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A 12-month, longitudinal, intervention study examining a tablet protein substitute preparation in the management of tyrosinemia

Anne Daly *, Sharon Evans, Alex Pinto, Catherine Ashmore, Anita MacDonald

Birmingham Women 's and Children's Hospital, Steelhouse Lane, Birmingham, UK

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ABSTRACT

Protein substitutes (PS) without tyrosine (Tyr) and phenylalanine (Phe), are an essential source of synthetic protein in the treatment of tyrosinemia (HT). In the UK, the only available protein substitutes for HT are Tyr/ Phe free amino acid liquid or powders or formulations based on glycomacropeptide (CGMP). A tablet Tyr/ Phe free amino acid supplement (AAT) has now been introduced. The aim of this two-part prospective, longitudinal intervention study was to assess the efficacy, acceptability, and tolerance of AAT in children aged >8 years with HTI. Part 1: was a 28-day acceptability/ tolerance study, part 2, was a 12-month extension study examining efficacy of AAT. Anthropometry and blood Tyr/ Phe were assessed. All subjects were taking NTBC [2-(2-nitro-4-triflourothybenzoyl) cyclohexane-1, 3-dione] with a Tyr restricted diet. Eight subjects with HTI were recruited 4 boys, and 4 girls with a median age of 14.3y (range 10.4–17.3); 3 were Caucasian and 5 of Pakistani origin. The median (range) protein equivalent from PS was 60 g/d (50–60), natural protein 20 g/d (15–30), and NTBC 30 mg/d (25–80). No subjects were taking Phe supplements. Five (63%) subjects completed part 1, with 4 taking all their PS requirements as AAT. Subjects reported AAT were tasteless and had no odour. No adverse gastrointestinal symptoms were recorded, with two reporting improvements in abdominal discomfort. At 12 months, 4 subjects had a non-significant decrease in blood Tyr/ Phe compared to the 12 months pre-treatment. Median blood Tyr (μmol/ L) pre-intervention was 500 (320–590); and at 12 months, 450 (290–530). Median blood Phe (μmol/L) pre-intervention was 40 (30–40); and at 12 months 30 (30–50). Median height z scores remained unchanged, but there was a small decrease in weight z score (pre-study weight − 0.1 (−1.4 to1.1), 12 m − 0.3 (−1.4 to 1.3) and BMI (pre- study BMI 0.2 (−2 to 1.4), and 12 m, −0.1 (−2.5 to 1.5)).
Conclusion: AAT were useful for some adolescents with HTI who struggled with the taste and volume of conventional powdered and liquid PS.

1. Introduction

Hereditary tyrosinemia type I (HTI) is caused by a deficiency of fumarylacetoacetate hydrolase, the last enzyme in the tyrosine pathway [1]. It is characterised by liver failure, renal Fanconi syndrome, and porphyria like neurological crises [2]. It is a rare disorder, with an estimated incidence of 1:100,000 to 1:120,000 births [3]. The first case report was almost seventy years ago, and the condition had a high mortality [4]. Fortunately, from 1992, outcome improved after the introduction of 2-(2-nitro-4-trifluromethylbenzoyl)-1,3cyclohexanedione (NTBC or Nitisinone) [5,6]. Nitisinone is a potent enzyme inhibitor of 4-hydroxyphenylpyruvate (oxidase) dioxygenase the third catabolic step in the pathway. It inhibits the formation of the toxic intermediary metabolites fumarylacetoacetate acid, maleylacetoacetate, succinyl acetoacetate and succinyl acetone preventing the development of liver cirrhosis and decreasing the risk of liver cancer. However, because of this pathway inhibition, blood Tyr increases so the recommended treatment is NTBC with a Tyr/Phe restricted diet [7]. Some children need Phe supplementation to prevent its deficiency commonly reported in HT1 [8–11].

Tyr is found in natural protein food sources. The amount of natural protein that is tolerated in HT1 is insufficient to sustain normal growth and prevent nutritional deficiencies so supplementation with a protein substitute that is low/free from Tyr/ Phe is essential. Protein substitutes have a multifunctional role supplying nitrogen for growth, lowering blood tyrosine concentrations, and improving tyrosine tolerance.

* Corresponding author.
E-mail addresses: a.daly3@nhs.net (A. Daly), sharon.morris6@nhs.net (S. Evans), alex.pinto@nhs.net (A. Pinto), catherine.ashmore@nhs.net (C. Ashmore), anita.macdonald@nhs.net (A. MacDonald).

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Protein substitutes should be taken at least three times a day and given evenly over the course of the day to maximize amino acid utilisation [12–14].

In the UK, due to the rarity of HTI, there are few suitable protein substitutes available. They are based on L-amino acids or low Tyr/ Phe CGMP. The choice of flavours and their presentation is limited, potentially causing taste fatigue, lowering acceptance and adherence [14,15]. Failure to take the prescribed amount of protein substitute is associated with poor metabolic control [16].

Although the availability of AAT was reported in phenylketonuria (PKU) almost 20 years ago [17], in 2022 the first Tyr/Phe free amino acid tablets (AAT) were introduced as a protein substitute for HT in the UK. However, there was no research describing their long-term efficacy and acceptability in HT1. We report a two-part prospective, longitudinal intervention study investigating the acceptability and efficacy of AAT in children with HTI.

2. Materials and methods

2.1. Study design

Part 1: a 28-day study examining the acceptability and tolerance of AAT in children with HTI. One or more of their usual daily doses of protein substitute was replaced with the same amount of protein equivalent from AAT (Fig. 1).

Part 2: an extension study that monitored the progress of the subjects for a further 12 months on the study product.

All subjects followed their prescribed Tyr/ Phe restricted diet throughout the study. The study was performed at one site, at Birmingham Children's Hospital, UK.

3. Materials and methods

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Subjects

Only subjects, aged ≥8 years, diagnosed with HTI and treated with Nitisinone and a Tyr/ Phe restricted diet, supplemented with a free or low Tyr/ Phe substitute (based on Tyr/ Phe free amino acids or low Tyr/ Phe CGMP) were eligible for inclusion in this study. Exclusion criteria were children <8 years of age, additional co-morbidities (diabetes, renal impairment) or unable to swallow the AAT.

3.2. Composition of study product

The AAT (Tyr easy tablets, Galen Ltd.®) consisted of essential and non-essential amino acids. Each AAT was coated with sodium alginate with an outer coating of hydroxypropyl-methyl cellulose and a steric film, to aid swallowing. AAT had a neutral/ cream colour, were oval shaped 2 cm long and 1 cm wide. The outer layer had a neutral flavour to help mask the taste and smell of amino acids. Eleven AAT contained 10 g of protein equivalent, 54 kcals, 2.9 g carbohydrate, 0.9 g fat and weighed 13.5 g (Table 1). They did not contain vitamins or minerals, so a separate comprehensive vitamin and mineral supplement was prescribed (Phlexy Vits, Nutricia Ltd.®).

3.3. Monitoring protein substitute intake and gastrointestinal symptoms

In study part I, one week pre-intervention and daily over the 28-day study period, caregivers/subjects completed a diary documenting the timing and amount of ATT taken each day together with their usual protein substitute. Any gastrointestinal symptoms were recorded daily. A gastrointestinal tolerance questionnaire included seven questions about the occurrence of diarrhoea, constipation, bloating/ abdominal pain, nausea, vomiting, burping/flatulence, regurgitation and abdominal discomfort or pain. This included a scoring system: zero for no symptoms, one for mild, two for moderate and three for severe.

At the end of study part 1, a questionnaire was completed on the overall acceptability, palatability and ease of taking AAT. Responses were measured by a one to five Likert scale (one was very acceptable; five very unacceptable).

3.4. Blood tyrosine/phenylalanine concentrations

Routine weekly fasting finger prick blood spot samples were collected for blood Tyr/ Phe during part 1 of the study, and weekly/ biweekly in study part 2. Trained parents/ caregivers took fasting early morning samples on filter cards; Perkin Elmer 226 (UK standard NBS). Blood cards were sent via first class post to Birmingham Children's Hospital Clinical Chemistry laboratory. All the cards had a standard thickness, and blood Tyr/ Phe concentrations were calculated on a 3.2 mm punch by MS/MS tandem mass spectrometry.

Target blood tyrosine ranges were 200 to 400 umol/L for children as recommended by an international working group on HT1 [18], with recommended early morning fasted Phe >50 μmol/L although this is not

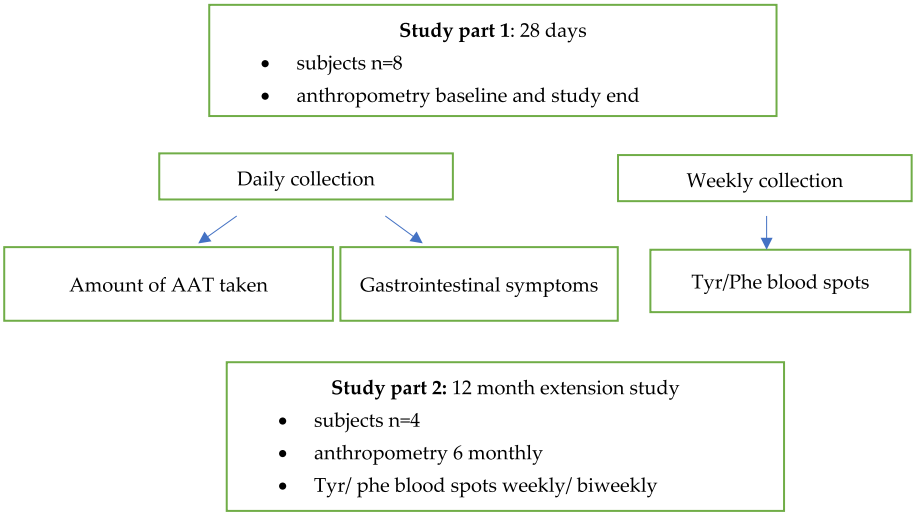

Fig. 1. Study methodology.

Table 1
Nutritional composition of ATT (Tyr Easy Tablets®) compared with current protein substitutes used in HT1.

Nutrient	Tyr Easy * Tablets (study product) 20 g PE (22 tablets/27 g)	Tyr cooler (174 mL) 20 g PE	Low Tyr Sphere (35 g) 20 g PE	Tyr Express (34 g) 20 g PE	Tyr Lophlex LQ (125 mL) 20 g PE
Manufacturer	Galen Ltd.®	Vitaflo International®	Vitaflo International®	Vitaflo International	Nutricia®
Energy Kcal/KJ	108/459	130/549	120/508	101/429	120/ 509
Protein equivalent g	20	20	20	20	20
Amino acids g	24	n/a	n/a	n/a	n/a
Fat g	0.9	1.6	1.6	0.07	0.4
Saturated fat g	0.9	0.3	0.4	0	0.1
DHA mg	0	134	110	0	150
Carbohydrate g	2.3	8.9	6.3	4.7	8.8
Sugar g	0.4	5.9	1.4	0.33	8.8
Fibre g	0.8	0	0	0	0.5
L-alanine g	1.4	1.6	0.8	1.44	1.1
L-arginine g	1.7	1.9	1.7	1.85	1.7
L-aspartic acid g	4.1	3.1	1.3	2.86	1.6
L-carnitine g	0.02	n/a	n/a	0	0.02
L-glutamine g	1.7	0	2.7	1.83	0
Glycine g	1.5	1.6	2.5	1.5	1.6
L-cystine g	0.6	0.7	0.2	0.69	0.8
L-histidine g	0.9	1.1	0.7	1.01	1.0
L-isoleucine g	1.5	1.8	1.4	1.68	1.6
L-leucine g	2.3	2.9	3.0	2.69	2.5
L-lysine g	1.7	2.1	1.0	1.91	1.8
L-methionine g	0.4	0.5	0.3	0.43	0.4
L-proline g	1.4	1.7	1.6	1.55	2.0
L-phenylalanine g	*0*	*0*	*30*	*0*	*0*
L-serine g	1.1	1.3	1.0	1.18	1.3
Taurine g	0.03	0	0	0.05	0.1
L-threonine g	1.1	1.4	2.3	1.29	1.3
L-tryptophan g	0.5	0.6	0.4	0.54	0.5
L-tyrosine g	*0*	*0*	*<10.5*	*0*	*0*
L-valine g	1.7	1.9	1.1	1.83	1.7

Abbreviations: *No vitamins, minerals and trace elements; PE protein equivalent; CGMP casein glycomacropeptide; Tyr tyrosine; Phe phenylalanine; DHA docosahexaenoic acid, NA no available information.

an internationally agreed [9]. For each subject, median blood Tyr and Phe concentrations were recorded for 12 months prior to the intervention and compared with median blood Tyr/ Phe collected during study part 1 (28 days) and part 2 (12 months).

3.5. Anthropometry

Weight, height and body mass index (BMI) were collected pre-intervention and at 28 days and at 6 and 12 months in study part 2. Measurements were taken at home or when subjects came to clinic. Weight was collected using calibrated digital Seca scales (Seca, Medical Measuring Systems and Scales, Birmingham, UK) measured in kilograms to the nearest 0.1 kg. Perpendicular height was collected using a Harpenden stadiometer (Holtain Ltd., Crymych, UK) measured to the nearest 0.1 cm. Body mass index was calculated using the formula weight divided by height squared [weight (kg) / height m^2].

3.6. Introduction of AAT

Each subject who expressed an interest in participating in the study was visited at home to explain the study in detail. They were shown the AAT and given information about the number required daily. In study part 1, each subject replaced at least one dose (10 or 20 g protein equivalent) of their current protein substitute with one dose of AAT (11 or 22 tablets). The AAT was taken with water or fruit squash. Some subjects ($n = 2$) administered the AAT over 4 doses. All subjects took the AAT in an upright position. Children aged 11 years and younger in primary school were supervised by an adult.

3.7. Ethical approval

Ethical approval was given by the West Midlands, South Birmingham Research Ethics Committee (REC Ref: 19/NI/0219; IRAS No 272118). After agreeing to participate in the 28-day study, caregivers signed a consent form and the children an assent form, being able to opt out of the study at any time.

3.8. Statistical analysis

Descriptive statistics were used to examine the demographics, gastrointestinal symptoms, metabolic control, blood Tyr/ Phe and any adverse events.

4. Results

4.1. Subjects

Eight of 11 children with HTI, who met the inclusion criteria, were recruited with a median age of 14.3 years (range 10.4–17.3y). There were 4 boys and 4 girls, 3 were British Caucasian and 5 British Pakistani origin. Before commencing AAT, the protein substitute types used were Tyr/ Phe free liquid or spoonable amino acids [Tyr Cooler 20 (Vitaflo Ltd), $n = 3$; Tyr express, (Vitaflo Ltd), $n = 1$] and low Tyr/ Phe CGMP [Tyr Sphere, (Vitaflo Ltd), $n = 4$]. The median protein equivalent from protein substitute was 60 g/ day (range 50-60 g), and median natural protein intake was 20 g/ day (15-30 g). None of the children were taking Phe supplements. Four children completed the 12-month extension study, 2 girls and 2 boys. The median age was 12.5 years (range 10-16y) Two children were British Caucasian and 2 British Pakistani origin

4.2. Blood Tyr and Phe

Study part 1: all subjects had lower median blood Tyr concentrations on the AAT than in the 12 months prior to intervention, although this was not statistically significant. Median blood Phe remained unchanged.

Study part 2 in the 4 children who completed study part 2, median blood Tyr was non-significantly ($p = 0.2$) lower than pre-intervention (Table 3). However, 3 of 4 subjects had median blood Tyr above the upper recommended target of 400 μmol/L both pre-intervention and with AAT. Median blood Phe was 40 μmol/L pre-intervention and 35 μmol/L at 12 months but lower than recommended by van Dam 2016 et al. [9].

4.2.1. Percentage of protein substitute taken as AAT

The percentage of protein substitute taken as AAT in study part 1 is shown in Table 2. Four subjects took the full amount and one subject 67% as AAT. In study part 2 all 4 subjectes took AAT exclusively.

4.3. Study part 1: Questionnaire results on acceptability of AAT during study part 1 (Tables 4a, 4b, 4c)

There were no negative scores for ATT for flavour, texture and palatability, in contrast to their usual protein substitute pre-intervention. One subject could not sustain taking the high number of AAT required and returned to their usual substitute after 28 days. However, the other subjects were able to take the higher number of daily AAT without difficulty.

Tables 4a, 4b, 4c. Comparing the acceptability of AAT with the usual protein substitute, individual subject responses.

4.4. Gastrointestinal tolerance

Pre-intervention, no subject reported diarrhoea, constipation or vomiting. Two subjects reported mild to moderate nausea and moderate to severe abdominal distension, discomfort and pain. Symptoms lessened or stopped with the AAT (Table 5). Two subjects who reported severe gastrointestinal symptoms pre-intervention remained on the AAT for 12 months and reported improved symptoms. Both subjects with moderate to severe gastrointestinal symptoms, found that powdered or liquid amino acid protein substitutes exacerbated their gastrointestinal issues. They had both tried CGMP which had improved their symptoms, but they struggled to take the required volume of liquid. The AAT led to improved adherence.

4.5. Ease of preparation

There was no preparation needed when taking the tablets.

4.6. Anthropometric measurements

Table 6 describes the anthropometric measurements for weight, height and BMI pre-intervention and over the 12 month extension study. All the subjects grew appropriately maintaining their height centiles. Two children had low BMIs both pre-intervention and with the AAT, but both had appropriate height growth.

Case studies describing the children's experiences who remained on AAT over 12 months are given in Appendix 1.

Table 2
Percentage of protein substitute and protein equivalent (g/day and g/kg/day) taken as AAT over study part 1 (28-day study).

Subject number	% protein substitute taken as AAT	Protein equivalent (g/ day) from AAT	Protein equivalent g/ kg/day from AAT
1	100	60	0.9
2	100	60	1.2
3	100	60	2.1
4	100	60	1.8
5	67	40	0.8
6	Withdrew from study part 1 after 7 days		
7			
8			

5. Discussion

In the UK, this was the first tablet protein substitute to be evaluated for HTI. Although only five children (63%) completed the 28-day short term study, with four continuing on the 12-month extension period, adherence with protein substitute improved. Blood Tyr showed a non-significant improvement at 12 months, with Tyr concentrations lower than pre-intervention. This tablet preparation helped this group of children who had struggled with the daily volume and taste of amino acids or CGMP substitutes. Although all the children in our cohort were interested in AAT, some found the amounts impracticable, and others having tried AAT preferred their familiar protein substitutes. The subjects reported that the AAT had no taste, smell or after taste and there was no associated satiety. Abdominal discomfort improved in two subjects.

Any strategies that improve protein substitute adherence are important. Amino acids hold important metabolic functions [19,20]. They are key elements of energy metabolism, providing precursors of intermediary substrates for the Krebs cycle, and provide nitrogenous compounds to produce nucleic acids. They also play a major role in brain metabolism. At the blood brain barrier, the large neutral amino acid transporter 1 (LAT1) is the major transport system for all large neutral amino acids. Disturbances in the amino acid ratios, low blood Phe and high Tyr concentrations may lead to an imbalance in brain biochemistry [21,22].

An emerging concern in HT1 is the growing evidence of neurocognitive impairments, the pathophysiology of which is not fully understood [23–25]. Tyr and Phe are important precursors of neurotransmitters, dopamine, serotonin and norepinephrine. The main source of Tyr and Phe is dietary, with small amounts contributed by low Tyr/ Phe CGMP and additional Phe supplements, if necessary, whilst protein substitutes provide all the other essential and large neutral amino acids. In HT1 blood Phe concentrations are commonly less than the clinical reference ranges [8,9] and there is a potential link between low blood Phe and neurocognitive outcomes [26,27] A protein substitute that could improve the ratio of Tyr to Phe, preventing low blood Phe and or high Tyr concentrations may improve the transport of these amino acids across the blood brain barrier.

The importance of slow-release protein substitute in amino acid disorders is gaining momentum. The AAT used in this study contained a sodium alginate base, but its kinetic properties in HT1 have not been investigated, so it is unknown if it slowly releases amino acids. Porta et al. [28] have shown that a slow-release AAT based on sodium alginate as a granulated formulation and containing higher amounts of Tyr significantly improved blood Tyr in subjects with PKU compared to traditional amino acid preparations. When using this slow-release product in PKU subjects, there was a sustained absorption of Tyr, improving its bioavailability. However, this study only measured blood Tyr in patients with PKU.

Two further studies in PKU have used slow release coated micro tablet protein substitutes, Giarratana et al. in an animal model [29] and Giovannini et al. in children with PKU [30]. Giovannini et al. [30] examined prolonged release micro tablets manufactured using sodium alginate, a hydrophilic carrier. The sodium alginate was further adapted using a wet granulation phase with an aqueous solution creating the micro tablets which enclosed the L-amino acids. The tablets were dried and then coated with hydroxypropyl methyl cellulose and a steric film (a coating applied to oral medication, acting as a barrier to prevent gastric erosion of the tablets) and pressed into small (4 mm) cylindrical shapes.

Table 3
Median blood Tyr and Phe concentrations pre-intervention and during part 1, (28-days) and part 2, (12-month extension study) for subjects.

	12 months pre-intervention		Part1:28-day acceptability study		Part 2: 12-month extension study	
Subject number	Median tyrosine (range) μmol/L	Median phenylalanine (range) μmol/L	Median tyrosine (range) μmol/L	Median phenylalanine (range) μmol/L	Median tyrosine (range) μmol/L	Median phenylalanine (range) μmol/L
1	460 (250–710)	40 (30–50)	330 (250–360)	50 (30–50)	440 (270–600)	40 (30–50)
2	590 (430–940)	40 (30–60)	500 (440–660)	50 (40–50)	530 (320–650)	30 (20–70)
3	560 (380–830)	30 (20–50)	490 (380–640)	40 (20–50)	510 (290–800)	30 (20–50)
4	320 (190–660)	40 (30–70)	300 (220–340)	40 (30–40)	290 (210–450)	50 (30–50)
5	330 (260–660)	50 (30–50)	300 (220–560)	40 (30–40)	Stopped after 28 days	
6						
7	Withdrew from study after 7 days					
8						

Table 4a
Likert scale response on palatability for individual subjects.

Subject	Palatability of protein substitute pre-intervention					Palatability of ATT				
	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant
1					✓	✓				
2					✓	✓				
3				✓			✓			
4					✓		✓			
5				✓				✓		

Table 4b
Likert scale response on texture for individual subjects.

Subject	Texture of protein substitute pre-intervention					Texture of ATT				
	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant
1					✓	✓				
2					✓	✓				
3				✓			✓			
4					✓		✓			
5				✓				✓		

Table 4c
Likert scale response on flavour for individual subjects.

Subject	Flavour of protein substitute pre-intervention					Flavour of the ATT				
	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant	Very pleasant	Pleasant	Neutral	Not very pleasant	Very unpleasant
1					✓	✓				
2					✓	✓				
3				✓			✓			
4					✓		✓			
5				✓				✓		

This technology improves the sodium alginate matrix theoretically slowly releasing the amino acids This study did not measure the kinetic properties of amino acids limiting the evidence in patients with PKU to support this concept. Giarratana [29] used a physiomimic technology based on sodium alginate and ethylcellulose. In this animal model study, the technology successfully showed that amino acids were slowly released compared to conventional protein substitutes. However, this product has not been kinetically tested in subjects with PKU, but it has been successfully studied in non PKU subjects suggesting slow-release properties. The AAT used in this study was based on a sodium alginate base only and there is no work to support that it slowly releases amino acids, but further work is warranted to answer this question.

One disadvantage of the AAT used in our study was that it was not supplemented with vitamins, minerals and trace elements, so an additional prescribed comprehensive vitamin and mineral supplement was necessary to ensure adequate intake. Previous work in PKU has shown that adherence to additional vitamin and mineral supplements is poor, increasing the risk of potential nutritional deficiencies [31].

There are limitations to this study. Only a small number of children were recruited limited by the rarity of the disorder and the age restriction. We did not collect any detailed weighed food intake or food frequency data. Similarly, biochemical nutritional bloods and information on adherence with vitamin and mineral supplements was not collected as this was not the primary objective of the study. Two children (brother and sister) had a negative BMI z score and had poor weight gain despite energy supplementation with a glucose polymer, but height growth was not compromised in either subject. Their lack of weight gain was a long-standing problem since early childhood and in part might have been

Table 5
Individual subject responses on gastrointestinal symptoms for gastrointestinal symptoms pre-intervention and during the 28 days on AAT.

Subject	Gastrointestinal symptoms pre-study on usual protein substitute						Gastrointestinal symptoms over 28 days on study product							
	Diarrhoea	Constipation	Vomiting	Nausea	Bloating abdominal distension	Regurgitation flatulence	Abdominal discomfort pain	Diarrhoea	Constipation	Vomiting	Nausea	Bloating abdominal distension	Regurgitation/ flatulence	Abdominal Discomfort/ pain
1	0	0	0	1 to 2	3	2	2 to 3	0	0	0	0	0	1 to 2	0 to 1
2	0	0	0	1 to 2	2	2	2 to 3	0	0	0	0	0	1 to 2	0 to 1
3	0	0	0	0	0	1	0	0	0	0	0	0	1	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6														
7	Withdrew from the study													
8														

Key 0 no symptoms; 1 = mild; 2 = moderate; 3 = severe or troublesome.

Table 6
Median z scores for height, weight and body mass index pre-intervention and during the 12-month extension study on the AAT.

Subjects	Median height z score Pre-study	End	Median weight z score Pre-study	End	Median BMI z score Pre-study	End
1	0	0	1.1	1.3	1.4	1.5
2	−0.3	0	−1.4	−1.4	−2.0	−2.5
3	0	0	−0.5	−1.0	−0.7	−1.0
4	−0.7	−0.7	0.3	0.3	1	0.8

familial as both parents were tall and underweight.

6. Conclusions

In HTI, for some pre-teenagers and teenagers trying to gain independence and peer acceptance, AAT was an appropriate choice of protein substitute. They viewed taking AAT as more 'acceptable' than other protein substitutes and this helped reduce parental burden. Ideally a formulation with added vitamins, minerals and micronutrients would be an advantage. Further research is needed to investigate the kinetic properties of AAT based on sodium alginate only, compared to preparations based on a modified sodium alginate matrix and liquid-based substitutes.

Informed consent

Written informed consent was received by all subjects.

Ethical approval was given by the West Midlands, South Birmingham Research Ethics Committee (REC Ref: 19/NI/0219; IRAS No 272118).

Consent for publication

All authors give their consent for publication. All authors have read and agreed to the published version of the manuscript.

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CRediT authorship contribution statement

Anne Daly: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sharon Evans:** Writing – review & editing. **Alex Pinto:** Writing – review & editing. **Catherine Ashmore:** Writing – review & editing. **Anita MacDonald:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

All the authors Anne Daly, Sharon Morris, Alex Pinto, Catherine Ashmore, Anita MacDonald have received grants for research, honoria for lectures or participated in medical reviews from Cambrookes, MetaHealth, Nutricia, Vitaflo, The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data availability

The data contains patient confidential information and therefore has not been deposited in a repository. As there are only a few patients with this condition patients might be identifiable. Therefore, all data is available by request to the author.

Appendix A. Appendix 1 Case studies of subjects remaining on a tablet preparation over 12 months

A.1. Case study 1

Subject 1: A 16-year-old female with neurocognitive dysfunction and a school educational health care plan, was diagnosed at 6 weeks of age. She struggled with protein substitute acceptance and commonly vomited when taking this until the age of 5 years. In her early years, her metabolic control was sub-optimal with low blood Phe despite supplementation. In later childhood, she tried a low Tyr/ Phe CGMP that was well accepted and associated with alleviation of long-standing constipation. However, she complained of satiety post substitute administration, affecting her appetite. She accepted the AAT well and self-administered 66 tablets/day in small frequent doses. She felt more in control of her dietary management and AAT helped her dietary adherence. Her parents no longer needed to cajole her to take protein substitute and she had no further abdominal discomfort.

A.2. Case study 2 and 3

Two siblings, a boy aged 15 years and a girl aged 10 years both diagnosed in infancy following a family history of HT1, had a long-standing issue with non-adherence with protein substitute. The girl struggled with powder and liquid amino acid protein substitutes and complained of constipation and frequent abdominal pain and nausea. The brother accepted his liquid protein substitute but disliked its taste and smell. Both changed onto a low Tyr/ Phe CGMP. On taking CGMP, the girl had improved gastrointestinal symptoms with a resolution of constipation and a decrease in abdominal distension and discomfort. Both siblings preferred its taste to conventional substitutes, but the girl struggled with the prescribed volume. The AAT had no associated taste and was well accepted by both children. They took the AAT without difficulty, and it replaced their entire daily dose of protein substitute. The boy took 66 tablets in 3 daily doses and the girl 60 tablets in 4 daily doses. Both siblings had appropriate height growth following the fiftieth percentile, but both had poor weight gain, despite calorie supplementation with a glucose polymer.

A.3. Case study 4

A 10-year-old boy diagnosed with HT1 via newborn screening (outside the UK), had excellent adherence with diet and protein substitute, with blood Tyr within the recommended treatment range. He frequently complained about the volume, taste and after-taste of liquid amino acids, taking 174 mL three times daily. His parents supervised him to ensure he completed the full prescribed amount. He changed to the AAT, taking 60 tablets in three divided doses, without difficulty. His adherence was better, and his parents commented that it was easier for him to complete each dose. All protein substitute requirements were met with the AAT, height continued to follow the twenty fifth percentile and weight the fiftieth percentile.

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