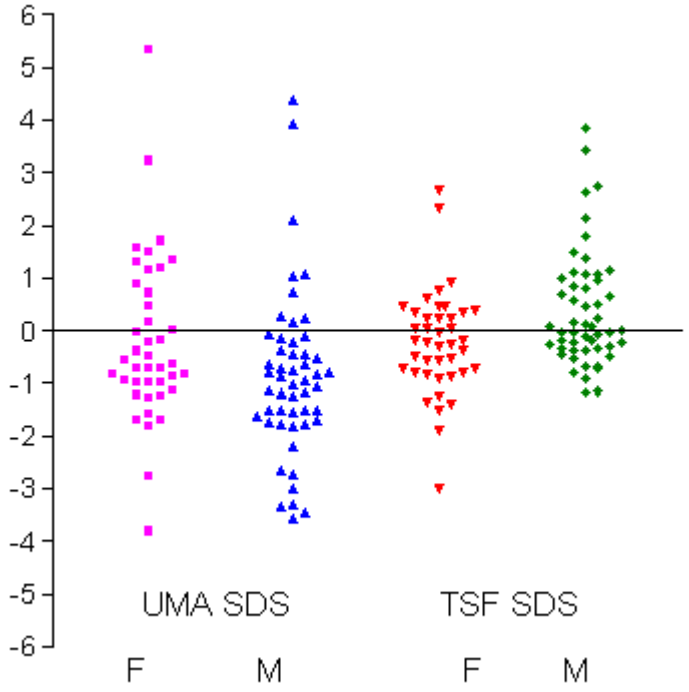

Figure 3-12 Distribution of UMA SDS and TSF SDS in children with IBD.

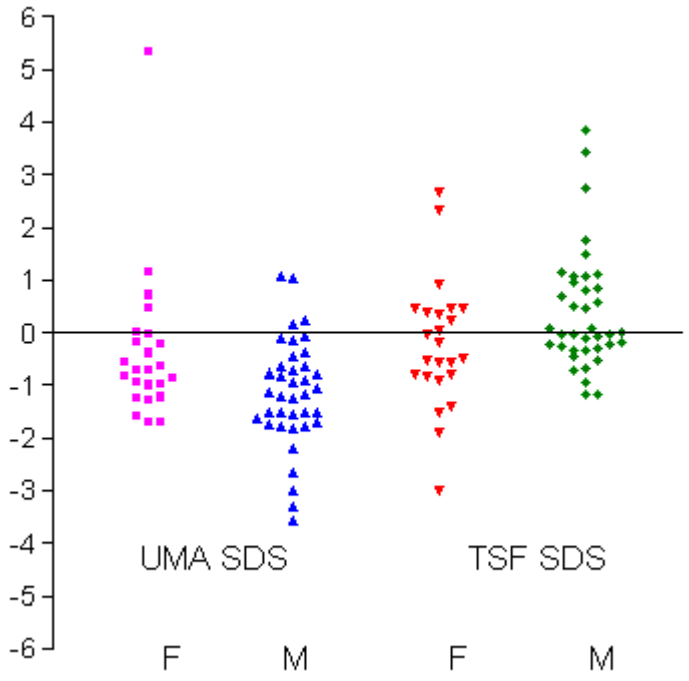

Figure 3-13 Distribution of UMA SDS and TSF SDS in children with CD.

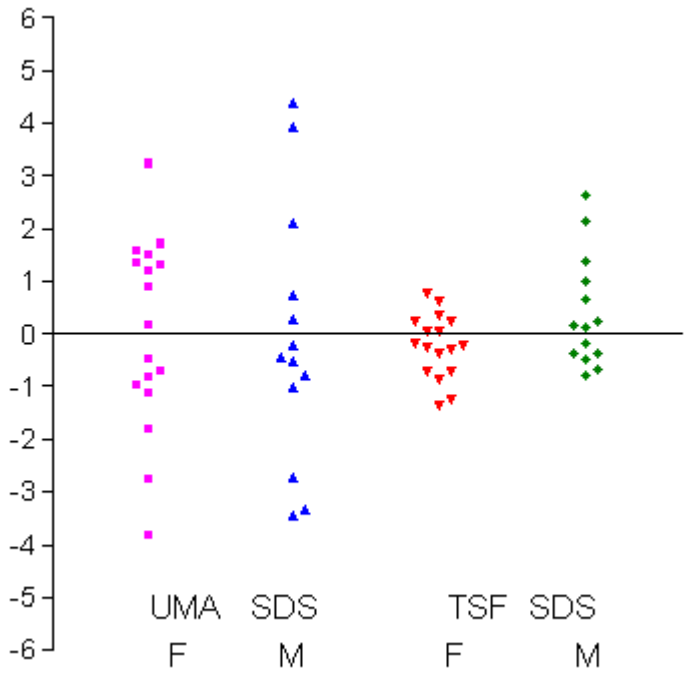

Figure 3-14 Distribution of UMA SDS and TSF SDS in children with UC.

Table 3-8 UMA SDS and TSF SDS in IBD children (and divided into UC and CD)

	N	Range	Mean	P Value
IBD				
UMA SDS	93	-3.81-5.35	-0.57 ± 1.59	0.001
TSF SDS	93	-3.00-3.85	0.12 ± 1.09	0.275
CD				
UMA SDS	63	-3.57- 5.30	-0.84± 1.25	0.00
TSF SDS	62	-3.00- 3.85	0.16 ± 1.19	0.287
UC				
UMA SDS	30	-1.36 -2.63	-0.02 ± 2.04	0.953
TSF SDS	31	-3.81 – 4.40	0.04 ± 0.88	0.759

As it can be seen from the figures, there is a wide scatter for UMA SDS and TSF SDS in IBD children. Five children with CD (5M) and, five children with UC (3F, 2M) had a UMA SDS< -2. 5 CD children (3M, 2 F), and 2 UC children (both M) had a TSF SDS> 2.

CD and UC children were compared in terms of their body composition measures and the results are summarized in Table 3-9.

Table 3-9 Comparison of UC and CD children in terms of UMA SDS and TSF SDS

	CD	UC	Mean difference(CD_UC)	P value
UMA SDS	-0.84± 1.25	-0.02 ± 2.04	-0.823	0.018
TSF SDS	0.16 ± 1.19	0.04 ± 0.88	0.113	0.640

The comparison demonstrates that the two groups are not significantly different in terms of UMA SDS (P value= 0.640), however, their TSF SDS is significantly different (P value= 0.018).

UMA SDS was plotted against BMI SDS and TSF SDS was plotted against BMI SDS to explore the extent to which differences in BMI were associated with differences in lean or fat mass

There were marked differences in TSF SDS and UMA SDS for any given BMI SDS in CD patients. For a BMI SDS between -0.5 and +0.5, TSF SDS ranged from -1.0 to 3.4 and UMA SDS ranged from -3.3 to 5.3 in CD patients .

In UC patients for a BMI between -0.5 and +0.5 TSF ranged from -0.87 to 0.76, however, UMA SDS ranged from -3.81 to 2.1, demonstrating larger differences of UMA SDS for any given BMI.

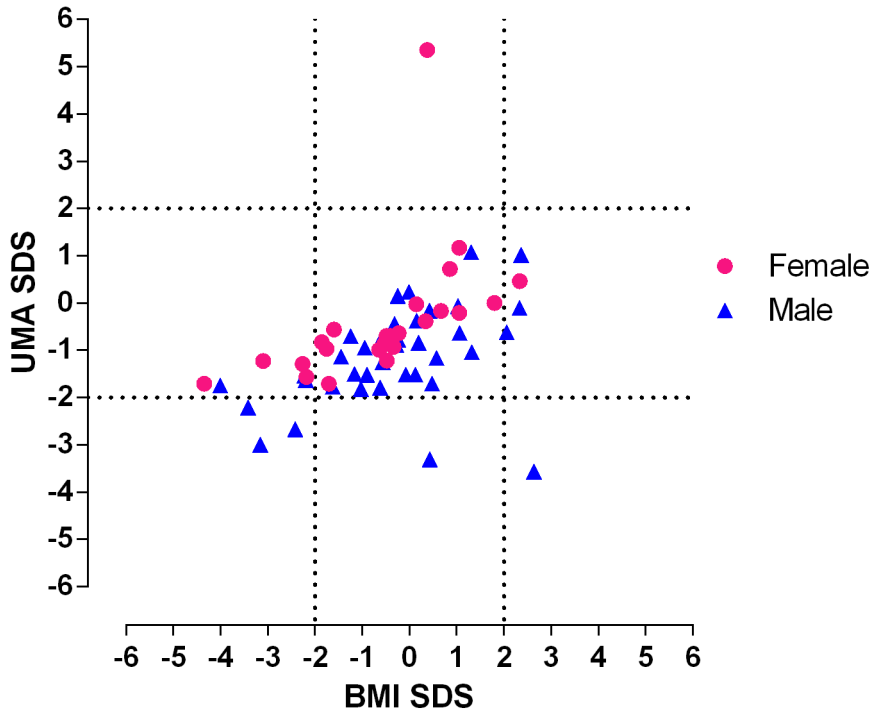

Figure 3-15 UMA SDS and BMI SDS cross plot in CD children.

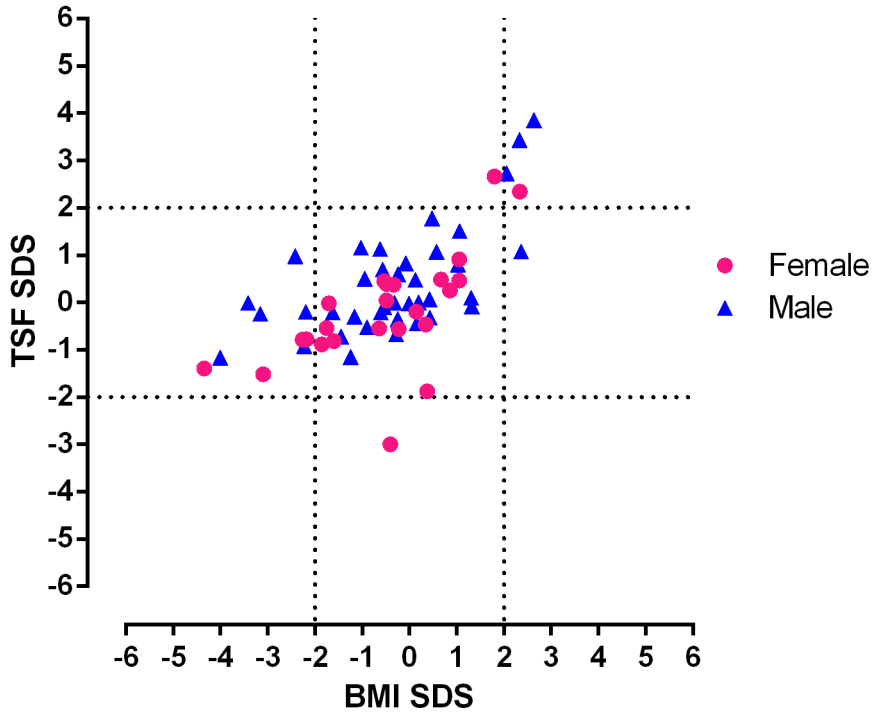

Figure 3-16 TSF SDS and BMI SDS cross plot in CD children.

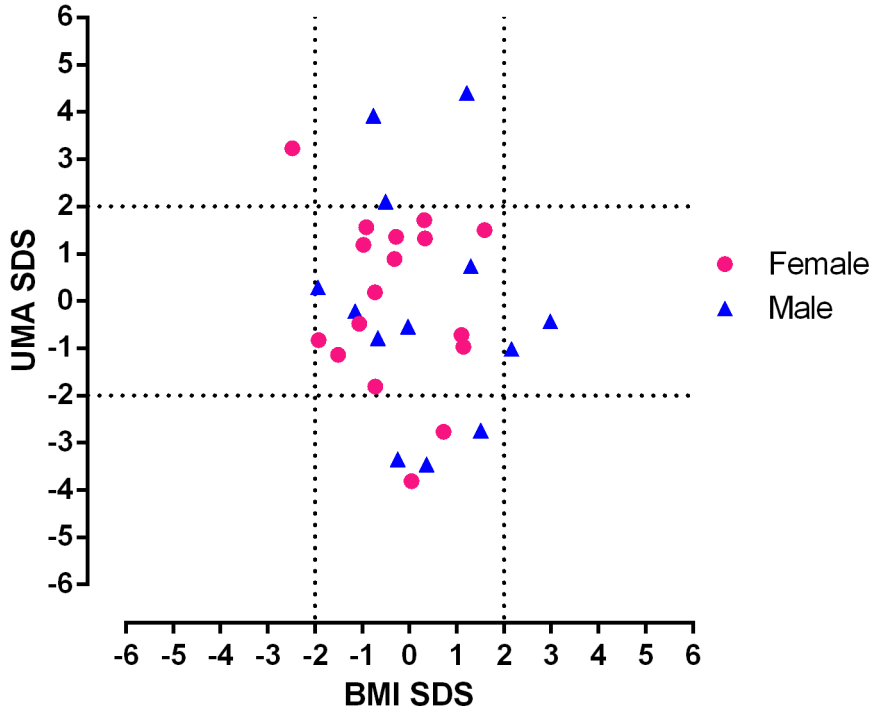

Figure 3-17 UMA SDS and BMI SDS cross plot in UC children.

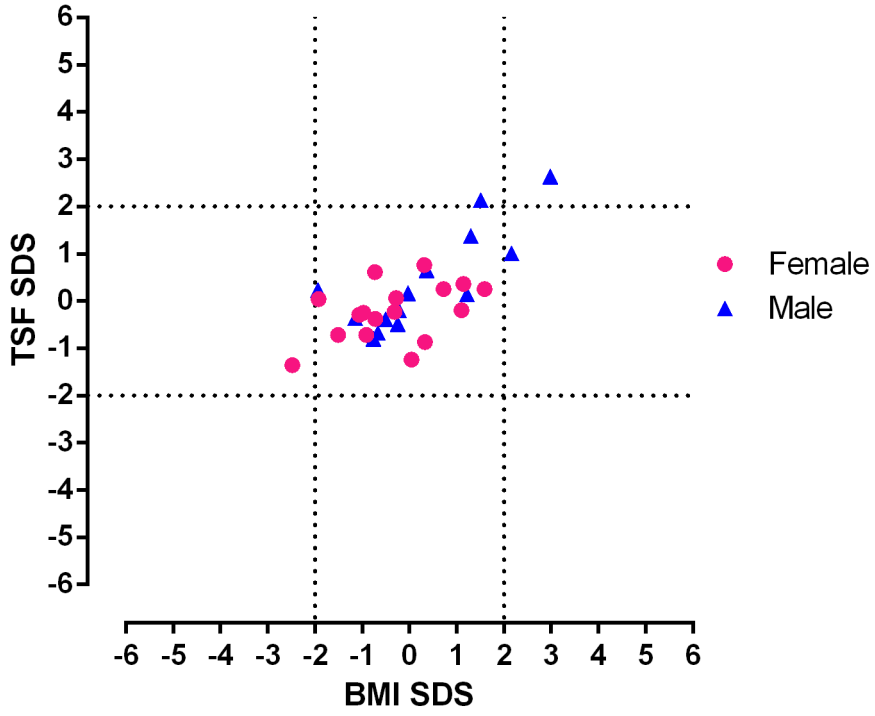

Figure 3-18 TSF SDS, and BMI SDS cross plot in UC children.

3.2.4 Relationship to disease activity:

The mean PCDAI of the children with CD was 16.93 ± 13.07 (range 0-55). After adjusting for age, height, and gender, there was a significant relationship between Fat Free Mass from UMA SDS and disease activity, measured by PCDAI(P value: 0.017), in children with CD

The mean disease activity measured by PUCDAI for children with UC was 23.62 ± 24.86 (range 0-85). After adjusting for age, height and gender , there was no significant relationship between Fat Free Mass from UMA SDS and disease activity, measured by PUCDAI (P value: 0.729) .

3.3.1 Discussion:

Growth and body composition abnormalities manifest themselves in children with IBD, but the aetiology is multifactorial and poorly understood. This study aimed to investigate the type of growth impairments that may exist in children with IBD and whether the extent of growth deficits varies between children with UC and CD. Another objective of this study was to examine whether body composition abnormalities exist in children with IBD, and whether UC and CD children differ in terms of their body composition.

This study demonstrates that IBD should not be considered as a single entity since children with CD and UC had more frequent and more severe growth deficits, especially in height, and to a lesser extent in weight and BMI. This difference between CD and UC may be explained by a much shorter interval between onset of symptoms and diagnosis in patients with UC. In addition, there is less systemic inflammation in UC and therefore less effect on bone growth, and less effect on appetite and nutritional status [83]. A related explanation is that more children with CD had active disease (less in remission) than UC. Unfortunately, inflammatory markers of disease activity since the onset of disease are lacking. The duration of illness could also influence the results, since growth failure is more likely to occur with long-term illness.

It appears that the observed weight deficits in our children with IBD are considerably smaller than those reported in the literature. For instance, *Sawczenko* et al had reported weight z- scores to be (-1.06, and -0.32 in CD and UC children respectively [126]. *Sawczenko et al* had reported the mean height SDS of UC children to be -0.12 which is significantly lower compared to subjects considered in the current study(mean height SDS: 0.46). The mean height SDS for CD children was reported -0.54 by *Sawczenko et al*, which was not significantly different from the values found in this study (-0.56). However this study was undertaken approximately 15 years ago, when the management of IBD differed from the current methods of treatment. With increasing awareness about the problems associated with IBD (resulting form availability of more guidelines, communication systems) it is possible that there is now earlier diagnosis and more aggressive treatment to control inflammation. Growth failure may be established before the diagnosis [167], [38]. For instance, *Kanof et al* [38] demonstrated in their study that a decrease in height velocity appears in 88% of children before diagnosis; therefore earlier diagnosis in current patients can be a possible reason for improved growth deficits. In addition, there has been change in type of treatment used. For example, there has been a tendency to increasingly use exclusive enteral nutrition without the use of corticosteroids which in the long tem can detrimentally affect weight and height gain. *Lambert et al* [168] followed up children with IBD divided into two groups of treatment, EEN and steroids, for duration of 24 months. They demonstrated that there was an increase in weight SDS over the 24 months of the study in the EEN group. However, children treated with steroids had a sharp increase in weight SDS over the first 6 months prior to decreasing again. Over the initial 12 months following diagnosis, mean height for age SD scores in both the EEN and steroid groups decreased, Over the second 12 months, however, the mean SD score in the EEN group increased, while that of the steroid group continued to decline. Therefore increased use of EEN might be another reason behind improvement of height and weight SD scores in children of IBD in the current studies compared to the old literature. However, the mean height SDS for CD children in our study was similar to that reported by *Sawczenko* (mean height SDS= -.054). Therefore, it seems that new management guidelines have not been successful in improving height deficits in children with CD whilst correcting their weight deficits.

This study also shows that children with IBD can vary considerably in their weight and height status, so that both over-weight and under-weight can co-exist in both CD (mean weight SDS: -0.56 ± 1.32) and UC (mean weight SDS: 0.15 ± 1.12). Indeed, this study illustrates the spectrum of growth abnormalities. Eight of CD children were categorized as wasted (height within ± 2sd and BMI <-2sd), three of them as stunted (BMI within ± 2sd and height <-2sd) and, five were obese (BMI SDS>2 and height SDS within ± 2 SDS). Perhaps these children had less aggressive disease. In UC patient's only one child that was wasted, two were obese, and the rest are within the normal range (± 2SD) for weight and height. This provides a striking contrast with the spectrum observed in CD. Whilst, the majority of attention is on growth impairments, obesity and it`s complications should not be overlooked. Both are important and both require attention. Whilst the proportion of obese individuals in CD was only 8% in our study it could pose problems in some individuals, as suggested by other CD cohorts [35].

The Health Surveys for England undertaken in recent years (2007-2010) show that the general population is heavier than that used to establish the UK 1990 growth charts. This explains the weight of all IBD children is significantly lower when it is compared with the current population of children but not when compared to establish the 1990 growth charts. This means that the impact of IBD on weight and BMI status and energy balance are greater than that suggested by comparison with the UK 1990 growth charts alone.

The causes of the differences in weight, height and BMI status (and body composition) between children with CD and UC (more modest deficits in children with UC) are unclear. However, one potential explanation is that CD, unlike UC, affects the small bowel and therefore absorption of nutrients that are necessary for growth and accretion of tissues. The presence of active disease in a greater proportion of children with CD (38%) compared to UC (16%) suggests that there may be impaired absorption of nutrients as well as impairment of appetite by systemic inflammation.

Little information exists in the literature about the effect of CD and UC on body composition. This study adds to the literature by demonstration that in comparison with a reference population, upper arm muscle area was reduced in children in IBD and to a greater extent in children with CD. Since triceps skinfold thickness was within the normal range obtained in the same reference population, it seems that

CD produces deficits in specific body compartments (muscle as a proxy of lean body mass, rather than triceps skinfold thickness as a proxy of fat mas). This may be due to disease activity, since upper arm muscle area was found to be inversely related to PCDAI and independent of any effect of height, and age. Such relationships were not observed with UC, which again emphasises the differential effects of the two conditions is not restricted to body mass but they extend to body composition. These observations suggest that at least in patients with CD deficits in muscle mass relative to fat mass are inadequately predicted weight or BMI alone. Since muscle mass is related to muscle strength, a depletion of muscle mass could have important functional consequences. It is not clear for this cross -sectional study whether a lower lean mass represents an acute loss of lean tissue in the presence of active disease or whether children with IBD fail to gain lean in the first place. A study by *Sylvester et al* demonstrated that lean deficits persist following treatment in children with CD [61].

Other studies in paediatric IBD have reported a lower FFM in children with CD compared to healthy controls [114], [59] and [56]. However, many have reported absolute measurements of FFM that were not corrected for height. This means that a low fat free mass may have been associated with shorter people who would be expected to have less lean tissue. In contrast, the present study adjusted for height and age and evidence is provided that the depletion is independent of these confounding variables.

3.4.1 Conclusion

Most children were fitted in the normal range for height and weight SDS. In the clinical settings less attention is given to management of overweight problems in IBD children. However, children with IBD may be at risk of obesity and in need of proper nutritional supervision. In clinical settings consideration of only BMI as a marker of nutritional status and well being may mask significant FFM deficits in the presence of normal or higher proportions of FM. Body composition should be an important consideration in planning and assessing growth and nutritional outcomes in children with CD. Performing bedside measurements of body composition is highly beneficial as it helps to interpret the pattern of weight gain, weight loss and

tissue accretion in CD children. As a result, these children can benefit from appropriate dietary interventions and growth management strategies.

There are many factors contributing to FFM and height, identifying these factors will enable us to better understand the nature of growth and body composition deficits in IBD children. Therefore, providing more appropriate strategies of controlling factors that contribute to FFM and height deficits will help us to minimize body composition deficits in these children.

Furthermore, the time of onset of IBD in relation to the age at peak height velocity may confer risk for impaired linear growth. These findings emphasize a need for greater attention to growth and development issues of those with disease diagnosed in childhood and early adolescence. Future studies of children with IBD are required to more fully realize the gender specific abnormalities and risk factors contributing to impaired growth.

As discussed growth and body composition deficits are more severe in children with CD. The main limitation of this study is that it is cross-sectional. Therefore, inferences about long-term impact on growth are limited. In order to overcome this limitation a prospective cohort study of children with CD was designed and conducted the Welcome Trust Clinical Research Facility at Southampton General Hospital.

These observations emphasize the need for a more complete consideration of body composition in the clinical assessment of growth in children with CD. Therefore in the following chapter s methods of assessing body composition will be discussed.

Chapter 4 Measuring body composition by different bio-electrical impedance devices

4.1.1 Introduction

Assessment of body composition, particularly, Fat Mass and Fat Free Mass, is a core marker of nutritional status in the management of nutritional complications of chronic diseases and public health problems, such as obesity. However, the use of some methods including densitometry, deuterium dilution technique, and dual x-ray absorptiometry are time consuming, expensive, and are not practical to use in routine clinical settings or population surveys.

Bioelectrical impedance analysis (BIA) is a rapid, non-invasive, and relatively inexpensive method for assessing body composition in clinical settings. With this method, low-level electrical current is passed through the body and the impedance (Z), or opposition to the flow of the current, is measures by BIA. The Total Body Water (TBW) of the subject can be estimated from the impedance measurement, as the electrolytes in body water are conductors of electrical current. When the volume of TBW is large, the current flows more easily through the body. The resistance to the current flow is greater in individuals with large amounts of body fat, as adipose tissue is poor conductor of electrical current due to it`s relatively small water content.

BIA is applicable in routine clinical settings, as it does not require a high degree of technician skill, and is comfortable for patients [145].

There are three main different devices of bioelectrical impedance analysis - Bioelectrical Impedance Spectroscopy (BIS), multi-frequency bioelectrical impedance (MFBIA) , and single frequency bioelectrical impedance analyser (BIA). These different devices may be used in clinical settings but care needs to be taken as whilst they measure impedance, the use of proprietary algorithms means that they may produce different results for TBW and subsequently lean mass and fat mass. There are no device-specific standard deviation scores available for fat mass and fat free mass, which makes it difficult to determine whether the derived values reflect a

lean deficit or fat mass excess in children normalized for height, age and gender compared to a normal population.

Several different devices were available to the author to use in the longitudinal study. One of which, the Tanita device (BC-418 MA), had been used previously in another centre to generate per cent body fat curves for children and so there was a need to determine whether the different devices generated comparable values for impedance and in turn, body composition, and how they related to the Tanita device [169]. Two independent studies were designed, one in adults and another in children with IBD. The aim of these studies was to examine the concurrent validity of different bioelectrical impedance devices for measures of impedence and body composition (i.e. can they be used interchangeably in clinical settings?).

4.1.2Methods and materials of the study in adults:

Nurses in the Welcome Trust Clinical Research Facility (WTCRF) of Southampton General Hospital and the staff of Institute of Human Nutrition, University of Southampton participated in this study. A total of 17 subjects were measured with each of four different types of devices (Impedimed SFB7, Quad 4000 (1), Quad 4000 (2) - the two Quad 4000 devices were the same model from one manufacturer but different version releases), and the Tanita BC-418 MA).

A summary of characteristics of subjects participating in this study are summarized in

Table 4-1. As BIA devices have only been shown to be valid up to a BMI of 34 kg/m^2 [170], the subjects in this study had a BMI range of 18-33 kg/m^2. Each person was measured with each device in one day and the measurements were completed in approximately 45 minutes, and the devices were ordered in a sequence which was randomly assigned. The measurement procedure was exactly followed as stated in the relevant SOP which is available in the appendix 3. All the measurements of this study were conducted by the author.

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Table 4-1 Characteristics of subjects participating in the study.

Number of subjects	17
Age	34.29 ± 10.00
Female/ Male	11/ 6
BMI (kg/m^2)	24.50 ± 3.67

Below is a summary of the four measuring devices:

Impedimed SFB7: The Impedimed SFB7 a multiple frequency bioelectrical impedance analysis instrument operating in tetra-polar (4 leads - 2 current sources and 2 voltage sensing) mode**.** The manufacturer claims that the device accurately measures current, voltage and phase angle, and calculates impedance, resistance and reactance. These measurements and calculations are used to estimate the body composition: fat-free mass (FFM) and fat mass (FM), and fluid distribution: Total Body Water (TBW), intracellular fluid (ICF), and extracellular fluid (ECF).The device was used in the *MFBIA Mode* - this is a Multiple Frequency Bioelectrical Impedance mode. Impedance readings are taken by applying all 256 frequencies sequentially in a very short time in the range of 4 kHz to 1000 kHz.

According to the user manual this device estimates extracellular fluid (ECF) at low frequency and Total Body Water (TBW) at high frequency; however the exact frequencies used are not stated in the manual or the manufacturer's website. Intra-cellular fluid (ICF) is then estimated by the difference between TBW and ECF. Fat free Mass (FFM) is estimated from measured TBW assuming the single hydration constant of a person as 73.2%. Fat Mass (FM) is estimated from the difference between body mass and fat free mass.

Quadscan 4000: The Quadscan 4000 is battery operated device multi-frequency bioelectrical impedance which will measure at 5, 50, 100 and 200 KHZ. At 50 KHz a proportion of the applied current is unable to penetrate the cell membranes and therefore passes only through the extra- cellular space. At this frequency BIA is believed to reflect TBW; however at low frequencies the current is unable to penetrate the cell membrane and so at 5 KHZ, ECW is predicted. At 200 KHz the cell

membrane is penetrated and both intra-and extra cellular fluid spaces can be accessed to determine TBW. By deducting ECW from TBW value at 200 KHz, ICW is then calculated. At the higher frequency of 200 KHz, the estimate of TBW is greater than that predicted at 50 KHz; the 3rd space water is the difference between the two estimates of total body water.

In this study two Quadscan 4000 devices (different version releases of the same model) were used to explore whether they produce identical results and therefore can be used interchangeably in clinical and research settings.

TANITA BC-418: The TANITA BC-418 is a whole body composition analyser that utilizes BIA to determine body fat percent and regional muscle mass. It measures whole body impedance using an electrode panel which the participant stands on and two electrode hand grips which the participant holds during the measurement procedure. It is a single frequency device which operates at 50 KHZ and at this frequency the BIA is not measuring TBW but a weighted some of ICW and ECW resistivities. The BIA permits to estimate FFM and TBW, but cannot determine differences in ICW.
Despite the manufacturer's assurance, there is insufficient evidence to assess the accuracy of either the measured impedance or the equations used to predict TBW.

4.1.3 Analysis:

The concurrent validity of these devices was assessed by comparison against the Impediment SFB7 device as the reference method. The SFB7 was chosen as the reference device as it is a spectroscopy device applying all 256 frequencies (4 kHz to 1000 kHz) and has provides the opportunity to access raw data across all frequencies and examine the underlying calculations (e.g. Cole-Cole plots). The other devices are multi-frequency (Quad4000) or single frequency (Tanita BC- 418). The comparisons of the measured values available for the two latter devices were compared against that of SFB7 to investigate whether Quadscans and Tanita generate the same results as SFB7.

Statistics: Correlations and P-values were derived from the paired sample T-Tests. Bland- Altman plots were plotted to demonstrate the degree of concordance between devices.

4.1.4 Results:

SFB7 & Quadscan (1)

The measured values and the predicted values of SFB7 and Quadscan (1) were compared. The measured values and the predicted values are summarized in Table 4-2 and Table 4-3. . There is good agreement between SFB7 and Quadscan (1) for the measured values. Although both devices generate similar results for TBW (L) and TBW (%), FFM and FM, they produce significantly different results for the ICW and ECW.

Table 4-2 The comparison of measured values by SFB7 & Quadscan(1).

	SFB7(mean± SD)	Quadscan 1(mean± SD)	Mean Difference	95% confidence interval of the difference	T-test P-value	correlation	Correlation p-value
Imp 5 kHz (Ohms)	636±75	631±77	5.48±20	-4.52 to 15.50	0.262	0.968	<0.001
Imp 50 kHz (Ohms)	555±77	543±74	11.63±37	-7.35 to 30.62	0.212	0.888	<0.001
IMP 100 kHz (ohms)	517±69	511±69	5.44±17	-3.09 to 13.98	0.195	0.973	<0.001
Imp 200 kHz (Ohms)	492±68	486±70	5.7±16	-2.73 to 14.13	0.171	0.972	<0.001
Resistance 50 kHz (Ohms)	539±70	539±74	-0.44±25	-13.05 to 12.16	0.941	0.944	<0.001
Reactance 50 kHz	62±8	63±8	-0.81±2	-1.87 to 0.24	0.124	0.967	<0.001

(Ohms)							

Table 4-3 The comparison of predicted values of SFB7 & Quadscan(1).

	SFB7	Quad 2b	Mean Difference	95% confidence interval of the difference	T-Test P-value	Correlation	Correlation P-value
TBW(L)	38.33±7.04	37.71±6.84	0.62 ±1.68	-0.24 to 1.48	0.147	0.971	<0.001
TBW (%)	55.14±6.86	54.53±6.06	0.60±3.02	-0.94 to 2.15	0.421	0.898	<0.001
ICW(L)	22.75±4.15	20.86±4.49	1.88±1.84	0.93 to 2.83	0.001	0.912	<0.001
ECW(L)	15.60±3.01	16.72±2.31	-1.12±0.86	-1.57 to -0.67	0.00	0.981	<0.001
Fat(kg)	17.57 ±8.36	17.51± 8.27	0.064±3.7	-1.83 to 2.00	.944	0.897	<0.001
Fat (%)	24.64±9.38	24.74±9.73	-0.10±5.30	-2.82 to 2.62	.939	0.847	<0.001
FFM(kg)	52.39± 9.63	52.57±10.88	-0.18± 3.55	-2.00 to 1.64	.835	0.947	<0.001
FFM (%)	75.35±9.38	75.25±9.73	0.10±5.30	-2.62 to 2.82	.939	.847	<0.001

SFB7 & Quadscan (2):

The two devices generally agree very well over the measured values and the majority of predicted values with the exception of ICW, ECW. The measured values and the predicted values are summarized in the Table 4-4 and Table 4-5.

Table 4-4 The comparison of measured values by SFB7 & Quadscan(2).

	SFB7	Quad Elia	Mean Difference	95% confidence interval of the difference	T-Test P-Value	Correlation	Correlation P-Value
Imp 5 KHz (Ohms)	636±75	623±85	-14±9	-32±5	.142	.905	0.00
IMP 50 KHz (Ohms)	555±77	542±78	-13 ±37	-32±6	.164	.888	0.00
IMP 100 KHz (Ohms)	517±69	509±76	-8±19	-17±2	.131	.969	0.00

IMP 200 KHz (Ohms)	492±68	485±74	-6±17	-16±3	.180	.969	0.00
Resistance 50 KHz(Ohms)	539±70	538±79	-1±24	-13±11	.885	.955	0.00
Reactance 50 KHz(Ohms)	62±8	59±16	-3±12	-10±3	.316	.613	.009

Table 4-5 The comparison of predicted values of SFB7 & Quadscan(2).

	SFB7	Quad Elia	Mean Differences	95% confidence interval of the difference	T-test P-Value	Correlation	Correlation P-Value
TBW (L)	38.33±7.04	37.81 ± 7.00	0.52 ± 1.70	-.035 to 1.39	.223	.971	0.00
TBW (%)	55.14±6.86	52.77± 10.16	2.36 ± 6.98	-1.22 to 5.95	.182	.728	0.00
ICW (L)	22.75±4.15	20.92 ± 4.61	1.82 ± 1.87	0.86 to	.001	.914	0.00
ECW (L)	15.60±3.01	17.02 ± 2.86	-1.42 ± 1.21	-2.05 to -0.80	0.00	.916	0.00
Fat (Kg)	17.57 ±8.36	17.35 ± 8.35	0.21 ± 3.64	-1.65 to 2.09	.809	.905	0.00
Fat (%)	24.64±9.38	24.50 ± 9.97	0.14 ± 5.09	-2.47 to 2.75	.907	.863	0.00
FFM(Kg)	52.39± 9.63	52.65 ± 11.05	-0.26 ± 3.63	-2.13 to 1.60	.768	.947	0.00
FFM (%)	75.35±9.38	75.50 ± 9.97	-0.14 ± 5.09	-2.76 to 2.47	.907	.863	0.00

SFB7 & Tanita:

The SFB7 and the Tanita produce significantly different results for impedance, Lean and fat values. However, they generate similar results for TBW. The measured and predicted values are summarized in table Table 4-6.

Table 4-6 The comparison of measured and predicted values from SFB7 & Tanita.

	SFB7	Tanita	Mean Difference	95% confidence interval of the difference	T-test P-Value	Correlation	Correlation P-Value
IMP 50 KHz(Ohms)	55 ±76	655 ±87	-101±40	-122±-81	0.00	.889	0.00
TBW (L)	38.33±7.04	37.33±7.17	0.96 to 2.46	-2±.3	.126	.940	0.00
Fat (Kg)	18.57 ± 8.36	19.38±9.30	-1.80 to 3.30	.1±4	.039	.935	0.00
Fat (%)	24.64±9.38	27.21±10.25	-2.56 to 4.67	.16±5	.038	.891	0.00
FFM (Kg)	52.39 ± 9.63	50.56 ±10.06	1.82 to 3.27	-4± -.14	.035	.964	0.00
FFM (%)	75.35±9.38	72.7±10.25	2.56 to 4.67	.16±5	.038	.891	0.00

4.2.1 Methods and materials of the validation study in children with IBD:

Body composition of children participating in routine clinical care study in paediatric IBD were measured using three different devices of bioelectrical impedance (Tanita BC-418, QuadScan 4000 (two otherwise comparable devices) and Impedimed SFB7) on the day of their normal visit to Welcome Trust Clinical Research Facility (WTCRF) of Southampton General Hospital. The three measurements were completed in 15 minutes and the measurement procedures were exactly followed as stated in the relevant SOP`s which are available in appendix 3.

Table 4-7. Characteristics of children taking part in the study.

Number of subjects	15
Age	18.99 ± 2.68
Female/ Male	5/10
BMI	18.99 ± 2.68

4.2.2Results:

SFB7 was set as the reference method, as it is a multi-frequency spectroscopy device and the measured and predicted values derived by Quadscan 400 and Tanita were compared against that of SFB7.

SFB7 & Quadscan4000:

Table 4-8 The comparison of measured and predicted values by SFB7 & Quadscan.

	Quadscan 4000	SFB7	Mean Difference	95% confidence interval of the difference	T-test P-value	correlation	Correlation p-value
Imp 5 KHz(Ohms)	701.42	688.71	12.71	-3.84 to 29.27	0.121	0.945	<0.001
IMP 50 KHz(Ohms)	631.07	607.48	23.58	5.88 to 41.28	0.013	0.918	<0.001
IMP 100 KHz(Ohms)	590.35	573.65	16.70	3.69 to 29.70	0.016	0.954	<0.001
IMP 200 KHZ(Ohms)	560.28	545.37	14.91	0.33 to 29.49	0.046	0.939	<0.001
Resistance 50 KHz(Ohms)	620.64	646.60	-25.96	-56.66to 4.74	0.091	0.845	<0.001
Reactance 50 KHz(Ohms)	61.42	43.43	17.99	7.94 to 28.03	0.002	0.403	0.153
TBW(L)	27.12	27.05	0.072	-0.53 to 0.68	0.800	0.987	<0.001
TBW (%)	60.38	60.32	0.059	-1.53 to 1.67	0.938	0.732	0.003
FFM(Kg)	35.98	36.76	-0.77	-1.84 to 0.28	0.137	0.979	<0.001
FFM (%)	79.79	82.27	-2.47	-4.81 to -0.13	0.39	0.720	0.004
Fat (Kg)	9.02	8.07	0.94	0.08 to 1.80	0.34	0.898	<0.001

Fat (%)	19.89	17.58	2.30	-0.01 to 0 4.63	0.52	0.696	0.006

The two devices generate significantly different results for all the measured values except for Imp 5 (KHz), however the two devices are in good agreement for the predicted values and as the t-test results illustrate the generated values are not significantly different. Bland- Altman plots for Impedance at 50 KHz (Ohms) and TBW are presented below in Figure 4-1 and Figure 4-2.

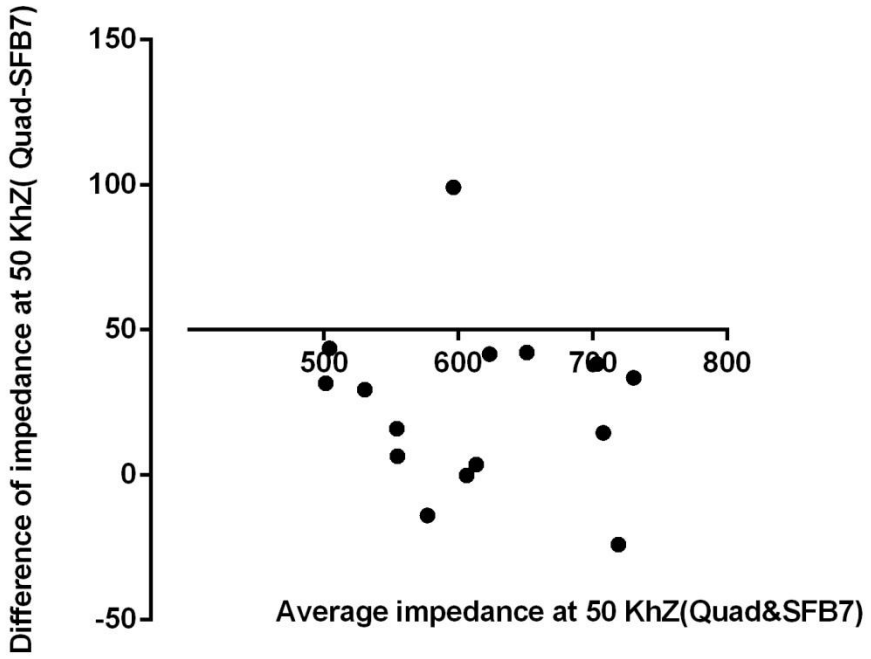


Figure 4-1 Bland-Altman plots of Impedance (Ohms) at 50 KHz by SFB7 and Quadscan

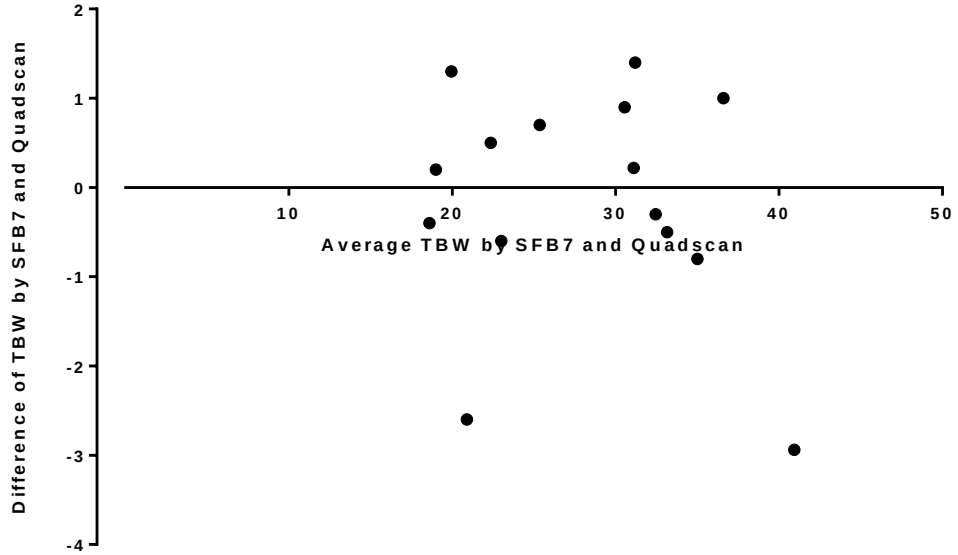


Figure 4-2 : Bland-Altman plots for TBW (litres) measured by SFB7 and Quadscan

Tanita & SFB7:

Table 4-9 The comparison of measured and predicted values of SFB7 & Quadscan.

	SFB7	Tanita	Mean Difference	95% confidence interval of the difference	T-test P-value	correlation	Correlation p-value
Imp 50kHz (Ohms)	607.48	735.28	-127.8	-148.5±-107.01	0.000	0.819	<0.001
TBW(L)	27.05	25.62	1.42	0.61±2.24	0.002	0.975	<0.001
TBW (%)	60.32	58.29	2.03	0.065±4.00	0.044	0.544	0.027
Lean (Kg)	36.76	35.00	1.75	0.44±3.07	0.013	0.967	0.028
Lean (%)	82.27	79.73	2.53	-0.27±5.3	0.073	0.525	0.059

				4			
Fat(Kg)	8.07	9.02	-0.95	-2.08±0.18	0.094	0.819	0.048
Fat (%)	17.58	20.26	-2.67	-5.32±-0.029	0.048	0.556	0.044

Different values for Imp 50(KHz) may be due to the fact that the two devices measure the impedance through different routes through the body. However the two devices are in general agreement for Lean (%), Fat (Kg), and Fat (%).

The SFB7 produces statistically significant higher values for TBW (L), TBW (%) and Lean (Kg) than the Tanita device. The significantly different values for FFM (kg) may be due to the fact that the subject`s weight has to be manually put in to SFB7. On the other hand, Tanita measures the weight of the subjects independently when they step on it to be measured for their impedance. Different recorded weight values may result in different values reported for FFM.

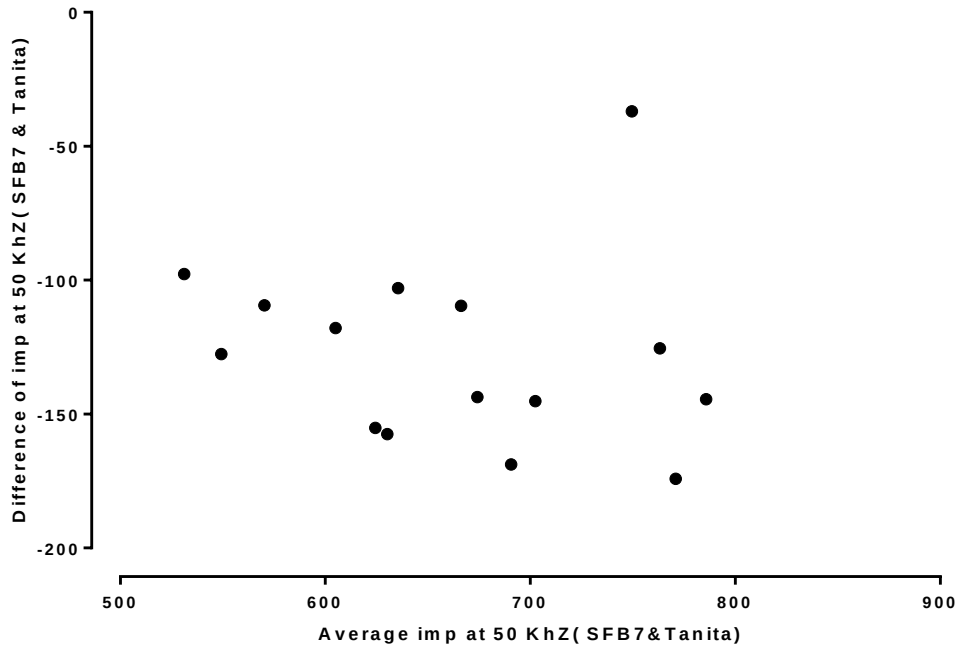


Figure 4-3. Bland_Altman plots of impedance (Ohms) at 50 KHz by SFB7 and Tanita

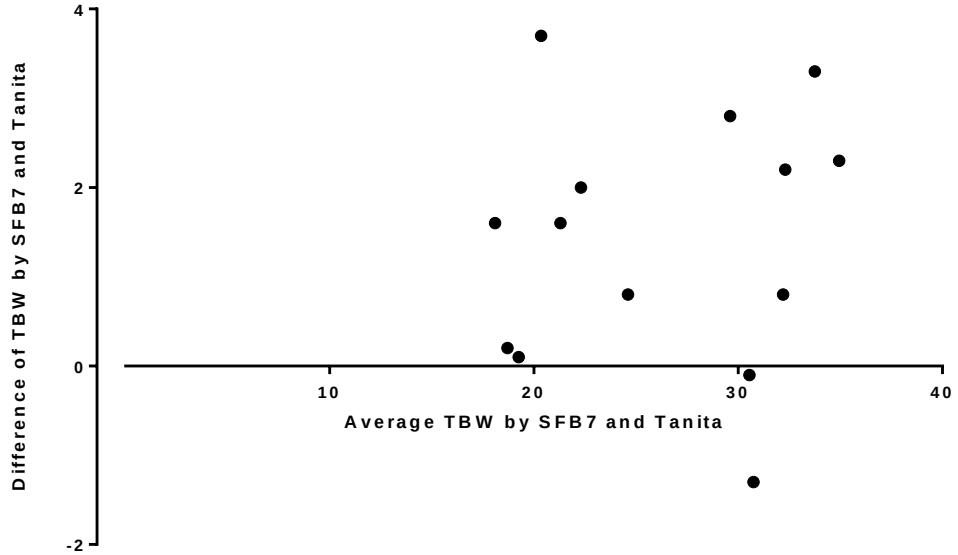


Figure 4-4. Bland-Altman plots for TBW (litres) measured by SFB7 and Tanita

4.3.1Discussion:

As BIA devices become more widely available and are used more widely in clinical settings, there is a need to determine the comparability of the results – measured and derived - generated by different types of devices. This study aimed to explore whether different devices of BIA produce similar results and therefore may be used interchangeably in clinical settings. The first question addressed was whether different devices generate similar values for direct measurements at common frequencies. The next question was whether different devices produce similar results for predicted values such as TBW.

Table 4-10: A summary of comparison of different devices of bio-electrical impedance in adults.

	SFB7	Quadscan(1)	Quadscan(2)	Tanita
Impedance at 50 KHz(Ohms)	555	543	542	656
TBW(l)	38.3	37.7	37.8	37.3
FFM(kg)	52.4	52.57	52.65	50.56

Table 4-11 : A summary of comparison of different devices of bio-electrical impedance in children.

	SFB7	Quadscan	Tanita
Impedance at 50 KHz(Ohms)	607.48	631.07	735.28
TBW(l)	27.05	27.12	25.62
FFM(kg)	36.76	35.98	35.00

It can be concluded from Table 4-10 that SFB7 and both Quad scans agree well over the measured and predicted values with the exception of ICW, and ECW in adults. The significant difference in the derived values for ICW and ECW by the two types of the device may be caused by their use of different equations to calculate ICW and ECW from TBW, as SFB7 and both Quad scans produce similar values for TBW. Over all, SFB7 and Quadscans may be used interchangeably to calculate TBW, FM and FFM in adults (with a BMI< 33). However, if ICW and ECW values are required, they should be calculated independently according to the relevant equations.

The SFB7 and Tanita produced significantly different results for impedance at 5 KHZ in adults. This difference may be due to the fact that Tanita is a foot-to-foot BIA and SFB7 is a hand-to-foot device, and they measure the impedance of different parts of the body. The two devices generate similar results for TBW; however, they predict significantly different results for FM and FFM. The difference in the predicted measures for FM and FFM may be caused by different equations in calculating FM and FFM from TBW. Therefore, the two devices may be used interchangeably to calculate TBW in adults (with a BMI< 33); however, FM and FFM should be calculated independently.

Previous studies on comparison between different BIA devices have reported mixed results. The current study supports previous findings by *Ritchie et al* [171]. *Ritchie et al* investigated the comparability of hand-to-foot BIA (BIA 310 Bio-impedance analyser) and foot-to-foot BIA (Tanita scale plus body fat monitor, model BF-556) among fifty older adults aged 56-94 with a mean BMI of 28.7 ± 4.9 kg /m^2. The results demonstrated a significant correlation between hand-to-foot and foot-to-foot BIA ($P<0.001$) suggesting that could be used interchangeably, with the foot-to-foot

device measuring a slightly higher mean FFM (36%) than the hand-to-foot device (35%) (P=0.13).

Another study conducted by *Ramsey et al* [172] compared body composition values measured with three different devices; the single frequency foot-to-foot BIA (300GS Tanita Corporation), single frequency hand-to-foot BIA(Imp DF50, Impedimed, Australia) and BIS(Imp SFB7, Impedimed , Australia). This study demonstrated that the two single frequency devices generated results for FFM that were not significantly different (P= 0.140). However, differences in FFM measured by SFB7 and the two single frequency devices were significant (P< 0.001 in both comparisons), suggesting that SFB7 and single frequency BIA devices can not be used interchangeably to measure body composition.

In children as can be concluded from Table 4-11, SFB7 produced significantly different results from Tanita and Quadscan 4000. This may be due to the fact that SFB7 does not apply equations specific to children and uses the fixed hydration factor (contribution of water to the FFM) of 73.2% for all ages. However, Tanita and Quadscan 4000 use their own specific hydrations factor that varies for age. Unfortunately, there have been no other studies comparing different devices of bioelectrical impedance in children.

The major limitation of this study was that no reference standard was used with which to compare our measures for body composition, consequently, the true values for TBW, FM and FFM are unknown. *Leahy et al* [173] compared a multi-frequency bio-electrical impedance analyser (Tanita MC_180 MA) which required the subjects to stand on it to DXA and reported that BIA underestimated %body fat when compared to DXA by 2.1 %(P value < 0.001). Another finding of this study was the underestimation by BIA became more evident as % body fat increased. *Ellegard et al* compared a Bioelectrical Impedance Spectroscopy (BIS) device (Xitron Hydra 4200) with DXA in cancer patients . It was found that BIS underestimates FFM by 1.6 % in cancer patients. They suggested that this bias may be due to weight, malnutrition and systematic inflammation as a fixed hydration factor of 73% was used.

Chapter 5 Deuterium dilution space technique

This chapter describes the analytical methods used in studies presented in this thesis measuring Deuterium Dilution Space (DDS) by SIFT-MS and IRMS.

The measurement of body water (Hydrometry) is central to any measurement of body composition and an appreciation of how differences in Total Body Water influence the estimate of the amount of lean and fat is required in order to interpret the results of the 2,3 or 4 compartment.

Water is the most abundant constituent of the body, typically comprising over 60% of the body's weight. Total Body Water can be sub-divided into extracellular (ECW) and intracellular water (ICW). It is presumed that water content of adipose tissue is close to zero; and that nearly all of the body water is distributed throughout lean compartment. Hydrometry is based on the dilution principle. This principle states that the volume of the solvent (body water) is equal to the amount of the compound (isotopic water) added to the solvent divided by the concentration of the compound in that solvent [141]. Put simply, if the same dose of tracer is dispersed in different distribution volumes, the concentration of the tracer in a larger volume will be less than that of the tracer in the smaller volume.

The calculation of FFM from Total Body Water is based on the assumption that the contribution of water to the FFM- the hydration factor -is constant in all adult subjects [91]. However, this assumption is not always appropriate in that individuals who either are dehydrated or have abnormal water metabolism leading to oedema would have lower or higher hydration factors respectively. The most commonly used hydration constant is 0.732 which was first recommended by *Pace and Rathbun* [174]

In this case, FFM is calculated using the following model

$$FFM(Kg) = TBW/0.732$$

Total Body Water is typically estimated via the measurement of dilution of isotope tracers such as deuterium oxide and ^{18}O isotopes in a reprehensive sample of biological fluids after equilibration. Water labelled with ^{18}O is not usually used to assess TBW given their high cost of tracer and general lack of availability, whereas Deuterium oxide (D_2O) is more widely used for measurement of TBW.

The conventional approach is to determine deuterium abundance in a given body fluid (saliva or urine) that is deemed to reflect the abundance seen in all compartments of water within the body (the Total Body Water). Isotope Ratio Mass Spectrometry (IRMS) is generally considered to be the most reliable and accurate method to determine deuterium abundance but it is costly, time consuming and requires access to instrumentation which is not usually available in the clinical settings. Fourier transform infrared spectroscopy (FTIR) is more accessible and considerably less costly, but requires much higher dose of tracer to achieve acceptable accuracy and precision, note the precision is less than that seen in IRMS(approximately 2% vs. < 1%). In both cases, obtaining a specimen of body fluid as saliva or urine can be practically challenging, particularly in children as it requires co-operation. The time-course to achieve equilibrium differs between water compartments and different approaches are offered as to how a plateau could be determined. However, in practice, few studies demonstrate that they have taken multiple time points in order to determine plateau enrichment and it is more usual for one or two samples to be collected after dosing at time points that is assumed to reflect equilibrium [141]. The International Atomic Energy Agency (IAEA) suggests that saliva and urine samples collected after three- and four hours respectively reflect attainment of equilibrium [141].

More recently, a new method has been introduced which offers for the first time, the opportunity to measure deuterium abundance that would readily advance the application of this technique in clinical practice. Selected ion flow tube mass spectrometry, SIFT-MS, is a technique for real-time measurement of concentrations of trace gases and vapours of volatile compounds in humid air including exhaled breath and headspace above biological fluids such as urine or saliva [175]. Using precursor (reagent) ions, such as H_3O^+, NO^+, and O_2^+, it is possible to enable ion/molecule reactions with the compounds in the breath vapour to proceed over an accurately defined time. Knowing the reaction rate constants, from an established library, makes it possible to use the ratios of ion count rates to calculate the absolute concentrations of trace gases with a limit of detection being typically 1 parts-per-billion, ppb. SIFT-MS has been used in several areas of research including non-invasive breath analysis for clinical diagnosis and for therapeutic monitoring, as well as environmental and security related research. An important development that stemmed from SIFT-MS is flowing afterglow mass spectrometry, FA-MS [176] which is exploited for the on-line, real time analysis of

the deuterium content of breath water vapour and the headspace of aqueous liquids. This technique relies on the accurate measurement of the of the water cluster ions signal ratio $H_3O+(H_2O)2HDO/H_3O+(H_2O)2H_2{}^{18}O$ as generated in an afterglow plasma from H3O+ precursor ions and the mixture of H_2O and HDO molecules present in a breath/headspace introduced into the plasma. Thus, following a known dose of D_2O, the deuterium disperses as HDO throughout the TBW and, at equilibrium, a measurement of the deuterium content of single breath exhalations provides a measure of the TBW [177]. The advantages over IRMS are that SIFT-MS does not require off-line sample preparation, and the results can be obtained in real-time so that it is possible to make as many replicate measures over time for no real additional cost. Whilst such an approach offers immediate practical advantages, it requires further examination.

There are three specific considerations that need to be addressed. Firstly, the extent to which deuterium abundance by SIFT-MS is the same in different biological samples such as breath, saliva and urine as it is possible that fractionation of tracer may occur between water pools or compartments such that different values for TBW would be obtained in the same subject dependent on which body fluid was assessed [141] . Secondly, the temporal pattern of change in abundance over time in breath compared to that determined in saliva or urine to explore whether equilibrium is achieved at the same points in time. Thirdly, there is a need to demonstrate that the values of deuterium abundance in saliva or urine by SIFT-MS was directly comparable to that derived by IRMS which is taken as the reference method. In addition, whether the value for TBW and FFM obtained from deuterium abundance measures by SIFT-MS was comparable to that derived by other approaches to predict TBW and FFM such as BIA or DXA. To date, there have been only limited reports that address each of the points.

5.1.1 Development and validation of the method:

This section describes the development of the Sift-MS method for assessing deuterium abundance in order to determine the deuterium dilution space technique for the prediction of TBW and body composition.

Method development:

The SIFT-MS was introduced into the Nutrition SCBR Mass Spectrometry Unit at Southampton General Hospital prior to the study and as this was the first occasion where it has been used for this purpose, required method development and validation prior to use.

As a first step of developing the methodology for this technique, a set of eight gravimetrically derived standards were prepared from stock (Sigma Deuterium Dioxide 99.99% APE) with theoretical deuterium abundance and measured in duplicate. Deuterium abundance measured by SIFT-MS was compared against the theoretical concentrations and the results are presented in Table 5-1

Table 5-1: weighed standards and measured values by SIFT-MS.

Weighed standards	Measurement 1	Measurement 2	Average measurement of SIFT-MS
155	156	145	150.5
340	330	321	328
490	489	425	462
590	548	512	540
155	151	169	165
767	708	743	723
1248	1176	1166	1171
1344	1215	1225	1232

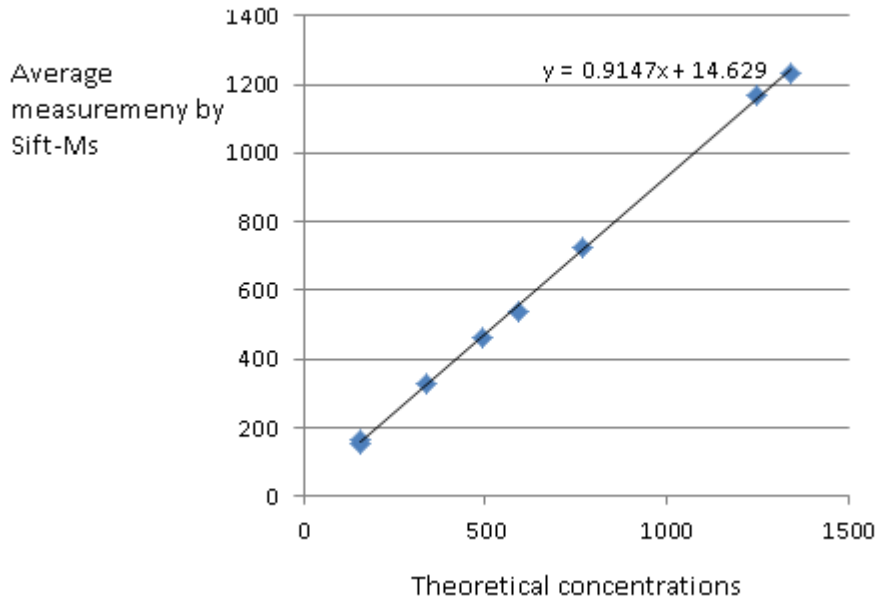


Figure 5-1 Deuterium abundance measured by SIFT-MS compared against the theoretical concentration(ppm).

As can be seen from the linear regression through the derived values, there is good linearity and minimal intercept, but the slope is significantly less than 1.00(P value<0.001In the second step, three sets of gravimetric standards with theoretical concentrations of D_2O (155 ppm, 337 ppm, 942 ppm) were prepared. All weightings were conducted on a balance reading to six decimal points. An accurately weighed amount of D_2O (99.99%) was diluted with an accurately weighed amount of tap water (approximately 40 ml), making sure the D_2O was minimally exposed to the ambient air. This diluted D_2O stock (Mix 1) was then used to prepare standards with deuterium contents of 155 ppm, 337 ppm, and 942 ppm. Each of the three standards was measured 12 times, and the results summarized in Table 5-2.

Table 5-2 This table summarizes repeated measures on 3 sets of standards(ppm).

	942 ppm	337 ppm	155 ppm
1	819	303	139
2	839	312	130
3	821	309	115
4	837	291	127

5	839	307	139
6	827	282	150
7	810	291	137
8	789	279	133
9	814	290	128
10	827	274	130
11	821	283	139
12	822	291	123

Table 5-3 : This table summarizes the standard deviation and the coefficient of variation of repeated measures of standards.

SD	13.41	11.90	8.72
CV / %	1.63	4.07	6.58

These results show firstly, that the measured deuterium abundance by SIFT-MS without correction is less than the theoretical abundance. Secondly, that the precision, expressed as the Coefficient of Variation (CV%; SD/Mean x100), at a level of abundance equivalent to that anticipated at equilibrium using the recommended dose of D_2O was < 2%, but at a level of abundance equivalent to baseline or natural abundance is about 6%. It appears as though the absolute precision in ppm does not vary markedly across the range, but that when expressed in relative terms appears greater at low values and lower at higher values.

In the third step, a gravimetric standard with a theoretical abundance of 692 ppm was prepared and used to determine whether differences in the sample volume of the same theoretical abundance had any impact on the measured deuterium

abundance by SIFT-MS. Repeated measurements were performed on different volumes of this standard (0.5ml, 1ml, 2ml, 5 ml, 10 ml, and 20 ml) – note that the volume of saliva samples acquired to measure TBW is usually about 1-2ml.

Table 5-4: This table summarizes the measured deuterium abundance in replicates of the same standard at different volumes of standard within the same 250 ml headspace vessel.

Replicate	0.5 ml / ppm	1.0 ml / ppm	2.0 ml / ppm	5.0 ml / ppm	10.0 ml / ppm	20.0 ml / ppm
1	529	542	551	567	572	567
2	572	587	544	546	550	571
3	597	621	580	557	564	585
4	625	589	518	578	572	607
5	595	629	568	584	584	558
6	586	594	576	616	605	587

Average	584	594	556	575	575	579
SD	32.1	30.8	23.4	24.5	18.7	17.5
CV	5.5	5.2	4.2	4.3	3.3	3.0

Table 5-5: This table summarizes the Standard deviation and the Co-efficient of variation of the repeated measures on different volumes.

These results show that firstly, once again, the measured deuterium abundance by SIFT-MS uncorrected was less than the theoretical abundance. Secondly, the precision estimate as Coefficient of Variation ranged from 3.0% at the largest sample volume rising to 5.5% at the smallest sample volume. This implies that the larger the sample volume: headspace vessel volume ratio, the better the precision.

5.2.0 Experimental study

The aim of this study was to compare the deuterium abundance determined by SIFT-MS in breath, urine and saliva specimens obtained concurrently from the same individual. Replicates of all urine and saliva specimens were also analysed independently in a blinded form by a commercial mass spectrometry laboratory (ISO-ANALYTICALS, Crewe) for comparison against the values obtained by SIFT-MS. Note that breath samples could not be analysed by IRMS.

5.2.1Methods and materials:

Protocol:

Samples of saliva, urine and breath were taken from a group of adult subjects, before and after dosing with deuterium oxide, in order to examine whether equilibration time and estimated total body water varied according to the type of fluid used. Informed written consent was obtained from all subjects (Ethical approval reference, SOMSEC095.11.) before the study was started.

The recruitment of subjects, anthropometry measurements, obtaining samples, and the analysis of the samples were conducted by the author.

Subjects:

12 healthy male subjects aged 18-60 years took part in the study. All the participants were either working in Southampton General Hospital or PhD students at the University of Southampton. They were studied at Welcome Trust Clinical Research Facility of Southampton General Hospital.

Clinical methods
Weight and height

Height and weight were measured according to the procedure indicated in the SOP (Appendix 4)

Total body water

Total body water was estimated using the water dilution technique according to SOP (Appendix 4).

Baseline samples of breath, saliva and urine were collected from the participants. Second, subjects were dosed with deuterium oxide (99.99% APE; Sigma Chemicals) according to their weight (see Table 5-6, with the aim of achieving deuterium-abundance after equilibration of between 400-800 ppm. The exact dose of deuterium oxide was given to the subjects non-diluted in a capped bottle and participants were asked to drink the entire dose using a straw. After drinking the full volume of deuterium oxide, the participants were asked to rinse their mouth with 20 ml of tap water to remove any residual deuterium. The container and the straw were re-weighed following oral ingestion of the dose to account for any dose that was retained within the container, to give the exact amount of dose ingested.

Further samples were obtained following times after dosing: at 1 h, 2h,3h, and 4h for breath; 1h,2h, 3h, for saliva; and2h,3h,and 4h for urine. The procedures for collecting samples varied with the type of fluid. Breath samples were obtained by asking the participants to blow through a disposable cardboard mouth piece which was connected directly to handheld probe of the SIFT-MS machine. Three consecutive breath samples were obtained at each time point

Saliva samples were collected using salivettes (Sarstedt) at intervals of 1, 2 and 3hs and centrifuged at 4˚ C for eight minutes at 2060×g (3000ppm). These samples were then split equally between two appropriately labelled glass vials using a pipette. One to be analysed by IRMS and the other by SIFT-MS.

Urine samples were collected at baseline 2, 3, and 4 hours. Subjects were asked to fill the pot provided for them and then void their bladders completely. Two aliquots of each sample were transferred to appropriately labelled glass vials using a pipette. One to be analysed by IRMS and the other by Sift-MS.

Table 5-6 :D_2O Dosing volumes according to weight

Subject weight	Dose of deuterium oxide
51-70 Kg	20 ml
71-100 Kg	29 ml
More than 100 Kg	33 ml

Impedance

Whole body impedance was measured according to SOP using the Impedimed SFB7 device (details Appendix 3). The measurements were made with the subjects in the supine position after resting for 5 minutes. Details of electrode placement and position of arms and legs are provided in the SOP.

Laboratory methods

Aliquots (up to 2ml) of standard, saliva or urine samples are placed into in 250ml glass bottles which are then placed in a water bath at 37 degrees centigrade; lead rings were used on top of the bottles to prevent them from floating (because the amount of the specimen in the bottles were small, the rest of the bottle would be filled by gas and therefore the bottle would float if a lead ring was not used). After 15 minutes thermal equilibration, a needle on the handheld probe is inserted into the fitting of the bottle in order to sample the headspace above the sample (the headspace would be approximately 248ml). The bottles were then left for 30 minutes and after that, sampling took place for 30 seconds. Duplicate measurements of urine and saliva samples were undertaken.

Breath samples were obtained from blowing at a fixed and constant rate through a cardboard mouth piece connected to handheld probe of the SIFT-MS. Three consecutive exhalations were analysed, each lasting for about ten seconds. Sample data were accumulated from the 74/75 ion count rate ratios and used to determine deuterium abundance.

Calculation of total body water

$TBW\ was\ calculated\ using\ the\ equations\ recommended\ by\ the\ IAEA$:

$$TBW\ (kg) = \ Dose\ 2H2O\ \frac{mg}{enrichment}\ 2H\ in\ saliva\ or\ urine(mg/kg)$$

$Should\ read$:

$$VD\ (kg) = \ Dose\ 2H2O\ \frac{mg}{enrichment}\ 2H\ in\ saliva\ or\ urine\ (mg/kg)$$

And

$$TBW\ (kg)\ = \ VD\ (kg)/1.041$$

Statistical analysis

Estimates of precision and coefficient of variation of measurements of enrichment and abundance of deuterium oxide in physiological fluids were established for repeated measurements on the same samples. Sequential measurements over time were analysed using paired-sample T tests, and repeated measures of ANOVA. Differences between different samples collected at the same time were also analysed by paired-sample T tests. The analyses were undertaken using IBM Statistics SPSS 19.

5.2.2 Results:

Precision:

As a first step the coefficient of variation of triplicate samples of breath, saliva and urine samples were calculated at each time point.

Table 5-7: The mean ± SD deuterium abundance (ppm) and coefficient of variation in within duplicate and triplicate samples at each time point..

	Mean	CV%
Baseline Breath	150.97 ±	4.67

	10.55	
Baseline Saliva	160.61 ± 14.04	6.12
Baseline Urine	152.04 ± 14.55	8.69
Breath post-dosing	722.46 ± 94.80	2.86
Saliva post-dosing	752.29 ± 108.69	2.17
Urine post-dosing	732.95 ± 108.16	3.97

To confirm the obtained C.V from duplicate and triplicate measurements, a study was conducted to measure the enrichment of a single urine and saliva sample and ten consecutive breath samples at four hours post dosing. Breath samples were obtained from ten consecutive exhalations. The C.Vs for these measurements is summarized in Table 5-8.

Table 5-8 The coefficient of variation of a urine and saliva sample and ten consecutive breath samples at four hours post dosing.

	C.V% single sample
Urine	3.7
Saliva	4.4

As it can be observed from the table that the C.V% range of single samples and within samples are similar.

To calculate TBW measurements of deuterium abundance were made in samples of saliva at 3h and in urine at 4h, as suggested by the IAEA. These results are presented below consider:

a) Change in abundance over time

b) Differences in abundance between fluids at the same time points

c) Difference in abundance between samples of breath and urine samples at various time points on the one hand and with saliva at 3 hours on the other hand (the latter value being recommended by the IAEA).

Change in abundance and enrichment of deuterium abundance over time

Table 5-9 shows the changes in abundance over time. Following a rapid increase in deuterium abundance from baseline, there was no significant change in deuterium abundance of breath samples (1 to 4 hours; repeated measures ANOVA) – suggesting that equilibrium occurred very rapidly and abundance values after 1h were equivalent. In contrast, the deuterium abundance of saliva rose rapidly to a highest value at 1h before progressively decreasing at 2 and 3h. There was a significant decrease in the abundance of saliva samples (at all measured time points between 1 and 3 hours). The deuterium abundance in urine increased more slowly with lower values at 2h and higher values, which were comparable, at 3 and 4h with a statistically significant increase in abundance in urine (from 2 to 3 hours) with no further increase at 4h.

Some of the discrepancies in the abundance values may be as a result of discrepancies in baseline values. Subsequently, changes in the enrichment of different samples over time were observed. The results are summarized in

Table 5-10 . The enrichment of breath samples did not change significantly over time (P-value: 0.117). The enrichment of saliva samples decreased significantly over all measured time points, demonstrating no evidence of an equilibration.

Table 5-9. Changes in the abundance of deuterium over time in breath, saliva and urine samples(ppm).

	1 hour	2 hour	3 hour	4 hour	P-value(repeated measures ANOVA)
A* Breath	713 ± 95	718+86	703+ 98	70+ 88	0.098
A * Saliva	776 ± 118	747 ± 103	730 ± 110		0.001
A * Urine	No sample	686± 108	750+ 111	754± 104	0.005
P-value repeated measures of anova	< 0.001	0.047	0.001	<0.001	

A*: Abundance of deuterium

Table 5-10 Changes in the enrichment of deuterium(ppm) in breath, saliva and urine samples over time.

	1 hour	2 hour	3 hour	4 hour	P-value(repeated measures ANOVA)
E* Breath	56±91	567.61±82.50	552.73± 95.13	552.94±83.48	0.117

E * Saliva	614 ± 119	585 ± 106	569 ± 114		0.001
E * Urine		534 ± 73	598 v 105.	602± 99.76	0.006
P-value repeated measures of ANOVA	0.002	0.098	0.011	< 0.001	

E*: Enrichment of deuterium

1) Differences in abundance and enrichment between fluids at the same time points

Differences between the abundance of different samples at each time point are demonstrated in Table 5-9(table should be looked at vertically). The abundance of deuterium in different samples differs significantly except for breath and urine abundance at two hours (P-value: 0.196), saliva and urine abundance at three hours (P-value: 0.125). Subsequently the enrichment in different samples was compared at the same time points, see

Table 5-10(table should be looked at vertically). The results demonstrated that the enrichment of different samples did not differ significantly at one, three and four hours but not at 2 hours. Further investigation showed that the enrichment of saliva and breath samples was not significantly different at 3 h (P-value: 0.304).

2) **Difference in abundance and enrichment between samples of breath and urine samples at near equilibration time points compared against saliva at 3 hours (the latter value being recommended by the IAEA to calculate TBW).**

The deuterium abundance in all samples were compared to saliva at 3h, which can be used as a 'reference'. Table 5-11 shows the results of baseline measurement of deuterium abundance and at time intervals after dosing with D_2O (see methods).

Table 5-11 : Mean values of abundance of D_2O (ppm) in urine and breath samples at different time points are compared with values obtained in saliva at 3 hours (N = 12 for each fluid at each time point)

	Mean	Difference from abundance in saliva at 3h* *	No. of samples (out of 12) in which abundance in saliva is greater than that of the alternative sample	P value***
A* breath 2 h	71±86	12 ± 31	7	0.205
A* breath 3 h	703± 98	27± 36	8	0.027
A* breath 4 h	703± 88	27 ± 29	8	0.008
A*urine 3 h	750± 111	-19 ± 40	1++	0.125
A* urine 4 h	742 ±117	-23 ± 20	2+	0.003

*A: Abundance
** Abundance in Saliva at 3h: 730.97 ± 110.54
+ p <0.05, ++ p <0.01 (sign test)
*** P value obtained by paired sample t-test

It can be seen from Table 2 that:

a) There is no significant difference between deuterium abundance in saliva at 3 hours on the one hand and breath at 2 hours and urine at 3 hours on the other.

b) There is lower abundance in saliva than urine both at 3 hours and 4 hours (significant at 4 hours only).

Since there were some differences in baseline abundance both within and between fluids (Table 5-12) the pattern of enrichment at 2, 3 and 4 hours differed a little from that of abundance: the enrichment in breath samples at 2, 3 and 4 hours became non-significantly lower than saliva at 3 hours; the urine samples at both 3 and 4 hours became significantly higher than saliva at 3 hours.

Since the abundance after dosing is due to baseline abundance and increment in enrichment after dosing, it is possible that the discrepancies in abundance between saliva and alternative samples at the stated times are due to differences in baseline values, differences in enrichment post-dosing, or a combination of both. The discrepancies have implications for the calculation of total body water.

To explore the cause of the discrepancy further, baseline abundance and increment in enrichment after dosing were examine separately. Table 5-12 shows the baseline abundance in breath and urine, both of which were compared to that of saliva.

Table 5-12: Mean values of deuterium abundance (ppm) in urine and breath samples at baseline compared with values obtained in baseline saliva samples. (N = 12 for each fluid)

	Mean	Difference from abundance in baseline saliva*	No. of samples(out of 12) in which the abundance in saliva is greater than that of the alternative sample	P value**
A* Baseline breath	150 $\pm$10	10 $\pm$ 21	9	0.101
A* Baseline Urine	152 $\pm$14	9$\pm$14	10+	0.042

*Abundance is saliva at baseline: 161.88$\pm$ 14.08

+P <0.05 (sign test)
**P value obtained by paired sample t-test

Baseline deuterium abundance in saliva was greater than that in breath and urine, but the difference only reached significance with urine. Therefore, the Abundance of d_2O in physiological fluids should not be used interchangeably since they could affect the calculation of total body water.

The small difference in abundance between saliva on the one hand and breath and urine (significant in the case of urine) on the other could contribute to the post-dosing discrepancy. As the abundance was greater in saliva than urine at baseline and smaller in saliva at 3 hours post-dosing, suggests that post-dosing enrichments in different fluids could also exist.

To evaluate the possibility that the differences in enrichment of urine and breath samples at specific time points are viable , the enrichment of urine and breath samples at different time points were compared against the enrichment in saliva at 3h. The results demonstrate that no significant differences exist between the

enrichment values of saliva at 3 hours and breath at 2, 3, and 4 hours. However, there is a significant difference between the enrichment values of saliva at 3 hours and urine at 3, and 4 hours.

Table 5-13 Mean values of deuterium enrichment (ppm) in urine and breath samples at different time points are compared with values obtained in saliva at 3 hours (N = 12 for each fluid at each time point)

	Mean	Difference from enrichment in saliva at 3h**	No. of samples(out of 12)in which abundance in saliva is greater than that of the alternative sample	P value***
E* breath 2 h	567.61±82.50	1.47 ± 46.59	7+	0.915
E* breath 3 h	552.73±95.13	16.35 ± 52.52	6	0.304
E* breath 4 h	552.94±83.48	16.13 ± 43.53	7	0.225
E* urine 3 h	598.14±105.85	-29.06 ± 45.72	1	<0.050
E* urine 4 h	602.01 ± 99.76	-32.93 ±31.10	2	0.004

E*: Deuterium Enrichment
**Enrichment of Saliva at 3h: 569.03 ± 114.92
***P value obtained by paired sample t-test.
+ P> 0.05 (Sign test)

Significant differences in enrichment between saliva at 3h and alternative samples at specific time points, will affect the estimation of TBW. Discrepancies compared with the 3h reference saliva sample are more likely to occur with urine collected at 3 and 4 hours, rather than breath at 2, 3 and 4 hours.

Table 5-14 shows the estimated TBW values.

Table 5-14 : Mean values of TBW (kg) from urine and breath samples at different time points are compared with TBW values obtained from saliva at 3 hours (N = 12 for each fluid at each time point)

-	Mean	Difference from TBW obtained from saliva at 3h**	No. of samples(out of 12) in which TBW from saliva is greater than that of the alternative value	P value***
TBW breath 2 h	51.53 ± 6.64	0.71 ± 5.29	5	0.651
TBW breath 3 h	53.30 ± 8.28	-1.06 ± 6.25	6	0.569
TBW breath 4 h	52.97 ± 7.12	-0.72 ± 4.87	5	0.616
TBW urine 3 h	49.34 ± 8.02	2.90 ± 5.24	11+	0.082
TBW urine 4 h	48.93± 7.94	3.31 ± 3.60	10++	0.009

** TBW from saliva at 3 h (52.24 ± 9.13)
+ p <0.05, ++ p <0.01 (sign test)

*** P value obtained by paired sample t-test

As expected, estimated TBW was lower using urine samples obtained at 3 and 4 hours than saliva samples obtained at 3 hours(this difference reached statistical significance for urine at 4 hours, P-value: 0.009), corresponding to about 6% and 7%, respectively. Furthermore, the discrepancy TBW estimated from saliva at 3h and

alternative samples (Table 5-14)in individual subjects is large e.g. standard deviation of the difference between saliva at 3 hours and breath samples at 2,3 and 4 hours ranges from 4.87 to 6.25 (corresponding to about 10-14% of difference). For urine the corresponding SD of the difference is 3.6 to 5.24 (about 7% to12 %)

Comparison of measurements of D2O abundance made by IRMS and SIFT-MS

Urine and saliva samples of all subjects were also analysed by IRMS method (48 urine samples, and 48 saliva samples).

Table 5-15: Comparison of deuterium abundance measured by Sift-Ms against IRMS in saliva and urine samples.

	Mean Sift	Mean IRMs	Mean Difference	95% confidence of intervals	P value
Baseline Saliva	160 ± 14	153 ± 3	-7 ± 14	-16 to 1	0.105
Enriched Saliva	752 ± 108	766 ± 96	14 ± 27	4 to 23	< 0.001
Baseline Urine	152 ± 14	152 ± 1	0.20 ± 14	-9 to 9	0.962
Enriched Urine	732 ± 108	736 ± 92	3 ± 24	-4 to 12	< 0.001

Table 5-15 shows that whilst the baseline measurements in saliva and urine were not significantly different from each other, the post dosing measurements (at all time points) were significantly higher with IRMS. Most noticeable is the wide standard deviation of the difference (the 95% confidence range corresponds to about 4% of the mean abundance/enrichment values).

As it can be observed IRMS and SIFT-MS produce results that are significantly different after enrichment. Bland- Altman plots are presented below in Figure 5-2 and Figure 5-3.

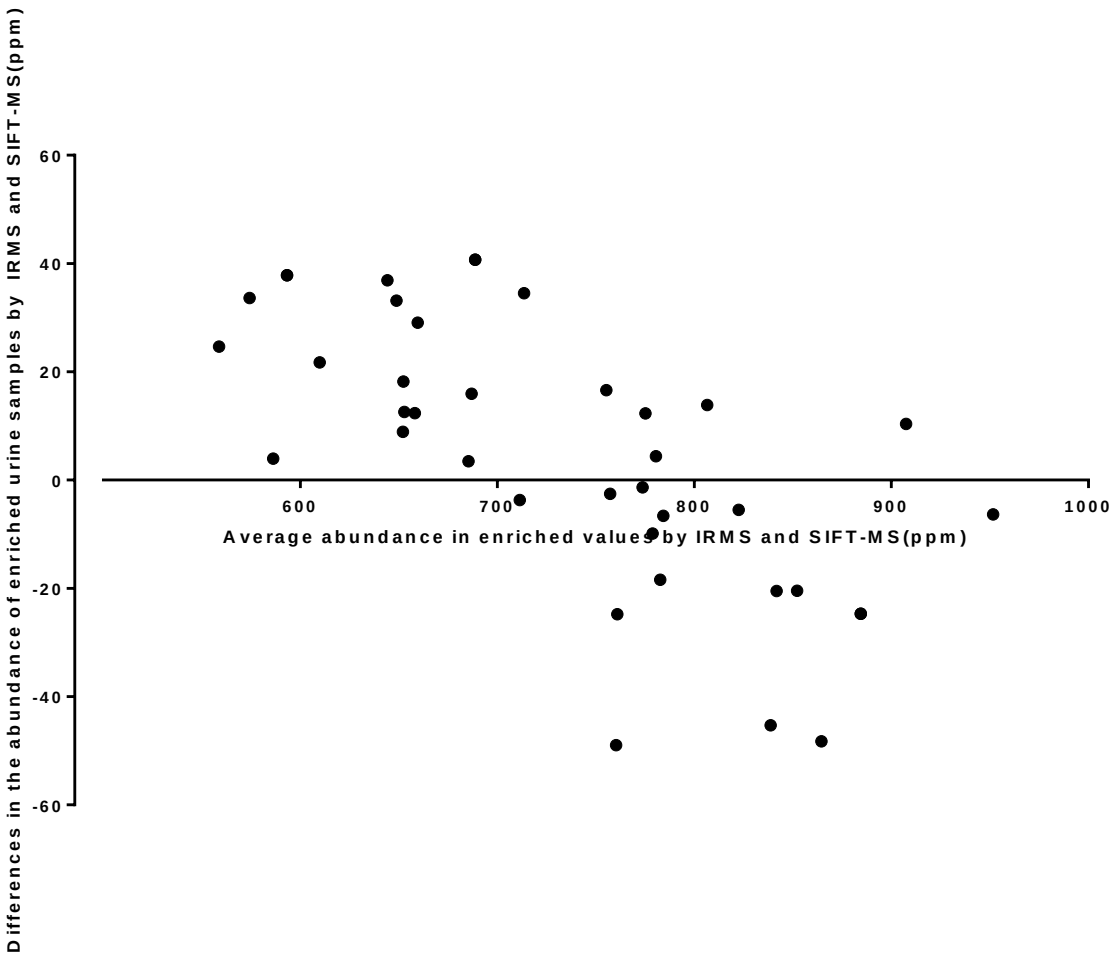


Figure 5-2: Bland-Altman plots of abundance of deuterium (ppm)in enriched urine samples by IRMS and SIFT_MS (ppm)

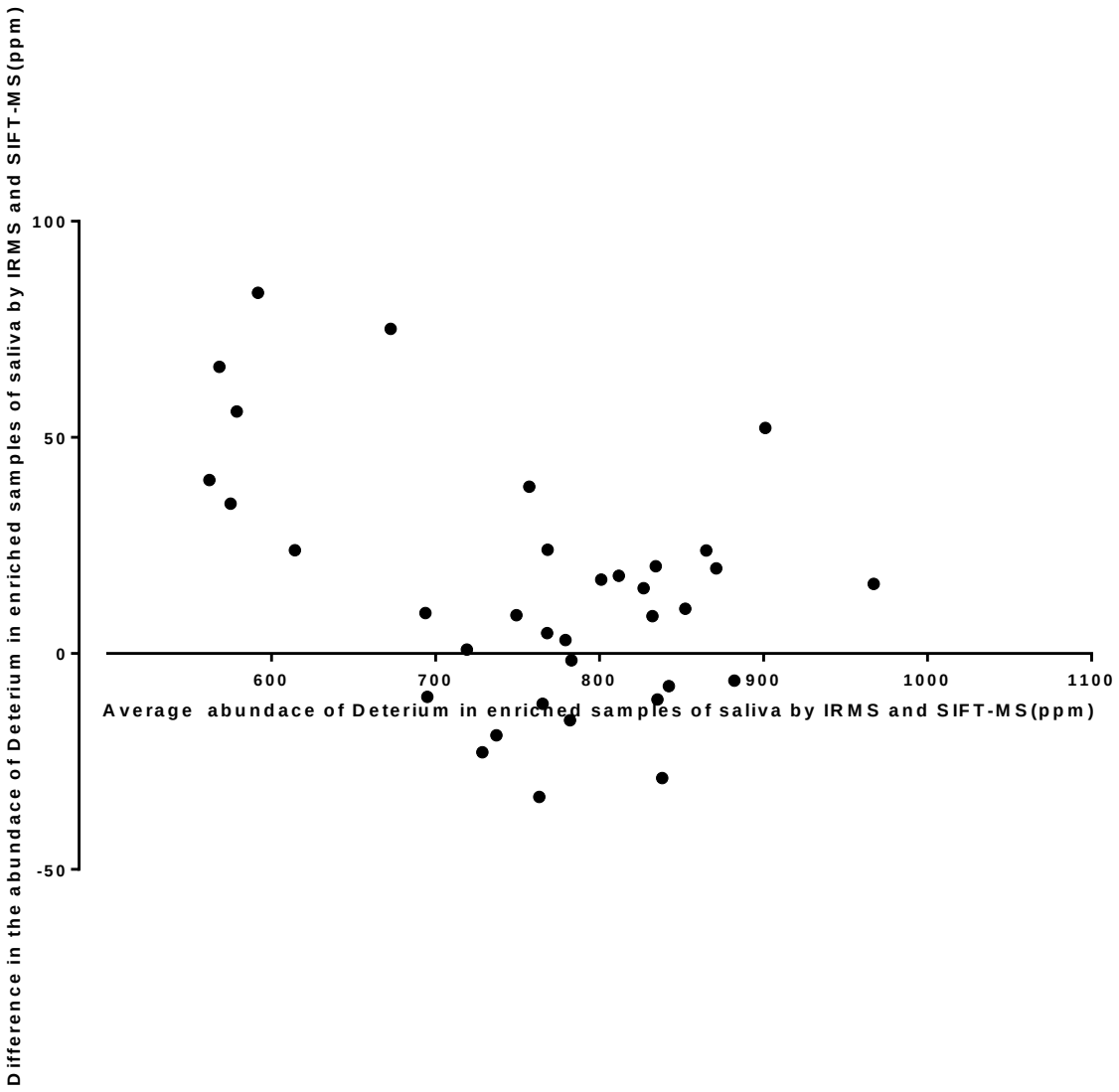


Figure 5-3 : Bland-Altman plots of abundance of deuterium (ppm)in enriched saliva samples by IRMS and SIFT_MS (ppm)

One of the aims of this study was to explore whether breath samples could be used to calculate TBW, therefore, breath samples at 3h analysed by SIFT-MS were compared against urine at 4 h and saliva at 3h analysed by IRMS.

Table 5-16 Comparison of deuterium abundance in breath at 3 hours by Sift-MS and deuterium abundance in saliva and urine by IRMS.

	IRMS	Sift-Ms	Mean Difference	95% confidence of intervals	P value
U4h IRMS-Breath 3h	748.25 ± 87.32	703.70 ± 98.34	44.55 27.76	26.91 to 62.19	< 0.001
S 3h IRMS-Breath 3 h	741.95 ± 87.68	741.95 ± 87.68	38.24 ± 25.26	22.19 to 54.30	< 0.001

The main reason for measuring deuterium abundance in human samples in this project is to predict TBW. Below are the mean values of TBW from different samples analysed by IRMS and SIFT-MS are shown in Table 5-17.

Table 5-17: Summary of values calculated for TBW by IRMS and Sift-MS

	Mean ± SD
TBW IRMS saliva 3h	49.65 ± 6.00
TBW IRMS urine 4h	49.04 ± 5.99
TBW SIFT-MS saliva 3h	53.30 ± 8.28
TBW SIFT-MS urine 4h	49.06 ± 8.01
TBW SIFT-MS breath 4h	52.11 ± 9.10

	Mean Difference	95% confidence of intervals	P value
TBW SIFT-MS Saliva 3 h -TBW IRMS Saliva 3 h	2.46 ± 5.47	-1.01 to 5.94	0.148
TBW SIFT-MS Urine 4 h -TBW IRMS Urine 4 h	0.01 ± 3.03	-1.91 to 1.94	0.987

Comparison of IRMS and BIA

The mean values for TBW derived from D_2O dilution technique from urine and saliva samples, and SFB7 bioelectrical impedance are summarized in Table 5-18.

Table 5-18: Summary of TBW values calculated by deuterium enrichment in saliva and urine by IRMS and bio-electrical impedance.

TBW by IRMS using Urine @ 4h (litres)		49.04
TBW by IRMS using Saliva @ 3h (litres)		49.65
TBW by bioelectrical impedance SFB7 (litres)		46.23

Table 5-19: Comparison of TBW values calculated by deuterium enrichment in saliva and urine by IRMS and bio-electrical impedance.

	Mean Difference	95% confidence of intervals	P- Value	Correlation
TBW by IRMS using saliva @ 3h- TBW by SFB7	3.42 ± 2.78	1.64 to 5.19	0.001	0.949
TBW by IRMS using Urine @ 4h- TBW by SFB7	2.81 ± 3.16	0.80 to 4.82	0.01	0.925

Although there were high correlations between estimates of TBW obtained by BIA and IRMS, BIA systematically and significantly underestimated TBW about 3 litres (Table 5-19) corresponding to about 6% of the mean TBW. In addition the 95% confidence range of the difference was about 8%.

Discussion:

The measurement of body is a key component in the assessment of body composition. Hydrometry is the reference method of measuring TBW. Body water can be sampled in the form of saliva, urine, plasma, breath or human milk, and the enrichment of deuterium can be measured by isotope ratio mass spectrometry (IRMS) or FTIR spectrometry. SIFT-MS is a newly developed device that can be used to measure deuterium abundance in urine, saliva and breath samples. Real-time determination of deuterium abundance using breath samples has clear advantages in research settings. The aims of this study were to first explore differences in abundance and enrichment of different samples over time. Second, compare enrichment and abundance values of different fluids at the same time points to investigate whether these samples can be used interchangeably. Third, to compare enrichment values from different samples produced by Sift-MS against values generated by IRMS as IRMS stands the reference device of measuring deuterium abundance.

Changes in abundance/enrichment over time

The study found that that the time course for deuterium enrichment in body fluids at ≥1hour varied according to type of fluid. No significant changes were found in breath after the first hour, but significant changes occurred in saliva and urine. The reasons for these differences are not clear but rapid equilibration might be expected to occur in breath due to the thin capillary membrane that separates the rapidly flowing blood through the lungs and the air perfusing the lungs. The slower equilibration in saliva and urine may be due to slower penetration and equilibration of labelled water in the salivary glands and kidneys. In saliva there may also be delay in storage of saliva before it is actively secreted. In the case of urine, the interval between voiding (hourly intervals between 2 and 4 hours) may have contributed to the apparently slow equilibration since the urine collected for analysis represented the mean value of all the urine collected over a period of time.

In contrast the breath sample was collected at a point in time. These differences in apparent equilibration time have implications for protocols for measuring TBW.

It would have been desirable to have extended the period of sampling beyond 3 hours for saliva and beyond 4 hours for urine, so that more definitive information about the equilibration could have been

Other studies show that equilibration of D_2O in saliva and plasma is reached by 3 hours [178] and [179] . The time required for equilibration of D_2O in the body water is a point of critical concern. *Lukaski et al* [180] showed that on the average plasma and saliva deuterium abundance peak by two hours following administration and reach equilibration after three hours. *Schloerb et al* [179] also reported an equilibration after three hours of D_2O ingestion in saliva samples. The lack of other data on the time course of equilibration in breath samples makes our data more novel. There is also a lack of data on equilibration times in urine samples, although these may depend on instructions to empty the bladder shortly before sampling (e.g. half an hour in the study of Jennings et al [181]).

Differences in abundance/enrichment between fluids at the same time point

Differences between the abundance and enrichment of different samples are of great importance .In the current study, there were significant differences in abundance between fluids at all individual time points (and also for enrichment, apart from 2h, where the differences did not reach statistical significance). Apart form the reasons about equilibration time, which are discussed in the previous section, there is some fractionation associated with the change from a liquid to a gaseous phase. Such fractionation tends to produce lower deuterium enrichment (heavier than H_2O) in the gaseous phase. This may contribute to the lower enrichment of isotope in breath compared to urine and saliva, in which water remains in the liquid phase.

Unfortunately no other studies have compared deuterium enrichment values in urine and saliva samples; however, a few studies have compared deuterium enrichment in saliva and serum samples. *Lukask*i et al [180] compared deuterium abundance at various times in plasma and saliva after oral administrations of D_2O and reported the deuterium abundances were higher in saliva than plasma during the first hour after ingestion of D_2O. However, during the remainder of the

sampling period the D_2O concentrations in plasma and saliva reached unity. *Taggart et al* [182] measured D_2O in pregnant women and reported three hour post-ingestion saliva/serum D_2O concentrations ranging from 1.05-1.34.

Comparison of enrichment/abundance in breath and urine samples near equilibration time points on the one hand with saliva at 3 hours on the other hand.

Since the IAEA recommends that D_2O measurements in saliva at three hours can be used for calculation of TBW, enrichment of samples of breath and urine obtained at various time points after dosing were compared against this 'reference'. For breath obtained at 2, 3, and 4 hours, there was no significant difference with the 'reference' saliva samples. For urine obtained at 3 and 4 hours, there were significant differences with the' reference' saliva samples. This suggests that not all types of fluids obtained at the same time can be used interchangeably for estimation of TBW (see above discussion for possible explanations).

Comparison of measurements of D_2O abundance made by IRMS and SIFT-MS

IRMS is generally considered to be a reliable (precision 1% according to IAEA) and accurate method for measuring D_2O enrichment in liquid samples. Although it is not generally available for routine use, and is expensive requiring skilled operators, it was used as a reference method to explore the extent of agreement obtained with results generated by SIFT-MS. The significant discrepancies in the mean results and wide discrepancies between individual differences (suggested by the large SD of the differences) raises important questions about their cause. However, inspection of the precision of SIFT-MS suggests (see section 4.1) that it can explain most of the difference between methods. However, the systematic bias between the two methods (difference between the mean results) remains unexplained.

Comparison of TBW measured from DDS by IRMS and BIA

BIA using the SFB7 machine and equations was found to underestimate TBW obtained by IRMS by about 3 litres (SD also about 3 L). The results are within the range of validation studies carried out by other workers. *Haroun et al,* found that Tanita underestimated TBW by 1.5 litres in black females [183].

The results of this study showed that, there was a significant difference between values of enrichment produced by saliva and SIFT-MS. SIFT-MS is much less

expensive and accessible; however more research is required to adjust the enrichment values to that of a reference method.

Chapter 6 Longitudinal study of children with CD

6.0.0Introduction:

Children with IBD present with varying degrees of under-nutrition, manifest by growth faltering, which may or may not be adequately corrected during treatment. Growth starts to falter in children with IBD often before gut symptoms such as pain and bleeding are recognised. By the time they present to a clinician generally two thirds of children with CD and one third with UC have lost weight. These children are relatively shorter, lighter and thinner (they have a lower Body Mass Index Standard Deviation Score) than their peers with delayed d skeletal maturation and onset of puberty. Our preliminary work has identified a wide spread of age-adjusted heights and weights in the clinic with some children showing profound height or weight deficits, with some being particularly thin, whilst others are relatively tall and overweight.

Nutritionally, the factors that contribute to poor growth and weight loss are some combination of inflammation, diarrhoea, direct effects of disease on gut function, loss of appetite and decreased food intake, poor food choices and specific nutrient deficiencies. Other factors such as corticosteroid therapy may also play a role. Each factor is recognised as contributing to the problem, but their relative importance and interactions are poorly defined.

In addition to growth deficits, body composition abnormalities may exist in children with IBD. The standard markers of growth (height and weight for age) do not provide a picture of the composition of tissue. If tissue is deposited disproportionally, the majority of the weight gain in these children may be due to gain in fat compartment of the body rather than the balanced pattern of tissue accretion. This would imply that the balance of energy and nutrients does not adequately meet the needs of the child and may lead to poor growth, worsening inflammatory state and increased risk of relapse.

Few studies have been conducted on body composition in children with IBD and so the extent to which the excess body fat and depleted lean mass affect children with IBD is largely unknown, which makes it difficult to assess their nutritional status. In addition, much of our knowledge in this area has been acquired using research methods that are not suitable for use in routine clinical settings. Further research is

required on changes that occur in terms of body composition in children with IBD. A greater understanding of these changes may make it possible to use body composition measurements as means of evaluating growth and, therefore, assessing the efficacy of different interventions in these children. In addition, there is a need to determine the utility of approaches that can be applied in clinical settings as part of the nutritional assessment of the patient.

The aim of this work is to first assess whether in addition to any differences in growth assessed by weight and height measurements, there are abnormalities in body composition (excess fat mass, and depleted lean mass) in children with IBD . In addition if such disturbances exist this work aims to examine whether they can be corrected by current treatments.

6.1.0Methods and materials:

6.1.1 Study design

This study was embedded within a much larger prospective cohort study. Ethics approval was granted from the Isle of Wight, Portsmouth and South-East Hampshire, local research ethics committee. LREC NO 09/H0501/70.

Twenty children were recruited between December 2010 and April 2012. Children, referred to the outpatient clinic were eligible for recruitment if the clinical and biochemical suspicion of Crohn's disease was high. Children were excluded if the diagnosis of Crohn's disease was not highly probable according to the lead physician's opinion or if logistics prohibited them completing the study protocol.

The first study visit occurred at the time of diagnostic upper and lower gastro-intestinal endoscopy. After this, children were started on 'usual' treatment; the choice of exclusive enteral nutrition or corticosteroids was at the discretion of the patient's lead clinician, the child and their family. Most of the children were treated with exclusive enteral nutrition and received approximately 8 weeks of Modulen® IBD (Nestlé HealthCare Nutrition, Croydon, UK) before starting a phased food re-introduction and gradual withdrawal of Modulen®. Visit 2 occurred approximately five days into initial treatment, usually while children were still in hospital. Visit 3 occurred after around two weeks of disease treatment. Visit 4 occurred at approximately seven weeks around the time of planning food re-introduction at the

end of exclusive enteral nutrition. Subsequent visits occurred at three, six and twelve months post diagnosis. Children treated with steroids received 3-5 days of intravenous treatment followed by 2 weeks high dose oral corticosteroid before weaning over 8 weeks, but were studied at the same time points as the children receiving EEN. Visit intervals over the first year of treatment are demonstrated in Figure 6-1.

In this thesis, the detailed measurements of body composition were made on enrolment and at 12 months.

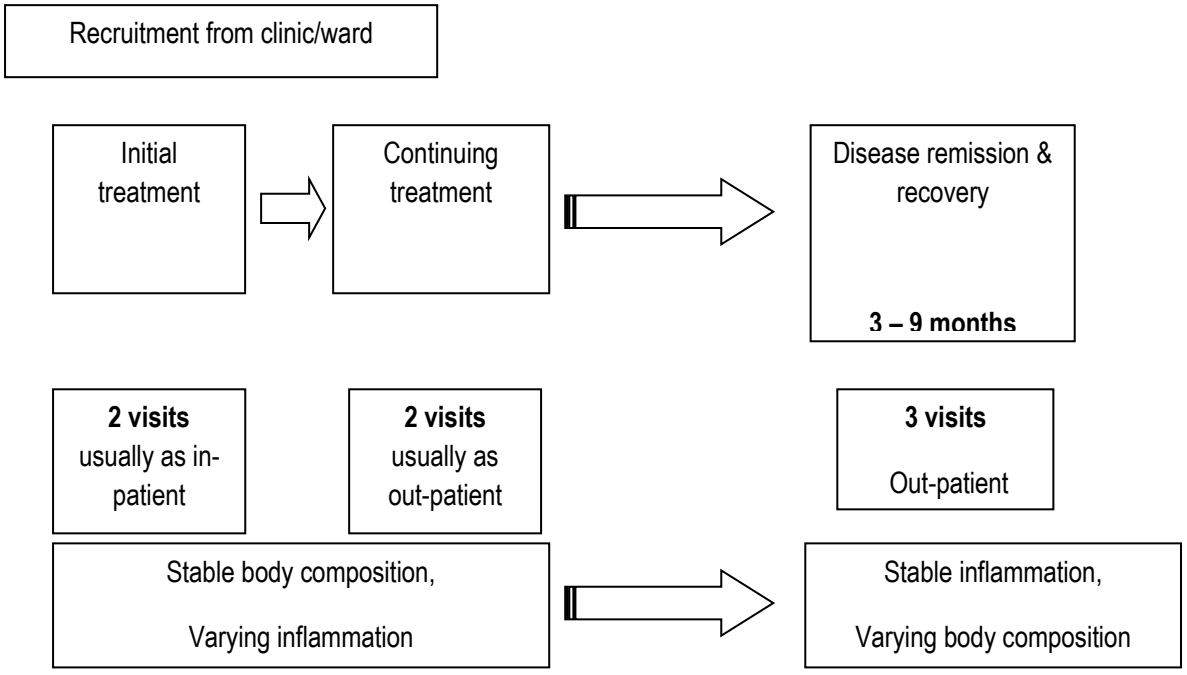


Figure 6-1 Visit intervals over the first year of the study.

Table 6-1: Drug therapy used at each visit by individuals.

ID	Visit	Treatment used
1	4th and 5th	5-ASA
2	0	5-ASA
6	0	5-ASA
10	1 and 5th	Prednisolone
15	0	5-ASA
18	0	5-ASA
21	0 and 2nd	5-ASA
22	0	5-ASA

6.1.2 Growth and Body composition measurements

Anthropometric measurements (height, weight, and skinfold thickness) together with a measure of multi-frequency bioelectrical impedance spectroscopy (BIS) to provide further information on body composition were conducted at each visit.

Height was measured with the head in the Frankfort plane and weight was measured in light clothing after voiding; both measures were performed on a single occasion at Southampton General Hospital Welcome Trust Clinical Research Facility using validated equipment (a digital scale to the nearest 0.1 KG and a stadiometer nearest 0.1 cm (Leicester Height Measure).). These measurements were made according to the related Statement of Purposes (SOP) available in Southampton Centre for Biomedical Research (SCBR) (see appendix 2)
Height and weight measurements were then converted to Standard Deviation Scores (SDS) against UK 1990 Growth Charts (LMS growth programme; Harlow Healthcare, South Shields UK; www.healthforallchildren.co.uk).

Mid upper arm circumference was measured on the non-dominant side of the body according to the existing SOP available in SCBR (see the Appendix 2). Skinfold thickness measurements at triceps, subscapular, biceps and suprailiac were taken in triplicate from the non-dominant side of the body using a skinfold calliper (Holtain Ltd. Crymych, UK) following the method of *Tanner and Whitehouse* [164] from the SOP available in SCBR, see the appendix. In all but one child, triceps skinfold (TSF) and mid-arm circumference (MUC) were also measured and used to determine Upper Arm Muscle Area (UMA); both TSF and UMA were then expressed as SD scores according to *Anthropometric standards for the assessment of growth and nutritional status developed by Frisancho* [165].

BIS was measured using SFB7 which is multiple frequency bioelectrical impedance analysis instrument operating in tetra-polar (4 leads - 2 current source and 2 voltage sensing) mode**.** The Impedimed SFB7 is a multiple frequency bioelectrical impedance analyser. The measurements were conducted using the relevant SOP that is available in SCBR, Southampton General Hospital (see appendix 3).

In addition , total body water by deuterium oxide dilution technique, body volume by air plesythmography (BodPod) and bone mass by DXA were measured on two occasions – once during active disease (Days 0-1) and again when in disease remission (approximately one year after the initial visit). The Standard Operating Procedure for each of the methods can be found in the appendix.

Deuterium oxide dilution technique:

Saliva samples were used to measure changes in deuterium abundance following the administration of a single dose of D_2O. For the initial visit 5.4 grams of D_2O was diluted in 49 mLs of tap water and 27 mLs of this stock solution was administrated to the patients. However, it was found during the study that whilst this level of dosing is appropriate for measurement by IRMS, and SIFT-MS was not sensitive enough to be used for calculation of TBW in this case. In the next measurement of deuterium oxide dilution space after one year, neat doses of D_2O were administrated according weight (both SOPs can be found in the (appendix 4).

Table 6-2 deuterium dosing volumes on the 7th visit

Subject weight	Dose of deuterium
51-70 Kg	20 ml
71-100 Kg	29 ml
More than 100 Kg	33 ml

6.1.3 Disease activity

Disease activity was scored using the Pediatric Crohn`s Disease Activity Index (PCDAI) [29] for children with CD. The PCDAI offers a score drawn from 11 variables (3 historical, 5 physical examination, and 3 laboratory) which ranges from 0 to 100, with higher scores demonstrating greater disease activity.

6.1.4 Statistics:

Power Calculation:

The primary outcome variable for the longitudinal study is the change in lean tissue over the initial year of treatment. As previous studies have not been able to consistently demonstrate an increase in lean tissue with initial treatment, there was little available to construct a secure power calculation. However, it was possible to predict the likely minimum effect size from that which could be expected in a group of otherwise healthy children growing normally over the study period of similar ages where the mean difference in FFM was 2kg, and the standard deviation of the difference was 1kg/year [101]Using these values in a paired t test , then a sample size of 6 children would have an 80% power to detect a 2kg difference in FFM, assuming a two-tailed test and $p<0.05$. Alternatively, using the predicted changes in Fat Free Mass Index of 0.5 kg/m^2 over 12 months, with a standard deviation of the difference to be 0.4 kg/m^2 [101] , then a sample size of 9 children would have an 80% power to detect a 0.5 kg/m^2 difference in Fat Free Mass Index, assuming a two-tailed test and $p<0.05$.

Apart from descriptive statistics, paired sample t -tests were used to examine differences between visits. One sample t -tests were used to compare differences from the UK 1990 reference SD scores. The analyses were undertaken using SPSS 18 (Chicago, USA). The results are presented as mean and SD.

6.2.1 Results:

The mean Height SDS, weight SDS, BMISDS, UMA SDS, TSF SDS, and PCDAI of all subjects at each visit are summarized in Table 6-3.

Table 6-3: Anthropometry data at each visit of the follow up.

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Height SDS	-0.52± 1.27	-0.55± 1.28	-0.49± 1.27	-0.42± 1.25	-0.48± 1.24	-0.46± 1.27	-0.35 ± 1.28
Weight SDS	-1.60± 1.47	-1.68± 1.46	-1.43± 1.47	-1.20± 1.29	-0.90 ± 1.29	-0.85 ± 1.36	-0.71 ± 1.44
BMI SDS	-1.85± 1.33	-1.93± 1.33	-1.61± 1.35	-0.71± 1.02	-0.79 ± 1.15	-0.69± 1.17	-0.63 ± 1.16
UMA SDS	- 1.38±0.77	-1.41± 0.71	-1.31± 0.81	-0.98 ± 0.90	-1.01± 0.83	-0.95 ±0.84	-0.90 ± 0.83
TSF SDS	-0.50± 0.57	-0.60± 0.46	-0.40 ± .55	0.10 ± 0.56	-0.05± 0.55	0.00 ± 0.75	0.43 ± 0.58

PCDAI	34± 11.24	20.32 ± 9.69	13.23 ± 11.38	9.00 ± 7.96	9.55 ± 8.11	11.26 ± 10.51	9.57± 8.37

Overall, it demonstrates that the subjects were short (HT SDS -0.52 ± 1.27) and underweight (BMI SDS -1.60 ± 1.47), with wide variations between subjects at diagnosis (Visit 1) - standard deviations are substantially greater than 1, which is by definition the value of the reference population. The standard deviation of the change was substantially greater for weight and BMI SD scores than height SD scores. In addition the anthropometry at diagnosis suggests that there is a greater depletion in muscle tissue compared to adipose tissue SD scores (UMA SDS: -1.38 ± 0.77 compared to TSF SDS: -0.50 ± 0.57).

The PCDAI at diagnosis (mean value is 34± 11) suggests that CD was active, since only a score < 10 indicates clinical remission. Following treatment the PCDAI progressively decreased until 4th visit (approximately 7 weeks), after which there was a small increase, but by the time of the final visit (approximately 48 weeks) the mean value was in the remission range.

During the seven visits, which took place over a 48 week period, there was a small non-significant improvement in height SDS, which was accompanied by greater and significant improvements in weight and BMI SD scores. There were also significant improvements in both UMA and TSF SD scores see Table 6-4.

The pattern of change of anthropometric measures in individual subjects is demonstrated in Table 6-4.

Table 6-4: changes in anthropometry between the initial and final visit.

	Visit 1	Visit 7	Mean difference	P value	95% confidence
Height SDS	-0.52± 1.27	-0.35 ± 1.28	0.17 ± 0.46	0.125	-0.05-0.39
Weight SDS	-1.60± 1.47	-0.71 ± 1.44	1.09 ± 0.80	0.001	0.56-1.20
BMI SDS	-1.85± 1.33	-0.63 ± 1.16	1.09 ± 69	0.001	0.14-1.53
UMA SDS	-1.38±0.77	-0.90 ± 0.83	0.36 ± 0.42	0.038	0.26-0.65
TSF SDS	-0.50± 0.57	0.43 ± 0.58	0.72 ± 0.50	0.004	0.30-0.74
PCDAI	34± 11	9 ± 8	-26 ± 13	0.000	-31.19-(-17.65)

Table 6-4 show that the change in height between subjects during the follow up period was variable. Three subjects appeared to show a rapid increase in SD scores (shown in the colour green in all the Figures); two subjects demonstrated a decrease in SD scores (shown in the colour red); and the remaining subjects showed little or no change in SD scores (shown in the colour blue). Regression analysis of the individual subjects confirmed the graphic display, two subjects significantly improved height SD scores over time; height SD scores decreased significantly in two subjects; and the remaining subjects showed no significant change with time. Absolute changes in height are shown in Figure 6-3. For comparison with SD scores see Figure 6-2. There was a concordance between the two indices of growth displayed on the figures. Subjects who grew rapidly according to increase in height SD scores also grew rapidly in absolute height. The two subjects who experience a decrease in their height SD scores demonstrated little or no increase in absolute height. The remaining subjects generally demonstrated intermediate change in absolute height.

All but 4 subjects had negative BMI and weight SD scores at diagnosis. Of the 11 subjects who were wasted (BMI SD score <-2) 2 were in group 1, 3 were in group 2 and the rest were in in group 3. There were only two children who were stunted and wasted (height SDS<2 and BMI SDS<2), one in group 2 and the other in group 3, and no children who were stunted alone. Initially wasted Individuals below a height SD score of less than 2 are classified as being short. There was no relationship between the changes in height SD scores and the initial height SD score according after conducting GLM.

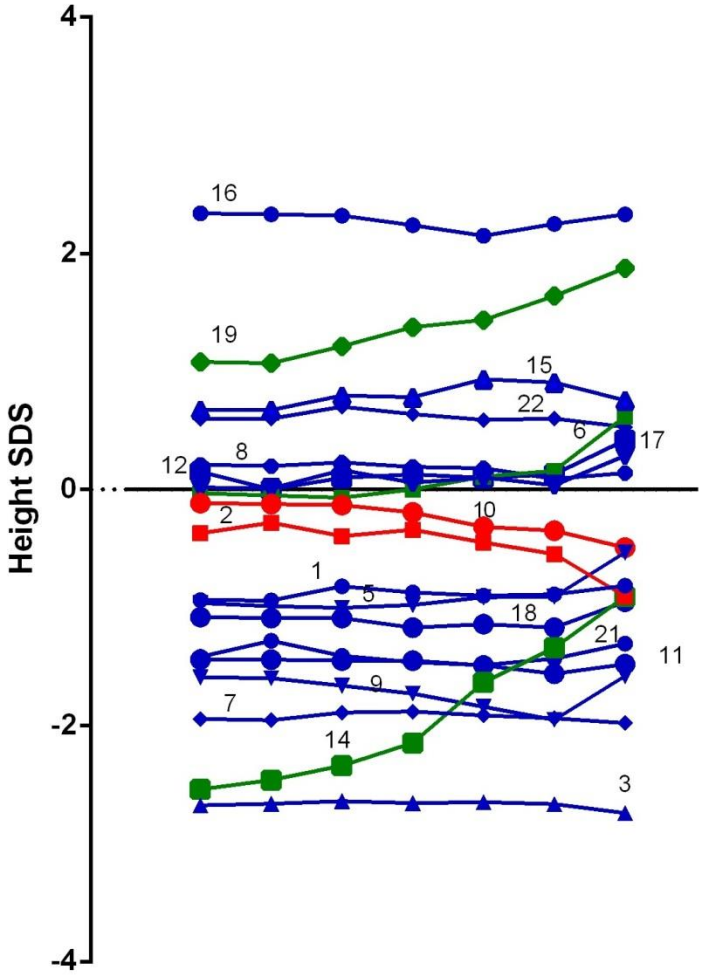


Figure 6-2 Changes in Height z-scores of each individual child in the 6 follow up visits

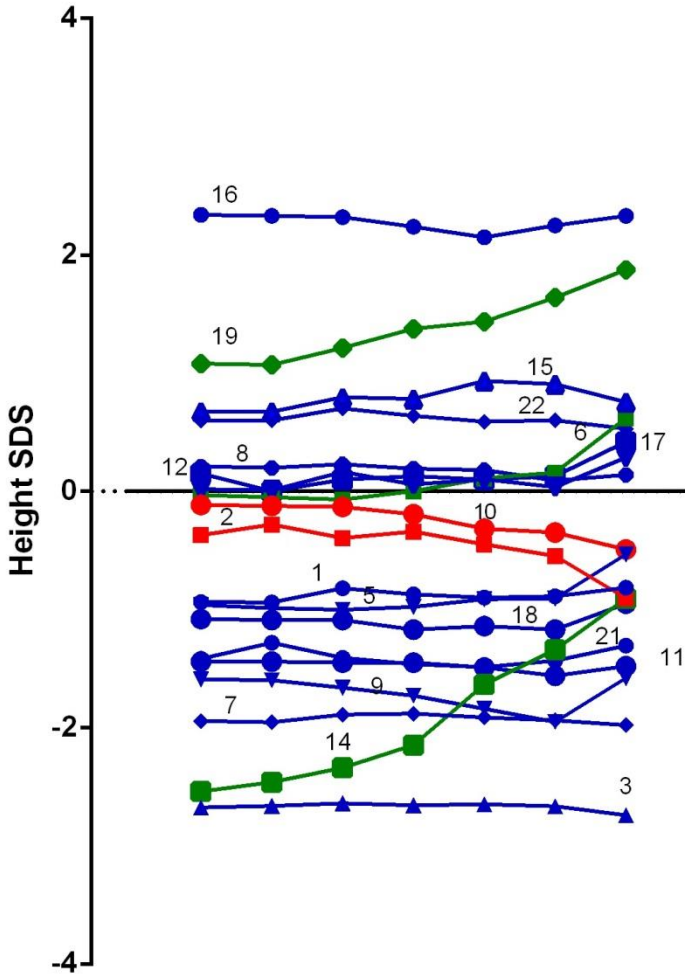


Figure 6-3 Changes in height (cm) of each individual child at the six follow up visit.

More detailed examination of data in a statistical model (General Linear Model, with subject as the fixed factor) showed that, there were significant differences between subjects in the changes in height SD scores over time, which persisted after adjustment for baseline height SD score and also after adjustment for observed PCDAI values at each visit. The same conclusion was reached when the model incorporated absolute height instead of height SD scores. The PCDAI was inversely related to changes in absolute height (P = 0.010) but the relationship was not significant when height SD scores were used in the model (P =0.205)

The changes in weight SD scores are shown in Table 6-4. In contrast to height SD scores, which showed no significant difference between the first and last visit (mean difference: 0.17), there was a highly significant associated increase in weight SD scores (mean difference: 1.09). The patterns of change in weight are shown in Figure 6-4 (SD scores) and Figure 6-5 (kg). Both graphs illustrate an increase in

weight, with a general concordance in the magnitude of the change. For comparisons with the changes in height SD scores, the same colour schemes are used (green subjects showed significant gain in their height SD scores, red subjects experienced a decrease in their height SD scores, and blue subjects showed little or no change in their height SD scores.).

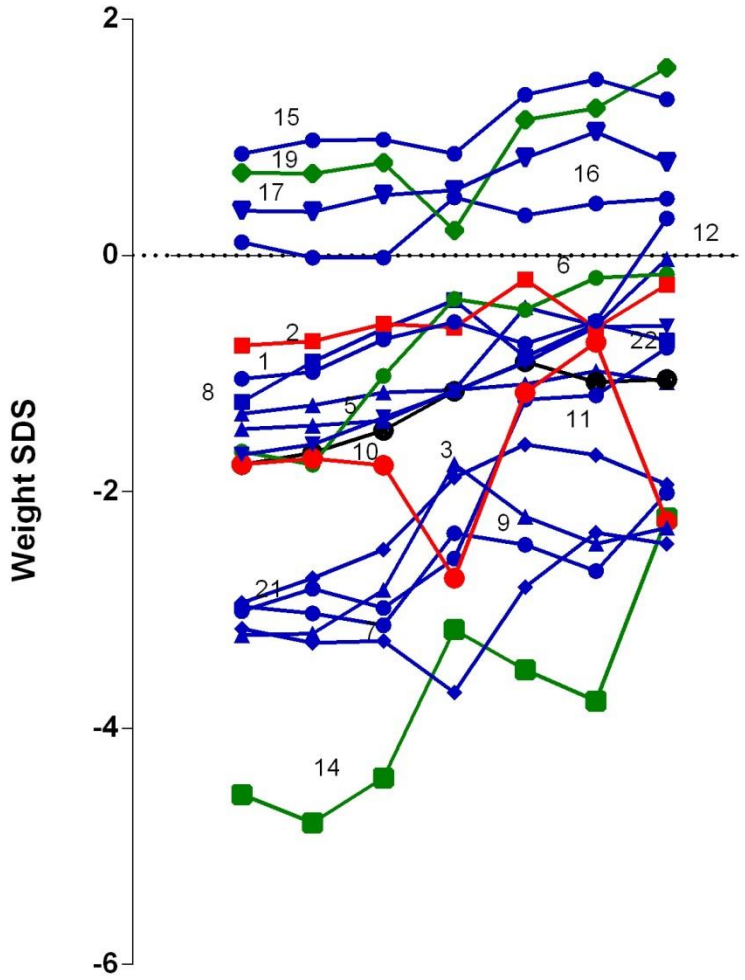


Figure 6-4 Changes in weight SD score of individuals in six follow up visits.

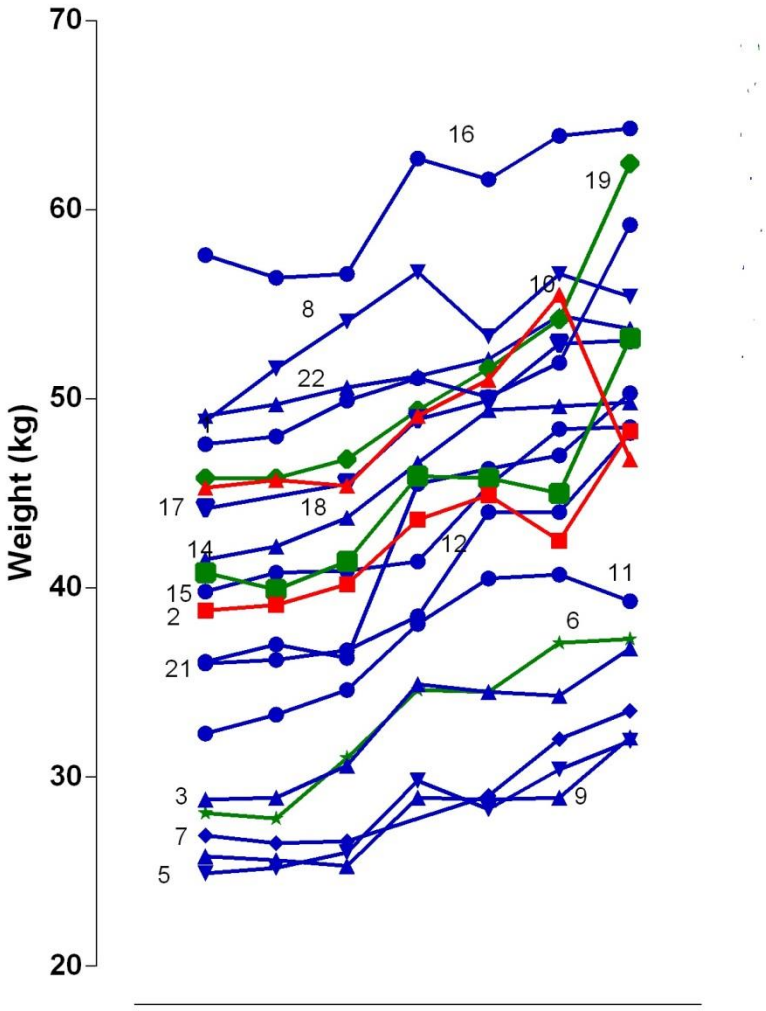


Figure 6-5 : changes in weight (kg) of individuals in six follow up visits.

Four observations about the changes in weight and height are noteworthy. First, there were greater intra-individual variations in weight SD scores over time than height SD scores. Second, there was a general concordance between increase in height SD scores and weight SD scores (compare Figure 6-2 and Figure 6-4). Third, there was sometimes dissociation between growth in height and weight. For instance, subject 5 and 21 had major increase in weight in the absence of a significant increase in height SD scores. Fourth, there were significant improvements in weight SD scores in all subjects, albeit to a lesser extent in the two subjects who did not grow in height, according to height SD scores (ID: 2 and 10).

Changes in body composition were also assessed using upper arm anthropometry and DXA.

The changes over time in UMA SDS and TSF SDS in individual subjects are shown in Figure 6-6 and Figure 6-8. As it can be observed from the figures subjects behave

differently in terms of their UMA SDS over the period of 48 weeks of follow up. More detailed examination of data in a statistical model (General Linear Model, with subject as fixed factor) showed that the change in UMA SDS is inversely related to PCDAI (P value= 0.010).

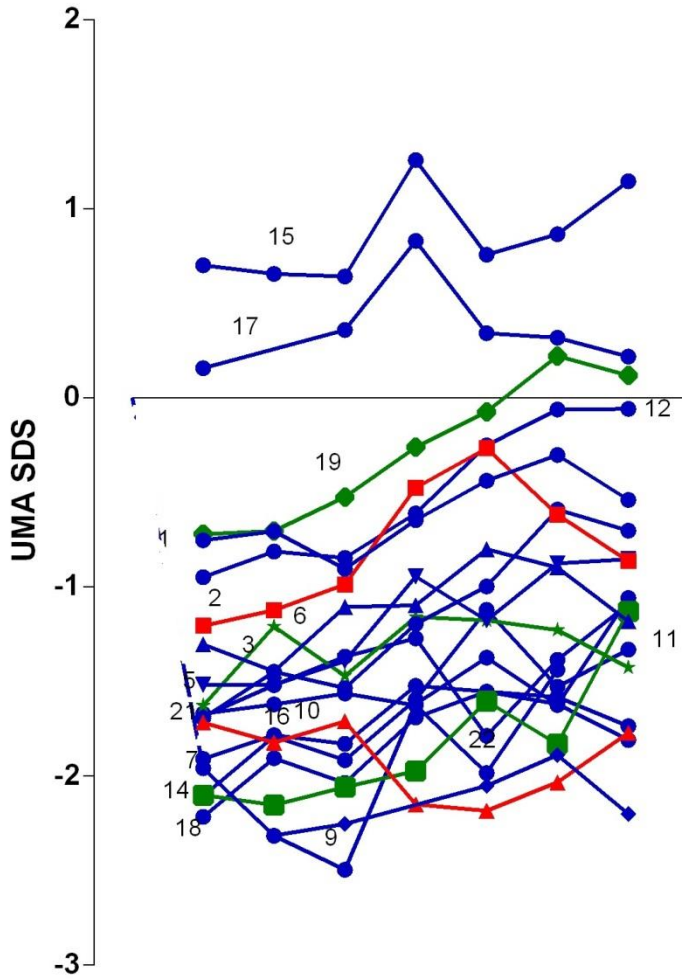


Figure 6-6 Changes in UMA SDS of individuals in the six follow up visits.

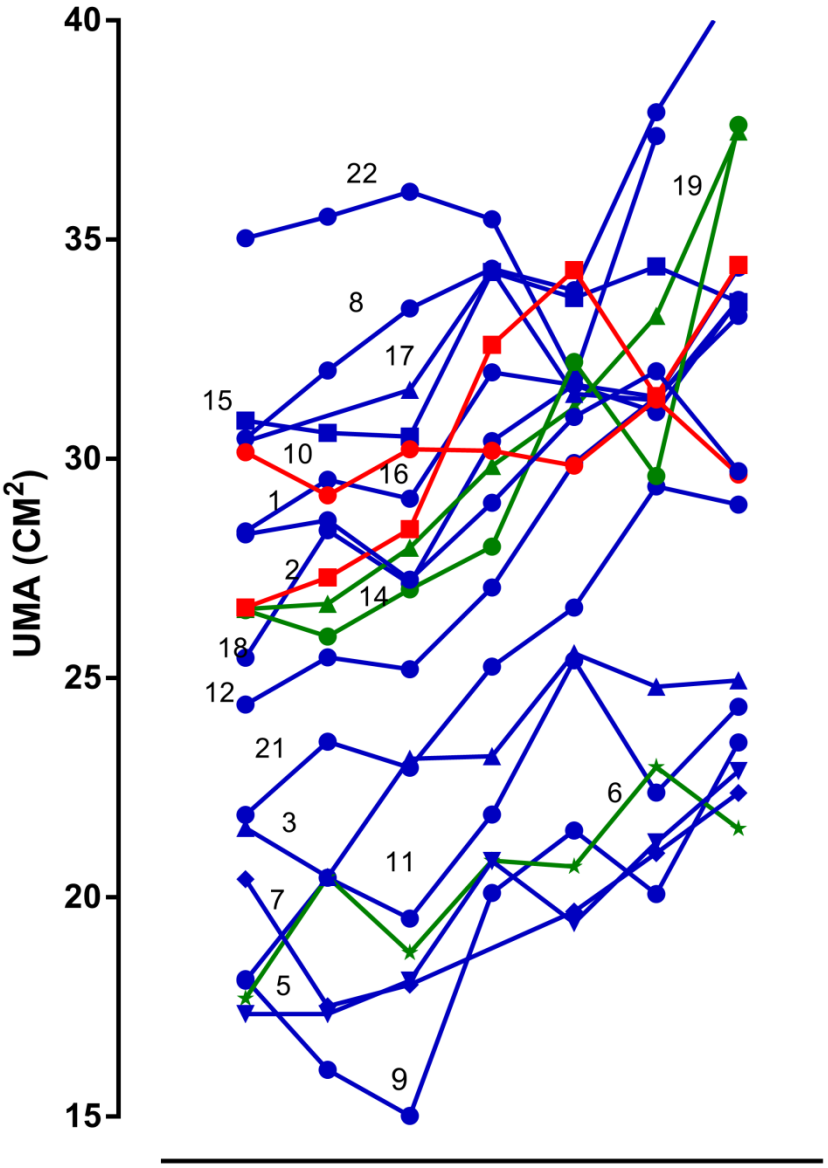


Figure 6-7 Changes in UMA (cm2) in the six follow up visits.

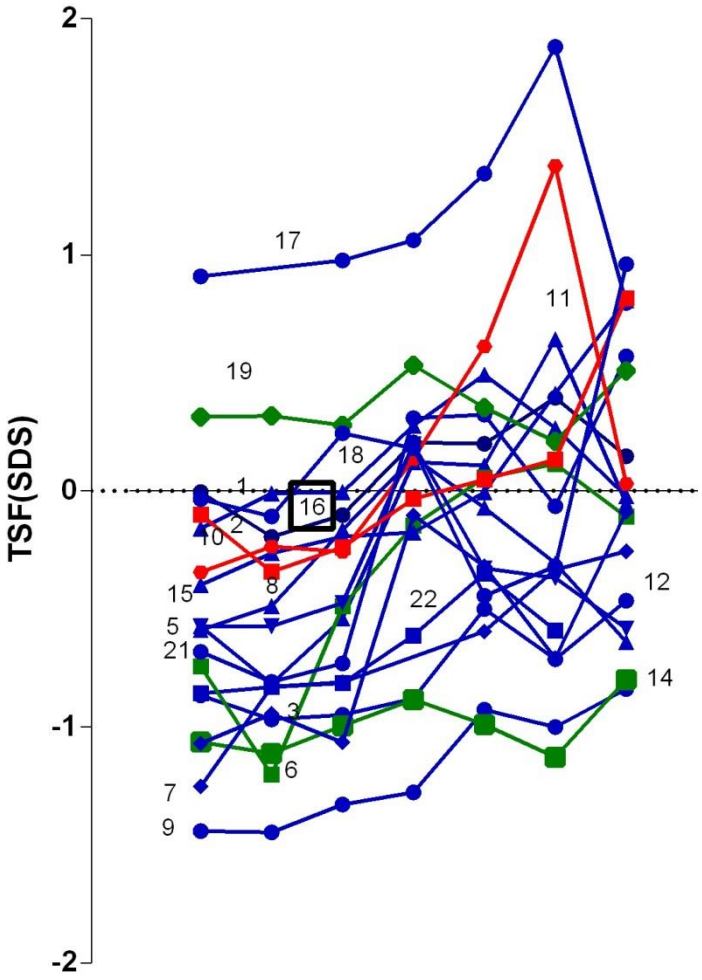


Figure 6-8: Changes in TSF SDS of individuals in the six follow up visits.

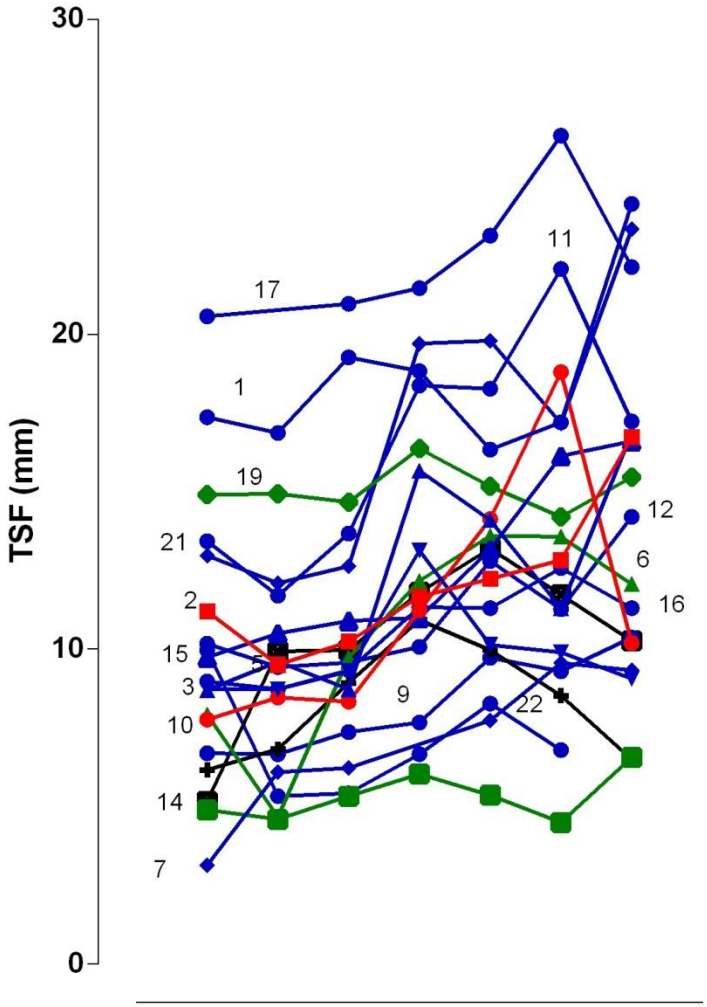


Figure 6-9 Changes in TSF (mm) of individuals in the six follow up visits.

DXA measurements of body composition undertaken in 18 subjects at the beginning and end of the follow up period showed a significant increase in lean mass (4.98 ± 2.95 kg; P = <0.001) fat mass (3.27 ± 1.50 kg; P =< 0.001) , and total body mineral content 0.089 kg). Lean body mass accounted for the largest proportion of weight gain (56.59% (Table 6-6). The changes in lean and fat mass in individual subjects are shown in

Figure 6-10 and Visit 1 Visit 7

Figure 6-11. As in previous figures, these figures also identify the subjects who had a significant increase in height SD score (colour green), those who had a significant decrease in SD score (colour red) and no significant change in height (colour blue). When subjects were divided into these three groups there was an overall

progressively greater deposition of lean body mass (and percent weight gain due to lean body mass) and total BMC to progressively from the group with the least gain in height to the group with the most gain in height. No such relationship was observed with fat accretion which was comparable in the three groups. The mean values for the PCDAI also progressively increased with the group but the differences were not significant (P (trend) = 0.108, without age adjustment and P = 0.158.

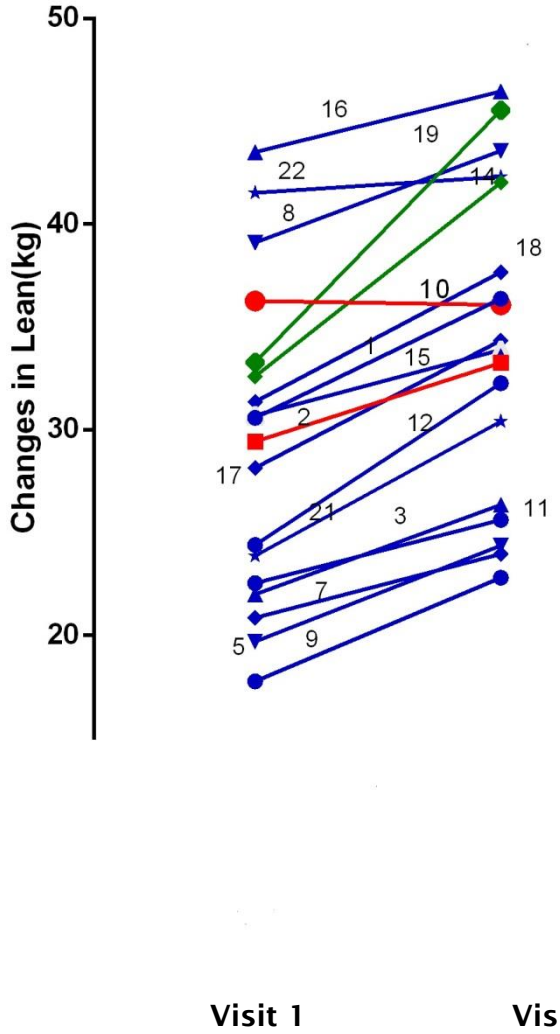


Figure 6-10 Changes in lean (kg) measured by DXA between visit 1 and visit 7.

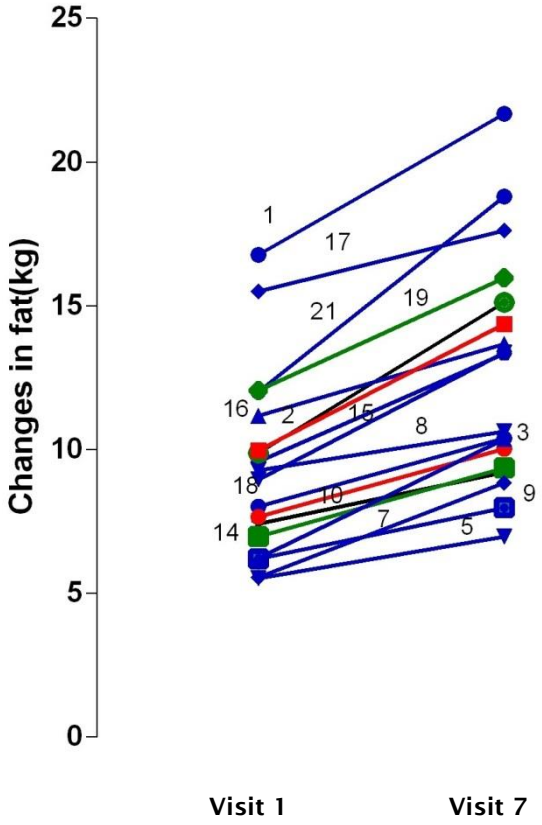


Figure 6-11 Changes in fat (kg) measured by DXA between visit 1 and visit 7.

DXA was used to assess changes in body composition between the first and last visit only. The individual changes of subjects in body composition measures performed by DXA are summarized in Table 6-5.

Table 6-5 Changes in individual body composition values (both in kg) measured by DXA at the initial and final visit.

ID	Lean 1*	Lean 7 **	Fat 1 +	Fat 7 ++
1	30.57	36.37	16.78	21.68
2	29.41	33.24	9.97	14.37
3	21.98	26.34	6.23	10.36
5	19.69	24.36	5.52	6.98
7	20.85	23.96	5.56	8.85
10	36.25	36.06	7.67	10.03
14	32.59	42.01	6.97	9.36
15	30.69	33.88	8.96	13.37
17	28.13	34.34	15.50	17.62
19	33.28	45.53	12.06	15.97

21	23.86	30.40	12.01	18.81
6	Missing data	Missing data	Missing data	Missing data
8	39.09	43.56	9.28	10.63
9	17.76	22.79	6.20	7.98
11	22.53	25.60	9.58	13.34
12	24.39	32.26	9.88	15.13
16	43.50	46.45	11.18	13.68
18	31.36	37.66	8.02	10.40
22	41.51	42.30	7.42	9.22

* Lean 1: lean (Kg) at visit 1.

** Lean 2: Lean (Kg) at visit 7.

+ Fat 1: Fat (Kg) at visit 1.

++ Fat 7: Fat (Kg) at visit 7.

Table 6-6 Changes in mean weight (by DXA), lean, fat, and BMC between visit 1 and visit 7.

	Visit 1	Visit 7	Mean difference	P value	95% confidence intervals
Weight (kg)	38.67 ± 8.93	46.93 ± 9.42	8.25 ± 3.46	<0.001	6.44 to 10.07
Lean(kg)	29.30 ± 7.59	34.28 ± 7.66	4.98 ± 2.95	<0.001	3.51 to 6.44
Fat(kg)	9.37 ± 3.20	12.65 ± 4.04	3.27 ± 1.50	<0.001	2.52 to 4.02
BMC (kg)	1.47 ± 0.34	1.56 ± 0.32	0.89 ± 0.13	0.013	0.2 to 0.15

SD scores for Bone Mineral Content (BMC), percent fat for age and FFM/H^2 were calculated using Baylor College of Medicine, body composition laboratory software

which is based on National Health and Nutrition Examination Survey (NHANES) - DXA data released in 2008.

Table 6-7: SD scores for, fat percent and FFMI SDS at visit 1 and visit 7.

	Percent fat SDS, visit 1	FFMI SDS, visit 1	Percent fat SDS visit 7	FFMISDS visit 7
1	0.4	-1.8	0.6	-0.4
2	0.1	-1.7	0.9	-1.6
3	-1.7	-2.3	-0.6	0
5	-0.8	-1.7	-0.7	-1
7	-0.3	-4.4	0.5	-2.5
10	-0.8	-3.2	0.4	-3.7
14	-0.9	-3.7	-0.8	-2.5
15	-0.7	0.4	0.2	1
17	-0.9	-1	0.2	0.1
19	0.1	-0.8	0.2	1.3
21	0	-4.3	0.6	-1.7
6	Missing data	Missing data	Missing data	Missing data
8	0.3	-3.3	0.4	-2.5
9	-0.1	-4.8	-0.3	-2.7
11	0.5	-4.7	0.9	-3.5
12	0.1	-3.7	0.4	-1.1
16	0.3	-3	0.7	-3.1
18	0.2	-3.7	0.5	-2.4
22	-2.4	-1.7	-1.9	-1.8

Table 6-8: Differences of SD scores for BMC, fat percent and FFMI SDS between visit 1 and visit 7.

	Mean difference	95% confidence of intervals	P value
BMC at V1_BMC at V7	0.8 ± 0.45	-0.25 to 0.69	0.486
Percent fat at V1_percent fat at V7	0.50 ± 0.43	0.29 to 0.71	<0.001
FFMI at V1- FFMI at V7	1.07 ± 0.98	0.58 to 1.56	<0.001

To be consistent, BMI SD scores where then calculated based on NHANES study 2008 and are presented in Table 6-9. Paired sample t-tests were conducted, and the results demonstrated significant improvements in FFM/H^2 z-scores and percent fat SD scores. Individual changes in FFM/H^2 SDS and BMI SDS are shown in Figure 6-12 and Figure 6-13.

Table 6-9 Changes in BMI SDS when calculated based on NHANES study 2008.

BMI according to NHANES at V1	BMI according to NHANES at V7	Mean difference	95% Confidence Interval of the Difference	P-value
-1.48 ± 1.14	-0.71 ± 1.07	0.77± 0.63	0.46 ± 1.08	< 0.001

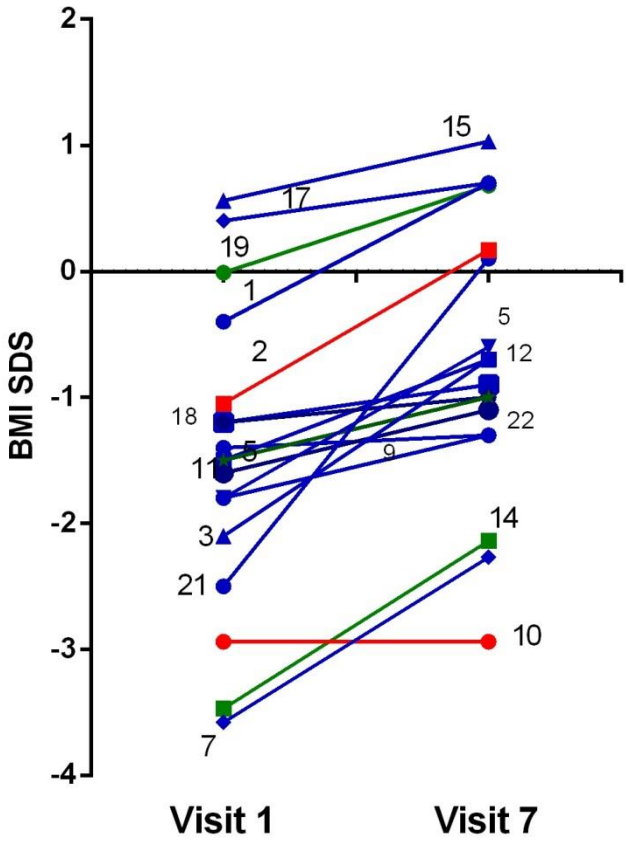


Figure 6-12 : Individual changes in BMI SDS by NHANES.

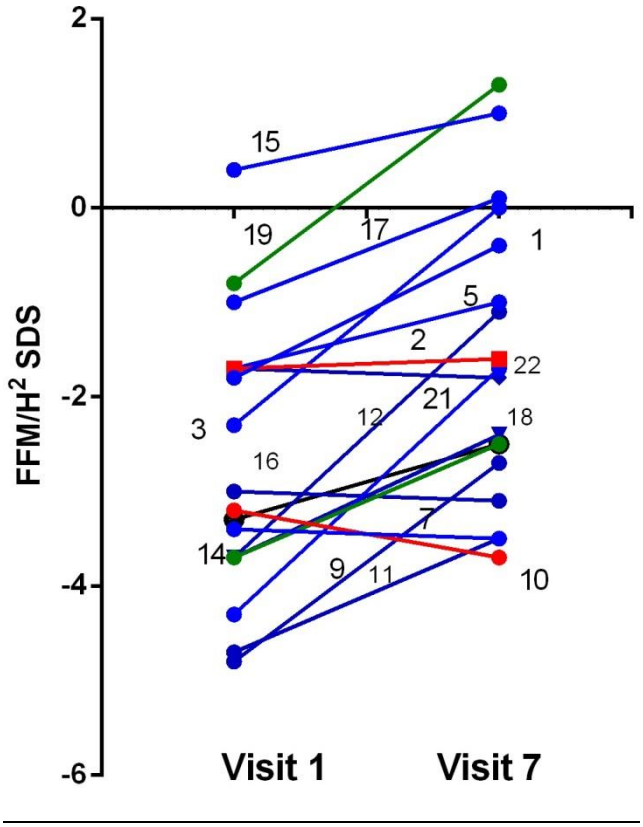


Figure 6-13 Individual changes in FFM/H^2 SDS.

Regression analysis was conducted to explore to what extent BMI SDS explains the differences of FFMI SDS at the initial visit, final visit and the change from initial visit to the final visit. The r^2 demonstrates that BMI SDS can explain 52% of the differences in FFMISDS at baseline, 35% at the final visit and the change in BMI SDS can explain 32% of the change in FFMISDS. However, it should be taken in to account that as the range of the 95% confidence of intervals shown in Figure 6-14, Figure 6-15 and Figure 6-16 is relatively large, therefore BMI is not an appropriate tool in predicting FFM.

Table 6-10: The results of the regression analysis.

	R^2	P-value
Initial visit	0.523	<0.001
Final first	0.357	0.011
Change	0.326	0.013

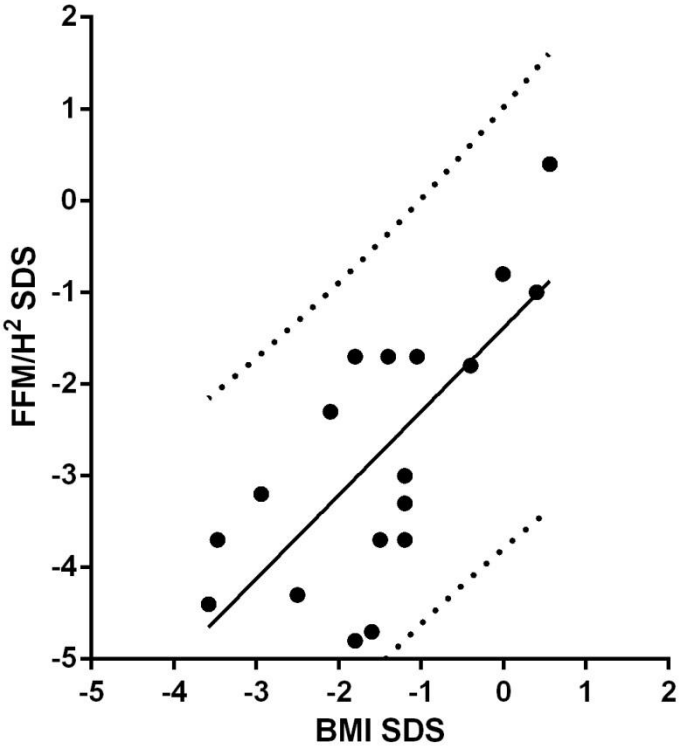


Figure 6-14 Scatter plot of regression analysis of FFM/H^2 SDS and BMI at baseline.

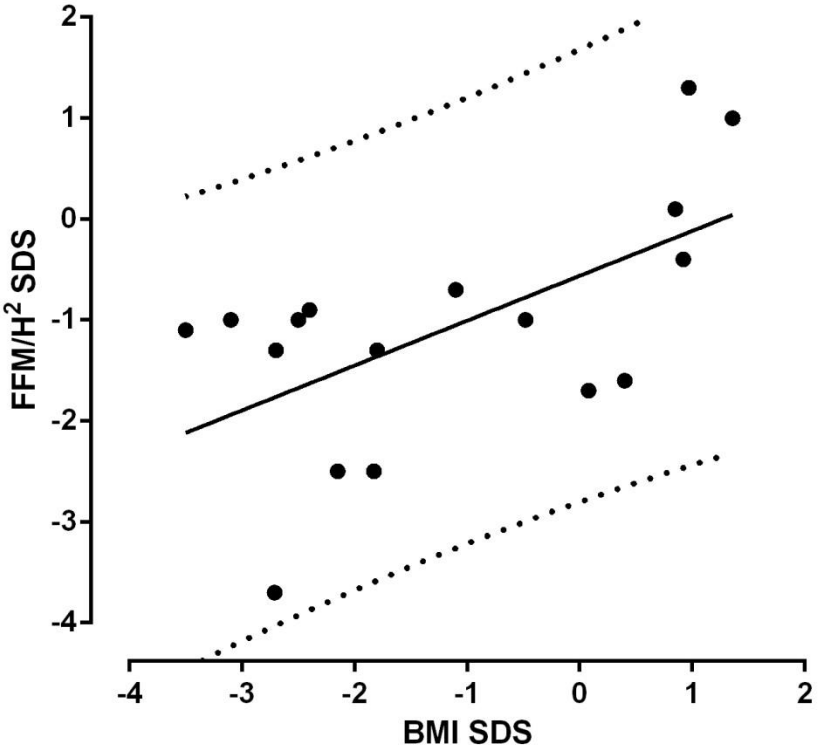


Figure 6-15 Scatter plot and regression analysis of FFM/H^2 SDS and BMI at visit 7.

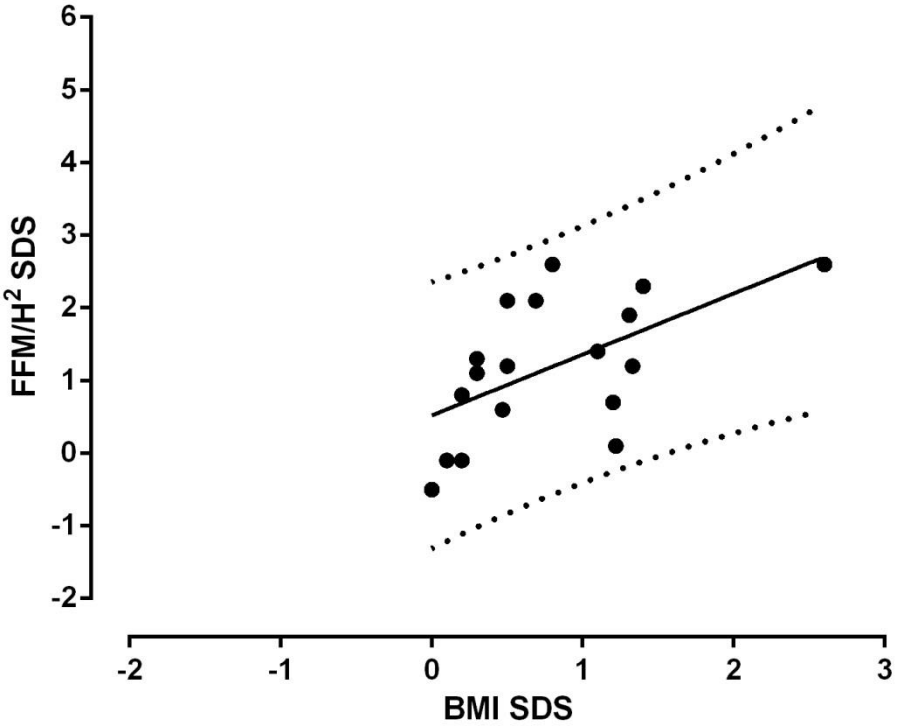


Figure 6-16 Scatter plot and regression analysis the change in of FFM/H^2 SDS and BMI.

Bio-electrical impedance analysis

Impedimed SFB7 was used to predict TBW, FM, and FFM in all subjects except one (subject number 7). The predicted values at the initial visit and visit 7 are summarized in Table 6-11. Paired sample T-tests were performed to demonstrate changes in TBW, FM, and FFM between the initial visit and visit 7.

Table 6-11: Individual changes in body composition measured by SFB7.

ID	TBW at visit 1(litre)	FFM at V1(kg)	FM at visit 1(kg)	TBW at V7(litre)	FFM at V7(kg)	FM at V7(kg)
1	26.58	36.31	8.69	29.84	40.77	4.23
2	24.48	33.45	11.55	26.49	36.18	8.82
3	24.29	33.18	11.82	27.56	37.65	7.35
5	22.82	31.17	13.83	28.05	38.32	6.68
7	Missing data	Missing data	Missing data	Missing data	Missing data	Missing data
10	25.73	35.14	9.86	25.61	34.99	10.01
14	26.66	36.42	8.58	30.41	41.54	3.46
15	28.82	39.37	5.63	29.88	40.82	4.18
17	26.04	35.57	9.43	26.89	36.74	8.26
19	25.69	35.1	9.9	29.7	40.58	4.42
21	21.13	28.87	16.13	28	38.25	6.75
6	17.00	23.20	4.90	22.10	30.20	8.10
8	Missing data	Missing data	Missing data	38.00	51.80	3.60
9	15.30	20.90	4.90	19.20	26.20	5.90
11	17.60	24.10	8.20	21.60	29.50	8.90
12	19.70	27.00	9.00	27.00	37.70	10.50
16	36.20	49.40	8.20	41.70	57.00	7.30
18	25.70	35.10	7.10	32.70	44.70	5.10
22	Missing data	Missing data	Missing data	35.10	47.90	5.80

Table 6-12: Differences between TBW, FFM and FM between visit 1 and visit 7.

	Mean difference	95% confidence interval of difference	P value
TBW2-TBW1	3.93 ± 2.21	2.75 to 5.11	<0.001
FFM2-FFM1	5.4 ± 3.11	3.77 to 7.08	< 0.001
FM2-FM1	-2.3 ± 3.4	-4.11 to -0.53	0.015

Unfortunately exact SD scores for body composition values by measuring bioelectrical impedance were not available. *Wells et al* have offered what they

believe is a universal reference for body composition data based on the 4 component model and propose that the values can be used across a variety of different methods of body composition assessment. They suggest that this reference data can be used to derive a SD score for Fat and Fat Free Mass Index derived using devices other than the one used in their setting. Approximate SD scores for FFM and FM based on a reference data for simple and reference techniques of body composition produced by *Wells et al* [184] are given in Table 6-13 [184].

Table 6-13 Changes in FFM SDS from SFB7 based on reference data produced by [184]

	FFM SDS Visit 1	FFM SDS Visit 7	FM SDS Visit 1	FM SDS Visit 7
1	-1.33	-.67	-1.33	-.67
2	-.67	.00	.67	.00
3	-.67	.00	.00	.00
5	1.33	>2.00	1.33	2.10
7	Missing data	Missing data		
10	-2.00	-<2.00	.00	-2.10
14	<-2.00	-2.00	-.67	-2.00
15	>2.0	>2.00	-.67	2.10
17	.67	.00	-.67	.00
19	.67	.67	.67	.67
21	<-2.00	-.67	.67	-.67
6.00	Missing data	-.67	-.67	-.67
8.00	Missing data	Missing data	Missing data	Missing data
9.00	<-2.00	<-2.00	-2.00	-2.10
11.00	<-2.00	<-2.00	-1.33	-2.10
12.00	<-2.00	.00	-.67	.00
16.00	.00	.67	.00	.67
18.00	-2.00	-1.33	-.67	-1.33
22.00	Missing data	Missing data	-1.33	-1.33

Calculation of TBW using deuterium dilution space technique

TBW was calculated from deuterium enrichment values in saliva at 4 hours at the initial and final visit. Age specific hydration factors [185] were used to calculate TBW from deuterium enrichment values at four hours.

Table 6-14 Individual changes in TBW, FFM, and FM between visit 1 and visit 7.

ID	TBW at	FFMat	FMat	TBW at	FFM at	FM at

	V1(litre)	V1(kg)	V1(kg)	V7(litre)	V7(kg)	V7(kg)
1	31.23	41.64	6.52	32.39	43.19	16.01
2	26.4	35.34	3.94	27.78	37.19	11.11
3	21.66	28.69	0.50	28.56	37.83	-1.03
5	18.8	24.67	0.56	22.93	30.41	1.49
7	26.77	35.84	-8.45	22.93	30.7	2.80
10	35.66	48.06	-2.11	33.71	45.43	1.37
14	30.73	41.53	-0.17	36.3	49.05	4.15
15	29.86	39.19	1.14	30.41	40.33	8.17
17	27.63	36.07	8.61	31.46	41.67	11.43
19	34.98	46.39	0.03	39.58	52.99	9.46
21	22.58	30.11	6.41	28.31	37.75	12.55
6	20.11	26.67	1.43	24.03	31.87	5.43
8	41.18	55.5	-6.7	42.37	57.1	-1.7
9	19.18	25.04	0.76	21.9	28.59	3.51
11	Missing data	Missing data	Missing data	Missing data	Missing data	Missing data
12	24.86	32.93	3.07	28.58	37.85	10.35
16	31.31	42.2	15.4	43.77	58.99	5.31
18	30.16	40.65	0.85	35.6	47.98	1.82
22	Missing data	Missing data	Missing data	Missing data	Missing data	Missing data

Summary of results

The purpose of the longitudinal study was to determine whether there was a deficit in lean tissue at diagnosis and whether treatment using 'usual care' which was predominantly Exclusive Enteral Nutrition (EEN) to induce remission would be associated with an increase in lean tissue over the initial 12 months of care. Lean mass was determined using a range of techniques from simple anthropometry and bioelectrical impedance, through to more advanced approaches using deuterium dilution or DXA, and expressed either a) in absolute terms in kilograms, or b) when compared against a reference range and expressed as a SD score. Table 6-15 provides a summary statement of the extent to which lean tissue measured using these different approaches increased over the initial 12 months of treatment.

Table 6-15: Summary of lean mass using different measurement approaches of children with CD at diagnosis (visit 1) and after 12 months treatment (visit 7).

Variable	Visit 1	Visit 7	difference	P value
FFM DXA (kg)	29.30 ± 7.59	34.28± 7.66	4.98± 2.95	<0.001
FFM D2O (kg)	36.76 ± 8.53	41.70 ± 9.21	4.93± 4.82	0.001
FFM BIA (kg)	32.76 ± 6.97	38.19 ± 6.96	5.42 ± 3.11	<0.001
FFM/Ht^2 SDS DXA	-2.68 ± 1.43	-1.61 ± 1.53	1.07 ± 0.98	<0.001
UMA SDS	-1.36± 0.79	-0.90 ± 0.83	0.46 ± 0.38	<0.001

FFM DXA: Fat Free Mass by DXA; FFM D2O: Fat Free Mass by deuterium dilution technique; FFM BIA: Fat Free Mass by BIA using Impedimed SFB7: FFM/Ht^2 SDS DXA: Fat Free Mass Index Standard Deviation Score by DXA; UMA SDS: Upper Arm Muscle Area Standard Deviation Score.

These results clearly demonstrate that lean mass, however it was determined in this series of children, increased from diagnosis (visit 1) to the end of the first year of treatment (visit 7). The extent of the increase in FFM in kg appears to be dependent on the method of assessment with the greatest apparent increases seen when FFM was estimated from deuterium dilution compared to that estimated from DXA or BIA. Equally of note, is that the FFM was comparable between deuterium dilution and DXA at diagnosis, but differed markedly at visit 7. In contrast, FFM by BIA was generally higher than that estimated from both DXA and deuterium dilution at diagnosis, but the increase in FFM was comparable to that estimated by DXA.

These changes in lean mass are even more striking when adjusted for height or expressed relative to the reference range either as FFM from DXA or by simple anthropometry as Upper Arm Muscle Area. Here, not only have the children gained lean mass, they also appear to have increased in lean mass greater than would have been expected for the gain in height or that what would be expected in healthy children growing normally (the reference range).

Taken together, these observations demonstrate a consistent pattern and support the view that these children have demonstrated an increase in lean tissue.

6.3.1Discussion:

This longitudinal study in children with CD provides insights into patterns of growth and changes in body composition over time and some of their determinants.

The baseline anthropometry suggested significant depletion compared to the reference data (1990 for weight, height and BMI). There were note worthy features of the baseline data. First, the mean weight SDS was not only significantly less than zero, but also significantly lower than the height SDS. (-1.60 v -0.52; P = 0.12). This could have resulted acutely from rapid loss of weight after the onset of CD. In contrast short periods of time in health and disease are not expected to affect height to any great extent. An alternative explanation is that CD could have preferentially affected growth in weight rather than height over prolonged periods of time. Detailed information about pattern of weight and height changes prior to the onset of CD would help resolve these issues, but such information was not available in the present study. Second, the standard deviation of the z scores for weight and height were increased (1.47 and 1.27 respectively compared to the reference population, which would be expected to have a z score of 1. This means there is greater variability in weight and height in children with CD than the reference population. This may be because of the variable severity and duration of CD. Indeed even at the time of the first visit, the PCDAI showed considerable variability (34 + 11.24). Rapid weight loss after the onset of CD in some children and not others would also tend to increase the variability and potentially explain the greater variation standard deviation scores for weight compared to height.

Changes over time

Height

Overall there was a small non-significant increase in mean height SDS (from -0.22 to -0.35), but this masks the wide inter individual variations and did not attain statistical significance. These findings support previous studies that reported little

or any change in height SDS following treatment [61], [150], and [128]. For the purposes of presentation and discussion three groups of height changes were established: group 1 (red) characterised by a decrease height SDS; group 2(blue) characterised by no significant change in height SDS; and group 3(green) characterised by an increase in height SDS. Various explanations can be put forward for this variability. One of these is that the variable baseline SDS score could influence growth. Children with impaired growth often show catch-up growth as a result of treatment. However, this was not evident with respect to height in this study. Furthermore, when adjustments were made for baseline height significant differences in changes in height SDS persisted between individuals. This suggests that other factors are involved in the variable changes in height SDS over time. Another possible explanation is that dietary intake was reduced and/or absorption impaired to a variable degree between subjects. The lack of dietary intake data and lack of data on the extent of malabsorption in this thesis precludes further exploration of this possibility in this thesis. However, this study provided evidence that growth in height SDS (and also in cm) was significantly related to the mean PCDAI during the 1 year follow-up period. Whilst this suggests that disease activity is responsible for the variable growth, it does not indicate whether it is due to poor intake, failure of absorption of nutrients, or an independent effect of the disease-related mediators on the growth plates of bones.

Weight

In contrast to changes in height SDS, which did not improve significantly over time, those of weight SDS did. One possible explanation is that growth in height (bone growth) is slower than growth in weight. The body has a very limited capacity to oxidise excess energy (beyond that associated with physical activity) so that extra energy is ingested and deposited, with the result that weight increases. On the other hand growth of bone plates and accrual of bone mass may be affected by lean deficits, reduced physical activity, nutrition malabsorption, consumption of corticosteroids, and persistent inflammation [51]. These factors have been discussed in detail before.

Body composition

To the author`s knowledge there here have only been three prospective longitudinal studies to assess changes in body composition following treatment in

children with CD. *Sylvester et al* [186] demonstrated that FFMI SD scores (using DXA) does not increase significantly following treatment with steroids in children with CD. *Thayu et al* [150] , found improvements in FFMI SD scores using DXA which was associated with inflimax therapy. *Gerasimidis et al* explored growth and body composition changes in children with active CD treated exclusively with a polymeric feed for 6-8 weeks [159]. Body composition was measured using Bioelectrical Impedance Analysis and converted to SD scores. The authors reported that weight SD scores, BMI SD scores and Lean index SD scores improved significantly following 30 days of EEN use. Treatment of active CD with exclusive enteral nutrition as the main therapy in this study suggests that there is a significant deposition of lean tissue, demonstrated by an increase in UMA (in absolute area and in SDS). This is the first study to have reported changes in UMA during treatment as both mentioned studies used DXA to report changes in FFMI SD scores. It is also demonstrated by the DXA measurements, which showed increase in lean body mass (both in SDS and in Kg of lean tissue). Indeed DXA suggested that the majority of tissue deposited was lean tissue (60%). Previous studies reporting failure of lean tissue accretion during the treatment of active CD involved the use of steroids which are known to have catabolic properties. It is possible that the deposition of lean tissue in the subject of the present study occurred because no steroids were used. Instead children were treated with exclusive enteral nutrition, which also provided a balanced mixture of nutrients.

The body composition measurements also showed that there was accretion of fat demonstrated by both an increase in TSFT and an increase in percent body fat from DXA. Again in fat and lean tissue in the same proportions as they exist in the body would not be associated with a change in fat percent. The results of this study suggest that a greater fat: lean ratio is deposited (0.4:0.6) compared to that which was present at baseline (0.24:0.76).

An analysis of the changes in body composition according to categories of height growth shows that the increase in lean body mass was least in the group with a significant decrease in height SD score (1.9 kg), intermediate in the group with no significant increase in height SD score (5.64 kg) and most in the group with a significant increase in height SD score (11.0 kg). Despite the small number of subjects studied, the observations suggest that an increase in height is expected to be associated with an increase in lean tissue, which would normally be expected to

be increased in healthy subjects during growth. However, since there was an inverse relationship between changes in height (SDS or cm) and PCDAI, the inflammatory mediators associated with CD could contribute growth failure and potentially produce additional catabolic effects.

Lean tissue has great functional importance. Its measurement could provide useful information for monitoring children with CD. However, such measurements would be of value if simple routine measurements provide accurate estimates of body composition. Body mass index has long been used as an index of body composition in adults, but in children it is further confounded by altered relationships according to age. Indeed the relationship between BMI SDS and FFMI SDS is associated with large 95% prediction band at baseline (Figure 6-14) at the end of the follow up period (Figure 6-15) and also for the change between baseline and the end of the follow up period (Figure 6-16), all with a 95% confidence limits of about 2.5 to -3 SD score. This suggests that BMI is an inadequate measure of body composition in children with CD. Decisions need to be whether measurements of body composition should be made in routine clinical practice.

TBW and FFM were also predicted using deuterium dilution technique. As it is evident TBW was predicted much higher than it`s real value. This could be due to several factors: a) subjects may have not consumed the whole dose given to them. b) The wrong amount of deuterium dose may have been recorded. c) The hydration factor used for children with CD was according to age specific hydration factors of normal children. As these children are usually under-nourished specific hydration factors should be used for them, which is unfortunately unavailable. *Patrick et al* [187] had previously reported that the ratio of TBW: weight is significantly different in malnourished children.

Chapter 7 General discussion

The central hypothesis of this thesis was that children with IBD present with) growth deficits and ii) lean deficits greater than that which can be simply attributed to their lack of height; and iii) that conventional therapy, including exclusive enteral nutrition, may not adequately correct the nutritional state and deficit of lean tissue. In order to test this hypothesis, the work conducted for this thesis was designed in three parts.

Firstly a cross-sectional study was designed to explore the extent of differences in height, weight, and BMI expressed as SD scores, together with simple measures of body composition using anthropometry. This study demonstrated that whilst both CD and UC children exhibit modest deficits in height, weight and BMI there was significant variance across the group with more distinct deficits in some children. Lower UMA SD scores and higher TSF SD scores were evidence of existing lean deficit and fat excess in children with IBD, even in children who were in the normal range for BMI(±2 SDS). The lean and height deficits were more severe in CD children compared to UC patients, therefore the prospective study investigating growth and body composition focused on CD children only.

The second part explored different approaches to assessing body composition by determining the concurrent and face validity of different bioelectrical impedance devices using deuterium dilution space as a reference method and ii) the potential of using SIFT-MS to conduct real-time near-patient measures of deuterium abundance on breath vapour was examined in comparison to measures of deuterium abundance in saliva and urine assessed by both SIFT-MS and IRMS. These studies demonstrated marked differences in lean mass were evident between devices. Deuterium abundance in saliva and urine by SIFT-MS was directly comparable to that by IRMS although higher levels of D_2O administration were required for optimal analytical performance; greater imprecision was evident in determining deuterium abundance in breath.

The third part described detailed measures of body composition (anthropometry, DXA, deuterium abundance in saliva by IRMS, and BIA) in a prospective inception cohort of eleven children with CD studied at diagnosis, and followed for the first year of treatment from active disease into remission using exclusive enteral nutrition. Lean deficits greater than that which could be attributed to shortness,

were evident at diagnosis using both DXA and Upper Arm Muscle Area, when compared against reference data. Treatment was associated with gains in height and weight, but in contrast to previous reports where corticosteroids were used to induce remission, gains in lean mass over the first year of treatment using exclusive enteral nutrition were observed which were greater than that which could be attributed to an increase in height that reflect at least a partial correction of the lean deficit.

The results of the studies reported in this thesis demonstrated that height; weight and BMI deficits at diagnosis were similar to the more recent studies which probably reflect the shorter time to diagnosis. In addition as reported by others, height gains with treatment are modest and whilst there was weight and BMI gain, there was a concern that a rise in BMI should not necessarily be interpreted as an improvement as BMI is an insufficient tool of measuring changes in body composition. Measurements of upper arm muscle area and triceps skinfold are simple, inexpensive and can easily be used in clinical settings if they are compared against a valid reference range. Measurements of FFM by deuterium dilution technique using SIFT-MS are a rapid approach; however it needs further refinement to improve precision before it can be usefully applied clinically. One of the limitations of using deuterium dilution technique to calculate FFM is lack of hydration factors for children in inflammatory conditions. Hydration factors for normal children are not appropriate to be used for children with inflammatory states, as observed in this programme of work FFM was overestimated and therefore predicting minus values for FM.

Only three longitudinal studies have been conducted to explore body composition changes following diagnosis in children with CD. *Selvester et al* studied 6-17 years old patients with CD and reported that BMI SD scores normalize following treatment with steroids however, FFMI SD scores measured by DXA do not improve. *Thayu et al* studied CD children 5-21 years and followed them up for 12 months, FFMI SD scores were generated based on a control population(n=212). They reported that height SD scores do not improve significantly following treatment, however, FFMI SD scores improved. The improvement in FFMI SD Scores was associated with infliximab therapy. In another study, *Gerasimidis et al* explored growth and body composition changes in children with active CD treated exclusively with a polymeric feed for 6-8 weeks [159]. Body composition was measured using bioelectrical impedance analysis. The authors reported that FFMI was significantly improved

following treatment. This is the first study to measure explore changes in body composition of children with CD using different methods. Improvements in FFM deficits in children with CD were confirmed using two different methods (UMA SDS and FFMI from DXA). In addition similar improvements in FFM (kg) can be observed in bio-electrical impedance measurements and also using deuterium dilution.

This is the first longitudinal study to explore changes in body composition of children with CD treated mainly with exclusive enteral nutrition using different methods. Findings of this study demonstrated that following treatment with exclusive enteral nutrition FFMISD scores improve significantly in children with CD, one of the reasons may be that EEN improves nutritional status as well as controlling inflammation and therefore may improve body composition deficits. The greatest improvements in UMA SD scores were between visit 3 and visit 4 which is usually after remission using EEN. In addition in the intervals of the 6th and 7th visit UMA SD scores decreased which may be as a result of disease relapse. This is also the first study that has different approaches to examine body composition in children with CD and used UMA SD score as a measure of body composition, and improvements in FFM are also confirmed by improvements in UMA SD scores.

Several potential mechanisms of EEN on growth have been proposed. Improving the nutritional status and correcting malnutrition is a potential factor for improvements in weight status and lean mass accrual. CD patients commonly have decreased oral intake [33], and EEN may improve energy and micronutrient deficiencies. In addition, improvements in nutritional status may increase physical activity and therefore contribute to lean mass deposition. Within the first week of EEN a rapid decrease of pro-inflammatory cytokines, such as CRP, ESR and IL-6 has been observed [188]. Decrease of inflammation may lead to normalization of bone modelling and lean mass accrual.

As stated previously BMI is an insufficient tool to demonstrate changes in body composition and nutritional status. Simple approaches of measuring body composition should be included in clinical settings. Bio-electrical impedance devices are applicable to use as a simple bed side measurement, however lack of reference data against which body composition measures can be compared make it more difficult to assess body composition in children.

The biggest caveat of the cross-sectional study and longitudinal study was the small number of study subjects which was limited by time constraints. Insufficient prior

evidence was available to derive secure power calculations. Therefore; this thesis provides the necessary information to inform future studies. Another major constraint is the absence of well characterised reference data sets for body composition measures in children. Body composition was measured using upper arm muscle area, bio-electrical impedance analysis, deuterium dilution space and Dual-energy X-ray absorptiometry in this study; however, SD scores could only be calculated for UMA and FFMI from DXA. Unfortunately there were no appropriate reference data for FFM using deuterium dilution or BIS, and DXA reference datasets used here to calculate SD scores is based on NHANES reference data which is an American reference data and SD scores calculated using the reference data developed by *Wells et al* [185] are approximate . Having a control group to compare the patients against would be very beneficial. Control group should be matched with patients in terms of age, sex, and race. Growth retardation has been linked with delayed puberty in children with IBD [60], assessing the pubertal stage at diagnosis and in follow-up sessions would be very advantageous, as it would help clarify whether changes in body composition and anthropometry are associated with pubertal stage or are as a result of treatment.

Further research is required to reinforce the observations found in this study and to investigate the mechanisms by which EEN can improve FFM deficits in children with CD. More investigations are required to evaluate the effect of EEN on lean mass and bone density development; it would be beneficial to compare the results of EEN group against a control group treated with steroids or biologicals. A greater number of patients measured at defined points in the care pathway for body composition assessment would make it possible to further investigate the effects of treatment and disease activity on tissue accretion. In addition, appropriate reference datasets of measuring body composition with bedside techniques such as bioelectrical impedance analysis and skinfold thickness could be very beneficial in investigating changes in body composition of children with CD. Furthermore, growth is a process that occurs over a long period of time, and prolonging the time of follow-up would be beneficial as lean mass deposition and bone density accrual take place over time.

Ultimately embedding quality assured measures of body composition in clinical settings will markedly advance our understanding and may make it possible to use changes in FFM as a prognostic marker in terms of predicting and/or being used as an outcome measure to evaluate the efficacy / effectiveness to treatment. It would

also be feasible to assess changes in body composition using different therapeutic interventions to investigate the efficacy of various treatments

Chapter 8 Appendices

Appendix 1:

Table 8-1 : A summary of studies reporting growth deficits in children with IBD.

Study Reference	Time of Assessment	Subject	Definition of Liner Growth Impairment	Percentage with Growth Impairment	Article Style
Kanof 1988	At diagnosis	Prepubertal CD (Tanner I or II) (n=50)	Decrease in height velocity	88%	Retrospective case control
Motil 1993	At diagnosis and yearly for 3 years	IBD (n=50)	Height/age < 95% centile	39%	Prospective cohort study
Griffiths 1993	During follow-up	Prepubertal CD (Tanner I or II) (n=100)	Height Velocity < 2 SD for age for > 2 years	49%	Retrospective cohort study
Markowitz 1993	At maturity (Tanner V)	IBD (38 CD, 10 UC) (n=48)	Height < 2 centiles below premorbid centile	60%	Retrospective cohort study
Hilderbrand 1994	From birth to adulthood	Population-based IBD (46 CD, 60 UC, 18 IC) (n=148)	Height, weight/ height & height velocity > 2 SDS.	65% CD, 34% UC	Retrospective cohort study
Sentongo 2000	During follow-up	CD (64% males) (n=132, control=66)	Statistical difference ($P < 0.05$); height/age z score, weight/age z score, pubertal stage.	All parameters significantly lower in males with CD v. controls	Retrospective cohort study
Spray 2001	At diagnosis	IBD (64 CD, 41 UC, 7 IC) (n=112)	Height / z score $\leq$ -2 SD	19% CD; 5% UC	Retrospective cohort study
Sawczenko 2003	At diagnosis	n=(431 CD, 211 UC)	Height below 3rd centile	13% CD, 3% UC	Prospective cohort
Burnham 2004	During follow-up	CD (n=104, controls=223)	Statistical difference ($P < 0.05$) v. controls; Height, lean-	All parameters significantly lower in CD compared	Retrospective cohort study

			mass/height z score	to controls	

Appendix 2:

Standard Operating Procedure for Measuring the Height of Children (Secca Stadiometer)

Accurate length/height measurement of infants and children is essential as an indicator for physical growth. Length and height measurements can be plotted on percentile charts and compared to the general population.

Procedure

- Only individuals who have been deemed competent in measuring the height of a child must undertake this procedure.

- Measuring SECA (stadiometer) must be checked and calibrated annually following manufacturer's guidelines. This must only be used with children who are able to stand independently (if child is unable to stand, the Kiddimeter must be used instead).

- Explain procedure to child and parent(s). Explain to the child you will want them to stand as tall and straight as possible.

- Take child's shoes off so that the measurer can see that heels are against the bar in the correct position.

- Undo or adjust hairstyles and remove hair accessories that interfere with measurement.

- Take the nappy off, if appropriate.

- The child must only wear light clothing.

- Ask the child to stand on the stadiometer, facing forwards as tall and straight as possible, arms hanging loosely at their sides.

- The child's feet must be positioned together and flat on the base plate of the stadiometer with their heels touching back plate.

- The child's knees must be straight, with buttocks and shoulders touching the stadiometer, but the child must not lean against it. This may require the assistance of a second nurse

- Position the head in "Frankfort plane", an imaginary line from the centre of the ear hole to the lower boarder of the eye socket. This is a midline position, child must look straight ahead, parallel to the floor.

- To ensure best position is achieved: explain to the child, what you are about to do.

- Cup the child's head in your hands, placing the heels of your palms either side of the face. Your fingers should come to rest on the mastoid process behind the ears. Firmly but gently, apply upward pressure lifting the child's head to their maximum height. Avoid jerky movements, perform the procedure smoothly and take care not to tilt the head at an angle.

- Check for any bending of the knees, slumping of shoulders or rising of heels. This may require a second nurse

- Lower headpiece of the stadiometer lightly onto child's head, ensuring it rests on the crown of the head, i.e. the top back half.

- Ask child to take a deep breath in, let it out (shoulders will relax) and then the nurse should read the measurement whilst still holding chin.

- Nurse's eyes should be level with counter/pointer (Red marker dividing the fixed and extendable measurer) read to the nearest millimetre. Record the measurement, plot on growth chart and sign. Take 3 measurements and use the average.

The child should be able to step off the stadiometer without ducking their head.

Standard Operating Procedure for Weighing Children (sit and stand on Secca Scales)

Accurate weight measurement of infants and children is essential for both calculating accurate drug dosages and as an indicator of physical growth. Weight values can be plotted on percentile charts and compared to the general population. Only Class III clinical electronic scales must be used in order to give accurate readings (RCPCH 2009).

Procedure

1. Only RGN/RSCN or RN Child who have been assessed as competent may weigh children.

2. The scales must be checked and calibrated annually following the manufacturer's guidelines. It must be checked that the scales have been calibrated prior to each use.

3. If using sit on scales it must be ensured that the brake has been secured.

4. Turn on the scale display as a check for the operational function and ensure that the scale is set to kilogram mode. Wait for the display to read 0.00 before asking the child to stand on the scales.

5. Explain procedure to child and parent(s)/carers. Explain to the child you will want them to sit/stand as still as possible. Ask the child to remove shoes and heavy clothing.

6. All children under the age of 3 years should be fully stripped for weighing, as per trust child protection policy. If child of any age ($\leq$ 3 or $\geq$3 years old) is to be weighed solely for a research purpose this requirement should be discussed with the Principal Investigator and senior nurse. All children over two who are not being fully stripped must be weighed wearing light clothing. (RCPCH 2009)

7. Ask the child to sit/stand in the centre of the scales with their arms hanging loosely at their sides, their head facing forward. Ensure they look straight-ahead and remain as still as possible. The posture and co-operation of the child is important as this ensures their weight is evenly spread, to achieve an accurate reading.

8. The display should then show a fixed weight.

9. The weight must be recorded in kilograms and be signed by the nurse taking the measurement. If available the reading must also be entered into parent child health record, plotting in pencil and recording measurement and date in ink.

Standard Operating Procedure for Measuring Biceps, Triceps, Suprailiac, Mid Axillary and Subscapular Skinfold Thickness

This Standard Operating Procedure is to be used for measuring skinfold thickness. Measurements of skinfold thickness can be used to assess the distribution and amount of subcutaneous body fat. Low amounts of body fat suggest that the individual is undernourished. However, in this country the opposite is more commonly seen and the accompanying health problems associated with this are well known. In addition to identifying the *amount* of subcutaneous body fat, its *distribution* on the body is also important and provides further information; for instance, a greater proportion of fat located

within the trunk can be predictive of future coronary heart disease and diabetes. Having obtained the skinfold measurement values, the amount of fat or fat mass may be calculated by inserting the raw data into a series of equations.

Measuring skinfold thickness is an easy and cost-effective way of assessing percentage body fat; however, it can be subject to significant intra- and inter-observer error. Therefore, steps must be taken when training staff, to ensure this source of error is kept to a minimum. By marking the area to be measured and taking the skinfold measurements in partnership with an already qualified person, more precise and reproducible measurements can be made.

Equipment

Measurements are taken using skinfold callipers (calipers = American English) figure 1, table 1). They measure the thickness of two layers of skin and the underlying subcutaneous fat (figure 2).

Figure 1. Holtain skinfold Callipers

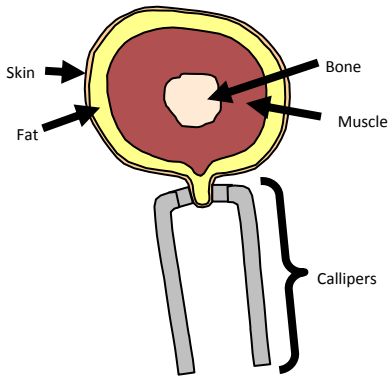


Figure 2. Measurement of skinfold thickness

(Adapted from Heyward and Wagner [2004])

The two most commonly used skinfold callipers in this country are the Harpenden and Holtain: The measuring precision of both are 0.2mm. They are delicate precision instruments and to maintain their measuring precision

they need to be treated with care; they are supplied in a carrying case which they should always be stored and transported in (figure 1).

This SOP has been written for use with **Holtain callipers** which are those used within the SCBR. The dial displays the results in 0.2 mm increments and the net weight of the callipers is 0.4 kg.

Calliper type	**Average pressure (g/mm^2)**	**Range (mm)**	**Scale precision (mm)**
Harpenden	8.2	0-55	0.2
Holtain	10.0	0-48	0.2

Table 1. Comparison of metal skinfold callipers

(Adapted from Heyward and Wagner [2004]).

The Holtain Callipers can be purchased from Chasmors Ltd. Tel: 020 7387 2060, email: sales@chasmors.com, website: http://www.chasmors.com. Callipers in the SCBR are checked for accuracy with machined metal blocks on a quarterly basis. Should they be found to be outside the 0.2mm calibration range, they can be sent back to the manufacturer but this is costly and should not be necessary if they are treated carefully.

Procedure

MAKING THE MEASUREMENTS – GENERAL POINTS

1. Where possible, make the measurements on the participant's non-dominant side.
2. It is advantageous for two people to be involved in making these measurements; one making the measurements and the other recording the results. The second person can also view the calliper dial and read/record the value when the callipers are being held at an angle which makes reading the dial more difficult. Also, the recorder can quickly advise the measurer if additional measurements need to be taken due to too much variability between the first three values.
3. Wash your hands and clean the calliper blades and tapes before use with a detergent wipe.

4. Explain to the participant what skinfold measuring involves. Reassure them that they will feel no pain and only a little discomfort. You can achieve this by showing them how the callipers operate and explaining the term “skinfold thickness” by picking up the skin on the back of the hand. Demonstrating the action of the callipers on their index finger will give them an idea of how it will feel on their skinfolds.
5. Ask the participant to remove upper body clothing (bras can be kept on).
6. The measurer and the participant should be positioned such that the measurer can easily read the dial without needing to twist/rotate the callipers whilst they are grasping the skinfold. This twisting action may be painful for the participant.
7. When measuring subscapular, triceps and biceps skinfold thickness, reading the dial may be difficult if the participant is taller than the measurer. In this instance the participant may be seated. Conversely, when measuring suprailiac and mid-axillary skinfold thickness, the participant must be standing and it will be necessary for the measurer to sit or crouch to be able to read the dial accurately.
8. Prior to making any measurements, mark all the sites with a cross using a ballpoint pen. If you are taking measurements at more than one skinfold site you should rotate sites between measures. For example, if making three measurements each of subscapular, suprailiac and triceps skinfold thickness, you should make one measurement of subscapular followed by one of suprailiac then one of triceps and repeat this pattern of measurement until you have a set of three values for each site.
9. For the technique of picking up the skinfold, the action is to sweep the index finger and thumb of both hands together over the surface of the skin from about 6 to 8 cm apart and collect the subcutaneous tissue pushed away from the underlying muscle fascia by this action. Massage up a tube of skin with the thumb and fingers of both hands. **One hand then remains holding the skinfold throughout the measurement** and the measurer picks up the callipers with the other hand.
10. The positioning of the calliper blades on the skinfold will vary according to the size of the fold of skin, but in general should be at least one blade-breadth in from the apex of the skinfold.
11. Difficulty in picking up the skinfold suggests that the measurement is being made against the natural contours of the site or that the location is not marked up correctly.
12. The callipers should be in the correct orientation for picking up the skinfold. When the callipers have been placed on the fold, wait five seconds before recording the value. During this five second period you may notice that the needle on the dial moves as the callipers settle on the skinfold. The callipers should be fully closed on the skin and the dial should be read at five seconds after that, even if the needle is still moving.
13. Do not drag the callipers off the fold at the end of the measurement, as this is uncomfortable and may damage the callipers.

Consciously open the jaws to remove them. Always return the calliper blades to their closed position by slow and controlled use of the handles. When the blades are made to "snap" together, the surface area of the calliper blades becomes worn, and consequently results in imprecise measurements.

14. Generally, at least three measurements are taken at each site, releasing the skinfold and picking it up again each time.
15. The technique is the same in males and females.

Site-specific Measurements

<u>Suprailiac site measurement</u>

1. Explain the procedure to the participant.
2. Ask the participant to stand with their back to the measurer with arms folded in front of them, as it will be easiest to find the suprailiac site this way.
3. The suprailiac site is just above the iliac crest in the midaxillary line. With the participant in the standing position, palpate the iliac crest (figure 3). Imagine the participant in the supine position when this bone which becomes more prominent, then mark this area in ball point pen with a cross. The measurer now needs to come round to be on the front (side) of the participant for greater ease. The actual mark is made 1 cm higher up from the mark made at the iliac crest and 2 cm across horizontally (proximal to the umbilicus). This is where the suprailiac skinfold measurement is to be made (figure 3).

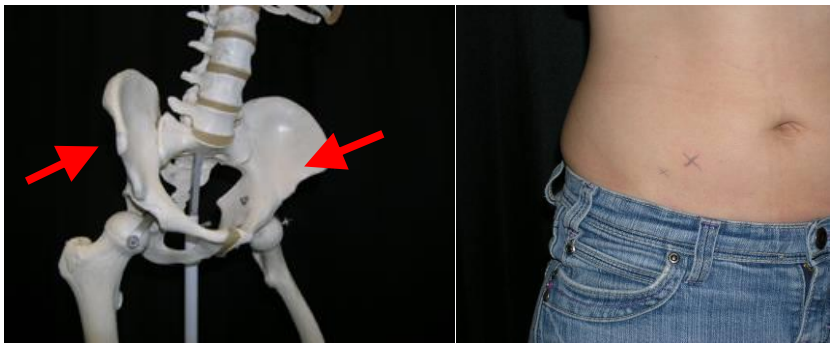


Figure 3. Identification of iliac crest and marking for suprailiac measurement

4. The measurer should position themselves to the side of the participant. By asking them to lean slightly forwards and bend from the waist towards you, it will help ease the tension on the skin, confirm the correct location and define the angle at which you need to pick up the skinfold. Pick up the skinfold with your fingers (figure 4).

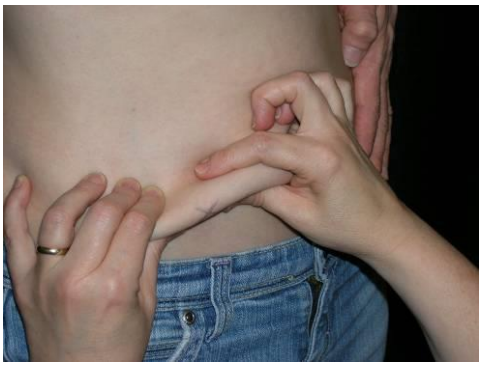


Figure 4. Picking up the suprailiac skinfold

5. Pick up the fold following the natural contours and apply the callipers at the level of the cross, closest to the umbilicus, (figure 5).

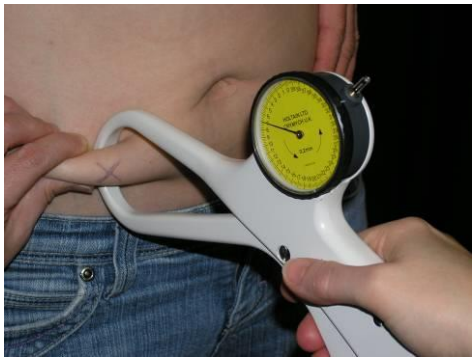


Figure 5. Measuring the suprailiac skinfold

6. Record three measurements of suprailiac skinfold thickness (in rotation if you are measuring more than just the one site).
7. Calculate the mean by adding the values together and dividing by three.

Subscapular site measurement

1. Explain the procedure to the participant.
2. Ask the participant to stand with their back to the measurer with shoulders and arms relaxed.
3. Palpate the lowermost tip of the scapula and mark with a cross (figure 6).

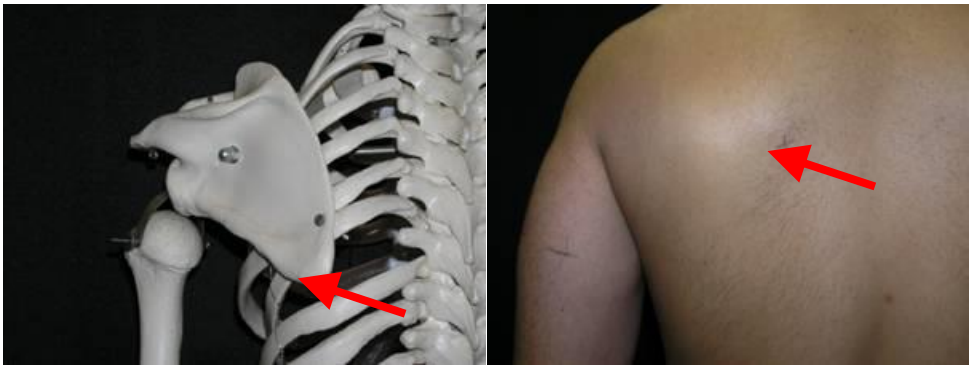


Figure 6. Identification of lowermost tip of scapular and marking for subscapular skinfold

4. It may help to follow the medial border of the scapula downwards until the inferior angle is felt. The scapula will be more easily identified if the arm is put behind the back, in a half-nelson position. Alternatively you can ask the participant to roll their shoulders back, which will also make the medial border of the scapular more prominent. Once identified, the arm must be relaxed again before the skin is marked.
5. The skinfold is picked up obliquely, following the natural contours of the skin and the callipers are applied at the level of the cross, with the cross at the apex of the fold (figure 7).

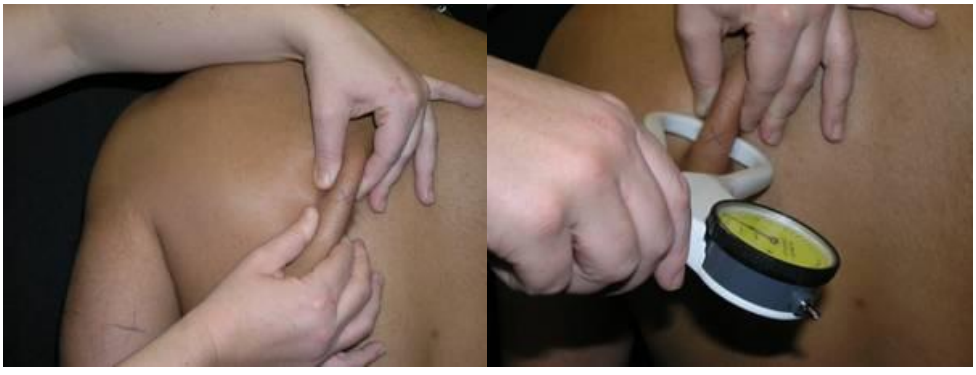


Figure 7. Picking up and measuring the subscapular skinfold

6. Record three measurements of subscapular skinfold thickness (in rotation if you are measuring more than just the one site).
7. Record the mean by adding the values together and dividing by three.

Mid Axillary site measurement

1. Explain the procedure to the participant.
2. Ask the participant to stand with their back to the measurer with arms folded in front of them.
3. The mid axillary site is located between the highest point of the pelvis and the lower costal margin (the lowest rib in the midaxillary line). With the participant in the standing position, palpate the top of the pelvis and mark this area with a cross. Palpate the lowest rib in the mid axillary line and mark with a cross (figure 8).
4. Put the tape measure on the mark on the lower costal margin and drop it down to the mark on the highest point of the pelvis in the mid axillary line. Read the exact half distance between the two points and mark the site with a cross. When you have completed all the measurements on the volunteer, wipe the length of the tape measure with a detergent wipe. Do not use alcohol wipes as this will damage the print on the equipment.

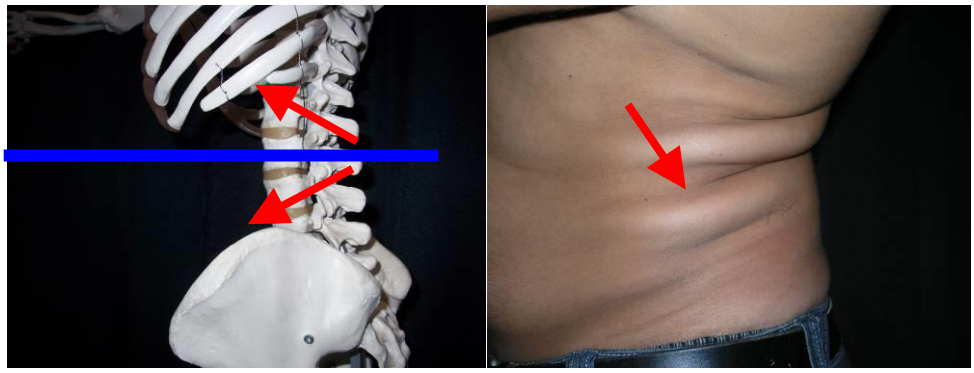


Figure 8. Identifying the lower costal margin and the highest point of the pelvis

5. When you are positioned at the participant's side, it will help you to identify the site by asking them to lean sideways towards you.

6. The skinfold is picked up obliquely when the participant is standing straight, at the mid distance between the highest and lowest crosses, following the natural contours of the skin. The callipers are then applied at the level of the marked mid distance (figure 9).
7. Record three measurements of subscapular skinfold thickness (in rotation if you are measuring more than just the one site).
8. Record the mean by adding the values together and dividing by three.

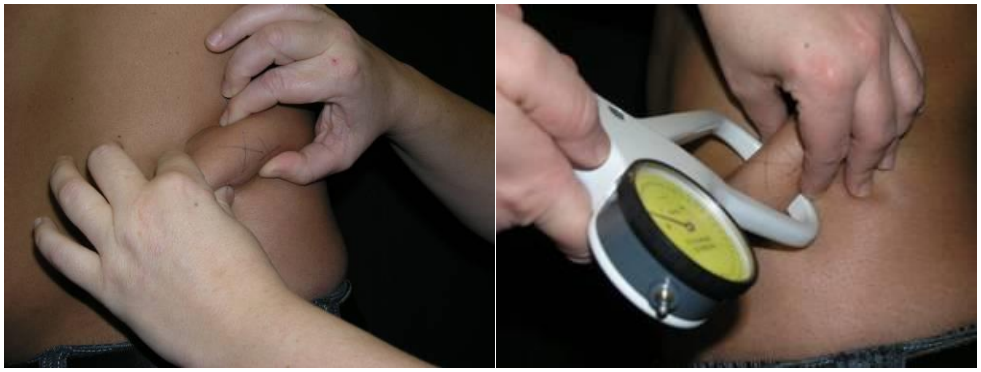


Figure 9. Picking up and measuring the mid axillary skinfold

Biceps and Triceps site measurement

Marking up sites for triceps and biceps skinfold thickness involves the **same** initial stages of establishing the exact distance between acromion and olecranon. This section is therefore to be followed for both triceps and biceps skinfold measurements.

1. Explain the procedure to the participant. It will mean marking the acromion and olecranon (figure 10).

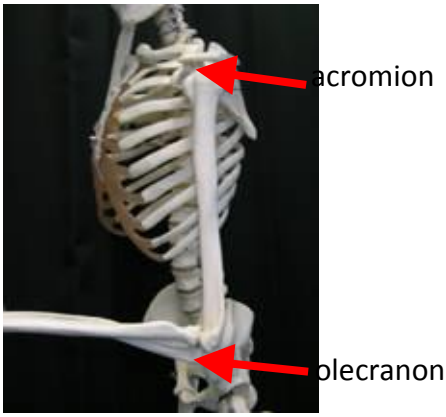


Figure 10. The acromion and olecranon

2. Ask the participant to stand with their back to the measurer and their arms hanging by their sides.
3. Palpate the tip of the acromion (the point of the shoulder) and mark with a cross (figure 11).

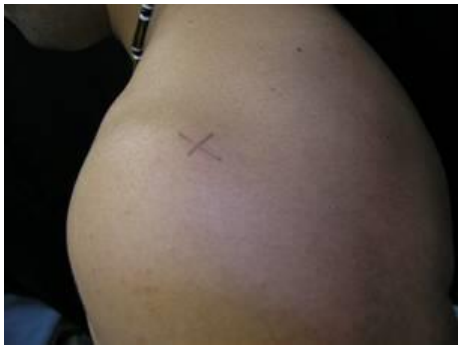


Figure 11. Marking the acromion

4. With the arm flexed at 90˚, as if holding a tray, palpate the olecranon (the tip of the elbow) and mark with a cross (figure 12).

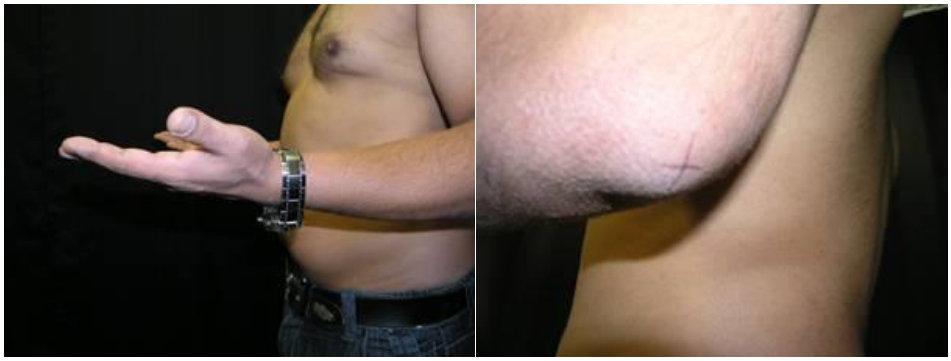


Figure 12. Position of participant and marking the olecranon

5. Put the tape measure on the mark on the acromion and drop it down to mark on the olecranon, by the side of the arm. Read the exact distance and mark a point on the arm halfway between the acromion and the olecranon (figure 13).

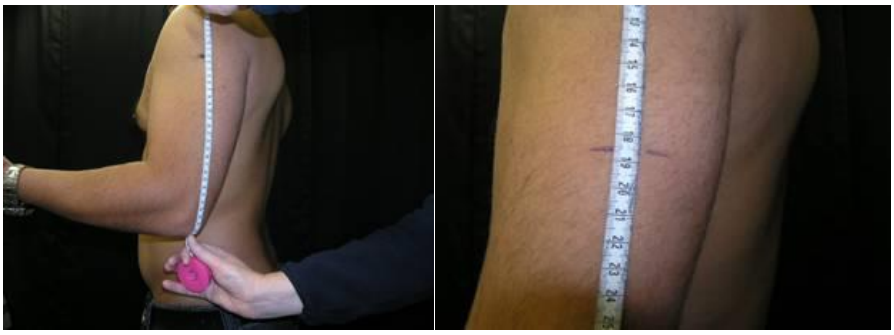


Figure 13. Marking the mid upper arm

6. This marks the **vertical level** at which the circumference will be measured.
7. Ask the participant to relax their arm by their side.
8. Place the tape measure around the upper arm with the **upper border of the tape at the level of the mark**, as if to measure mid-upper arm circumference. With the tape in position, draw a line on the skin posteriorly and anteriorly to the level of the first mark (figure 14). When you have completed all the measurements on the volunteer, wipe the length of the tape measure with a detergent wipe. Do not use alcohol wipes as this will damage the print on the equipment.

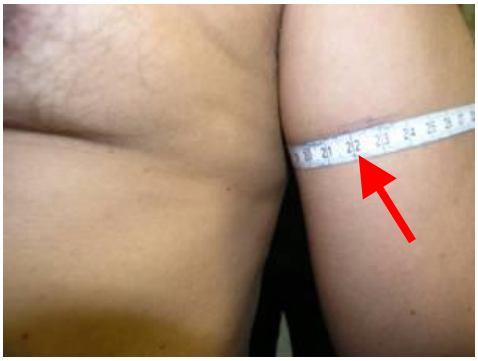


Figure 14. mid-upper arm circumference

9. The posterior line is used for the triceps fold and the anterior line is used for the biceps fold.

Biceps site measurement

1. Ask the participant to stand with their arms by their sides and their palms facing forwards. To determine where to take the skinfold measurement, "eyeball" the mid-point of the part which sticks out furthest. Make a vertical mark to form a cross.
2. The skin is picked up in a vertical tube with two hands, at least 1cm above and below the cross. The skinfold callipers are applied at the level of the cross, with the cross on the apex of the fold (figure 15).

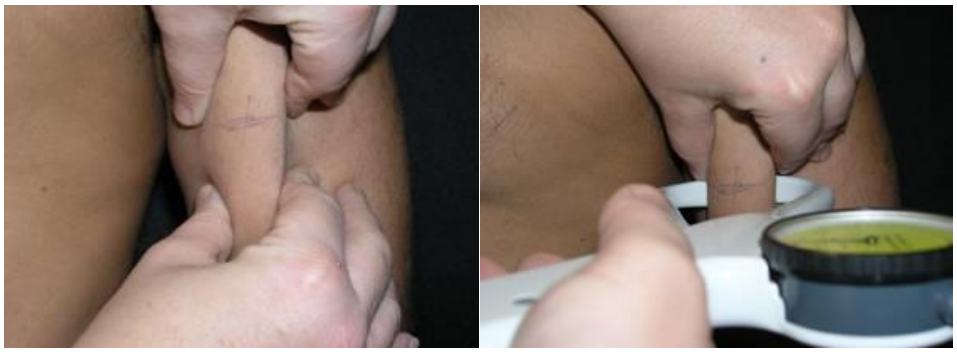


Figure 15. Picking up and measuring the biceps skinfold

3. Record three measurements of biceps skinfold thickness (unless you need to rotate with biceps, triceps etc measurements).

4. Record the mean by adding the values together and dividing by three.

Triceps site measurement

1. To determine where the skinfold is measured, "eyeball" the mid-point and the most dorsal (the part which sticks out furthest) part of the upper arm at the level of the horizontal mark. Make a vertical mark to form a cross.
2. The skin is picked up in a vertical tube with two hands, at least 1cm above and below the cross. The skinfold callipers are applied at the level of the cross, with the cross on the apex of the fold (figure 16).

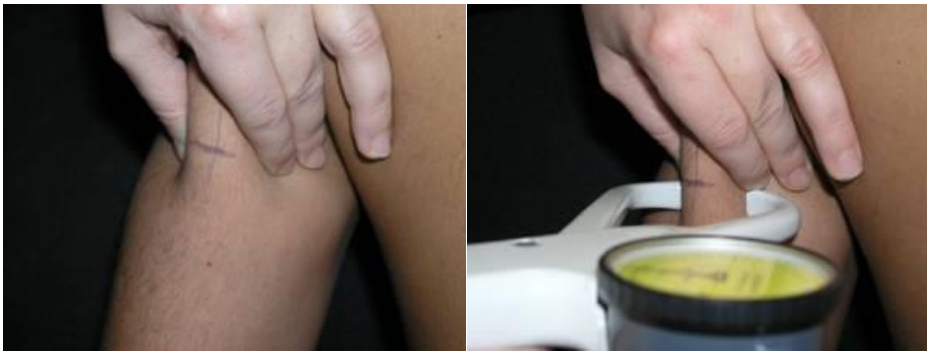


Figure 16. Picking up and measuring triceps skinfold

3. Record three measurements of triceps skinfold thickness (unless you need to rotate with biceps, triceps etc measurements).
4. Record the mean by adding the values together and dividing by three.

Appendix 3:

Standard Operating Procedure for Measuring Bioelectrical Impedance Using the ImpediMed SFB7

This Standard Operating Procedure is to be used for measuring bioelectrical impedance (BI) using the ImpediMed SFB7 equipment. BI and Bioelectrical Impedance Spectroscopy (BIS) are methods designed for measuring body composition and are based on the observation that the body's lean compartment (which includes muscle, bone and water), conducts electricity far better than the body's fat compartment which is low in body water.

The ImpediMed SFB7 is a single channel – tetra polar device that scans 256 frequencies between 4 and 1000 kHz. Cole modelling with Hanai mixture theory are used to determine total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF) from impedance data and additional data can be generated both by the equipment directly and/or using the software supplied with the device.

1. PROCEDURE
ImpediMed SFB7

Do not use this device on patients with active implanted medical devices, e.g cardiac pacemakers, defibrillators or patients connected to electronic life support devices. The ImpediMed SFB7 has yet to be clinically validated for use with pregnant patients; however, bioelectrical impedance technology has been shown to have no adverse affects.

Before testing

The operator should be mindful of the fact that certain situations are known to affect body water concentration:

- Just prior, during and just after menstruation.
- Use of diuretics.
- Renal or heart failure.
- Excessive exercise 2h prior to bioimpedance analysis.
- Consumption of excessive alcohol within 12h prior to analysis.

Preparing the volunteer

Prior to analysis the volunteer should:

1. Remove all jewellery (rings on fingers may be left on).
2. Remove stockings/tights/socks
3. Have an empty bladder
4. Be accurately measured for height (to the nearest 0.5 cm) and weight (to the nearest 0.1 kg).
5. Lie in the supine position for 5 minutes.
6. Ensure that their feet are not in contact with the bed frame (if present).
7. Extend their arms and legs making sure that they are not in contact with one another or touching/resting on any other part of the body.

Before using the ImpediMed SFB7:

1. **Do not use the machine when it is plugged in to the mains. The measurements are meant to be made when the machine is operating on battery power.**
2. Perform a calibration check on the machine (See "Calibration of the ImpediMed SFB7" section of this SOP). You will need to remove the alligator clips at the ends of the leads to do this.
3. Replace the alligator clips after performing the calibration test.
4. The manufacturers recommend the leads remain plugged in to the back of the device. Continual plugging and unplugging of the leads into the back of the machine is more likely to damage the leads over time.
5. Check the expiry date of the electrodes.
6. Remember to always use the stylus end (non-writing end) of the ImpediMed supplied pen to operate the touch screen. This pen is kept in the lid part of the ImpediMed carrying case.
7. Check the battery status in advance of seeing the patient. By selecting "setup" on the start screen you can check, by looking at the battery indicator, whether there is sufficient battery power to complete your series of measurements. To do this, select "setup" and look at the large battery symbol on the right side of the screen. To fully charge a depleted battery it will need to be

plugged in for 6 hours (during which time the equipment ***may not*** be used). A fully charged battery supplies 4-8 hours worth of operating time before recharging is needed.

8. Wash your hands and explain the procedure to the volunteer.

9. Obtain accurate measurements of the volunteer's height (in cm, to the nearest 0.1 of a cm) and weight (in kg, to the nearest 0.1 of a kg) following appropriate SOPs.

10. Instruct the volunteer to remove their shoe and sock from their right foot, remove any watches or bracelets on the right wrist which may impede the correct placing of electrodes, and lie in the supine position for 5 minutes before taking the measurements.

11. Ensure that the legs and arms are spread out so they are not in contact with any other part of the body.

12. Thoroughly wipe (using sterettes) the area of the skin where the electrodes are to be attached (as products such as body moisturiser, can affect the results).

Using the ImpediMed SFB7:

17. Turn on the SFB7 machine by pressing the on/off button on the front of the main unit (figure 1).

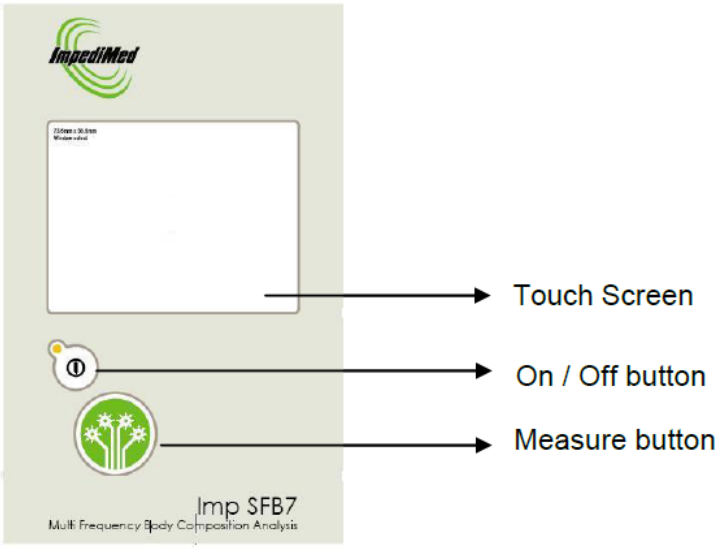


Figure 1. The front of the ImpediMed SFB7 unit.

18. To select whether you want the device to take measurements in BIS (bioimpedance spectroscopy) mode or in SFBI (selected

frequencies) mode, tap “setup” (figure 2) on the start screen and then select “modules” (circled red, figure 3).

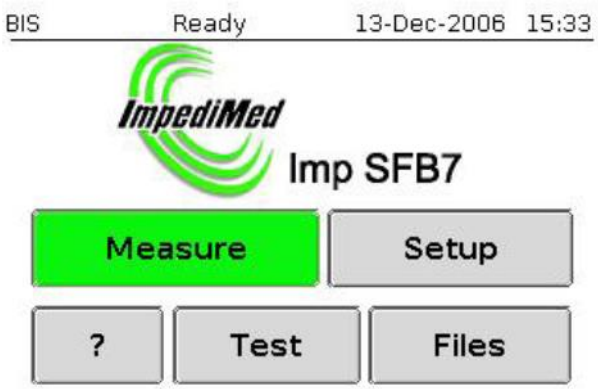


Figure 2. The start screen.

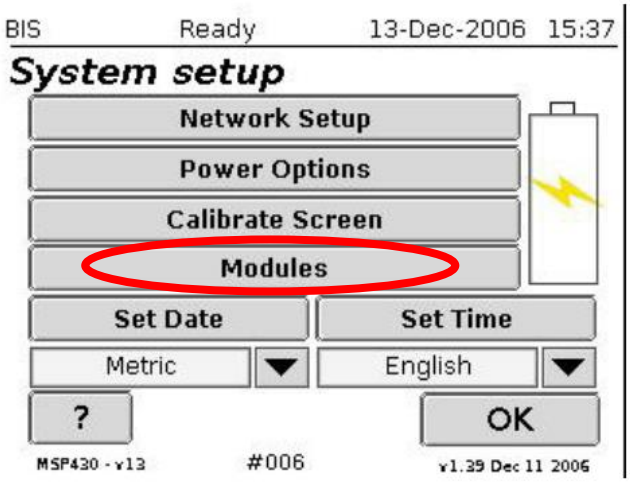


Figure 3. Setup screen

After tapping “modules”, choose either BIS or SFBI by tapping the screen. The one you have selected to use, from the “modules” screen will be marked with a cross. BIS will then be displayed on the upper left of the ImpediMed machine screen (circled red, figure 4).

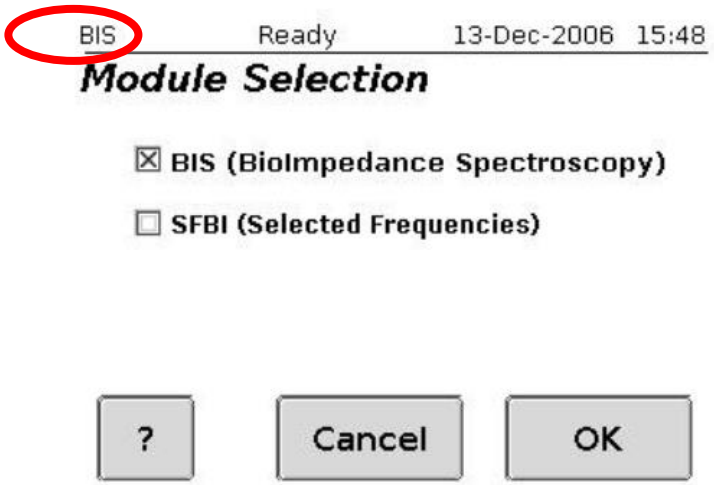


Figure 4. The module selection screen

19. Place two electrodes on both the right hand and the right foot with the tabs of the electrodes facing outwards (away from the volunteer) and connect the alligator clips on each lead to the appropriate electrode, following the instructions and diagrams below. There needs to be 5cm of free skin between the two electrodes. Use a ruler to measure this – there is one in the zip pocket of the ImpediMed machine case.

20. *Note that the placement of the electrodes to which the red lead and the black lead are attached is further away from the knuckles and toes than that for the Bodystat QuadScan 4000 and the Bodystat 1500.*

The yellow lead

The yellow lead end is attached to the electrode on the right hand, on the wrist next to the ulnar head (wrist joint, figure 5).

The red lead

The red lead is attached to the electrode on the dorsal surface of the right hand (figure 5).

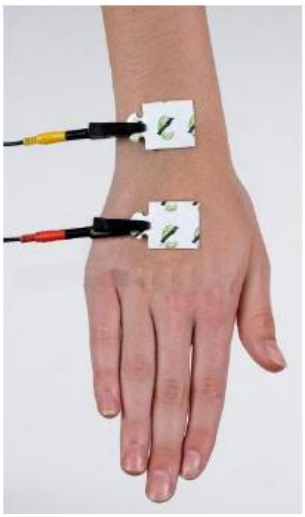


***Figure 5**. Placement of electrodes on hand*

The blue lead

The blue lead end is attached to the electrode on the dorsal surface of the right foot, on the ankle at the level of the medial and lateral malleoli (large protruding bones on the side of the ankle, figure 6).

[redacted]

The black lead end is attached to the electrode on the dorsal surface of the right foot (figure 6).

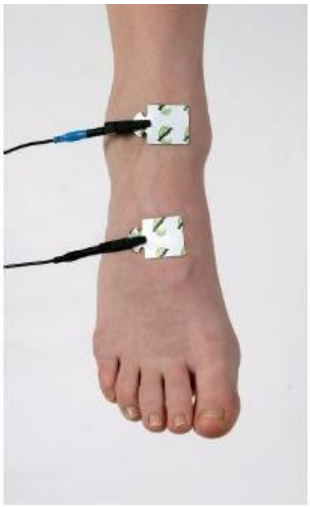


Figure 6. Placement of electrodes on foot

Making measurements in BIS mode

21. Select BIS mode by following the instructions above (no. 2, figure 3 and 4)

22. Tap "measure" on the starting screen to take you to the BIS "measurement setup" (figure 7) screen and tap on the "file name" box. This will bring up a key pad where you can type in the file name and/or number of your choice. After choosing a name for your file tap "ok" ***(If at this stage, the machine switches itself off, please follow the instructions on the laminated letter from ImpediMed [in a pocket of the ImpediMed machine carry case], which describes how to avoid this).***

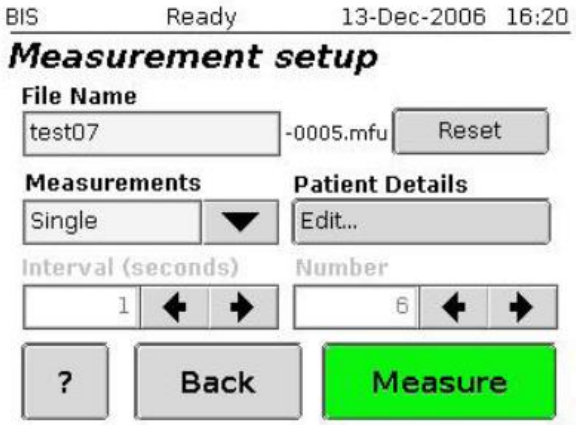


Figure 7. Measurement setup screen for BIS

23. Tap "patient details" and select the volunteer's gender, height, weight and age (figure 8) and then tap "ok". If you tap on the area circled red in figure 8 (instead of changing the value using the arrows), you can enter a more accurate height and weight value using the numerical key pad on the screen.

Figure 8. Patient details screen for BIS

24. Tap the down arrow to the right of the "measurements" box and select "continuous" and "3" (figure 7 – showing "single").

25. Check that all the alligator clips are correctly attached and that the volunteer is lying in the correct position and then press the measure button on the front of the device (large circular green button) or tap the "measure" box on the touch screen (figure 1 and 7).

26. Then tap "start" to begin.

Making measurements in SFBI mode *– note: you do not need to enter details of weight, height, age and gender when using the SFBI mode only.*

27. Select SFBI mode by following the instructions above (no. 2, figure 3 and 4)

28. Tap "measure" on the starting screen to take you to the SFBI "measurement setup" screen and tap on the "file name" box. This will bring up a key pad where you can type in the file name and/or number of your choice. After choosing a name for your file tap "ok" ***(If at this stage, the machine switches itself off, please follow the instructions on the laminated letter from ImpediMed [in a pocket of the ImpediMed machine carry case], which describes how to avoid this).***

29. Tap the down arrow to the left of the "measurements" box and select "single" (figure 9).

30. Now the machine is set to SFBI, the box that said patient details in the BIS mode now says "Selected frequencies" and SFBI is displayed in the upper left of the screen (circled red, figure 9).

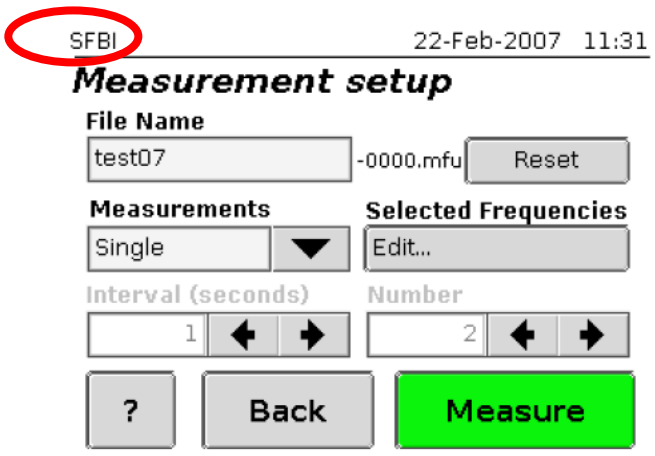


Figure 9. Measurement setup screen for SFBI

31. Tapping on the "selected frequencies" box will take you to the "frequency selection" screen where you may then select which frequencies you would like the machine to use for the measurements (figure 10). Tap on the boxes to put a cross in the frequencies you want to use. This section also gives you the opportunity to select frequencies of your own choice.

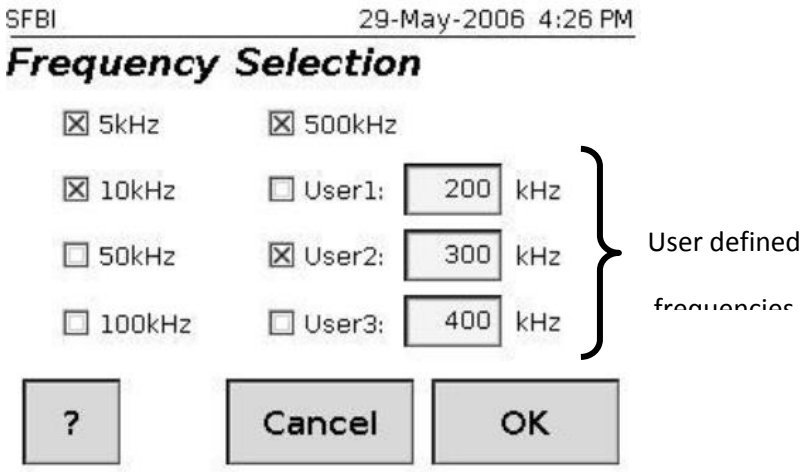


Figure 10. Frequency selection screen for SFBI

32. To make measurements at user-defined frequencies, tap on the box containing the numerical values, adjacent to the User1, User2 and User3 boxes. By doing so, you will be taken to the number keypad where you can enter the kHz value of your choice. Ensure that the boxes next to User1, User2 and User3 are marked with crosses or the machine will not make the measurements at your chosen frequencies.

33. Check that all the alligator clips are correctly attached and that the volunteer is lying in the correct position and then press the measure button on the front of the device (large circular green button) or tap the "measure" box on the touch screen (figure 1 and 7).

Calibration of the ImpediMed SFB7

ImpediMed recommends that calibration checks be performed at the start of each day of use. This is done using the RRC Test Cell, supplied with the machine.

1. Switch on the machine and set it to BIS mode, by following the instructions above (no. 2, figure 3 and 4).
2. Remove the alligator clips from the ends of the leads.
3. Plug the leads into the appropriate sockets of the test cell (figure 11).

Figure 11. The leads connected to the calibration Test Cell

4. From the starting screen, tap “test”
5. Then tap “start”.
6. The screen will display “Calculating”.
7. Followed by “passed” in green text or “failed” in red text.
8. If the test fails, an error code will be shown. In thtis instance, contact ImpediMed, your distributor or an authorised service centre, quoting the error code to arrange for service or repair.
9. By tapping “more” you can view graphs.

The *Rzero* value should be 604 ohms (± 5) and the *Rinf* value should be 403 (± 5)

Standard Operating Procedure for Bioelectrical Impedance Analysis using the Bodystat QuadScan 4000

This Standard Operating Procedure is to be used for measuring bioelectrical impedance using the *Bodystat QuadScan 4000*. Bioelectrical Impedance Analysis (BIA) is a method designed for measuring body composition. It is quick and easy to use and relatively cheap. The principle is based on the observation that the body's lean compartment (which includes muscle, bone and water), conducts electricity far better than the body's fat compartment because this is low in body water.

Different components of the body have varying levels of resistance (impedance) in response to different frequencies of electrical signals. The *Bodystat QuadScan 4000*, will provide actual resistance/impedance values from measurements taken at frequencies of 5, 50, 100 and 200 kHz and additionally calculate several *estimates* of body composition.

EQUIPMENT

http://www.bodystat.com/products/quadscan-4000/

What information can the Bodystat QuadScan 4000 give you?

- ECW % and ECW Volume
- ICW% and ICW Volume
- TBW % and TBW
- 3rd Space Water
- Body Cell Mass
- ECW/TBW Nutrition Index
- Plus Normal % Levels
- Body Fat % and Fat Weight
- Body Lean Mass
- Dry Lean Mass
- Basal Metabolic Rate (BMR)
- BMR/Body Weight
- Average Daily Calorie Requirement
- Waist/Hip Ratio
- Body Mass Index - Plus Normal Range
- Body Fat Mass Index (BFMI)
- Fat Free Mass Index (FFMI)
- Illness Marker™
- Impedance Values at 5, 50, 100 and 200 kHz
- Resistance 50 kHz/Reactance 50 kHz
- Phase Angle 50 kHz

Bodystat QuadScan 4000

Not recommended for females in the early stages of **pregnancy** or for participants with **pacemakers**.

Before testing

For accurate and reproducible results on repeat tests, it is important to ensure that the participant is as normally hydrated as possible. This should be based on a clinical assessment of the patient by the research nurse. They should refrain from:

- Eating for 4-5 hours before the test.
- Exercise for 12 hours before the test.

- Caffeine (i.e. normal tea, coffee and energy drinks) and alcohol consumption 24 hours before the test.

Before using the Bodystat QuadScan 4000:

1. Explain the procedure to the participant.
2. Clean the machine using a detergent wipe and then wash your hands.
3. Obtain accurate measurements of the participant's height (in cm, rounded-up or -down to the nearest whole number) and weight (in kg, to the nearest 0.1 of a kg) following appropriate SOPs.
4. Check that there is sufficient battery power in the machine prior to commencing by switching on the machine and checking the battery indicator (series of bars on the left of the display).
5. Instruct the participant to remove their shoe and sock from their right foot, remove any watches or bracelets on the right wrist which may impede the correct placing of electrodes,
6. Lie participant in the **supine position for 5 minutes** before taking the measurements.
7. Ensure that the legs and arms are spread out so they are not in contact with any other part of the body.
8. Thoroughly wipe (using alcohol wipes/sterettes) the area of the skin where the electrodes are to be attached as products such as body moisturiser, can affect the results.

PROCEDURE

Using the Bodystat QuadScan 4000:

1. Place two electrodes on to the right foot (it might be helpful to draw an imaginary straight line between the protruding bones on the ankle, then place each electrode in the centre of that line). Place one behind the second toe and the other on the ankle between the medial and lateral malleoli (the large protruding bones on the side of the ankle) (figure 1).

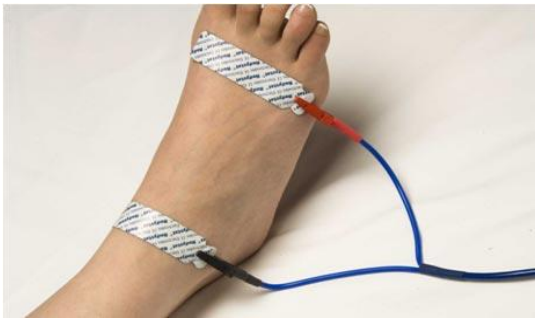


Figure 1. Placement of electrodes on foot

Foot electrodes: *Electrodes are placed sideways so that the non-stick electrode connector point is facing the researcher. One electrode is placed centrally, directly where the second and third toe meet the foot. Place the second (black) electrode at the crease of the ankle (midline to the 'boney' landmarks).*

2. Attach two alligator clips to the electrodes (red to the electrode nearest the toes, figure 1).
3. Place two electrodes on to the right hand (it might be helpful to draw an imaginary straight line between the protruding bones on the wrist, then place each electrode in the centre of that line). Place one behind the knuckle of the middle finger and the other on the wrist next to the ulnar head (figure 2).

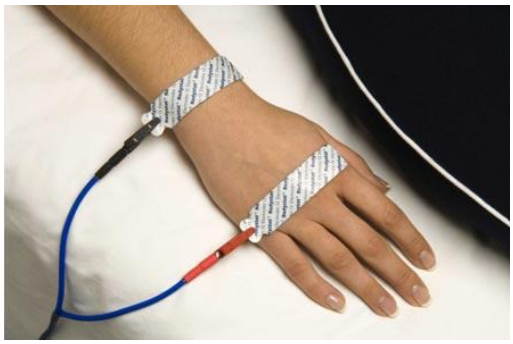


Figure 2. Placement of electrodes on hand

Hand electrodes: *Electrodes are placed sideways so that the non- stick electrode connector point is facing the researcher. One electrode is placed centrally, directly below the third knuckle of the middle finger. The second (black) electrode is placed on the crease of the wrist (midline to the 'boney' landmarks).*

4. Attach two alligator clips to the electrodes (red to the electrode nearest the fingers, figure 2).
5. Turn on the *Bodystat QuadScan 4000.*
6. 'test number' appears on the screen (used to identify subjects). Make a note of the number and then press enter ↵ to continue
7. Key in accurate data using the up and down buttons (↑/↓) (age, height, weight, and measurements of hip and waist circumference).

8. It is **not necessary** to enter waist and hip measurements or to select an activity level. You can leave these as the default setting.
9. Ensure that the participant has been in the supine position for 5 minutes.
10. Press enter to perform the measurement.
11. The *Bodystat Quad Scan 4000* will tell you to 'connect electrodes'.
12. Press enter again to commence the measurement and 'measuring' will appear on the screen.
13. The results will appear on the screen, the top line will display the actual measured result and the bottom line displays the recommended range.

Calibration of the *Bodystat QuadScan 4000*

The *Bodystat QuadScan 4000* should be calibrated at the beginning of each day of use.

The calibrator is supplied with the *Bodystat QuadScan 4000* machine. Calibration is performed following the instructions below:

1. Attach one pair of red and black leads to any one terminal of the calibration unit.
2. Attach the other pair of red and black leads to the other terminal of the calibration unit.
3. Switch the unit on and make a note of the test number.
4. Accept the "default" values on the display.
5. Continue as normal to make the measurement.
6. Scroll using the down arrow to the values of impedance. You should find that the results at 5 kHz, 50 kHz, 100 kHz and 200 kHz should reflect readings of between 496 to 503, approximately a 0.5% variance on either side of the high precision 500 ohm resistor in the Bodystat calibrator.
7. If the results are incorrect, replace the battery with a Duracell or Procell. If this does not rectify the problem, contact *Bodystat QuadScan 4000* or your local supplier.

Standard Operating Procedure for Measuring Bioelectrical Impedance UsingTanita

1. PROCEDURE

5.1 EQUIPMENT

http://www.cranlea.co.uk/index.php?option=com_content&view=category&layout=blog&id=3&Itemid=1

What information can the Tanita BC-418 MA give you?

Total body measurements:

- Weight
- BMI
- Body Fat %
- Fat Mass
- Fat Free Mass
- Muscle Mass
- Total Body Water Kg
- Total Body Water %
- Basal Metabolic Rate7

Segmental Measurements:

- Segmental Body Fat %
- Segmental Fat Mass
- Segmental Fat Free Mass
- Segmental Muscle Mass
- Segmental Impedance
- Goal Setter Calculation

5.2 PROCEDURE

Prior to using the Tanita equipment, the participant's height must be measured, following the appropriate SOP. The Tanita machine will measure the weight for you!

When measurements are made using this equipment you should ensure that:

- The participant stands barefoot on the electrode panels with their feet parallel.
- They let their arms hang naturally.
- They do not stand with bent knees or elbows.

- The participant is facing forwards.
- They do not lean on the main unit.

1. Do not use this equipment within 2 metres of products that emit electromagnetic waves. Certain lighting equipment, medical instruments, and communications equipment (such as inverter fluorescent lighting, microwave therapy devices, and mobile phones) may cause interference.
2. Place feet correctly on the electrode panel for measurement. Incorrect positioning may result in a lower fat % than is actually the case or an error message. Their feet should be positioned on the heel and toe electrodes on each side.
3. Place arms straight down during the measurement as other positions may induce a lower than actual fat %, or an error message.
4. If possible, measurements should be taken under the same conditions each day in order to determine any fluctuations (not during the three hours after getting up in the morning, or three hours after meals).
5. Any dust or dirt should be cleaned from the feet prior to measurement as this may interfere with the results.
6. Avoid skin contact between the arms and torso, and between the inner thighs. A dry towel may be placed between the arms and torso/inner thighs if there is skin-to-skin contact in these areas.

For installation

- Do not position in close proximity to stored chemicals, or where gas is generated.
- Do not position in direct sunlight, in areas subject to airflow directly from an air conditioner, or near a heater.
- Do not position in locations subject to significant temperature fluctuation (use the machine at 5-35˚C).
- Do not position in locations subject to high humidity or near a water supply (use the machine at 30-80% humidity).
- Do not position on surfaces subject to strong vibration.

For handling

- Do not wash with water. Wipe clean with a detergent wipe.
- Avoid subjecting the equipment to excessive shock or vibration.
- Do not unplug the power cable by pulling on the cord.

- When it is not to be used for a prolonged period, turn off the power, and unplug the power cord.

For storage

- Do not store on a surface subject to strong vibration.
- Do not store in locations subject to high humidity (storage range = 10%-90%) or dust.
- Do not store in locations subject to temperatures outside its storage range (storage range = -10-50˚C).
- When it has not been used for a long time, confirm it can be operated correctly and safely before use.
- Cover the main unit to prevent it from becoming dirty.

For cleaning

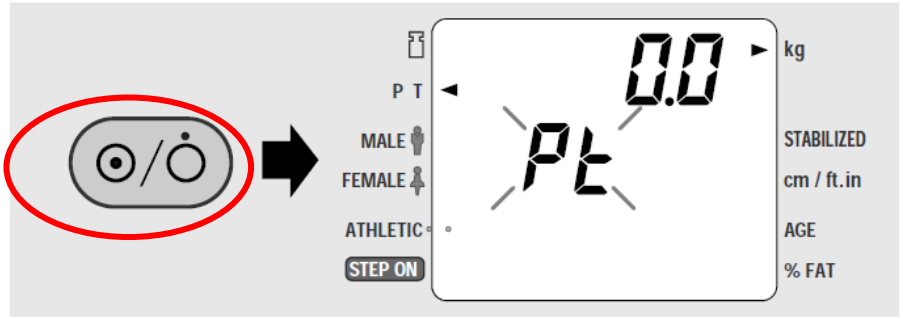
- Please use a detergent wipe to clean the equipment, and wipe it dry with a cloth immediately afterwards.

* **Definition of the athletic body type**

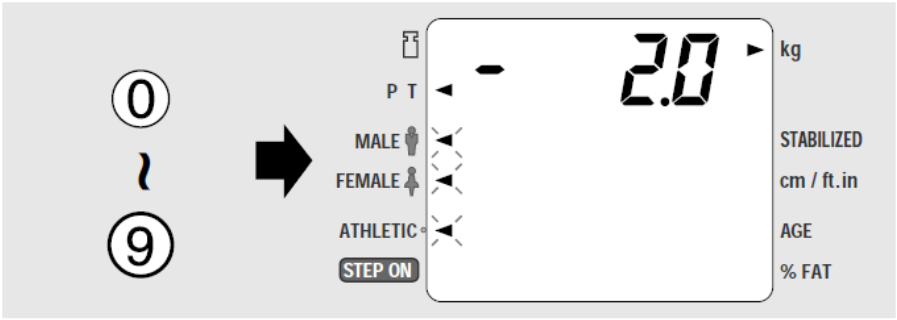
It is recommended that “Athletic” should be selected for individuals aged 18 or over who consider themselves to be athletes or to whom the following conditions apply:

- People who do 12 or more hours of training (exercise) a week.
- Members of gymnastics or sports organizations aiming to participate competitively.
- People such as bodybuilders who undergo training to build up muscles.
- Sports professionals.

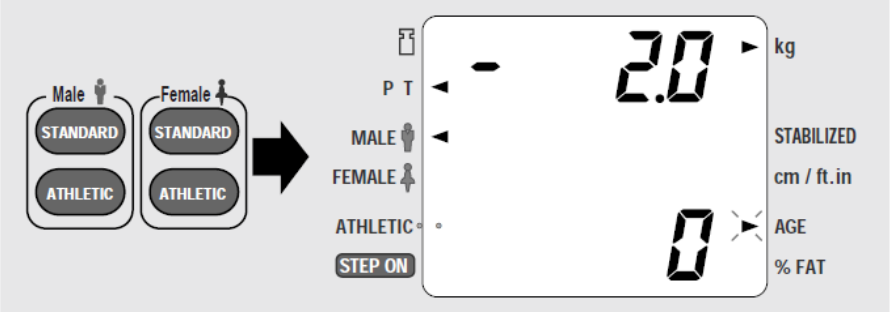
1. Remove the dust cover from the Tanita machine and wipe the hand pieces and foot plates with an alcohol wipe. Dry with a paper towel to prevent the participant from slipping.
2. Turn on the Tanita machine: 0.0 and Pt should both appear on the screen.



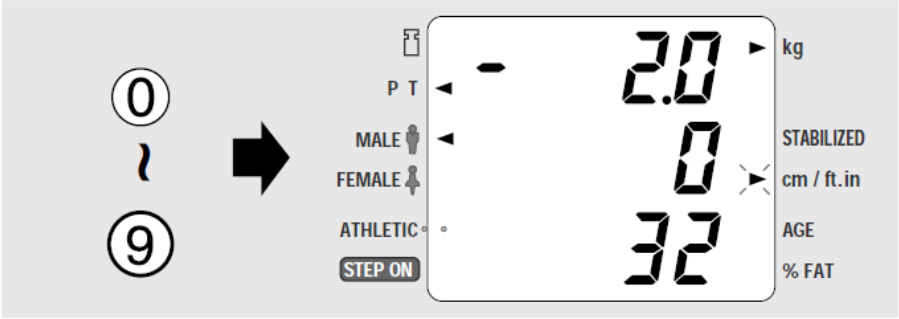
3. The 0.0 indicates the weight of clothes. Enter **1kg** for clothes weight (when entered this will be displayed as a minus value. In this example, clothes weight is set for 2kg).



4. Next, key in the participants age.



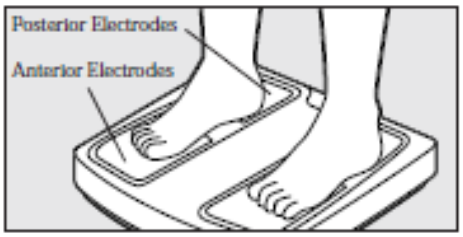
5. Then the participants height (in cm).



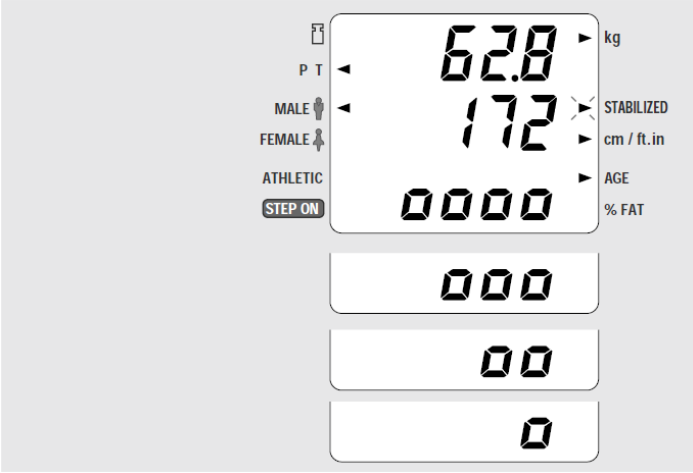
6. If the function has not been switched off, the machine may next require you to key in the participants target body fat ratio, if you are not asked to do this, it will go directly to the

measurement step and ask you to step on to the platform. Feet should be positioned on the heel and toe electrodes on each side.

7. Without them holding the handgrips (as this section is only measuring weight), ask the participant to step on the platform and stand in a stable position without bending their legs. Continue to stand, without holding the handgrips, until the word "stabilised" appears on the right side of the screen.



8. Do not step off the platform. Reach the handgrips and grasp one in each hand. "*o o o o*" will appear on the bottom left of the screen. Remain in the same position, grasping the handgrips until the "*o*" symbols have disappeared.



9. When all the measurements have been completed, the overall body fat percentage will be shown at the bottom of the screen. If the printer is on, the results will be printed out.

For turning off the equipment:

Clean the hand-held electrodes and foot plates with alcohol wipes.

NEVER unplug or turn off the TRANSFORMER (blue battery sized box attached to the equipment).

If you want to alter the number of printouts and/or the printout items

follow the instructions below:

To alter the number of printouts, press and hold the [0] key and at the same time, press the start "key" (circled red in point no. 2) and then release the keys after "Prt-1" is displayed. Select the number of printouts using the numerical keys.

After pressing a number to indicate the number of printouts you want, the screen will display "Lng-1". This indicates the language, press 1 for English. The screen will then move on to SPEC – (on the first line), 0.5h (on the second line, turned on and off by pressing the number 1 key) and 1.Ln (on the third line, turned on and off by pressing the number 0 key). If you press the number 0 key so that 1.Ln is no longer displayed then the machine will print out a shortened version of the results consisting of only whole body impedance. If you press the number 1 key which will remove the 0.5h then the machine will print out both whole body and segmental analysis results.

Standard Operating Procedure for Measuring Adult Height

This Standard Operating Procedure is to be used for measuring adult height. Height measurement can be affected by posture, foot wear, feet and head positioning. It is necessary therefore to have a technique to measure height that can be replicated by other measurers; over time; and in the same subject. For the purposes of both longitudinal follow-up studies of individuals or populations, and cross-sectional group studies, accurate and reproducible measurements of height are essential.

PROCEDURE

Stadiometers: www.white-medical.co.uk

Stadiometers are devices specifically designed for the accurate measurement of height and when used with care yield data of the highest quality. There are a few different stadiometer models in the SCBR. The Leicester Height Measure is very "user friendly" but all the stadiometer models in the SCBR can yield equally accurate and precise results if the measurer adheres strictly to the SOP.

The 'Leicester Height Measure' is lightweight and portable and allows measurement accuracy of height to the nearest 1mm. The range is from 0 – 2.07m, in 1mm gradations. It comes in the form of a plastic measuring rod, in four sections which slot together. There are unique codes at each end of each rod (i.e. star shape, square, circle etc.) which line up with each other to ensure that sections are slotted together properly. It has a base plate for the individual to stand on, two stabilising side arms that make contact with the wall and a head plate with arrows indicating the point at which the measurement should be read. Each rod is marked in metric (centimetres and millimetres) and imperial (feet and inches) units.

You will require **two practitioners**, one holding the participant's head in the correct position, the other reading the value.

1. Ensure the stadiometer is checked and validated using metal rods of known height.
2. Ensure that the stadiometer is wiped clean before use.
3. Wash your hands and explain the procedure to the participant. Explain you will want them to stand as tall and straight as possible and that you will be making 3 measurements of their height.
4. Ensure that heavy outer clothing and shoes are removed and if necessary, trousers / jeans are rolled up to enable the measurer to check the position of the heels.
5. Undo or adjust hairstyles and remove hair accessories that interfere with measurement.

   If the person has a hairstyle that can not be adjusted (e.g. braids/dreadlocks), an implement of a known length (such as a short metal rod) can be placed on the crown of the head between the braids/dreadlocks when the head is in the Frankfort plane. The total height of participant *plus* rod can then be measured and the length of the rod can be subtracted from the result in order to obtain a height

measurement. The same approach applies for individuals wearing turbans. You may ask ladies wearing headscarves if they would mind removing them. If they are unhappy to do this, you can ask to feel the top of their head/ask them how many layers of material are on top of the head and how their hair is arranged beneath the scarf. Make a note in the participant's medical notes if you have had to do any of these.

6. Aim to measure wearing light clothing.
7. Ask the participant to stand on the stadiometer, facing forwards as tall and straight as possible with their arms hanging loosely at their sides.
8. Their feet should be flat on the base plate of the stadiometer and positioned slightly apart, in line with their hips, to aid balance. There is an outline of feet on the base plate but it is not necessary for the participant to stand on the feet marks. There will be some exceptions (e.g. participants with a larger chest/belly) but when possible their heels should be touching the back plate.
9. Their knees should be straight and their buttocks and shoulders should touch the stadiometer. Again, there may be some exceptions (e.g. participants with a bigger bottom) but if they are able to do so then they should.
10. Ensure the participant's head is in the "Frankfort plane". This position is an imaginary line from the centre of the ear hole to the lower boarder of the eye socket. This is a midline position.
11. If will be necessary for one person to manipulate the participant's head in your hands by placing the heels of your palms either side of the face and the fingers of each hand resting on the back of the skull above the neck. Your fingers should come to rest on the mastoid process behind the ears. Firmly but gently, apply upward pressure lifting their head to the maximum height. Avoid jerky movements, perform the procedure smoothly and take care not to tilt the head at an angle.
12. The other measurer should stand to the side to double check the Frankfort Plane is correct. Both measurers can check for any bending of the knees, slumping of shoulders or raising of heels.
13. Ask the participant to take a deep breath and hold.
14. The assisting measurer standing at the side should then bring the head plate down onto the head, ensuring it rests on the crown of the head, i.e. the top back half.
15. The nurse should then read the measurement.
16. Nurse's eyes should be level with counter/pointer and measurement read to the nearest 1mm (this may require a stool/small ladder). Record the measurement, plot on growth chart and sign/initial with date.

17. The participant should be able to step off the stadiometer without ducking their head.
18. Make three measurements of height, asking the participant to stand off the stadiometer between each measurement.
19. The three measurements should all fall within 2mm of one another. If the first three measurements do not fall within this 2mm limit then you must perform measurements of height until the 3 **most recent** results are within 2mm of one another. Cherry-picking the best 3 results from a choice of more than 3 measurements is not permitted.
20. Record the three most recent results and calculate the mean by adding the three values together and dividing by 3.
21. Should you be making repeated measurements on the same individual on different days, it is advisable to measure at the same time of day if possible. During the day our height decreases due to compression of the spine.

Standard Operating Procedure for measuring adult weight

This Standard Operating Procedure is to be used for measuring weight of adults. Weight measurement can be a useful guide to nutrition, fluid retention and assessment of BMI and body composition. The measurement of weight can serve as an indicator of a person's general health and well-being. It may also help to diagnose current medical conditions or serve to predict or identify future health problems. It is very important that the measurement is taken using the same method and in the same conditions in order to ensure uniformity between participants and in the same participant over time. For the purposes of both longitudinal follow-up studies of individual children or populations, and cross-sectional group studies, accurate and reproducible measurements of weight are essential.

EQUIPMENT

Any equipment used for measuring weight must comply Trust policy with a recommended maximum error allowance of 0.1kg. Electronic weighing scales designed for medical purposes are generally better than the simple mechanical weighing scales which should be avoided for two reasons – firstly, the mechanisms can wear and secondly, they have the capacity for adjustment and are prone to 'fiddle'.

There are many different weighing scales available, but the scales that are best suited to this purpose are those which are:

- valid
- can be routinely checked through calibration
- have an in-built spirit level to ensure that scales are horizontal
- have a remote display so that the individual cannot see the reading
- are portable, light and easy to transport
- display weight to 100g over the range 0-150 kg and have both a hold and tare facility

Mechanical bathroom scales must not be used because of innate inaccuracies and errors that may be introduced with use over time.

The weighing equipment must be calibrated and this should be done by trained personnel annually. Calibration sticker and spirit level must be checked at the start of each measuring session.

Once every three month the scales should be weight checked by the relevant person within WTCRF-BRU. In order to check the equipment, you should use two different masses of known weight (i.e. 25 and 50kg - see Trading Standards). You should set the scales to zero and place each of the weights, in turn, on to the scales to check that the weighing equipment records the same weight as the portable weight. If the error goes beyond 100g, the scales must be sent for calibration.

PROCEDURE

1. The scales need to be checked and calibrated annually following the manufacturer's guidelines.
2. Turn on the scale's display as a check for the operational function, check the spirit level is balanced and ensure that scales are in kilogram mode. Wait for the display of 0.00 before asking the participant to stand on the scales.
3. Ensure shoes and any heavy clothing is removed and ask the participant to stand as still as possible.
4. Ensure the participant stands in the centre of the scales with their arms hanging loosely at their sides, their head facing forward. They must look straight-ahead and remain as still as possible. The posture is important as this ensures their weight is evenly distributed, to achieve an accurate reading.
5. The display should then show a fixed weight.
6. The weight should be recorded in kilograms and should be signed /dated by the nurse taking the measurement.

7. Weight should be recorded as displayed (i.e. to the nearest 100g or 0.1kg) They should then step off the scale and repeat the process a further 2 times to get a total of three readings.
8. If these readings are within 100g of each other, then record these readings as well as the average of the three readings.
9. If you do not have three readings that are within 100g of each other, then continue to repeat the process until you do.

Appendix 4:

Standard Operating Procedure for preparation and administration of deuterated (^2H_2O) Water and collection of saliva samples for analysis by FA-MS (Flowing Afterglow-Mass Spectrometry)

This Standard Operating Procedure is to be used for preparation and administration of labelled water and the processing of resulting samples. Deuterated (heavy) water for the purpose of measuring total body water (TBW) has been used for over forty years. The principle states that the volume of TBW is equal to the amount of deuterium consumed, divided by the concentration of deuterium in the body after equilibration.

EQUIPMENT

Deuterium oxide 99.9 atom % can be bought from Sigma Aldrich in different volumes ranging from 10g to 4kg. The product code is 151882

Salivettes are from Sarstedt. "Cotton swab salivettes without preparation". Product code is 51.1534 for 500/case (100/bag).

PROCEDURE

The deuterium concentration in the resulting saliva samples makes these samples suitable for analysis by FA-MS (Flowing Afterglow Mass Spectrometry) or IR-MS (Isotope Ratio Mass Spectrometry), on sample dilution.

Deuterium is not a hazardous substance and the drink should be made up in a <u>kitchen</u> NOT a <u>laboratory</u>.

Preparation of the water

1. Label an empty dosing bottle with the date, participant's details and your contact details.

2. With gloved hands, label and then weigh an empty dosing bottle with the lid and a drinking straw on the nutrition kitchen scales (to 3 decimal places).

3. Record the weight of the bottle, lid and straw.

4. Transfer the appropriate dose of deuterium based on the participant's body weight (table 1), into the weighed and labelled dosing bottle.

Subject Weight	**Dose of deuterium to drink through straw**
Less than 30 kg	10 ml
30 – 40 kg	12 ml
41 – 50 kg	15 ml
51 – 70 kg	20 ml
71 – 100 kg	29 ml
More than 100 kg	33 ml

Table 1. Dosing volumes in ml based on body weight in kg

5. Label another dosing bottle with the date, participant's details and your contact details and put in 20ml of tap water.

6. Wash your hands and explain the procedure to the participant.

7. Check that the participant has not had anything to drink for 2 hours prior to administration and has not brushed their teeth for at least 30 minutes before drinking the water.

8. Ask them to empty their bladder.

9. Take the cotton swab from the first of 2 salivettes (labelled with Pre-dose, date, subject identification and visit number).

10. Ask the participant to move the swab around their mouth until wet and then replace the wet swab into the upper portion of the salivettes. The swab should **not** be chewed. Repeat this using the second salivette so that you have obtained 2 saliva-soaked cotton wool swabs.

11. Centrifuge the collected saliva samples following the instructions in the section on "centrifugation of saliva samples" below (5.2.2).

12. Ask the participant to drink the full volume of deuterium through the straw that was weighed with the bottle.

13. When they have ingested all the solution, ask them to rinse their mouth with 20ml of tap water to remove any residual deuterium.

14. Record the time that the dose was given.

15. Keep the straw in the bottle and weigh the bottle and lid with the straw remaining in the bottle.

16. Calculate the weight drunk by subtracting this value from the weight of the dosing bottle containing the deuterated water drink, lid and straw. Record these details.

17. Obtain three more sets of saliva samples ideally from 2 salivettes per time-point: 2, 3 and 4 hours post-dose.

18. If the participant finds it difficult to soak 2 cotton swabs with saliva then one well soaked one should be satisfactory for the 2, 3 and 4 hour time points. It is essential to ensure there is enough saliva collected from the participant before they drink the deuterium (pre-dose saliva sample) so 2 salivettes worth of saliva at this time-point is essential. No less that 0.5 ml of saliva from any time-point should be stored in each glass vial.

Centrifugation of saliva samples

Perform this in the WTCRF sample processing laboratory "category 2" laboratory

19. Take the cotton swab after the participant has moved it around their mouth to thoroughly wet it and place it back into the salivette.

20. Making sure that you have balanced the weight in the centrifuge using the scales by the side of the centrifuge in the WTCRF sample processing lab, place the salivettes into the appropriate centrifuge buckets.

21. Centrifuge at 4°C for 8 minutes at 2060 x g (3000 rpm) following the WTCRF SOP for use of the "Beckman Counter Allegra 6R" centrifuge.

22. Remove from the centrifuge and check that all the saliva is now in the bottom portion of the salivette. Remove the top half (comprising the lid, upper plastic section and swab) and discard.

23. Split the saliva sample (in the bottom portion of the salivette) equally between 2 appropriately labelled glass vials using a pipette. Parafilm the lid to prevent any evaporation prior to analysis, whilst in storage.

24. Store the samples at -80˚C

Appendix 5:

Standard Operating Procedure for Child Dual-Energy X-Ray Absorptiometry (DXA)

This Standard Operating Procedure is to be used for Dual-energy X-ray Absorptiometry (DXA) scans on Children. Bone density scanning, also called dual-energy x-ray absorptiometry (DXA or DEXA) or bone densitometry, is an enhanced form of radiographic technology that is used to measure bone loss, bone mineral accrual and body composition. DXA is today's established standard for measuring bone mineral density (BMD). A radiograph is a painless medical test that helps physicians diagnose and treat medical conditions.

EQUIPMENT

The system used is a Hologic Discovery DXA System, manufactured by Vertec Ltd., who also support servicing, updates and repairs.

www.vertec.co.uk

The equipment is calibrated with quality assurance checks using a spine phantom before every use by appropriately qualified and trained staff. In addition, a weekly step-wedge check is performed for system accuracy. The machine will not operate if these checks are not passed. The staff using the equipment will be qualified in radiation protection procedures and use the equipment to the specified guidelines produced by the manufacturer. Only the volunteers are exposed to ionising radiation (x-rays) during the procedure and the approximate radiation doses are recorded in the patient's hospital notes.

Procedure

Contraindications to scanning

1) Pregnancy
2) Any investigation carried out in the last 10 days using a radioisotope, as these will affect the body composition results.
3) Presence of internal metal artefacts, as these will affect the body composition results.

Whole Body DXA scan in children:

1. The child will need to have their height measured first. As this forms the basis of determining the scan length.

2. The child will need to wear clothing that does not have any buckles, belt or metal fasteners. All jewellery must be removed if possible. .
3. The child should always be moved to and from the table with the scan arm to the left (foot-end) of the table, for stability and access.
4. The child should be lying on their back, with their head at the right end of the table and within the scan limit borders marked on the mattress. For any patients taller than the length of the scanning table, their feet can hang over the edge of the bed.
5. The patients arms should at their sides, with palms downwards but not touching their thighs. This maybe difficult to achieve in large/obese patients, in this case, the arms should be at their sides with palms touching their thighs
6. The patient's feet are rotated inwards slightly, leaving a gap between the toes. If movement may be a problem, thin tape may be placed around the toes to support the legs in position. In young children, it may be perferable to allow their legs to relax outwards, in order for them to keep still for the entire scan.
7. The patient is asked to remain still and to breathe normally.
8. Scanning is started in accordance to the operating procedure described in the Hologic Discovery Training Manual. The researcher should inform the patient that the table and scan arm will move over them in 7 passes and to keep as still as possible. The whole body scan will take approximately 5 - 7minutes, depending on the height of child.
9. The DXA machine is operated using the settings and techniques described in the Hologic Discovery Training Manual, which is kept in the scan room. In children, the scan length will be adjusted to a suitable size, depending on the height of the child.
10. The scan is then analysed using the Whole Body Fan Beam analysis algorithm and the results are printed.

Data storage: After each scan is performed and analysed the data is archived on the machine's hard drive and an in addition, the scans are backed up onto an external hard drive.

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