ORIGINAL ARTICLE

Randomised controlled trial of essential fatty acid supplementation in phenylketonuria

MA Cleary¹, F Feillet², FJ White³, M Vidailhet², A MacDonald⁴, A Grimsley⁵, N Maurin⁶,
H Ogier de Baulny⁷ and PJ Rutherford⁸

¹Department of Metabolic Medicine, Great Ormond St. Hospital for Sick Children, London, UK; ²Department of Pediatrics, Hôpital D'enfants Braboïs, Nancy, France; ³Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester, UK; ⁴Birmingham Children's Hospital, Birmingham, UK; ⁵Royal Belfast Hospital for Sick Children, Belfast, UK; ⁶Hôpital Timone Enfants, Marseille, France; ⁷Hôpital Robert Debré, Paris, France and ⁸Alder Hey Children's Hospital, Liverpool, UK

Objective: The long-chain polyunsaturated fatty acids (LC-PUFA) status of children with PKU is often compromised. LC-PUFA, which are important fatty acids in the development of the CNS, can be synthesised endogenously from the parent essential fatty acids (EFA) provided dietary intakes are adequate. This study was designed to assess the biochemical effect over a 20-week period of a phe-free protein substitute that has been supplemented with a balanced blend of n-3 and n-6 EFAs on LC-PUFA status of children with PKU.

Design, setting and subjects: Fifty three community-living children aged 1–10 years diagnosed with PKU in the newborn period were recruited from seven tertiary centres in the UK and France and randomised to a fat-free control formula or the EFA-supplemented test-treatment formula in an open, prospective study. Forty four children completed the study (20 controls, 24 test-treatments). Fatty acid status was assessed at entry and 20-weeks follow-up. Three day dietary diaries were recorded at 20 weeks' follow-up. The safety, efficacy and palatability of the test-treatment formula were also assessed.

Results: The test-treatment group had significantly higher intakes of fat and EFA than the control group. There was a significant between group difference ($P=0.04$) in increases in median docosahexaenoic acid (DHA) concentrations in erythrocyte phospholipids, which increased by 19% in the test-treatment group and by 0.5% in the control group over the study period. Growth and phe control were satisfactory in all subjects.

Conclusions: Supplementing the diets of children with PKU with a balanced blend of n-6 and n-3 EFA improves DHA status without compromising AA status.

European Journal of Clinical Nutrition (2006) 60, 915–920. doi:10.1038/sj.ejcn.1602401; published online 8 March 2006

Keywords: phenylketonuria; docosahexaenoic acid; arachidonic acid; long-chain polyunsaturated fatty acids

Introduction

Phenylketonuria (PKU) (McKusick 261600) is a rare, recessively inherited inborn error of metabolism in which the

conversion of phenylalanine (phe) to tyrosine is impaired. Treatment begins in early infancy, when affected infants are identified at newborn screening, and now increasingly continues into adulthood. A low-protein diet supplemented with specially manufactured phe-free protein substitutes is the cornerstone of management, the aim of which is to maintain plasma phe concentrations ideally between 120 and 360 $\mu\text{mol/l}$ (MRC, 1993) to minimise the neurological damage caused by excess levels of circulating phe. The phe-free protein substitutes usually provide most of the patient's protein requirements (around 80% of their total protein intakes) and make a substantial contribution to the intakes of other essential nutrients.

The dietary therapy necessary for the effective management of PKU, in particular the restriction of high-protein animal-origin foods, compromise patients' intakes of fat and distort the ratio of n-3: n-6 essential fatty acids (EFA) in the

Correspondence: Dr MA Cleary, Consultant Paediatrician, Department of Metabolic Medicine, 7th Floor Southwood Building, Great Ormond St Hospital for Sick Children, London WC1N 3JH, UK.

E-mail: Clearym@gosh.nhs.uk

Sources of financial support: SHS International Ltd, 100 Wavertree Boulevard, Wavertree Technology Park, Liverpool, L7 9PT, UK.

Guarantor: MA Cleary.

Contributors: All contributors are clinicians or dietitians from the seven recruiting centres. All contributors were involved in the development of the study protocol. The principal investigator (MC) produced the initial draft of the manuscript and the dietitians (FW, AM, AG, PR) collected and analysed the diet diaries. The other contributors provided additional information and comments on the drafts of the manuscript.

Received 9 July 2005; revised 8 December 2005; accepted 19 December 2005; published online 8 March 2006

diet. The docosahexaenoic acid (DHA, C22: 6 n-3) status of patients with PKU has been found to be poor compared to population norms, a consequence of poor n-3 EFA intakes (α -linolenic acid (C18: 3 n-3), which is endogenously converted to DHA), and the absence of preformed dietary sources of DHA. In this study, we sought to establish whether the DHA status of children with PKU could be improved by incorporating an EFA-supplemented phe-free protein substitute (Minaphlex[®]/Anamix[®] (The product is marketed under 2 brand names), SHS International Limited, Liverpool, UK) into their diets, without compromising their AA status. The efficacy of supplementing PKU diets with preformed DHA and arachidonic acid (AA, C20: 4 n-6) has been established (Agostoni *et al.*, 1995, 2000; Beblo *et al.*, 2001), whereas the effects of increasing intakes of parent EFAs, in particular α -linolenic acid, and normalising the ratio of dietary n6: n3 EFA has not been studied. EFA source oils are more palatable than LC-PUFA source oils, allowing their incorporation into the protein substitute and thereby obviating the need to take an additional supplement.

Subjects and methods

Children aged 1–10 years with PKU were recruited from four UK and three French tertiary centres responsible for the management of children with metabolic disorders. Subjects were randomised either to their current fat-free protein substitute ('control group') or the EFA-supplemented protein substitute ('test-treatment group') in an open-labeled study and followed-up over 20 weeks. The macronutrient and EFA composition of the test-treatment formula is detailed in Table 1. It was not possible to blind the study because the test-treatment formula had a distinctly different taste and mouthfeel to the fat-free control formulas. The inclusion criteria were (1) diagnosis of PKU in the newborn period treated by a low phe diet, (2) good compliance with the dietary regime as assessed by means of blood phe concentrations (70% of results being within the

MRC guidelines during the previous 3 months) and (3) acceptability of the test-treatment formula (An entry criterion because many PKU patients are known to be reluctant to change their usual protein substitutes for new products. Subjects only entered the study if, on a single tasting of the test-treatment formula, they agreed in principle that they would be prepared to take the product for the duration of the study in the event that they were randomised to the test-treatment group. This did not affect their right to withdraw from the study). Subjects who had taken LC-PUFA supplements (e.g., cod liver oil) at any time during the previous 5 months, infants or children who were being weaned onto the next stage of phe-free protein substitute (e.g., from phe-free infant formula to phe-free protein substitute) and those on fat-containing energy supplements were excluded. The volume of protein substitute consumed was dependent on the age, weight, severity of the condition and the clinics' standard practices.

Fatty acid status

DHA and AA levels in erythrocyte membrane phospholipids have been found to be a reliable marker of the levels in brain tissue (Makrides *et al.*, 1994). Blood samples collected at entry and 20 weeks' follow-up were analysed by means of GCMS using a quadripole mass filter (Electron Ionisation mode and split mode ratio 20:1, Agilent 5972 series). After detection, each fatty acid was expressed as a percentage fraction of the total fatty acids.

Growth and nutritional intakes

Height and weight were assessed at entry to the study and at 4, 12 and 20 weeks' follow-up and converted to z-scores (Freeman *et al.*, 1993). Diaries recording food consumption were kept over a 3-day period at 20 weeks' follow-up. Mean daily intakes of energy, fat and EFA from foods consumed were calculated using Microdiet[®] (Downlee Systems Limited, England) (Data on the EFA content of foods in the Microdiet[®] food tables are incomplete. The food tables were amended such that all foods providing >1 g fat/100 g recorded in the diaries had values for linoleic acid and α -linolenic acid).

Phenylalanine control

The clinics' normal practices for monitoring blood phe concentrations were followed, and generally this was weekly for children under 5 years and every 2 weeks for older children. As a minimum, assessments were carried out every 4 weeks. Blood specimens were analysed for phe using high-pressure liquid chromatography in all of the centres, except for Manchester, which used tandem mass spectrometry.

Table 1 Energy, macronutrient and essential fatty acid composition of the test-treatment formula

<i>Per 100 g powder</i>	
Energy (kcal)	390 (374)
Protein equivalent (g)	29
Carbohydrate (g)	38 (34)
Fat (g)	13.5
Of which PUFA (g)	2.8
<i>g/100 g fatty acids</i>	
Linoleic acid	17.2
α -Linolenic acid	4.5

Figures in brackets are for the flavoured version. Ingredient sources of fat: safflower oil, canola oil and coconut oil.

Acceptability

Subjects were asked to complete a questionnaire about the ease of use, acceptability and palatability of the protein substitute they had been randomised to at the end of the study.

Data analysis

Subjects, who were randomised but failed to complete the study were excluded from the data analysis. A two-sample *t*-test for equality of control and test-treatment groups was used to compare differences in age, phe and baseline fatty acid concentrations between groups. A χ^2 test for association was used for sex difference. As a means of correcting for the variability in fatty acid status of individual children at entry to the study, percent changes in the concentrations observed at 20 weeks compared to baseline were used in assessing the effect of EFA supplementation. A nonparametric test (Mann–Whitney) was used to establish whether median percentage changes in the concentrations of fatty acids in plasma and erythrocyte membrane phospholipids and total plasma lipids between baseline and 20 weeks follow-up differed significantly between the groups. A level of $P < 0.05$ was considered significant.

Ethics

The respective review boards at each site approved this study, and written informed consent was obtained from the children's parents.

Results

Subjects

Of the 53 subjects recruited to the study, 44 (Three subjects found the test-treatment formula unacceptable. Two subjects withdrew because of vomiting episodes while taking the test-

treatment formula, although we are unclear as to whether this was due to intercurrent illness. Four subjects found the control formula unacceptable. These subjects were improperly randomised to a predetermined control formula rather than their current protein substitute) (24 in the test-treatment group, 20 in the control group) completed the study. The study groups were comparable in that the age, sex and phe control did not differ significantly between the groups.

Fatty acid status

Concentrations of key fatty acids in erythrocyte membrane phospholipids from 30 (nine children from the control group and five children from the test-treatment group were excluded because blood samples at follow-up were unavailable) children (11 from the control group, 19 from the test-treatment group) were analysed at baseline and 20-weeks follow-up. At baseline, fatty acid profiles were similar in that children in both groups had low concentrations of erythrocyte AA and DHA compared to normal ranges (Table 2).

Median DHA concentrations in erythrocyte membrane phospholipids increased by 19% in the test-treatment group and by 0.5% in the control group ($P = 0.04$). Median AA concentrations increased from baseline concentrations by 0.5% in the test-treatment group and 7.6% in the control group, although these increases were not significantly different to one another ($P = 0.06$).

Growth and nutritional intakes

Within each group, changes in weight and height z-scores over the study period were not significant, although the sample size was too small to establish equivalence and the duration of the study was short. All children grew at a normal rate for their age and centile.

Table 2 Key fatty acids in erythrocyte membrane phospholipids in subjects in the test-treatment and control groups at entry and at 20 weeks follow-up

	% Of total fatty acids, means (s.d.)						Median percentage change in fatty acids from entry to 20 weeks follow-up	
	Typical normal value ^a	At entry means (s.d.)		At 20 weeks follow-up means (s.d.)		Control (n = 11)	Supplemented (n = 19)	P-value
		Control (n = 11)	Supplemented (n = 19)	Control (n = 11)	Supplemented (n = 19)			
Linoleic acid (C18:2n-6)	12.9	9.93 (1.5)	10.79 (1.9)	10.52 (1.3)	10.99 (1.7)	8.00	5.88	0.68
α -linolenic acid (C18:3n-3)	NA	0.05 (0.1)	0.06 (0.1)	0.05 (0.1)	0.11 (0.1)	0.00	40.00	0.06
Arachidonic acid (C20:4n-6)	15.6	13.98 (1.4)	14.17 (1.8)	15.41 (1.7)	13.76 (1.7)	7.64	0.54	0.06
Eicosapentanoic acid (C20:5n-3)	0.5	0.16 (0.2)	0.24 (0.2)	0.20 (0.2)	0.34 (0.2)	17.86	50.00	0.59
Docosahexaenoic acid (C22:6n-3)	4.5	1.60 (0.3)	1.77 (0.6)	1.64 (0.4)	2.07 (0.8)	0.46	19.23	0.04 ^b

NA not available.

^aPersonal communication with Dr J Farquharson, Department of Biochemistry, Royal Hospital for Sick Children, Yorkhill, Glasgow.

^bSignificant difference ($P < 0.05$) between control and test-treatment groups.

Table 3 Energy and fat content of the diets of children in the study and healthy subjects

	Sample means (s.d.)			
	Control (n = 19)	Test-treatment (n = 24)	P-value	Non-PKU subjects ^a
Energy intake (kcal/day)	1303 (337)	1390 (323)	0.39	1334
Energy intakes as a % of EAR ^b	88 (15.3)	95 (15.3)	0.16	90
Fat as a % of energy	24.3 (5.6)	32.4 (6.7)	0.0001*	35.9
Fat (g/day)	35.9 (14.8)	50.3 (17.8)	0.007*	53.1
Linoleic acid as a % of energy	3.4 (1.5)	5.3 (2.2)	0.002*	4.3 ^c
Linoleic acid (g/day)	5.3 (2.9)	8.2 (4.0)	0.01*	6.6 ^c
α -Linolenic acid as a % of energy	0.4 (0.2)	0.8 (0.2)	<0.0001*	0.7 ^d
α -Linolenic acid (g/day)	0.5 (0.2)	1.3 (0.4)	<0.0001*	1.1 ^d
Linoleic acid: α -Linolenic acid ratio	10:1	6.5:1	0.004*	6:1

Abbreviations: EAR, estimated average requirement for the UK.

^aExtrapolated from mean values for healthy children from two UK diet and nutrition surveys (Gregory *et al.*, 1995; Gregory and Lowe, 2000), age-matched for children in this study.

^bDepartment of Health. 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. RHSS No. 41. London: HMSO; 1991.

^cValues represent total n-6 PUFA, which comprise around 95% linoleic acid in normal diets.

^dValues represent total n-3 PUFA, which comprise around 95% α -linolenic acid in normal diets.

* $P < 0.05$.

Subjects in the test-treatment group had significantly higher intakes of fat and EFA than did subjects in the control group (Table 3).

Phenylalanine control

All subjects showed satisfactory phe control (MRC, 1993), with concentrations lying between 180 and 360 $\mu\text{mol/l}$ at almost every assessment. For some subjects, elevated phe concentrations were occasionally found, but these were explicable, for example, because of intermittent infection. There was no difference in the phe concentrations between the groups (control group mean 323 $\mu\text{mol/l}$ (s.d. 192), test-treatment group mean 262 $\mu\text{mol/l}$ (s.d. 82)).

Acceptability

Thirty-nine subjects completed questionnaires on the acceptability of the phe free protein substitutes taken (16 from the control group, 23 from the test-treatment group). Assessments of the test-treatment formula were generally more favourable than those for the control formulas. The majority of respondents taking the test-treatment formula found the product to be more acceptable (70%) and easier to mix (83%) than the fat-free protein substitutes they were taking before entry to the clinical trial.

Discussion

Treatment of PKU with a low-phe diet has been very successful in avoiding the severe mental retardation associated with untreated PKU seen prior to newborn screening and treatment for this condition. The relationship

between good phe control and intelligence in PKU is well established (Burgard, 2000), and early and strict treatment is the most effective means of optimising the neurological outcome. However, even in patients who are well controlled during childhood, neurological deterioration has been observed (Villasana *et al.*, 1989; Thompson *et al.*, 1990; Cleary *et al.*, 1994; Weglage *et al.*, 1995) and children with PKU have, on average, an IQ score that is 0.5 s.d. less than the general population (Smith *et al.*, 1991). The reason for this may be the effects of raised phe on brain myelination but the relative deficiency of DHA supply may also contribute to a poorer intellectual outcome (Cockburn *et al.*, 1996).

Fat is an essential component of all diets as a source of energy and in meeting requirements for n-3 and n-6 EFA. Compared to healthy children, children with PKU have been found to have markedly poorer intakes of fat, α -linolenic acid and preformed LC-PUFA, resulting in poor LC-PUFA status, in particular poor DHA status (Galli *et al.*, 1991; Sanjurjo *et al.*, 1994; Cockburn *et al.*, 1996; Moseley *et al.*, 2002). The disproportionately high intakes of linoleic acid relative to poor α -linolenic acid intakes may further compromise the conversion of dietary α -linolenic acid to DHA as linoleic acid and α -linolenic acid compete for the same desaturase enzymes (Infante and Huszagh, 2001). On average, fat provided only 24% of energy intakes in the control group, compared to a population average of approximately 36% (Gregory *et al.*, 1995; Gregory and Lowe, 2000) and the test-treatment group average of 32%. Mean intake of α -linolenic acid by the test-treatment group was substantially higher than that of the control group, at 1.3 g/day and 0.5 g/day ($P < 0.0001$), respectively, bringing intakes up to those of non-PKU children of the same age of around 1.1 g/day (Gregory *et al.*, 1995; Gregory and Lowe, 2000). The diet for

PKU provides a higher ratio of linoleic acid: α -linolenic acid than non-PKU diets because of disproportionately high intakes of linoleic acid. The relatively low linoleic acid: α -linolenic acid ratio of the test-treatment formula (4:1) normalised the ratio of these EFA in the diets of the test-treatment group (means 6.5:1 for the test-treatment group compared to 10:1 for the control group, $P=0.004$), bringing the ratio closer to that provided by the diets of non-PKU children of around 6:1 (Gregory *et al.*, 1995; Gregory and Lowe, 2000).

There has been some debate over the most suitable combination of supplementary fatty acids in the diets of children with PKU. The test-treatment formula was supplemented with EFA rather than preformed LC-PUFA because of the unpalatable nature of LC-PUFA ingredient sources and the detrimental effect their incorporation may have had on compliance. The oils used in the test-treatment formula improved the product's reconstitution properties and overall palatability, the principal difference between the formulas being the absence/presence of fat. This was the first study that we are aware of demonstrating a positive biochemical effect of EFA supplementation as opposed to preformed LC-PUFA supplementation on the fatty acid status of children with PKU. The effectiveness of EFA supplementation depends upon the child's ability to endogenously synthesise an adequate supply of LC-PUFA from EFA precursors. Subjects in the test-treatment group had greater increases in erythrocyte DHA concentrations over the 20-week study period than those in the control group, although their status was still relatively poor compared to population norms. Baseline DHA concentrations in both groups were lower than those reported elsewhere for children with PKU (Sanjurjo *et al.*, 1994; Cockburn *et al.*, 1996), and the extent to which the test-treatment formula is capable of affecting a clinically significant improvement in DHA status among subjects with higher baseline concentrations is not known. Reports on the AA status of this group have been less consistent, although there is convergence on the finding that the AA status of PKU patients is not adversely affected, probably because there is an abundance of good sources of linoleic acid in their diets, the precursor for AA (Galli *et al.*, 1991; Sanjurjo *et al.*, 1994; Cockburn *et al.*, 1996). In this study, the aim of supplementation was to improve DHA status without compromising AA status, and we achieved this.

We did not assess the functional consequences of EFA supplementation in this study. Previous studies on the effects of LC-PUFA supplementation have shown an effect on visual function (Agostoni *et al.*, 2000; Beblo *et al.*, 2001). Study of the long-term neurological development of children taking EFA-supplemented diets compared to children taking unsupplemented diets would provide useful data in advancing the understanding of this area. Nonetheless, the biochemical findings of this study demonstrate that supplementing the diets of children with PKU with EFA improves DHA status.

Acknowledgements

The authors thank the parents, children and the health care professionals at the following centres who participated in the trial: Hôpital D'enfants Brabois, Nancy, France; Royal Manchester Children's Hospital, Manchester, UK; Birmingham Children's Hospital, Birmingham, UK; R Thom, ER Trimble, Royal Belfast Hospital for Sick Children, Belfast, UK; J Sarles, Hôpital Timone Enfants, Marseille, France; Hôpital Robert Debré, Paris, France; DC Davidson, Alder Hey Children's Hospital, Liverpool, UK. The authors are grateful to David Percy (School of Accounting, Economics and Management Science, University of Salford, Manchester, UK) who carried out the statistical analysis of the data and Helen Rose and Catherine Deering (R&D Department, SHS International Ltd, Liverpool, UK) for their assistance with the preparation of this article.

References

- Agostoni C, Masetto N, Biasucci G, Rottoli A, Bonvissuto M, Bruzzese M-G *et al.* (2000). Effects of long-chain polyunsaturated fatty acid supplementation on fatty acid status and visual function in treated children with hyperphenylalaninemia. *J Pediatr* **137**, 504–509.
- Agostoni C, Riva E, Biasucci G, Luotti D, Bruzzese MG, Marangoni F *et al.* (1995). The effects of n-3 and n-6 polyunsaturated fatty acids on plasma lipids and fatty acids of treated phenylketonuric children. *Prostaglandins Leukot Essent Fatty Acids* **53**, 401–404.
- Beblo S, Reinhardt H, Muntau AC, Mueller-Felber W, Roscher AA, Koletzko B (2001). Fish oil supplementation improves visual evoked potentials in children with phenylketonuria. *Neurology* **57**, 1488–1491.
- Burgard P. (2000). Development of intelligence in early treated phenylketonuria. *Eur J Pediatr* **159** (Suppl 2), S74–S79.
- Cleary MA, Walter JH, Wraith JE, Jenkins JP, Alani SM, Tyler K *et al.* (1994). Magnetic Resonance Imaging of the brain in phenylketonuria. *Lancet* **344**, 87–90.
- Cockburn F, Clark BJ, Caine EA, Harvie A, Farquharson J, Jamieson EC *et al.* (1996). Fatty acids in the stability of the neuronal membrane: Relevance to PKU. *Int Pediatr* **11**, 56–60.
- Freeman JV, Cole TJ, Chinn S, Jones PRM, White EM, Preece MA (1993). Cross-sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child* **73**, 17–24.
- Galli C, Agostoni C, Mosconi C, Riva E, Salari P-C, Giovannini M (1991). Reduced plasma C-20 and C-22 polyunsaturated fatty acids in children with phenylketonuria during dietary intervention. *J Pediatr* **119**, 562–567.
- Gregory J, Lowe S (2000). *National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey.* HMSO: London.
- Gregory JR, Collins DL, Davies PSW, Hughes JM, Clarke PC (1995). *National Diet and Nutrition Survey: children aged 1½–4½ years. Volume 1: Report of the diet and nutrition survey.* HMSO: London.
- Infante JP, Huszagh V (2001). Impaired arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid synthesis by phenylalanine metabolites as etiological factors in the neuropathology of phenylketonuria. *Mol Genet Metabol* **72**, 185–198.
- Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA (1994). Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr* **60**, 189–194.

- Medical Research Council Working Party on Phenylketonuria (1993). Recommendations on the Dietary Management of Phenylketonuria. *Arch Dis Child* **68**, 426–427.
- Moseley K, Koch R, Moser AB (2002). Lipid status and long-chain polyunsaturated fatty acid concentrations in adults and adolescents with phenylketonuria on phenylalanine-restricted diet. *J Inher Metab Dis* **25**, 56–64.
- Sanjurjo P, Perteagudo L, Rodriguez Soriano J, Vilaseca A, Campistol J (1994). Polyunsaturated fatty acid status in patients with phenylketonuria. *J Inher Metab Dis* **17**, 704–709.
- Smith I, Beasley MG, Ades AE (1991). Effect on intelligence of relaxing the low phenylalanine diet in phenylketonuria. *Arch Dis Child* **66**, 311–316.
- Thompson AJ, Smith I, Brenton D, Youl BD, Rylance G, Davidson DC *et al.* (1990). Neurological deterioration in young adults with phenylketonuria. *Lancet* **336**, 602–605.
- Villasana D, Butler IJ, Williams JC, Roongta SM (1989). Neurological deterioration in adult phenylketonuria. *J Inher Metab Dis* **12**, 451–459.
- Weglage J, Pietsch M, Funders B, Koch HG, Ullrich K (1995). Neurological findings in early treated phenylketonuria. *Acta Paediatr* **84**, 411–415.