MONASH University

Long term effects of medically prescribed diets on growth and body composition in children with inborn errors of metabolism

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List of abbreviations

IEM	inborn error of metabolism
IEIPM	inborn error of intermediary protein metabolism
MMA	methylmalonic acidaemia
PA	propionic acidaemia
IVA	isovaleric acidaemia
UCD	urea cycle disorder
MSUD	maple syrup urine disease
PKU	phenylketonuria
BH_4	tetrahydrobiopterin
GSD	glycogen storage disease
LC-FAOD	long chain fatty acid oxidation disorder
VLCAD	very long chain acyl dehydrogenase deficiency
AAF	amino acid formula
EEA	essential amino acid
P:E ratio	protein to energy ratio
BMI	body mass index
BMR	basal metabolic rate
PAL	physical activity level
BIA	bioelectrical impedance analysis
FFM	fat free mass
TBW	total body water
%fatmass	percentage body fat mass
P%cals	energy from protein as a percentage of total energy
E%BMR	energy intake as a percentage of basal metabolic rate

Abstract

Background: Dietary therapies are the mainstay of treatment for many inborn errors of metabolism (IEM) to avoid accumulation of toxic products that may cause damage to body organs. Dietary recommendations for patients with IEM are based on, or extrapolated from, estimated requirements for healthy populations including recommendations from the WHO/FAO/UNU. However, as IEM diets often differ in natural protein and energy intake from these recommendations, their impact on longer term growth and body composition needs ongoing evaluation. The implementation of specific tools, strategies or recommendations to better prescribe diets and monitor patients with IEM would therefore be of great benefit to clinicians working in this area.

Aims:

1. To explore longitudinal growth and body composition patterns of patients with IEM who require dietary therapy that may directly or indirectly modify dietary protein intake
2. To document the dietary protein and energy intake of patients, and the protein to energy ratio (P:E ratio) of diets, and explore the relationship between dietary intake and growth and body composition outcomes.
3. To examine the validity of bioelectrical impedance analysis (BIA) to measure body composition in patients with phenylketonuria (PKU).

Methods:

In a retrospective longitudinal study: data on growth and dietary intake were collected through a systematic review of all health records, including dietary records of patients (n= 195) with: isovaleric acidaemia (IVA; n=7), methylmalonic acidaemia/propionic acidaemia (MMA/PA; n= 14), urea-cycle defects (UCD; n=44), maple syrup urine disease (MSUD; n=10), GSD I and III (n=19), very-long-chain acyl-dehydrogenase-deficiency (VLCAD) (n=22) and PKU (n=79). In a subset of patients, prospective longitudinal data on growth, dietary intake and body composition from 71 patients: IVA (n=5), MMA/PA (n=6), UCD (n=7), MSUD (n=3), GSD I (n=4), VLCAD (n=9), PKU (n= 37) were collected at clinic visits. Growth and body composition was collected in healthy controls (n=21) to compare to children with PKU.

In a prospective study, patients with PKU (n=16) had total body water (TBW) and fat-free mass (FFM) measured by BIA and compared to the criterion method of deuterium dilution.

Results: Growth patterns comparable to 'healthy population' standards were observed for patients with IVA, UCD, VLCAD and PKU. Reduced height growth was observed in patients with MMA/PA/, MSUD, and GSD I and III.

In patients with IVA/MMA/PA/UCD natural protein intake was adequate for all disorders. Natural protein intake above recommendations was not associated with increased height growth, but higher natural and total protein intake were significantly associated with lower body fat levels. A P:E ratio associated with improved growth and body composition outcomes was determined for patients with MMA/PA/IVA and UCD and PKU.

No predictive bias was seen between TBW or FFM measured by deuterium dilution and BIA, confirming BIA as a reliable method to measure body composition in PKU patients.

Conclusions:

Dietary recommendations in IEM should encourage natural protein intake to tolerance. The P:E ratio may be used as a additional clinical tool to prescribe and monitor these diets. Ongoing outpatient-clinic assessment of patients with PKU can include BIA to reliably measure body composition.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: . 

Print Name: ..MAUREEN EVANS

Date: .....10/11/2017.....

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 4 original papers published in peer reviewed journals and 1 publication in press. The core theme of the thesis is; Dietary intake and growth and body composition in children with inborn errors of metabolism. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Faculty of Medicine, Nursing and Health Sciences under the supervision of Professor Helen Truby and Professor Avihu Boneh.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2, 3, 5, 7, 8 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status**	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	New ways of defining protein and energy relationships in inborn errors of metabolism	Published *Molecular Genetics and Metabolism* 112 (2014) 247–258	80%: Concept, literature review, manuscript preparation including writing all drafts	1) Helen Truby, input into manuscript 10% 2) Avihu Boneh Input into manuscript 10%	No No

3	The relationship between dietary intake, growth, and body composition in inborn errors of intermediary protein metabolism	Published *The Journal of Pediatrics* 188 (2017) 163-172	80%: Study concept, design, data collection, and analysis and interpretation of results. Manuscript preparation including writing of all drafts	1) Helen Truby input into manuscript 10% 2) Avihu Boneh input into manuscript 10%	No No
5	VLCAD deficiency: follow-up and outcome of patients diagnosed through newborn screening in Victoria	Published *Molecular Genetics and Metabolism* 118 (2016) 282–287	40%: concept development Anthropometric & dietary intake data collection, analysis. manuscript drafting and contributing to all drafts	1) Brage S. Andresen Mutation testing and input into manuscript 20% 2) Judy Nation input into manuscript 5% 3) Avihu Boneh clinical data collection and input into manuscript 35%	No No No
7	The validity of bioelectrical impedance analysis to measure body composition in Phenylketonuria	Accepted for publication on 10/11/17 *Journal of Inherited Metabolic Disease: Reports BOLI-D-17-00347K1*	70% study concept, data and sample collection and analysis, interpretation of results and manuscript preparation including writing of all drafts	1) Kay Nguo, Sample analysis and input into manuscript 20% 2) Avihu Boneh. Input into manuscript 5% 3) Helen Truby, Input into manuscript 5%	No No

8	The relationship between dietary intake, growth and body composition in Phenylketonuria	Published *Molecular Genetics and Metabolism* 122 (2017) 36-42	80% Study concept, design, data collection, and analysis and interpretation of results. Manuscript preparation including writing of all drafts	1) Helen Truby, input into manuscript 10% 2) Avihu Boneh, input into manuscript 10%	No No

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date: 10/11/2017**

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: [redacted] **Date: 10/11/2017**

Publications during candidature

This thesis was completed on a part-time basis commencing between 2010 and 2017

The publications that are submitted as a part of this thesis are

Chapter 2:

New ways of defining protein and energy relationships in inborn errors of metabolism

Maureen Humphrey, Helen Truby, Avihu Boneh.

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Chapter 3

The relationship between dietary intake, growth and body composition in Inborn Errors of Intermediary Protein Metabolism

Maureen Evans, Helen Truby, Avihu Boneh

The Journal of Pediatrics 188 (2017) 163-172

The above paper was discussed in The Journal of Pediatrics Editorial:

Dietary guidelines for inborn errors of metabolism

Hans C. Andersson. The Journal of Pediatrics 188 (2017) 1-2

Chapter 6:

VLCAD deficiency: Follow-up and outcome of patients diagnosed through newborn screening in Victoria

Maureen Evans, Brage S. Andresen, Judy Nation, Avihu Boneh

Molecular Genetics and Metabolism 118 (2016) 282–287

Chapter 7

The validity of bioelectrical impedance analysis to measure body composition in children with Phenylketonuria

Maureen Evans, Kay Nguo, Avihu Boneh, Helen Truby

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Chapter 8

The relationship between dietary intake, growth and body composition in Phenylketonuria

Maureen Evans, Helen Truby, Avihu Boneh

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The following papers were co-authored and published during the period of candidature, but are not included in this thesis

Early clinical manifestations and eating patterns in patients with urea cycle disorders

Gardeitchik, T., Humphrey, M., Nation, J., Boneh, A.

Journal of Pediatrics 161 (2) 2012, 328-32

Linear Growth in Children on a Ketogenic Diet: Does the Protein-to-Energy Ratio Matter?

Nation J, Humphrey M, MacKay M, Boneh A.

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Infrastructure Support Program who provided early financial support.

Preface

This thesis by publication describes dietary intake and longitudinal growth and body composition patterns of children who require medically prescribed diets due to an inborn error of metabolism (IEM). In particular, we have investigated the relationship between dietary protein quality and quantity, the dietary protein to energy ratio (P:E ratio), and growth and body composition outcomes. This has been examined across three categories of dietary intervention that may affect protein intake.

The first category includes conditions that require a direct reduction in total protein intake without the routine use of additional amino acid formula (AAF) to meet estimated protein needs. The second category includes conditions where the dietary intervention may result in an indirect reduction in natural protein intake due to a focus on increased carbohydrate and/or fat intake. The third category includes conditions that require a low natural protein intake with additional amino acid supplementation to meet estimated protein and nutrient needs.

The study methodologies employed were a retrospective case history analysis using longitudinal data, with a subset of patients enrolled in a prospective longitudinal study when body composition was measured using bioelectrical impedance analysis (BIA). To validate the use of BIA in patients with phenylketonuria (PKU), a study comparing the measurement of total body water (TBW) and fat free mass (FFM) against the criterion method of deuterium dilution was conducted.

The findings of this thesis indicate that a higher natural protein intake is associated with improved body composition, and that a P:E ratio associated with improved growth and body composition can be described for some disorders. In addition, the use of BIA as a method of measuring body composition has been validated in PKU.

Roadmap:

Section 1:

Background literature.

Chapter 1:
Literature review regarding the principles of dietary management in IEM, the evolution of the P:E ratio concept and its potential application in IEM.

Chapter 2: Published paper

This paper provides details of the methods for, and limitations of, determining protein and energy requirements in IEM and the potential application of the P:E ratio as a clinical tool

Section 2:

The relationship between dietary intake, growth and body composition in disorders where dietary interventions result in either a direct or indirect reduction in natural protein intake.

Chapter 3: Published paper

The focus of this paper is on growth, body composition and dietary intake, and the correlations between them, in inborn errors of intermediary protein metabolism, including organic acidaemias and urea cycle disorders. Recommendations for the P:E ratio in the diet are made.

Chapter 4

This chapter provides detail of the growth and dietary intake in glycogen storage disorder types I and III where carbohydrate intake may affect the P:E ratio.

Chapter 5: Published paper

This paper provides details of growth, and body composition outcomes of patients with very long chain acyl-CoA dehydrogenase (VLCAD) deficiency.

Section 3:

Phenylketonuria (PKU)

The relationship between dietary intake, growth and body composition in a disorder where intake of natural protein intake is low and equivalent dietary protein needs are derived predominantly from amino acid formula.

Chapter 6

The focus of this chapter is a retrospective analysis of the longitudinal growth patterns and age related natural protein tolerance of patients with PKU

Chapter 7: paper in press

The focus of this paper is to describe the validation of bioelectrical impedance analysis to measure body composition in patients with PKU.

Chapter 8: Published paper

In this paper, the relationships between dietary intake and growth and body composition in patients with PKU are explored prospectively. Recommendations for natural protein intake and P:E ratio are made.

Section 4

Discussion and future directions.

Chapter 9

Overall discussion and future directions

Section 1: Background literature

Section 1: Aims

Aim 1: To provide an overview of dietary management principles in inborn errors of metabolism (IEM) (Chapter 1)

Aim 2: To describe the evolution and potential application of the Protein to Energy ratio (P:E ratio) (Chapter 1)

Aim 3: To describe methods for estimating protein and energy requirements in IEM (Chapter 2)

Aim 4: To describe the potential application of the P:E ratio as a clinical tool in IEM (Chapter 2)

Chapter 1

Supportive literature regarding dietary therapy in inborn errors of metabolism.

The evolution and potential application of a protein to energy ratio to describe dietary adequacy

1.1 Dietary therapy in inborn errors of metabolism.

An Inborn error of metabolism (IEM) is an inherited disorder of body chemistry, due to genetic mutations that affects the production or function of a specific enzyme, transporter or channel, which is responsible for a metabolic process. Consequently, this may result in a clinically significant block in a metabolic pathway resulting in the accumulation of the substrate proximal to the block, a deficiency of a critical intermediary product and a deficiency of the specific final product. Inborn errors of intermediary metabolism can occur in the pathways affecting protein metabolism: including amino acidopathies, organic acidaemias and urea cycle disorders; carbohydrate metabolism: including galactosaemia and glycogen storage disorders; and fat metabolism: including disorders of mitochondrial fatty acid oxidation.

In many IEMs, dietary therapy is the mainstay of treatment to reduce the intake of the offending substrate that cannot be efficiently metabolised, and to supply the deficient metabolites for normal function of the pathway. Precise dietary manipulations are particularly critical in disorders that lead to acute and recurrent intoxication, or chronic and progressive intoxication from the accumulation of toxic compounds. Effective treatment requires an understanding of both the biochemistry of the defect and the nutritional requirements of the individual, (1) to provide an adequate intake and maintain metabolic stability.

The approach to dietary therapy is specific to each metabolic disorder, but the principles are identical. For example, in inborn errors of intermediary protein metabolism (IEIPM), protein metabolic products: amino acid(s), organic acids or ammonia form the offending substrate(s). The dietary approach is to directly restrict the quantity of natural protein to ‘tolerance’, that is, the amount that maintains metabolic stability with or without the use of precursor free amino acid formulas (AAF) to provide additional protein.(2) The provision of adequate energy is also essential to promote anabolism and prevent catabolism of body tissue that can also result in an increase in the toxic metabolite. For IEIPM including organic acidaemias such as

Methylmalonic Acidaemia (MMA), Propionic Acidaemia (PA) and urea cycle disorders (UCD), the routine use of AAF or essential amino acid supplements (EAA), may not be necessary, as tolerance to natural protein, although low, usually meets estimated requirements. However, the use of AAF is considered critical for amino-acidopathies such as Phenylketonuria, when except for the mildest of cases, the natural protein tolerance is inadequate to meet estimated protein needs.

In some IEM protein is not directly restricted or modified, but the dietary approach required to maintain metabolic stability may cause an indirect effect on protein intake. In some Glycogen Storage Disorders (GSD), there may be lacticaemia associated with hypoglycaemia and treatment focuses on adequate carbohydrate for energy. In Long-Chain Fatty Acid Oxidation Disorders (LC-FAOD) there is accumulation of toxic acyl-carnitines as beta oxidation of long chain fatty acids and ketone production is limited and treatment requires adequate carbohydrates with medium chain triglyceride supplementation to supply adequate energy. In both groups of disorders, it is critical to avoid decompensation due to catabolism and overtreatment with carbohydrates may occur and result in poorer dietary intake due to an indirect reduction in protein intake.(3)

Because of these manipulations, these dietary regimens are often extremely restrictive with significant deviations of protein and total energy intake from that of the healthy population. Protein intake may be considerably altered in quantity or quality, and the diet may be high in carbohydrate and/or fat. Micronutrient intake is often affected. Moreover, during periods of physical stress such as intercurrent illness an acute change in diet is often required to prevent metabolic decompensation, particularly in disorders that result in an acute intoxication. This may result in an exaggeration of the original diet, with periods of nil or minimal protein intake and high energy intakes, thus increasing periods of nutritional imbalance.

Literature reports of adverse nutritional status such as reduced height growth, increased prevalence of overweight, increased fat mass, decreased lean body mass and reduced bone density, pose a dilemma for the long term clinical management of children with these IEM. Provision of adequate protein and energy is essential for growth, development and metabolic stability; however excessive energy intake may also contribute to the high weight for height and body fat observed. In addition, patients on the most restrictive diets, or those with recurrent intercurrent illness or frequent metabolic decompensation, carry the most nutritional risk.

Studies on the effect of low protein intake on body composition are found in the obesity literature, the premise being that lower protein and higher carbohydrate diets hinder satiety and energy expenditure and inhibit fat oxidation by increasing insulin response (4). This may result in increased hunger, which promotes food intake and the accumulation of body fat.(4) Consequently, the long-term effects of prescribed diets for IEM are likely to include the 'Metabolic Syndrome', a combination of symptoms including central obesity, insulin resistance and hyperlipidaemia resulting in increased risk of cardiovascular disease and diabetes.(5)

Despite these risks, it is accepted that these restrictive diets are fully justified in IEM as they are critical for metabolic stability. However, evidence is lacking in regard to the exact prescription of macronutrients that is required for overall management or that required to promote optimal growth and body composition outcomes. Moreover, in daily clinical practice, tolerance of natural protein is generally described in terms of metabolic stability rather than other health outcomes.

Given the careful balance that is required to maintain metabolic stability but still maximise growth potential, a novel way to describe and prescribe diets in this group, may be the use of a single index, the Protein to Energy ratio (P:E ratio). To determine if this concept may have some validity for use in patients in whom protein and energy intake is so carefully controlled,

we aimed to document longitudinal growth, body composition and dietary intake in our patients and explore the relationships that may exist between them.

1.2 The evolution and potential application of a protein to energy ratio to describe dietary adequacy

An adequate diet is one that meets the nutritional, cultural, economic and social needs of an individual, group or population. From a nutritional perspective, dietary adequacy, or 'dietary quality', is determined by the answer to the question: "If an individual or group consumes this diet, in amounts that will satisfy energy needs, will the concentration of nutrients be high enough to meet their nutritional needs?"(6) Consequently, this can be expressed as a nutrient density of a food per energy unit, or a Nutrient:Energy ratio.

Protein density, or the protein concentration in a given amount of food energy, can be expressed as a ratio of Protein to Energy (P:E ratio) and is used to describe dietary quality by defining safe levels of protein intake when a diet is consumed to meet energy needs.(7) In this context, the P:E ratio can been expressed as a 'numerical' relationship including grams of protein per 100kcal, or energy from protein as a percentage of total energy (P%cals).

The P:E ratio may be used to set dietary goals or dietary guidelines(6, 8, 9) as an adequate P:E ratio implies sufficient protein, as well as protein associated micronutrients.(10) However, P:E ratios are generally not applied to individuals, but rather recommended for groups such as infants, pregnant women, and the elderly, or those assuming a similar intake such as in nursing homes, child-care centres, and hospitals; its use being most advantageous in 'at-risk groups' such as malnourished patients.(7)

To date, incorporating both energy and protein requirements together in a 'single expression' has proven difficult, as calculating safe P:E ratios requires the determination and use of the appropriate values of both protein and energy requirements.(11, 12) However, whilst the energy requirements of infants and children are evidence based(13), evidence is lacking for a correlation between protein intake and requirement in healthy individuals, or of a drive for

protein intake in its own right.(6, 7) To date, the identification of a safe or reference P:E ratio involves the assumption that intake is determined by a ‘drive for energy’ rather than an intrinsic ‘drive for protein’.(10)

The ‘Protein Leverage Hypothesis’, has recently challenged thinking in this area. This concept explores the evidence for determinants of protein intake and advances the possibility of an intrinsic drive to maintain a target protein intake (through a specific appetite for protein based foods). It is based on epidemiological evidence in humans for a steady intake of protein of approximately 15% of dietary energy over time despite variations in carbohydrate or fat intake. The concept is supported by animal studies that demonstrate a prioritisation of protein intake with consequent change in energy intake when dietary macronutrients are modified.(14, 15) These observations have led to a proposed relationship between dietary protein and dietary energy intake and obesity rates in humans, in which Simpson hypothesises that a modern diet with high consumption of low P:E dense foods will lead individuals to increase fat and carbohydrate consumption and thus energy intake if protein targets are to be met. Conversely, if foods with a high P:E ratio are consumed, then energy intake is curtailed due to satiation when the target protein intake is achieved.(15) The Protein Leverage Hypothesis, in its suggestion that protein may exert a stronger influence on consumption patterns than energy intake alone, challenges our whole conceptual thinking from which safe or adequate P:E ratios have been based. This further suggests an advantage in developing food based recommendations, to primarily address our protein needs and thus promote an appropriate P:E ratio to avoid an excessive energy intake.

This review aims to describe the development and use of the P:E ratio within the context of theoretical constructs of protein metabolism and requirement that highlight the discrepancy between what the body ‘needs’, versus what the body ‘wants’. It highlights the implications

of protein recommendations and their application in a clinical setting such as for individuals on medically prescribed protein restricted diets.

1.3 Evolution of the concept of a P:E ratio

The conceptualisation, derivation and application of a P:E ratio has been the source of debate for several decades , often necessitated by food restrictions or rationing in times of war and hardship (Table 1). Early use of protein expressed as a proportion of total calories is found in data from Macrae et al, from which the net protein utilisation (NPU) and likely adequacy of foods and diets at four Royal Air Force stations during World War II was described.(16) In 1951 Munro reviewed the relationship between protein and other dietary factors, suggesting an early reference to P:E ratios(17, 18) and in 1954 Calloway and Spector examined the relationship between ideal protein and calorie intake in order to design a single unit army survival ration.(19) This concept was further developed, focusing on the use of dietary protein concentration as a mode to assess the adequacy of diets where the efficiency and concentration of protein was combined and termed the "Net Dietary Protein Value" (NDPV).(18) Based on the consideration that food intake is mostly determined by calorie needs, protein concentration was then expressed in terms of food calories derived from protein using the term NDPcals%.(20) Early attempts to describe a reference P:E ratio calculated as the simple ratio of protein requirements to energy requirements were criticised for not considering the variability in both protein and energy requirements and the extent to which they may be independent of each other.(11) Nevertheless, the underlying concept was widely accepted.(11, 21)

The Food and Agricultural organisation (FAO) and later the World Health Organisation (WHO) and United Nations University (UNU) have been meeting regularly since the late

1940s to discuss the derivation and interpretation of nutrient, protein and energy requirements, incorporating new research findings and technologies into population recommendations. Although based on the best available evidence, by today's standards this early work would be considered relatively poor-quality and consisted of a few small studies with considerable emphasis on the opinions of experts (Table 2). One of the concerns about protein requirements, has been their expression as a P:E ratio when the ratio of protein is expressed as its energy content in relation to the energy requirement.(22) however, support for defining a P:E ratio that would allow a useful appraisal of dietary quality was discussed in this context and despite the methodological assumptions involved, a calculation of 'safe P:E ratios', incorporating recognition of the existence of both a distribution of requirements and of intakes was endorsed by the 1985 FAO/WHO/UNU report on energy and protein requirements.(6, 23) Since then, there has been ongoing interest and discussion about the calculation methodology and potential application of the P:E ratio to define dietary quality and risk of protein deficiency, but consensus is still lacking.

1.4 Determinants of a P:E ratio

A distinction between the biological requirement for protein and the recommended protein intake is crucial.(8, 24) The apparent true biological requirement for protein is the lowest intake required to maintain the functional needs of an individual, but the recommended protein intake is defined in terms of a 'Safe Level of Intake'. It is equivalent to the average + two standard deviations (2SD) of the requirement. It meets or exceeds the needs of practically all individuals in a specific group, assuming energy balance and normal physical activity and considering individual variation in requirement. Differing patho-physiological states, including infection, injury or recovery from malnutrition, are recognised to lead to a variation

in protein requirements of up to 20-50% in individuals.(24) During infancy, a period of rapid growth, there is a change in both energy and protein requirements per unit of body size of differing magnitudes so the appropriate dietary P:E ratio recommendation will change over time.(24) The composition of weight gain, whether muscle or fat, also markedly affects the relationship between energy and protein requirements. This can be demonstrated in calculating the 'optimal' P:E ratio of the diet in children requiring catch up growth. As the body requires a different amount of energy and/or protein for deposition of muscle and fat than for lean tissue, so the P:E ratio necessary to achieve this may vary.(24)

Fomon suggested that 'the safe protein intake must apply to the diet as fed and is best stated in terms of the Protein:Energy ratio'.(10, 25) Moreover, protein quantity alone is not enough, and protein quality is equally important.(26) As such, the P:E ratio would be controversial if it solely describes 'quality' as protein density, without considering the biological value of the proteins.(9) Thus, by default the definition of a safe protein intake refers to high quality protein, which depends mainly on its amino acid profile, digestibility and utilisation efficiency.(6, 8, 27) In some mixed diets the 'safe' level may need to be adjusted to account for digestibility and the amino acid composition of foods consumed when determining a 'recommended intake'.

The efficiency of protein utilisation depends on both the quality and quantity of the protein and the adequacy of the total diet, including energy intake and expenditure above or below metabolic needs. (10, 26-29) It also depends on digestibility.(6) Millward and Jackson argued that variability in protein digestibility would reduce the amino acids available for metabolism and thus lower the efficiency of utilisation of food protein.(7)

In this context, the type of food available for consumption also determines the P:E ratio of the diet. The P:E ratio falls as animal protein intake (high quality, digestible and utilisable)

decreases, creating difficulties for implementation of protein recommendations which are based on a 'reference' animal protein, to diets of vegetable or mixed protein sources. In this instance, recommendations are that the numerator (P) be increased in relation to the denominator (E) to account for this lower quality protein. (12) In marginal or vegetarian diets, dietary fibre may cause a reduction of 2-3% of available energy, which can also affect the P:E ratio.(6, 10) Some vegetarian diets have such low energy density and high bulkiness that children and the elderly may be unable to eat enough to meet their energy needs, even when the calculated P:E ratio is adequate. The actual P:E ratio of these diets will then be lower than the P:E ratio of the food before consumption,(7) and the nutritional value of dietary protein will have to be re-calculated.(7, 26)

1.5 Relationships between protein and energy intake

Traditionally, food consumption and energy intake are believed to be mostly determined by energy expenditure, as a function of basal metabolic rate (BMR) and physical activity level (PAL). Protein intake is also determined by these factors, via energy intake.(7, 10, 12, 30) Nitrogen balance has been shown to be sensitive to changes in energy intake over a range of protein intake from low to high.(6, 10, 19, 29) An increase in energy intake improves nitrogen balance and nitrogen retention when protein intake is fixed at any level, reaching a plateau that represents the limitations of the dietary protein content.(8, 12, 26-33) Increasing energy intake also enhances protein synthesis and reduces amino acid oxidation.(6, 8, 27, 34) On the other hand, energy utilisation can be further extended by increasing protein intake.(6) Yet defining the maintenance requirements for energy and protein under different metabolic conditions is difficult.(34)

The clinical implications of the relationship between protein and energy intake under different metabolic conditions are significant and need to be considered when making recommendations at an individual level. These can be summarised as follows:

- Adequate protein and energy intake: This allows maintenance of body weight in adults or a normal growth rate in infants and children.
- Adequate protein but inadequate energy intake: When total energy intake is inadequate, this results in inadequate amino acid utilisation, a loss of body protein in adults and reduction in growth rate in children. Although inadequate energy indirectly affects protein requirements, increasing protein intake further without increasing energy intake is ineffective as energy requirement and utilisation are intimately related to protein requirement and synthesis.(28) This relationship is complex in children as protein deposition is partly endogenously regulated and a positive balance can occur in negative energy balance.(12)
- Inadequate protein but adequate energy intake: The results of this will depend on the degree of protein inadequacy. In children, this may lead to deterioration in labile protein stores, decreased albumin pool and an accumulation of hepatic fat as seen in Kwashiorkor rather than a significant effect on weight or protein synthesis.(35) When protein intake is almost adequate and energy is excessive, lean body mass might be maintained, but excess energy is directed to fat synthesis resulting in obesity.(35) An intake of <7% of total energy from protein has been associated with higher rates of fat deposition.(24) In a study of infants fed an iso-energetic lower P:E ratio formula versus a higher P:E ratio, the former consumed more volume with a resulting increased BMI and increased fat deposition, leading to the hypothesis of a 'compensation' for the lower protein density, and providing some support for the 'Protein Leverage Hypothesis' in humans.(36) Alternatively, weight gain velocity

may be reduced in order to maintain body composition, suggesting that protein intake may determine energy utilisation (see section “Adaptation”).(35) Very low protein intake in adults leads to extra energy being dissipated as heat, effectively increasing the actual P:E ratio of the remaining diet. (33, 37) A negative effect on body weight of adequate energy intake and lower protein intake in adults has also been shown.(29, 32)

- Inadequate protein and inadequate energy intake: This results in weight loss and risk of malnutrition, unless energy intake is only mildly inadequate and pre-existing stores from adipose tissue can be utilised. Additional energy will improve nitrogen balance, most significantly when nitrogen intake is closer to the maintenance level rather than severely restricted.(33) In adults, prolonged energy and protein restriction may result in some adaptation through a reduction in energy output.(8)
- Excessive protein but adequate or inadequate energy intake: Protein intake above that required to maintain nitrogen balance influences muscle and bone through an anabolic drive of amino acids.(26) Many population groups consume protein at levels well above the recommended level, with data indicating worldwide protein intake of between 10-15% of dietary energy.(6, 15, 21) While individuals may tolerate high levels of protein as a percentage of dietary energy, intake >25 – 35% of energy are associated with potential health risks such as urinary calcium loss which may predispose to bone loss, and kidney disease particularly in those with diabetes and cancer, although data remain inconclusive.(38) Low Carbohydrate diets which are higher in protein and fat have been used for weight loss, although a restriction in energy intake is also required. The benefit of a high protein diet may be the enhanced satiation resulting in a reduced food intake, (39) and an increase in total energy expenditure by increasing the thermic effect of feeding, although this effect is

considered small.(30, 38, 40) In children, a protein intake above requirements for adequate growth does not immediately increase weight gain further when energy intake is adequate.(35) Excessively high protein intake in young infants may result in increased urea production and metabolic stress on the kidneys as observed in formula fed infants compared to breast-fed infants.(41) Recent reports suggest an association between a high protein intake above metabolic requirements in early childhood and an increased risk of obesity in later life,(42) while other reports suggest an association with body size but not body fat content.(43, 44)

- Excessive protein and energy intake: This results in overconsumption and risk of obesity if excessive energy intake is not met by an increase metabolic demand or in expenditure.(45)

1.6 Adaptation

Adaptation is a process by which a new steady state is reached in response to a change in intake. Protein adaptation refers to "adjusting rates of amino acid oxidation, urea production and protein synthesis and proteolysis to the low protein test diets fed'' in balance studies (46), but may include adaptation to an excess dietary protein. Body protein undergoes continuous breakdown and resynthesis with reutilisation of amino acids. During periods of rapid growth or recovery, an increased efficiency of utilisation may be the equivalent of an improved biological value of protein. At sub-maintenance levels of protein intake, the tissue protein pool may reach a new steady state with lower turnover and reduced catabolic rate, an important mechanism for populations where risk of deficiency is high.(6, 7) Millward proposed a 'Metabolic Demand Model', in which protein requirements are a combination of a small fixed component, combined with a variable adaptive component that is relatively

insensitive to changes in acute food or protein intake, changing slowly with a sustained change in intake, enabling nitrogen equilibrium to be maintained.(46)

The mechanisms and benefits of adaptation to a chronically low protein intake are unclear, nor is it known whether adaptation to a low intake could result in any functional impairment, despite an intake that is sufficient to achieve nitrogen balance and normal growth. Current recommendations for protein intake do not consider adaption, although there is recognition for allowing individuals time to adapt to a new level of intake and for intra-individual variability of the requirement involving a range of intake within which protein homeostasis could be maintained.(46, 47) Inoue et al (1973) showed that feeding adults a very low protein diet and excessive energy reduced the loss of labile protein in the early stages of adaptation but improved nitrogen balance with adaptation.(29) Nigerian low income farm workers accustomed to eating diets with mixed protein similar to the minimum or 'safe level of intake ' used absorbed nitrogen more efficiently than US students who habitually consumed a much higher protein intake, suggesting that "man possesses mechanisms of intermediary metabolism that allow him to adjust to low levels of protein intake."(48, 49) These factors have implications for P:E ratio recommendations particularly when applied to individuals or patient groups where a longstanding low protein diet has been consumed, as potential for adaptation to this intake may result in a lower safe P:E ratio than predicted. However, if the Protein leverage theory is applied to populations at risk of protein deficiency due to low P:E ratios, then there may be an advantage in an overall restriction in food supply rather than provide additional energy as carbohydrate and fat, so that the P:E ratio of the diet (P%cals) is maintained and a negative effect on body composition is minimised.

1.7 Clinical Studies

Clinical studies using P:E ratios have mostly been limited to infants, where the effect of feeding infant formulas of varying P:E ratios has been examined (Table 3). Several authors have attempted to quantify an adequate P:E ratio for infant formula, expressed as grams protein per 100kcal, and others have examined feeding at different P:E ratios in order to define minimum safe ratios.(25, 36, 50-52) An 'adequate P:E ratio' in infant formula has been defined as one that permits growth similar to infants fed relatively generous P:E ratios and that yields serum concentrations of albumin and urea nitrogen that are no less than those observed in breast fed infants. A 'safe P:E ratio' has been defined as one with no detectable adverse side effects including poor growth, low albumin, or obesity.(36) These studies have served as evidenced based resources for the recommendations summarised in Table 4.

Recommendations for the P:E ratios of diets in children requiring catch up growth have also been suggested depending on the composition of the required tissue to be deposited.(34, 53, 54) During recovery from infection, more rapid catch up growth has been demonstrated in children fed a higher protein diet (15% energy from protein) compared with children on an iso-energetic lower protein diet (7.5% energy from protein).(55)

Studies in adults examined the effect of varying protein and energy intake on nitrogen balance, protein utilisation and body weight, and highlighted the implications for those on very low protein diets. (19, 29, 31-33, 37, 56) (Table 5)

1.8 Clinical Implementation and Limitations

The P:E ratio of breast milk may represent the lower range of adequate or safe P:E ratios partly because of its unique properties which allow for a more complete digestion and absorption of its protein. A widely accepted use of a P:E ratio has been applied to infant

formula where one food provides total nutrition, and the P:E ratio is unchanging. After weaning, mixed protein sources from food with variable digestion and absorption may dictate the need for an increase in the percentage of energy required from protein necessitating an increase P:E ratio.

Waterlow suggested an ‘Operational approach’ for determining protein requirements in infants, using the P:E ratio of human milk, which is assumed to have the ideal P:E ratio for infants up to 4 months of age, and multiplying this figure by a factor determined by calculating the protein requirement at different ages as a fraction of the requirement for infants of 3-4 months.(57) Although simple in concept, this approach has been criticised as it fails to address issues of determining minimum protein requirements.(24)

Concern that the application of a P:E ratio is mostly limited to diets with a predictable and non-variable P:E ratio, unaffected by factors such as digestibility and quality, suggests difficulties with its widespread use. However, in clinical situations where the prescribed protein intake is marginal or closer to the true biological requirement, or when energy requirements are excessively low such as in disability or immobility, the use of the P:E ratio may prove important. When protein intake is prescribed at a low level, then the addition of energy as carbohydrate or fat may result in a dilution of protein and a decreased and potentially inadequate P:E ratio. On the other hand, in clinical situations where energy requirements are considerably low, a higher P:E ratio is required to ensure an adequate protein intake without excessive energy that can cause disproportionate weight gain. The most vulnerable clinical groups will therefore be those requiring a manipulation of macronutrient intakes such as individuals with disorders of protein metabolism including organic acidaemias or urea cycle disorders, or amino acid disorders, or potentially those where there may be an indirect manipulation of protein intake such as glycogen storage disorders (high carbohydrate diets), fatty acid oxidation disorders (high carbohydrate, fat

modified diets), epilepsy (high fat ketogenic diets) or disorders of energy metabolism (high fat diets). In these situations, the utilisation of a P:E ratio prescription may be extremely useful to maintain adequate growth and optimal body composition, although this has remained untested.

Criticism of the use of the P:E ratio relates mostly to the complexity in calculating a meaningful index. Conceptual disagreement relates to the lack of a simple linear relationship between protein and energy requirements. The P:E ratio does not imply a constant relationship between protein and energy requirements, and will change as these requirements change,(12) making it difficult to use.(21) Failure to recognise that P:E ratios are situation-specific and that they change with activity and lifestyle may also be a limitation.(11) Calculations of 'safe P:E ratios' to be compared with actual P:E ratios appears especially difficult, so too are differences between ratios applicable to an individual diet versus one applicable to the average diet of a group. Finally, uncertainty also exists as to the use of either the existing energy intake or the energy requirement associated with a desired state of health as this could result in very different ratio recommendations.(6)

Millward and Jackson proposed an alternative method to Waterlow's operational approach, whereby they calculated a P:E ratio to judge adequacy of a population intake. This firstly required the construction of a set of P:E ratios of requirements for varying levels of energy expenditure, then the creation of a set of reference P:E ratios that would represent a safe intake for that population group, and a comparison of the reference P:E ratio with the protein quality corrected ratios of the diets consumed.(7) Using this model, they compared different population groups and concluded that the reference P:E ratio for men and women increases with age; is higher for females than males; is lower for small compared with large adults; and decreases with physical activity as energy requirements increase.(7) With aging, the reference P:E ratio rises due to a progressive fall in energy needs in proportion to body size rather than

an increase in the protein need per kg, and is sensitive to levels of physical activity. However, when this model was used to indicate risk of deficiency using the available dietary and requirement data, it indicated a higher than expected risk for apparently well-nourished populations, prompting the suggestion of methodological error and a caution that using P:E ratios may create uncertainty over what action to take if they predict a high risk of deficiency.(7)

Diets with a higher P:E ratio are not necessarily of superior nutritional quality, provided that the nutrient and P:E ratio of the food is adequate when eaten to meet energy needs. The exception may be with marginal diets if energy demands change significantly.(9, 10) In some situations, the P:E ratio of the diet may be adequate for one individual and not another. For example, breast milk has a P:E ratio that satisfies both the protein and energy needs of a young infant, but becomes inadequate in protein for older children when consumed in quantities to meet energy needs.(12) While a moderately high protein intake does not appear to have serious side effects, recommendations for an unnecessarily high P:E ratio may influence food policy unreasonably and be costly to implement.(9)

If a goal in the use of P:E ratios is to enhance long term health, then the significance of recent research and the emergence of the protein leverage theory cannot be ignored. Incorporating new theories and emerging research will be required if we are to use P:E ratios of diets effectively. This includes both the apparently opposing evidence for a negative effect of high P:E ratios in infants and the risk this may pose for future weight and size, compared with the apparent positive benefits of the higher protein, lower carbohydrate diet for weight management and improved body composition with age. Breast milk with a lower P:E ratio of ~6- 8% (P%cals) continues to be considered the gold standard for infants for the first few months of life(57), however this is significantly less than the 14-15 (P%cals) suggested by the ‘Protein Leverage Hypothesis’ as the amount that humans may have a ‘drive’ to consume.

(15) This discrepancy in intakes may lend further credence to the early modelling work by Millward, which highlights the need for differing P:E ratios at different ages, body size and physical activity levels to minimise risk of protein deficiency.

1.9 Conclusion

While the use of a P:E ratio to describe the adequacy of a diet appears to have clear benefits at a population level, its clinical application remains challenging. To date, there is a no consensus about the most acceptable way to calculate, to interpret and apply such a ratio. A simple measure that will relate dietary intake and dietary requirements with consideration of within and between individual variations and allow for the determination of risk of protein deficiency or dietary adequacy, could have wide application for populations where there is a clinical justification for a reduction in protein intake or an indirect reduction in protein intake due to manipulation of other macronutrients. The application of a 'safe' P:E ratio as a clinical tool for individuals with medical conditions requiring highly regulated low protein diets, such as individuals with inborn errors of protein metabolism, or those on low energy diets, may improve metabolic stability and nutritional outcomes. If the 'drive' for protein requires a protein intake well above that previously considered necessary, then this has the potential to change the way we currently view P:E ratios and the relationship between protein and energy requirements, which are based on biological need. It may be that the protein intake well above biological requirements, as seen in most developed nations, does not actually represent excess, or result only from food availability and eating style, but denotes a better way to maintain optimal physical health and body composition. Studies that measure clinical outcomes associated with the P:E ratio are scant. By utilising longitudinal data of growth and body composition measurements, the value and use of the P:E ratio in the IEM clinical

environment will be examined and this thesis will address these unanswered questions to improve our knowledge.

Table 1. Factors considered in the development of P:E ratios

Factors	**Year**	**reference**
Adequacy of diets dependent of the percentage of energy derived from protein.	1943	(16)
Carbohydrate and fat have a protein sparing effect on protein	1951	(17)
Technique for determining net protein utilisation (NPU).	1955	(58)
Efficiency and concentration of protein combined in a single index: net dietary protein value (NDPv)	1958	(18)
Termed: Net dietary protein value calorie percentage (NDPvcals%) for the utilisable protein of the diet in proportion to its energy content.	1961	(20)
Protein to calorie ratio as a description of dietary quality. Calculated a reference P:E ratio.	1961	(20)
P:E ratios must account for individual variability in energy needs and the extent to which these may be independent of variability in protein requirements.	1975	(11)
Developed a mathematical model, based upon the bivariate distribution, for the prediction of the risk of protein deficiency associated with specific ratios of protein:calories in human diets.	1974	(21)
Operational approach to calculate P:E ratios	1990	(57)
An approach to calculate from distribution curves of intake and requirements the proportion of a population that is protein deficient.	1999	(59)

Table 2. History of committee meetings including FAO/WHO/UNU and development of P:E ratios

Meeting	Outcome
1949 1st FAO Expert Committee: energy requirements	Focus on energy only
1955 2nd FAO expert consultation: protein requirements (report published 1957):	Requirements determined relative to 'reference protein'.
1958 3rd 2nd expert consultation: energy requirement	Focus on energy only
1963 Collaboration of FAO and WHO: protein requirements	Protein requirement determined by rate of obligatory N loss in protein free diet. Protein requirement requires adequate energy. Defined protein calories in terms of total calories.
1971 Joint FAO/WHO ad hoc expert committee: energy and protein requirements first time both were considered. (report published 1973)	Differences in estimation of protein and energy requirement noted. 'Safe level of protein intake' versus 'average requirement' for energy. Requirements vary between individuals. No correlation between requirements and intake.

1975 WHO/FAO informal meeting to consider issues arising from the 1971 meeting and 1973 report.	Considered requirements for situations including catch up growth or the effects of frequent infections.
1977 WHO/FAO informal meeting to review process begun in 1975	Identified areas for consideration for the formal 1981 meeting.
1981 WHO/FAO/UNU Collaboration: protein and energy requirements (report published 1985)	Noted effect of energy on N balance and of inadequate energy dense diets on intake of nutrients (P:E and nutrient:energy ratios). P:E ratio a measure of dietary quality.
2002 WHO/FAO/UNU Expert consultation: Protein and amino acid requirements in human nutrition. Report published in 2007	Reviewed data from healthy populations and relevance to developing countries, requirements for amino acids, and digestibility of proteins in a mixed diet. Concept of adaptation to a low protein diets considered.

Table 3. Infant and children studies on the effect of feeding at diets with differing P:E ratios

Study	Population	P:E ratio/dietary intake	Result	Author
Effect of protein intake on energy utilisation.	6 children 4-17mths post recovery from malnutrition	Energy intake: 125-150kcal/kg/day. Protein provided: 4.0, 5.3, 6.4-6.7, or 8% of energy.	Energy utilisation sensitive to quantity and quality protein. At constant energy, protein intake effects rate and composition weight gain and serum albumin, (varies). 8% energy as protein intake: rise in albumin but not further rate weight gain.	(35)
Effect of energy on protein utilisation, & protein &energy on diet induced thermogenesis and composition of weight gain.	LBW infants 900-1750gm. 3 groups fed at 180ml/kg until reached 2200gms.	Group 1: 2.8gm/kg and 119kcal/kg (2.4gm/100kcal) Group 2: 3.8gm/kg and 120kcal/kg (3.2gm/100kcal) Group 3: 3.9gm/kg 142kcal/kg (2.8gm/100kcal)	Rates of weight gain/ nitrogen retention highest for infants fed higher protein or energy. Higher protein intake increased BUN and plasma amino acids. High energy caused increased fat deposition. Use protein better when energy intake high.	(52)

Adequacy of P:E ratios believed to be near safe value of milk-based infant formulas.	Experimental group (EG) males 8 – 112 days. Control and reference groups.	Experimental: 1.56g/100kcal D 8 – 27 1.25g/100kcal 84 – 111 days) Control: 2.2gm/100kcal Reference group higher P:E ratio.	Weight gain, albumin lower for EG significantly different all groups. Length gain lower EG, only significantly less than reference group. Serum urea nitrogen significantly less EG group. P:E ratio experimental formula not safe.	(25)
Hypothesis that P:E ratio of 1.7g /100kcal safe and adequate.	Study: males day 8-112. Reference: milk formula. Breast fed reference group	Study group casein-predominant formula P:E 1.7g /100kcal D 8–112 reference group 1.8 – 2.7g /100kcal	Study group energy intake D 8 – 55 significantly higher than formula reference group and significantly increased weight gain. BMI significantly higher than other groups. Albumin and urea nitrogen same as BF infants. Study formula not safe.	(36)
Feeding improved protein quality and content formula allows normal growth and urea concentration.	Healthy infants birth weight 2500-4500gms. Breast fed or assigned to formula fed groups	Formula groups P:E ratio 2.2g/100kcal or 1.8g /100kcal (Whey dominant vs. whey modified). All formula iso-caloric.	No difference in 4 groups for weight/ length gains/BMI. No differences for energy intake, protein intake less in infants fed 1.8gm/100kcal formula. Plasma urea levels for infants fed 1.8gm/100kcal closer to BF infants. Study formula safe and adequate.	(50)

Suitability, safety modified protein formula compared to conventional	Breast fed controls study formula: randomised to casein or iso-caloric whey	Conventional formula P:E ratio 2.6g/100kcal Study formula 1.8gm/100kcal.	Formula fed groups increased weight gain compared with BF infants. Formula with P:E ratio of 1.8gm/100kcal considered safe.	(51)
Effectiveness of nutrient-dense formula with energy-dense formula in infants with faltering growth	49 infants with FTT	Nutrient dense formula (NDF) 2.4g/100kcal or energy dense formula (EDF) 1.4g/100kcal.	No significant differences in tolerance, volumes, energy intake, mean weight z score. Median protein intake NDF 3.7g/kg/d and EDF 2.0g/kg/d. Blood urea ESF group fell 50% ESF group significant fall in length z score, NDF no change	(12, 53)

Table 4. Recommendations for the P:E ratio of infant formulas

Milk/age	Source of Recommendation	Recommended P:E ratio
Infant formula	FDA (1985) and ESPGAN (1977)(60)	Minimum level 1.8gm/100kcal
Infant formula infants < 3 months	Fomon, 1991(60)	minimum level of 2.2gm/100kcal
Infant formula infants > 3 months	Fomon, 1991(60)	Minimum 1.6gm/100kcal
Infant formula infants 3 – 4 months	(23)	Minimum of 1.7gm/100kcal
Infant formula 56-83days of age	(25)	Suggested safe level >1.44g/100kcal
Infant formula: 84-111days of age	(25)	Minimum >1.25gm/100kcal
Cow milk infant formula	European Commission Directive 91/321/EEC	Minimum 1.8/100kcal.
Soy milk infant formula	European Commission Directive 91/321/EEC	Minimum 2.25g/100kcal.
Cow milk infant formula	US FDA (1981) (also endorsed by ESPGEN (1977), Codex Alimentarius (1994) and the Committee on Nutrition of the American Academy of Pediatrics (1976)	1.8gm/100kcal

Note: Human milk contains a P:E ratio of 1.2 – 1.9gm protein/100kcal depending on stage of lactation (24)

Table 5. Adult studies examining the effects of variation of protein and energy intake

Study	Population	P:E ratio/dietary intake	Result	Reference
Effect of overfeeding a high or low protein diet on weight gain	16 young adults	Fed for periods of 4-8 weeks, diets ~ 2.8 or 15% of protein calories, and excess of 1,400 kcal/day above normal intake.	Mean weight gain of low and high -protein groups lower than theoretical estimate. No significant change in body composition or PAL. Excess caloric intake disposed of by increased heat production.	(61)
Effect of excess dietary energy on adaptation to a low protein diet	28 healthy males 20-27 yrs. old studied over a 2-year period	Initial period of > 1 week of standard diet then 4 groups with maintenance or excess energy and protein from 0.28 to 0.76gm/kg	At maintenance energy/ low protein a significant decrease weight and increased N loss. At excess energy: no weight loss/ loss labile protein, reduced time for adaptation to low protein diet, increased availability of ingested protein, reduction in protein requirement	(29)
Adequacy of the 1973 FAO/WHO egg protein allowance for men	6 Caucasian young male students	Subjects fed constant protein intake with increasing energy intake as required above estimated until positive nitrogen balance achieved.	5/6 subjects in negative N balance at EER. Increased energy intake resulted in positive N balance and weight gain.	(56)

Relationship between energy intake and efficiency of utilisation during long term balance studies	4 young men 21-23yrs of age	Subjects fed 1973 FAO/WHO safe level of egg protein (0.57g/kg) at several levels of dietary energy	Dietary protein quality and N metabolism significantly altered by changes in energy intake above and below maintenance needs. Increased energy intake improved N balance. Higher energy intake above requirements needed to achieve N balance	(32)
Adequacy of the 1973 FAO/WHO egg protein allowance for men with added N	4 young men 20-21yrs of age studied 58-79 days	Diet provided 0.57g/kg of egg protein with 0.23gm/kg of non-essential amino acids.	Significantly lower energy intake than in previous studies required to maintain N balance when additional protein as non-essential amino acids provided	(62)
Effect of different levels of energy intake on requirement and utilisation of egg protein.	46 young Japanese men.	6 groups 1 week normal diet then 2 weeks low protein diet at 2 energy levels 40kcal/kg/day and 48kcal/kg/day and 3 levels protein restriction; 0.2, 0.4, 0.6gm/kg/day	Sub maintenance energy: all negative N balance and lost weight. Maintenance energy only lowest protein intake lost weight. N Balance and NPU affected by N and energy intake.	(33)

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Chapter 2: New ways of defining protein and energy relationships in inborn errors of metabolism

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Minireview

New ways of defining protein and energy relationships in inborn errors of metabolism

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ABSTRACT

Dietary restrictions required to manage individuals with inborn errors of metabolism (IEM) are essential for metabolic control, however may result in an increased risk to both short and long-term nutritional status. Dietary factors most likely to influence nutritional status include energy intake, protein quality and quantity, micronutrient intake and the frequency and extent to which the diet must be altered during periods of increased physical or metabolic stress. Patients on the most restrictive diets, including those with intakes consisting of low levels of natural protein or those with recurrent illness or frequent metabolic decompensation carry the most nutritional risk. Due to the difficulties in determining condition specific requirements, dietary intake recommendations and nutritional monitoring tools used in patients with IEM are the same as, or extrapolated from, those used in healthy populations. As a consequence, evidence is lacking for the safest dietary prescriptions required to manage these patients long term, as tolerance to dietary therapy is generally described in terms of metabolic stability rather than long term nutritional and health outcomes. As the most frequent therapeutic dietary manipulation in IEM is alteration in dietary protein, and as protein status is critically dependent on adequate energy provision, the use of a Protein to Energy ratio (P:E ratio) as an additional tool will better define the relationship between these critical components. This could accurately define dietary quality and ensure that not only an adequate, but also a safe and balanced intake is provided.

Contents

Abbreviations: P:E ratio, protein to energy ratio; P%cals, energy from protein as a percentage of total calories.

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1. Introduction

In many Inborn Errors of Metabolism (IEM), particularly those of intermediary metabolism, dietary therapy is the mainstay of treatment in order to reduce the intake of the offending substrate that cannot be efficiently metabolised, and to help supply essential nutrients for normal cellular function. It is widely accepted that these diets are vital to maintain short and medium-term metabolic stability. Traditionally during childhood, the focus in prescribing these diets has been on maintaining metabolic control while enabling normal growth. However, these diets are often extremely restrictive in type of food or food group and their implementation may override normal variations in appetite and eating behaviour.

Dietary regimes include altered macronutrient manipulations comprising reduced natural protein in disorders of protein metabolism such as Urea Cycle Disorders (UCD), altered fat source in disorders such as Fatty Acid Oxidation Defects (FAOD) and carbohydrate modification in disorders such as Glycogen Storage Diseases (GSD). Disorders of protein metabolism pose unique challenges. The potentially detrimental long term effect of the protein restriction used in the treatment of these diseases is underscored by the emerging evidence for longer term functional benefits of protein intake for some groups above that required for maintenance [1], growth [2] or above Recommended Dietary Intake (RDI) [3–7]. Moreover, protein restricted diets may consist of poor quality proteins resulting in inadequate essential amino acid and micronutrient intake. The dietary prescription may be further exacerbated by acute modifications to nutritional intake, which may occur frequently in some patients. Most often this occurs during the management of an intercurrent illness when the risk of metabolic decompensation is high. In this instance the substrate that cannot be efficiently metabolised must be reduced, and an increase in dietary energy is provided to drive anabolism [8].

These clinical situations provide the greatest challenge in maintaining a balance between energy and protein intake. In practice, a great deal of attention has been paid to energy intake and less to protein intake in these situations. However, not only does a low energy intake lead to catabolism; a low protein intake may also result in increased (or persistent) catabolism and a load on the metabolic pathway [9,10]. On the other hand, the cumulative effect of an excessive energy intake over a substantial fraction of the year due to recurrent illnesses, without the provision of adequate protein, may negatively affect body composition and have implications for long term health.

In view of the growing recognition of the proven and potential long-term adverse effects of the current IEM diets, and to ensure adequacy and safety, the development of newer dietary therapies that promote dietary protein and energy balance and tools to address adverse outcomes remain a necessity. This review aims to describe the foundation of dietary advice used in IEM, which is derived predominantly from clinical practice with a limited evidence base, and to provide an alternative view on potential clinical tools to promote improved nutritional outcomes.

2. Current tools for establishing dietary recommendations

Many dietary recommendations and monitoring tools of nutritional status of patients with IEM are the same as used for healthy populations and include RDI for energy, protein and other macro- and micronutrients, and reference growth standards for head circumference, weight and linear growth. The RDI for protein and energy in the general population is based on the assumption that food intake is determined by a 'drive for energy', to meet a caloric goal [11]. This is critically important as nitrogen balance is sensitive to changes in energy intake over a range of protein intakes from low to high [11–14]. An increase in energy intake improves nitrogen balance and retention when protein intake is fixed at any level, reaching a plateau dependent on protein intake [1,4,13,15–21]. Increasing energy intake also enhances protein synthesis and reduces amino acid oxidation [14,16,17,22]. Conversely, energy utilisation can be further extended by increasing protein intake [14]. However energy requirement estimates are imprecise. Current requirements in children up to 2 years of age are determined from studies using doubly labelled water to estimate total daily energy expenditure. However, for children, adolescents and adults, energy requirements are extrapolated from predictive equations to estimate basal metabolic rate, with a factor for the level of physical activity, which can vary considerably with lifestyle [23]. These recommendations are relevant for healthy populations rather than those with chronic disease, or in particular individuals with IEM.

When making protein recommendations, a distinction between the biological requirement, which is the lowest intake required to maintain normal function, and RDI, which is defined as 'safe level of intake', and equals the average + two standard deviations of the mean requirement, is necessary [2,17]. It should be noted that protein RDI assumes that an adequate energy intake is consumed and some high quality (animal) protein intake, defined by its amino acid profile, digestibility and utilisation efficiency [14,16,17]. As the protein RDI meets or exceeds the needs of 97% of the population, it may be excessive for many children, including some with IEM [9] such as those with UCD. Yet a protein intake approximating RDI still represents a significantly lower target as compared with many western diets as measured via dietary surveys [5,24].

The nutritional risks associated with an imbalance of protein and energy intake are summarised in Table 1. In children an imbalance of either component may result in reduced growth and potential for abnormal body composition and in adults a loss of body protein, abnormal body composition and weight loss. Excess of either or both components has potential to impact body weight and body composition.

3. Nutritional outcomes in IEM

Data on the effect of IEM diets on longer term nutritional status and body composition highlight their adverse effects. Young adults with IEM, on chronically low protein intakes, have been shown to have reduced stature, higher Body Mass Index (BMI) and percentage body fat and lower skeletal muscle mass. A self-restriction of protein intake below prescription was also observed in some patients [35]. Studies in individuals with Organic Acidaemias (OA) have documented an increased weight for height [36–38], a higher proportion of body fat than controls [39], and a Resting Energy Expenditure (REE) lower than predicted [36,37,39,40]. Reports of energy intake in OA show adequate growth on intakes as low as 53% of Recommended Dietary Allowance (RDA) for energy suggesting excessive energy prescriptions may aggravate the body composition abnormalities seen [39,40].

While there is recognition that these long-term effects are undesirable, little has been published regarding measures to overcome these adverse effects. Infants and children with Urea Cycle Disorders (UCD) who were placed on a protocol to increase total protein intake with supplemental EAA's, showed improved growth and protein status from baseline levels when adequate energy was also consumed. However, many patients still failed to meet protein and energy goals and some continued to have growth faltering [41].

Nutritional studies in PKU provide inconsistent outcomes. Early reports indicated poorer growth in children consuming predominantly amino acid based diets at levels close to FAO/WHO recommendations for healthy children [42,43]. A positive effect on nutritional outcomes including head circumference [44] and a positive correlation with higher fat free mass have been associated with natural protein intake [45]. In a controlled study where protein source varied but total protein intake was similar, children with PKU on diet had lower total body nitrogen when predicted from lean body mass, weight and age, suggesting dietary protein type may be critical [46]. Body composition studies

Table 1
Potential nutritional consequences of an imbalance or inadequacy of protein and/or energy intake.

Protein and energy intake	Possible effects
Adequate protein Adequate energy	Adequate growth [14]
Adequate protein Inadequate energy	Dietary protein used for energy [1] Adults: Loss of body protein [18,25,26] Children: reduced growth [27] No value in increasing protein without increase in energy
Inadequate protein Adequate energy	Reduced body protein stores and weight loss [13,20] Children: reduced growth [27,28] Potential increased body fat [2,27,29,30]
Inadequate protein Inadequate energy	Weight loss or poor growth if too low [31] May 'adapt' and result in decreased energy expenditure to conserve stores
Excessive protein Adequate or inadequate energy	May impact bone health if too disproportionate [6] Infants: risk of metabolic and renal stress [2] High protein/low CHO diets have benefits during weight loss [32,26,33,34]
Excessive protein Excessive energy	Overweight and obesity [1,27]

have shown an increased percentage fat mass in PKU [47] while others showed no difference from controls [45,48,49]. Observations of reduced body nitrogen level versus controls [48] and increased risk of low bone mineral content [50–54] and reduced plasma prealbumin [55,56] indicate nutritional risk with a PKU dietary regime. While a recent report in PKU suggests no increased risk of metabolic syndrome in patients on diet compared with controls, the incidence of overweight and obesity in both the PKU and healthy population were unacceptably high (32.6% vs. 24.1%) [57].

The high carbohydrate intake prescribed for many GSD may reduce daily protein intake and dietary quality including micronutrient intake, particularly when corn-starch therapy is used. A possible reduction in whole body protein synthesis and delayed pubertal growth spurt may negatively affect growth, muscle and bone development overall [58]. Indeed, a reduced adult height has been demonstrated in GSD [36].

The benefits of prescribing a higher protein, lower carbohydrate (30% and 48% energy) versus lower protein, higher carbohydrate diet (11% and 67% energy) in FAOD has been studied prospectively [59]. At baseline, patients showed higher than predicted percentage fat mass index (5.4 ± 3.0 vs. 3.9 ± 2.8 kg/m^2) and lower percentage lean mass index (12.7 ± 2.1 vs. 15.3 ± 2.4 kg/m^2) compared to published reference data, regardless of BMI percentile. Despite the short study time, increased REE with a reduced total energy intake was documented on a higher protein diet, attributable perhaps to protein's greater satiating properties. Longer term follow up will be required to determine if these dietary factors impact on body composition.

4. Limitations in the application of current nutritional tools in IEM diets

It is assumed that the limitations inherent in the development of general paediatric standards for RDI and growth also apply in IEM. Despite the complex nutritional needs of individuals with IEM, available evidence for precise energy and protein requirements is limited, mainly due to small patient numbers, which prevent unequivocal conclusions being made, and the difficulty in performing energy balance studies in patients where fasting tolerance is limited. As a result, many reports on dietary intake are retrospective and describe nutritional outcomes limited to small numbers of centre based protocols. While there have been attempts to accurately determine energy needs by measuring resting energy expenditure in some conditions including phenylketonuria (PKU), organic acidaemias (OA) and FAOD [36,37,39,46,59–62], in practice, recommendations are frequently estimated using RDI, which may have differences between country of origin. While consideration of individual growth patterns and allowances to prevent catabolism and metabolic decompensation are included, these may represent an under- or over-estimation of actual needs [8].

There is even less evidence for protein requirements in children with IEM and internationally practice varies widely [63–65]. It cannot be assumed that individuals with IEM receiving protein at the RDI level have no or only minimal risk of protein deficiency and the following must be considered:

1. Protein quality: A low protein diet restricts consumption of animal protein thus limiting essential amino acid intake. Diets with protein composed mainly of synthetic amino acids will have an artificially increased requirement due to altered absorption and oxidation rates compared with natural foods [58]. For these diets, consensus suggests that protein intake greater than RDI be provided [42,45,66–71].
2. The frequency with which the diet must be modified to manage the risk of metabolic decompensation may have a negative and a cumulative effect on protein status if it is already marginal. This includes situations where natural protein intake is temporarily reduced and energy intake is increased particularly by increased fat and carbohydrate effectively diluting protein concentration.
3. Children with some IEM (e.g. UCD) experience an aversion to protein dense foods and struggle to meet even low intake goals [72], a unique situation as opposed to the forms of anorexia existing within the general population. Despite large individual and disorder-related variability, the natural or total protein tolerated or consumed may be self-restricted to below the RDI or even below the mean requirement (which meets needs of 50% of population), and therefore presents a risk for protein malnutrition.
4. Children with some IEM (e.g. OA) may have altered appetite or reduced mobility affecting energy requirement and intake [37,39,40,73] This may demand an evaluation of individual protein needs.
5. A factor rarely considered is the extent of adaptation to a chronically low protein intake. Adaptation is a process by which a new steady state is reached over time in response to a change in intake. The mechanisms and benefits of adaptation to a chronically low protein intake are unclear, and it is unknown if functional impairment may result despite an intake sufficient to achieve nitrogen balance and normal growth. Current protein recommendations do not account for adaption although its potential influence on intra-individual variability in protein requirement to maintain homeostasis is recognised [74,75]. This may be pertinent in conditions where total protein intake is chronically restricted, as true adaptation to a low intake may mean that RDI or 'safe intakes' overestimate protein needs for a greater proportion of patients than would be expected. In this instance a lower protein to energy ratio may be tolerated.

5. A strategy to address protein and energy imbalance

A diet for a patient with IEM must consider not only the immediate goals of metabolic stability and longer term goals of growth and development, but also take into consideration body composition and long term health risks such as metabolic syndrome. In consideration of the limitations regarding protein and energy provision in IEM, effective clinical tools to implement dietary therapies and monitor safety are necessary. In clinical practice, recommendations for energy and protein intake required for metabolic stability, adequate growth and development are made separately, with limited tools that consider their inter-dependence. However, a method for combining them into the one recommendation may prove advantageous. The protein to energy ratio (P:E ratio) (Fig. 1), which describes the amount of protein in a given amount of food energy, has potential to provide a new 'requirement' to apply in the most marginal of diets. It answers the question: 'If a particular diet is eaten to satisfy energy needs, will it also provide adequate protein?' P:E ratios are generally recommended for groups, or those assuming a similar intake, such as in institutions and hospitals; its use being most advantageous in 'at-risk groups' such as malnourished patients [76]. In this context the P:E ratio can be expressed as a 'numerical' relationship or density including grammes of protein per 100 kcal, or energy from protein as a percentage of total energy (P%cals). The unique dietary regimes used in IEM provide an opportunity to explore this concept in a new clinical framework.

Paediatric protein requirements are normally calculated as g/kg/day. However, this results in a fixed value that does not allow protein targets to be adjusted if energy needs change. Given the interdependence between these components and as IEM prescriptions are often close to the margins of safety, then the P:E ratio may give greater individual tailoring and flexibility without compromising safety. For example, RDI protein of 0.94 g/kg in a 20 kg 6 yr old with energy requirement of 1570 kcal/day provides 4.8% protein energy and a P:E of 1.2 g:100 kcal. If activity increases, energy requirement increases to 1760 kcal, protein at this level is reduced to 4.3% energy and P:E ratio is reduced to 1.1 g:100 kcal which may be inadequate.

6. The evolution of P:E ratios in healthy adults

Historically, the P:E ratio has described dietary quality by defining safe levels of protein intake when a particular diet is consumed to meet energy needs [76]. Early expression of the proportion of energy derived from protein in the diet is found in data from Macrae et al., from which the net protein utilisation (NPU) and adequacy of diets at Royal Air Force stations during World War II was described [77]. In 1951 Munro reviewed the relationship between protein and other dietary factors, making early reference to P:E ratios [78,79] and in 1954 Calloway and Spector examined the relationship between ideal protein and energy intake in order to design a single unit army survival ration [12]. Further development focused on the use of dietary protein concentration as a mode to assess dietary adequacy combining the efficiency and concentration of protein termed the "Net Dietary Protein Value" (NDPV) [79]. With the assumption that energy needs determine food intake, protein concentration was then expressed in terms of food calories derived from protein using the term NDPcals% [80]. While early attempts to describe a reference P:E ratio as the simple ratio of protein requirements to energy requirements were criticised for ignoring their variability and the extent of their independence, the concept was widely accepted [81,82].

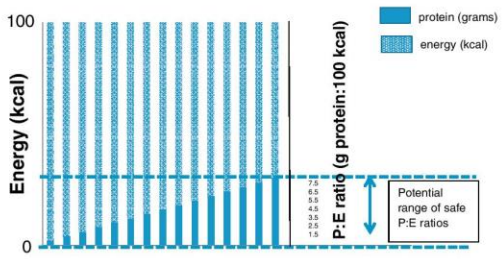

Fig. 1. The P:E ratio is represented as the number of grammes of protein, depicted by the solid areas, per 100 kcal. Energy (100 kcal) is depicted by the shaded bars. The potential safe ratio (g protein: 100 kcal) is described as a value between the dashed lines and depicted by the arrow.

The current assumption is that energy rather than protein needs drive intake [11]. The 'Protein Leverage Hypothesis', challenges this thinking, as it explores the evidence for determinants of protein intake and suggests an intrinsic drive to maintain a target protein intake [83,84]. This theory is based on long-term epidemiological evidence for a steady protein intake in humans of ~15% of energy, despite changing dietary trends. According to this hypothesis, overconsumption and obesity are the likely result of a diet with a lower P:E ratio as excessive energy will be consumed in an attempt to achieve protein intake goals [84]. This hypothesis, suggesting that protein is more influential than energy on consumption patterns, implies an advantage in promoting an appropriate P:E ratio to the public to reduce the risk of overweight and obesity.

The Food and Agricultural organisation (FAO), World Health Organisation (WHO) and United Nations University (UNU) have considered the derivation and application of P:E ratio over several decades and despite the methodological assumptions and complexity involved, endorsed a calculation of 'safe P:E ratios', in the 1985 report on protein and energy requirements [14,85]. However since that time its adoption in practice has been limited.

Table 2 describes the relationship between the P:E ratio based on RDI for protein and energy for males up to 18 years of age. Calculation is for 50%ile weight with a light activity level (BMR × 1.6). In this instance the calculated dietary P:E ratio does not consider individual variation in requirements. The P:E ratio is further described as the percentage of energy from protein if RDI for protein and energy (P%cals) are consumed.

Criticism of the use of P:E ratio relates mostly to its complexity and meaningful application. As illustrated in Table 2, a linear relationship between protein and energy requirements does not exist, i.e. protein requirements do not alter as energy requirements do with age, growth or activity change. Consequently the P:E ratio will change as energy needs change [1] making its use and interpretation more challenging in clinical practice with IEM patients [81]. Suggestions that P:E ratio application is limited to diets that are predictable and non-variable also reduces its application to the general population.

7. Implementation of P:E ratios in children

Breast milk provides a reference against which the ideal P:E ratio for infants and children could be estimated [86]. Clinical studies report the effects of feeding infants at varying P:E ratios, in order to quantify a safe minimum ratio for infant formula [28,29,87–89]. An 'adequate P:E ratio' is defined if it permits similar growth to infants fed higher ratio formulas while maintaining serum concentrations of albumin and urea nitrogen no less than observed in breast fed infants.

Clinical recommendations for P:E ratios for malnourished children have also been suggested depending on composition of the required tissue to be deposited (stunted versus wasted) [22,90,91]. Higher protein iso-energetic diets have demonstrated more rapid catch up growth and higher increases in fat free mass in malnourished children than lower protein diets (15% versus 7.5% energy from protein) [92,93].

In challenging clinical situations where preservation of nutritional status and growth are difficult to achieve such as with critically ill infants, using the P:E ratio may provide a better strategy than only

Table 2
Protein and Energy relationships calculated using RDI.

Age (years)	50% ile weight for age (kg)	RDI protein (g/kg/d)	Total protein (g/day)	RDI energy (kcal/kg/d)	Total energy (kcal/d)	P:E ratio (gprotein:100 kcal)	P%cals (%)
0.5	7.9	1.43	11.3	81	643	1.8	7.0
1	10.3	1.6	16.5	81	833	2	7.9
2	12.7	1.08	13.7	83	1048	1.3	5.2
3	14.3	1.08	15.4	93	1333	1.2	4.6
4	16.2	0.91	14.7	87	1405	1	4.2
5	18.4	0.91	16.7	80	1476	1.1	4.5
6	20.7	0.91	18.8	76	1571	1.2	4.8
7	23.1	0.91	21	72	1667	1.3	5.0
8	25.6	0.91	23.3	68	1738	1.3	5.4
9	28.6	0.91	26	65	1857	1.4	5.6
10	31.9	0.94	30	62	1976	1.5	6.1
11	35.9	0.94	33.7	58	2095	1.6	6.4
12	40.5	0.94	38.1	55	2214	1.7	6.9
13	45.6	0.94	43.9	52	2381	1.8	7.4
14	51	0.99	50.5	50	2534	2	8.0
15	56.3	0.99	55.7	47	2667	2.1	8.4
16	60.9	0.99	60.3	46	2810	2.1	8.6
17	64.6	0.99	64	45	2905	2.2	8.7
18	67.2	0.99	66.5	44	2976	2.2	8.9

Source: Nutrient Reference Values for Australia and New Zealand (2005) [23].

meeting recommendations for protein and energy intake. More recent evidence for its use is provided from studies in the intensive care unit (ICU), in which infants with bronchiolitis showed significantly higher protein balance due to increased protein synthesis when fed a formula with a high P:E ratio [94]. We have recently shown that a group of children on the classical Ketogenic Diet with intakes meeting 100% recommendations for energy and for protein but with a P:E ratio ≤1.5 g protein:100 kcal had poor linear growth [96]. Thus in meeting protein and energy targets, the suggestion that one enteral feed with a fixed protein to energy ratio is adequate is refuted, particularly as energy requirements, body composition and level of illness vary [95]. Incorporating this clinical application into daily practice when prescribing this dietary regime may enable better growth outcomes.

7.1. Clinical use of P:E ratios in children with IEM

A search in Medline (1980–2014) using the terms 'protein recommendation', 'protein requirement', 'protein intake' or 'energy recommendation', 'energy requirement', 'energy intake,' or 'protein to energy ratio', and limited to consensus statements or studies with n = ≥ 5 yielded, 15 reports with dietary recommendations, 27 reports of dietary intake and only 3 reports describing a protocol or intake using protein and energy as a ratio, however with no recommendations about this ratio. Depending on location, recommended intakes are referred to as RDA, D-A-C-H, FAO/WHO/UNU, DRI, DRV or RDI which are all comparable (Appendix 1).

In a smaller longitudinal study of 3 infants with MMA to determine the response to varying levels of protein intake on growth, nitrogen balance and organic acid metabolism, the P:E relationship was highlighted, demonstrating an increased protein tolerance when a high energy intake was provided, particularly during catch up growth [97]. However no specific clinical recommendations were made. Likewise, in a retrospective study on protein tolerance in MSUD, OA and PKU up to 3 years of age, the authors describe intake as a P:E ratio: (g protein/100 kcal) but make no conclusions of the effects of differing ratios on metabolic or nutritional outcomes [73].

A small increase in P:E ratio from 2.74 g:100 kcal to 3.12 g:100 kcal in patients with PKU over 6 months from birth resulted in better growth in infants fed the higher P:E ratio than the lower ratio, despite both groups consuming a similar energy intake [66]. This study also highlights the issue of protein quality in IEM as studies in healthy infants indicate adequate growth when fed natural protein formulas with ratios of 1.8 g/100 kcal [88].

7.2. Potential application of the P:E ratio in IEM

Recommendations for macronutrient distribution to treat hepatic GSD include protein intake of 10–15% of dietary energy [98], however emphasis on achieving adequate carbohydrate of up to 65% of dietary energy to manage hypoglycaemia could limit protein intake. A report of dietary intake of 20 patients with GSD1, described a wide range of carbohydrate and protein intakes from 55.9 to 77.4% and 6–18% of energy respectively [99]. A higher protein intake in some GSDs that affect muscle is recommended, with evidence of improved growth [100]. A case report showed a dramatic improvement in cardiomyopathy when dietary protein was increased to 30% of total energy. The authors suggest that this higher protein intake may prove effective in both the prevention, treatment and reversal of cardiomyopathy, and the use of a P:E ratio when initiating and monitoring these patients may be advantageous [101].

The use of P:E ratios could also provide evidence to change practice in IEM where current practice is that protein would be eliminated for a short period to manage or prevent metabolic decompensation such as during critical illness. For example evidence suggests that plasma amino acid levels may be low in patients with UCD at presentation of acute illness [102] and they may benefit from adequate energy supplements in combination with essential amino acids at a minimum P:E ratio, rather than zero protein.

8. Conclusions and future perspectives

Although the application of a P:E ratio is likely to primarily benefit those with protein intakes directly manipulated in quantity and/or quality, its use should be considered for all IEM diets that require macronutrient manipulation in which protein intake can also be indirectly affected (e.g. GSD). Studies in healthy subjects suggest that lower protein/higher carbohydrate diets hinder satiety and energy expenditure and inhibit fat oxidation by increasing insulin response, resulting in increased hunger, promoting food intake and leading to increased body fat. This may contribute to longer term risk for inflammatory response and metabolic syndrome [103–106]. Likewise dietary manipulations that reduce the P:E ratio may be similarly detrimental to body composition in individuals with IEM as well as result in reduced bone mineralisation. The determination of a clinically applied minimum safe ratio may protect against the increased risk of marginal protein status caused by overzealous dietary restrictions previously suggested [58,107].

Clinical P:E ratios need to be disease-specific and situation-specific which will enable greater flexibility than the use of RDI's. Diets in IEM are often prescribed within a defined intake range, have similar construction, and are highly prescribed and monitored providing a sound basis from which appropriate P:E ratio estimations could be made. Future studies will need to address the disease-specific and child-specific P:E ratio in the diet and to determine safe ratios, and a level below which nutritional or metabolic status maybe compromised. Due to variations in age, gender and activity levels there is likely to be a range of safe P:E ratios which may prove more difficult to establish. Studies are required for the elucidation of patient-related safe P:E ratios in patients with IEM.

This review provides a theoretical argument of how P:E ratios may be used to address the inherent shortcomings of use of RDI in IEM patients. A current limitation is the lack of experience and published reports using the P:E ratio. Therefore prospective studies will be required for any development of dietary guidelines and suggested P:E ratios. Implementation studies could then evaluate the longer term nutritional and metabolic outcomes of prescribed ratios to validate their safety.

Conflict of interest

The authors have no financial or other relations that could lead to conflict of interest.

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Appendix 1

Table 1
IEM reports with protein or energy recommendations.

First author and year	Condition	Reporting country	Protein recommendation	Energy recommendation	Benefit/other
Fernandes J. 1988 [108]	GSD	Europe/Israel	GSD 1A and 1B: adolescents and adults. Energy: protein 10–15% Fat 25%, CHO > 60% energy GSDIII: energy: protein 25%, fat (MCT included) 20–25%, CHO 50–50%,	–	GSDIII: 'liberal protein to serve as substrate for gluconeogenesis'
Kindt E. 1988 [109]	PKU (2 groups) Protein at RDA n = 8 Protein at FAO/WHO 1973 n = 8	Norway	FAO/WHO 1973 recommendations for protein intake provide insufficient amino acids in PKU	–	FAO/WHO protein intake ~30% lower than RDA resulted in decreased length/growth velocity for some.
Medical Research Council Working Party on PKU 1993 [110]	PKU	UK	Protein substitute recommendations <2 yrs: >3 g/kg/day >2 yrs: 2 g/kg/day	–	
Przyrembel H. 1996 [111]	PKU	Germany	FAO/WHO safe levels of protein intake corrected for digestibility and amino acid score.	"Adequate energy"	
Acosta PB. 1996 [112]	PKU	USA	A range of protein intakes required. Protein > RDA required due to the source.	Range of intakes required	Consider increased intakes during intercurrent illness/infection
Cockburn F. 1996 [51]	PKU	UK	MRC recommendations (1993) adjusted for health and Phe and Tyr levels. >10 yr protein substitute amounts based on Recommended Intake (RNI) + 25–50%	–	MRC recommendations difficult to achieve with respect to amino acid intakes and may result in excessive energy intake.

Appendix Table 1 (*continued*)

First author and year	Condition	Reporting country	Protein recommendation	Energy recommendation	Benefit/other
Urea Cycles Disorders Conference Group 2001 [113]		USA	Long term management: reduced protein intake necessary. RDA may be greater than required to achieve normal growth in UCD. Severely affected patients may need EAAs at 25-50% protein intake	–	Based on theoretical considerations and anecdotal experience.
Berry G. 2001 [114]	UCD	USA	Infants with OTC/CPS/arginase deficiency: 0.7 g/kg natural protein and 0.7 g/kg EAA ASS/ASL: 1.5–2.0 g/kg natural	-	Individually tailored to patients.
Leonard J. 2001 [9]	UCD	USA/UK	FAO/WHO/UNU (1985) safe intakes likely excessive. Revised safe values [2] adopted. Consider 25–50% protein as EAAs if severe.	'High energy during illness'	Protein intake should be adjusted to the IEM and individual patient characteristics.
Rake JP. 2002 [115]	GSD I	Europe	10–15% total energy from protein	60–65% energy from CHO	
Yannicelli S 2006 [58]	PA/MMA	USA	0.0–<0.5 yrs: ~2.5 g/kg 0.5–<1.0 yrs: ~2.5 g/kg 1–<4 yrs: >30 g/d 4–<7 yrs: >35 g/d 7–<11 yrs: ~40 g/d 11–<19 yrs: ~50 g/d	100–125% RDA	Recommendations assume ~50% natural protein, ~50% amino acid supplements, >RDA due to protein source. Some may not require amino acid supplements and total protein can be lower. Protein amount titrated to tolerance, anabolism and growth.
Singh R. 2007 [10]	UCD	USA	Total protein (natural + EAA supplement): 0 to <3 months g/kg: 2.21–1.25 3–6 months: 2.0–1.15 9 to <12 months: 1.6–0.9 g/day: 1–<4 yrs: 8–12 4 to <7 yrs: 12–15 7 to <11 yrs: 14–17 Women g/day 11–<15 yrs: 20–23 15–<19 yrs: 20–23 >19 yrs: 22–25 Men g/day 11–<15 yrs: 20–23 15–<19 yrs: 21–24 >19 yrs: 23–32	Kcal/kg: 150–125 140–120 120–110 kcal/day: 945–1890 1365–241 1730–346 Women kcal/day 1575–3150 1260–3150 1785–2625 Men kcal/day 2100–3885 2200–4095 2625–3465	Review protein recommendations based on Protocol 24 — UCD. The Ross Metabolic Formula System Nutrition Support Protocols 2001.
Kishnani P. 2010 [116]	GSDIII	USA		Energy: 20–30% protein 35–55% CHO 20–35% fat -	Consensus guideline
Kölker S. 2011 [117]	GA1	International Collaboration/ Guideline	Lysine from natural protein as mg/lysine per day plus amino acid mixtures of g/kg/d 0–6 months: 1.3–0.8 7–12 months: 1.0–0.8 1–3 yrs: 0.8 4–6 yrs: 0.8 >6 yrs: 'Safe levels of natural protein'	–	Revised guideline. Acknowledges differing approaches including low protein/low lysine diet without amino acid supplement and low lysine diet or low protein diet with amino acid supplements.
Häberle J. 2012 [118]	UCD	Europe	Acute illness cessation of protein 24–48 h Long term: WHO/FAO/UNU 'safe intakes' as guide. EAA supplements if natural protein intake inadequate.	100–120% age adjusted energy requirements Long term use WHO/FAO/UNU recommendations	Consensus based guideline.

Table 2
Studies describing protein or energy intakes.

First author and year	Condition	Reporting country	Protein intake	Energy intake	Other
Acosta PB 1994 [119]	PKU N = 25 from diagnosis Period 1 = 0–3 months age Period 2 = 3–6 months age	USA	Fed protein substitute Group A 3.12 g/100 kcal Group B 2.74 g/100 kcal	Group A Period 1 580 ± 28 kcal and Period 2 680 ± 30 kcal/kg Group B Period 1 591 ± 30 kcal and Period 2 709 ± 34 kcal/kg	Prospective longitudinal study. Group A infants tolerated increase Phe. Group A improved growth vs Group B
Schäfer F. 1994 [120]	PKU N = 82 (0–6 yrs)	Germany	Natural/total protein g/kg/d <2 yrs ~0.7 ± 0.1/2.2 ± 0.25 3–6 yrs ~0.5 ± 0.1/2 ± 0.25 g/kg/d	–	Retrospective review: Some growth retardation in first 2 years.
Thomas E. 1994 [121]	PA N = 12 Dx (3–790 days)	Saudi Arabia	Initial consult 1.0–3 g/kg Final consult 1.6–3 g/kg	115–145 kcal/kg 99–273 kcal/kg	Retrospective review. Protein recommendations include amino acid supplements. Intakes prescribed based on Ross nutrition support protocol.
Schulz B. 1995 [122]	PKU N = 99 (12–29 yrs). Groups ± protein supplement	Germany	Group with protein supplement: 112–138% RDA Group without protein supplement: 90–104% RDA	Protein:8–20% energy Fat: 11–40% CHO: 36–80% Protein: 5–16% Fat: 26–47% CHO: 43–66%	Retrospective review: Dietary survey. Group without protein supplement had inadequate intake some nutrients and higher Phe levels.
MacDonald A 1996 [123]	PKU N = 19 (1–16 yrs)	UK	Allocated protein g/kg/d <1 yr: 3 2–5 yrs: 2.5 6–10 yrs: 2.0 >11 yrs: 1.5	Mean (SD, range) 105% (17%, 77%–178%) EAR	Prospective longitudinal study. No correlation with energy intake and plasma Phe level. Distribution of protein substitute affects 24 h Phe variability
Krauch G. 1996 [124]	PKU Low and high tolerance groups at various ages	Germany	Recommended protein intake: 3 months: 2.2 g/kg 10 months: 2.0 g/kg 3 yrs: 1.7 g/kg 8 yrs: 1.4 g/kg 12 yrs: 1.1 g/kg 16 yrs: 0.9 g/kg	'Average energy intake'	Excess of several amino acid for some patients when fed protein at these levels.
Acosta PB. 1998 [68]	PKU N = 35 (0.5–6 months)	USA	Mean 17.3 ± 0.6 g	660 ± 18 kcal	Prospective longitudinal study. Normal growth seen, supporting MRC recommendations for supplementary protein of 3 g/kg/day
Thomas J. 2000 [40]	Organic acidaemia N = 6	USA		All children consumed energy <RDA for age.	Retrospective review. Adequate growth seen at <RDA energy. May be related to decreased mobility.
Arnold G. 2002 [55]	PKU N = 28 (2–18 yrs)	USA	Total protein g/d > RDA: 2–4 yrs: 30 4–7 yrs: 35 g 7–11 yrs: 40 >12 yrs: 50–55	–	Retrospective chart review. No general growth impairment. Hypoprealbuminaemia predicted linear growth restriction.
Yannicelli 2003 [125]	MMA/PA N = 16 (0.03–3 yrs)	USA	Total protein g: mean ± SD: <6 months: 15 ± 0.9 6–<12 months: 18.3 ± 1.1 1–<4 yrs: 25.1 ± 2.46	Energy kcal Mean ± SD: <6 months: 645 ± 10 6–<12 months: 741 ± 92 1–<4 yrs: 1062 ± 100	Multicentre outpatient study. Those increasing in length achieved 98% and 115% of WHO/FAO/UNU energy and protein intakes respectively. Those that did not achieved 87% and 104% respectively.
Gillingham M. 2003 [126]	LCHAD/TFP deficiency N = 10 (1–10 yrs)		Mean 2.5 g/kg/d (1.3–5 g/kg/d)	Protein 12% energy: MCT: 12 LCT: 11 CHO: 66	Prospective study. No growth deficiencies observed.
Dobbelaere D. 2003 [49]	PKU N = 20 (0.7–7 yrs)	France	Mean ± SD Total protein: 1.67 ± 0.23 g/kg/d Natural protein mean 9.8 g/d	67–12% RDA (Mean 89%)	Prospective, cross sectional study. Patients shorted and lighter than reference populations. No relationship between protein and calorie intake and growth retardation.
MacDonald A. 2004 [127]	PKU N = 25 (2–10 yrs)	UK	Protein equivalent at 2 g/kg and 1.2 g/kg	–	Randomised cross over study. Poorer Phe control seen with lower amount of protein substitute.

Appendix Table 2 (*continued*)

First author and year	Condition	Reporting country	Protein intake	Energy intake	Other
Hoeksma M. 2005 [44]	PKU N = 174 (0–36 months)	Netherlands	Mean ± SD Total protein: 2.33 ± 0.42 g/kg/d Natural protein 0.99 ± 0.34 g/kg/d	Mean ± SD For first year of life 27 kJ ± 2.6 kJ/kg/d	Retrospective study. Height growth not clearly related to protein intake. Natural protein rather than total protein correlated with head circumference.
Acosta PB. 2005 [41]	UCD N = 17 Median 4.4 months (0.22–38.84 months) Protocol: Protein ~50% FAO/WHO/UNU or as tolerated. 40–70% of protein from medical food.	USA	Intakes as % FAO/WHO/UNU 0–<6 months: 70 6–<12 months: 62 12–<24 months: 89 24–<36 months: 59 36–<48 months: 35, 81	Intakes as %FAO/WHO/UNU 0–< 6 months: 110 6–< 12 months: 110 12–<24 months: 89 24–<36 months: 45.132 36–<48 months: 70.89	Longitudinal study. Adequate intakes resulted in anabolism and linear growth without increasing ammonia. Some still failed to ingest recommended protein and energy intakes.
Touati G. 2006 [128]	MMA and PA N = 137 (85 MMA, 52 PA) n = 56 Dx 1970–1987 n = 81 Dx 1988–2005 n = 39 severe disorder Dx >1988	France	Severe patients on amino acid (AA) supps: 40% at 3 yrs, 50% 6–11 yrs. Intake at 3, 6, 11 yrs g/kg/d: Group without AA supps: Natural protein: 0.92, 0.78 0.77 Group with AA supps: Natural protein: 0.75, 0.74 0.54 Total protein:1.29, 1.17, 0.89	Energy intake kcal/kg/d No AA supps vs. AA supps: 3 yrs: 93.1 vs. 85.9 6 yrs: 80.7 vs. 70.2 11 yrs: 66.4 vs. 52.2	Retrospective review: Since 1988 all patients treated with low protein diet to tolerance and only occasional use of AA supps. Metabolic control not different between groups.
Nagasaka H. 2006 [129]	OTC N = 7 Follow up age 3–5 yrs	Japan	Infancy 1.3–2.0 g/kg Older children 0.7–1.1 g/kg	According to age requirements 1350–1660 kcal	Prospective study to determine effect of reintroduction of L-arginine on nutrition, growth, and urea cycle function.
Heumer M. 2007 [45]	PKU N = 34 Mean 8.7 yrs (2–15 yrs)	Austria	Mean total protein intake g/kg/d: 1.2 ± 0.3 124% (77–19) DACH 2000 Natural protein 0.3 ± 2 g/kg/day	–	Prospective longitudinal study with cross sectional component. A significant correlation of fat free mass with intake of natural protein rather than total protein
Singh R. 2007 [10]	UCD	USA	EAA supplement to 50% protein intake: 0 to <3 months: 2.1–1.4 g/kg/d 3–6 months: 1.5–1.2 9 to <12 months: 1.2–1.1 1–<4 yrs: 18.6–12.5 g/d 4 to <7 yrs: 21.0–19.0 7 to <11 yrs: 22.0–24.0	 150–101 kcal/kg 100-80 80–75 800–1040 kcal/d 1196–1435 1199–1693	Intake data from patient charts from author's clinic. Diets must be individualised depending on severity of disorder
Ahring K. 2009 [130]	PKU 10 Centre survey	Europe	Amino acid supplementation decreased with age (g/kg/d) Infancy: ~2–3 1–10 yrs: ~1.2-2 1–10 yrs: ~2–3 (40% centres) >10 yrs: ~1.0–1.5	'Normal' energy intake according to national or European recommendations	Structured questionnaire to assess practice differences. Substantial variation in dietary guidelines among countries and within countries. No consensus opinion based on solid scientific rationale.
Hauser N. 2011 [39]	MMA N = 29 (2–35 yrs) Natural protein ± amino acid supplements ± isoleucine or valine	USA	Total protein 0.38-2.94 g/kg/d Natural protein 0.29–2.12 g/kg/d (33–265% RDA) 18/29 had amino acid supplements 0.21–1.95 g/kg/day	23–86 kcal/kg/d	Prospective study. Wide variation in the dietary treatment of MMA. REE may be overestimated with standard equations due to altered body composition.
Rocha J. 2012 [57]	PKU N = 89 (3–30 yrs)	Portugal	Protein (g/kg/d): <10 yrs: Natural protein 1.03 ± 0.51 Protein suppl.: 1.46 ± 0.47 10–16 yrs: Natural protein 0.65 ± 0.36 Protein suppl. 1.42 ± 0.43 >16 yrs: Natural protein 0.53 ± 0.35 Protein suppl. 1.07 ± 0.42	Energy kcal/d: <10 yrs: 2171 ± 375 10–16 yrs: 2467 ± 256 >16 yrs: 2415 ± 375	Prospective study. Prevalence of overweight and obesity, body fat percentage and central obesity were comparable to controls, however higher than ideal.
Gokmen-Ozel H. 2012 [131]	GA1 N = 20 N = 9 without encephalopathic crisis (EC) N = 11 with EC (2.2–24.1 yrs)	UK	Patients without EC: Protein intake (median, range) Natural: 4/6 (1.3, 1.3–1.7 g/kg) Protein substitute (1.6,1.3–1.7 g/kg) 2/6 given general protein restriction. Patients with EC: Treatment varied. Low protein diet only. Protein substitute ceased over time.	–	Retrospective review. Dietary treatment dependent on age of diagnosis and symptom severity.
Adam S. 2012 [132,133]	UCD 16 IMD centres N = 175 N = 123 (0–16 yrs) N = 52 (>16 yrs)	UK	Prescribed protein intake as WHO/FAO/UNU 2007 safe level titrated to metabolic control (g/kg/d): 0–6 months: 2 7–12 months: 1.6 1–10 yrs: 1.3	–	Cross-sectional data detailing dietary practices from 16 IMD centres in the UK, collected by questionnaire

(*continued on next page*)

Appendix Table 2 (*continued*)

First author and year	Condition	Reporting country	Protein intake	Energy intake	Other
			11–16 yrs: 0.9 >16 yrs: 0.8 Variable use of EAA		
Adam S 2013 [133]	UCD N = 464	Europe	Variable for each condition/age/country Use of EAA supplements varied		Survey: Dietary treatment varies widely between centres.
Boy N. 2013 [134]	GA1 N = 33 (0–6 yrs) Asymptomatic n = 29 Dystonic n = 4	Germany	Asymptomatic patients Natural protein: Mean 109% (median 115%, SD 20%) DACH recommendations Amino acid supplement Mean 108% (median 110%, SD 14%) of GA1 guideline recommendations Dystonic patients: Mean 121% (median 122%, SD 8%) of DACH recommendations Amino acid supplement Mean 104% (median 103%, SD 7%) of GA1 guideline recommendations	Asymptomatic patients: 106% (median 102% SD 13%) of DACH recommendations. Dystonic patients (mean 110%, median 108%, SD 8%)	Prospective longitudinal study. Amino acid supplement and energy intake decreased with age in asymptomatic group. Normal weight gain in asymptomatic group but impaired in dystonic group. Reduction in height z-score for both groups.
Aldámiz-Echevarría L. 2013 [135]	PKU BH4 n = 38 Diet only n = 76 Followed up for 2 and 5 years	Spain	2 yr follow up group g/kg/d Diet only: Natural protein 0.4 (0.3–0.5) Total protein 1.4 (1.0–2.4) BH4 group: Natural protein 0.8 (0.5–1.0) Total protein 1.5 (0.7–2.2) 5 year follow up group final intake: Diet only Natural protein 0.3 (0.2-0.4) Total protein 1.6 (1.2-1.9) BH4 group: Natural protein 0.9 (0.7–1.1) Total protein 1.2 (0.7–1)	–	Retrospective review. Growth impairment also identified in patients on BH4 despite higher intakes of natural protein.

Table 3
Studies incorporating protein and energy as a P:E ratio.

First author and year	Condition	Reporting country	Protein: energy statement	Benefit/other
Acosta PB 1994 [119]	PKU N = 25 from diagnosis	USA	Fed protein substitute Group A 3.12 g/100 kcal Group B 2.74 g/100 kcal	Prospective longitudinal study. Formulas described as protein content in relation to energy content.
Acosta PB. 2003 [70]	PKU N = 58 from diagnosis (2–12.2 yrs)	USA	2–<4 yrs: 2.3 g/100 kcal 4–<7 yrs: 2.0 g/100 kcal 7–<11 yrs: 2.1 g/100 kcal Females 11–<13 yrs: 2.3 g/100 kcal Males 7–<11 yrs: 2.0 g/100 kcal	Study treatment protocol dictated protein goals expressed as P:E ratio.
Ogier De Baulny H. 2005 [73]	MMA/PA	France	Natural protein intake of patients on low protein Diets described graphically as g protein/100 kcal	Minimum protein requirement estimated as 70% of total requirement during first weeks of life, reducing to 50% at 6 months.

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Section 2: Direct and indirect modifications of total protein intake

Section 2: Aims

Aim 1:

a) To describe longitudinal patterns of growth and body composition and dietary intake in children requiring a direct modification to protein intake. This includes children with organic acidaemias (OA) including Methylmalonic acidaemia (MMA), Propionic Acidaemia (PA), Isovaleric Acidaemia (IVA) and Maple Syrup Urine Disease (MSUD), urea cycle disorders (UCD). (Chapter 3)
b) To describe longitudinal patterns of growth and body composition and dietary intake in children who may have an indirect modification to protein intake due to dietary manipulations that focus on carbohydrate and/or fat intake. This includes children with Glycogen Storage Disorder (GSD) Type I and Type III (Chapter 4), and Very Long Chain Acyl-Dehydrogenase deficiency (VLCAD).(Chapter 5).

Aim 2: To investigate the relationship between protein quantity and/or quality on growth and body composition in children with OA and UCD and GSD and VLCAD. (Chapter 3-5)

Aim 3: To determine if there is an optimal P:E ratio for prescribing dietary recommendations in children with OA and UCD. (Chapter 3)

Chapter 3: The relationship between dietary intake, growth, and body composition in inborn errors of intermediary protein metabolism

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ORIGINAL ARTICLES

The Relationship between Dietary Intake, Growth, and Body Composition in Inborn Errors of Intermediary Protein Metabolism

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Objectives To examine relationships between dietary intake, growth and body composition patterns in patients with inborn errors of intermediary protein metabolism and to determine a safe protein:energy ratio (P:E ratio) associated with optimal growth outcomes.

Study design Retrospective longitudinal data of growth and dietary intake in patients (n = 75) with isovaleric acidemia (IVA; n = 7), methylmalonic acidemia/propionic acidemia (MMA/PA; n = 14), urea cycle defects (UCD; n = 44), classical maple syrup urine disease (MSUD; n = 10) were collected. Prospective longitudinal data of growth, dietary intake, and body composition from 21 patients: IVA (n = 5), MMA/PA (n = 6), UCD (n = 7), and MSUD (n = 3) were collected at clinic visits.

Results Fifty-two of 75 (66%), 49 of 74 (68%), and 44 of 65 (68%) patients had a *z*-score of 0 (±1) for lifetime weight, height, and body mass index, respectively. Patients with MMA/PA had the lowest median height and weight *z*-scores, and MSUD patients had highest median body mass index *z*-score at all ages. In IVA, MMA/PA, and UCD, total natural protein intake met or exceeded the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO)/United Nations University (UNU) recommended safe levels. Median percentage fat mass was 17.6% in IVA, 20.7% in MMA/PA, 19.4% in UCD, and 17.8% in MSUD. There was a significant negative correlation between percentage fat mass and total protein intake in IVA, MMA/PA, and UCD ($r = -0.737$; $P = .010$). The correlation between the P:E ratio and growth variables in IVA, MMA/PA, and UCD suggest a safe P:E ratio (>1.5 to < 2.9) g protein:100 kcal/day.

Conclusion Growth outcomes in inborn errors of intermediary protein metabolism are not always ideal. Most patients with IVA, MMA/PA, and UCD consume sufficient natural protein to meet FAO/WHO/UNU recommendations. A P:E ratio range of (>1.5 to < 2.9)g protein/100 kcal/day correlates with optimal growth outcomes. *(J Pediatr 2017;188:163-72).*

The goals of dietary treatment in inborn errors of intermediary protein metabolism are to secure metabolic stability and promote normal growth. Recently, attention has also been directed toward attaining long-term ideal body composition. However, there is a lack of evidence-based research to inform best practice in the management of inborn errors of intermediary protein metabolism. Therefore, treatment modalities are still reliant on expert opinion rather than large-scale, clinical studies that could inform specific nutrient needs. Indeed, dietary management of patients with inborn errors of intermediary protein metabolism differs worldwide. Consensus exists regarding reduced natural protein intake and for the use of amino acid–based formulas (AAFs) for disorders such as maple syrup urine disease (MSUD), in which natural protein tolerance is below requirements.[1] In contrast, in isovaleric acidemia (IVA), in methyl-malonic acidemia and propionic acidemia (MMA/PA), and urea cycle disorders (UCDs), the consumption of AAF in combination with a lower intake of natural protein is debated. For example, published guidelines for UCD recommend a low natural protein diet without the use of essential amino

%fatmass	Percentage body fat mass
AAF	Amino acid–based formula
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BMR	Basal metabolic rate
E%BMR	Energy intake as a percentage of basal metabolic rate
EAAS	Essential amino acid supplement
FAO	Food and Agriculture Organization of the United Nations
FFM	Fat-free mass
IVA	Isovaleric acidemia
MMA/PA	Methyl-malonic acidemia and propionic acidemia
MSUD	Maple syrup urine disease
P:E ratio	Protein:energy ratio
REE	Resting energy expenditure
UCD	Urea cycle disorders
UNU	United Nations University
WHO	World Health Organization

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acid supplements (EAASs) for those who can adequately meet estimated requirements from foods and are metabolically stable,[2] yet EAASs are still advocated for regular use in some centers.[3] Consequently, studies that compare nutritional outcomes in children with disorders who consume natural protein only versus those requiring natural protein and AAF are required.

Dietary factors that contribute to nutritional outcomes are likely to be multifactorial and include the quality and quantity of protein tolerated, the frequency of further protein restriction and high nonprotein energy intake during metabolic decompensation[1,2,4,5] and the abnormal feeding behaviors and food aversion observed in patients with these disorders.[6,7] Taken together, these protein-restricted regimens may result in short- and long-term nutritional risks.

Exact nutritional requirements for patients with inborn errors of intermediary protein metabolism have not been studied systematically, partly owing to variability in individual tolerance and, therefore, are not well-defined. Consequently, dietary adequacy is often measured against recommendations for healthy populations. The application of these reference recommendations for children with potentially different requirements is, therefore, problematic. Population-based protein requirements also assume an adequate energy intake to ensure efficient protein use, which presents an additional challenge in children prescribed highly modified diets. The model of how protein and energy requirements are interdependent has evolved over decades of nutritional research,[8-13] and has been incorporated into the 1985 consensus statement from the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), and United Nations University (UNU).[14] This document summarizes the concept of the protein:energy ratio (P:E ratio), which describes the proportion of dietary energy derived from protein, and has traditionally been used to answer the question: If an individual or group consume this diet, in amounts that will satisfy energy needs, will the concentration (density) of protein also be high enough to meet protein needs?[14] This concept is both relevant and challenging in inborn errors of intermediary protein metabolism, where protein intake may be marginal owing to treatment requirements or protein aversion. Furthermore, the value of AAF as a protein alternative must also be considered given the differences in their absorption and bioavailability compared with natural protein.[15] Consequently, nutritional outcomes, including reduced height, increased incidence of overweight, and abnormal body composition are documented in inborn errors of intermediary protein metabolism.[6,16-19]

Current dietary prescriptions in inborn errors of intermediary protein metabolism do not consider the relationship between protein and energy intake formally. We hypothesized that the use of the P:E ratio in the dietary management of inborn errors of intermediary protein metabolism may have value and provide clinicians with additional guidance when making dietary prescriptions.

The aim of this study was to answer the following questions: Do patients with inborn errors of intermediary protein metabolism consume adequate protein and energy? What relationships exist between dietary intake and growth patterns in patients with inborn errors of intermediary protein metabolism? Finally, can we define a safe P:E ratio to be used as an additional clinical tool in the management of patients with inborn errors of intermediary protein metabolism?

Methods

This study was approved by the Royal Children's Hospital Human Research Ethic Committee (HREC: 30066B).

We collected longitudinal data on dietary intake and growth of patients born between 1976 and December 2014 (n = 75; 30 males, 45 females) with IVA (n = 7), MMA/PA (n = 14), UCD (n = 44), and classical MSUD (n = 10). Data were collected from medical and dietetic clinic records when patients were metabolically stable. Dietary data consisted of dietary recall, food diaries, and dietary history. The data represent reported rather than prescribed intake.

Parents provided written consent for inclusion of their children. We collected longitudinal data on dietary intake and growth, body composition measurements of patients born between January 1995 and December 2014 (n = 21; 8 males, 13 females): IVA (n = 5), MMA/PA (n = 6), UCD (n = 7), and MSUD (n = 3) currently under our care. Data were collected over a 2-year period and included between 1 and 6 separate measurements of body composition and dietary intake for individuals, depending on the age at diagnosis, time to consent, frequency of appointments, metabolic stability, and compliance with data collection requirements.

Weight and length for children under 2 years of age were obtained by standard techniques using digital baby weighing scales and crown-heel length on a scaled length board. Height and weight of children greater than 2 years of age were measured using a combined stadiometer and digital weight measuring station (Seca 284, Seca, Hamburg, Germany). Participants were in light clothing with no shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were recorded. Body mass index (BMI) was calculated using the equation kg/m^2. Measurements were performed by the dietitian or clinic nurse.

Anthropometric measurements were expressed as age- and sex-specific *z*-scores, using the epidemiological software package Epi Info (version 3.5.1), based on the Centers for Disease Control and Prevention (Atlanta, Georgia) 2002 reference database.

Dietary data collected from 3-day food diaries were analyzed by the same metabolic dietitian using the dietary analysis program Foodworks (Xyris, Version 7.0.3016, Kenmore Hills, Australia). Dietary intake of protein in grams per kilograms per day was compared with FAO/WHO/UNU recommended safe levels.[20] Energy intake was expressed as a percentage of the basal metabolic rate (BMR) calculated using predictive equations according to Schofield,[21] based on age, sex, height, and weight. The P:E ratio was expressed as g protein/100 kcal/day and compared with the P:E ratio calculated from the 1985 FAO/WHO/UNU equation:[14]

$$\text{Average P:E}_{\text{requirement}} = \frac{\text{estimated average requirement protein g/kg/day} \times 16.7\ \text{kJ/g} \times 100}{\text{estimated energy requirement (kJ/kg/day)}}$$

For comparison purposes, protein and energy requirement values were combined for both sexes up to 8 years of age and an assumption that the estimated energy requirement = BMR × physical activity level 1.6 was made, because this corresponds with accepted values for light activity.[22]

Body composition was measured indirectly by bioelectrical impedance analysis (BIA) using the QuadScan 400, Bodystat (Isle of White LTD, England, United Kingdom) per manufacturer's instructions. Participants were instructed to fast for at least 90 minutes and to avoid exercise before the BIA assessment. Measurements were taken twice. The first reading was used when the second was within 1% of the first reading. If there was a greater variance, a third reading was taken and the average between the 2 readings within 1% was used.

Fat-free mass (FFM) and fat mass were estimated using raw impedance values using the equation of Houtkooper[23]:

$$\text{FFM (kg)} = \left(0.61 \times \left(\text{height [cm]}^2 / \text{impedance at 50 kHz}\right)\right) + (0.25 \times \text{weight [kg]}) + 1.31$$

Fat mass was calculated as total mass (kg) − FFM (kg). Then, percentage fat mass (%) was calculated as (fat mass [kg]/total weight [kg]) × 100. Comparisons were made between disorders at key childhood ages of 3, 5, 10, and 14 years of age.

Statistical Analyses

Statistical analyses were performed using SPSS for Windows software version 23 (IBM, Chicago, Illinois). Significance was set at $P < .05$. Continuous variables including *z*-scores for body weight, height, and BMI; protein and energy intake; and P:E ratio are presented as median and range. Nonparametric tests used included the Kruskal-Wallis test for 1-way between-group analysis of variance, the Mann-Whitney *U* test for differences between 2 independent groups on a continuous measure, and the Wilcoxon signed-rank test for variance between 2 measures in the same subjects. The Spearman correlation coefficient was used to evaluate associations between independent variables.

Results

Fifty-two of 75 (66%), 49 of 74 (68%), and 44 of 65 (68%) patients had a *z*-score of 0 (±1) for lifetime weight, height, and BMI, respectively (**Table** and **Figure 1**). Longitudinal growth patterns differed between the groups. Children with IVA had essentially normal growth patterns. In children with MMA/PA, median height *z*-scores were persistently less than −1 after 5 years of age. In children with UCD, growth patterns were normal, although male patients had significantly higher BMI *z*-scores than female patients at 3 years of age (n = 22; $P = .038$) and 10 years of age (n = 16; $P = .011$). In children with MSUD median height *z*-scores were persistently less than −1 in 3 of 9 patients.

Table. Growth data and dietary intake of patients by disorder

Disorders	Variable	Total no. of data points (range per patient)	Lifetime *z*-score		
			<−1, n/N (%)	0 (±1), n/N (%)	>1, n/N (%)
IVA (n = 7)	Weight: *z*-score	139 (3-28)	1/7 (14)	5/7 (71)	1/7 (14)
	Height: *z*-score	111 (2-26)	1/7 (14)	4/7 (57)	2/7 (29)
	BMI *z*-score		0/7	6/6 (100)	0/7
	Total protein	91 (0-23)			
	Total energy	57 (0-18)			
	Patients having hospital admissions*	6			
MMA/PA (n = 14)	Weight: *z*-score	593 (3-74)	5/14 (36)	8/14 (57)	1/14 (7)
	Height: *z*-score	407 (6-59)	7/14 (50)	7/14 (50)	0/14
	BMI: *z*-score		0/14	8/14 (57)	6/14 (43)
	Total protein†	481 (9-67)			
	Total energy	232 (7-36)			
	Patients having hospital admissions*	14			
UCD (n = 44)	Weight: *z*-score	936 (3-80)	4/44 (9)	31/44 (70)	9/44 (20)
	Height: *z*-score	685 (2-66)	6/44 (14)	33/44 (75)	5/44 (11)
	BMI: *z*-score		1/36 (3)	24/36 (67)	11/36 (31)
	Total protein	496 (1-39)			
	Total energy	247 (0-18)			
	Patients having hospital admissions*	34			
MSUD (n = 10)	Weight: *z*-score	461 (16-83)	1/10 (10)	8/10 (80)	1/10 (10)
	Height: *z*-score	261 (9-65)	3/9 (33)	5/9 (56)	1/9 (11)
	BMI: *z*-score		0/9	6/9 (67)	3/9 (33)
	Total protein	433 (11-73)			
	Energy	111 (6-14)			
	Patients having hospital admissions*	10			

*Table 1 hospital admissions for metabolic decompensation or other causes with dietary modifications.
†Total protein intake including 30 data points from before 1991 with AAF contributing to total protein intake.

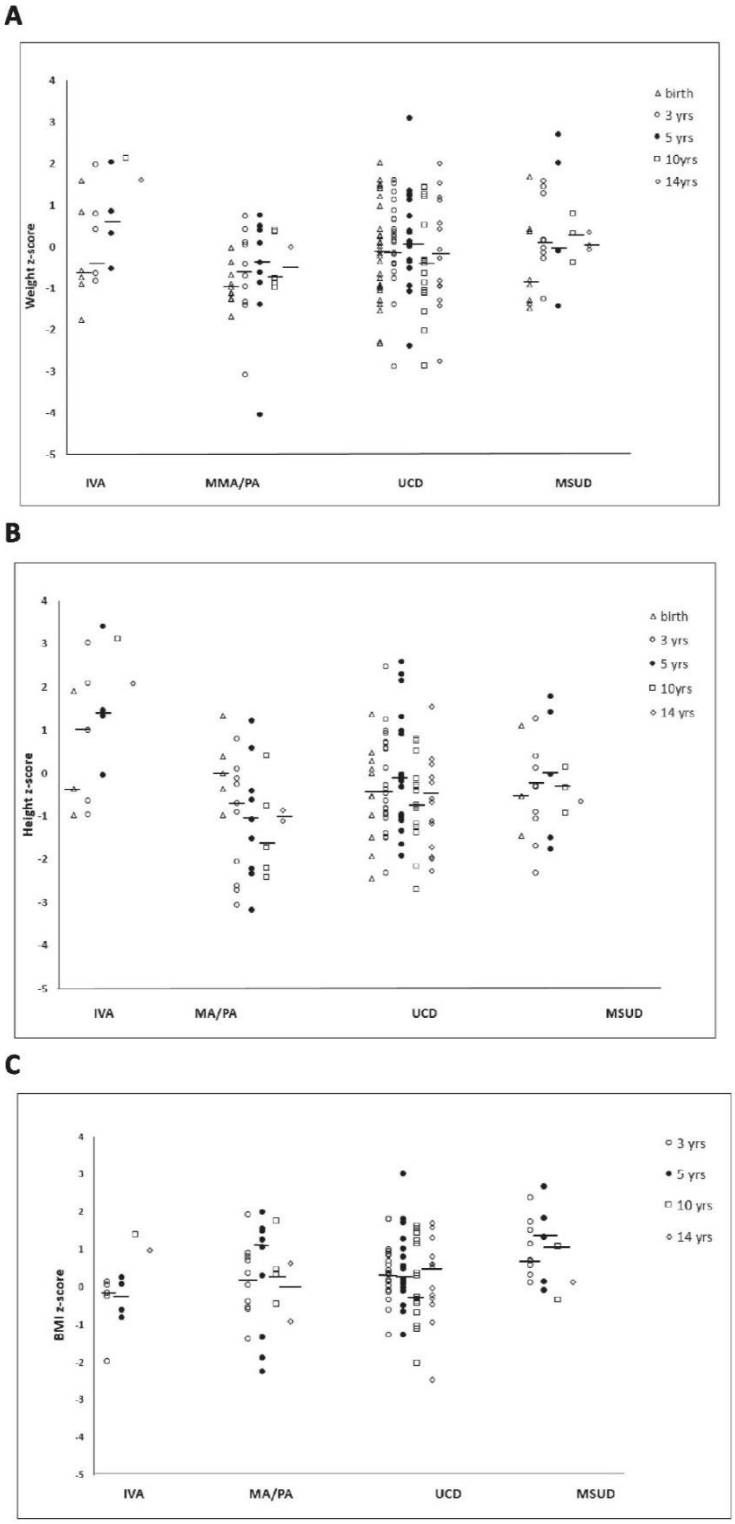

Figure 1. Anthropometric variables at birth, 3, 5, 10, and 14 years of age for groups of patients with MSUD, MMA/PA, IVA, and UCD. **A,** Body weight. **B,** Body length. **C,** BMI. Each point represents an individual patient's *z*-score. *Black bars* represent the median score for the group.

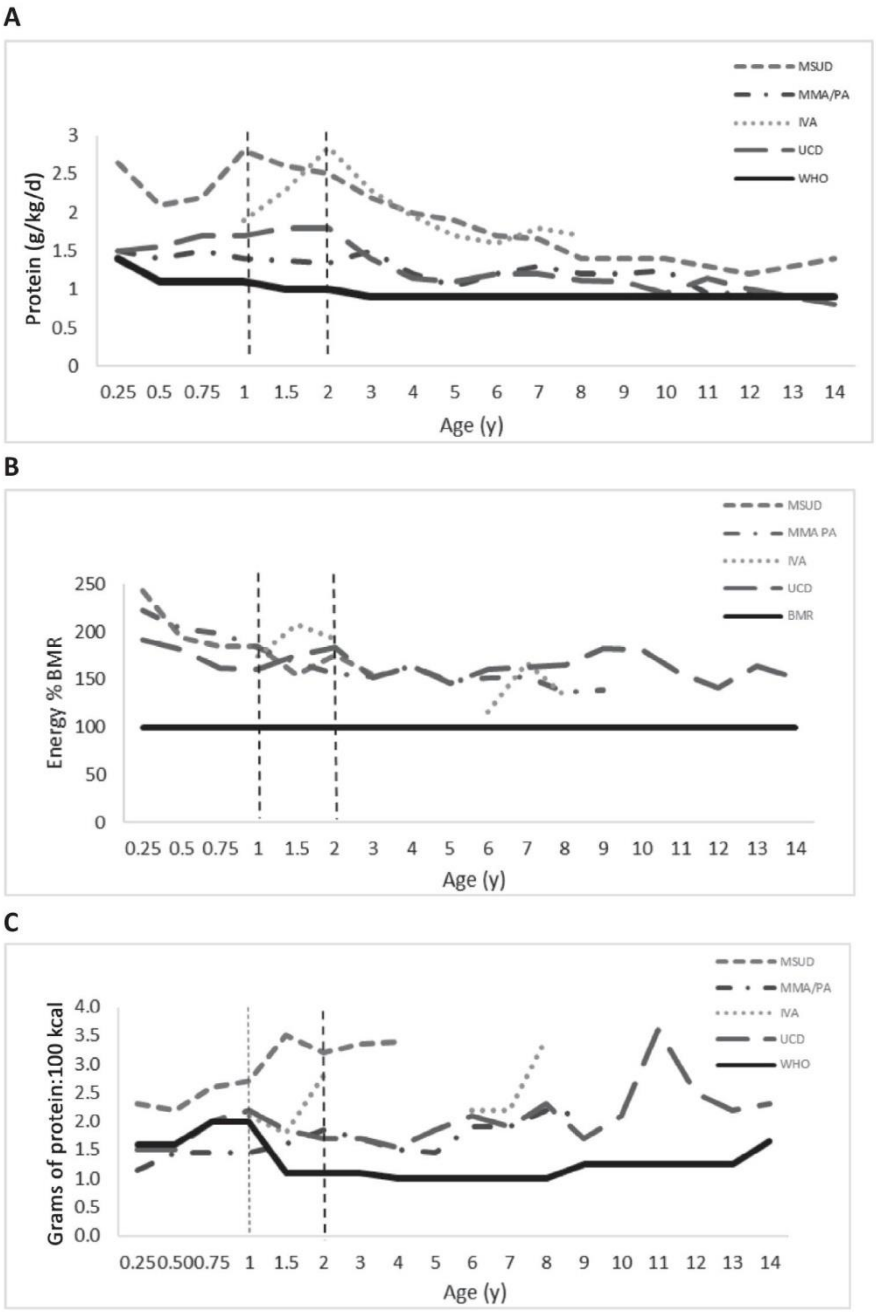

Figure 2. Dietary intake. **A,** Total protein intake expressed as median grams per kilogram per day and compared with the FAO/WHO/UNU recommended safe protein intake. **B,** Total energy intake expressed as a percentage of BMR calculated from the predictive equations of Schofield and compared with 100% of BMR. **C,** The P:E ratio expressed as median grams of protein per 100 kcal and compared with the P:E ratio for each age according to the FAO/WHO/UNU equation for estimating a reference P:E ratio.

There were 1501 data points for total protein intake available for analysis (**Table**). Total natural protein intake (g/kg/day) decreased with age in all patients, yet median protein intake in all disorders up to age 14 years met or exceeded the FAO/WHO/UNU recommended safe level except for UCD patients at 14 years, whose median protein intake was 0.8 g/kg/day (WHO recommendation of 0.9g/kg/day) (**Figure 2**, A). Patients with IVA consumed natural protein only. Patients with

MMA/PA in our center have not been treated with AAF since 1991. However, the final data analysis included 30 data points of patients with MMA/PA treated before 1991, for whom AAF were a component of the total protein intake; patients with UCD consumed natural protein, with EAAS for some patients only during intercurrent illness or when metabolically unstable. In MSUD, natural protein intake contributed 17%, 15%, 14%, and 20% of total protein intake at 3, 5, 10, and 14 years of age, respectively.

Overall, 16 of 75 patients reported 1 or more episodes where protein intake was less than FAO/WHO/UNU recommendations, including 1 of 7 with IVA, a boy with autism and significant protein aversion; 3 of 14 with MMA/PA, all born between 1976 and 1984; and 12 of 44 with UCD, 7 of 12 born before 1987, 3 of 12 with persistent metabolic instability, 1 of 12 with extreme protein aversion, and 1 of 12 with an eating disorder.

The median energy intake over the data collection period was as follows: IVA, 116-208 energy intake as a percentage of basal metabolic rate (E%BMR); MMA/PA, 137-222 E%BMR; UCD, 141-192 E%BMR; and MSUD, 155-243 E%BMR (**Figure 2**, B). Only 3 of 40 data points (7.5%) from 2 of 7 patients with IVA, and 6 of 215 data points (2.3%) from 6 of 44 UCD patients indicated energy intake of less than 100% of the BMR. Energy intake was not different between the groups at key ages.

Dietary P:E ratio increased with age in all disorders, and was greater than the P:E ratio calculated from the 1985 FAO/WHO/UNU Consultation equation (**Figure 2**, C) in all diets, except in children with MMA/PA under 1.5 years of age. To calculate an apparent safe P:E ratio (**Figure 3**), stepwise statistical correlations were made between weight, height, and BMI, respectively, and (1) data points from the lowest P:E ratio to the highest (which yielded a trajectory with a strong correlation) and (2) data points from the highest P:E ratio to the lowest (which yielded a trajectory with no correlation). The point of most extreme statistical difference between the 2 trajectories, at which they cross, represents the apparent safe P:E ratio (ie, there is a strong positive correlation between lower P:E ratio and lower weight, height, and BMI, respectively, and no correlation between higher P:E ratio and these variables, respectively).

At baseline measurement, the median %fat mass was 20.7 in MMA/PA, 19.4 in UCD, 17.8 in MSUD, and 17.6 in IVA patients. There was no difference in %fat mass across the disorder groups at all ages or for patients <10 years of age. Body composition measurements were not different over 2 or more measurements.

Correlations between Dietary Variables

Protein, Energy, and P:E Ratio and Growth Variables. Weight, height, and BMI *z*-scores were assessed at key ages (3, 5, and 10 years). The small number of patients with IVA precluded correlation analysis and no major trends were observed. In MMA and PA, there was a trend for a positive correlation between total protein intake and weight *z*-score at 3 years (n = 10; r = 0.567; $P = .087$), and 5 years of age (n = 8; r = 0.395; $P = .333$) and between height *z*-score at 3 years of age (n = 10; r = 0.506; $P = .136$), 5 years of age (n = 8; r = 0.287; $P = .490$), and 10 years of age (n = 5; r = 0.205; $P = .741$), that weakened with age. There was no consistent correlation between energy intake and height or weight *z*-score, suggesting that excessive energy intake (>180% BMR) did not improve growth in this group. In UCD patients, dietary variables were not consistently associated with height or weight *z*-score. However, there was a significant negative correlation between total protein intake and BMI at 10 years of age (n = 10; r = –0.665; $P = .036$), suggesting a reduced BMI in those on higher protein intakes. In MSUD, there was a significant correlation between total protein, which included AAF intake, and height *z*-score at 3 years of age only (n = 9; r = 0.689; $P = .040$).

There was a significant negative correlation between natural protein intake and %fat mass at baseline BIA measurement in IVA patients (n = 5; r = –0.900; $P = .037$) and a trend for such a correlation in MMA/PA patients (n = 6; r = –0.522; $P = .288$), but not UCD patients. Small patient numbers precluded assessment in MSUD. When all disorders were combined, including MSUD, there was a significant negative correlation with %fat mass and total protein intake at baseline BIA (n = 21; r = –0.515; $P = .017$) and measurement 2 (n = 14; r = –0.562; $P = .036$). In IVA, MMA/PA, and UCD patients, who consumed protein from natural sources only, higher protein intake was somewhat correlated with lower %fat mass at the baseline BIA measurement (n = 18; r = –0.463; $P = .053$) and significant at BIA measurement 2 (n = 11; r = –0.737; $P = .010$). The median difference in time between measurements was 0.54 years of age (range, 0.34-1.00).

P:E Ratio and %Fat Mass. In IVA patients, there was a trend for lower %fat mass at baseline BIA measurement when the P:E ratio was higher (n = 5; r = –0.600; $P = .285$), but this trend was not observed in MMA/PA or UCD patients.

When all disorders were combined, there was a trend for a positive correlation between BMI and %fat mass at baseline BIA measurement (n = 15; r = 0.464; $P = .081$) and at measurement 2 (n = 11; r = 0.395; $P = .230$). When MSUD was excluded from the analysis, the relationship was significant at baseline BIA (n = 13; r = 0.599; $P = .031$) and measurement 2 (n = 9; r = 0.683; $P = .042$).

To determine an apparent "safe" P:E for use in IVA, MMA/PA, and UCD, the lifetime median weight, height, and BMI *z*-scores were correlated with the lifetime median P:E ratio for each patient (**Figure 3**). Patients with MSUD were excluded owing to their small number and total protein intake comprising less than 80% AAF. The results suggest that all growth variables are stable and lie at *z*-score 0 (±1) when the P:E ratio is (>1.5-<2.9)g protein/100 kcal/day (ie, 6%-12% of dietary energy is provided from protein). The value of higher P:E ratios could not be determined owing to a small number of data points. Stability was defined as an r of approximately 0, that is, no correlation between P:E ratios (>1.5-<2.9) and weight, height, and BMI *z*-scores. Low weight and height *z*-scores were recorded as the P:E ratio decreased to less than 1.5 g/100 kcal/day. Median %fatmass of children with P:E ratios (>1.5-<2.9)

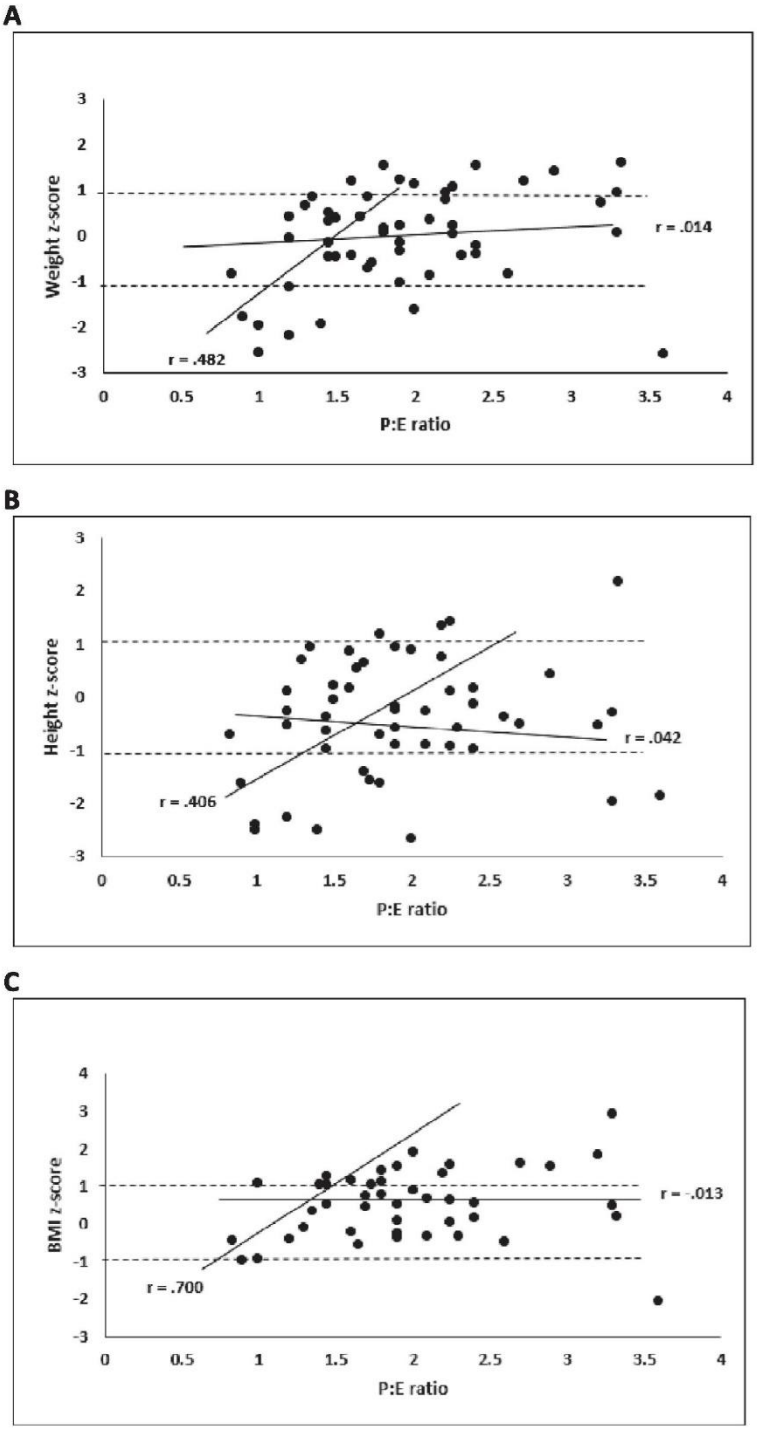

Figure 3. Correlations between anthropometric measures and P:E ratio to determine a safe P:E ratio. **A,** Weight. **B,** Height. **C,** BMI. The *lines across* the data points represent 2 trajectories of stepwise statistical correlations between weight, height, and BMI and P:E ratios when calculated from the lowest P:E ratio forward, and from the highest P:E ratio, backward. For further details, see the text.

was 21.3%, which meets accepted age standards.[24] More data are needed to draw conclusions regarding the effect of P:E ratio of greater than 2.9 on growth and body composition.

Discussion

Growth patterns in inborn errors of intermediary protein metabolism may be affected by both disease pathophysiology and the nutritional adequacy of consumed or prescribed diets. In this study, we assessed dietary adequacy and explored associated nutritional outcomes to define an apparent safe P:E ratio to be used as an additional clinical tool in the management of these patients. A strength of our study is that the protein intake reported was the estimated amount actually consumed rather than prescribed. In addition, longitudinal data were retrieved from a single center with a relatively stable treatment policy over many years. An obvious limitation of a study of this kind is the relatively small number of patients. In addition, energy requirements were estimated from predictive equations and not actually measured. Feeding behaviors, satiety levels, and dietary compliance, all of which may contribute to variations in outcome, were also not evaluated. Nevertheless, the inclusion of more than 4000 data points of growth variables and reported nutritional intake in 75 patients over 28 years enabled us to confidently document growth and dietary intake patterns and explore relationships between them.

Chronic protein restriction and frequent catabolic events during which patients have high energy and minimal protein intakes are likely to play a crucial role in their growth patterns. Despite this circumstance, the majority of patients had normal growth. Patients with IVA had growth patterns similar to healthy children, confirming results from a recent European report.[18] Patients with MMA/PA showed similar growth patterns to those previously reported,[16,18,19] with reduced weight and height *z*-scores during infancy, and a greater weight than height *z*-score throughout childhood, predisposing children to an increased risk of obesity.[6,16,18,19] We speculate that the slight worsening in height *z*-score from 5 to 10 years of age before improving later in adolescence may reflect a skewed pubertal growth in MMA/PA, but this hypothesis will need confirmation in a larger longitudinal study. Median height *z*-score scores in patients with UCD were 0 throughout childhood, in line with previous reports of adequate weight gain velocity but not height velocity in patients with UCD.[17] We also observed a mildly delayed pubertal pattern. In a large European cohort, the variability in growth patterns for different UCD was attributed to the actual disorder.[18] Patients with MSUD showed an increase in growth velocity in the first 3 years of life, which then stabilized, but increased weight versus height *z*-score resulted in the highest incidence of overweight. Contributing factors to early and continuous overweight in MSUD may include the high energy intakes to prevent catabolism particularly during illness,[1] and the large amounts of AAF consumed daily to meet nutritional requirements and provide metabolic stability. There are few reports about %fat mass in patients with inborn errors of intermediary protein metabolism. High mean fat mass, up to approximately 40% of body mass, has been documented in MMA/PA patients.[16,19] In our cohort, median %fatmass in patients with MMA/PA was approximately 21%, and 19% in patients with UCD. The difference between our patients and those previously reported could be owing to disease severity or the benefit of natural protein intake rather than AAF, and should be further explored.

Dietary protein intake in IVA, MMA/PA, and UCD is determined by tolerance and rate of dietary modifications during intercurrent illnesses and metabolic decompensation; therefore, in severely affected individuals, this could result in a diet marginal in adequacy. Nevertheless, patients with IVA, MMA/PA, and UCD had median protein intake at or above the FAO/WHO/UNU recommended safe intakes, confirming that AAF or daily EAAS were not required for adequate protein intake. Total protein intake decreased with age in all groups, similar to previous reports.[6,25-27] Patients with IVA tolerated more protein than MMA/PA, whose protein intake was similar to patients with UCD. In a retrospective review of patients with MMA/PA, a total natural protein intake of 0.92, 0.78, and 0.77g/kg/day was documented at 3, 6, and 11 years of age, respectively,[6] which is lower than our patients who consumed a median 1.5, 1.2, and 0.95 g/kg/day, respectively, at the same ages. Taken together with a recent report on adverse outcomes in relation to the use of AAF by Manoli et al,[19] our results support the call for a review of the necessity of the use of AAF for MMA, as suggested. Protein intake in UCD patients showed greater individual variation than in MMA/PA, possibly owing to the variability in OTC activity in female patients. Most of the episodes of protein intake below the current FAO/WHO/UNU safe intake recommendations occurred in teenage patients born in the 1970s and early 1980s, when accepted safe levels were lower.[28] Because the protein intakes in our study were "actual" rather than prescribed, they may also reflect the protein aversion well-documented in UCD.[7] Total protein/AAF intake of MSUD patients in the current study was more than 120%-140% the safe intake, which meets published guidelines when the source of protein is predominantly AAF.[1]

Energy requirements in patients with inborn errors of intermediary protein metabolism are currently not well-defined, yet it is recommended to increase energy intake to promote anabolism during illness. Prolonged or frequently high energy intakes can increase the risk of overweight, particularly in children with more frequent admissions or metabolic decompensations, or those with reduced energy requirements owing to decreased mobility. In MMA/PA, Feillet et al[29] determined the resting energy expenditure (REE) to be 80% of that from the predictive Schofield equations. Indeed, appropriate growth velocity was documented in patients with OA despite energy intake considerably less than the recommended daily allowance.[30] By contrast, Van Hagen[31] found no difference between measured and predicted REE. Energy intakes lower than US recommendations were also reported in UCD.[27] Energy intake in our patients with MMA/PA and MSUD in the early years was more than 200% of predicted BMR, thus potentially contributing to the long-term increased BMI and %fat mass. Although energy intake is critical to metabolic stability

in IVA and UCD, we did not document excessive energy intakes in these disorders.

We demonstrate a consistent correlation between higher protein intake and lower %fatmass in all patients studied. Although an increase in natural protein intake may not be possible in many patients with inborn errors of intermediary protein metabolism, determining the maximum natural dietary tolerance for individuals could improve their body composition. Several mechanisms have been proposed to explain a benefit from increased protein intake on weight and body composition, including a reduction in dietary energy intake mediated by an effect on satiety,[32] an increase in REE owing to a greater diet-induced thermogenesis,[33] an influence on growth hormone and insulin-like growth factor-1 production on body composition,[34] and a stimulatory effect on muscle protein anabolism favoring the retention of lean muscle mass.[35,36]

The increased thermogenic effect of protein is thought to be mediated via an increase in protein turnover owing to the lack of flexible storage capacity and consequent increased oxidation when supplied with increased intake, and an upregulation of uncoupling proteins increasing thermogenesis and energy expenditure.[37] In a short-term study in patients with long chain fatty acid oxidation disorders, the REE was significantly higher when patients were prescribed a higher protein versus a higher carbohydrate diet.[38] Total protein intake has also been associated with body composition changes.[34] Over a 6-year period, an inverse relationship between dietary protein intake and change in fat mass index was observed in lean girls, via a decrease in body fat gain and increase in FFM gain.

The physiological adaptations to a low protein intake may include a decrease in the obligatory nitrogen loss, an increase in the efficiency of protein use and a decrease in lean body mass.[39] This finding may partly explain the higher %fatmass in those on the lowest protein intakes in our group. Chronic metabolic acidosis may potentially override this response by directly stimulating skeletal muscle proteolysis and accelerating nitrogen losses and impairing growth.[40] This observation may explain partly why our patients with MMA/PA showed the most severe growth deficits, given their increased risk of chronic acidosis. Additional factors contributing to compromised linear growth could be related to hormonal factors or to neurologic abnormalities in some patients,[41] but these factors have not been explored in the current study.

The application of a P:E ratio as a measure of dietary quality for a population is limited owing to the difficulty of incorporating individual variation in requirements affected by sex, age, size, and activity into the value.[42] The limitations and implications of the use of the P:E ratio for patients with inborn errors of intermediary protein metabolism have been reviewed recently,[43] and further prospective study is required to explore the contribution of sex, age, and physical activity.

The P:E ratio in the diets of our patients meets or exceeds the calculated ratios for individuals estimated from the FAO/WHO/UNU equations, except in patients with MMA/PA who are less than 1.5 years of age, most likely owing to their very high energy intakes at this age. Our apparent safe P:E ratio for diets for children with inborn errors of intermediary protein metabolism is based on optimal growth outcome, and is in line with our previous report of reduced linear growth in children on the classical ketogenic diet with a P:E ratio of less than 1.5 g protein/100 kcal.[44] It should be noted that our calculated safe P:E ratio is based on dietary and growth data disregarding periods of further dietary modifications, that is, lower protein and higher calorie intake, which could amount to a considerable number of days per year in some patients. Therefore, we consider it a safe rather than a minimum P:E ratio.

In conclusion, we have demonstrated that, despite adequate protein and energy intake, growth outcomes in patients with inborn errors of intermediary protein metabolism are not always ideal. We show that in patients with IVA, MMA/PA, and UCD the short-term natural protein intake meets the FAO/WHO/UNU recommendations. We also show that a P:E ratio range of >1.5-<2.9 g protein/100 kcal/day correlates with an optimal growth, BMI, and %fatmass in those with inborn errors of intermediary protein metabolism. This ratio could be used as an additional clinical tool when constructing diets for patients with inborn errors of intermediary protein metabolism. ■

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Note: The sentence in the discussion page 84, midway through the second paragraph should read: "Median height z-scores in patients with UCD were close to 0 throughout childhood, which compares favourably to previous reports of adequate weight gain velocity but not height velocity in patients with UCD[17]."

The publication of this paper was accompanied by an Editorial by Dr Hans Anderson who described the importance and value of this study regarding evaluating dietary intake and outcome to allow a review of current nutritional practices considering new evidence.

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This Month In The JOURNAL of PEDIATRICS

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Remember to play and play to remember

— Paul G. Fisher, MD

Much attention has been paid recently to the effects of aerobic exercise on cognitive function. Do dance and swim lessons, running, cycling, skating, or just playing outside school improve working memory later in life?

In this volume of *The Journal*, López-Vicente et al report their findings from 1400 Spanish children in a prospective cohort study evaluating the relationship between extracurricular physical activity and sedentary behavior at 4 and 6 years of age with working memory at 7 and 14 years of age. While the association between low extracurricular physical activity with poorer working memory at 7 years of age did not reach statistical significance, decreased physical activity and increased sedentary behaviors by 6 years of age did predict lower working memory at 14 years of age. Interestingly, television viewing was not associated with working memory.

While the authors acknowledge the limitations of their study, particularly the possibility of confounding from unmeasured variables such as family income or educational status, their results point to low physical activity in early childhood resulting in worse working memory among adolescents. This important line of research needs further confirmation. Aerobic exercise has been increasingly implicated in the development and integrity of brain white matter, and could provide an underlying biological mechanism to explain the findings from López-Vicente et al. Regardless, childhood play is well established to promote socialization and overall physical health. Pediatricians and parents would be well served to remember to encourage our children to play and not just to sit still. Perhaps this play will help them remember more in school and elsewhere.

Article page 35 ►

Dietary guidelines for inborn errors of metabolism

— Hans C. Andersson, MD

Among the most vexing decisions in the management of metabolic diseases that require restriction of dietary components is the determination of adequate and sufficient diet. In metabolic diseases such as urea cycle disorders or organic acidemias, under-restriction of protein can result in hyperammonemia or acidosis whereas over-restriction can lead to poor growth. Each condition may have unique dietary allowances and patients can be variably affected. The field of metabolism is re-examining the rigid guidelines of metabolic formulas that have allowed these patients to survive. A new approach to each condition's specific dietary requirements is occurring and a few groups have suggested that some conditions may be allowed greater natural protein amounts than previously considered safe.

Evans et al, in this volume of *The Journal,* have examined the relationship of dietary protein to energy consumption and growth outcomes over age ranges from birth to 14 years of age in 75 patients with 5 rare inborn errors of metabolism. Their findings strongly support a specific range of dietary protein:energy—1.5-2.9 g protein:100 kcal/day—for optimal growth outcomes. Such long-term outcome studies in rare disorders are valuable for patients whose dietary restrictions may result in poor growth. The comparisons between patients with urea cycle and those with methylmalonic academia/propionic academia make clear the differences in growth outcomes for these disorders under the best circumstances. Such studies are revising the approaches of metabolic physicians to better understand the broad nutritional effects of dietary restrictions which have allowed these children to live otherwise healthy lives.

Article page 163 ►

Chapter 4: Longitudinal growth and dietary intake in patients with Glycogen Storage Disease Type I and Type III

4.1 Introduction

Glycogen storage diseases (GSD) are a group of rare genetic disorders characterised by accumulation of glycogen in various tissues, particularly liver and muscle. The most common hepatic GSD are Glycogen Storage Disease Type I (GSD I) and type III (GSD III) which are both characterised by hypoglycaemia, hepatomegaly, poor physical growth and an abnormal biochemical profile.(1) GSD I results from a deficiency of the hydrolytic enzyme glucose-6-phosphatase (G-6-P) activity (GSD Ia), or deficiency of the transporter protein G-6-P translocase (GSD Ib).(2) GSD III results from a deficiency of glycogen debrancher enzyme and may involve liver and muscle (GSD IIIa) or liver only (GSD IIIb). The gluconeogenic pathway from alanine is blocked in GSD I but it is intact in GSD III. Consequently, patients with GSD I cannot use protein to supply glucose but patients with GSD III can.

Patients with GSD I may show symptoms during the neonatal period; however, presentation is often between 3 to 6 months of age (3) as this corresponds with a change in feeding pattern, with a longer fasting period precipitating hypoglycaemia. There are significant complications associated with GSD I. Short term complications include hypoglycaemia, hyperlipidaemia, hyperlactataemia, and hyperuricaemia. Longer term complications include growth faltering with delayed puberty, liver adenomas and risk of hepatocellular carcinoma, impaired platelet function, anaemia, osteoporosis and osteopenia, renal dysfunction and pulmonary hypertension.(4-6). In GSD Ib there are unique problems of neutropenia and impaired neutrophil function and Crohn's disease-like enterocolitis in some patients.(7)

Patients with GSD III usually present later than those with GSD I as fasting tolerance may be longer and hypoglycaemia may not be as severe. However, despite their different enzymatic bases the disorders are sometimes difficult to distinguish on clinical grounds

alone. Recognised short and long-term complications of GSD III include recurrent hypoglycaemia, liver adenomas, hepatocellular carcinoma, cardiomyopathy, myopathy, growth failure, osteoporosis and osteopenia.(8)

4.1.1 What are the aims of dietary therapy in GSD?

Dietary management is the mainstay of treatment for both GSD I (9-11)and GSD III.(3, 8) Although hypoglycaemia in these disorders may become less severe with age, inadequate therapy can result in impaired physical growth and a delay in pubertal onset (11) and may not prevent the other complications mentioned above.(8) The recognised primary aims of therapy in GSD I and III are to correct hypoglycaemia and achieve normal growth and development, and prevent or delay the longer-term complications of the disorders.(2, 3, 11) However, effective lifelong management of GSD I and III is not just about avoiding hypoglycaemia, but about maintaining normoglycaemia. If the goal is to just avoid hypoglycaemia, then overtreatment with carbohydrates may occur, which also increases the risk of higher insulin levels and obesity.(12)

4.1.2 What is the recommended dietary therapy in GSD I?

Dietary recommendations and treatment strategies for GSD I have improved significantly over the years.(9) The concepts of management are generally agreed upon, and focus primarily on the avoidance of fasting both day and night. Carbohydrate intake is calculated to supply glucose at approximately 8-10mg/kg/min in infants and 4-8 mg/kg/min in older children, which is considered adequate to prevent hypoglycaemia and suppress lacticacidaemia.(11) Energy distribution is recommended at approximately 10-15% from protein (P%cals) to provide the daily recommended intake, 25-30% from fat and 60-70%

carbohydrate.(2, 11) The provision of fuel is based on small frequent feeds/meals, which ideally contain slow-released carbohydrates, at regular intervals.(13) In infants, feeding frequency may be 2-3 hourly, although this usually extends with age. Overnight, a gastric drip feed of regular daytime formula is often required in infants. However, even in older children, the demands of regular feeding may still mean gastric drip feeding is required at night.(2)

Uncooked corn starch (UCCS) is a branching polymer with a high amylo: amylopectin ratio, which slowly releases glucose into the circulation under the action of pancreatic amylase.(14) UCCS is generally introduced around twelve months of age due to the reduced activity of pancreatic amylase in children less than two years of age. Patients require a slow introduction of UCCS to promote gastrointestinal tolerance and dosing is individual and variable but is generally between 1.6-2.5g/kg every 3-6 hours, depending on age.(11, 14) UCCS is used successfully during the daytime and at night-time particularly in older patients.(15) A systematic literature review and meta-analysis concluded that both intermittent UCCS and continuous glucose feeds could safely maintain blood glucose levels overnight (16), and thus practise should remain individualised and address what is most effective and convenient for the patient and family. More recently, a commercially available starch preparation that can prolong normoglycaemia has become available and may be used as a substitute for UCCS.(10, 11)

While the use of overnight UCCS (17) or glucose polymer is suggested (18), more nutritious ("complete") feeds that provide some benefit regarding protein and micronutrient intake could be used, although energy intake must be considered. However, the risk of technical problems with overnight gastric feeds has been highlighted and recognised as an impediment to the use of this treatment mode.

Recent attention has been directed toward the use of medium chain (MCT) fats to replace long chain (LCT) fats as a means of generating acetyl CoA to inhibit glycolysis and enhance ketone production.(19) This recommendation has not yet been endorsed in practice guidelines.(11) The dietary management for GSD Ia and Ib is essentially the same, however patients with GSD Ib may require further dietary manipulations secondary to enterocolitis, if it occurs.(11)

It should be noted that despite the accepted general concepts, there is controversy in clinical approach to dietary therapy in GSD I with regards to the minimisation or avoidance of foods containing fructose and lactose to reduce lacticacidaemia, as these sugars are not metabolised into the gluconeogenic pathway.(2, 9, 10)

4.1.3 What is the recommended dietary therapy in GSD III?

While the treatment goals are similar for GSD I and for GSD III there are some key differences in nutritional strategies. In principle, recommendations for GSD III describe a dietary regimen like that for GSD I regarding regular daytime carbohydrates, but UCCS doses required may be lower than in GSD I.(3) In younger children, night-time continuous feeds to prevent hypoglycaemia are likely to still be required, but this lessens with age.(3) In older patients and those with milder manifestations, a bedtime snack may be all that is needed to prevent night time hypoglycaemia, as fasting tolerance improves.(3)

As patients with GSD III have an intact gluconeogenic pathway from alanine, the importance of a higher protein intake to allow the conversion of protein derived alanine to glucose during fasting has been a key difference between dietary recommendations in GSD III compared with those for GSD I. Nevertheless, debate has existed for some time as to whether the

benefit from a high protein diet for prevention of hypoglycaemia and improved growth exceeds that from a continuous source of glucose.(1, 20)

A higher protein intake may provide multiple benefits in the overall management of GSD III, but its role may be under-appreciated.(8) Benefits have been shown to include a slowing in the progression of the associated (cardio)myopathy in those with muscle involvement,(20-23) improvement in liver function, enhanced muscle protein synthesis and improved muscle function (strength and endurance),(21) decreased endogenous proteolysis by skeletal muscle breakdown, decreased hypertriglyceridaemia,(1) and the prevention of excess deposits of glycogen in the tissues by less carbohydrate consumption.(3, 21)

Although the ideal distribution of energy from carbohydrates, protein and fats for children with GSD III is unclear, recommendations for adults are for protein to supply ~ 25% dietary energy (P%cals),(2, 21) as overtreatment with carbohydrates may cause some additional harm including obesity.(8) Thus, treatment with a low-carbohydrate-high-protein diet is accepted practice for adults with GSD III.(3) This strategy has also shown benefit in children with the use of a high protein, high fat, low carbohydrate (Modified Atkins) diet, particularly in regard to skeletal and heart muscle function.(24) Given that the onset of myopathy in GSD IIIa, which involves both liver and muscle, can occur at an early age, it is recommended that a higher protein diet should not be delayed at the expense of providing carbohydrates. Additionally, for some patients with GSD III, the longer-term muscular and cardiac complications and the crucial role for mitochondrial fatty acid oxidation in these organs suggest dietary fat increase (and thereby ketone bodies) should be further investigated.(8)

4.1.4 What are the risks associated with dietary therapy in GSD?

When designing, and managing the diet for patients with GSD I or III, it is critical to focus on the quality of the whole diet rather than a sole focus carbohydrate intake and distribution.

Potential nutritional complications arising in GSD I and GSD III may include: excessive or deficient energy intake that could result in poor growth or obesity, hyperlipidaemia, hyperlactataemia, essential fatty acid and micronutrient deficiencies including vitamin D,(3, 10, 11) and vitamin B_{12}.(25) Overtreatment with carbohydrates may also be harmful and may result in poorer dietary intake due to an indirect reduction in protein intake.(3, 8) A carbohydrate intake that exceeds that needed to assure normo-glycaemia, may lead to increased glycogen deposition and overtime contribute to growth retardation or increased weight for height.(26)

Regardless of what constitutes the best nutritional therapy, it seems likely that compliance with this demanding, rigorous dietary and monitoring regimen is difficult long-term, and may cause altered feeding behaviours and disrupted eating patterns, although these are not well studied.(10)

4.1.5 What do we know about nutritional outcomes in GSD?

There is a recognized growth pattern in children with GSD I that includes short stature with delayed puberty and a tendency to obesity.(27) While there are fewer reports of nutritional outcome in GSD III, growth failure was also reported for some patients,(20, 28) and published guidelines outline the need for serial measurement of growth and development.(3) The problem of poor growth in GSD is likely to be multifactorial, although underlying mechanisms are not completely understood. As well as growth abnormalities, truncal obesity,

muscle wasting and thin limbs, osteopenia and low bone mineral density(3) may be present longer term in poorly controlled patients.(26)

Whilst it is accepted that intensive dietary therapy will improve clinical outcomes in GSD I, it is also recognized that it will not completely correct clinical and biochemical status.(9) However, when hypoglycaemia is prevented by providing adequate glucose throughout the day and night, the biochemical abnormalities diminish and growth improves.(17, 29, 30) Patients with the best metabolic control appear less susceptible to complications.(9, 17, 18, 31)

4.1.6 What are the aims of the current study?

The aim of this study was to contribute to our understanding of longitudinal growth and dietary patterns in children with GSD I and GSD III. More specifically we aimed to answer the following questions from retrospective longitudinal data:

1. What are the longitudinal patterns of weight, height and BMI in children with GSD?
2. Is there a difference in growth and dietary intake between children with GSD I and GSD III?
3. Is total protein intake and energy intake adequate in children with GSD I and III?
4. What is the P:E ratio of the diets of children with GSD I and III? Can it serve as an additional tool to evaluate the adequacy of the diet in these children?

A limitation of a retrospective study is the lack of complete data with which to draw firm conclusions. Therefore, we mainly restricted the current study to a descriptive observation of longitudinal anthropometric parameters and dietary intake patterns.

We also conducted a small prospective longitudinal study in four patients with GSD I with the aim to document changes in body composition with an adjustment in dietary protein intake in these patients.

4.2 Methods

This study was approved by the RCH Human Research Ethic Committee: VICIEM HREC # 30066B. We collected retrospective longitudinal data on dietary intake and growth parameters of all patients diagnosed with GSD I and GSD III and treated in our metabolic specialist clinic between July 1995 and March 2017. Data were analysed in patients with GSD I (n=11; 8 males, 3 females), and patients with GSD III (n=8; 2 males, 6 females). Data on weight and height were collected from medical and dietetic clinic records when patients were well. Regular clinic visits occurred at least every 3-4 monthly after diagnosis in infants and younger children and at 6 monthly intervals thereafter. Body Mass Index (BMI) was calculated using the equation weight (kg)/ height (m^2). All anthropometric measurements were expressed as age and gender-specific z-scores, by entering weight and height data into the epidemiological software package Epi Info (version 3.5.1), based on the Centres for Disease Control and Prevention (Atlanta, GA) 2002 reference database. Criteria to determine overweight were based on the CDC Growth Charts (2000).(32)

Dietary data were collected from dietary and medical records and consisted of parent or patient report, 24-hour recall, dietary history and 3-day food diaries. Dietary data represented actual intake rather than prescribed intake. Dietary intake of protein in g/kg/d was compared with the FAO/WHO/UNU recommended safe levels (33). Protein intake was also compared to specific recommendations for GSD I and GSD III.

Energy intake was expressed as a percentage of basal metabolic rate (E%BMR) calculated for each patient using the BMR predictive equations of Schofield (34). We estimated a physical

activity level (PAL) of 1.6, which equates to an energy intake of ~160% of BMR, to be the upper level of intake likely to be required for this group of children. This energy intake is similar to that determined by WHO for children up to 11 years of age who engage in a light physical activity level (35).

4.2.1 Body composition

Four patients with GSD I participated in a larger prospective study. This study was approved by the RCH Human Research Ethic Committee (HREC: 32056A). Written consent was provided by parents for the additional measure of body composition to be completed at routine clinic visits. Body composition was measured by Bioelectrical Impedance Analysis (BIA) using the QuadScan 400, Bodystat® (Isle of White LTD) as per the manufacturer's instructions. Participants were instructed to fast for at least 90 minutes and to not exercise prior to the BIA assessment. Percent fat-free mass (%FFM) and percent fat mass (%fatmass) were estimated using raw impedance values using the equation of Houtkooper.(36) Data on body composition and dietary intake in these patients is reported here.

4.2.2 Statistical analysis

Statistical analyses were performed using SPSS for Windows software version 23 (IBM, Illinois, Chicago, IL). Continuous variables including z-scores for weight, height and BMI, protein (g/kg/d) and energy intake (%BMR) and P:E ratio (g protein/100kcal/d) are presented as median and range for the groups. Weight- height- and BMI z-scores were analysed for each child from time of diagnosis until last documented episode (treatment period), and were presented as the mean for the individual. Statistical comparisons were not made between GSD I and GSD III patients due to low patient numbers and data is presented descriptively.

4.3 Results

4.3.1 Age at diagnosis

GSD I: 3/11 patients were diagnosed at < 1 month of age, 1/11 patient was diagnosed between 1 and 6 months of age, 3/11 were diagnosed between 6 and 12 months of age and 4 /11 were diagnosed > 12 months of age. Median age at diagnosis was 0.6 years of age (0.01 – 3.3 years) (Table 1, see page 110).

GSD III: 2/8 patients were diagnosed between 1 and 6 months of age and 6/8 patients were diagnosed > 12 months of age. Median age at diagnosis was 2.1 years of age (0.6 – 7.8 years) (Table 1, see page 110).

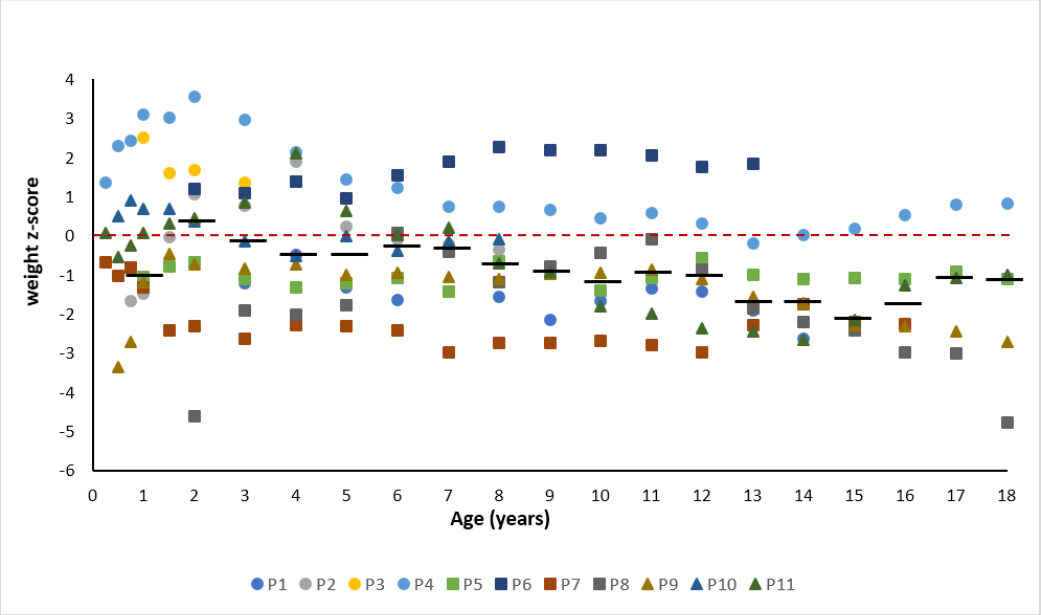
4.3.2 Growth patterns

A total of 18/19 patients were assessed. One patient, recently diagnosed with GSD III, was not included for longitudinal growth assessment. Length of treatment ranged from 1 year to 17.75yrs (median 11 years). Of these patients 9/18, all with GSD I, had overnight continuous feeding (nasogastric or gastrostomy). For 2/18 patients, both females, one with GSD Ia and one with GSD III, medical induction of puberty commenced prior to the last measurement.

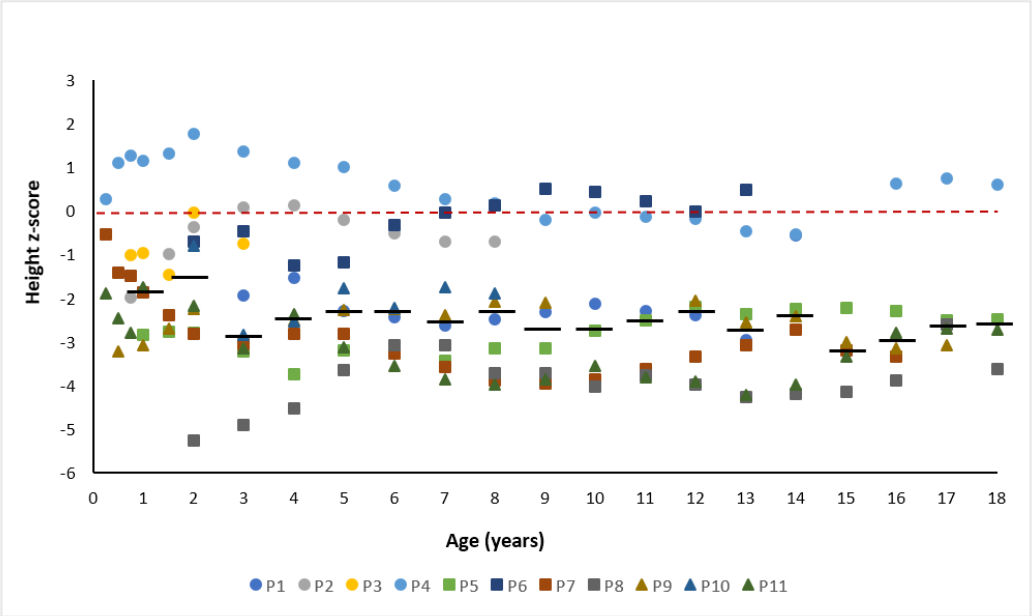
GSD I: In all patients, individual growth trajectory was assessed from diagnosis until the last measurement (Figure 1). At diagnosis 10/11 patients had a height z-score <0, median -1.89 (-5.25 – 0.27). At the last measurement taken, 9/11 patients still had a height z-score < 0, median -1.89 (-3.62 – 0.61). In 3/11 patient's height z-score worsened over treatment (n=2 with GSD Ib, n=1 with GSD Ia) (Figure 2). All three of these patients received a liver transplant after the last included measurement.

Figure 1 depicts the longitudinal growth patterns in (a) weight z-score, (b) height z-score (c) BMI z-score for individual patients with GSD I from diagnosis to last assessment. Median z-score for the group is shown by the black bar. Patients 1-7 inclusive have GSD 1a and patients 8-11 inclusive have GSD 1b. Median weight z -score for the whole group remained <0 after 3 years of age (Figure 1a). Median height z-score was < 0 at all ages (Figure 1b) and median BMI z-score was > 0 for all ages except 14 and 15 years of age (Figure 1c). The overall change in height z-score is shown in Figure 2.

1a)



1b)


Height z-score
3
2
1
0
-1
-2
-3
-4
-5
-6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11

1c)

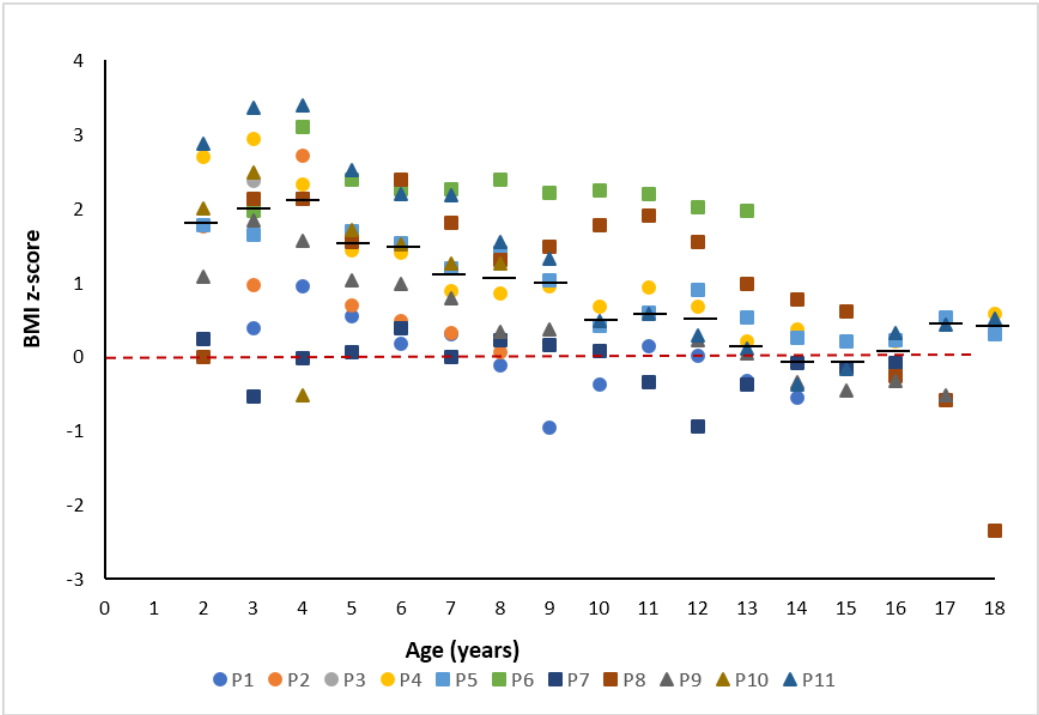
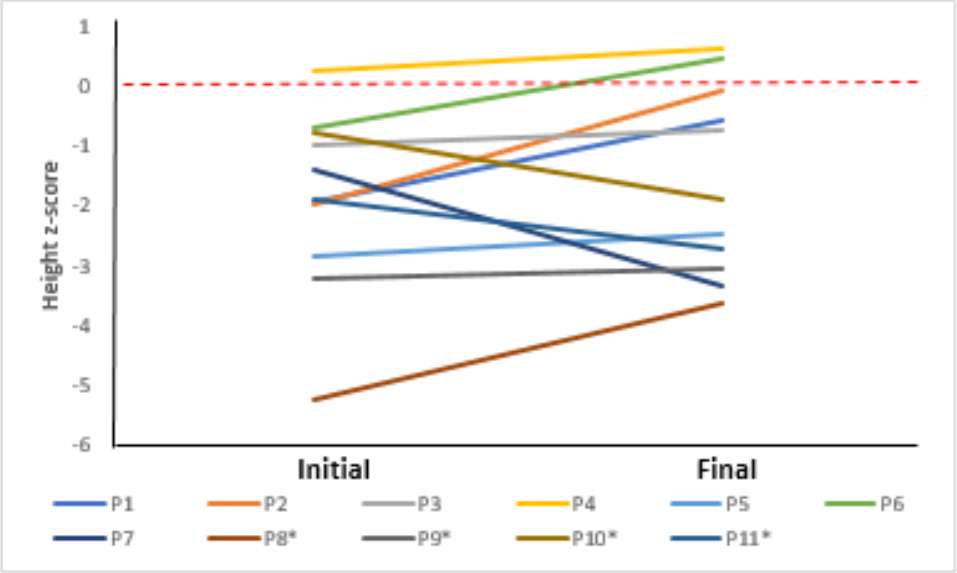

BMI z-score
4
3
2
1
0
-1
-2
-3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11

Figure 2: GSD I: Overall change in height z-score over treatment.

* indicates patients with GSD1b



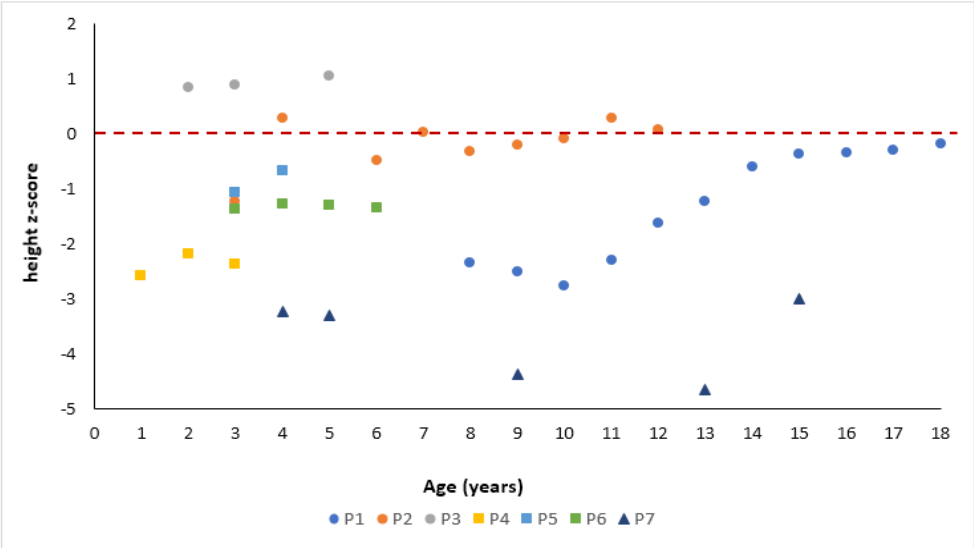
Mean z-scores for data points collected over the patient's treatment period from diagnosis until the latest data collection episode for weight, height and BMI, were categorised by z-scores: <-2, -2 to <-1, -1 to 1, >1 to 2 and > 2. The results are shown in Table 2. Mean weight-, height-, and BMI z-score was 0(±1) in 3/11, 4/11 and 5/11 of patients, respectively. Height growth was more affected than weight, with 6/11 of patients with a mean height z-score < -2.0. Poor height growth is likely to contribute more to the high weight-for-height ratio observed, with 4/11 of patients meeting criteria for overweight (BMI z-score >1-2) and 2/11 of patients meeting criteria for obesity (BMI z-score >2).

Table 2: Anthropometric outcomes for GSD I and GSD III patients

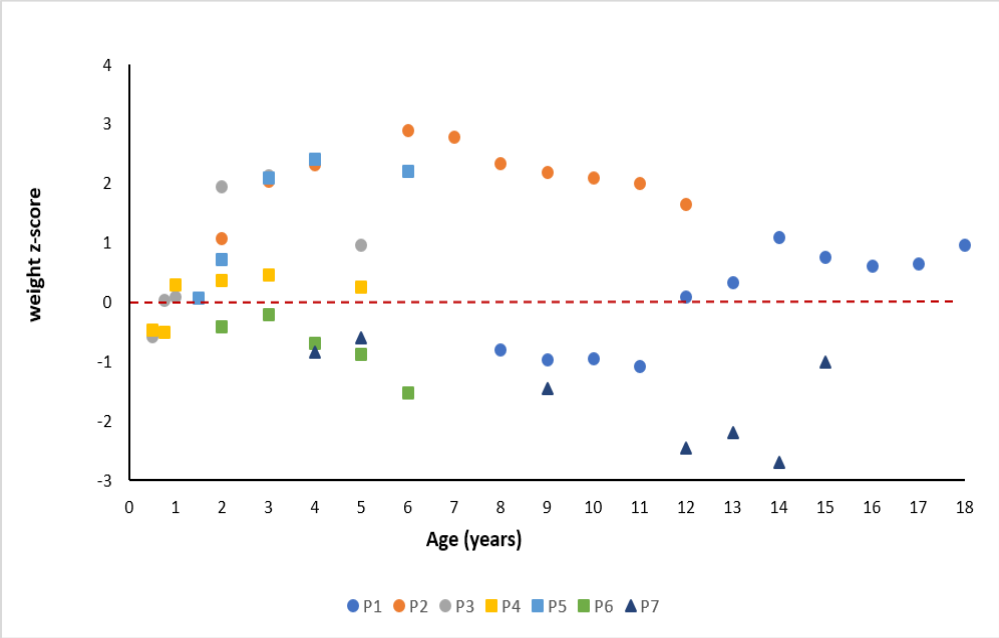
		Mean z-score over treatment period				
Disorder	**Growth parameter**	**<-2**	**-2 to <-1**	**-1 to 1**	**>1 to 2**	**>2**
GSD I	Weight: *z*-score	1/11	4/11	3/11	3/11	
n=11	Height: *z*-score	6/11	1/11	4/11		
	BMI: *z*-score			5/11	4/11	2/11
GSD III	Weight: *z*-score	1/8	1/8	4/8	1/8	1/8
n=8	Height: *z*-score	3/8	2/8	3/8		
	BMI: *z*-score			2/7	1/7	4/7

GSD III: Individual growth trajectories in 7/8 patients were assessed from diagnosis until the last measurement. Figure 3 depicts longitudinal (a) weight z-score (b) height z-score (c) BMI z-score for individual patients with GSD III from diagnosis until the last assessment. At diagnosis 7/8 patients had a height z-score <0, median -1.86 (-3.22 – 0.85). At the last measurement 6/8 patients still had a height z-score < 0, median -1.00 (-2.99 – 1.05). Due to small patient numbers, the median for the group has not been calculated for each year of age. The overall change in height z-score is shown in Figure 4. Height z-score improved in all patients after treatment except one recently diagnosed infant who has continued tracking (Figure 4).

3a)


height z-score
2
1
0
-1
-2
-3
-4
-5
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)
P1 P2 P3 P4 P5 P6 P7

3b)


weight z-score
4
3
2
1
0
-1
-2
-3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)
P1 P2 P3 P4 P5 P6 P7

3c)

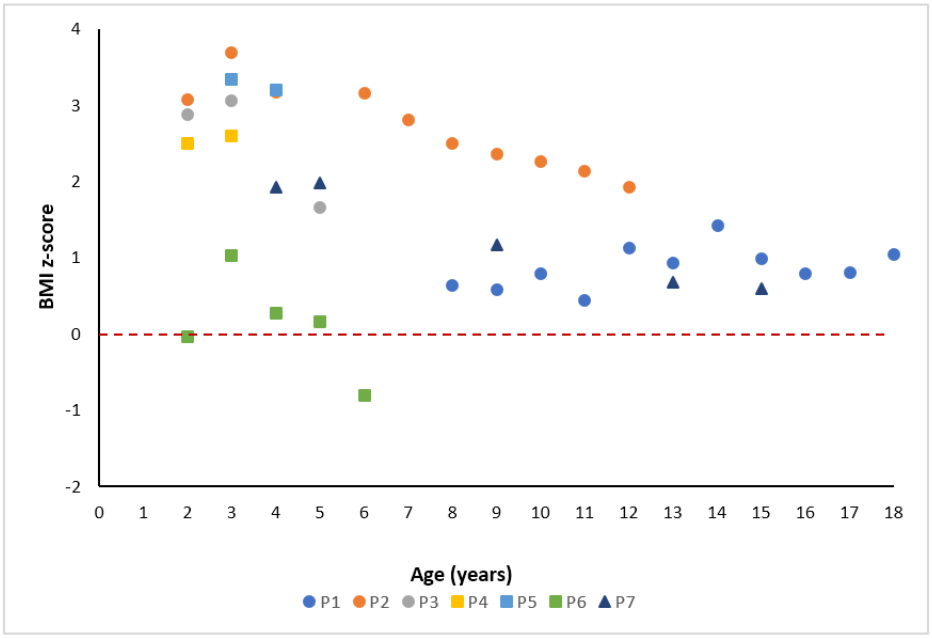
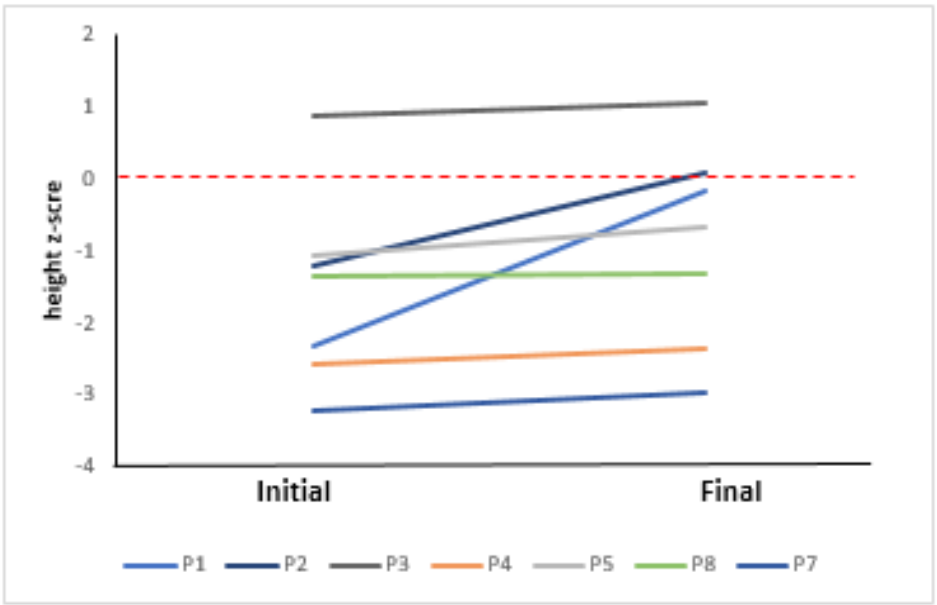


Figure 4: GSD III: Overall change in height z-score over treatment



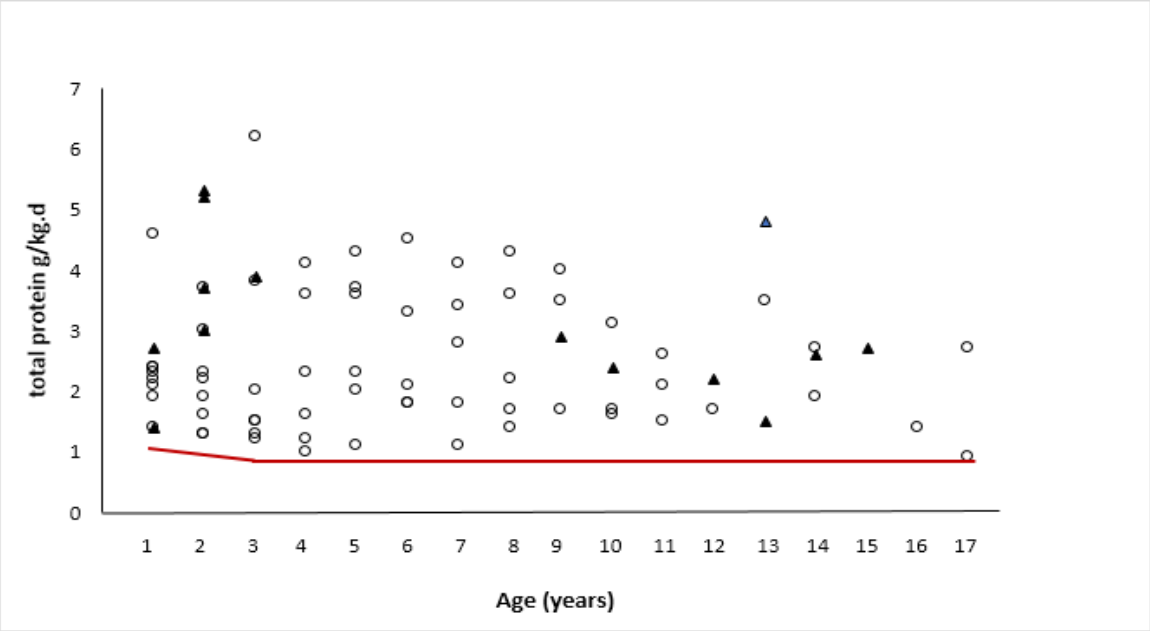
Patients with GSD III had better growth compared to GSD I. When data were analysed for the whole treatment period, mean weight-, height- and BMI z-scores were 0(±1) in 4/8, 3/8 and 2/7 of patients, respectively. Longitudinal height growth was still significantly affected with 2/8 having a height z-score between -2 and <-1 and 3/8 with a height z-score <-2. A greater proportion of patients, 4/7, met the criteria for obesity, however, interpretation is limited due to small patient numbers (Table 1).

4.3.3 What was the protein intake of our patients?

There was a total of 80 data points of protein intake (g/kg/d). Data points from 12 months of age and above were included for analysis for patients with GSD I and GSD III.

Figure 5 depicts the range of protein intake for the GSD I and GSD III patients from the first year of age until 17 years of age. Total protein intake exceeded the FAO/WHO/UNU recommended safe levels in all patients at all ages. From 12 months of age the approximate protein intake recommendation is 1g/kg/d.

Figure 5: protein intake in g/kg/d. The solid red line represents the WHO/FAO/UNU safe protein intake for age. GSD III patients are represented by the symbol▲.



When compared to FAO/WHO/UNU recommendations:

In GSD I patients, 15/64 data points indicated protein intake were between 1.0 and 1.5g/kg (i.e. 100 -150% requirement), 30/64 data points were between 1.5 and ≤ 3g/kg/d (i.e. ~150-300% requirement), and 19/64 data points for > 3.0g/kg/d (i.e. >300% requirement).

In GSD III patients, 2/16 data points were between 1.0 and 1.5g/kg, 7/16 data points were between 1.5 and ≤ 3g/kg/d and 7/16 data points were > 3.0g/kg/d.

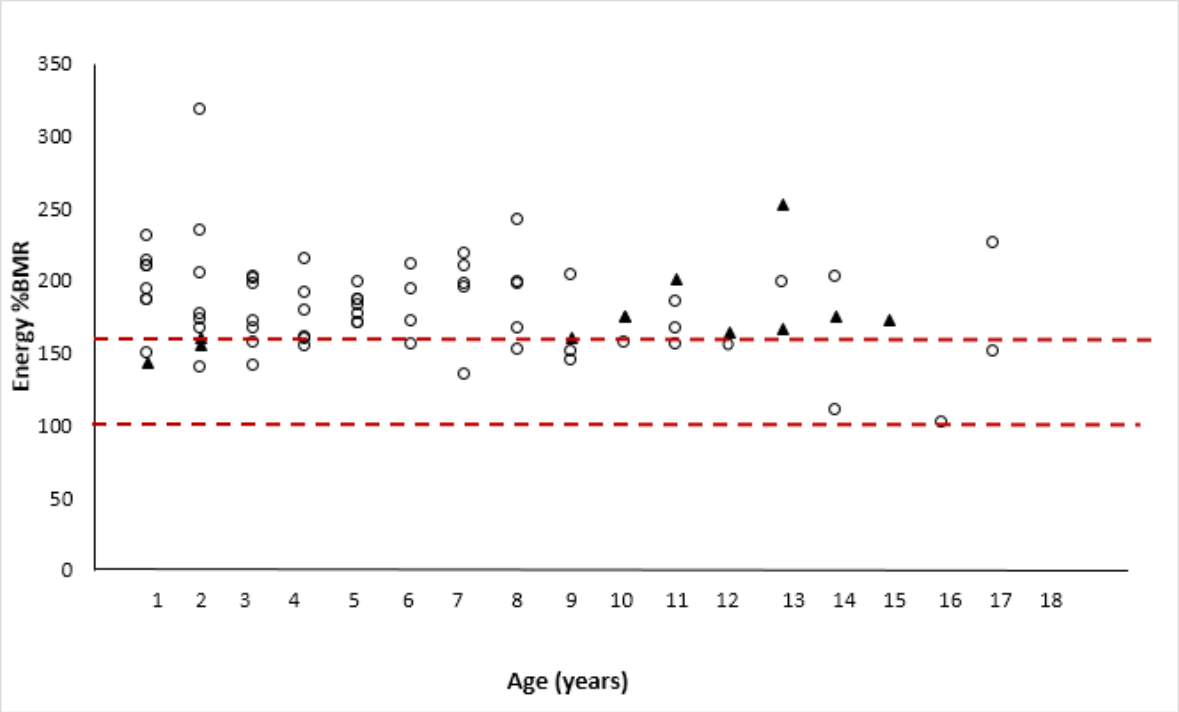
4.3.4 What was the energy intake of our patients?

There was a total of 75 data points of energy intake (Energy %BMR). Data points from 12 months of age and above were included for analysis for patients with GSD I and GSD III.

Figure 6 depicts the range of energy intakes expressed as a percentage of BMR for the GSD I and GSD III patients from 1 year of age until 17 years of age. An intake associated with 100% of BMR and 160% of BMR is indicated by the red dashed lines.

In GSD I, 16/64 individual data points for energy intake were between 100 – 160 %BMR, and in GSD III 4/11 individual data points for energy intake were within that range. All other data points were above 160%.

Figure 6: Energy intake expressed as a percentage of basal metabolic rate (%BMR). GSD III patients are indicated by the symbol ▲.



4.3.5 What was the P:E ratio of the diets in our patients?

There was a total of 63 data points of P:E ratio available for analysis for GSD I and GSD III patients.

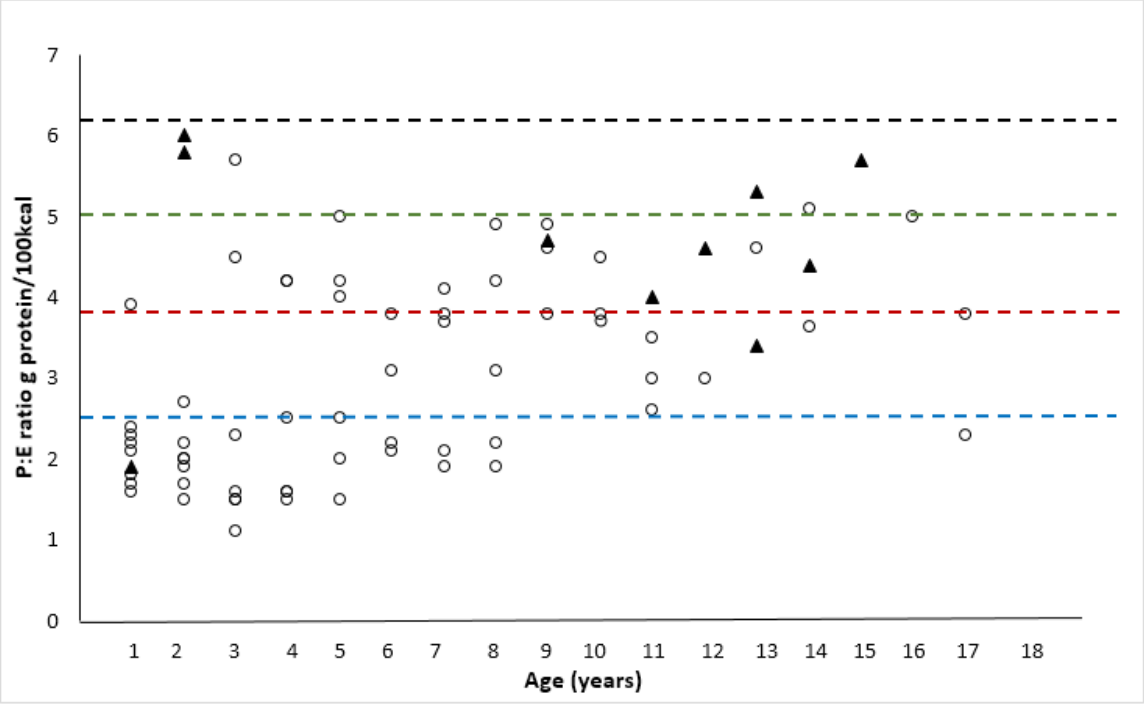
Figure 7 depicts the range of P:E ratio (g protein/100kcal) for the GSD I and GSD III patients from 12 months of age until 17 years of age. GSD I recommendations of intake of between 10 to 15 P%cals is equivalent to a P:E ratio of between 2. 5 and 3.8 g protein/100kcal and is indicated by the blue and red dashed lines, respectively; GSD III recommendations of

between 20 to 25 P%cals is equivalent to a P:E ratio between 5 and 6.3 g protein/100kcal and is indicated by the green and the black dashed lines, respectively.

In GSD I, 30/63 individual data points indicated the diet consumed consisted of a P:E ratio < 2.5 g protein/100kcal, and 16/63 individual data points indicated the diet consumed consisted of a P:E ratio between 2. 5 and 3.8 g protein/100kcal, and 2/63 data points indicated the diet consisted of a P:E ratio was >5.0 g protein/100kcal.

In GSD III, 1/11 data points indicated the diet consumed consisted of a P:E ratio < 3.8 g protein/100kcal, and 4/11 data points indicated the diet consumed consisted of a P:E ratio >5.0 g protein/100kcal.

Figure 7: Protein to energy ratio of the diet (g protein/100kcal). GSD III patients are represented by the symbol▲.



4.3.6 What is the body composition of our patients?

The results of the prospective study of serial measurements of body composition (%body fat) and dietary intake assessed by 3-day food diaries assessed in 4 patients with GSD I are shown in Table 3.

Table 3: Body composition and dietary intake in GSD I

	Patient 1: DV	Patient 2: GM	Patient : LJ	Patient 4: RT
Type of GSD	GSD Ia	GSD Ib	GSD Ia	GSD Ia
Measurement 1				
Age (years)	3.9	6.1	9.6	13.1
%body fat	**32.3**	**27.7**	**35.7**	**14.4**
BMI z-score	2.7	1.5	2.3	-0.4
Protein intake(g/kg)	1.2	1.8	1.7	3.5
P:E ratio	**1.6**	**2.2**	**3.8**	**4.6**
Measurement 2				
Age (years)	4.7	6.7	10.6	
%body fat	**30.7**	**28.6**	**35.4**	
BMI z-score	1.3	1.3	2.1	
Protein intake(g/kg)	2.7	4.1	1.4	
P:E ratio	**4.0**	**4.0**	**4.0**	
Measurement 3				
Age (years)	5.3	7.8	**11.1**	
%body fat	**28.1**	**29.9**	**35.7**	
BMI z-score	0.7	1.2	2.2	
Protein intake(g/kg)	3.7	4.3	1.5	
P:E ratio	**4.2**	**4.2**	**3.4**	

In patient 1, %body fat and BMI z-score decreased over time, as protein intake (g/kg/d) increased and P:E ratio increased from 1.6 to 4.2 (g protein/100kcal). The estimated percentage of energy from protein increased accordingly from 6.4% to 16% and to 16.8%.

In patient 2, %body fat remained constant over time but BMI z-score decreased as protein intake (g/kg/d) and P:E ratio increased from 2.2 to 4.2 (g protein/100kcal). The estimated percentage of energy from protein increased from 8.8% to 16% and to 16.8%

In patient 3, %body fat and BMI z-score remained constant, as diet protein intake (g/kg/d) and P:E ratio also remained constant. The estimated percentage of energy from protein was 15.2%, 16% and 13.6%.

In patient 4, only one measurement was taken, but %body fat was normal (37) and BMI z-score was normal on high protein intake (g/kg/d) and high P:E ratio of 4.6 (g protein/100kcal) that represented ~ 18.4% energy as protein.

Table 1: Anthropometric parameters at diagnosis and over treatment period for individual patients

GSD I (GSD Ib*)										
ID (Gender)	**Overnight Feeds/UCCS**	**Age Diagnosis (years)**	**Age last height measure (years)**	**Weight z-score diagnosis**	**Weight z-score last measure**	**Height z-score diagnosis**	**Height z-score last measure**	**Treatment period: Weight z-score Mean (range)**	**Treatment period: Height z-score Mean (range)**	**Treatment period: BMI z-score Mean (range)**
1 (F)	UCCS	3	14	-1.22	-2.64	-1.94	-0.56	-1.57 (-2.64 - -0.5)	-2.16 (-2.95 - -0.56)	0.02 (-0.96 - 0.95)
2 (M)	Feeds	0.6	8	-1.67	-0.35	-1.98	-0.70	0.01 (-1.67-1.06)	-0.58 (-1.98 – 0.13)	1.00 (0.06 - 2.71)
3 (F)	Feeds	.8	2.8	0.85	1.36	-1.0	-0.74	1.60 (0.85-2.5)	-0.84 (-1.0 - -0.04)	2.37 (2.37)
4 (M)	Feed/UCCS	Birth	18	1.35	0.81	0.27	0.61	1.32 (-0.02-3.55)	0.56 (-0.53 – 1.77)	1.10 (0.20 - 2.93)
5 (M)	Feed/UCCS	1.1	18	-1.06	-1.1	-2.83	-2.47	-1.03 (-1.42- -.057)	-2.79 (-3.73 - -2.19)	0.96 (0.21 - 2.13)
6 (M)	Feeds	2.1	9.9	1.2	1.83	-0.69	0.48	1.70 (0.95-2.28)	-0.18 (-1.25 – 0.51)	2.27 (1.96 - 3.10)
7 (M)	UCCS	Birth	15.8	-0.69	-2.27	-1.41	-3.34	-2.18 (-2.99- -0.69)	-2.85 (-3.94 - -1.41)	-0.09 (-0.94 - 0.39)
8* (M)	Feeds	3.3	18	-4.63	-4.78	-5.25	-3.62	-1.84 (-4.78-0.06)	-3.90 (-5.25 - -2.59)	1.01 (-2.35 - 2.38)
9* (M)	Feeds	.6	18	-3.35	-2.72	-3.22	-3.06	-1.48 (-3.35- -0.46)	-2.59 (-3.22 - -2.05)	0.47 (-0.52 - 1.84)
10* (M)	Feeds	0.2	7.8	0.51	-0.08	-0.79	-1.88	0.17 (-0.52-0.89)	-1.96 (-2.84 - -0.79)	1.38 (-0.52 - 2.48)
11* (F)	Feeds	Birth	18.1	0.08	-0.99	-1.89	-2.72	-0.66 (-2.67-2.1)	-3.14 (-4.22 - -1.74)	1.27 (-0.37 - 3.39)
GSD III										
ID (Gender)	**Overnight Feeds/UCCS**	**Age Diagnosis (years)**	**Age last height Measure (years)**	**Weight z-score diagnosis**	**Weight z-score last measure**	**Height z-score diagnosis**	**Height z-score last measure**	**Treatment period: Weight z-score Mean (range)**	**Treatment period: Height z-score Mean (range)**	**Treatment period: BMI z-score (range)**
1 (F)	UCCS	7.8	18	-0.81	0.96	-2.35	-0.17	0.06 (-1.09-0.96)	-1.32 (-2.75 - -0.17)	0.86 (0.44 - 1.41)
2 (M)	UCCS	2.7	11.9	1.07	1.64	-1.22	0.08	2.13 (1.07-2.89)	-0.18 (-1.22 -0.29)	2.70 (1.92 - 3.68)
3 (F)	Feed/UCCS	0.6	4.7	-0.59	0.96	0.85	1.05	0.76 (-0.59-2.13)	0.93 (0.85 – 1.05)	2.53 (1.65 - 3.06)
4 (F)	Feed/UCCS	0.6	4.7	-0.47	0.26	-2.57	-2.36	0.07 (-0.5-0.46)	-2.37 (-2.57 - -2.19)	2.55 (2.50 - 2.60)
5 (F)	Feed/UCCS	2	4	0.06	2.2	-1.07	-0.67	1.49 (0.06-2.4)	-0.87 (-1.07 - -0.67)	3.27 (3.20 - 3.33)
6 (F)	Feeds	0.9	1.2	-2.54	-2.54	-2.39	-2.39	-2.54 (2.54)	-2.39	
7 (F)	UCCS	2.9	15.8	-0.85	-1	-3.22	-2.99	-1.60 (-2.69- -0.6)	-3.70 (-4.65 - -2.99)	1.27 (0.59 - 1.98)
8 (M)	Feeds	2.2	6	-0.21	-1.52	-1.37	-1.33	-0.75 (-1.52- -0.21)	-1.32 (-1.37 - -1.27)	0.12 (-0.81 - 1.02)

4.4 Discussion

Abnormal growth in children with GSD, which includes short stature, delayed puberty and a tendency to obesity(27), has been documented even with early treatment and follow up.(17, 28, 30) In a large outcome study, 50% of adult patients had a height z-score < -2.0 .(38) The prevalence of adult stunting has been shown to be more obvious in GSD Ib than GSD Ia patients.(39) Delayed puberty was also observed, with adult height reached at a median of 21 years in males and 20 years in females.(9) A more recent study of outcomes in GSD Ia, suggests that with improved treatments over time, height growth can become near normal.(31) Additionally, a follow up study of patients with GSD III suggests that growth retardation is severe in the early childhood years, but patients may eventually reach a normal adult height.(28)

Poor linear growth is likely to be multifactorial and its underlying mechanisms are not completely understood, but alterations in hormonal patterns are likely to contribute. A previous study has shown that patients who had normal pubertal- or bone development showed less stunted adult height than those with delayed puberty.(9) When hormonal factors were investigated to explain potential mechanisms to account for a wide range in height outcomes, patients with higher BMI had the lowest serum growth hormone (GH) level but normal insulin like growth factor (IGF-1).(40) Additionally, patients with the poorest growth had lower insulin response to glucose load, GH insensitivity and a higher mean plasma cortisol level.(40) The authors concluded that the disturbance of the GH-IGF-1 axis is responsible for the poor growth of those individuals with the lowest height z-score, but also that the endocrine status of some treated patients is like that expected in untreated patients. Consequently, some ambiguity remains as to whether growth can be significantly improved in GSD I, and if this would be achieved through either improved treatment or

adherence to current treatment.(40) Patients with GSD Ib also have been shown to have lower serum IGF-1 and an impaired growth hormone secretion.(39)

The primary focus of dietary intervention in GSD is to determine a regimen most likely to result in optimal metabolic control. Although this is critical for longer term health, the dietary rigour that is necessary to maintain normoglycemia may detract from the attention required to provide a nutritionally adequate total dietary intake.(11) Indeed, the risk to overall deficiency in micro- and macro- nutrient intake have been acknowledged.(10) It is possible that the dietary therapies necessary to achieve this control may conflict with requirements to maximise long term growth and physical development in this unique group. In this respect, it is important to note the debate regarding the use of overnight drip feeding (glucose polymer or complete enteral feeds) versus UCCS therapy, which has the potential to significantly influence dietary quality. In a review of adult patients with GSD Ia who had received long term corn-starch therapy, both during the day and night time, the mean height z-score was still low at -1.2 ± 1.3, significantly less than the mean target height, and patients had increased weight-for-height ratio with mean BMI of 0.7 ± 1.0.(17)

In summary, it is possible that current dietary practices contribute to the abnormal growth patterns observed in patients with GSD, although these have not been extensively investigated. It is also possible that as this study includes several sibling pairs, that the influence of genetic growth potential may have influenced results, however this has not been measured or controlled for in the analysis.

Herein we aimed to further explore nutritional factors that may contribute to the growth patterns in these patients and that are amenable to dietary manipulation, particularly regarding protein intake.

4.4.1 What are the longitudinal patterns of weight and height gains and BMI in children with GSD?

Our results are in line with previous reports with the most notable finding being a reduced height z-score across the lifespan for some patients.(17, 28, 30) While the pattern of growth abnormalities was similar in GSD I and GSD III, the most obvious growth deficits were documented in patients with GSD I, particularly Ib. As weight z-scores were not as low as height z-scores, this resulted in an increased prevalence of overweight and obesity.

As previously reported, after a period of treatment improved linear growth was noted in most of our patients.(18) This 'catch-up' growth has been reported in patients with over one year of good metabolic control, including adequate blood glucose and lactate levels.(18) In our group, two of the three patients who did not show improvement in height growth on treatment had GSD Ib.

4.4.2 Are total protein intake and energy intake adequate?

When compared to WHO/FAO/UNU recommendations, our results suggest that total protein intake expressed as g/kg/d was adequate in all patients and met or exceeded the recommended safe levels.(33)

Our data also suggest that GSD I patients had a lower number of episodes of dietary intake at the highest intake (> 300%) of the FAO/WHO/UNU protein recommendations (> 3g/kg/d) compared to GSD III patients. Overall, protein intake in our GSD III patients was between 1.4 and 5.2 g/kg/d, similar to that recently reported in a large multicentre follow up study.(28)

When protein intake was expressed as a percentage of energy intake (P%cals), for comparison with the GSD specific recommendations, many patients fell short of achieving these recommendations, particularly the youngest children and those with GSD I.

Protein recommendations in GSD I are not well described beyond meeting an intake comparable to the 'healthy population' of 10-15 P%cals.(2, 11) Protein recommendations for a high protein diet in GSD III are based on the provision of glucose via gluconeogenesis rather than the benefits to dietary intake and growth and body composition.(8) While evidence suggests a higher protein intake has benefit in GSD III,(3, 8) the actual protein intake of 20 to 25 P%cals recommendation is largely based on case reports (21-24, 41) and not on a systematic study. More importantly, if protein recommendations are to be applied in this way, then it is necessary for patients to consume adequate but not deficient or excessive energy intake. When compared to GSD specific recommendations, approximately one half to two third of episodes of dietary protein intake for GSD I, and two thirds of episodes of dietary intake for GSD III patients were below recommendations.

We confirmed that energy intake is adequate in our patients, and that some patients consume an energy intake above their estimated average requirement, similar to another report.(10) There is a lack of information about energy requirement in GSD. In an early study of resting energy expenditure (REE) in 7 adults with GSD Ia, measured REE was higher in the GSD patients than predicted, when compared to healthy controls.(27) The authors hypothesised that for patients with nephro-hepatomegaly, this observation may be due to organ specific increased REE. Of note, approximately three quarters of our patients were consuming an energy intake greater than 160% BMR. This equates to an intake above a physical activity level of 1.6, and thus would be considered excessive in individuals who did not participate in high levels of physical activity.(42) This energy intake is likely to be disproportionately high in a group of short statured children. However, estimation of BMR assumes normal body composition and the abnormal growth pattern observed in GSD may mean this assessment is even less accurate. Therefore, these data preclude the possibility that low energy intake is a contributing factor to poor height growth in our patients and suggests that promoting a high-

energy intake to improve growth is likely to be ineffective and result in further overweight or obesity.

4.4.3 What is the P:E ratio of the diets of children with GSD I and III?

A high total energy intake is common in patients with GSD, particularly from carbohydrate, which is the consequence of the need to maintain normoglycaemia. This is particularly true for GSD I. It is therefore possible that the excessive energy intake of these patients dilutes the protein in the diet when expressed P%cals, as has been documented previously.(10) This is also shown by the low P:E ratio, an alternative way to express P%cals, calculated for our patients. Overall, a greater proportion of our patients with GSD I had a documented episode of a lower P:E ratio than patients with GSD III.

We have previously shown that poorer growth resulted in patients on a ketogenic diet when the P:E ratio of the diet was < 1.5 g/protein/100kcal (< 6 P%cals).(43) We have also shown that patients with MMA/PA/IVA/UCD have the best growth and body composition outcomes when the P:E ratio of the diet is >1.5 - < 2.9 (6 – 11.6 P%cals) (Chapter 3). In our group only six episodes of dietary intake in patients with GSD I had a P:E ratio ≤ 1.5 g protein/100kcal.

GSD I specific guidelines for protein intake as 10 to 15 P%cals would suggest that the 'target' P:E ratio of the diet would be 2.5 to 3.8 g protein/100kcal. For GSD III specific guidelines of 20 to 25 P%cals, the 'target' P:E ratio would be 5 to 6.3 g protein/100kcal. In our group, approximately one half of dietary episodes for patients with GSD I indicated a P:E ratio < 2.5 g protein/100kcal, and two thirds were < 3.8g protein /100kcal. For our GSD III patients, only one third of dietary episodes indicated a P:E ratio > 5.0 g protein/100kcal.

Our preliminary observation that body composition and BMI z-score improved in some children with GSD I on a higher protein intake and a higher P:E ratio above this 'target" P:E

ratio supports the importance of a higher P:E ratio to improve nutritional status. It also provides an opportunity to extend our thinking about an 'ideal' protein intake to inform recommendations for patients with GSD I, although this needs confirmation with larger patient numbers. Interestingly, of the four patients studied, the child with GSD Ib showed an improvement in BMI z-score, without a similar benefit in body fat levels, when dietary protein intake and P:E ratio increased. This patient also had a decrease in height z-score over time, despite exceeding WHO/FAO/UNU protein recommendations, having UCCS treatment and feeding schedule in line with GSD I recommendations day and night. This may support the premise, as discussed previously, that patients with GSD Ib have a different growth pattern, and may require different or more intensive dietary therapy and long-term monitoring.

It is possible that an improvement in P:E ratio could be achieved by either reducing energy intake to better meet estimated energy needs, or increasing protein intake. In the context of the controversy regarding night time feeding, providing more nutritious ("complete") feeds overnight, rather than just glucose polymer or UCCS could improve growth parameters and body composition.

4.5 Limitations

Limitations of this study are that it was mostly retrospective and includes only a small number of patients and corresponding a small dietary data set. We had insufficient data to make comparisons between GSD I and III, or to do extensive statistical analyses, thus firm conclusions could not be made. As data were collected during regular clinic reviews, metabolic control was assumed, but this has not been consistently documented. Dietary changes for some children may occur between clinic visits, particularly for unstable or younger children, and these are not accounted for in the methodology employed.

Currently we do not have adequate data to more closely or conclusively examine the relationship between total protein intake and P:E ratio and growth parameters in GSD, and this would be important to determine in future studies.

4.6 Conclusions

Our study provides some important observations that deserve consideration regarding dietary factors and their potential effect on growth in GSD. However, due to small patient numbers and the amount of dietary data available, we acknowledge that this study provides a preliminary exploration only. It is possible that dietary factors including high energy intake and a low P:E ratio of the diet consumed may contribute to poorer growth and body composition outcomes.

Our preliminary results suggest that if a positive relationship between P:E ratio and growth outcomes could be confirmed in more patients; the use of this ratio would provide a useful clinical tool when determining an appropriate dietary strategy to confirm optimal metabolic control and maximise growth and physical development in patients with GSD I and III.

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Chapter 5: VLCAD deficiency: Follow up and outcome of patients born through newborn screening in Victoria

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VLCAD deficiency: Follow-up and outcome of patients diagnosed through newborn screening in Victoria

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ABSTRACT

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is an inherited metabolic disorder of fatty acid oxidation. Treatment practices of the disorder have changed over the past 10–15 years since this disorder was included in newborn screening programs and patients were diagnosed pre-symptomatically. A genotype-phenotype correlation has been suggested but the discovery of novel mutations make this knowledge limited. Herein, we describe our experience in treating patients (n = 22) diagnosed through newborn screening and mutational confirmation and followed up over a median period of 104 months. We report five novel mutations. In 2013 we formalised our treatment protocol, which essentially follows a European consensus paper from 2009 and our own experience. The prescribed low natural fat diet is relaxed for patients who are asymptomatic when reaching age 5 years but medium-chain triglyceride oil is recommended before and after physical activity regardless of age. Metabolic stability, growth, development and cardiac function are satisfactory in all patients. There were no episodes of encephalopathy or hypoglycaemia but three patients had episodes of muscle pain with our without rhabdomyolysis. Body composition studies showed a negative association between dietary protein intake and percent body fat.

Larger patient cohort and longer follow up time are required for further elucidation of genotype-phenotype correlations and for establishing the role of dietary protein in metabolic stability and long-term healthier body composition in patients with VLCAD deficiency.

1. Introduction

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is an inherited metabolic disorder of fatty acid oxidation. Based on clinical presentation, three phenotypes of VLCAD deficiency have been identified: an early onset insidious type that causes a potentially lethal cardiomyopathy, a later onset type that presents with hypoketotic hypoglycaemia and an adult onset type that mainly causes muscular symptoms [1]. The inclusion of VLCAD deficiency in newborn screening programs enables early initiation of treatment and improved outcome [2], altering the natural history of this disorder, at least in those patients who would have presented in childhood. On the other hand, "patients" who may never need treatment may be identified, making the decision of whether to treat or not, and how, complex.

Treatment practices of VLCAD deficiency have changed somewhat over the past 10–15 years since this disorder was included in newborn screening programs and the number of diagnosed patients has risen dramatically [2,3]. Concerns regarding hypoglycaemia led to a general recommendation to avoid prolonged fasting and, at times, to prescribing carbohydrate-rich drinks to patients. The early practice of prescribing medium chain triglyceride (MCT) supplementation on a regular basis and chronically [2] has been revisited. In a multi-centre collaborative study that focused on management and outcome of patients with VLCAD deficiency there were 32 patients (18 identified by newborn screening and treated from infancy) who were asymptomatic [4]. These asymptomatic infants were treated with fat-modified diet, either a combination of breast-milk and MCT-rich formula or a formula low in long- chain triglycerides (LCT) and high in MCT [4]. Current recommendations include prevention of fasting for long periods of time, a diet that is low in LCT, and MCT supplementation at times of physical activity [5]. Some centres include carnitine, but this has not been shown to make any improvements to clinical outcome [6,7].

Our practice in the management of VLCAD deficiency has also changed over the years in parallel with the changes noted in the literature [5]. In 2013 we formalised our approach to treatment based on the published consensus papers from 2009 [4,5] and our own experience.

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The purpose of this study was to review our experience with the management of patients with VLCAD deficiency from birth to their current age. We also wished to assess the patients' outcome in light of their mutations, in order to evaluate a possible prognostic value of mutation testing beyond the actual confirmation of the diagnosis. Clinical outcome parameters included: 1) metabolic stability, as measured by the number and type of admissions as well as the patients' symptoms; 2) growth; and 3) body composition. The neuropsychological outcome of some of our patients has been previously reported [8].

2. Methods

We conducted a retrospective review of all health records, including dietary records, of all patients with VLCAD deficiency diagnosed and treated at our centre since the implementation of the expanded newborn screening program in 2002. Data were collected in VICIEM, a RedCap-based in-house databank for patients with metabolic disorders. Ethics approval for collection of data in the databank (HREC #30066B) and specifically for publication (HREC #DA005-2015-11) was granted from the RCH Human Research Ethics Committee.

2.1. Mutation analysis

PCR amplification of all exons, including part of the flanking intron sequences, of the human VLCAD gene, was carried out as previously described [1] using a GeneAmp(r) PCR system 9700 (Applied Biosystems). Sequence analysis with M13 forward and reverse primers was performed using the BigDye(r) Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) and a 3100-Avant genetic analyser (Applied Biosystems). Sequence data were analysed with the Sequencer v3.1.1 software (Gene Codes Corporation). Sequencing was performed at Research Unit for Molecular Medicine, Skejby Sygehus, Aarhus, Denmark.

2.2. Management protocol

The original management protocol of patients with VLCAD deficiency included a very low 'natural'-fat (LCT) diet (~10% of total energy intake) and supplementation with MCT (~20% total energy intake). Adequate intake of protein, micronutrients, essential fatty acids (EFA) and calories were prescribed as were specific age appropriate sick day regimes. Patients were advised to consume carbohydrate-rich drinks or food at regular times when unwell or at times of increased physical activity but in 2010 we changed this practice to MCT oil (see below), in line with the recommendations in the literature [4]. In response to emerging evidence that a lifelong fat restricted diet may not be necessary for patients who remained asymptomatic [5] and in line with our experience, we reviewed the outcome of our patients diagnosed since 2002 and formalised these modifications in a new protocol (Table 1). Asymptomatic infants are encouraged to continue breastfeeding after diagnosis as per current recommendations [4]. MCT containing formula is not prescribed unless infants require top up feeding. MCT are added with the establishment of solid foods intake (>6 months of age). In addition to the well documented benefits of breastfeeding, this minimises the risk of EFA deficiency, which has been documented in infants fed exclusively on MCT based formula [9]. LCT intake is relaxed to ~30% total energy intake in patients who are asymptomatic at 5 years of age (for practical reasons, before starting school), with MCT supplementation (0.25 g/kg/bodyweight) recommended at times of increased energy demand such as physical activity, as this may still provide protection [4, 10]. We advise parents to use an arbitrary 'rule of thumb' regarding fasting tolerance at night: feeding every 3 h in the first months of life and 'adding' a possible fasting hour for each month over 4 months of age, up to a full overnight fast at age 10–12 months (it should be noted that this general guide is individualised, e.g. if the infant is small). Carnitine supplementation was prescribed during 2002–2003 but stopped in 2003. Anthropometric growth parameters are routinely assessed at regular clinic visits with body composition measurements using BIA currently being incorporated into our regular clinical care. Patients have formal annual cardiac reviews.

Table 1
Dietary protocol, formalised 2013.

Asymptomatic patients	
0–6 months	Breast feed +/− MCT formula
6–12 months	Breast feed +/− MCT formula + low LCT diet (~15% energy)
1–5 years	Low LCT diet: ~15–20% energy, MCT: 15–20% energy
5+ years	Healthy 'low- fat' diet (30% energy total fat, MCT used only at times of increased requirements; activity/illness)
Symptomatic patients	
0–6 months	MCT formula
6–12 months	MCT formula + very low LCT diet (~10% energy)
1–5 years	Very low LCT diet (10% energy), MCT: 20–25% energy
5+ years	Very low LCT diet (10% energy), MCT: 20–25% energy

2.3. Metabolic stability

Admissions were recorded according to four causative categories: Metabolic Decompensation (high plasma creatine kinase (CK) with or without rhabdomyolysis, and/or metabolic acidosis, and/or hypoglycaemia), Prophylactic (the child is unwell but metabolically stable), Procedure (imaging, allergy testing, any cause of anaesthesia etc.), and 'Other' (other paediatric or miscellaneous causes for admission). The results of serial cardiac assessments were collated.

2.4. Nutrition, growth and z-scores

Dietary intake was typically assessed through clinic reviews. Nutritional intake data including breastfeeding, LCT and MCT recommendations and actual intake were collected. The age at which recommendations to liberalise dietary fat intake were given was noted. Dietary intakes from 3-day food diaries were analysed quantitatively using Foodworks Version 7, 2012 (Xyris Software Australia Pty. Ltd.) Growth data, including height and weight taken at routine clinic visits when children were well, were collated and converted to standard deviation (z-scores) using the CDC z-score calculator.

2.5. Body composition

Body composition was assessed in nine children, as part of a larger study on nutrition in IEM (Ethics approval HREC #32056), by multi-frequency Bioelectrical Impedance Analysis (BIA) using the Quadscan 4000 (Bodystat Ltd. Isle of Man). Measurements were taken as per the manufacturer's instructions at routine clinic review when children were well, at approximately 6 monthly intervals. Analysis was performed at time points where both dietary intake and body composition measurements were available. Body composition analyses were done using raw impedance values at 50 kHz and the predictive equations of Houtkooper [11] to estimate fat-free mass and fat mass, and then converted to percentage of body weight. All measurements were completed by the same practitioner (ME). Pearson's correlation test was used where applicable using the Statistical package for the Social Sciences (SPSS) software version 23 (IBM, USA).

3. Results

There were 23 patients (13 male/10 female). All patients were picked up by newborn screening and their diagnosis was confirmed by mutation analysis (Table 2). Patient 18 is a sibling of patient 17 and patient 21 is a sibling of patient 20. Follow up period ranged from 14 months to 16 years and 7 months (median: 104 months).

Table 2
Patients, mutations and admissions (number and type).

Patient	Genotype				Clinical	
	Mutation 1	Mutation 2	Reference (first report)	Additional patients	Follow-up (m)	Admissions
1	c.1077G>A (p.A359A)	c.1077G>A (p.A359A)	Miller et al. [12]	Mutations found in other newborns; unpublished	66	Prophylactic 1
2	c.685C>T (p.R229*)	c.1711G>A (p.G571R)	Andresen et al. [1] Miller et al. [12]		87	Prophylactic 1
3[a]	c.889-91delGAG (p.E297del)	c.1246G>T (p.A416S)	Brown et al. [8]	Mutations found in other newborns; unpublished	121	Prophylactic 3
4	c.848T>C (p.V283A)	c.1097G>A (p.R366H)	Andresen et al. [1,13,14]		55	Decompensation-Neonatal 1
5	c.848T>C (p.V283A)	c.343delG (p.E115Kfs*2)	Andresen et al. [1], Strauss et al. [23]		112	Prophylactic 4
7	c.1019G>T (p.G340V)	c.753-27C>T	Miller et al. [12] Novel		27	None
8	c.559A>G (p.K187E)	c.1077+15C>T, c.1678+23C>T	Novel		47	None
9	c.1226C>T (p.T409M)	c.1226C>T (p.T409M)	Ryder et al. [17]	Prevalent in Maori or Pacific ethnicity	95	None
10[a]	c.481G>A (p.A161T)	c.1349G>A (p.R450H)	Boneh et al. [24], Smelt et al. [25]		139	Prophylactic 3 Procedure 1
11[a]	c.848T>C (p.V283A)	c.753-2A>C	Andresen et al. [1,13,14]		137	None
12	c.848T>C (p.V283A)	c.1405C>T (p.R469W)	Andresen et al. [1,13,14]		27	None
13[a]	c.848T>C (p.V283A)	c.476A>G (p.Q159R)	Andresen et al. [1]		111	Prophylactic 3
14	c.848 T>C (p.V283A)	c.950 T>C (p.V317A) c.1097 G>A (p.R366H)	Andresen et al. [1,13,14]		14	Prophylactic 1
15	c.848T>C (p.V283A)	c.1405C>T (p.R469W)	Andresen et al. [1,13,14]		88	Prophylactic 1
16[a]	c.848T>C (p.V283A)	c.848T>C (p.V283A)	Andresen et al. [13,14]		157	Prophylactic 2 Procedure 1 Other 1
17[a]	c.1117A>T (p.I373F)	c.1153C>T (p.R385W)	Boneh et al. [24]		135	Prophylactic 5
18	c.1117A>T (p.I373F)	c.1153C>T (p.R385W)	Boneh et al. [24]		102	Prophylactic 6 Procedure 3 Other 2
19[a]	c.1097G>A (p.R366H)	c.1322G>A (p.G441D)	Andresen et al. [1,13,14]		144	Decompensation-Neonatal 1 Decompensation 8 Procedure 1 Other 1
20[a]	c.1500-1502delCCT (p.L501del)	c.1500-1502delCCT (p.L501del)	Boneh et al. [24]	Another patient known; presented clinically; unpublished	144	Decompensation 3
21	c.1500-1502delCCT (p.L501del)	c.1500-1502delCCT (p.L501del)	Boneh et al. [24]		100	Prophylactic 2 Decompensation 1
22	c.848T>C (p.V283A)	c.865G>A (p.G289R)	Andresen et al. [13,14] Spiekerkoetter et al. [2]	Adult patient with heterozygous c.865G>A mutation known; muscular presentation; unpublished	32	Prophylactic 3 Other 2
23	c.848T>C (p.V283A)	c.1923G>C (p.L641P)	Andresen et al. [13,14] Novel	1923.G>C found along with known disease-causing mutations in two other patients; possibly 'mild'; unpublished	57	Prophylactic 4

[a] Patients previously reported. [8,24].

3.1. Mutation analysis

Details of all mutations identified in our patients are shown in Table 2. We detected 13 sequence variants that had previously been observed in patients who presented clinically, supporting their disease-causing nature. The c.848T>C variant was present in 11 alleles corresponding to ~30% (12 of 40 alleles) in the identified newborns, confirming that this is the most frequent sequence variant in patients with VLCAD deficiency. Other variants have only been found in newborns detected by screening. Of these, six (c.481G>A, c.888-891delGAG, c.1019G>T, c.1077G>A, c.1246G>T and c.1711G>A) were recently identified in newborns with an abnormal acylcarnitine profile suggestive of VLCAD deficiency [12]. Since all of these variants are extremely rare in the ExAc data we suspect that they are deleterious. One patient was homozygous for the c.1226C>T variant, a very frequent variant in newborns of Maori or Pacific origin, which appears to be benign and has not been associated with clinical symptoms [17]. Five variants (c.559A>G, c.753-27C>T, c.1077+15C>T, c.1678+23C>T and c.1923G>C) have not been previously reported.

Two patients had more than two sequence variants: Patient 7 is heterozygous for three previously unreported variations: c.559A>G, c.1077+15C>T and c.1678+23C>T. It is not possible to assign the different variants to individual alleles. The c.559A>G variant is extremely rare and is not present in the ExAc database, whereas the

c.1077 + 15C>T and c.1678 + 23C>T variants are rare with allele frequencies of 0.0007740 and 0.001725, respectively. The c.559A>G mutation in exon 7 causes a p.K147E change in the mature VLCAD protein (corresponding to p.K187E in precursor VLCAD) and has not been previously observed in patients, controls or newborns. This patient is also heterozygous for the two intronic mutations: c.1077 + 15C>T in intron 10 and c.1678 + 23C>T in intron 17. Both of these mutations may influence pre-mRNA splicing, but neither of them abolishes or creates splice site consensus sequences, thus any effect on splicing would be due to the mutations creating/abolishing other splicing regulatory sequences that regulate recognition of the weak donor splice sites of intron 10 or intron 16, respectively. Patient 13 is heterozygous for the prevalent disease associated c.848T>C variant and a c.950T>C variant previously reported to be located in *cis* with c.848T>C [13,14]. This patient is also heterozygous for the c.1097G>A variant in the other allele.

Patient 1 is homozygous for the c.1077G>A (p.A359A) variant, which does not change an amino acid codon. However, the G>A change is located in the last nucleotide in exon 10 and it is thus likely to cause aberrant splicing of this exon thereby disrupting VLCAD activity [15]. This variant was also identified by Miller et al. [12] in a newborn with an abnormal acyl-carnitine profile and it is extremely rare (1/120.748 samples) in ExAc. The c.1019G>T variant was also reported in several newborn samples [12], and is very rare in the ExAc database.

3.2. Metabolic stability

Patients 4 and 18 presented with neonatal hypoglycaemia and were admitted to the neonatal unit before the results of newborn screening were available. After diagnosis, there were 65 admissions in total. Of these, only 14 were due to metabolic decompensation and were noted in only four patients, including two episodes during the neonatal period (Table 2). These admissions followed either an intercurrent infection or excessive physical activity. Patient 17 had several admissions for investigation of allergy. No patient had any episode of hypoglycaemia or 'encephalopathy' during the follow-up period. All cardiac assessments were normal.

3.3. Nutritional information

Longitudinal dietary information, including data on dietary fat intake, was available on 21/22 patients (one patient moved interstate intermittently with poor clinic attendance). Of the 22 patients, 18 were breastfed with or without MCT-based formula top ups (mean duration 8.1 months; median 4.0, range 0.5–36 months), two were not breastfed at all, and data regarding breastfeeding are missing on three. At commencement of solid food intake 20/22 children were treated with very low fat diet (~10% total energy intake) with MCT supplementation (~20% total energy intake) including MCT-based formula, MCT-oil for cooking or supplements added to foods. One patient was initially advised to follow a "healthy fat diet" (~30% energy from LCT) only, due to social issues, but MCT-oil was introduced later, along with better compliance. Diet was subsequently relaxed to a 'healthy' low fat diet with MCT supplements during exercise only (~0.25 g/kg/dose) in eight patients who have remained symptom free up until age 5 years. Twelve patients currently remain on low LCT diet with MCT supplements: nine who are asymptomatic but are <5 years of age, and three who are symptomatic.

3.4. Growth and body composition

The mean weight z score for all ages was −0.12 (median: −0.135, range: −2.52 to +2.42). The mean height z score for all ages was −0.06 (median: −0.08, range: −2.69 to +1.93). No child failed to grow linearly based on initial height z score. BMI was calculated for children > 2 years of age (n = 16), but not at all time points due to some missing data. The mean BMI z-score for the group was 0.05 (median: −0.09, range: −1.52 to 2.75). One of these children met the criteria for the diagnosis 'obese' (BMI z-score > 2) and two for 'overweight' (BMI z-score 1–2). Two of these three children were symptomatic.

Table 3
Body composition of patients with VLCAD deficiency.

Patient	Sex	Age (years)	BMI z score	%body fat (BIA)	Percentile[a]
3	M	7.5	0.09	22.1	91
5	M	5.9	0.20	15.4	25–50
13	M	6.3	−0.86	9.5	<2
15	M	4.5	−0.58	12.4	N/A
17	M	8.3	0.87	22	85–91
18	F	5.6	−0.32	17	25–50
19	M	10	−0.86	12.6	2
20	F	9.1	1.05	28.6	85–91
21	M	5.5	2.24	18.5	85

[a] Based on: McCarthy et al. [16]: <2%ile underfat, 2–85%ile normal fat, >85%ile overfat, and >95%ile obese.

Nine children had simultaneous dietary intake assessments and BIA measurements (mean age: 6.9 years; median: 6.3, range: 4.5–10.0 years). The mean percentage body fat in these patients was 17.6% (median: 17%, range: 9.5–28.6%). When compared with body fat percentile standards for healthy children [16], four children had body fat levels within the normal range, three had body fat levels above the normal range, including one who was symptomatic, and one had body fat levels below the normal range (Table 3). There was a trend towards a correlation between percentage body fat and BMI (r = 0.647, p = 0.0.6). More importantly, there was a significant negative correlation between protein intake (represented as gm protein/kg bodyweight/day) and percentage body fat (r = −0.677 and p = 0.045) (Fig. 1). There was also a trend for higher Protein to Energy ratio (P:E ratio, gm protein/100 kcal) to be associated with lower body fat (r = −0.626, p = 0.071).

4. Discussion

The identification of patients with VLCAD deficiency through newborn screening poses questions regarding the optimal treatment for these patients, particularly since prediction of severity of disease and outcome are difficult. There are hardly any biochemical predictive markers that can be used to suggest who would need treatment and who wouldn't. Not surprisingly, the newborn screening blood results in our patients were not predictive of symptomatology, either in the

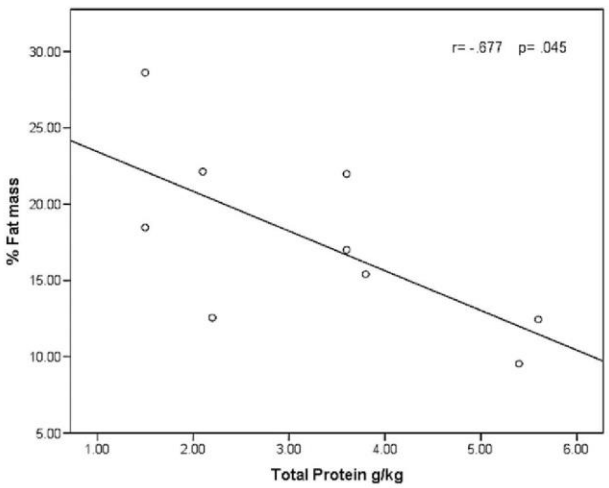

Fig. 1. Percent body fat relative to daily total protein intake. Body fat was assessed by BIA. Pearson Correlation Coefficient was calculated using SPSS. For further details see text.

neonatal period or later. Whether presentation in the neonatal period is predictive of symptomatology thereafter is very questionable, and the very small number of patients who presented in the first days of life in our cohort does not provide a clear answer to this question. Some mutations have been previously associated with symptomatology in childhood or in adulthood [1] or with no symptomatology at all (c.1226C>T; [17]). However, the number of novel mutations of which not much is known is constantly increasing. Miller et al. have recently reported several novel mutations in infants picked up by newborn screening [12] and in this study we found patients with some of these mutations and three patients with additional novel mutations. It may therefore be concluded that mutation analysis could be considered as a predictive test with valuable but often incomplete predictive value for the need of treatment and that collaborative studies describing large cohorts of patients and their mutations, and long term follow up (into adulthood) may shed further light on the genotype-phenotype correlation.

In theory, it is conceivable that tolerance to breast-feeding (i.e. to tolerance to breast milk, which is relatively high in natural fat) may predict that the infant will not need fat restriction (i.e. 'treatment'). However, this is assumption requires proof, because likewise, it is theoretically possible that infants tolerate breast-milk fat but would not tolerate natural fat at a later age. We have not explored this possibility thus far; yet it would be worth exploring with large groups of patients. Such studies will need to be carefully structured to avoid misinterpretation of results. In this regards, it should be noted that patients 20 and 21 were admitted for decompensation in childhood despite tolerating breast-milk in infancy.

Despite the relaxation of dietary fat intake, we recommend the use of MCT oil before physical activity regardless of age and symptomatology, as suggested in the consensus paper [5]. This is important particularly as children grow up and exercise becomes more competitive and structured. The use of MCT oil for asymptomatic patients during periods of increased energy requirement is supported by evidence for its utilisation as an alternative fuel for skeletal and cardiac muscle in individuals undergoing similar physical stress [10,18].

We found a trend towards a positive correlation between BMI z-score and body fat percentage. Increased percent body fat and lower percent lean body mass were previously found in children with Long-Chain 3 Hydroxy-acyl-CoA Dehydrogenase (LCHADD) or Trifunctional Protein Deficiency (TFP) regardless of BMI [19]. In that study, measured resting energy expenditure was not lower than in control subjects but total energy expenditure, measured by doubly labelled water analysis, was lower in some patients [19]. It is suggested that the risk of overweight and for higher than normal body fat may be associated with a high carbohydrate intake, the lack of fasting tolerance and the need for frequent feedings and a decrease in physical activity [19,20]. Given that recommendations have changed from carbohydrate to MCT supplementation before physical activity and not as part of an on-going daily diet, it is likely that prospective follow-up studies will clarify whether this issue is resolved. However, compliance issues and the preference for carbohydrate-rich drinks (e.g. patients 20 and 21 in our cohort) may impact on the results.

Finally, we observed a negative correlation between protein intake (represented as gm protein/kg bodyweight/day or protein as percent of total daily energy intake) and percentage body fat, suggesting that higher protein intake may be beneficial to body composition in the long term. In a previous report on a short term randomised cross over study, which was also controlled for fat intake, subjects with LCHAD or TFP deficiency had an increase in energy expenditure when given a higher protein versus higher carbohydrate diet [21]. It is possible, at least in theory, that the potential anaplerotic effect of protein as a source of acetyl-CoA for the Krebs cycle may contribute to metabolic stability. It would be of interest to study the effect of high protein diet on metabolic control, growth and body composition prospectively in patients with VLCAD deficiency and to consider inclusion of a recommendation for high protein diets in disorders of long-chain fatty acid oxidation.

5. Conclusions

There are currently no biochemical markers for prediction of disease severity and for the need for treatment in VLCAD deficiency. Mutation analysis may offer predictive value but this may not be robust enough for a large proportion of those mutations that have not been previously reported in clinically affected patients. Following the current guidelines for treatment of patients with VLCAD deficiency as per the European consensus paper [5] seems effective and safe during childhood. We propose that the diet of asymptomatic patients can be relaxed at age 5 years and that the possibility of prescribing high protein daily intake be further explored in terms of its contribution to metabolic stability and to improved body composition. Long term follow up of a large number of patients is needed to elucidate possible genotype-phenotype correlation and better prediction of age-related symptoms after age 8 years [22], particularly if their mutation has been associated with symptoms at a later age.

Conflict of interest

Maureen Evans, Brage S. Andresen and Avihu Boneh have no conflict of interest to declare. Judy Nation is currently an employee of Vitaflo but was employed by the Royal Children's Hospital, Melbourne, at the time of data collection and has no conflict of interest to report.

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Section 3: Phenylketonuria

Direct modification of natural protein intake with amino acid supplementation.

Section 3: Aims

Aim 1: To describe longitudinal patterns of growth and body composition and dietary intake in children and adolescents with PKU. (Chapter 6)

Aim 2: To investigate the relationship between protein quantity and quality on growth trajectory in children and adolescents with PKU. (Chapter 6 and 8)

Aim 3: To determine the optimal P:E ratio for prescribing dietary recommendations in children and adolescents with PKU (Chapter 8)

Aim 4: To determine the validity of using Bioelectrical Impedance Analysis methodology to measure body composition in children and adolescents with PKU. (Chapter 7)

Chapter 6: Longitudinal growth and body composition in Phenylketonuria

6.1 Introduction

Phenylketonuria (PKU) is an inborn error of protein metabolism that results from perturbation in the enzyme phenylalanine hydroxylase activity leading to elevated blood and tissue levels of phenylalanine (phe). As elevated phe levels have a toxic effect on the brain, treatment with a diet low in phe needs to commence as soon as possible after birth. Untreated PKU is associated with significant morbidity, most commonly progressive and irreversible intellectual impairment.(1) An expectation for all newly diagnosed and treated infants and children with PKU is to promote normal neurocognitive development and to maximise the potential for normal growth and body composition. There is a recognised spectrum in PKU ranging from 'severe', when individuals have a very low phe tolerance, to milder forms when individuals have a higher phe tolerance, which is defined as the phenylalanine intake that is compatible with optimal blood phe control. The use of cofactor tetrahydrobiopterin (BH_4),(2) in PKU patients who respond to this treatment, form a special group in dietary terms, as they have high phe tolerance, hence, tolerate a higher natural protein intake. In some cases, this may enable them to consume a normal diet.(3, 4)

Lifelong goals of treatment in PKU are to maintain phe levels within the target range to achieve optimal neurocognitive outcomes. Nutritional goals are the same as for the general population, that is, to achieve "satisfactory growth and the avoidance of deficiency states".(5-7) Recent attention has been directed towards attaining long term ideal body composition in children and adults with PKU. (8, 9)

6.1.1 What is the recommended dietary therapy?

Consensus exists regarding the need for reduced natural-protein intake and supplementation with phe- free amino acid based formulae (AAF), as natural-protein tolerance is mostly below

safe requirements in most patients.(10, 11) As the nitrogen requirement for individuals with PKU is considered the same as for the healthy population,(12) published recommendations for 'total-protein intake' in PKU, defined as natural-protein intake plus AAF, are based on healthy population nutritional recommendations with an additional estimated factor to account for the apparent difference in quality between natural-protein and AAF. (10) These recommendations consider the potential risk for poor nutritional outcome when consuming only exact protein recommendations. This was demonstrated when children with PKU consumed a protein intake equivalent to the FAO/WHO 1973 'safe level of protein intake' compared to children consuming the RDA protein recommendation which was 30% higher.(13) In that study, those consuming the FAO/WHO recommendations, showed growth faltering despite adequate energy intake. In addition, consideration is given to the role of AAF in achieving optimal phe levels and micronutrient intake.(8, 14) Consequently, total protein intake may be determined by factors other than meeting nutritional requirement, and overall protein quality is determined by both by phe tolerance, and the quantity of AAF necessary to meet protein targets.

Total energy intake mostly depends on the amount and type of natural protein and AAF consumed, as these can vary significantly in fat and carbohydrate content, in addition to the protein 'free' food products eaten.(15) It follows that nutritional outcomes in PKU are likely to be affected by both the quality and quantity of protein consumed and total energy intake.

6.1.2 What are the risks to nutritional intake associated with this diet?

Recommended dietary intakes can be imprecise, particularly for those with different physiological needs and consuming highly modified diets, and this requires some degree of flexibility and common sense in their application. Moreover, the monitoring of nutritional

status to assess the impact of dietary recommendations, is an essential component of care.(16) Dietary prescriptions for PKU are demanding, exacting and complex,(17) and risk to nutritional intake may come from both the restrictive and semi-synthetic nature of the diet itself, or difficulty with adhering to the dietary prescriptions long term. Importantly in a condition such as PKU, there is a likelihood that protein intake may at times be marginal due to illness, changes in appetite, and failure to take the prescribed amount of AAF.

At the more severe end of the spectrum of PKU, AAF is the primary source of dietary protein and is also designed to provide recommended intakes of micronutrients when consumed in amounts to meet protein needs.(10) On the other hand, patients at the milder end of the spectrum, who tolerate higher amounts of natural protein and require less AAF to meet protein recommendations, may also be at risk of inadequate intakes depending on the nutrient density and quality of the foods consumed.(18) Even at the milder end of the spectrum of PKU, such as those responsive to BH4, many patients still require a natural protein restricted diet, compared to free living healthy children, and a proportion of their total protein intake is still derived from AAF. Thus, it is possible that nutritional outcomes could still be compromised in all patients with PKU. While a carefully constructed diet should effectively meet all nutrient requirements, non-compliance with prescribed amounts of AAF is likely to significantly impact on both phe control and nutritional intake.(17) Dietary non-compliance, whether intentional or non-intentional, may result in altered nutritional intake and increase the risk of nutritional imbalance. However, while dietary non-compliance in PKU is acknowledged, it is not well studied,(17) and non-compliance is often reported in terms of phe control, adherence to blood monitoring requirements and clinic attendance.(19-21)

In children with fussy eating behaviours there is an additional risk to energy intake that may be problematic to manage, and may range from an inadequate to an excessive energy intake. Contributing factors to low energy intake include the limited range of foods available for

consumption and the lower energy density of some AAF. Factors that may result in excessive energy intake include the high carbohydrate, high fat and energy dense 'protein free' foods,(22) as these may be perceived by carers and individuals with PKU as being 'unrestricted' in the diet due to their negligible protein content. The higher energy density of some AAF may also contribute significantly to energy intake.

6.1.3 What do we know about the nutritional outcomes?

Despite some subtle changes in neurological outcomes, early and continuous treatment has proved effective for individuals born with PKU. Consequently, outcomes are primarily described in terms of neuropsychological performance and executive function, as deficits are still identified in those who have received early and continuous treatment.(23) Reports describing patterns of growth in PKU have been inconsistent in the outcomes documented, and there is evidence of growth retardation and poor nutritional status in some cohorts.(6, 24, 25) Common abnormal findings have included a low height for age,(15, 26-30) and increased prevalence of overweight compared with either sex and age matched controls or comparable large-scale population groups.(31-33) On the other hand, there are numerous reports of normal growth patterns in patients with PKU.(34-36)

Growth outcomes have generally improved over the years with adjustments to dietary treatments and expectation nowadays should be for normal growth outcomes in children with PKU. In early reports of growth and nutritional deficits in PKU were mainly attributed to inadequate total protein, particularly when a significant amount was derived from AAF.(37) With the advent in the 1990's of the 'diet for life' approach to PKU treatment, there has been an increased focus on addressing the long term nutritional adequacy of the diet and recommendations for total protein intake have been proposed to better address the altered

source of protein in the diet.(38) Changes to practice have included an allowance for many fruits and vegetables to be consumed ‘freely’ without being included in the calculated daily phe-intake allowance. These foods may contain additional nutrients and ‘oligo-elements’ that could contribute to improved growth.(39) There has been a substantial increase in the number and improved formation of AAF, resulting additional and increased levels of compounds such as essential fatty acid content and preformed DHA that also support growth and development. Children in our clinic are closely monitored, with frequent phone review and face-to face outpatient meetings. The provision of intensive dietetic consultation should ensure that any growth discrepancy can be identified early and dietary adjustments can be made to ensure no longer term growth faltering or weight acceleration.

Increasingly, efforts have been made to establish a relationship between growth outcomes and dietary intake to help guide dietary recommendations. This include studies that report a relationship between higher total protein intake and improved growth,(37) higher natural protein/phe intake and improved growth(30) and studies that report no relationship between the quantity or quality of dietary protein intake and growth.(27, 39-43) Nowadays, nutritional status is more closely monitored, with assessment recommendations detailed in published clinical practice guidelines,(7, 11) and nutritional outcomes are increasingly well studied and documented.

Consideration of the differences between protein sources have been incorporated into guidelines for dietary management of PKU for some time.(38, 44) It has been acknowledged that whole-body protein metabolism may be better supported by intact dietary protein rather than amino acids. The promotion of protein synthesis is less efficient with amino acids, with evidence for a lower nitrogen excretion with dietary protein rather than amino acids, even when the same energy is consumed.(25) Moreover, efficient utilisation of amino acids for synthesis of body protein is influenced by additional factors including the rate of protein

digestion and amino acids transport into the blood, and requires the presence of all the essential and no-essential amino acids at the same time.(45)

6.1.4 What are the aims of the current study?

The aim of this retrospective study was to contribute to our understanding of longitudinal growth and dietary patterns in children with PKU. A limitation of a retrospective study is the lack of compete data with which to draw firm conclusions. Therefore, we restricted the current study to the contribution of protein quality and quantity to longitudinal anthropometric parameters in PKU. More specifically, we aimed to answer the following questions:

1. What are the longitudinal patterns of weight and height gains and BMI in children with PKU?

2. Does total protein intake, or type of protein, impact on growth trajectory in children with PKU?

6.2 Methods

We collected retrospective longitudinal data on dietary intake and growth of all patients diagnosed with PKU by newborn screening and treated in our metabolic specialist clinic. We included patients who were born between January 1995 and December 2014. We did not include patients diagnosed with hyperphenylalaninaemia, defined as those with untreated phe levels <400μmol/L, as these patients did not require dietary intervention. Patients were excluded if they were born <32 weeks gestation (n=1), or had a comorbidity known to affect growth (n=2), or who had poor compliance with AAF intake (n=3).

Data were analysed in patients with PKU treated with phe restricted diet (D-PKU) (n=79; 31 males, 48 females), and patients with PKU treated with tetrahydrobiopterin (BH_4) ± phe restricted diet (BH_4-PKU) (n=14; 8 males, 6 females). For certain calculations, data have also been combined and denoted as all-PKU which represent the spectrum of PKU and the range of protein tolerance.

Data on weight and height were collected from medical and dietetic clinic records when patients were well. Regular clinic visits occurred every 3 to 4 months for the first 2 years of life and then approximately every six months thereafter. Body Mass Index (BMI) was calculated using the equation kg/m^2. All anthropometric measurements were expressed as age and gender-specific z-scores, by entering weight and height data into the epidemiological software package Epi Info (version 3.5.1), based on the Centres for Disease Control and Prevention (Atlanta, GA) 2002 reference database. Criteria to determine overweight were based on the CDC Growth Charts (2000).(46)

Dietary data were collected from dietary and medical records and consisted of parent or patient report, 24-hour recall, dietary history and food diaries. Dietary intake of protein in g/kg/d was compared with FAO/WHO/UNU recommended safe levels.(47) One gram of natural-protein was considered to provide an average equivalent of 50mg phenylalanine. While the amount of protein in human milk changes over the period of lactation, assumptions about the protein content of breast milk of ~1g/100ml were made(48) as this was not directly measured. An estimation of total natural protein intake was made based on available data in babies consuming breast milk. In children who were breast feeding (i.e. not expressed breast milk) or the number of breast feeds per day was not quantified, no estimation of natural or total protein intake were made. Therefore, the relationships between total protein intake, natural protein intake and growth parameters were not made for infants <12 months of life.

Statistical comparisons were made between D-PKU and BH_4-PKU up to 9 years of age only due to limited numbers of older patients treated with BH4. More detailed analysis of all-PKU at key childhood ages (3, 6, 9, 12, 15 years) was made to determine if a correlation between dietary intake: total-protein, natural-protein and AAF, and anthropometric variables: weight-, height-, and BMI- z-scores could be established over childhood. Several factors influenced the choice of these ages: at 3 years of age children's growth velocity is steadier, eating patterns are better established and the frequency of inter-current illnesses reduces and thus natural protein intake is more reflective of consistent phe tolerance. BMI reference standards exist for children greater than 24 months of age, however as these data were collected retrospectively the use of 3 years of age when height measurements are routinely taken in the standing position improves the accuracy of this measurement.

Age 5-6 years represents the age at which children start to attend school and increase independence, and 9 years of age represents the age preceding the increase in growth velocity and pubertal changes associated with adolescence. Until recently, recommendations for these patients in our centre, for target Phenylalanine control also increase from 200 – 400 µmol/l to 200-700 µmol/l after the age of 10 years. This increase in the upper end of the range for phe allows an increased natural protein intake, which would negate comparisons of natural protein intake across ages.

6.2.1 Statistical analysis

Statistical analyses were performed using SPSS for Windows software version 23 (IBM, Illinois, Chicago, IL). Significance was set at $p< 0.05$. Continuous variables including z-scores for weight, height and BMI, protein (g/kg/d) and energy intake (%BMR) and P:E ratio (g protein/100kcal/d) are presented as median and range for the groups. Weight- height- and

BMI z-scores were analysed for each child over their lifetime, from 3 months of age to the last data collection episode, and were presented as the mean for the individual. Non-parametric tests included: Kruskal-Wallis test for one-way between-group analysis of variance; Mann-Whitney U test for differences between two independent groups on a continuous measure; Friedman test for variance between multiple measures in the same subjects. Spearman correlation coefficient Rho (r_s) was used to evaluate associations between categorical variables.

6.3 Results

6.3.1 What were the growth patterns of our patients?

Growth: Longitudinal growth patterns for D-PKU and BH_4-PKU are similar to the reference population. (Table 1, see pages 162-166). When data were analysed for each child over their lifetime, results suggest that in D-PKU patients mean weight-, height- BMI- z-score was 0(±1) in 80%, 86% and 73% of patients, respectively. In BH_4-PKU patients mean weight-, height- BMI- z-score was 0(±1) for 93%, 77%, and 80% of patients, respectively. In the D-PKU group 10/73 (14%) of patients had a lifetime mean BMI z-score >1 and considered overweight and 5/73 (7%) considered obese with lifetime mean BMI z-score >2. In the BH4-PKU group 2/10 (20%) of patients were considered overweight. When groups were combined 17/83 (20%) patients were considered overweight or obese. (Table 2).

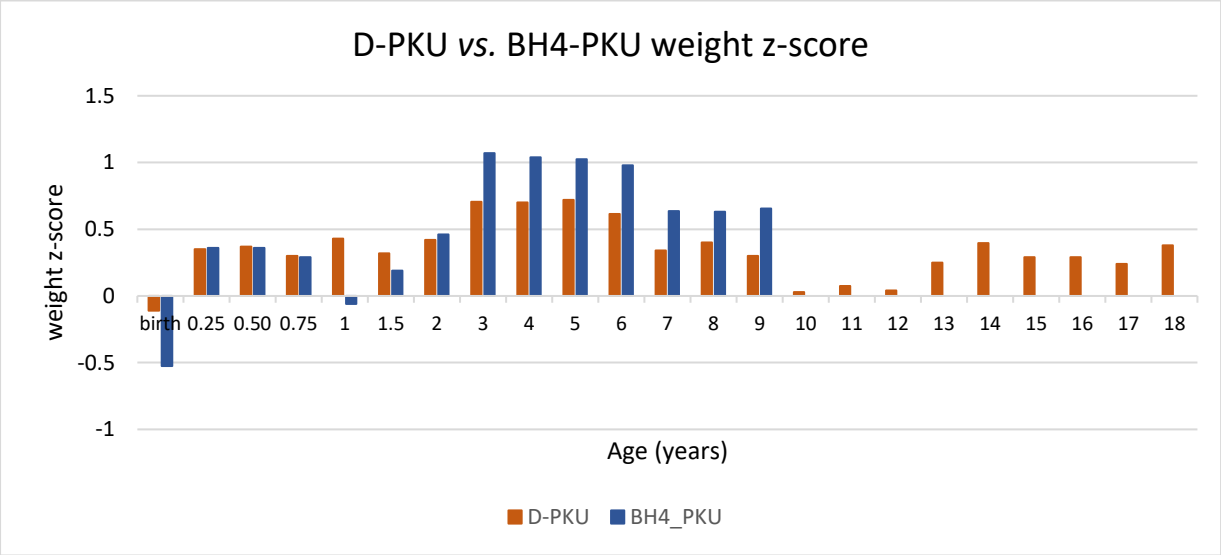
Table 2: Lifetime anthropometric outcomes for D-PKU and BH4-PKU patients: mean z-score for all data points collected over the patient's lifetime until the latest data collection episode for weight, height and BMI, categorised by z-scores: <-1, -1 to 1, >1-2 and >2

		Mean lifetime z-score			
Disorder	Growth parameter	<-1 n (%)	-1 to 1 n (%)	>1-2 n (%)	>2 n (%)
D-PKU	Weight: *z*-score	5/79 (6)	63/79 (80)	11/79 (14)	0/79
	Height: *z*-score	4/79 (5)	68/79 (86)	7/79 (9)	0/79
	BMI: *z*-score	1/73 (1)	57/73 (73)	10/73 (14)	5/73 (7)
BH4-PKU	Weight: *z*-score	0/14	13/14 (93)	1/14 (7)	0/14
	Height: *z*-score	0/13	10/13 (77)	3/13 (23)	0/13
	BMI: *z*-score	0/10	8/10 (80)	2/10 (20)	0/10
All-PKU	Weight: *z*-score	5/93 (5)	76/93 (82)	12/93 (13)	0/94
	Height: *z*-score	4/92 (4)	78/92 (85)	10/92 (11)	0/92
	BMI: *z*-score	1/83 (1)	65/83 (78)	12/83 (14)	5/83 (6)

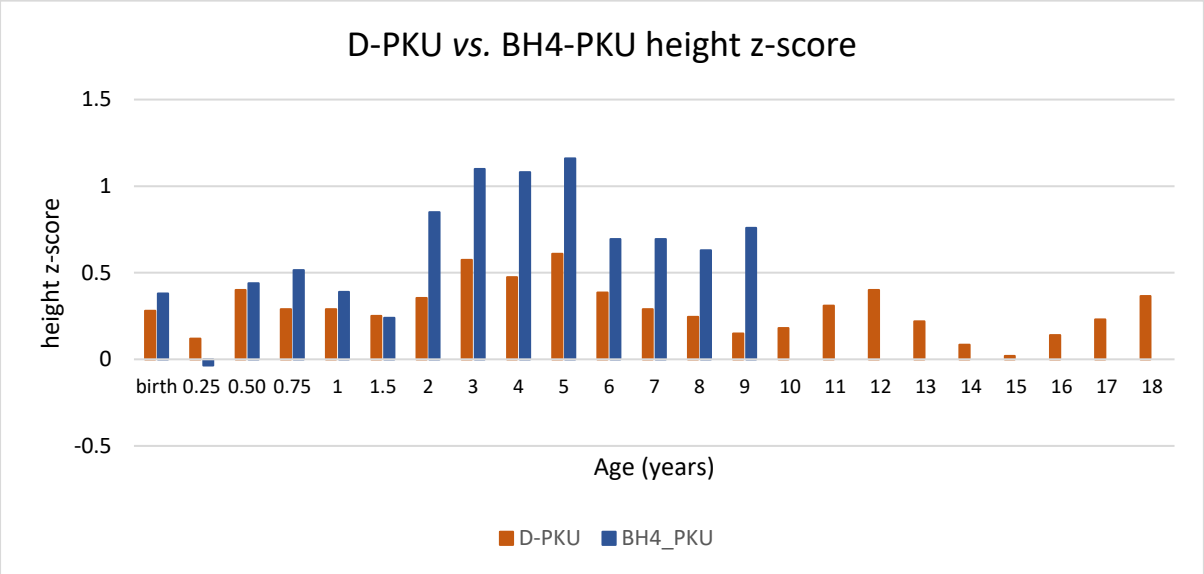
In both groups, median weight and BMI z-score was highest between 3 and 6 years of age (Table 1, Figure 1). Children in the BH_4-PKU group were significantly taller than children in the D-PKU group between 2 and 5 years of age (2 years: p=.007, 3 years: p=.049, 4 years: p=.024, 5 years: p=.021). There was no significant difference in weight score over the period of treatment, however D-PKU were significantly heavier at birth (p=.047), although the clinical significance of this is not clear. There was no significant difference in BMI-z-score at any age up to 9 years of age for which there are data.

Figure 1 depicts the median weight- 1a), height- 1b), and BMI- z-score 1c) of D-PKU and BH4-PKU patients from birth until 18 years of age.

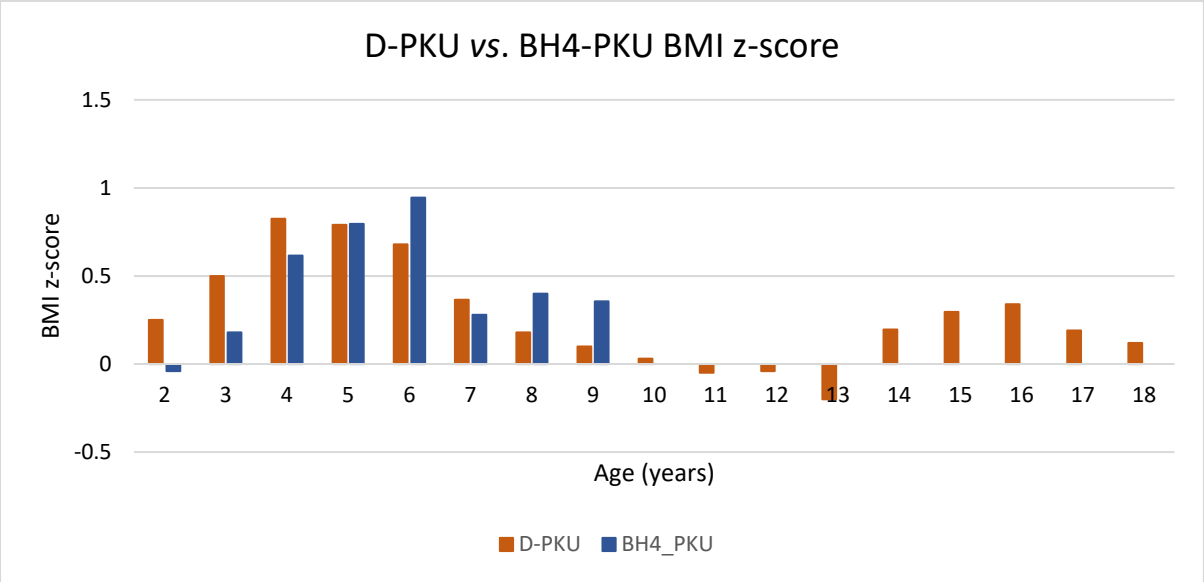
1a)



1b)



1c)

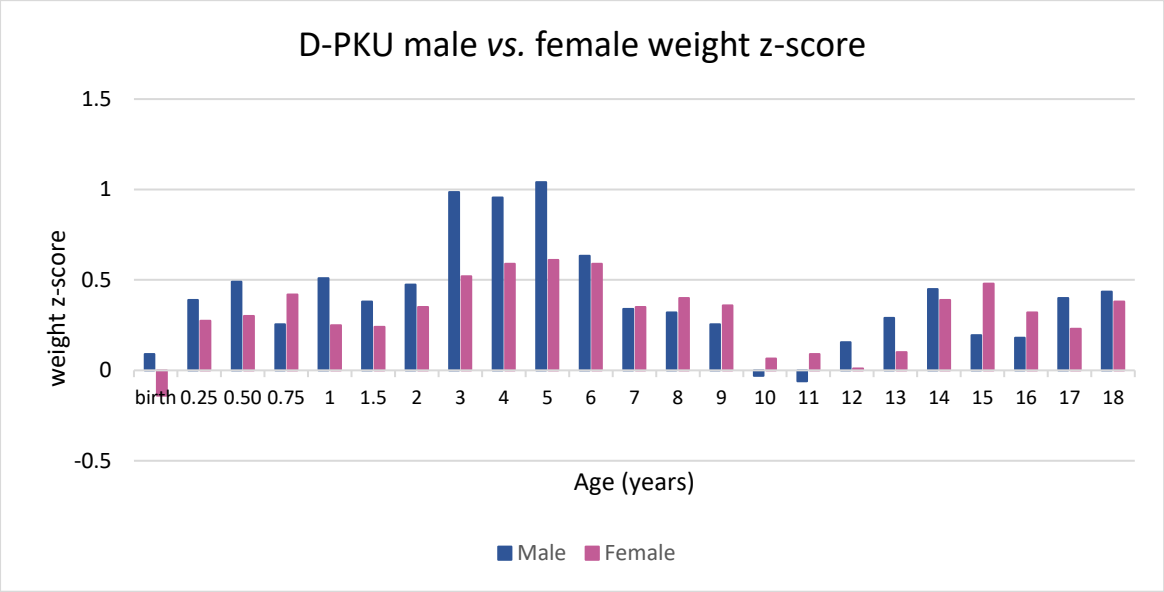


6.3.2 Does growth vary with gender?

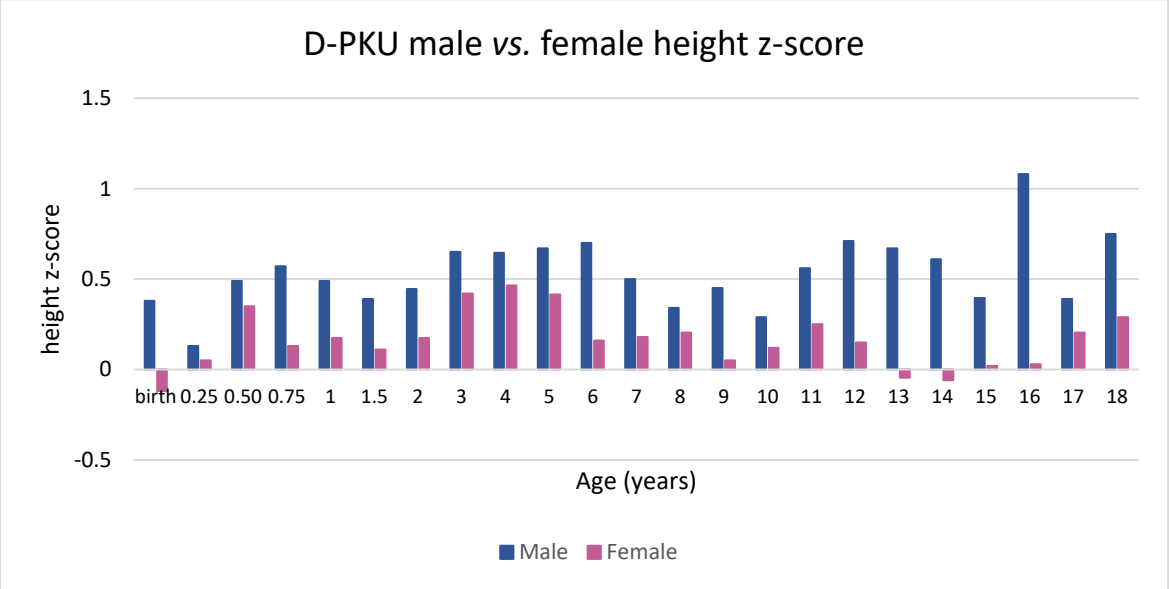
When the children in the D-PKU group were analysed by gender (Figure 2), there was no significant difference in weight z-score at any age except 3 years (p=.05), although males tended to have a higher median weight z-score than females There was no significant difference between males and females for height z-score, but males tended to have higher height z-scores than females. BMI z-score was significantly greater for boys at 3 years of age (p=.044). Smaller patient numbers during the adolescent years mean absolute conclusions cannot be drawn. (Figure 3)

Figure 2 depicts the median weight- 1a), height- 1b), and BMI- z-score 1c) of D-PKU male and D-PKU female patients from birth until 18 years of age.

2a)



2b)



2c)

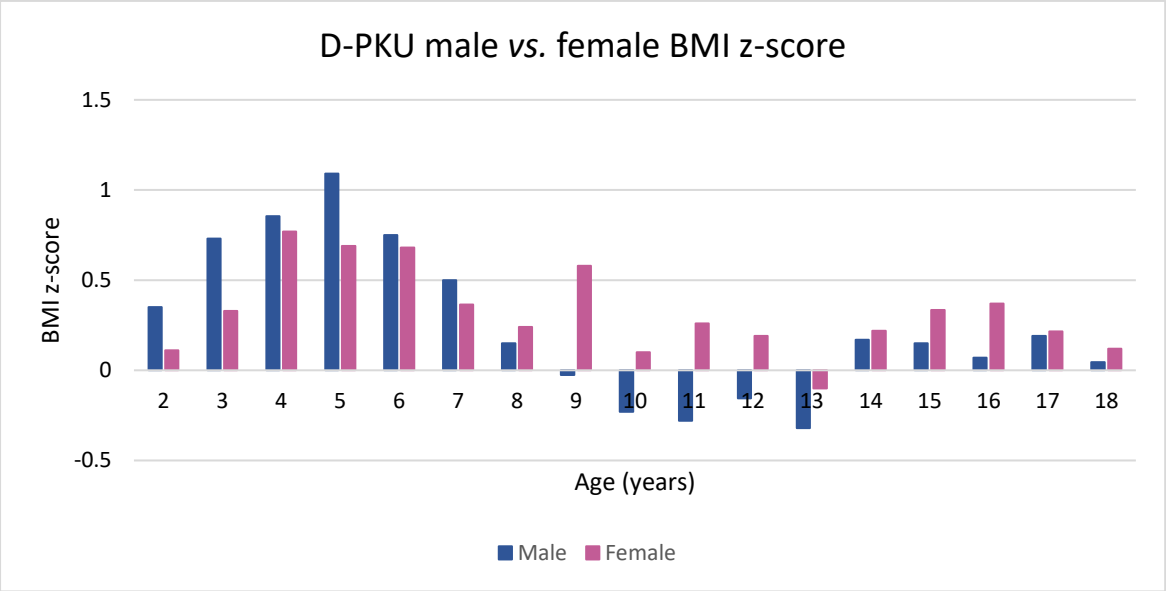
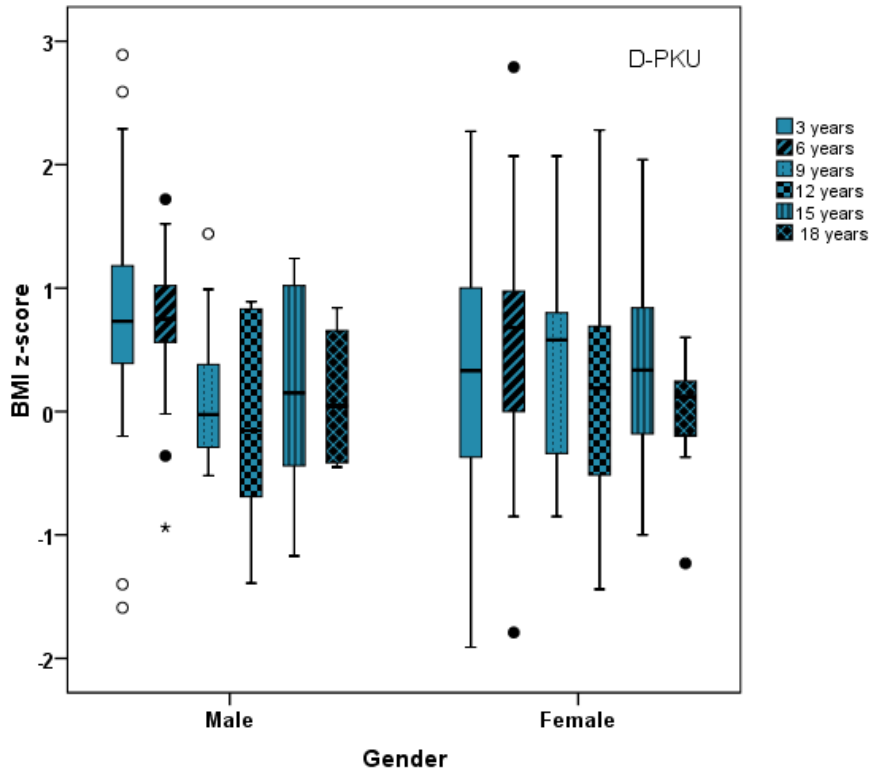


Figure 3 depicts the BMI z-score at key ages for the patients in the D-PKU group. The ends of the box represent the upper (Q3) and lower quartiles (Q1) and the median is marked by the vertical line inside the box. The whiskers represent the highest and lowest observations.



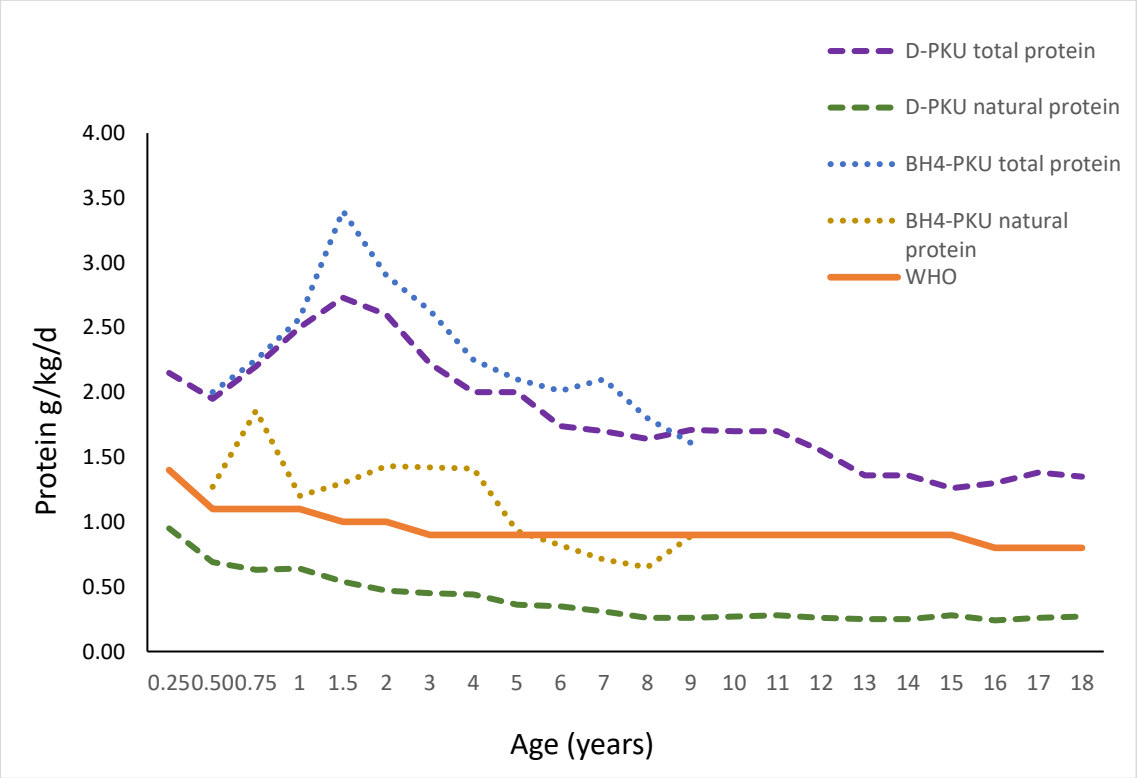
6.3.3 Is the prevalence of overweight increasing?

As BMI z-score is highest in the youngest age groups, we determined if overweight is an emerging problem and increasing in prevalence over time. We assessed the difference in BMI z-scores in children in the D-PKU group born pre-and post-2007, and found no significant difference in BMI z-score up to 6 years of age in those born in the last decade.

6.3.4 What was the dietary protein intake of our patients?

Figure 4 depicts the median total and natural protein intake for the D-PKU and BH4-PKU groups from 3 months of age until 18 years of age.

Figure 4



A total of 61/94 (65%) of patients consumed breast milk after diagnosis. Recorded duration of breastfeeding ranged from 2 weeks to 17 months. Duration of breast milk consumption was not documented for all patients as data were collected from clinic visits and breast milk consumption may have ceased between these times. A total of 22/94 (23%) of patients were not breast fed at time of diagnosis, and it is unclear if 11/94 (12%) infants were breast fed at all.

Median total-protein intake exceeded the FAO/WHO/UNU recommended safe levels at all ages Total protein intake was significantly higher in the BH_4-PKU group than in the D-PKU group at 3yrs (p=.020) only. Natural protein intake was significantly higher for BH_4-PKU group than D-PKU group at all ages except 8 years of age (1-5yrs p<0.001, 6yrs p=.001, 7yrs p=.011, 9yrs p=.008), and exceeded the FAO/WHO/UNU recommended safe levels until 5 years of age (Figure 4).

The amount of AAF consumed was significantly greater for D-PKU group than BH4-PKU group at all ages from 1 to 9 years of age (p<.005) (table 1). When patients who were BH4 responsive and who did not consume AAF were removed from this analysis, children with D-PKU still consumed significantly more AAF than children with BH4-PKU at all ages between 1 and 9 years except 2 years of age (1year: p<.0001, 3 years: p=.027, 4 years: p=.029, 5 years: p=.004, 6 years: p=.003, 7 years: p=.029, 8 years: p=.007, 9 years: p=.005).

6.3.5 What are the correlations between dietary variables and growth variables?

Table 3 depicts the correlation matrix between the growth variables: height-, weight-, BMI z-score and dietary variables: total protein, natural protein and AAF at key childhood ages of 3, 6, 9, 12 and 15 years of age. Analysis was completed for the all-PKU group (Table 3a), D-

PKU group (Table 3b) and BH4-PKU group (Table 3c). From 12 years of age, the all-PKU group includes only 1 patient treated with BH4

1. *Relationship between protein intake and weight*

There was a trend for a lower weight z-score with higher total protein intake that was consistent for D-PKU and BH4-PKU and when combined: all-PKU. In the all-PKU group a higher total-protein intake was correlated with a lower weight z-score at all key ages, and was significant at 3 years (p=.001) and 12 years (p=.001). In the D-PKU group this relationship was significant at 3 years (p< .0001), 6 years (p=.013), 9 years (p=.034) and 12 years (.001). The relationship was not significant for BH4-PKU at 3 or 6 years. There was insufficient data to explore this relationship after 6 years of age.

There were no significant relationships between natural protein intake and weight z-score for all-PKU or BH4-PKU. This relationship was significant for D-PKU at 3 years (p=.030) and 6 years (p= .007)

There was a significant relationship between AAF and lower weight z-score for all-PKU at 3 years (p=.002), D-PKU at 3 years (p=.003). For BH4-PKU there was a significant relationship between AAF and higher weight z-score at 6 years (p=.023)

2. *Relationship between protein intake and height*

There was no significant relationship between total protein intake or natural protein intake and height z-score at any key age for all-PKU, D-PKU or BH4-PKU. There was a significant relationship between AAF and lower height z-score for all-PKU at 3 years (p=.028), and 12 years (p=.002), for D-PKU at 12 years (p=.016).

3. Relationship between protein intake and BMI

Higher total-protein intake was correlated with a lower BMI z-score at all key ages for all-PKU, and this was significant at 3 years (p<.0001) and 12 years (p=.008). The same pattern was observed for D-PKU with significance observed at 3 years (p=.001) and 12 years (p=.007). The relationship was significant in BH4-PKU at 3 years of age (p=.045).

There was also a trend for lower BMI z-score with a higher natural protein intake for all groups. This was significant at 6 years of age for both all-PKU (p=.033) and D-PKU (p=.006).

There was a trend for a lower BMI z-score with a higher AAF for all groups and ages, except for BH4-PKU at 6 years. The relationship was significant for all-PKU at 3 years (p=.007), and D-PKU at 3 years (p=.005).

Table 3: Correlation matrix for weight-, height-, BMI- z-score and dietary intake at key childhood ages a) all-PKU b) D-PKU c) BH4-PKU. significant correlations are bolded include corresponding p value

a)

All-PKU					
Variables	**3 years**	**6 years**	**9 years**	**12 years**	**15 years**
	r_s	r_s	r_s	r_s	r_s
Weight z-score					
total protein	**-.371 (p=.001)**	-.242	-.263	**-.551 (p=.001)**	-.386
natural protein	-.157	-.216	-.130	-.046	-.264
AAF	**-.349 (p=.002)**	-.254	-.230	**-.516 (p=.002)**	-.222
Height Z-score					
total protein	-.063	-.022	-.151	-.336	-.308
natural protein	.080	.097	.114	.305	.071
AAF	**-.257 (p=.028)**	-.254	-.216	**-.454 (p=.012)**	-.078
BMI z-score					
total protein	**-.407 (p<.0001)**	-.200	-.224	**-.474 (p=.008)**	-.249
natural protein	-.201	**-.297 (p=.033)**	-.209	-.198	-.333
AAF	**-.313 (p=.007)**	-.145	-.125	-.330	-.075

b)

D-PKU					
Variables	**3 years**	**6 years**	**9 years**	**12 years**	**15 years**
	r_s	r_s	r_s	r_s	r_s
Weight z-score					
total protein	**-.451 (p<.0001)**	**-.372 (p=.013)**	**-.327 (p=.034)**	**-.566 (p=.001)**	-.386
natural protein	**-.267 (p=.030)**	**-.399 (p=.007)**	-.248	-.069	-.264
AAF	**-.362 (p=.003)**	-.233	-.173	-.505	-.222
Height Z-score					
total protein	-.193	-.127	-.149	-.323	-.308
natural protein	-.099	-.072	.073	.310	-.073
AAF	-.180	-.084	-.151	**-.444 (p=.016)**	-.078
BMI z-score					
total protein	**-.413 (p=.001)**	-.279	-.278	**-.489 (p=.007)**	-.249
natural protein	-.210	**-.276 (p=.006)**	-.305	-.244	-.333
AAF	**-.349 (p=.005)**	-.133	-.097	-.309	-.075

c)

BH4-PKU		
Variables	**3 years**	**6 years**
	r_s	r_s
Weight z-score		
total protein	-.176	-.073
natural protein	-.248	-.286
AAF	.080	**.778 (p=.023)**
Height Z-score		
total protein	.251	.236
natural protein	.283	.036
AAF	-.142	.539
BMI z-score		
total protein	**-.678 (p=.045)**	-.127
natural protein	-.517	-.321
AAF	-.126	.623

6.4 Discussion

There is a recognised spectrum of severity in individuals with PKU. In addition to phenylalanine control for best neurocognitive development, measuring and attaining optimal growth outcomes are a key focus in clinical management and long-term follow up in PKU. To date, reports on physical development and growth outcomes documented in PKU have

varied.(25) Early reports documented that anthropometric parameters in PKU may be compromised due to the dietary restrictions required to maintain blood phe levels in the range associated with best neurocognitive outcomes. Proposed 'gold standard' assessments include comprehensive dietary, growth, body composition, social and biochemical evaluations to monitor the evolution, rather than just the prevalence, of overweight and obesity and associated morbidities including metabolic syndrome.(9)

6.4.1 What are the longitudinal patterns of growth in children with PKU?

In this study, we retrospectively evaluated longitudinal growth patterns and dietary protein intake in children across the spectrum of severity of PKU. The results confirm reports of essentially normal growth in children with PKU.(34-36)

Recent studies have documented longitudinal growth from birth to 18 years of age. Belanger-Quintana et.al. compared growth to age-matched reference values and found no significant differences in growth parameters except for females >13yrs with more 'severe' PKU who had increased weight and BMI z-scores.(39) Aldamaz-Echevarria et.al. documented a fall in height z-score in their patient group from birth to 2 years of age and again on reaching adulthood.(30) More recently, no significant differences were seen between patients with PKU and mild hyperphenylalaninaemia and the general population, however, height was slightly lower and weight was slightly higher than in the general population.(36)

Early childhood overweight has been documented in PKU.(31, 33, 37, 49, 50) In our cohort, we also observed that the highest median BMI z-scores in both the D-PKU and BH_4-PKU groups were in children < 6 years of age. The prevalence of overweight did not appear to be worsening over the last decade: No difference was observed in BMI z-score up to 6 years of age in children born pre- versus post- 2007 in D-PKU, however in all-PKU, which included

those treated with BH_4, children born post 2007 had a significantly higher BMI z-score only at 5 years of age.

When assessed by lifetime mean BMI z-score, 17/83 (20%) in the all-PKU group had a mean lifetime BMI z-score >1, meeting the CDC classification of 'overweight' (46). This is slightly lower than a recent estimation of 26% overweight and obesity rates in Australian children 2-17 years of age.(51) Our results compare favourably to a retrospective chart review of 85 paediatric PKU patients (2-20 years of age) from two centres in the USA where 40% of patients were overweight or obese.(33) The level of overweight in our group were also lower than that reported in a prospective cross-sectional analysis of 89 Portuguese patients (3-30 years of age), where the prevalence of overweight and obesity was 32.6% compared to 24% in the control group.(52) Potential causes for overweight in PKU include: excessive energy intake or inadequate energy expenditure (37), parental overweight and early BMI rebound.(50) An important cause is non-compliance with a low-phenylalanine diet and AAF (33), as adequate AAF is suggested to induce satiety and help reduce energy intake from protein free foods. This hypothesis has some support from a report demonstrating a lower, but non-significantly different calorie intake from foods in children with PKU when consuming a higher energy dense formula than a lower energy dense formula.(53) In an additional study of 133 children aged 2-10 years, weight for height ratio was also associated with higher phe levels and poor dietary adherence.(32) The authors proposed that higher phe levels meant a higher energy intake from food consumed, however this was not measured. Furthermore, the challenges of maintaining a strict dietary regimen from a young age, and the consequent affect this may have on eating behaviours (54) is also a potential risk factor for overweight. Patients with known AAF non-compliance were excluded from our analysis, and interpretation of causes of overweight in our patient group is limited as long-term energy intake data are not available on all patients.

In contrast to other reports,(26, 27, 30) we did not find any growth impairment in the first years of life. During these early years, when blood-phe monitoring is most frequent and very tight phe control is emphasised, there is a risk that dietary intake of essential nutrients or protein could be more restricted.(26, 38) Several earlier reports documented a moderate reduction in longitudinal growth patterns,(26, 28, 30, 42) with suggestions that this may be related to lower phenylalanine or tyrosine intake,(26, 30) lower lipid intake,(28) an undetermined nutrient deficiency such as zinc(42) and protein insufficiency as documented by low pre-albumin levels.(55) Actual determinants associated with these finding have been inconclusive with other studies showing no association with intake of phe or tyr.(27, 40, 41) In this regard, our findings in the BH4-PKU group are intriguing. These children consume a higher natural protein intake (some are on an unrestricted diet). Despite the smaller number of patients in this group, we observed that some of these children were significantly taller between 2-5 years of age than children on diet alone. As BH_4 has only been used as a treatment for PKU in our clinic since 2003, there are fewer patients with BH_4-PKU and this has meant that firm conclusions could not be drawn regarding differences in growth between these groups. Nevertheless, this result differs from a previous report where no difference was found.(4) While the reasons for this are unclear and greater patient numbers and dietary intake data is necessary to confirm these finding and provide further insight.

6.4.2 Does amount of total protein intake, or type of protein impact on growth trajectory in children with PKU?

Total-protein intake in all-PKU met or exceeded WHO safe protein recommendations(47) but did not meet PKU specific recommendations in children <3 months of age.(10) Median natural-protein intake for age in our cohort of D-PKU is comparable to other reports,(30, 35)

and not surprisingly natural-protein intake in this groups did not meet WHO safe protein intakes at any age. However, comparison of natural-protein intake and phe tolerance between centers requires careful interpretation as it may be influenced by differing phe targets based on location.(56, 57) Natural protein intake may also vary depending on practices and control measures that do not maximise phe tolerance, such as allowing phe levels to stay below the target ranges.(57) Allowing natural protein to phe tolerance requires ongoing assessment and adjustment with age and changes in body weight, and an increased phe tolerance above the phe-intake prescribed has been documented in adults.(58) This is important as increased phe tolerance has also been associated with improved compliance in adults.(59) The high AAF in our BH_4-PKU patients may reflect either over-prescription, over-consumption or the need to maximise BH_4 dose, which would allow a greater natural-protein intake and a reduction in AAF. Overall, high total-protein intakes in all-PKU are likely to reflect the need for additional AAF to control phe levels, rather than just meet nutritional requirements. A move to standarisation of phe targets will help to make future comparisons of natural protein and phe intake and phe tolerance more meaningful.(11) Australasian Guidelines for blood-phe targets that correspond with recently endorsed European guidelines,(11) and new guidelines for the use of BH4 are currently being considered by all clinics where individuals with PKU are treated. If implemented this will also allow more direct comparisons between PKU populations.

6.4.3 What are the challenges of protein intake in the first few months of life?

Protein recommendations of 2.5-3g/kg/d in the first months of life may be difficult to achieve in breastfed infants without providing an excessive energy intake or over-riding natural satiety. We documented that approximately 65% of infants continued to consume some breastmilk after diagnosis, though its duration varied widely. Statistics from Australian

surveys suggest that although 96% of mothers initiate breast feeding, only 39% of babies are still exclusively breast fed until 3 months (< 4 months of age),(60) and that only 28% of children are still being breastfed at 12 months of age.(61) Breastfeeding has been shown to increase IQ significantly in children with PKU compared to infants who received only formula feeding,(62) however since this report, AAF have improved significantly in their composition particularly in the addition of pre-formed LCPUFA's including DHA. Although breastfeeding is recommended in children with PKU, breastfeeding rates are rarely reported. Agostini et.al reported that the breast-feeding rate for hyperphenlalanaemic infants in their cohort was lower than for the reference Italian population.(63)

Increased AAF 'protein' in early childhood can be achieved from the introduction of more amino acid dense transitional formulas,(64) and specific dietary management of intercurrent illnesses.(10)

6.4.4 What is the relationship between protein intake and growth?

Attempts to investigate the effect of dietary treatment on growth have proved inconclusive. While some authors have concluded that total protein intake, if adequate, is not related to growth outcomes,(12, 42, 65) the possibility that either the natural protein intake or the amino acid component of the diet exerts a greater influence on this outcome is acknowledged.(25, 30, 34, 43)

In contrast to the study by Aldámiz-Echevarría et.al.,(4) we did not observe a statistically significant correlation between greater median natural-protein intake and increased height z-score at any key childhood ages in all-PKU or D-PKU, neither did we observe this correlation in BH4-PKU children despite their higher median natural-protein intake. Our results support those of Hoeksma et.al., who observed that neither protein nor energy intake correlated with

linear growth; yet in that study there was a statistically significant association between head circumference growth and natural and total-protein intake.(43)

Our results support the view that strategies to enhance maximum natural-protein tolerance may be important to enhancing normal growth in PKU. We document a consistent trend for a lower weight z-score with higher total protein intake and higher natural protein intake, which was significant at some key ages (Table 3). Our results support the view that higher AAF intake is associated with lower weight z-scores. This may be because AAF may aid satiety and reduce appetite for other foods.(54) However, consumption of AAF is only one aspect of dietary compliance and one cannot assume that this is the only factor affecting weight gain.

Although adequate AAF is necessary to ensure target phe levels are attained in PKU, the amounts required may be variable and individual,(14) and overconsumption may contribute to an excessive protein and energy intake. We did not see this trend in our patients, rather, we observed a trend for lower weight z-score and lower BMI-z score in those with higher total protein intakes and AAF. Nevertheless, this notion deserves ongoing consideration in view of the debate regarding an association between high protein intake in the first year of life and risk of later overweight in healthy children.(66) The potential negative effect of high protein intake has been reviewed previously in PKU.(50) In that study, no relationship between incidence of overweight at 8 years of age and protein intake at 1 year of life was observed, in children requiring dietary therapy as well as those with mild hyperphenylalaninaemia, who consumed less protein than healthy Italian children.

Overall PKU dietary intake is defined by natural protein tolerance, that is the amount of natural protein that can be consumed while maintaining blood phenylalanine levels. Consequently, natural protein intake is individualised, highly monitored and more controlled than AAF. Higher natural protein intake confers some advantage to the patient

as it allows for a greater dietary variety and more 'whole' or 'natural' foods to be included. Consequently, more natural protein could be considered more 'desirable' from a patient perspective. While AAF is associated with favourable weight status, the value of a higher natural protein intake is key to the food choices available to the patient and should be prioritised. We observed that the higher use of AAF is in fact associated with higher weight z-score for 6 years old children in the BH4-PKU group, however strong conclusions cannot be drawn due to small patient numbers and we acknowledge the limitations of a retrospective study.

6.4.5 What feeding patterns could contribute to improved weight status in PKU?

While PKU patients appear to handle dietary protein in a similar manner to healthy individuals,(25) there are still several key differences between the protein intake of children with PKU and healthy children. These may allow these children to tolerate higher amounts of protein without a negative effect on body weight, or may promote a positive effect, despite the body's limited capacity for storing protein for later anabolic use. In healthy adults, there is a body of evidence that suggests that increased protein above the recommended intakes may convey health benefits, particularly for muscle mass and functional capacity beyond those who just consume the recommended intake.(67, 68) However, a notable difference between the diet of the general population and the PKU diet is the mealtime distribution of protein. Except in infants, when feeding patterns and therefore protein distribution are similar to those in healthy infants, the protein distribution at meals in older children and adolescents is likely to be considerably different. For individuals with PKU, it is recommended that protein, both natural and AAF, be spread evenly over the day as this supports metabolic control,(69) whereas the traditional western diet tends to have a lower protein, carbohydrate rich breakfast with an increased protein intake at the evening meal. Data from the NHANES

survey, documented a protein intake at the evening meal to be approximately 3 times that consumed at breakfast for US adults.(70) The effects of daily protein distribution have been described in several studies. An increased 24-h muscle protein synthesis of ~ 25% was observed in a study of young adults comparing the same total protein intake distributed evenly across main meals versus an intake skewed towards a more protein dense evening meal.(70) The benefit to improved preservation of lean tissue mass has also been demonstrated when the protein intake was supplemented at breakfast and lunch in healthy adults.(71) Moreover, consuming moderate amounts of high-quality protein at each meal may also provide a dietary platform that not only supports the maintenance of muscle mass and function, but promotes healthy weight management.(72) A less recognised benefit that may be derived from the distribution patterns of protein in PKU patients, is the idea that pre-sleep protein intake may also support overnight muscle protein synthesis.(73) While this effect has been measured in adults only after a bout of resistance training, it may be that consumption of pre-bed protein does convey some benefit beyond exercise recovery. To promote better blood phe and tyrosine levels, we recommend that children in our clinic limit the number of hours without AAF to up to12 hours overnight, which often means a dose of AAF pre-bed. While this is suggested to improve metabolic control, there may also be some benefits to body weight and body composition that have not been appreciated. While most studies examining the effect of protein distribution on muscle protein synthesis have been in adults, it may also be relevant to children, however this will need further examination.

6.4.7 What are the mechanisms suggested for the effect of protein on body weight and composition?

Several mechanisms have been proposed to explain the benefit from increased protein intake in the healthy population, on weight and body composition, including a reduction in dietary

energy intake mediated by an effect on satiety;(74) an increase in REE due to a greater diet-induced thermogenesis;(75) an influence on growth hormone and IGF-1 production on body composition;(76) and a stimulatory effect on muscle protein anabolism favouring the retention of lean muscle mass.(77) Over a 6-year period, an inverse relationship between dietary protein intake and change in fat mass index was observed in lean girls, via a decrease in body fat gain and increase fat free mass gain.(76) Benefit of a high protein intake has been documented in individuals with inborn errors of long chain fatty acid disorders (LCFAOD).(78) In a short-term study REE was significantly higher when patients with LCFAOD were prescribed a higher protein (30% of total energy) versus a higher carbohydrate diet. Another potential mechanism for the effect of protein intake on weight and body composition including in patients with PKU is via a hormonal response. It has been shown that: "protein intake stimulates insulin and insulin like growth factor 1 metabolism (IGF1), which consequently leads to increased cell proliferation, growth and increased adipose tissue".(66) As the major source of protein in PKU is L-amino acids that are more rapidly absorbed and oxidised compared to intact protein, this may contribute to an altered metabolic or hormonal response. Currently there is no evidence to suggest this is the case, as normal IGF1, IGFBP3 and thyroid hormones levels are reported,(42) and insulin levels in PKU were not different to a group of controls.(52)

Another possible mechanism is the P:E ratio, because protein and energy intake are co-dependent as discussed in chapters 1-2. However, in the current study we do not have adequate data on longitudinal energy intake which limits the ability to explore this. Therefore, to investigate the relationship between protein and energy intake and the value of AAF as protein, we needed a prospective study. In Chapter 8 we present the results of this study.

Table 1. Anthropometric and dietary intake data from birth to 18 years of age for D-PKU and BH4-PKU patients

			Birth		*3 months*		*6 months*		*9 months*		*12 months*
Disorder	Variable	n	Median range	n	Median range	n	Median range	n	Median range	n	Median range
PKU	Weight: *z*-score	66	-.11(-1.85, 1.94)	66	.35 (-2.04, 2.34)	64	.37 (-1.72, 2.13)	63	.30 (-2.63, 2.13)	67	.43 (-2.13, 1.61)
	Height: *z*-score	34	.28 (-1.85, 2.77)	51	.12 (-1.82, 2.65)	55	.40 (-2.17, 3.39)	48	.29 (-1.81, 3.18)	57	.29 (-1.97, 2.09)
	BMI: *z*-score										
	Total protein: g/kg/d			56	2.15 (1.4, 4.04)	53	1.95 (1.4,3.73)	57	2.2 (1.2, 3.39)	64	2.5 (1.6, 5.8)
	Nat protein: g/kg/d			56	.95 (.51, 1.33)	54	.69 (.42, 2.09)	57	.63 (.28, 1.19)	64	.64 (.36, 1.71)
	AAF: g/kg/d			63	1.25 (.4, 3.02)	61	1.13 (.23, 3.04)	61	1.61 (.52, 2.6)	66	1.87 (.76, 4.0)
	Energy: %BMR			52	195 (122, 288)	33	163 (108, 216)	5	147 (113, 188)	0	
	P:E ratio			50	2.5 (1.7, 2.9)	31	2.5 (1.6, 3.3)	4	3.05 (2.5, 4.7)	0	
BH4	Weight: *z*-score	10	-.53 (-1.88, .78)	11	.36 (-.92, 1.49)	12	.36 (-1.31, 1.55)	11	.29 (-1.74, 1.3)	13	-.06 (-1.34, 1.45)
responsive	Height: *z*-score	5	.38 (-.37, 1.21)	4	-.035 (-1.39, .78)	5	.44 (-1.23, 1.13)	8	.52 (-.72, 2.28)	10	.39 (-1.82, 2.22)
PKU	BMI: *z*-score										
	Total protein: g/kg/d			2	1.67 (1.34, 2.0)	8	2.0 (1.4, 3.6)	8	2.25 (1.4, 4.3)	12	2.6 (1.4, 5.2)
	Nat protein: g/kg/d			2	1.4 (1.3, 1.45)	8	1.3 (.9, 3.1)	8	1.9 (.6, 3.2)	12	1.2 (.5, 4.7)
	AAF: g/kg/d			6	.0 (0.0, 2.38)	12	.5 (0.0, 1.33)	10	.4 (0.0, 1.4)	12	1.0 (0.0, 1.7)
	Energy: %BMR			2	140 (106, 173)	2	172 (165, 178)	2	228 (198, 257)	0	
	P:E ratio			2	2.35 (2.3, 2.4)	2	2.04 (2.0, 2.08)	2	2.0 (1.6, 2.3)	0	

			18 months		*2 years*		*3 years*		*4 years*		*5 years*
Disorder	Variable	n	Median range	n	Median range	n	Median range	n	Median range	n	Median range
PKU	Weight: *z*-score	67	.32 (-1.96, 1.77)	67	.42 (-2.55, 2.04)	66	.71 (-1.58, 1.96)	59	.70 (-1.20, 2.14)	55	.72 (-1.87, 2.63)
	Height: *z*-score	57	.25 (-1.9, 1.82)	62	.36 (-1.84, 2.64)	64	.58 (-1.39, 2.22)	58	.48 (-2.28, 2.21)	53	.61 (-2.3, 2.27)
	BMI: *z*-score			48	.25 (-3.38, 1.73)	64	.50 (-1.91, 2.89)	58	.33 (-1.47, 3.26)	53	.79 (-2.06, 2.99)
	Total protein: g/kg/d	64	2.73 (1.7, 4.07)	66	2.6 (1.7, 5.5)	66	2.22 (1.65, 3.8)	59	2.0 (1.4, 3.9)	55	2.0 (1.4, 3.9)
	Nat protein: g/kg/d	64	.54 (.21, 1.55)	66	.47 (.21, 1.58)	66	.45 (.13, 1.47)	59	.44 (.11, 1.09)	55	.36 (.10, 1.0)
	AAF: g/kg/d	64	2.15 (1.07, 3.43)	66	2.06 (1.12, 5.11)	66	1.79 (.8, 3.16)	59	1.6 (.86, 2.86)	55	1.51 (.94, 2.63)
	Energy: %BMR	2	171 (161, 181)	6	148 (137, 167)	4	164 (143, 180)	5	144 (134, 212)	3	159 (137, 161)
	P:E ratio	2	3.6 (3.4, 3.7)	6	3.35 (3.1, 3.9)	4	3.1 (3.0, 3.6)	5	3.2 (2.5, 3.6)	3	2.9 (2.8, 3.8)
BH4	Weight: *z*-score	11	.19 (-.08, 1.34)	10	.46 (-.13, 1.87)	10	1.07 (.08, 2.21)	10	1.05 (.07, 2.21)	10	1.05 (-.15, 2.02)
responsive	Height: *z*-score	9	.24 (-1.26, 1.26)	9	.85 (-.03, 1.72)	9	1.1 (-.54, 1.92)	10	1.09 (.1, 2.17)	10	1.16 (.24, 2.08)
PKU	BMI: *z*-score			6	-.04 (-1.13, 1.88)	9	.18 (-.29, 2.06)	10	.62 (-.53, 1.95)	10	.80 (-1.64, 2.17)
	Total protein: g/kg/d	11	3.4 (1.85, 4.8)	9	2.9 (2.3, 3.8)	10	2.6 (2.1, 4.0)	10	2.2 (1.7, 3.7)	9	2.1 (1.5, 3.5)
	Nat protein: g/kg/d	11	1.3 (.6, 3.9)	9	1.4 (.7, 2.85)	9	1.4 (.7, 2.9)	10	1.4 (.4, 2.9)	9	.93 (.34, 2.6)
	AAF: g/kg/d	11	1.5 (0.0, 2.6)	10	1.3 (0.0, 2.4)	10	1.0 (0.0, 2.4)	10	1.0 (0.0, 2.2)	10	.9 (0.0, 1.94)
	Energy: %BMR	0		0		0		0		0	
	P:E ratio	0		0		0		0		0	

			6 years		*7 years*		*8 years*		*9 years*		*10 years*
Disorder	Variable	n	Median range	n	Median range	n	Median range	n	Median range	n	Median range
PKU	Weight: *z*-score	46	.62 (-1.47, 2.44)	45	.34 (-1.46, 2.54)	44	.40 (-1.45, 2.33)	42	.30 (-1.66, 1.65)	36	-.03 (-1.94, 1.59)
	Height: *z*-score	46	.39 (-2.62, 2.05)	44	.29 (-1.75, 1.56)	44	.25 (-1.93, 1.82)	41	.15 (-1.92, 2.12)	36	.18 (-2.13, 1.97)
	BMI: *z*-score	46	.70 (-1.79, 2.79)	44	.37 (-1.17, 2.32)	44	.18 (-.96, 2.52)	41	.10 (-.85, 2.07)	36	.03 (-1.77, 1.93)
	Total protein: g/kg/d	44	1.74 (1.09, 2.35)	45	1.7 (1.2, 2.2)	44	1.64 (1.1, 2.35)	42	1.71 (1.2, 2.5)	35	1.7 (1.1, 3.37)
	Nat protein: g/kg/d	45	.35 (.10, .70)	45	.31 (.10, .68)	44	.26 (.09, .88)	42	.26 (.08, .68)	35	.27 (.10, .79)
	AAF: g/kg/d	45	1.42 (.9, 2.26)	45	1.36 (.91, 1.88)	44	1.39 (.85, 2.14)	42	1.39 (.85, 2.4)	35	1.41 (.95, 2.2)
	Energy: %BMR			3	165 (152, 185)	3	134 (122, 171)	6	134 (124, 204)	7	175 (150, 241)
	P:E ratio			3	2.4 (1.9, 2.5)	3	3.0 (2.6, 5.0)	6	4.1 (3.2, 4.8)	7	3.0 (2.6, 4.2)
BH4 responsive PKU	Weight: *z*-score	8	.98 (.18, 2.14)	8	.64 (-.5, 2.12)	6	.63 (.53, 1.98)	4	.63 (.53, 1.98)	2	.96 (.89, 1.03)
	Height: *z*-score	8	.70 (.20, 2.44)	8	.70 (.03, 1.97)	6	.63 (-.06, 1.11)	4	.76 (.1, .97)	2	.33 (.31, .35)
	BMI: *z*-score	8	.95 (-.40, 1.94)	8	.28 (-1.6, 2.03)	6	.40 (-.02, 2.21)	4	.36 (-.33, 1.33)	2	1.09 (1.0, 1.17)
	Total protein: g/kg/d	7	2.0 (1.3, 2.7)	5	2.1 (1.4, 3.4)	4	1.8 (.9, 2.3)	3	1.61 (1.4, 2.1)	2	1.6 (1.6, 1.7)
	Nat protein: g/kg/d	7	.8 (.3, 2.7)	5	.7 (.2, 3.4)	4	.65 (.1, 2.3)	3	.9 (.4, 2.1)	2	.93 (.34, 2.63)
	AAF: g/kg/d	9	.71 (.00, 1.52)	8	.8 (0.0, 1.42)	6	.73 (0.0, 1.21)	4	.53 (0.0, .96)	2	.6 (0.0, 1.14)
	Energy: %BMR			1	138	1	181	1	154	1	122
	P:E ratio			1	4.7	1	3.0	1	2.8	1	4

			11 years		*12 years*		*13 years*		*14 years*
Disorder	Variable	n	Median range	n	Median range	n	Median range	n	Median range
PKU	Weight: *z*-score	34	.08 (-2.04, 1.59)	31	.04 (-2.1, 2.0)	23	.25 (-2.45, 2.02)	22	.4 (-2.62, 1.76)
	Height: *z*-score	34	.31 (-2.12, 2.31)	29	.40 (-2.3, 2.07)	23	.22 (-2.55, 1.46)	22	.09 (-2.81, 1.57)
	BMI: *z*-score	34	-.05 (-1.68, 1.78)	29	-.04 (-1.44, 2.28)	23	-.20 (-1.53, 2.34)	22	.20 (-1.1, 2.09)
	Total protein: g/kg/d	34	1.7 (1.1, 2.87)	31	1.55 (.97, 2.3)	23	1.36 (.84, 2.51)	22	1.36 (.84, 2.51)
	Nat protein: g/kg/d	34	.28 (.09, .69)	31	.26 (.09, .78)	23	.25 (.08, .61)	22	.25 (.07, .83)
	AAF: g/kg/d	34	1.31 (.86, 2.27)	31	1.28 (.76, 1.7)	23	1.08(.16, 2.01)	22	1.09 (.81, 1.84)
	Energy: %BMR	6	157 (125, 218)			4	134 (99, 163)	6	125 (107, 188)
	P:E ratio	6	3.75 (3.2, 5.0)			4	4.4 (3.9, 4.7)	6	4.4 (2.8, 5.4)
BH4 responsive PKU	Weight: *z*-score	2	1.02 (.95, 1.08)	1	1.14				
	Height: *z*-score	2	.35 (.17, .52)	1	.64				
	BMI: *z*-score	2	1.21 (1.21, 1.21)	1	1.24				
	Total protein: g/kg/d	1	1.4	1	1.6				
	Nat protein: g/kg/d	1	.4	1	.40				
	AAF: g/kg/d	2	.5 (0.0, 1.0)	1	1.2				
	Energy: %BMR	0							
	P:E ratio	0							

			15 years		*16 years*		*17 years*		*18 years*
Disorder	Variable	n	Median range	n	Median range	n	Median range	n	Median range
PKU	Weight: *z*-score	20	.29 (-.77, 1.76)	16	.29 (-.69, 1.67)	13	.24 (-.91, 1.62)	12	.38 (-.86, 1.57)
	Height: *z*-score	20	.02 (-1.54, 1.78)	16	.14 (-1.68, 2.42)	13	.23 (-1.14, 2.19)	12	.37 (-.99, 2.12)
	BMI: *z*-score	20	.30 (-1.17, 2.04)	16	.34 (-1.61, 2.12)	13	.19 (-1.24, 1.51)	12	.12 (-1.23, .84)
	Total protein: g/kg/d	20	1.26 (.96, 1.82)	15	1.3 (.90, 1.75)	13	1.38 (1.09, 1.72)	12	1.35 (1.04, 1.67)
	Nat protein: g/kg/d	20	.28 (.11, .77)	15	.24 (.09, .62)	13	.26 (.09, .74)	12	.27 (.15, .79)
	AAF: g/kg/d	20	.96 (.15, 1.59)	16	.98 (.76, 1.28)	13	1.09(.73, 1.26)	12	1.02(.85, 1.32)
	Energy: %BMR	3	113 (88, 180)	2	131 (100, 161)	2	120 (105, 134)	3	123 (118, 159)
	P:E ratio	3	4.9 (3.4, 6.8)	2	4.45 (4.0, 4.9)	2	5.0 (4.5, 5.4)	3	4.6 (3.3, 4.7)
BH4 responsive PKU	Weight: *z*-score								
	Height: *z*-score								
	BMI: *z*-score								
	Total protein: g/kg/d								
	Nat protein: g/kg/d								
	AAF: g/kg/d								
	Energy: %BMR								
	P:E ratio								

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Chapter 7: The validity of bioelectrical impedance analysis to measure body composition in Phenylketonuria.

This chapter has been submitted to the Journal of Inherited Metabolic Disease (JIMD): Reports and was accepted for publication on 10/11/17

Title:

The validity of bioelectrical impedance analysis to measure body composition in Phenylketonuria.

Abstract:

Aim: To compare the measurement of total body water (TBW) and fat free mass (FFM) using the criterion method of deuterium dilution space (^2H_2O) with bioelectrical impedance analysis (BIA) using a portable QuadScan 4000, Bodystat® in children and adolescents with Phenylketonuria (PKU).

Methods: Sixteen patients with PKU, median age 12.5 (range 5 – 20.6) years were recruited into this cross-sectional study. TBW was measured by both deuterium dilution and BIA on the same occasion as per a standard protocol. FFM was estimated from predictive equations.

Results: There was no significant difference between TBW_{Deut} and TBW_{BIA} ($p=.344$), or FFM_{Deut} and FFM_{BIA} ($p= .111$). TBW_{Deut} and TBW_{BIA} were highly correlated ($r=.990$ $p<.0001$), as were FFM_{Deut} and FFM_{BIA} ($r=.984$, $p< .0001$). Bland-Altman plots demonstrated that there was no proportional bias between the criterion method, TBW_{Deut}, and the test method TBW_{BIA}, in estimating TBW ($\beta = -.056$, adjusted $r^2 =.069$ $p=.169$), or FFM ($\beta = -.089$, adjusted $r^2 =.142$ $p=.083$).

Conclusion: Our results suggest that when compared with the criterion method, the QuadScan 4000, Bodystat® can reliably be used to predict TBW and FFM in patients with PKU. We suggest that due to the portability and non-invasive approach, this method can reliably be used to monitor body composition in the outpatient clinic setting, to further improve the monitoring and assessment of nutritional status in PKU.

Introduction

Phenylketonuria (PKU; MIM ID # 261600) is a rare inborn error of protein metabolism. Lifelong goals of management are to maintain blood phenylalanine (Phe) levels within a recommended target range associated with optimal neurocognitive outcome, and maintain normal growth and development (Singh et al 2016; van Spronsen et al 2017). This requires adherence to a diet low in natural protein and supplemented with phe-free L-amino-acid based formula, to meet estimated protein and micronutrient requirements (van Spronsen et al 2017). The dietary alterations involved may increase the risk of decreased linear growth (Dobbelaere et al 2003; Aldámiz-Echevarría et al 2014), and increase prevalence of overweight (Scaglioni et al 2004; Burrage et al 2012) with changes in body composition such as higher percentage of body fat (Albersen et al 2010).

The measurement of body composition is a valuable tool in the evaluation of the effects of modified diets, and in particular protein modified diets, on somatic development (Huemer et al 2007). The value of body composition measurement in patients with PKU, in addition to other anthropometric parameters, including BMI and waist circumference, is now acknowledged (Albersen et al 2010; MacDonald et al 2011). Of the four body compartments used to assess body composition; fat, water, mineral and protein (dry lean mass), water is the largest component (Wells and Fewtrell 2006). Measuring additional components of body composition beyond just body fat mass is becoming progressively more important in clinical practice with increasing recognition of their effect on health outcomes (Wells and Fewtrell 2006). Multicompartment models, such as dual-energy x-ray absorptiometry (DEXA), that measure body composition are most accurate with good acceptability of measurements, but are expensive, require exposure to radioactivity and are used predominantly in specialist research and does not specifically measure total body water (Wells and Fewtrell 2006). Deuterium dilution is a criterion or reference method to measure total body water (TBW)

(TBW_{Deut}), and subsequently fat free mass (FFM) (FFM_{Deut}) can be derived by using well validated predictive equations (International Atomic Energy Agency 2010). This method is highly technical in its application and as such is not practical as a routine bedside method of measuring body composition and remains a research tool.

Currently there is no agreed or validated method of measuring body composition in PKU, and several methods have been reported, including bio-electrical impedance analysis (BIA) (Dobbelaere et al 2003; Rocha et al 2013), body air-displacement plethysmography using a BodPod^{TM} (Albersen et al 2010), anthropometric skin fold measurements (Allen et al 1996), and total-body electrical conductivity (TOBEC) (Huemer et al 2007). More recently, it has been recommended that methods such as BIA could be used to monitor longitudinal changes in body compartments in PKU, due to the ease and speed in performing this assessment in the clinical setting (Rocha et al 2016). However, to date the method of BIA has not been validated for use in children with PKU.

BIA is a rapid, non-invasive, safe, and inexpensive method to estimate body composition via accurate estimation of total body water (Böhm and Heitmann 2013; Mulasi et al 2015). BIA methodology measures impedance to the flow of electrical current through the water component of body cells and uses empirical linear regression models to measure TBW and predict FFM. It offers the advantage of relative simplicity in obtaining results repeatedly with an instrument that is both functionally robust and physically portable. However, there are limitations in its use, particularly in populations with abnormal hydration status and/or 'body geometry', (Mulasi et al 2015) and it is important that this method be applied critically with consideration of factors that might lead to variable results (Jackson et al 2013).

The purpose of this study was to compare the performance of a multi-frequency BIA machine (QuadScan 400, Bodystat®) to measure TBW (TBW_{BIA}) and FFM (FFM_{BIA}), compared with the criterion method, deuterium dilution, in a group of patients with PKU.

Participants and methods

This study was approved by the RCH Human Research Ethic Committee: HREC # 32056D. Sixteen patients with PKU (7 males, 9 females) were recruited after signed consents were obtained from parents and/or participants. All participants had early and continuous treatment with a low-phe diet and phe-free amino acid formula. Patients over 4 years of age and who were continent of urine, and who had no known co-morbidities that that may affect hydration stats were considered eligible. In this cross-sectional study, all measurements (anthropometric, BIA and urine for deuterium dilution analysis) were collected on the same day for individual patients. Patients were well with no sign of illness or infection. Urine samples were collected and measurements were taken and recorded by a single experienced practioner (ME) using a standardised operating procedure. Data were collected between July 2016 and March 2017. Deuterium dilution analysis was performed by a trained technician (KN).

Anthropometry and BIA measurements

Patients were instructed to eat and drink normally the day prior to measurement, but fast from food and fluids from bedtime until the morning of the measurements. These occurred in the patient's home and close to usual waking time after they had voided their bladder. Height was measured to the nearest 0.1cm using a stadiometer and weight was measured to the nearest 0.1kg using a digital weight measuring scale. Participants were in light clothing with no shoes. All anthropometric measurements were expressed as age- and gender-specific z-scores, using the epidemiological software package Epi Info (version 3.5.1), based on the Centres for Disease Control and Prevention (Atlanta, GA) 2002 reference database.

Body composition was then measured by BIA in patients lying in the supine position, and with electrodes in the tetrapolar (wrist-ankle) arrangement using the multi-frequency BIA analyser QuadScan 4000, Bodystat® (Isle of White, United Kingdom LTD.) as per the manufacturer's instructions. This analyser measures impedance at 5-, 50-, 100- and 200- kHz and uses the 50-kHz frequency to predict the value of TBW and FFM. An undisclosed proprietary equation developed by the manufacturer calculated TBW. The BIA analyser measures FFM using predictive linear regression equations including the equation of Houtkooper for children (Houtkooper et al 1992). Measurements were taken in duplicate over approximately one minute. After the measurements, all impedance, water values and lean weight (FFM) values were recorded.

Criterion method: deuterium isotopic dilution

TBW was measured using the deuterium dilution technique according to the International Atomic Energy Association standard procedures (International Atomic Energy Agency 2010). The baseline fasting spot urine sample was collected for determination of background isotope enrichment. Participants were then provided a dose of 1:10 dilution of deuterium oxide (99.8 atom % excess; Sercon Ltd, Crewe, UK) following the recommended doses for participants of different body weights. The bottle containing the dose was rinsed with 50 mL tap water to ensure no labelled water remained in the bottle. Patients were advised to drink, eat and move normally after samples had been collected but avoid exercise. A spot mid flow urine sample was collected at 5 hours and total urine output was measured from dosing with the isotope until the end of the 5-hour equilibration period. Equilibration is the process whereby the deuterium oxide is evenly mixed throughout the body water resulting in all compartments of body water containing equal concentrations of deuterium. The Spot urine samples were stored frozen at -20 °C for batch analysis.

Analysis of deuterium enrichment was determined with an Isoprime Dual Inlet Isotope Ratio Mass Spectrometer (Isoprime, Manchester, UK) coupled in-line with a Multiprep Gilson Autosampler. Hydrogen analyses were completed by an overnight equilibration with hydrogen gas at 40° C using Hokko Coils. All samples were analysed in duplicate and laboratory standards were calibrated using the international standards USGS45, USGS46 and GFLES-4. Results were reported in ‰ (delta per mil units) relative to Standard Mean Ocean Water (SMOW). TBW was calculated assuming that the deuterium oxide space is 4.1% higher than TBW due to exchange of hydrogen with non-aqueous hydrogen in the body. TBW was then converted to fat-free mass using Lohman's age- and sex-specific ‘constants’ for the hydration of fat-free mass (Lohman 1993).

Statistical analysis

Shapiro-Wilk’s test ($p>0.05$) was used to explore data distribution. Normally distributed data were examined using Pearson correlation co-efficient and Lin’s concordance co-efficient to evaluate the relationship between TBW and FFM determined by the two methods. Paired samples t-test was used to evaluate the difference between the mean values of TBW_{BIA} and $TBW_{Deut,}$ and between the mean values of FFM_{BIA} and FFM_{Deut}. The Bland-Altman method was used to compare two measurements of the same variable and thus used to evaluate agreement between the TBW_{BIA} and TBW_{Deut} and between the FFM_{BIA} and FFM_{Deut}. This method calculated the mean difference between the two methods of measurement (the ‘bias’), and 95% limits of agreement as the mean difference (1.96 SD). A Bland-Altman plot was then constructed to explore the difference scores of the two measurements against the mean for each subject. To test for proportional bias, a linear regression of difference between measurements on the mean of the measurements was completed. Statistical analyses were

performed using SPSS for Windows software version 23 (IBM, Illinois, Chicago, IL). Significance was set at $p< 0.05$. Data are expressed as mean (SD), and median (range).

Results

Participants were 16 patients with PKU (7 males, 9 females). Median age 12.5 years (range; 5 to 20.6 years). Participants' anthropometric results are summarised in Table 1. Measurements of TBW and FFM taken from the duplicate BIA readings were identical in 14/16 participants and within 1% for 2/16 patients and the mean of these values was used.

Table 1: Participants' anthropometric characteristics and results summary

Measurement/Analyses	**Value**	**P value**
weight kg, z-score: median (range) height cm, z-score: median (range) BMI, score, z-score: median (range)	43.9 (19.6 to 74.5), 0.42 (-2.58 to 1.85) 154 (114 to 171.2), 0.35 (-2.52 to 1.67) 18.0 (13.37 to 26.87), -0.13 (-2.13 to 1.79)	
Total body water (TBW) kg: mean (SD)	TBW_{Deut} Mean 24.08 (± 9.37) TBW_{BIA} Mean 24.44 (± 9.91)	p = 0.344
Fat free mass (FFM) kg: mean (SD)	FFM_{Deut} Mean 31.81 (± 12.77) FFM_{BIA} Mean 32.93 (± 13.93)	p = 0.111
TBW correlation between methods Lin's Concordance Co-efficient FFM correlation between methods Lin's Concordance Co-efficient	TBW_{Deut} and TBW_{BIA} TBW_{Deut} and TBW_{BIA} FFM_{Deut} and FFM_{BIA} FFM_{Deut} and FFM_{BIA}	p < .0001 Rc = .987 p < .0001 Rc = .969
Bland –Altman analyses	$TBW_{Deut,}$ and TBW_{BIA} FFM_{Deut} and FFM_{BIA}	p = .169 p = .083
Shapiro-Wilks analysis	TBW_{Deut} TBW_{BIA}	p = .098 p =.198

Total body water

Values are summarised in Table 1. A Shapiro-Wilk's test ($p>0.05$) demonstrated that the TBW_{Deut} ($p=.098$) and TBW_{BIA} ($p=.198$) results were both normally distributed. When comparing the variance in TBW values between those obtained without correcting for urine output during the 5 hours after deuterium dosing, i.e. 'uncorrected' and those values obtained after 'correction' for urine output, the difference observed was minimal (median of 1.5%; 0.3-6.6%). Given this small difference, uncorrected values were used in the subsequent analysis. Figure 1a, depicts the relationship between TBW_{Deut} and TBW_{BIA} ($r=.990$ $p< .0001$). Lin's concordance co-efficient confirmed the significance of the correlation; Rc = 0.987, 95% CI [0.967 to 0.995]. One-sample T-test of the difference between TBW_{Deut} and TBW_{BIA} was not significant ($p=.344$).

Figure 1a

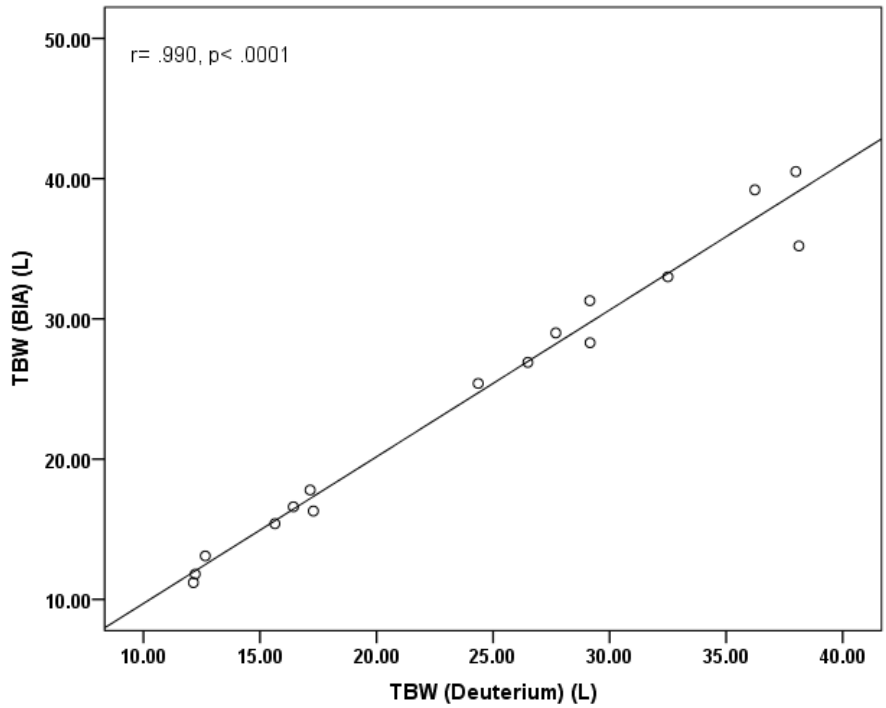

Figure 1a: Correlation between TBW calculated from BIA versus TBW calculated from deuterium dilution

Paired samples t-test showed no significant difference between the means of the TBW_{Deut} and the TBW_{BIA} measurements (p=.344) (Table 1). Variability between TBW_{Deut} and TBW_{BIA} measurements for individuals showed a median of 4.32% with range 0.45 – 9.1%.

Bland-Altman analysis and subsequent plot of the difference between the TBW measurement and the mean of the TBW measurements is depicted in Figure 1b. Results indicate that there was no significant proportional bias between the criterion method, $TBW_{Deut,}$ and the test method TBW_{BIA}, to measure TBW (β = -.056, adjusted r^2 =.069 p=.169).

Figure 1b

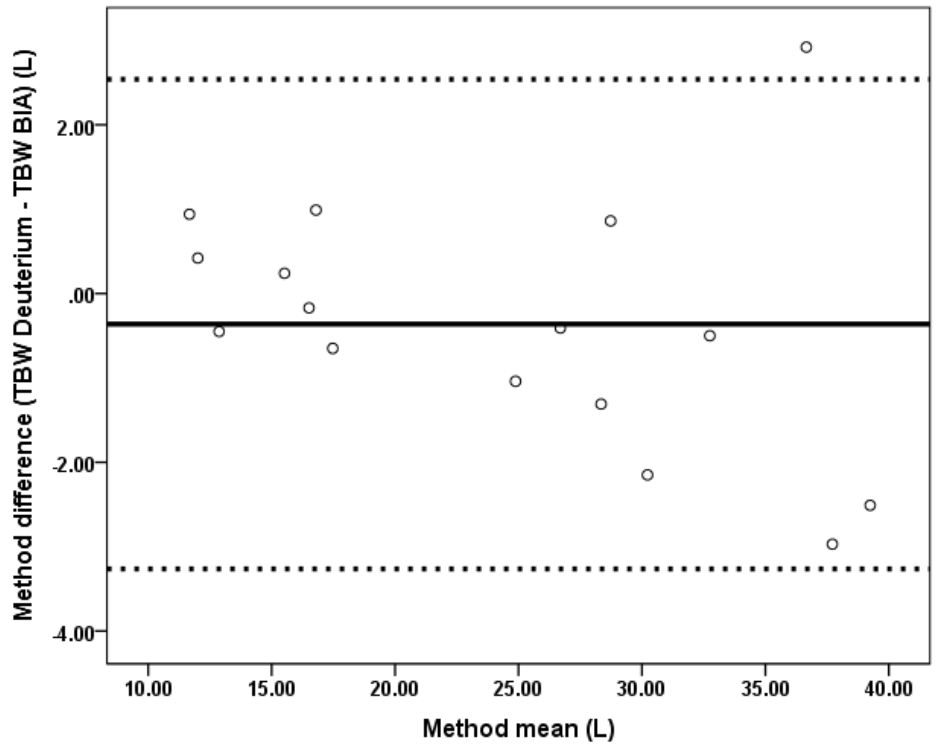

Figure 1b: The solid line indicates the mean; the dashed lines represent the upper (mean + (1.96 SD)) and lower (mean – (1.96SD)) levels of the 95% CI. Each dot represents the method difference versus the method mean for individuals.

Fat free mass determination

Correlation analysis showed that FFM calculated from BIA correlated significantly with FFM calculated from TBW_{Deut} using the equation FFM = TBW/Hydration co-efficient (r=.984, p< .0001) (Figure 2a). Lin's concordance co-efficient confirmed the significance of the correlation; Rc = .969, 95% CI [0.924 to 0.988].

Figure 2a

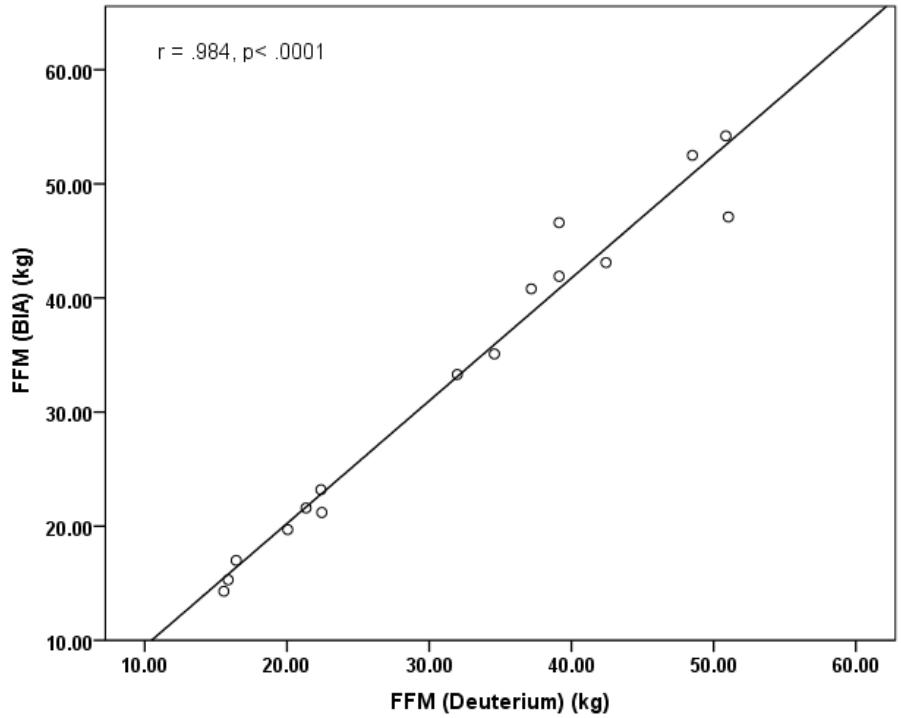

Figure 2a: Correlation between FFM calculated from BIA versus FFM calculated from deuterium dilution.

One-sample T-test of the difference between FFM_{Deut} and FFM_{BIA} was not significant (p=.111). Paired samples t-test showed no significant difference between the means of the FFM_{Deut} and the FFM_{BIA} measurements (p=.111) (see Table 1).

Bland-Altman analysis and plot is depicted in Figure 2b. Results indicate no proportional bias between the deuterium dilution and BIA methods to measure FFM (β = -.089, r^2 =.142 p=.083).

Figure 2b

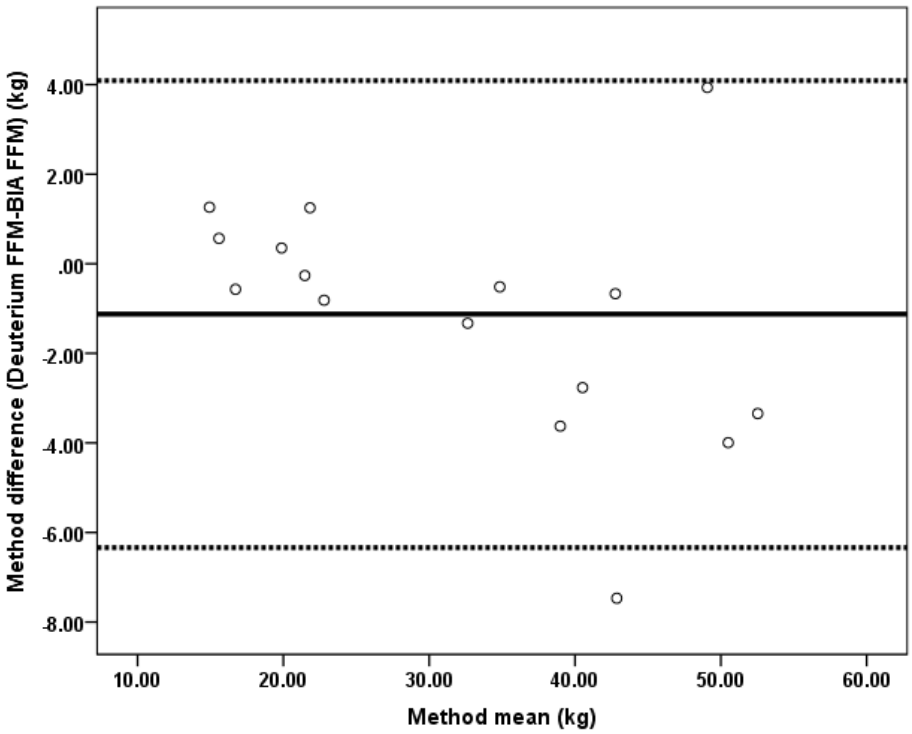

Figure 2b: The solid line indicates the mean; the dashed lines represent the upper (mean + (1.96 SD)) and lower (mean – (1.96SD)) levels of the 95% CI. Each dot represents the method difference versus the method mean for individuals.

Discussion

Anthropometric assessment of height and weight and the subsequent calculation of BMI are valuable clinical tools that monitor growth against standards and allow screening for overweight. However, they are not sufficient on their own for the comprehensive assessment of nutritional status and body composition in health and disease (Battezzati et al 2003). Longitudinal body composition monitoring in PKU in an outpatient setting may allow individualised nutritional management strategies based on lean body mass rather than the relatively blunt instrument of body weight (MacDonald et al 2011). It also provides important information in relation to disorder specific management strategies in the context of overall

longer-term good health (Albersen et al 2010) by enabling a better understanding of the course of an individual's anthropometric and body composition profiles (Rocha et al 2013).

The implementation of a reliable, quick and easy method to measure body composition in the outpatient clinic setting would be advantageous for clinicians managing individuals with PKU. Bio-electrical impedance machines are very useful due to their non-invasive nature, safety, ease of use, portability and relatively low cost compared to other clinically available methods of measuring body composition (Mulasi et al 2015). However, the proprietary and confidential nature of each manufacturer's algorithm equations from which body compartments are derived make it essential that each machine is validated for use in a population of interest. In this study, we compared the performance of the QuadScan 4000, Bodystat® against a criterion method, deuterium dilution, to determine TBW in a group of 16 patients with PKU. As both TBW values determined by deuterium dilution and the impedance values determined by BIA can be used to calculate FFM (Cleary et al 2008), we compared these methods for the estimation of FFM.

When comparing mean values of both methods, we found no significant difference between TBW_{Deut} and TBW_{BIA} and between FFM_{Deut} and FFM_{BIA}. We also show a significant correlation between TBW_{Deut} and TBW_{BIA} and between FFM_{Deut} and FFM_{BIA}. Bland-Altman analysis confirmed that there was no significant proportional bias between the methods (Martin Bland and Altman 1986), although there were slight biases for TBW_{Deut} to be greater than TBW_{BIA}, and for FFM_{BIA} to be greater than $FFM_{Deut.}$ Consequently, we conclude that deuterium dilution and BIA using the QuadScan 4000, Bodystat® can be used to measure TBW and estimate FFM in patients with PKU.

We observed individual differences in measurements between TBW_{BIA} and TBW_{Deut}, and three of four participants with the greatest difference were 13 and 14-year-old males who are

entering puberty and therefore likely to be undergoing a rapid change in body composition with changes in hydration status and increased FFM deposition. It is possible that because deuterium dose is based on body weight alone, this measurement was not precise enough to account for the potential body composition and hydration status changes in these boys. A study that included individuals of a similar age and pubertal stage may better address this potential issue. However, as the outliers in the difference between the measurements were minimal, this demonstrated a strength in the methods tested.

Other studies have reported differences in TBW values obtained for individuals from deuterium dilution and from BIA. In a BIA validation study performed in pregnant women with or without HIV-infection, a systematic predictive bias was seen in TBW using BIA at each time point during the pregnancy despite TBW_{Deut} and TBW_{BIA} being highly correlated (Kupka et al 2011).

We also show a strong and statistically significant correlation between FFM results obtained using both methods. We suggest therefore, that the proprietary regression equations within the QuadScan 4000, Bodystat® analyser, which have been developed for the healthy population, are valid in individuals with PKU. It is possible that no predictive bias was seen between the methods in our study because our participants with PKU are free living with normal physical development.

While it has been shown that BIA alone can be used as a surrogate to measure FFM in a paediatric population (Pietrobelli et al 2003), predictive equations used in BIA analysis have also been validated in a several population groups including healthy children (Cordain et al 1988) (Ellis et al 1999) overweight and obese children (Cleary et al 2008), and young female gymnasts (Eckerson et al 1997). Population specific BIA equations have also been developed when required, such as race combined equations for large epidemiological studies (Sun et al

2003). A study of healthy individuals aged 4- 24 years showed that while height ^{2}/impedance was a strong predictor of lean mass, some variability was observed in the younger years and older adolescent years, suggesting that no single BIA equation may be applied over all age groups (Montagnese et al 2013).

This study is limited by the relatively small number of participants and their wide age range, which included adolescents likely to be experiencing pubertal body composition changes. Ongoing evaluation of BIA methodology to measure body composition could be done to ensure that predictive equations using raw impedance values to estimate FFM can be applied across all ages. With more data, it may be possible to develop PKU specific equations if required in the future. Further study may also produce predictive equations for other metabolic disorders requiring dietary modification.

In summary, our results show no significant differences between the criterion deuterium dilution method and BIA in measuring TBW and predicting FFM in a group of children and adolescents with PKU. We suggest that BIA using QuadScan 4000, Bodystat® can be used to measure body composition in the outpatient clinic setting, to further improve the assessment of nutritional outcomes for patients with PKU.

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Chapter 8: The relationship between dietary intake, growth, and body composition in Phenylketonuria

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The relationship between dietary intake, growth and body composition in Phenylketonuria

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ABSTRACT

Aim: Phenylketonuria (PKU) is an inborn error of protein metabolism that results from perturbation in phenylalanine hydroxylase activity leading to elevated blood levels of phenylalanine (phe). We aimed to explore the relationships between dietary patterns (total-protein, natural-protein, amino-acid formula), and the ratio of protein to energy intake with growth and body composition.

Method: Longitudinal prospective data (1–6 measurements) of growth, dietary intake and body composition in patients treated with phe-restricted diet only (D-PKU; n = 32), and tetrahydrobiopterin (BH_4) ± phe-restricted diet (BH_4-PKU; n = 5) were collected over a two-year period. Healthy siblings provided control data (n = 21).

Results: There were no significant differences in weight-, height-, BMI z-score or percent body fat mass (% fatmass) between the D-PKU, BH_4-PKU and control groups or between the all-types of PKU combined and controls, which confirmed 'normal' growth in the PKU cohort. Total-protein intake in the all-types of PKU group met or exceeded WHO safe protein recommendations. There were no significant relationships between anthropometric and dietary variables. Significant negative correlations were found in body composition: %fatmass and total-protein intake ($r_s = -0.690$, $p \leq 0.001$), natural-protein intake ($r_s = -0.534$, $p = 0.001$), and AAF intake ($r_s = -0.510$, $p = 0.001$). Age was significantly correlated with %fatmass ($r_s = 0.493$, $p = 0.002$) A total-protein intake of 1.5–2.6 g/kg/day and natural-protein intake > 0.5 g/kg/day were associated with improved body composition. An apparent safe P:E ratio of 3.0–4.5 g protein/100 kcal was strongly associated with appropriate growth outcomes.

Conclusions: Clinical decision-making needs to consider both the enhancement of natural-protein tolerance and the application of an apparent 'safe' protein to energy ratio to support optimal growth and body composition in PKU.

1. Introduction

Phenylketonuria (PKU) is an inborn error of protein metabolism that results from perturbation in phenylalanine hydroxylase activity leading to elevated blood levels of phenylalanine (phe). As elevated phe levels have a toxic effect on the brain, treatment with a diet low in natural protein needs to commence as soon as possible after birth. Lifelong goals of treatment in PKU are to maintain phe levels within the target range to achieve optimal neurocognitive outcomes and maintain normal physical growth and development [1,2]. Recent attention has been directed towards attaining long term ideal body weight and composition in children and adults with PKU [3,4]. There is a recognised spectrum in PKU ranging from 'severe' when individuals have a very low phe tolerance and therefore require a severely restricted natural-protein intake, to milder forms when individuals have a higher phe tolerance.

Consensus exists regarding the need for reduced natural-protein intake and supplementation with precursor free amino acid based formulae (AAF), as natural-protein tolerance is mostly below safe requirements [5,6]. The emergence of cofactor therapy tetrahydrobiopterin (BH_4) [7], has meant that the group of PKU patients who respond to this treatment form a special group in dietary terms, as

Abbreviations: PKU, Phenylketonuria; phe, phenylalanine; %fatmass, percent body fat mass; %FFM, percent fat free mass; P:E ratio, protein to energy ratio; BH_4, tetrahydrobiopterin; AAF, amino acid formula; E%BMR, energy intake as a percentage of basal metabolic rate; BIA, bioelectric impedance analysis; %PE, percentage of dietary energy from protein

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they tolerate a higher natural protein intake which in some cases may result in a normal diet [5]. Currently, published recommendations for 'total-protein intake' in PKU, defined as natural-protein intake plus AAF, are based on healthy population nutritional recommendations with an additional estimated factor to account for the apparent difference in quality between natural-protein and AAF [6]. In addition, consideration is given to the role of AAF in achieving optimal phe levels and micronutrient intake [3,8]. Logically it follows that nutritional outcomes in PKU are likely to be affected by both the quality and quantity of protein consumed and total energy intake, however this needs further documentation.

We have shown previously that the protein:energy ratio (P:E ratio) of the diet has a pivotal role in long term nutritional outcomes, including body composition, in children with inborn errors of intermediary protein metabolism [9] and children on the ketogenic diet [10]. The concept of P:E ratio considers the inter-dependence of protein and energy intakes as it describes the proportion of dietary energy derived from protein. The concept has been incorporated into WHO/FAO/UNU recommendations to consider when describing the risk of protein insufficiency particularly in those consuming marginal diets [11]. We hypothesised that the P:E ratio may have benefit as a clinical monitoring tool in PKU due to the specific features of a PKU diet which include restricted consumption of protein of high biological value and reliance on the use of AAF as a 'protein substitute' to meet nutritional requirements.

The aim of this study was to answer the following questions: Does the intake of protein, impact on the growth and body composition trajectory in children with PKU? Is there an optimal P:E ratio for prescribing dietary intake for children with PKU?

2. Methods

This study was approved by the RCH Human Research Ethic Committee (HREC: 32056A) Written consent was provided by parents for the additional measure of body composition to be completed at routine clinic visits.

We collected prospective longitudinal data of growth, dietary intake and body composition in patients born between January 1996 and December 2014 who attend our specialist treatment centre in Melbourne Australia over a 2-year period. The initial measurement being denoted as the 'baseline' measurement.

Data were analysed in patients with: PKU treated with phe restricted diet D-PKU (n = 32; 10 males, 22 females), PKU treated with tetrahydrobiopterin (BH_4) ± phe restricted diet (BH_4-PKU) (n = 5; 3 males, 2 females). D-PKU patients were not categorised by type based on newborn peak phenylalanine levels. Data have also been combined and denoted as all-PKU which represent the spectrum of PKU and the range of protein tolerance. To incorporate every available measurement (all-measurements), the mean of each growth and dietary variable was calculated for each individual patient and the median then calculated for the group. The mean value was compared to the baseline measurement of each patient.

Controls: a single height, weight and body composition measurement was collected from healthy sex and aged matched sibling controls (n = 21; males 8, females 13).

Weight and length for children < 2 years of age were obtained by standard operating procedures using digital baby weighing scales and crown-heel length on a scaled length board. Height and weight of children > 2 years of age were measured using a combined stadiometer and digital weight measuring station (Seca 284). Participants were in light clothing with no shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were recorded. Body Mass Index (BMI) was calculated using the equation kg/m^2. Measurements were performed by the dietitian (ME) or clinic nurse.

All anthropometric measurements were expressed as age and gender-specific z-scores, using the epidemiological software package Epi Info (version 3.5.1), based on the Centres for Disease Control and Prevention (Atlanta, GA) 2002 reference database.

Dietary data from food diaries were analysed by a single metabolic dietitian (ME) using the dietary analysis program Foodworks (Xyris, Version 7.0.3016, Kenmore Hills, Australia). Food diaries were reviewed with parents to clarify content for analysis [12]. A subset of diaries was independently analysed by two dietitians for energy intake to ensure reliability. Dietary intake of protein in g/kg/d was compared with FAO/WHO/UNU recommended safe levels [11]. One gram of natural-protein was considered equivalent to 50 mg phenylalanine. This included all phe containing foods and 'uncounted' foods that contain small amounts of phenylalanine such as fruits and vegetables that are allowed freely, yet may increase natural-protein intake by up to 49% [13]. Energy intake was expressed as a percentage of basal metabolic rate (E%BMR) calculated for each patient using the BMR predictive equations of Schofield [14]. This calculation considered a physical activity level (PAL) defined by number of sessions undertaken per week of formal exercise activity, and classified as low, medium and high PAL. Mean energy intake was calculated for individual patients and median energy intake was calculated for the group. P:E ratio was expressed as gram protein/100 kcal/d.

Body composition was measured by Bioelectrical Impedance Analysis (BIA) using the QuadScan 400, Bodystat® (Isle of White LTD) as per the manufacturer's instructions. Participants were instructed to fast for at least 90 min and to not exercise prior to the BIA assessment. Percent fat-free mass (%FFM) and percent fat mass (%fatmass) were estimated using raw impedance values using the equation of Houtkooper [15].

3. Statistical analysis

Statistical analyses were performed using SPSS for Windows software version 23 (IBM, Illinois, Chicago, IL). Significance was set at $p < 0.05$. Continuous variables including z-scores for weight, height and BMI, protein and energy intake and P:E ratio are presented as median and range. Non-parametric tests included: Kruskal-Wallis test for one-way between-group analysis of variance; Mann-Whitney *U* test for differences between two independent groups on a continuous measure; Friedman test for variance between multiple measures in the same subjects. Spearman correlation coefficient Rho (r_s) was used to evaluate associations between categorical variables. Stepwise multiple linear regression analysis was performed with anthropometric parameters and body composition as the dependent variable and dietary parameters as the independent variables.

4. Results

There was no difference in age or gender distribution between PKU and control groups (Table 1).

4.1. Growth and body composition

There was no significant difference in weight-, height-, BMI z-score or %fatmass between the D-PKU, BH_4-PKU and control groups or between the all-PKU group and control group (Table 1). Low numbers of BH_4-PKU patients precluded comparisons with D-PKU patients.

4.2. Dietary intake

Median total-protein intake exceeded the FAO/WHO/UNU recommended safe levels, (data not shown). As expected, natural-protein intake was higher for BH_4-PKU patients. Diaries were independently analysed by two dietitians for energy intake and the maximum variation in energy was 10% with 34% having only 1–2% variation. Validity of energy intake was calculated as a % BMR, with valid records being defined as those with a reported energy intake between 2.1 and

Table 1
Participant characteristics at baseline measurement.

Subject characteristics	D-PKU	BH_4-PKU	All-PKU baseline	Controls	All-PKU vs. controls
Number of subjects	32	5	37	21	
Age years ± SD (range)	9.2 ± 4.7 (0.83–18.0)	6.8 ± 3.7 (0.64–10.9)	8.8 ± 4.6 (0.6–18.0)	8.8 ± 4.8 (0.87–17.5)	p = 0.878
Gender male	10	3	13	8	p = 0.823
female	22	2	24	13	
Growth:					
median ± SD (range)					
Weight z-score	0.33 ± 1.01 (−2.45–1.88)	0.76 ± 0.70 (0.26–2.14)	0.64 ± 1.00 (−2.45–2.14)	0.23 ± 1.05 (−2.08–2.09)	p = 0.159
Height z-score	0.30 ± 0.88 (−2.55–1.31)	0.44 ± 0.71 (0.21–1.93)	0.38 ± 0.87 (−2.55–1.93)	0.13 ± 0.90 (−1.52, 1.71)	p = 0.533
BMI z-score	0.03 ± 0.96 (−1.43–1.95)	1.00 ± 0.72 (0.14–1.85)	0.33 ± 0.96 (−1.43–1.95)	−0.06 ± 0.84 (−1.52–1.18)	p = 0.155
Body composition					
median ± SD (range)					
%fatmass	15.9 ± 7.38 (7.9–35.9)	16.5 ± 3.1 (12.5–19.7)	16.0 ± 7.0 (7.9–35.9)	19.4 ± 5.1 (13.8–32.7)	p = 0.101
%FFM	84.1 ± 7.4 (64.1–92.1)	83.5 ± 3.1 (80.3–87.5)	84.0 ± 7.0 (64.0–92.1)	80.9 ± 4.2 (71.8–86.2)	p = 0.148
Dietary intake:					
median ± SD (range)					
Total protein: g/kg/d	2.05 ± 0.60 (1.00–3.50)	1.90 ± 0.16 (1.70–2.10)	2.00 ± 0.56 (1.00–3.50)		
Natural protein: g/kg/d	0.50 ± 0.18 (0.18–0.80)	1.10 ± 0.60 (0.55–2.00)	0.50 ± 0.37 (0.17–2.00)		
AAF g/kg/d	1.54 ± 0.50 (0.80–2.70)	1.00 ± 0.61 (0.00–1.30)	1.43 ± 0.59 (0.00–2.70)		
Energy: %BMR	145 ± 31 (107–241)	157 ± 30 (122–181)	146 ± 31 (107–241)		
P:E ratio gprotein/100 kcal	3.6 ± 0.9 (1.9–5.5)	3.3 ± 0.8 (2.5–4.0)	3.6 ± 0.9 (1.9–5.5)		

Significance (p-value) was calculated to compare all-PKU and control groups.

1.1 × BMR. A higher energy %BMR was accepted in only one patient who played several hours of daily competitive sports with training. In a previous study, a minimum value set of 1.06 × BMR was used as the acceptable cut-off in children with PKU reporting intake based on 4 day weighed food records [16]. At baseline measurement, energy intake was 146 E%BMR, and when all food diaries (1–6 per patient) were combined, median energy intake was 143 E%BMR. At baseline measurement, median P:E ratio was 3.6 g protein/100 kcal when all-measurements were combined, median P:E ratio was 3.8 g protein/100 kcal. In six food diaries, energy and P:E ratio calculations could not be made due to imprecise recording of some protein free food.

4.3. Body composition

At baseline, there was no statistically significant difference in % fatmass or %FFM between groups (Table 1). There was also no statistically significant difference between %fatmass or %FFM in all-PKU at baseline measurement and when all-measurements were combined. At baseline measurement, %fatmass was correlated with age (r_s = 0.493, p = 0.002).

4.3.1. Relationships between age and dietary intake and body composition

All-PKU: at baseline, there was a significant correlation between age and: total protein intake (r_s = −0.658, p < 0.0001), AAF (r_s = −0.507, p = 0.001), P:E ratio (r_s = 0.386, p = 0.032) and % fatmass (r_s = 0.493, p = 0.002).

4.3.2. Relationships between dietary intake and growth and body composition

All-PKU: at baseline, there were no significant correlations between growth (weight-, height- and BMI z-score) and dietary variables (total-protein, natural-protein, AAF, E%BMR and P:E ratio). By contrast there was a significant negative correlation between %fatmass and total-protein intake, natural-protein intake, and AAF intake at baseline measurement (Table 2).

In a multiple linear regression analysis using baseline data, with % fatmass as the dependent variable and natural-protein, AAF and age as independent variables, the final model combined natural-protein and AAF to predict 44.5% of the variance in %fatmass (R^2 = 0.445, p < 0.0001). In this model, natural-protein contributed 31.1% to the variance. Age was not a significant predictor in this model. Simple linear regression showed that neither %EBMR nor P:E ratio contributed significantly to %fatmass or %FFM.

Table 2
Correlation matrix describing the relationships (r_s) between growth, body composition and dietary intake using all baseline data.

	Weight z-score r_s	Height z-score r_s	BMI z-score r_s	%fatmass r_s
Total protein	−0.098	−0.002	−0.182	−0.690*
Natural protein	−0.108	0.074	−0.133	−0.534*
AAF	−0.148	−0.065	−0.260	−0.510*
E%BMR	−0.096	0.73	−0.059	−0.292
P:E ratio	−0.037	−0.097	−0.138	0.206

* p < 0.05.

4.3.3. Determining an 'ideal' total and natural-protein intake associated with optimal body composition outcomes

To determine the optimal relationship between body composition and total protein intake, a series of bivariate correlations were plotted between %fatmass and total-protein intake (Fig. 1a), and %fatmass and natural-protein intake (Fig. 1b). These demonstrate the strong relationship between higher %fatmass with a total-protein intake of 1–1.5 g/kg/d (n = 5, r_s = −0.821, p = 0.089). There was a modest correlation between lower %fatmass and total-protein intake in the range 1.5–2.6 g/kg/d (n = 28, r_s = −0.366, p = 0.056), and no correlation between %fatmass and total-protein intake between 2.6 and 3.5 g/kg/d (n = 6, r_s = 0.209, p = 0.957) (Fig. 1a). 5/6 children with protein intake between 2.5 and 3.5 g/kg were < 3.4 years of age. Results also suggest a strong correlation between higher %fat mass and a natural-protein intake < 0.48 g/kg/d, and no correlation between % fatmass and a natural-protein intake ≥ 0.5 g/kg/d (Fig. 1b).

4.3.4. Determining a P:E ratio associated with optimal growth outcomes

To calculate an apparent safe P:E ratio, stepwise statistical correlations were made between weight-, height-, BMI- z scores, %fatmass and P:E ratio from the lowest P:E ratio towards the highest, until a final P:E range could be determined at which no correlation ($r_s \sim 0$) with the particular growth variable was observed (Fig. 2). Resulting P:E ratio ranges at which r_s was closest to 0 were: weight z-score: 2.5- < 4.9 (n = 28, r_s = −0.019, p = 0.923), height z-score: 1.9- < 4.5 (n = 23, r_s = −0.026, p = 0.907), BMI z-score: 2.5- < 4.9 (n = 27, r_s = −0.068, p = 0.737), %fatmass: > 3.0–5.0 (n = 23, r_s = 0.011, p = 0.962). The overall apparent safe P:E ratio was estimated from the

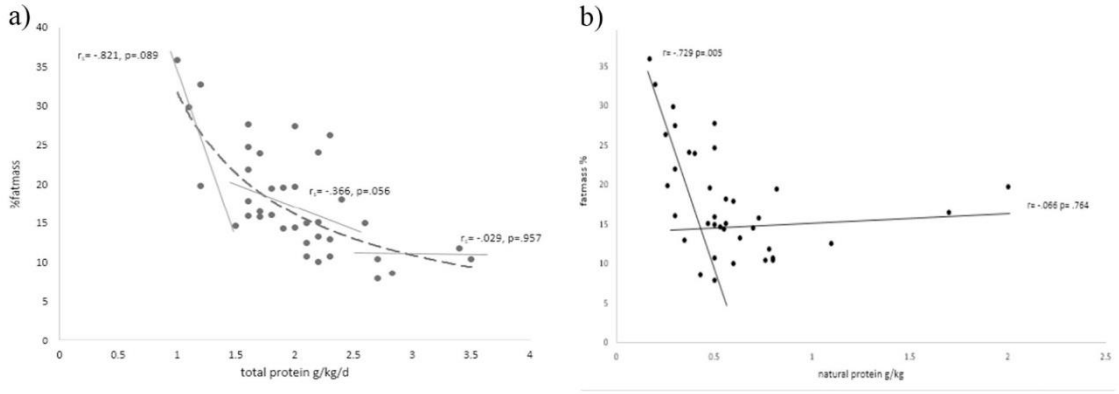

Fig. 1. Association between total-protein intake and body composition (%fatmass) (1a) and natural-protein intake and %fatmass (1b). Statistical bivariate correlations were made across the range of both total-protein and natural-protein intakes, to determine an intake at which the correlation between was the strongest towards the correlation at which the relationship was weakest.

overlap between the P:E ranges for each growth variable. The results suggest that in all-PKU the apparent 'safe' P:E ratio is 3.0–4.5 g protein/100 kcal/d. (~12–18% of energy from protein) (Fig. 2). We further explored the correlation between higher %fatmass and higher P:E ratio observed at baseline and determined that there was a trend for higher % fatmass when the P:E ratio was < 3 g protein/100 kcal (Fig. 2d). There was a lack of data for P:E ratios > 5 g protein/100 kcal to draw conclusions.

5. Discussion

This unique prospective study enables us to explore the relationships between anthropometric parameters and total and natural-protein intake and P:E ratio in the diet of patients with PKU over a 2 year period. The inclusion of BH_4-PKU enabled analysis of protein intake in the full spectrum of PKU severity. In addition, we have included longitudinal body composition measurements for each individual. These parameters enabled us to determine an 'ideal' total-protein and natural-protein intake associated with optimal body composition and an apparent 'safe' P:E ratio for consideration in the management of these patients.

Our results confirm recent reports of essentially normal growth in children with PKU [17–19]. While earlier reports documented a reduction in linear growth [20–22], the median height z-score in our patients was normal at + 0.38. Although small patient numbers limit the weight of our conclusions we confirm that there was no significant difference in height z-score between D-PKU and BH_4-PKU [23].

Early childhood overweight with a prevalence of 24.7 [24] to 40% [25] have been documented in PKU. In our all-PKU group 10/37 (27%) had a baseline BMI z-score > 1, meeting the CDC classification of

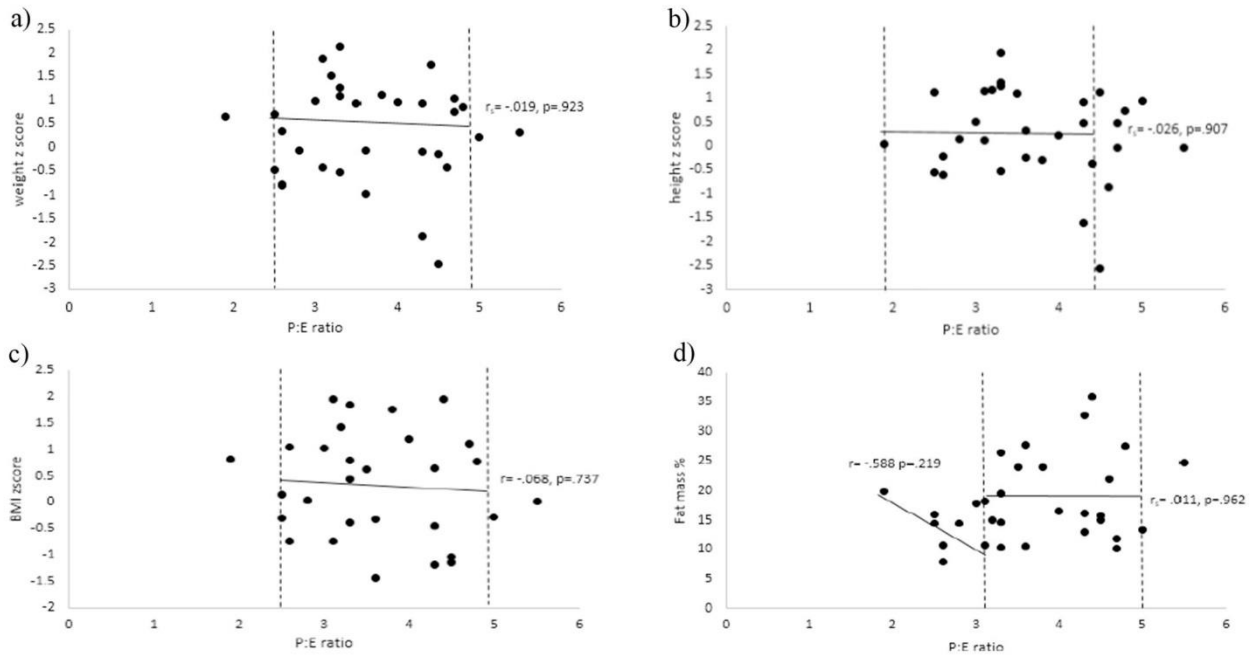

Fig. 2. Association between baseline anthropometric measures and P:E ratio to determine a safe P:E ratio. Weight (b) Height (c) BMI (d) %fatmass. Stepwise statistical correlations were made between weight-, height-, BMI- z scores, %fatmass and P:E ratio from the lowest P:E ratio towards the highest, until a final P:E range could be determined at which no correlation ($r_s \sim 0$) with the particular growth variable was observed.

'overweight' [26]. This compares with a recent estimation of 26% overweight and obesity rates in Australian children 2–17 years of age [27]. Potential causes of overweight include excessive energy intake or inadequate energy expenditure [28], parental overweight and early BMI rebound [24] as well as non-compliance with a low-phenylalanine diet and AAF supplement [25]. It should be noted that we did not find a relationship between energy intake and weight-, height- or BMI z-score or %fatmass in our patients. It is recognised that it is more important to assess body composition in children who are overweight [29] and by using portable bioelectrical impedance in this study we can confirm that it was due to an excess of body fat. Although higher %fatmass than controls has been observed in patients with PKU [30], our study confirms that it was not different from a group of healthy age and sex matched controls [17,21,31]. This suggests that those with PKU are subject to a similar environmental impact on obesity as other children. Although the incidence of overweight in PKU mirrors that of the general population, it is higher than ideal, and suggests that strategies to address excessive energy consumption are required.

Total-protein intake in the all-PKU group met or exceeded WHO safe protein recommendations [11]. Median natural-protein intake in our cohort is comparable to other reports [18,22]. We did not observe a significant correlation between greater natural-protein intake and increased height z-score in in the D-PKU or BH_4-PKU groups, in contrast to the study by Aldámiz-Echevarría et al. [23]. Instead our results support those of Hoeksma et al., who observed that neither protein nor energy intake correlated with linear growth [32]. Conversely, we demonstrate significant relationships between higher total-protein, natural-protein and AAF intake and lower %fatmass, with natural-protein intake contributing more than AAF intake in the model to best predict %fatmass. This concurs with results of Huemer et al., who found a significant correlation between FFM and natural-protein intake in PKU [17]. These findings suggest that a total-protein intake of > 1.5–2.6 g/kg/d is associated with improved body composition, and that there was no additional benefit to body composition when the total-protein intake is higher (between 2.6 and 3.5 g/kg/d). A total-protein intake of 1.5–2.6 g/kg/d is achievable for most children with PKU, including those for whom adequate AAF is required to maintain target phe levels. Total protein prescription and AFF supplementation in our clinic is consistent with recommendations for PKU, as a guide patients consume 3–4 AAF drinks per day.

In light of ongoing debate regarding an association between high protein intakes in the first year of life and risk of later overweight in healthy children [33], overconsumption of AAF may contribute to an excessive protein and energy intake. Adequate AAF is necessary to ensure target phe levels are attained in PKU [8], and therefore high total-protein intakes in some patients with D-PKU may reflect the need for additional AAF to control phe levels rather than meet theoretical nutritional requirements. Moreover, increased AAF 'protein' in early childhood can result from the introduction of more amino acid dense transitional formulas [34], and management of frequent illnesses [6].

We also determined that 0.5/kg/d natural-protein intake is the apparent 'ideal' intake to support healthy body composition, but realistically, many individuals with classical PKU will not maintain target phe levels with a natural-protein intake > 0.5 g/kg. Nevertheless, our results support the view that strategies to enhance maximum natural-protein tolerance may be key to enhancing healthy body composition in PKU.

Several mechanisms have been proposed to explain the benefit from increased protein intake for weight and body composition, including a reduction in dietary energy intake mediated by an effect on satiety [35]; an increase in REE due to a greater diet-induced thermogenesis [36]; an influence on growth hormone and IGF-1 production on body composition [37]; and a stimulatory effect on muscle protein anabolism favouring the retention of lean muscle mass [38].

Energy intakes, expressed as a percentage of BMR, permit a flexible allowance for physical activity level (PAL). Median baseline energy intake in the all-PKU group was similar to that determined by WHO for children up to 11 years of age who engage in a light physical activity level [39]. In addition, children with PKU have been shown to have similar energy requirements to healthy individuals in a study measuring resting energy expenditure (REE) [40]. Although families with PKU are proficient at keeping food records due to the rigors of maintaining such a restricted and carefully monitored diet, it is possible that the energy intakes in our children represent under-reporting [41], however there is evidence that parents of children with chronic disease are more accurate and adept at dietary reporting [42].

We determined that the relationship between growth variables: weight-, height-, BMI z-scores and %fatmass and P:E ratio are stable between 3.0 and 4.5 g protein/100 kcal. We have limited data from patients with a P:E ratio > 5 g protein/100 kcal. This apparent 'safe' ratio still allows for an adequate total-protein and AAF intake to support phe control. The median P:E ratio of 3.8 g protein/100 kcal from all dietary assessments and the optimal range of 3.0–4.5 g protein/100 kcal confirm and expand the value previously described in a study that documented improved growth in infants fed AAF with 3.12 g protein/100 kcal versus 2.74 g protein/100 kcal with adequate total-protein intake [43]. The range of P:E ratio observed in our patients equates to approximately 12–18% of dietary energy intake as protein (%PE). This is similar to the %PE that is provided by an omnivore diet containing animal sourced foods, and is also considered adequate to ensure a diet with minimal risk of protein deficiency [44]. While the observed P:E ratios provide evidence of adequate total-protein in our patients, the equivalent value of AAF compared to natural-protein is not defined. As the PKU diet is highly constructed around control of protein intake, it is assumed that meeting energy requirements will drive satiation, that is, individuals will cease eating once energy needs are met. In contrast to this assumption, the 'Protein Leverage Hypothesis' suggests that protein may exert a stronger influence on consumption patterns than energy alone, through an intrinsic drive to maintain a target protein intake. It is based on epidemiological evidence in humans for a steady intake of protein of approximately 15% of dietary energy over time, despite variations in carbohydrate or fat intake. These observations have led to a proposed relationship between dietary protein and dietary energy intake and obesity prevalence. The resulting hypothesis is that a modern diet with high consumption of low P:E dense foods may result in an excessive energy intake if protein targets are to be met [45]. Conversely, if foods with a high P:E ratio are consumed, then energy intake is curtailed due to satiety when target protein intake is achieved [45]. As many low protein foods available for patients with PKU are already highly carbohydrate- and energy-dense [46], this also supports the use of higher protein, lower energy dense AAF to achieve such a protein target.

6. Limitations

A limitation of this study is the smaller number, and younger age, of BH_4-PKU patients and the lack of correlation between protein intake and phe levels. In addition, our understanding of the exact protein equivalent 'value' of AAF is unknown, limiting our precision when evaluating the adequacy of 'total-protein intake'. D-PKU patients are not classified by type based on newborn peak phenylalanine level. A description of natural protein intake by age is not intended to define the difference between PKU severity, but to discuss the effect of dietary intake across the spectrum of PKU.

7. Conclusions

Children with PKU demonstrate growth patterns comparable to healthy children. While phe intake is a major focus of the PKU diet, our results suggest that efforts to enhance natural-protein tolerance are important. It may allow for a modest reduction in AAF consumption, and protect from excessively high P:E ratios. We suggest that clinical

decision-making consider the application of an apparent 'safe' P:E ratio which may promote optimal body composition and contribute to a reduced longer term health risk. We also suggest that with further validation, it may be possible to provide total-protein recommendations in PKU with both lower and upper levels of intake, and that clinical practice adopt measurement of body composition as a part of routine clinical care.

Conflict of interest

The authors have no financial or other relations that could lead to conflict of interest.

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Section 4: (Chapter 9) Discussion and Future Directions

The overall management of patients with IEM is aimed to maintain metabolic stability and to meet individual genetic potential for growth and development. The purpose of this study was to explore the relationships between dietary intake and growth and body composition in IEMs in which nutritional therapy is a key component of management and to explore novel strategies to best guide clinical decision making to improve nutritional outcomes. More specifically, we wanted to explore whether the P:E ratio in the consumed diet can be useful in clinical practice, combined with traditional methods of recommending protein and energy intake (i.e. protein g/kg/day and energy kcal/kg/day). In addition, we explored whether inclusion of body composition measures in clinical practice is feasible and helpful in monitoring growth and physical development.

We conducted a retrospective study that included a large number of data points collected from records of many patient-years and a prospective study on a smaller number of patients but with a more rigorous data collection protocol. We segregated our patients into groups based on the type of diet prescribed: patients on a low natural protein diet without supplementations (organic acidaemias MMA/PA/IVA and urea cycle disorders), patients on a very restricted protein diet with amino acid formula supplementation (PKU), and disorders in which protein intake or the P:E ratio may be altered secondarily to modifications in carbohydrate or fat intake (GSD and VLCAD deficiency).

Studies that monitor longitudinal growth contribute extensive information about the effect of dietary restrictions across the lifespan, as well as extending our understanding of the natural history of these rare disorders. The use of a lifelong monitored low protein diet is rarely documented outside of the field of IEM. A unique feature of this study is that it provides extensive longitudinal growth and dietary intake data from a relatively large number of patients with rare disorders of protein metabolism or disorders that may affect overall protein intake. Importantly, dietary data reflect actual intake rather than prescribed intake. Studying this

population provided valuable information on long-term physical development in individuals who consume protein intakes close to age dependent estimated requirements, but which are lower than the general population. This contributes to our overall understanding of the application and determination of protein requirement 'in real life', given that methods to determine these requirements are based on short term interventions such as nitrogen-balance studies, amino acid oxidation assessments etc. These data enabled us to confirm the importance of the P:E ratio in designing the diet as a targeted intervention to anticipate and address problems that may occur in growth or body composition.

Outcomes from nutritional studies with small numbers of patients with rare disorders on complex dietary prescriptions must be interpreted carefully. Our results suggest that there is no simple explanation for the lack of a consistent correlation with energy intake and growth parameters which requires consideration. Poor growth is seen in some IEM, even with what may appear an adequate dietary energy intake, while in other conditions excessive weight gain may be seen with relatively low energy intakes.

Consequently, we attempted to better explore the relationships between intake and outcome as this is likely multifactorial and not just a consequence of energy imbalance. For example, if we assume that most patients are in adequate energy balance to maintain metabolic stability, a core principle in IEM, then we would expect to see relatively normal growth distribution for all conditions, and this is clearly not the case.

However, the lack of any significant consistent correlation between energy intake on its own and growth and body composition in these patients is interesting. Beyond under-reporting, which is always possible, a significant factor that could contribute to this includes methodological inconsistencies including the lack of dietary data collected or included during periods of illness or decompensation when energy intake is likely to be increased significantly. For some patients this is likely to result in periods of energy excess that may contribute to

increase weight or body fat, but that is not accounted for when data is collected in the 'well' phase. In patients in the early years of life who are more likely to suffer from frequent childhood illnesses or those patients who have a more severe phenotype, this may have a measurable cumulative effect. These dietary manipulations are also likely to distort the P:E ratio and this has not been accounted for. We have only been able to consider data collected at limited time points and not include periods of dietary manipulations that may influence outcomes.

Correlation analysis is not a measure of cause and effect and on its own must be interpreted with caution. The relatively small patient numbers in this study do limit the type and power of statistical analysis that can be performed. With larger patient numbers and more extensive data, multiple linear regression analysis will be possible and extend our understanding of these relationships.

This study aimed to explore beyond energy intake or protein intake as independent predictors of nutritional outcome, and consider the relationship between them through the concept of the P:E ratio to determine its clinical applicability. Its limitations are acknowledged, but its value described. In the clinical environment it is possible to make assumptions about the effect of energy intake on protein utilisation when it is common practice to prescribe them separately. It is also common in practice to focus only on protein intake which is based on individual tolerance or dietary recommendations and assume energy is naturally regulated through appetite alone. These assumptions may result in a distorted energy intake for some individuals. The identification of a safe P:E ratio that has been correlated with optimal growth and body composition for different IEM diet may help to better control this.

We acknowledge that the variability in protein and energy requirement and intake for individuals is likely to have implications for the P:E ratio and that there will be a range of safe P:E ratios depending on age, activity and gender. This will allow for flexibility in dietary

prescriptions as documented in Chapters 3 and 8 with different P:E ratios for different conditions and diet types.

There are disagreements regarding the dietary therapy for inborn errors of protein metabolism, yet there are only few long-term longitudinal studies that allow comparison of different dietary approaches to management. We provide a new perspective on the effectiveness of dietary management practices that will ideally contribute to future comparisons of outcomes from children on different nutritional interventions. For example, there is a debate about the need or value of routine use of AAF and EAA in the management of MMA/PA/IVA and UCD. Our study contributes data that confirm that natural protein tolerance is adequate to meet estimated protein needs, good metabolic stability and appropriate growth outcomes. There is also a debate regarding the type of night feeds or UCCS use in patients with GSD. Although we did not have sufficient data to draw firm conclusions our preliminary results suggest that increasing protein intake in these patients may be beneficial. This will not only inform best practice, but determine if dietary treatments, although variable, can still be equally effective and produce comparable outcomes.

This research contributes to dietary management of IEM in that it has tested and shown benefit for an underutilised nutritional clinical tool, namely the P:E ratio. We provide recommendations regarding an apparent safe P:E ratio in different disorders, which clinicians can review and consider in context of their own practice. This is pertinent as individuals with IEM represent a group of vulnerable patients with severe dietary restrictions, but for whom to date, many dietary recommendations must be extrapolated from healthy populations. This tool allows for flexibility when determining the safety of a therapeutic approach for the management of a patient with IEM. Importantly, this also creates greater choice and confidence in management decisions, given the rarity of these disorders.

Finally, this research confirms the importance of body composition as a valuable component of long term follow up of dietary outcome, given improved life expectancy in IEM. It describes the use of a reliable, easy to access, non-invasive and quick methodology that could be incorporated seamlessly into clinical practice.

This study has some additional limitations. It includes patients from one metabolic centre only. This conveys some advantage in terms of follow up, constancy of practice and management protocols. However, this limits the size of the study population, and subsequent statistical analysis options. There may be some error when making correlations at multiple time points in the same small group of patients which may skew patterns of growth. We did not control for the influence of genetic bias in growth by estimating the difference between predicted and observed height in the retrospective studies including siblings. There are inherent limitations in both a retrospective and prospective analysis. These include the reliability and validity of some data using retrospective methodology. There was a lack of retrospective energy data for some disorders, which limited the analysis applied to substantiate growth outcome. There is selection bias in a prospective study in that it requires consent, as this may represent the most compliant group and may misrepresent the variability of outcomes in a small population.

When collecting reported rather than prescribed intake we assumed that dietary intake was relatively constant between clinic visits. Patients with intoxication type IEM often require a restricted protein intake during periods of metabolic stress, such as illness, that may have a cumulative effect on growth and body composition and which were not accounted for. Our analysis methods also considered that dietary intake was the only factor that affected growth. We did not explicitly correlate with metabolic control, and assumed it was adequate as children had data collected during periods of wellness, in clinic visits where monitoring occurred. As well, we did not further investigate the effect of potential micronutrient deficiencies that may occur in these children beyond regular clinical monitoring.

The validity and reliability associated with measuring dietary intake also has well documented limitations. Overall however, dietary review and education sessions with patients with IEM are frequent, and parents are highly motivated to comply with nutritional prescriptions, particularly in their child's younger years when it easier to monitor their child's intake.

9.1 Implementation

Our findings validate our current dietary regimens of patients with OA and UCD but point to some changes that are required in the management of patients with GSD. We determined an apparent 'safe' P:E ratio, associated with improved anthropometric and body composition outcomes, that can be used as an additional clinical tool when prescribing dietary intake in MMA/PA/IVA and UCD and PKU. We identified the potential benefit or P:E ratio in GSD. In all disorders examined, we determined that a higher natural protein intake was associated with lower body fat levels, suggesting that whenever possible, an increase in the intake of natural protein should be attempted. We confirmed that BIA can be used in regular clinical practice to measure of body composition to better monitor longitudinal nutritional status.

This work can be immediately translated to clinical practice. In doing so, we will be able to establish clinical protocols that include the implementation of apparent safe P:E ratios in the various disorders to improve long-term outcome. It can also contribute to any review of the current 'accepted' dietary prescriptions for disorders in which protein may be directly or indirectly modified. The concept of the P:E ratio can be used to provide guidance regarding the quality and composition of formulas used for some conditions, particularly those in which total nutrition can be supplied in the one preparation.

9.2 Future directions

Providing and promoting evidenced based therapies and guidelines is dependent on systematically collecting and evaluating data. “Whatever is not recorded is wasted” (Leonard J.V. Journal Inherit Metab Dis (2006) 29:275-278).

Standardisation of methods for data collection and data mining would be of immense benefit in IEM. When such restrictive and highly modified dietary therapies that can impact growth and development are initiated in the newborn period, it is paramount that their long-term safety is continually evaluated and confirmed.

Collection of prospective data consisting of complete dietary intake including natural protein intake and P:E ratio, growth and body composition measurements will be required in order to establish an apparent safe P:E ratio in GSD (for which we had only preliminary results) and to extend and strengthen the value of our findings and conclusions in the other disorders studied. In particular studies with much larger numbers of patients will allow fine tuning of safe P:E ratios. Existing recommendations for dietary intake of protein and energy vary with age and this may also apply to age-related safe P:E ratios. Larger studies, particularly in PKU, may allow analysis based on severity within the non-BH4 responsive group.

Additional research will be required to determine estimated energy requirements during both “healthy” and “sick” phases, in order to test the hypothesis that patients with these disorders require the same energy intake as healthy age and gender matched individuals. Consideration should be given to using pedometers or the currently popular wrist digital activity trackers to more scientifically assess activity.

In the era if personalised specific treatments that are based on identification of mutations and their known phenotypic correlation, our results put new questions to the forth: How can our understanding of new therapies that aim to manage specific genotypes, such as the use of BH4

in PKU, help us identify new criteria for dietary intervention? How does our understanding of the correlation between genotype and clinical outcome in disorders such as VLCAD, help us to personalise and predict nutritional interventions and outcomes?

The data collected and analysed in this thesis will help contribute to future evidence based standards of care in IEM and prompt international collaboration to validate and expand our results and to answer new questions that arise from these results.

List of related presentations during candidature

Department of Nutrition and Dietetics, Monash Medical Centre

Professional Development Workshop. Melbourne

Inborn Errors of Metabolism: Principles of Treatment and Nutritional Management.

Human Genetics Society of Australia Conference. Gold Coast. July 2011

Effect of BH4 on Phe/Tyr Ratios and Variation in Phe Levels in BH4 Responsive PKU Patients

Mitochondrial Research Group. Genetic Health Services Victoria Melbourne. September 2011

Adequacy of Medically Prescribed Diets: Does the Protein:Energy Ratio Matter?

Metabolic Dietary Disorders Conference. Gold Coast October 2011

Adequacy of Medically Prescribed Diets: Does the Protein:Energy Ratio Matter?

Undergraduate and Postgraduate Student lectures in Inborn Errors of Metabolism

Post Graduate Certificate in Paediatric Nutrition. Royal Children's Hospital Melbourne

Monash University 3rd and 4th Year Nutrition and Dietetics. 2010-2017

Murdoch Children's Research Institute retreat: 3-minute Thesis presentation November 2012

Long term effects of medically prescribed diets on growth, body composition, and nutritional markers in children with Inborn Errors of Metabolism (IEM)

International Congress of Inborn Errors of Metabolism (ICIEM): Barcelona Spain. September 2013

Invited speaker: New Ways of Defining Protein and Energy Relationships in IEM

6th National Conference on New Technology and Progress in Newborn Screening. August 2014 Huangshan, China.

Invited Plenary Speaker: Dietary management of Protein Disorders Diagnosed by Newborn Screening

Australasian Metabolic Clinicians Group Meeting. Melbourne March 2014

Invited speaker: Longitudinal Growth and dietary intake in children with IEM

Mitochondrial Research Group. Murdoch Children's Research Institute Melbourne. Nov 2014

Invited speaker: Calorie Utilisation and Weight Gain in Mitochondrial Oxidative Phosphorylation Defects

Society for the study of inborn errors of metabolism (SSIEM): Lyon France September 2015

Invited speaker: Emergency Dietary Management in Urea Cycle Disorders

Monash University November 2015: Inaugural SCS-Hudson PhD Symposium

Nominated speaker: The relationship between protein and energy intake and nutritional outcome in Inborn Errors of Metabolism.

Genetic Metabolic Dietitians International Conference: Arizona, USA. April 2016

Invited Speaker: The relationship between protein and energy intake and nutritional outcome in Inborn Errors of Metabolism.

'Live Life Well' Australasian Metabolic Dietitians meeting: Coogee, NSW. March 2016

Invited speaker: The relationship between protein and energy intake and nutritional outcome in Inborn Errors of Metabolism.

Biomarin PKU Advisory Board Meeting: Sydney, NSW. November 2016

Invited speaker: Guidelines for the management of phenylalanine hydroxylase deficiency

'Live Life Well 2' Australasian Metabolic Dietitians meeting: Adelaide, South Australia March 2017

Invited speaker: The relationship between dietary intake, growth and body composition in Inborn errors of metabolism

'Choose your own adventure'. Metabolic Dietary Disorders Association Parent's Retreat. Kalorama Victoria. October 2017

Invited speaker: Long term effects of protein modified diets on growth and body composition in children with inborn errors of metabolism

List of related achievements during candidature

Supervised Honours student from the Department of Nutrition and Dietetics Monash University 2015. Thesis: The Association between dietary intakes and body composition in children with PKU

Invited reviewer: Australasian consensus guidelines for the management of phenylketonuria (PKU) through the lifespan. June 2017

Courses completed

Expert seminar series MRGS/MPA:

- Thesis by Publication
- Writing Skills, Critical Analysis of Literature
- Turbo Charge your writing
- Confirmation of Candidature
- Excel Basic and Intermediate courses

Clinical Epidemiology and Biostatistics Unit/ Murdoch Childrens research Institute and Department of Paediatrics University of Melbourne

Data management:

- Introduction to Epidata

Monash University Department of Nutrition and Dietetics

- Biosafety training

Related Seminars/Conferences attended:

- Human Genetics Society of Australasia Conference (including the Australasian Society of Inborn Errors of Metabolism special interest group meeting). Melbourne 2010
- Human Genetics Society of Australasia Conference (including the Australasian Society of Inborn Errors of Metabolism special interest group meeting). Gold Coast 2011
- Higher Protein Diets. What's new? Meat and Livestock Corporation Sponsored DAA Seminar March 2012
- Genetic Metabolic Dietitians Conference: Challenging Issues and All that Jazz. New Orleans 2012
- ICIEM: International Congress of Inborn Errors of Metabolism. Barcelona Spain Sep 2013
- Australasian Metabolic Clinicians Group Meeting. Melbourne March 2014
- 6th National Conference on New Technology and Progress in Newborn Screening. August 2014 Huangshan, China.
- Nestle Paediatric Clinicians meeting Montreux, Switzerland July 2015
- SSIEM: Society for the study of inborn errors of metabolism. Lyon, France September 2015
- Genetic Metabolic Dietitians International Conference: Arizona, USA. April 2016
- 'Live Life Well' Australasian Metabolic Dietitians meeting: Coogee, NSW. March 2016
- 2016 Annual Multidisciplinary European Phenylketonuria Symposium: Amsterdam, the Netherlands October 2016 (Invited to attend)
- Australasian Metabolic Dietitians meeting: Adelaide, South Australia March 2017