Title: Characterising the nutritional status of children with primary ciliary dyskinesia

Authors: Marino LV[1,2], Harris A[3], Johnstone C[1], Friend A[3], Newell C[2], Miles EA[4], Lucas JS[2,3,5], Calder PC[2,4], Walker WT[2,3,5]

Affiliations: Department of Dietetics/Speech and Language Therapy, University Hospital Southampton NHS Foundation Trust, Southampton, UK[1], NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, UK[2], Primary Ciliary Dyskinesia Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK[3], Human Development & Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK[4], Clinical and Experimental Sciences Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK[5].

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Corresponding author: Luise Marino, University Hospital Southampton NHS Foundation Trust, Southampton, UK S016 6YD Tel: + 44 (0) 23 8079 6000 Email: Luise.Marino@uhs.nhs.uk

Abstract

Introduction: Primary ciliary dyskinesia (PCD) is a rare, heterogeneous genetic disorder where impaired mucociliary clearance is caused by dysfunctional motile cilia leading to bronchiectasis. There is limited evidence characterising the nutritional status of children with PCD, although lower body mass index (BMI) z-score has been associated with worse lung function (FEV_1).

Methods: All children (n=43) with PCD, aged <16 years, from a single tertiary centre were prospectively enrolled. Information on clinical phenotype and nutritional status including bioelectrical impedance spectroscopy (BIS) phase-angle was collected.

Results: There was a weak positive association between height-for-age z-score (HAZ) and FEV_1 z-score (n=28, r=0.4, p=0.049). Those with a low fat free mass index (<-2 z scores) had a lower BMI z score (-1.3±1.2 vs. 0.8±0.7, p=0.0002). BIS phase angle identified more patients at nutritional risk than using moderate malnutrition cut-offs of either HAZ or BMI ≤-2 z scores alone (21% vs. 4.6% vs. 6.9% respectively). PCD patients had a higher incidence of vitamin D insufficiency (<50 nmol/L) (54%) and deficiency (<30 nmol/L) (26%) than healthy children.

Conclusions We have characterised the nutritional phenotype of a cohort of children with PCD. Monitoring vitamin D levels is important in PCD patients. There is a weak association between lung function and nutritional status, and measures of BIS phase-angle. The use of BIS phase-angle may allow for early identification of at risk children and may therefore be of benefit for nutritional assessments in the clinical setting. These findings will help inform a future nutritional intervention strategy in children with PCD.

What we know:

- There is a positive association between lung function and body mass index in PCD.
- A low bioelectrical impendence spectroscopy phase angle is associated with poorer nutritional status, which may precede anthropometric changes and impact on clinical outcomes.

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What this study adds:

- In children with PCD, there were weak associations between BIS phase angle, nutritional status and clinical outcomes
- In PCD, the routine use of BIS phase angle may add a sensitivity and specificity in identifying children with nutritional risk earlier than using anthropometry alone
- Vitamin D insufficiency may be more common amongst children with PCD

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Introduction

Primary ciliary dyskinesia (PCD) is a rare, heterogeneous genetic disorder where impaired mucociliary clearance is caused by dysfunctional motile cilia. Infants with PCD usually have neonatal respiratory distress, and they go on to have persistent sinopulmonary infections, which leads on to bronchiectasis in over half of children and almost all adults [1]. Historically PCD has been considered a fairly mild respiratory condition; however, lung function (force expiratory volume on one second (FEV_1)) has recently been shown to decline at a similar rate to that seen in patients with cystic fibrosis (CF) throughout childhood [2]. Despite their recurrent sinopulmonary infections, no biomarkers of chronic inflammation have been identified in PCD patients to date. Further to sinus and lung involvement, the majority of PCD patients have recurrent glue ear with conductive hearing loss, approximately half have *situs inversus totalis*, with a proportion having complex congenital heart disease; most men are infertile due to immotile sperm and gastrointestinal, renal and neurological sequelae have been described [1].

Until recently there has been a paucity of evidence characterising the nutritional status of children with PCD. However, one large international cross-sectional study has recently demonstrated lower height-for-age z-scores (HAZ) in PCD patients [3], with another showing a progressive longitudinal decline in linear growth throughout childhood resulting in a mean loss of body height of approximately 0.5–1 standard deviations by the age of 17 years [4]. The cause of this growth failure is likely to be multifactorial [4, 5] and may be as a result of increased respiratory effort, possible effects of chronic inflammation and suboptimal nutrition intake, although to our knowledge this has yet to be described. It is therefore important to use nutritional measurements that both identify accurately and, where possible, early those children at nutritional risk who would benefit from nutritional intervention.

In CF, it is well-established that patients with a lower body mass index z score (BMIZ) have poorer respiratory outcomes (specifically FEV_1) [6]. This has led to intensive dietetic input into this cohort of patients and is a key factor in the improvements in life expectancy and clinical outcome measures seen in these patients over recent decades [7]. As a result, a body mass index (BMI) ≤ 0.67 z score (≤25^{th} percentile) is generally used as a marker for children at nutritional risk [8]. However, in more recent years, the use of BMI as a stand-alone measure has been questioned as it does not distinguish between fat mass (i.e. adipose tissue) or fat free mass (i.e. lean body mass). As a result, children may have persistent chronic malnutrition (i.e. be short) but have a normal BMI as their weight to height ratio is normal. As such, utilising other methods of body composition analysis, such as bioelectrical impedance spectroscopy (BIS), may add a sensitivity and specificity to nutritional assessment over the use of BMI alone [9], identifying those children with declining nutritional status who may benefit from early nutritional support [10].

BIS measurements have been used in a variety of settings to quantify body composition [11]. BIS uses a weak, alternating electric current at a range of radiofrequencies to characterise the conductive and non-conductive tissue and fluid components of the body [12]. BIS phase angle depends on the opposition to the flow of electrical current (resistance) and the effect of the capacitive ability of cell membranes to impede the current (reactance) [13, 14]. A low BIS phase angle 50^0 has been shown to predict clinical outcomes relating to morbidity and mortality, in critical care, renal disease and HIV/AIDS [15-17]. More recently BIS phase angle $200/5^0$ has been shown to be a marker of clinical outcomes and nutritional status in critically ill adults [18]. Furthermore, in this setting, BIS phase angle $200/5^0$ also appears to be a measure of functional outcome, e.g. with lower BIS phase angle values seen in patients with poor handgrip strength [18, 19]. In our work in children with congenital heart disease we have demonstrated a

relationship between pre-operative BIS phase angle $200/5^0$ measures and post-operative and intensive care unit length of stay [20, 21]. These results suggest that BIS phase angle 50^0 and $200/5^0$ may be useful in identifying changes to cellular resistance, alterations in cell membrane integrity and total body water which occur independently of and precede anthropometric changes in children [21-23]. BIS phase angle is increasingly being considered as a useful surrogate measure of overall cellular health and resilience, in addition to being a marker of nutritional status [21].

The aims of this study were to consider whether there were any associations between nutritional status and lung function in a cohort of children with PCD; to consider whether other measures, such as BIS phase angle 50^0 and $200/5^0$, might increase the sensitivity and specificity of identifying those patients at nutritional risk; to look for biomarkers of chronic inflammation in a PCD cohort; and finally, to measure a wide range of micro-nutrients, fatty acids and vitamins, in order to identify specific deficiencies that would need to be corrected in subsequent stratified nutritional interventions in this cohort of children.

Materials and Methods

The study was approved by the National Research Ethics Service (South Central (A) Committee 07/Q1702/109) and all participants provided written informed consent.

Participants

All children with PCD, aged between 0 and 16 years, from a single tertiary centre, University Hospital Southampton (UHS) NHS Foundation Trust (Southampton, UK), seen in outpatients between September 2016 and April 2017 were prospectively enrolled. The diagnosis of PCD had been confirmed according to the ERS consensus guidelines [24]. Data on clinical phenotype, anthropometry, BIS phase angle, and nutritional intake and blood samples were collected during a single study visit.

Spirometry measurement

Spirometry measurements were performed using CareFusion Microlab 3500 Mk8 (CareFusion, California, USA) following calibration, according to the manufacturers' instructions. All measurements were obtained according to ERS/ATS guidance [25]. Global lung initiative (GLI) equations were used to estimate z scores for FEV_1 and forced vital capacity (FVC); ethnicity specific equations were used where available [26].

Anthropometry

Anthropometric measurements were performed and recorded in accordance with World Health Organisation (WHO) guidelines [27]. Infants aged $\leq$1 year were weighed naked and children aged $\geq$1 year with minimal clothing; weight was measured to the nearest 0.1 kg using a digital scale. Recumbent length was measured to the nearest 0.1 cm for all children aged $\leq$2 years using an infantometer (Seca 416; Birmingham, UK) and standing in older children under a stadiometer (Seca 213: Birmingham, UK). Reference values for fat free mass index (FFMI) were used to quantify z scores for children over the age of 2 years [28]. Z-scores were calculated using WHO Anthro software version 3.3.3 2011 [29] for participants aged $\leq$5 years and WHO AnthroPlus 3.2 [30] for those aged ≥5 years. WHO growth reference interpretation of cut offs for malnutrition were used. Moderate malnutrition was defined as a height-for-age, weight for height, BMI or FFMI of ≤-2 z-scores below the mean of the WHO child growth standards [27].

Bioelectrical impedance spectroscopy measurements

BIS measurements were made using ImpediMedSFB7 (Pinkenba, QLD 4008 Australia), a single-channel tetra-polar device able to measure resistance and reactance across 256 frequencies. The machine was calibrated before use with a circuit of known impedance provided by the manufacturer. Measurements were conducted using a standard tetrapolar electrodes distribution; the inner arm electrode (sensor) was placed on the dorsal surface of the right wrist

and the leg electrode was placed on the anterior surface of the right ankle. Measurements were completed in unfasted subjects. Data files were processed using specialist software (Bioimp, ImpediMed), with data points rejected if they met any of the following criteria; i) positive X centre (Xc) values, ii) negative resistance values. Measurements were taken in triplicate and the mean used. BIS phase angle at a current frequency of 5^0, 50^0, 200^0 and $200/5^0$ were analysed [31].

Nutritional intake data collection

A 3-day estimated food diary (2 weekdays and 1 weekend day) was recorded by carers/ children following the clinical appointment. Carers were provided with detailed instructions on how to complete the diary accurately and were requested to return the diary via a prepaid postal envelope. Nutritional intake data were assessed using CompEat Pro (Visual Informatics Systems Ltd., Oxon, UK). Dietary intake for energy and protein were compared to the UK Dietary Reference Values using the reference nutrient intake (RNI) for protein and estimated average requirements (EARs) for energy [14]. As recommended by the Scientific Advisory Committee on Nutrition in the United Kingdom (SACN), insufficient protein was defined as an intake <100 % of the lower reference nutrient intake (LRNI—meeting nutrient requirements for 2.5 % of population), sufficient intake was between the LRNI 100% and ≤200 % of the RNI and excessive intake ≥200 % of the RNI [14]. For energy intake, the RNI was not used because it signifies an excess energy intake for the majority of the population, as highlighted by the SACN [14]. As advised by SACN, the EARs were used with children consuming ≤ 67 % classified as low energy intake, between 67% EAR and 110 % as sufficient intake and excessive intake ≥ 110 % of the EAR [15].

Blood analysis

Using standard laboratory techniques, routine clinical variables of interest were measured including C-reactive protein (CRP), selenium, zinc, copper, folate, vitamin B12 and vitamin D. Vitamin B6 concentrations were measured by high performance liquid chromatography with fluorescence detection as previously described [32]. Erythrocyte fatty acid levels were measured using a Hewlett Packard 6890 gas chromatograph (Hewlett-Packard; Avondale, PA, USA), as previously described [33]. Fatty acids are expressed as weight % of total fatty acids present. Plasma concentrations of interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor alpha (TNF-α), and vascular endothelial growth factor 1 (VEGF-1) were measured using the FCSTM09-06 high sensitivity magnetic bead cytokine panel kit (Bio-Techne, R&D Systems Luminex Performance, Abingdon, UK). Plasma concentrations of prostaglandin E_2 (PGE_2), 6-keto prostaglandin F_1 alpha (6-$KPGF_{1\alpha}$) and 8-isoprostane were measured by ELISA (Cayman Chemical, Cambridge Biosciences, Cambridge UK). For all kits the manufacturer's instructions were followed and the plates were read on a Bioplex 200 (Bio-Rad, Watford, UK).

Statistical analysis

An a priori statistical analysis plan was determined. SPSS version 24 (Chicago, IL) was used for conducting statistical analysis. Associations between BIS phase angle, anthropometry and clinical outcomes of FEV_1 z scores and FVC z scores were investigated. Relationships between inflammatory mediators, markers of oxidative stress, nutritional intake and plasma/serum levels were also investigated. Data was tested for normality. For data that was normally distributed parametric tests were used, otherwise non-parametric tests were chosen. T-tests and one-way ANOVA with Dunn post test comparison were used to investigate differences between continuous variables of anthropometry, phase angle and clinical outcomes FEV_1 and FVC z scores. Relationships between variables of interest were further investigated

using Spearman's correlations considering anthropometry, BIS phase angle clinical outcomes FEV_1 and FVC z scores. A p value of <0.05 was used to define statistical significance. Where values were found to be normally distributed they are shown as mean and standard deviation or otherwise as median and inter-quartile range.

Results

43 children were included in this study; of these 51% (n=22) were male. The average age at diagnosis was 2.7±3.8 years and at the time of study was 7.0±3.6 years. Patient demographics, nutritional status, spirometry values and blood results are described in Table 1.

Nutritional status and lung function

FEV_1 z scores were available in children older than 6 years of age (n=28). There was a weak positive correlation between HAZ and FEV_1 z score (n=28, r=0.4, p=0.049). There was also an association between BMIZ and FEV_1 but this was not statistically significant (Figure 1a and b). There was a positive association with HAZ and FEV_1 z score; children children with a low FEV_1 z score of <-2 were also shorter compared to >-2 (mean HAZ -0.49±1.1 vs 0.2±0.7, p=0.05).

Early detection of nutrition depletion by BIS phase angle

Only 4.6% (n=2) of children had HAZ <-2 z scores and 6.9% (n=3) of children had a BMI <-2 z scores. There were 29 children, older than 2 years of age, for whom BIS phase angle data were available to calculate FFMI; of these, 21% (n=6) had a FFMI <-2 z scores (Table 3). Children with a FFMI of < -2 z scores had a lower phase angle 5^o (p=0.03) and phase angle 50^o (p=0.0002) compared to those with a FFMI of > -2 z scores (n=23) (Figure 2). Those with a low FFMI (<-2 z scores) had a significantly lower FVC z score (-1.5±1.0 vs. 0.3±1.3 (p=0.01)) and a lower BMI z score (-1.3±1.2 vs. 0.8±0.7 (p=0.0002)) (Table 3).

Biomarkers of chronic inflammation

All inflammatory and oxidative stress markers were detectable in all samples available (n=35; Figure 3). Mean plasma concentrations of the pro-inflammatory cytokines assessed (IL-1β, IL-2, IL-6, IL-8 and TNF-α) were not raised compared to available normative data [34, 35] (Figure 3). There were weak positive associations between VEGF-1 and FEV_1 z score (n=27, r=0.41, p=0.035), VEGF-1 and BMI z score (n=35, r=0.48, p=0.001), IL-2 and BIS phase angle 50^0 (n=35, r=0.38, p=-0.026), and IL-2 and BIS phase angle $200/5^0$ (n=35, r=0.37, p=-0.031).

Red blood cells contained a mean of 14.2% linoleic acid (an essential fatty acid), 13.3% arachidonic acid, 0.5% eicosapentaenoic acid and 2.8% docosahexaenoic acid (Table 4). There were weak positive correlations between 6-keto $PGF_{1\alpha}$ and erythrocyte 18:1n-7 (n=35, r= 0.26, p=0.03) and 18:2n-6 (n=35, r=0.39, p=0.02). There were weak positive associations between PGE_2 and erythrocyte 14:0 (n=22, r=0.58, p=0.005), 16:0 (n=22, r=0.58, p=0.005), 18:1n-7 (n=22, r=0.4, p=0.02), 20:0 (n=22, r=0.39, p=0.03), 20:1n-9 (n=22, r=0.36, p=0.04), 22: 6n-3 (n=22, r=0.4, p=0.02) and 24:0 (n=22, r=0.37, p=0.04).

Dietary intake, fatty acids, vitamins and micronutrients

None of the children studied had a low energy intake (defined as consuming less than 67 % of EAR) but 63% (n=14) had excessive intake (defined as consuming greater than 110% of EAR). 6% of children consumed inadequate amounts of protein (≤100 % of RNI); 22% of children consumed adequate amounts of protein (LRNI to ≤200 % of the RNI) and 72% had an intake of protein ≥200 % of the RNI [15]. There were no associations between energy and protein intake, with respect to BMI, height for age or BIS phase angle (Table 5). Whilst there were no statistically significant differences between the groups, FEV_1 z score was higher in those with a protein intake ≥200% of RNI compared to those children with an adequate intake of protein (LRNI to ≤200% RNI) (-2.5±1.4 vs -1.4±1.1, p=0.2).

231 Vitamin D insufficiency (<50 nmol/l) was present in 54% (19/35) of those in the cohort
232 where plasma concentrations were available, of which 26% (n=9/35) were deficient (<30
233 nmol/l), requiring therapeutic supplementation. A weakly significant association was seen
234 between low vitamin D status and FFMI z score (n=29, r=0.4, p=0.02). All other vitamin and
235 micronutrient levels assessed were considered normal.

236 **Discussion**

237 We found a weak correlation between low HAZ and BMIZ and FEV_1 z score. Children
238 with a lower FEV_1 z score were also more likely to be shorter, which was consistent with findings
239 published in a large international cohort of children with PCD [24], and as such may be clinically
240 meaningful. BIS phase angle 50^0 was able to identify children with declining nutritional status
241 earlier than using standard measures of anthropometry alone e.g. HAZ and BMIZ ≤-2. Children in
242 our cohort commonly had insufficient vitamin D levels but all other micronutrient levels were
243 within the normal range. However, despite recruiting all children seen in the National PCD
244 Management Service at our centre over a 6-month recruitment window, due to the rarity of
245 PCD, only 43 children were enrolled, and, as such, our results should be interpreted with
246 caution.

247 The observation that poorer nutritional status is associated with poorer lung function in
248 PCD patients, although weak, is similar to that seen in patients with CF [7, 36], with a
249 relationship described between FEV_1 and BMI [3, 37]. However in CF the main nutritional issues
250 are thought to be due to malabsorption secondary to pancreatic insufficiency and PCD patients
251 have normal pancreatic function. This may imply that having chronic suppurative lung disease, in
252 isolation from pancreatic involvement, may have a negative impact on nutritional status.
253 However, although this does not imply causality as it is possible that other clinical and

254 nutritional factors may impact on linear growth [38], it would be an important relationship to
255 explore in a future larger study.
256 Improving nutritional status is one of the cornerstones of the management of children
257 with CF and is widely thought to have contributed significantly to the improvements in mortality
258 seen over the past decades [7, 36]. We, therefore, hypothesise that similar benefits may be seen
259 in children with PCD and postulate that if stratified nutritional interventions were developed to
260 provide appropriate amounts of both macro- and micronutrients for those patients displaying
261 early nutritional decline, longer term outcomes may be improved [39].
262 Although not statistically significantly different, FEV_1 z score was higher in those with a
263 protein intake ≥200% of RNI compared to those with an adequate intake of protein (LRNI to
264 ≤200% RNI). Whilst nutritional interventions have been successful in improving some outcomes
265 for children with chronic diseases such as CF [40], strategies have focused on increasing energy
266 intake at the expense of considering the need for type II nutrients, which support linear growth
267 such as zinc, sulphur (protein) and magnesium [41-43]. Children with chronic diseases are
268 surviving longer. As such, it is essential that excess adiposity, through targeting energy intake
269 alone, is not promoted with the aim of achieving a target BMI, without considering what is
270 happening to linear growth and lean body mass deposition [10].
271 Anthropometrical measures of height and weight are used to determine nutrition risk,
272 with WHO cut offs <-2 used for moderate malnutrition [27]. In this cohort using <-2 HAZ and
273 BMIZ scores 4.6% vs. 6.9% cases respectively would have been identified as being at nutritional
274 risk. FFMI and FMI z scores are increasingly being used to quantify body composition, which
275 when used in conjunction with BIS phase angle 50^0, might offer a method for early identification
276 of patients with declining nutritional status, as described in children with CF [44, 45]. In this
277 study 21% of children had a FFMI z score of <-2. This group of children also had a lower BIS

278 phase angle 50^0 of 4.3±0.4 compared to children with a FFMI z score of > -2 (n=23) whose BIS
279 phase angle 50^0 was 4.9±0.8 (p=0.0002). However, their BMI z score was -1.3±1.2 suggesting
280 they were normally nourished. As such, had nutritional cut offs of <-2 z scores only been used to
281 identify nutritional risk, these children may not have been recognised as being at risk. Therefore
282 it may be that BIS phase angle 50^0 may increase the sensitivity and specificity of identifying
283 those children at risk of nutritional decline earlier than routine measures of anthropometry,
284 such as height and weight currently afford and described amongst children with congenital heart
285 disease [21]. Children with lower BIS phase angle 50^0 had lower lung function, suggesting this
286 measure may potentially be associated with other independent and clinically important
287 outcomes, as seen amongst other children with lung disease [44, 45]. However, we acknowledge
288 the numbers in this study are small and further work amongst a larger cohort of children with
289 PCD will be required to confirm this observation as well as determining the causality as it may be
290 an incidental finding within this cohort.
291 In this study BIS phase angle 50^0 was measured using an ImpediMedSFB7 device. BIS
292 technology is easy to use in a clinical setting as it is non-invasive. Interest is increasing in trying
293 to develop BIS phase angle reference values, which would allow for the comparison of derived
294 BIS phase angle 50^0 measures amongst children who vary in age, gender and clinical condition.
295 This has the potential to allow for the earlier identification of those with declining nutritional
296 status, presenting an opportunity for earlier intervention [21]. Limitations for all studies,
297 including ours, conducted in this area are the use of differing machines, varying populations and
298 the various methods and approaches used to determining a value for phase angle [46]. This
299 makes it challenging to apply a single defined cut-off for phase angle, which may have been
300 defined for a specific study. Therefore, like others, our values should only be considered in the
301 context of the condition and population studied. In order to strengthen the application of this

technology, the utility of phase angle should be studied longitudinally, in a considerably larger cohort across multiple centres, with the aim of developing cut-off values and creating reference z scores for phase angle to be used in the context of PCD [47].

A wide array of micronutrients and vitamins were measured in order to identify any specific deficiencies that might need to be considered when planning future nutritional intervention strategies. The only noteworthy finding seen was that 54% of the cohort in whom plasma concentrations were available, had insufficient vitamin D levels (<50 nmol/L), with 26% being deficient (<30 nnol/L), needing treatment. This is three times the prevalence seen in an Italian study assessing 22 PCD patients, which found 18% had vitamin D levels <50 nmol/L [48]. Furthermore, it is higher than the prevalence of vitamin D insufficiency seen in UK children, shown to be 35% in one large population-based study [49]. This is particularly evident once geographical location is considered as the reported mean vitamin D levels of UK children living in Southern England was 62.3 nmol/L [49] whereas in our cohort it was 52.5 nmol/L. There was also a weak association between low vitamin D status and FFMI. Vitamin D has previously been shown to be essential for calcium transport, lean body mass development and protein metabolism. In vitamin D deficient children and adults with a classical Fontan circulation lean body mass is significantly lower than in those with normal levels [50]. The relationship between vitamin D status and body composition should be further investigated in future nutritional studies.

Patients with PCD have persistent and recurrent sinopulmonary infections. However, to our knowledge, there have been no studies to date considering blood biomarkers of inflammation in PCD patients. Many chronic conditions have an inflammatory component and low-grade inflammation can affect metabolism and body composition. Cytokines have a pivotal role in orchestrating the inflammatory response to infection. One group studied levels of IL-8 in

326 the sputum of children with PCD, finding it to be three times greater than in the sputum of
327 children with CF [51]. Furthermore, it has been shown that monocytes from PCD patients
328 produce higher levels of pro-inflammatory cytokines (IL-1β and TNF-α) in response to
329 lipopolysaccharide than those from healthy individuals [52]. It was interesting, therefore, that
330 mean plasma levels of the pro-inflammatory cytokines assessed were not raised in our cohort
331 compared to the available, albeit limited, normative data [34, 35]. We had hypothesised that
332 those patients with evidence of inflammation may have poorer nutrition outcomes. It was
333 therefore noteworthy that we only observed a weak correlation between the pro-inflammatory
334 cytokine IL-2 and BIS phase angle. The function of IL-2 is not completely understood but
335 elevated levels are associated with cancer cachexia and weight loss [53]. This finding is
336 hypothesis generating and further research into IL-2 and its association with nutritional status
337 would be of interest.
338 We also considered relationships between markers of oxidative stress (6-keto-$PGF_{1\alpha}$ and
339 8-isoprostane), various fatty acids and BIS phase angle. Red blood cells are considered stable
340 markers of status of polyunsaturated fatty acids and are reflective of dietary intake patterns.
341 The composition reported here is similar to compositions reported in children in other settings
342 [54]. N-3 fatty acids, especially EPA and DHA are anti-inflammatory and capable of inhibiting
343 inflammatory processes including leukocyte chemotaxis, adhesion molecule expression,
344 leukocyte-endothelial adhesive interactions and production of inflammatory cytokines and lipid
345 mediators. Dietary fat intake in the UK is high in n-6 fatty acids which facilitate the production of
346 eicosanoids like prostaglandins and leukotrienes from arachidonic acid (n-6 fatty acid cascade)
347 and subsequently are considered to promote inflammation and oxidative stress [55]. We found
348 a weak positive relationship between linoleic acid, an n-6 fatty acid, in red blood cells and

plasma 6-keto $PGF_{1\alpha}$, which would be of interest to investigate further in future nutrition studies.

There are a number of limitations of this study. Firstly, there are limitations to the accuracy of dietary intake methods, which are highlighted in many studies [56, 57]. In our study we chose to use a 3-day semi quantitative food diary as oppose to a 7-day diary in order to reduce the fatigue effect of recording dietary information for such a long time. However, future studies in children with PCD may benefit form adding a second dietary intake method to ensure that dietary intake recorded is accurate [56]. In addition, the observation regarding the potential benefit of BIS phase angle 50^0 in early identification of children with nutritional problems will need further assessment in larger cohorts before stronger conclusions can be drawn. In order to assess the benefit of this and any subsequent nutritional intervention, it is likely that a multi-centre design will be required.

Conclusion

We have characterised the nutritional phenotype of a cohort of children with PCD. There appears to be a weak relationship between lung function and nutritional status, in addition to measures of BIS phase angle 50^0. The use of BIS phase angle 50^0 may be better at identifying early nutritional decline in children with PCD and may therefore be of benefit for nutritional assessments in the clinical setting. These findings may help inform a future nutritional intervention strategy in children with PCD.

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Statement of authorship

All authors have made substantial contributions to the following areas of this manuscript: LVM and WW designed the study. EAM completed the cytokine, pro-oxidant and fatty acid analyses. WW, ALH, LVM and CJ carried out the data collection. ALH, LVM, BL, WW and PCC completed the data and statistical analyses and drafted the manuscript. All authors edited, read and approved the final manuscript.

Conflict of interest

None of the authors has any conflict of interest to declare in relation to this research.

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585

586 **Table 1: Demographics, nutritional status, spirometry values and blood results of children with**

587 **PCD**

Variables	**n=**	Mean ± SD
Age at diagnosis (years)	43	2.7±3.6
Age at time of study (years)	43	7.0±5.2
Forced Expiratory Volume in 1 second (FEV_1) predicted (%)	29	79.3±20.4
FEV_1 z score	28	-1.7±1.7
Forced Volume Capacity (FVC) (L)	29	2.2±0.9
FVC predicted (%)	29	90.9±19.1
FVC z score	28	-0.8±1.7
Oral antibiotic courses per year (n)	40	3.5±1.8
Gender	43	Male n=22; Female n=21
Weight (kg)	43	30.3±19.8
Height (m)	43	1.2±0.3
BMI (kg/m^2)	43	17.2±4.6
Weight for age z score (WAZ)	26	0.1±1.1
Height for age z score (HAZ)	43	-0.2±1.1
Mid upper arm circumference (MUAC) (cm)	33	19.7±4.7
Vitamin D (nmol/L)	35	52.5±29.2
Selenium (μmol/L) (ref range 0.2- 0.9)	35	1.4±2.3
Zinc (μmol/L) (ref range 11 – 24)	35	14.2±2.1
Copper (μmol/L) (ref range 10-22)	35	20.0±4.2
Ferritin (μg/L) (ref range 20-200)	36	24.0±13.4
Folate (nmol/mL) (ref range 5-21)	36	13.0±6.6
Vitamin B12 (pg/mL) (ref range 180 – 1000)	36	623.5±271
Vitamin B6 (μg/L) (ref range 5-50)	19	75.7±42.0
Iron (μmol/L) (ref range 5 – 31)	36	12.0±5.3
Transferrin (g/L) (ref range 1.8 - 3.3 g/L)	36	3.0±0.4
Transferrin iron saturation (%) (ref range <16)	36	19.5±9.4
Haemoglobin (g/L) (ref range 95-135)	36	129.9±13.8
Albumin (g/L) (ref range 35 – 45)	36	41.4±3.5
Calcium (mmol/L) (ref range 2.20-2.60)	22	2.3±0.1
C-reactive protein (g/L) (ref range 0 – 3.6)	36	5.9±17.4
Phosphate (mmol/L) (ref range 1.25-2.10)	36	1.5±0.3
Magnesium (mmol/L) (ref range 0.70-1.00)	36	0.9±0.8

Alkaline Phosphatase (U/L) (ref range 145-420)	35	260±96

588

589 **Table 2: Body composition measure of fat mass, fat free mass and BIS phase angle 50^{o} and**
590 **plasma cytokine concentrations in children with PCD**

	N	Mean ± SD
BIS phase angle 50^{o}	36	4.5 ± 0.9
Fat mass (kg)	36	5.9 ± 6.7
Fat free mass (kg)	36	22.8 ± 13.6
Fat mass (%)	36	19.2 ± 20.7
Fat free mass (%)	36	80.7 ± 79.2
IL-1β (pg/ml)	41	0.6 ± 1.1
IL-2 (pg/ml)	41	0.4 ± 0.8
IL-6 (pg/ml)	41	2.5 ± 4.1
IL-8 (pg/ml)	41	5.3 ± 4.5
TNFα (pg/ml)	41	7.7 ± 4.6
VEGF-1 (pg/ml)	41	30.7 ± 31.9
C-reactive protein (mg/L)	34	5.9±17.4

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Table 3: Comparison of children with PCD over the age of 2years with a fat free mass index <-2 z scores to those with a fat free mass index > -2 z scores

	Age (years)	FEV1 z score	FVC z score	HAZ score	BMI z score	Fat Mass Index	Fat Mass (%)	Fat free mass index	Fat Free Mass (%)	Total body water (%)	Extra cellular Fluid %	Intra cellular Fluid %	R200/5 HZ2	Phase angle 5 Hz	Phase angle 50 Hz	Phase angle 200 Hz
Fat free mass index < 2 (n=6)																
Mean±SD	5.9±3.5	-2.5±1.0	-1.5±1.0	0.0±1.4	-1.3±1.2	-2.7±0.5	12.4±10.6	-3.0±0.0	87.6±10.6	64.1±7.8	47.7±2.1	52.3±2.1	0.9±0.0	1.5±0.1	4.3±0.4	5.2±0.3
Fat free mass index > - 2 (n=23)																
Mean±SD	9.3±4.5	-1.2±1.4	-0.3±1.3	-0.2±0.9	0.8±0.7	-0.3±2.2	19.4±10.5	1.5±0.8	80.6±10.5	59.0±7.7	43.7±4.4	56.3±4.4	0.8±0.0	1.8±0.3	4.9±0.8	5.2±0.8
p values	0.1	0.09	0.01	0.9	0.0002	0.04	0.2	0.0002	0,2	0.2	0.0002	0.03	0.07	0.03	0.0002	0.9

Table 4: Erythrocyte fatty acids (% of total erythrocyte fatty acids) and measures of oxidative stress (pg/ml) in children with PCD

% erythrocyte fatty acids & markers of oxidative stress	N=	Mean ±standard deviation
14:0	22	0.4±0.2
16:0	22	22.7±1.7
16:1n-7	22	0.3±0.1
18:0	22	15.7±1.3
18:1n-9	22	17.2±1.4
18:1n-7	22	1.5±0.5
18:2n-6	22	14.2±1.7
18:3n-6	22	0.06±0.5
18:3n-3	22	0.2±0.1
20:0	22	0.1±0.6
20:1n-9	22	0.3±0.07
20:2n-6	22	0.2±0.07
20:3n-6	22	1.9±0.4
20:4n-6	22	13.3±1.5
22:0	22	0.09±0.04
20:4n-3	22	0.1±0.04
20:5n-3	22	0.5±0.2
24:0	22	2.9±0.6
24:1n-9	22	0.3±0.06
22:5n-3	22	1.7±0.3
22:6n-3	22	2.8±0.8
Prostaglandin E_2 (PGE_2) (pg/ml)	34	96.9±110
8-isoprostane (pg/ml)	34	45.9±79.7
6-keto $PGF_{1\alpha}$ (pg/ml)	36	95.1±120

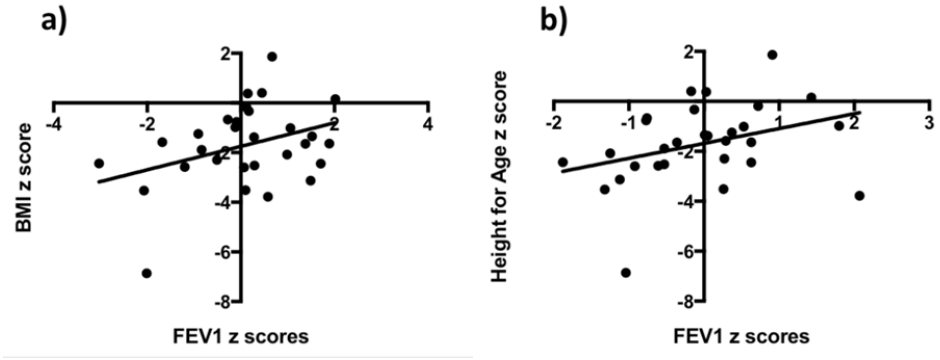
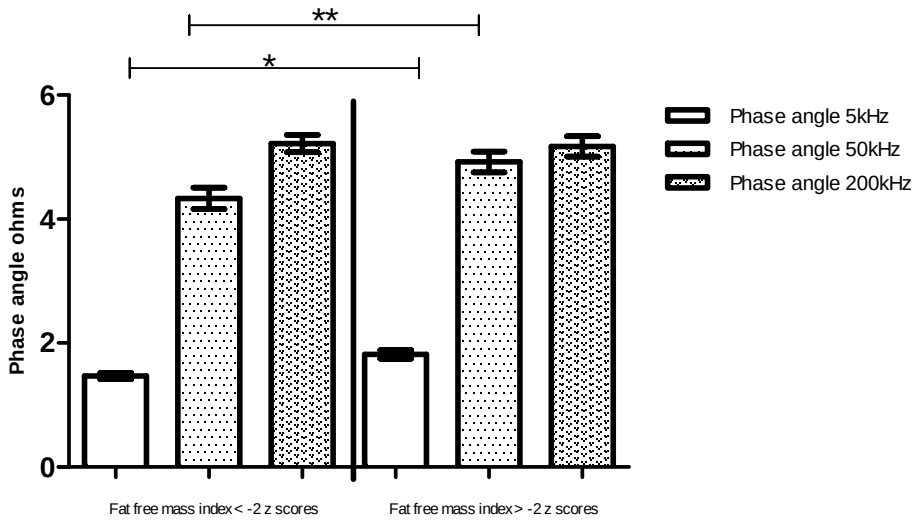


Figure 1: Relationship between FEV1 z score and a) BMI z score (n=29; r=0.36, p=0.055) and b) height for age z score (n=29; r=0.4, p=0.049) in children with PCD.



613

614 **Figure 2:** Children with a fat free mass index of <-2 z scores (n=6) had a significantly lower phase

615 angle 5^{o} (p=0.03) and phase angle 50^{o} (p=0.0002) compared to those with a fat free mass index

616 of > - 2 scores (n=23)

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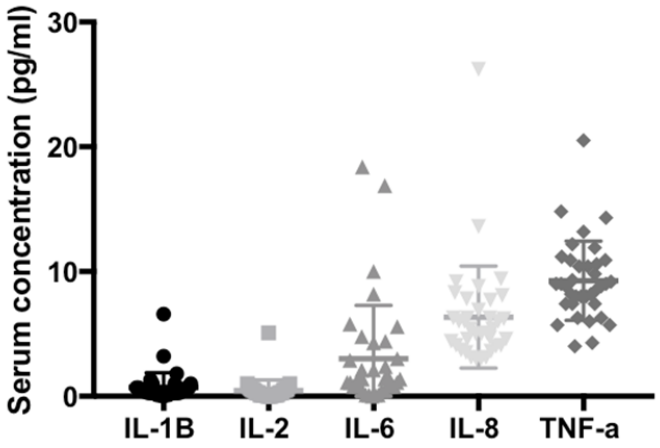

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620

621 **Figure 3** Concentrations of five pro-inflammatory cytokines (IL-1B, IL-2, IL-6, IL-8 & TNF-α) in

622 children with PCD (n=41) (Mean (SD), measured in pg/ml)