Molecular Genetics and Metabolism Reports 13 (2017) 99–104

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Refining low protein modular feeds for children on low protein tube feeds with organic acidaemias☆,☆☆,☆☆☆

A. Daly*, S. Evans, C. Ashmore, S. Chahal, S. Santra, A. MacDonald

Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom

ARTICLE INFO

Keywords:
Enteral feeding
Protein-free feeds
Organic acidaemia

ABSTRACT

Children with inherited metabolic disorders (IMD) who are dependent on tube feeding and require a protein restriction are commonly fed by 'modular tube feeds' consisting of several ingredients. A longitudinal, prospective two-phase study, conducted over 18 months assessed the long-term efficacy of a pre-measured protein-free composite feed. This was specifically designed to meet the non-protein nutritional requirements of children (aged over 1 year) with organic acidaemias on low protein enteral feeds and to be used as a supplement with an enteral feeding protein source.

Methodology: All non-protein individual feed ingredients were replaced with one protein-free composite feed supplying fat, carbohydrate, and micronutrients. Thirteen subjects, median age 7.4y (3–15.5y), all nutritionally tube dependent (supplying nutritional intake: ≥ 90%, n = 12; 75%, n = 1), and diagnosed with organic acidaemias (Propionic acidaemia, n = 6; Vitamin B_{12} non-responsive methyl malonic acidaemia, n = 4; Isovaleric acidaemia, n = 2; Glutaric aciduria type1, n = 1); were studied. Nutritional intake, biochemistry and anthropometry were monitored at week − 8, 0, 12, 26 and 79.

Results: Energy intake remained unchanged, providing 76% of estimated energy requirements. Dietary intakes of vitamins, minerals and essential fatty acids significantly increased from week 0 to week 79, but sodium, potassium, magnesium, decosahexanoic acid and fibre did not meet suggested requirements. Plasma zinc, selenium, haemoglobin and MCV significantly improved, and growth remained satisfactory. Natural protein intake met WHO/FAO/UNU 2007 recommendations.

Conclusions: A protein-free composite feed formulated to meet the non-protein nutritional requirements of children aged over 1 year improved nutritional intake, biochemical nutritional status, and simplified enteral tube feeding regimens in children with organic acidaemias.

1. Introduction

Enteral feeds for children with severe organic acidaemias, (propionic acidaemia, [PA]; vitamin B_{12} non-responsive methyl malonic acidaemia, [MMA B_{12}nr]; isovaleric acidaemia, [IVA]; glutaric aciduria type 1, [GA1]); dependent on tube feeding are complex. Protein-free composite feeds supplying adequate macro and micronutrients are unavailable for children over 12 months of age, necessitating feeding regimens to consist of multiple ingredients to meet the non-protein nutritional requirements. Adaptation of enteral formulations designed for other clinical conditions may lead to nutritional imbalance, suboptimal growth and body composition in children who are at risk of metabolic decompensation. It is essential to provide an age appropriate, protein-free composite feed to supplement sources of natural and precursor-free L-amino acid supplements for enteral feeds in this vulnerable group.

Our hypothesis was that an age appropriate protein-free composite feed (supplemented with fat, carbohydrate, vitamins, minerals and essential fatty acids) formulated for children over the age of 1 year with IMD would improve nutritional status and simplify enteral tube feeding regimens for children requiring low protein diets with organic acidaemias. The protein-free composite feed was designed to accompany a protein containing feed, which supplied the protein source.

We have previously reported the short-term efficacy and tolerance

☆ Authorship of all authors: All authors contributed to the manuscript, by helping in the design, assimilation, analysis, interpretation and critique of the final paper.

☆☆ Conflict of interest: The study was funded as part of PhD grant. The authors: Daly A, Evans S, MacDonald A, have received research grants and lecturing honoraria, consulting fees from various nutritional companies (Nutricia Ltd, Vitaflo Ltd, Merck Serono, Bio Marin, Firstplay dietary foods, Mevalia, Cambrook Foods). Santra S, Chahal S declare no conflict of interests.

☆☆☆ Funding source: This work was funded as part of a PhD grant by Vitaflo Ltd.

* Corresponding author.
E-mail address: a.daly3@nhs.net (A. Daly).

http://dx.doi.org/10.1016/j.ymgmr.2017.08.003
Received 1 May 2017; Received in revised form 14 August 2017; Accepted 14 August 2017
Available online 07 October 2017

of a powdered pre-measured protein-free composite feed [Basecal 200 Vitaflo Ltd. Liverpool UK], containing fat, carbohydrate and micronutrients designed for children with organic acidaemias, over 1 year of age. The children required a protein restriction, and were tube feeding dependent. In this open, extension study, we report the nutritional efficacy and caregiver feed preparation data after the protein-free composite feed formulated for children aged over 1 year of age had been used for 18 months when patients were fully transitioned onto the protein-free composite feed. This protein- free feed provided energy and an important source of carbohydrate, fat, essential fatty acids, vitamins and minerals. The protein requirements were provided by a separate feed and we were not aiming to study the impact on protein nutrition.

2. Methods

2.1. Study design

An 18-month, longitudinal prospective open-label, intervention, extension study using a protein-free composite feed (study feed) developed for children over 1 year of age. The introduction of the study feed in the first 6 months (phase 1) has been reported [1]. In phase 2, patients with organic acidaemias on tube feeds were studied for a further 12 months, when all individual modular energy sources were replaced with only one ingredient, the study feed

2.2. Study formulation (Table 1)

The pre-measured study feed has a nutritional profile that meets the nutritional requirements of children over 1 year of age when reconstituted at 1 kcal/ml. It contains carbohydrate, fat (including long chain polyunsaturated fatty acids), vitamins, minerals, and trace elements. It does not contain fibre. Each sachet (43 g) mixed with 200 ml water, provides 200 kcals (1 kcal/ml).

2.3. Inclusion criteria

Children were recruited if they had a proven organic acidaemia, aged over 1 years of age or weighed between 8 and 31 kg, and were taking a modular feed providing ≥75% of enteral feed intake. All children had taken part in study phase 1 [1].

2.4. Phased introduction of study feed (Table 2)

At the start of the study, when analysis is based on the follow-up subjects (n = 13), enteral feeds consisted of a median of 4 ingredients comprising of: a natural protein source, precursor-free L-amino acids (disorder specific) and energy modules (either an infant energy module [± additional energy modules] or individual energy modules).

2.4.1. Phase 1 (week 0–26)

In this phase, the aim was to ensure children tolerated the study feed. This feed replaced the infant protein-free composite feed (Energivit [Nutricia Ltd]). However, to maintain a constant energy intake supporting metabolic stability, other energy sources [glucose polymer (n = 8) and 50% fat emulsion (n = 1)] remained in the feed recipes. All precursor-free L-amino acids were changed to pre-measured sachets. (Vitaflo International Ltd)

2.4.2. Phase 2 (week 27–79)

The study protein-free composite feed completely replaced the remaining energy sources (glucose polymer and 50% fat emulsion).

2.5. Protein prescription

Changes were made to protein (intake/sources) as children increased in age (e.g. natural protein source changed from infant formula to standard enteral feed). Natural protein was prescribed according to WHO/FAO/UNU (2007) [2] safe levels of protein intake for MMA/PA and IVA and in GA1 according to European guidelines [3]. In MMA/PA, an additional 15 to 20% of total protein intake was prescribed from MMA/PA precursor-free L-amino acids (AA); in GA1, lysine-free, low tryptophan AA supplements were prescribed according to GA1 European guidelines [3]. No precursor-free AA supplements were prescribed for patients with IVA.

Table 1
A comparison of the nutritional composition (per 100 ml and per 100 Kcal) of study protein-free module ingredient (Basecal) with a protein-free infant feed (Energivit).

	Basecal 200 (Vitaflo Ltd) per 100 Kcal equivalent to 100 ml	Energivit (Nutricia) per 100 ml (15% dilution as recommended by manufacturer)	Energivit (Nutricia) per 100 Kcal equivalent to 20% dilution. NB. This is in excess of manufacturers recommendation
Energy Kcal/kJ	100/420	74/309	100/420
Protein g	0	0	0
Carbohydrate g	15	10	13.3
Fat g	4.5	3.8	5.1
Vitamin A μg	65	58.8	78.4
Vitamin D μg	1.7	1.3	1.7
Vitamin E mg	2	1	1.3
Vitamin C mg	15	7.4	10
Vitamin K μg	5.7	5.6	7.5
Thiamin mg	0.11	0.08	0.1
Riboflavin mg	0.16	0.08	0.1
Niacin mg	1.1	1.1	1.5
Vitamin B6 mg	0.13	0.08	0.1
Folic acid μg	18.5	8.3	11.1
Vitamin B 12 μg	0.28	0.2	0.27
Iron mg	1	1.2	1.6
Zinc mg	1	0.9	1.2
Copper mg	0.07	0.07	0.09
Selenium μg	3	2.3	3
Magnesium mg	10.2	8.7	11.6
Manganese mg	0.1	0.06	0.08
Biotin μg	3.5	2.7	3.6
Pantothenic acid mg	0.55	0.4	0.5
Sodium mmol	1.8	1.2	1.6
Potassium mmol	2.5	1.9	2.5
Chloride mmol	1.6	1.5	2
Phosphorous mg	1.3	1.5	2
Iodine μg	17	12.5	17
Molybdenum μg	4.8	1.8	2.4
Choline mg	20.6	13.7	18.3
Inositol mg	11	14.7	19.6
DHA mg	16	0	0
AAmg	16	0	0

2.6. Dietary analysis

For 3 days during week 79, caregivers recorded the volume of modular feed consumed and any oral food and drink intake (n = 1) by weighing dietary intake. Dietary analysis was calculated using the software program *Electronic Dietetic Manager* (EDM 2000™ Microman 2000 PO box 3721 Newport Pagnell UK) and McCance and Widdowson's 'The Composition of Foods.' [4]. The intake of energy, total protein (natural protein and protein equivalent from precursor-free L-amino acids), fibre, calcium, magnesium, iron, zinc, selenium, sodium, potassium, vitamin B_{12}, vitamin D, essential fatty acids (DHA and AA) and fluid was assessed. Each nutrient (except protein, essential fatty acids (DHA and AA), fluids and fibre) was compared as a percentage of the DH 1991 reference nutrient intakes (RNI) or estimated average requirement (EAR) for energy [5] Protein intake was compared

Table 2
Type of feed ingredients used and method of measurement at baseline phase 1 and phase 2 for n = 13 subjects.

	Feed ingredient	Measurement	Pre study (week 8)	Phase (week 0–26)	Phase 2 (week 27–79)
Natural protein source	Nutrini multifibre[a]	Liquid	n = 9	n = 9	n = 11
	Tentrini multifibre[a]	Liquid	n = 2	n = 2	n = 2
	Standard whey based infant formula (Cow and Gate)[a]	Scales	n = 2	n = 2	n = 0
Precursor-free L-amino acids	MMA/PA amino 5[b]	Sachet	n = 6	n = 10	n = 10
	GA amino acids[b]	Sachet	n = 1	n = 1	n = 1
	MMA/PA anamix infant	Scales	n = 4		
Energy source	Energivit[a]	Scales	n = 11		
	Study feed[b]	Sachets		n = 13	n = 13
	Glucose polymer, (Maxijul)[a]	Scales	n = 9		
	Glucose polymer, (SOS)[b]	Sachets		n = 8	
	50% fat emulsion, (Calogen)[a]	Liquid	n = 1	n = 1	
Vitamins and mineral supplement	Vitamin and mineral supplement, Paediatric Seravit[a]	Scales	n = 1		
Fibre	Soluble fibre, Optifibre[c]	Sachet	n = 1	n = 1	n = 4
Median number (range) of feed ingredients			n = 4 (1–5)	n = 3 (1–4)	n = 3 (1–4) *p = 0.05

Feed ingredients.
[a] Nutricia Ltd.
[b] Vitaflo Ltd.
[c] Nestle Ltd.
* Significant difference in core module ingredients between baeline and phase 2.

Table 3
DNA mutation analysis results for subjects participating in the modular feed study.

Subject number	DNA mutation confirmation	Diagnosis
1.	Homozygous c.937C > T (p.Arg313X)	PA
2.	Homozygous c.937C > T p.(Arg313*)	PA
3.	Homozygous c.1288C > T	PA
4.	Homozygous C.11.120786 c.1746 + 1G > A	PA
5.	Homozygous C.1498 + 2T > C	PA
6.	Confirmed by biochemical testing	PA
7.	Homozygous c.09.4018 MUT gene c.1106G > A p.(Arg369His)	MMAmut^0
8.	Two homozygous variants in Mut gene c.689C > G p (Thr230Arg) and c. 1991C > T p.(Ala664 Val)	MMA mut^0
9.	Skin biopsy activities of holomutase severely deficient both in fiboblasts cultured in MEM with or without B12. Defect of methymalonyl CoA mutase apoenzyme.	MMA mut^0
10.	Confirmed by biochemical testing	MMA
11.	Compound heterozygous in GCDH gene c.572T > C and c.304delG	GA1
12.	Skin biopsy isovaleric acid incorporation analysis	IVA
13.	Confirmed by biochemical testing	IVA

with the WHO/FAO/UNU safe levels of protein intake [2] and fibre with the Scientific Advisory Committee on Nutrition [6]. Nationally agreed definitions for DHA and AA intake for children in the UK are not available. A comparison was made with World Health Organization [7] global recommendations.

2.7. Anthropometric measurements

Weight, height/length, z-scores were collected at outpatient clinic appointments. In children ≥ 2 years of age, weight was estimated by: *standing* and *sitting* Seca electronic scales (accurate to two decimal places); and height by a calibrated stadiometer (accurate to one decimal place). In children < 2 years of age, measurements were taken naked: weight by Seca infant scales accurate to two decimal places, supine length on a Seca measuring mat accurate to one decimal place.

2.8. Illness episodes

The number of hospital admissions, reason for admission and length of stay were recorded from phase 1 (week 0–26) and phase 2 (27–79).

2.9. Nutritional blood biochemistry

Venous blood samples were collected for glucose, electrolytes, full blood count, plasma selenium glutathione peroxidase, zinc, iron, ferritin, vitamin B_{12}, plasma MMA, and C-reactive protein (CRP) at week − 8, 0, 12, 26. At week 79, venous samples were taken for full blood count, zinc, selenium, ferritin and CRP. Additionally, a random urine test analyzing sodium and potassium concentrations was collected at week 79, as dietary sodium intake was low in phase 1. Blood samples were taken after a minimum of 3 h fasting

2.10. Statistics

The median nutritional intake from the different feeding regimens was compared at baseline, week 26 and week 79. The statistical tests used were non-parametric paired Wilcoxon signed rank tests. The same statistical tests were applied to analyse for significant differences in biochemistry, haematology and anthropometric results.

3. Results

3.1. Subjects (Table 3)

Thirteen children (6 boys, 7 girls) were followed from phase 1. Confirmation of the organic acidaemia by DNA analysis is available for nine of the thirteen subjects. One subject had confirmation via skin biopsy and isovaleric acid incorporation analysis; the remaining three subjects were diagnosed by routine biochemical testing. Their median age (range) was 7.4y (3–15.5y). All had organic acidaemias: PA n = 6; MMA B_{12}nr, n = 4; IVA n = 2; and GA1, n = 1. Eight children were of Asian Pakistani origin, one Afro-Caribbean, one Arabic, one Bangladeshi and two European. All children received ≥ 75% of their enteral nutritional intake via tube feeds (nutritional intake: ≥ 90%, n = 12; 75%, n = 1). Two children, both with PA were withdrawn from the study after completing phase 1 (one died post liver transplantation and one required long term hospital intensive care management).

Table 4
Comparison of daily nutritional intake and reference nutrient intakes, median (range) for all nutrients measured at baseline, phase 1 (when study feed replaced one ingredient), and phase 2 (when all modular ingredients were replaced).

Nutrients	Mean nutrient intake (range)		Significance Pre-study to phase 1 n = 13	Mean nutrient intake (range) Phase 2 (week 79)	Significance Baseline to phase 2 n = 13	% reference nutrient intake		Significance Pre-study to phase 1 n = 13	% reference nutrient intake Phase 2 (week 79)	Significance Baseline to phase 2 n = 13
	Pre-study (week 8)	Phase 1 (week 26)				Baseline (week 8)	Phase 1 (week 26)			
Energy kcal/kg/d*	59 (32–82)	61 (34–87)	p = 0.87	48 (33–62)	p = 0.002	76 (47–103)	78 (46–98)	p = 0.06	76 (48–86)	p = 0.4
Total protein g/kg (natural and precursor-free L-amino acids) g/kg/d	1.2 (0.7–1.5)	1.2 (0.8–1.4)	p = 0.48	1.0 (0.6–2.1)	p = 0.002	129 (78–153)	127 (88–164)	p = 0.32	111 (67–200)	p = 0.002
Natural protein** g/kg/d	0.85 (0.5–1.2)	0.85 (0.5–1.2)	p = 0.3	0.9 (0.6–1.1)	p = 0.8	94 (56–141)	91 (56–118)	p = 0.56	100 (82–130)	p = 0.8
Precursor-free L-amino acids g/kg/d	0.35 (0.2–0.7)	0.4 (0.2–0.6)	p = 0.8	0.2 (0–1)	p = 0.002	n/a	n/a		n/a	
Calcium mg/d***	693 (426–848)	718 (498–1102)	p = 0.39	804 (569–1673)	p = 0.02	145 (89–242)	152 (95–247)	p = 0.43	153 (88–200)	p = 0.9
Iron mg/d***	12 (6–17)	12 (6–18)	p = 0.75	13.7 (10–27)	p = 0.02	142 (87–232)	147 (87–197)	p = 0.75	161 (95–260)	p = 0.4
Zinc mg/d***	10.5 (6–14)	11.5 (6–17)	p = 0.15	13.7 (10–26)	p = 0.0005	149 (92–240)	179 (120–243)	p = 0.15	183 (138–289	p = 0.06
Selenium µg/d***	30.2 (15–46)	35.5 (17–68)	p = 0.12	41 (29–71)	p = 0.005	148 (87–247)	168 (84–213)	p = 0.1	137 (93–195)	p = 0.6
Phosphorous mg/d***	508 (308–749)	553 (291–832)	p = 0.37	634 (445–1231)	p = 0.001	144 (93–214)	148 (95–211)	p = 0.36	153 (95–250)	p = 0.8
Potassium mmol/d***	26.9 (16–46)	28 (15–48)	p = 0.2	37 (26–63)	p = 0.005	91 (28–164)	102 (33–164)	p = 0.06	74 (49–143)	p = 0.4
Vitamin D µg/d***	13 (8–17)	15.7 (12–24)	p = 0.04	16 (11–29)	p = 0.14	179 (114–242)	214 (171–257)	p = 0.1	na$	na
Magnesium mg/d***	100 (67–173)	121 (57–278)	p = 0.02	145 (102–392)	p = 0.005	77 (43–144)	102 (49–135)	p = 0.02	89 (54–140)	P = 0.08
Vitamin B 12 µg/d***	2.7 (1.7–3.6)	3.0 (2–5)	p = 0.01	3.0 (3–6)	p = 0.003	339 (200–580)	388 (280–580)	p = 0.04	375 (200–600)	p = 0.6
Sodium mmol/d***	20 (8–33)	25 (9–36)	p = 0.002	31 (22–42)	p = 0.001	61 (29–110)	72 (34–110)	p = 0.005	80 (53–127)	p = 0.03
Docosahexaenoic acid (mg)§	0 (0–340)	96 (32–340)	p = 0.01	113 (44–369)	p = 0.0001	0 (0–170)	48 (16–170)	p = 0.005	57 (22–185)	p = 0.002
Arachidonic acid (mg)	0 (0–91)	96 (32–128)	p = 0.001	95 (45–121)	p = 0.0005	n/a	n/a		n/a	
Fluid ml/kg/d§§	1087 (630–1430)	1100 (700–1430)	p = 0.1	1200 (900–1890)	p = 0.0005	68 (39–89)	69 (44–89)	p = 0.2	70 (53–98)	p = 0.9
Fibre g/d§§§	4 (0 − 12)	4 (0–12)	p = 1	4 (0–12)	p = 1	20 (0–60)	20 (0 = 60)	p = 1	20 (0 = 60)	p = 1

n/a not available, * estimated average requirement ** WHO/FAO/UNU 2007 recommendations *** reference nutrient intake, §food and agriculture organization of united nations (FAO), §§based on Holliday Segar formula for 20 kg child, §§§SACN2015, $no vitamin D RNI available for children > 3 yr.

Twelve children had a gastrostomy tube/button and one had a nasogastric tube. Eleven families had one caregiver who spoke and read English fluently. Diagnosis was mainly in the neonatal period (including three siblings of index cases), but 2 were diagnosed later in infancy (7 months [$n = 1$] and 10 months [$n = 1$]).

3.2. Feed preparation changes (Table 2)

The number of enteral feed ingredients decreased significantly in phase 2: median number of ingredients baseline, n = 4; phase 1, n = 3; and phase 2, n = 3; [p = 0.05]. In phase 2, no caregivers used scales or scoops to measure ingredients.

3.3. Nutrient intake (Table 4)

3.3.1. Energy, protein and fluid intake

Energy intake (median of 76%) as a percentage of the estimated average requirement (EAR) for age [5] remained unchanged between baseline, phase 1 and phase 2. The energy intake was closely controlled to avoid excessive weight gain.

3.3.2. Protein intake

The median natural protein intake remained constant at 0.9 g/kg/day throughout the 18-month study period. Total protein intake expressed as g/kg/day significantly decreased, (p = 0.002) associated with decreasing natural protein requirements with age (WHO/FAO/UNU 2007) [2] and a corresponding decrease in precursor-free L-AA (p = 0.002) from baseline to week 79. Natural protein intake was provided from a standard paediatric feed Nutrini multifibre (Nutricia Ltd., Trowbridge, UK), meeting safe levels of protein intake. Precursor-free amino acids supplemented natural protein intake, providing an additional 15 to 20% of total protein intake.

3.3.3. Vitamin, mineral and long chain fatty acid intake (Table 4)

From baseline to week 79 there was a significant increase in the median daily intake of: calcium [p = 0.02], iron [p = 0.02], zinc [p = 0.0005], selenium [0.005], phosphorous [0.001], vitamin B_{12} [p = 0.003], potassium [0.005], magnesium [p = 0.005], and sodium [p = 0.001]. The intake of both docosahexaenoic acid (DHA) [p = 0.0001] and arachidonic acid (AA) [p = 0.0005] significantly improved. Nutrients failing to meet reference intakes were potassium (74%), magnesium (89%), sodium (80%), docosahexaenoic acid (DHA) 57% and fibre (20%).

In phase 1, dietary intake of only magnesium [p = 0.02], sodium [p = 0.002], Vitamin B_{12} (p = 0.01), Vitamin D (p = 0.04), docosahexaenoic acid (DHA) [p = 0.01] and arachidonic acid (AA) [p = 0.001] significantly improved. All except sodium (72%), DHA (48%) and fibre (20%) failed to meet reference guideline intakes.

3.4. Nutritional biochemical and haematology results (Table 5)

Over the 18-month period (baseline to week 79), there was a significant increase in plasma zinc [p = 0.03], selenium [p = 0.01], haemoglobin [p = 0.05] and MCV [p = 0.005]. All the other measured analytes, ferritin and CRP remained within the reference ranges and were unchanged. Median urinary sodium was 80 mmol/L (22 − 300) and potassium was 41 mmol/L (13–236), with an appropriate ratio of 2:1.

In phase 1, only plasma selenium [p = 0.002] and whole blood glutathione peroxide [p = 0.02] significantly improved (baseline to week 26).

3.5. Anthropometry

There were no significant differences in median height or weight z scores throughout the study. Median weight z score: baseline, 0.3 (− 3.1 to 2.5); phase 2, 0.2 (− 2.3 to 1.8); height z score: baseline, − 1.3 (− 2.6 to 1.5); phase 2, − 0.9 (− 2.9 to 0.4).

3.6. Hospital admissions

The median number of hospital admission days was: phase 1, 3 (range 2–12); and phase 2, 3 (range 1–86). The hospital admissions were associated with vomiting, chest infections or pancreatitis (n = 1) causing metabolic decompensation. No admissions were reported because of feed changes.

3.7. Medications

The median number of daily medications was 4, range (1–10): including L-carnitine (n = 13), metronidazole (n = 7), domperidone (n = 2), ondansetron (n = 4), omeprazole (n = 4), and ranitidine (n = 1). Six subjects were prescribed regular laxatives (movicol and lactulose) and 4 had fibre added to their feeds for constipation. One child was prescribed sodium supplements because of previous low blood concentrations. Only one child was routinely prescribed sodium benzoate due to frequent metabolic decompensation; no other patients were prescribed ammonia lowering drugs.

4. Discussion

In this longitudinal 18 month study, the use of a pre-measured protein-free composite feed designed for children aged over 1 year used in combination with protein sources (natural protein and precursor-free L-AA) to meet individualized protein requirements improved the nutrient intake and biochemical status in children with organic acidaemias. Replacing individual energy and fat modules with one comprehensive protein-free composite feed simplified the feeding regimens,

Table 5
Median biochemical and haematological (range) values with study feed replacing one ingredient and after 18 months when all modular ingredients replaced.

	Pre-study (week − 8) median (range)	Phase 1 (week 26) median (range)	Statistical significance pre-study to phase 1 n = 13	Phase 2 (week 79) median (range)	Statistical significance pre-study to phase 2 n = 13	Reference range
Plasma zinc μmol/L	10.9 (8.9–14.6)	10.7 (7–15.3)	p = 0.98	12.5 (7–16.5)	p = 0.03	10–18
Plasma selenium μmol/L	0.96 (0.6–1.2)	1.11 (0.8–1.4)	p = 0.002	1.2 (0.9–1.5)	p = 0.01	0.8–8.2
Ferritin μg/L	34 (5.3–121)	39.2 (7.8–167)	p = 0.12	38.5 (9–93)	p = 0.8	32–233
Vitamin B12 ng/L	957 (208–1049)	977 (166–2121)	p = 0.95	760 (114–1500)	p = 0.2	259–823
Haemoglobin g/dL	122.9 (103–139)	123.8 (114–136)	p = 0.84	130 (109–161)	p = 0.05	112–130
MCV g/L	75 (65–83)	76 (68–84)	p = 0.22	85 (73–94)	p = 0.005	75–90
C reactive protein mg/L	2.6 (1 − 13)	2.9 (1–19)	p = 0.9	2.3 (1 − 12)	p = 0.9	< 10
Urine sodium mmol/L	–			80 (22–300)		40–220
Urine potassium mmol/L	–			41 (13–236)		10–60

and overall, lowered the number of core ingredients used.

Over 18 months, there was a significant improvement in the intake of essential fatty acids, vitamins and minerals (calcium, iron, zinc, selenium, phosphorous, vitamin B_{12}, potassium, magnesium, sodium and docosahexaenoic and arachidonic acid), which led to an improvement in biochemical nutritional status for plasma zinc, selenium, haemoglobin and MCV. These results were better than we reported in phase 1 of this study. However, in phase 2 (out of controlled study conditions) we were we able to replace all energy supplied by glucose polymer supplements with the nutrient supplemented protein-free composite feed leading to further improvements in nutrient intake. It is essential that patients with organic acidaemias on low protein diets receive optimal nutrition from enteral feeds. They have frequent and unpredictable hospital admissions due to metabolic decompensation and require glucose-polymer based emergency feeds (with a low nutrient density) during intercurrent illness. This interrupts macro and micronutrient supply.

The nutrient composition of the study feed may require further optimization. Magnesium, potassium and sodium intakes were still below the UK RNI [5]. Only one child, with PA received sodium containing nitrogen scavenger drugs. Although, random sodium and potassium urine analysis in phase 2 suggested an overall satisfactory biochemical status, dietary intakes would imply some children might be borderline deficient. The long-term clinical consequences of these suboptimal intakes are unknown. Magnesium is physiologically important for skeletal development and maintenance of electrical potential in nerve and muscle membranes [8]. Potassium is required for lean muscle synthesis, normal cell function and maintenance of total body fluid volume, acid and electrolyte balance. The WHO guideline on potassium intake for adults and children [9] suggested a conditional recommendation of at least 90 mmol/day, with an adjustment downwards for children based on their energy requirements.

The study feed did not contain a source of fibre. In MMA and PA, the amount and optimal blend of fibre(s) that should be added to enteral feeds is unknown. Mixed fibre supplements in enteral feeds normalises transit time, improves gut barrier function, and increases production of short chain fatty acids especially propionate [10]. It is possible that adding mixed fibres, particularly those containing neutral sugars to enteral feeds, may increase concentrations of propionate from short chain fatty acids, which may be detrimental in children with PA and MMA [11–14]. More information is required about the composition of gut flora in children with OA before optimal fibre(s) amounts and composition can be defined [15,16]. A high number of our study group had chronic issues with constipation and were prescribed regular laxatives.

There were a number of limitations with this study. Due to the rarity of the conditions, it only included a small number of children. We did not measure biochemical fatty acid status. We have also not examined organic acids, protein status or amino acid status as this was not the purpose of this study. Plasma sodium and potassium are not sensitive indices of total body sodium and potassium. Although we did take measurements for urinary sodium and potassium, this was only performed at week 79, and only a single, random sample was taken rather than a 24-h collection to measure 24-h excretion as recommended by the World Health Organization report [17]. Overall this study cohort was followed up consistently, blood sample timing standardized and any feed changes monitored closely.

This long term follow up study successfully demonstrated that a premeasured protein-free composite feed used in combination with an enteral protein source for children aged over 1 year with organic acidaemias on low protein enteral feeds, significantly improved nutrient intake, biochemical nutritional status and simplified the feed making process. However, further changes to the nutrient composition of this protein-free composite feed are necessary to deliver the optimal nutritional intake in this group of children, whose nutrient intake may be frequently disrupted as a consequence of illness and metabolic decompensation.

Conflict of interest

The study was funded as part of PhD grant. The authors: Daly A, Evans S, MacDonald A, have received research grants and lecturing honoraria, consulting fees from various nutritional companies (Nutricia Ltd., Vitaflo Ltd., Merck Serono, Bio Marin, Firstplay dietary foods, Mevalia, Cambrook Foods). Santra S, Chahal S declare no conflict of interests

Transparency declaration

‘The lead author confirms that this manuscript is an honest, accurate and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and registered with the ethics committee) have been explained. The reporting of this work is compliant with STROBE guidelines.’

References

[1] A. Daly, S. Evans, C. Ashmore, S. Chahal, S. Santra, A. M, The challenge of nutritional profiling of a protein-free feed module for children on low protein tube feeds with organic acidaemias, J. Hum. Nutr. Diet. (2017) (In press).
[2] Protein and amino acid requirements in human nutrition, World Health Organ. Tech. Rep. Ser. (935) (2007) 1–265 (back cover).
[3] S. Kolker, E. Christensen, J.V. Leonard, C.R. Greenberg, A. Boneh, A.B. Burlina, et al., Diagnosis and management of glutaric aciduria type I–revised recommendations, J. Inherit. Metab. Dis. 34 (3) (2011) 677–694.
[4] R.A. McCance, E.M. Widdowson, A.A. Paul, D.A.T. Southgate, Mccance and Widdowson's The Composition of Foods, 4th rev. and extended ed., xii Elsevier/North Holland Biomedical Press, London Amsterdam; New York: H.M. Stationery Off., 1978, p. 417.
[5] Dietary reference values for food energy and nutrients for the United Kingdom. Report of the panel on dietary reference values of the committee on medical aspects of food policy, Rep. Health Soc. Subj. 41 (1991) 1–210 (Lond).
[6] Nutrition SACo, Carbohydrates and Health, (2015).
[7] Fats and fatty acids in human nutrition. Report of an expert consultation, FAO Food Nutr. Pap. 91 (2010) 1–166.
[8] H. Dimke, L. Monnens, J.G. Hoenderop, R.J. Bindels, Evaluation of hypomagnesemia: lessons from disorders of tubular transport, Am. J. Kidney Dis. 62 (2) (2013) 377–383.
[9] World Health Organization, Guideline: Potassium Intake for Adults and Children. WHO Guidelines Approved by the Guidelines Review Committee, World Health Organization (WHO), Geneva, 2017.
[10] E. Boets, S.V. Gomand, L. Deroover, T. Preston, K. Vermeulen, V. De Preter, et al., Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study, J. Physiol. 595 (2) (2017) 541–555.
[11] G.T. Macfarlane, S. Macfarlane, Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut, Curr. Opin. Biotechnol. 18 (2) (2007) 156–162.
[12] G.T. Macfarlane, S. Macfarlane, Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics, J. Clin. Gastroenterol. 45 (Suppl) (2011) S120-7.
[13] G.T. Macfarlane, S. Macfarlane, Bacteria, colonic fermentation, and gastrointestinal health, J. AOAC Int. 95 (1) (2012) 50–60.
[14] R. Sharp, G.T. Macfarlane, Chemostat enrichments of human feces with resistant starch are selective for adherent butyrate-producing clostridia at high dilution rates, Appl. Environ. Microbiol. 66 (10) (2000) 4212–4221.
[15] Human Microbiome Project C, Structure, function and diversity of the healthy human microbiome, Nature 486 (7402) (2012) 207–214.
[16] J. Qin, R. Li, J. Raes, M. Arumugam, K.S. Burgdorf, C. Manichanh, et al., A human gut microbial gene catalogue established by metagenomic sequencing, Nature 464 (7285) (2010) 59–65.
[17] Strategies to monitor and evaluate population sodium consumption and sources of sodium in the diet, Report of a Joint Technical Meeting Convened by WHO and the Government of Cananda Geneva Switzerland, World Health Organization, 2011, p. 42.