Is phenylalanine requirement in infants and children related to protein intake?

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1. Two groups of children with phenylketonuria (PKU) received protein at two different levels. The protein scource was a protein hydrolysate, devoid of phenylalanine, and intact protein from milk, vegetables and fruit. One group (RDA group) was given protein at a level based on the recommendations of the (US) Food and Nutrition Board (1974, 1980). The other group (FAO group) was given protein at the level of intake corresponding to the Joint FAO/WHO *ad hoc* Expert Committee (1973) safe levels of intake of egg or milk protein. The children were monitored very closely for several years. From an earlier study evaluating the protein intake of the two groups it was suspected that the Joint FAO/WHO *ad hoc* Expert Committee (1973) recommendations were marginal.

2. In the present study the phenylalanine intake of the two groups required to maintain the plasma phenylalanine concentration at the required level was established. The results showed that the RDA group required more phenylalanine than the FAO group. This difference was statistically significant from the age of 5–15 months.

3. We have interpreted the greater requirement for phenylalanine in the RDA group as a result of a greater nitrogen intake and thus a more rapid chemical maturation of N (increase in protein concentration of the body with age). It is known that up to the age of 6 months the chemical maturation of N is related to the N intake. In the present study we have found that this difference in chemical maturation lasted up to the age of 15 months. The conclusion drawn from the study was that a protein intake slightly higher than the Joint FAO/WHO *ad hoc* Expert Committee (1973) recommendations might be desirable.

The protein content of the body increases during infancy and up to the age of 4 years. The phenylalanine required for protein synthesis in early childhood includes, therefore, the phenylalanine required for the increment in weight as well as for the increase with time in the body's protein concentration. This increase in protein, and consequently in phenylalanine, is called chemical maturation. Little is known about the speed of this process, but it is assumed that it takes place mostly during the first year of life and is fully accomplished at about 4 years of age. According to Fomon (1976), the increase in protein content represents both more fat-free body mass and less fat, as well as a greater percentage of protein in the fat-free body mass. In the newborn the protein content is 11·4% of the whole body and 12·8% of the fat-free body mass. According to Fomon's (1976) calculations, the protein content of the whole body is increased to 16·4% and of the fat-free body mass to 20·0% at the age of 36 months, and reaches the adult value of 20·5% of the fat-free body mass at the age of 4 years. Fomon (1961) gave infants undiluted cow's milk and found that the growth of the infants was within normal limits but that nitrogen retention was much higher than in infants given human milk. His conclusion was that this result indicated a change in body composition. His only concern was that values from the study gave a body N content which was too high. By carcass analyses of piglets it has been shown that a high level of dietary N gives a higher body N content than a low level of dietary N (Filer & Churella, 1963). This higher body N content is represented by a more rapid weight gain, a lower percentage of body-weight as fat and a higher percentage of protein in the fat-free body mass.

We have studied the problem of chemical maturation in a long-term study of children with phenylketonuria (PKU). These children were on a very strict dietary regimen, which

made it possible to calculate exactly what the child ate over many years. Kindt *et al.* (1983) have previously reported the results of a comparison of two groups of children with classical PKU (Tourian & Sidbury, 1974) receiving diets differing only in the amount of protein. The group with the higher intake (RDA group) received protein at a level based on that recommended by the (US) Food and Nutrition Board (1974, 1980). The group with the lower intake (FAO group) received protein at the level recommended by the Joint FAO/WHO *ad hoc* Expert Committee (1973). The result of the study suggested that the Joint FAO/WHO (1973) recommendations were borderline and possibly too low. Thus we investigated other criteria that would help clarify our findings. As a well-regulated plasma phenylalanine concentration is the objective in the treatment of children with PKU, we measured the phenylalanine intake of the two groups necessary to maintain the plasma level within the required range. The results suggested that the RDA group required more phenylalanine than the FAO group, and that possibly more N was retained in the RDA group than in the FAO group.

MATERIALS AND METHODS

Subjects

All children with PKU born in Norway from January 1975 to February 1979 were included in the study. The RDA group consisted of eight children born from January 1975 to August 1976. The FAO group consisted of eight children born from August 1977 to February 1979. The children started the diet between 15 and 18 d of age, mean 16 d (see Table 1, p. 438). One child in the RDA group received only human milk during the first 6 months of life and values from this period have been omitted. Blood samples were taken regularly for routine haematological and chemical analyses including total proteins, albumin, α_1-, α_2-, β- and γ-globulins, transferrin, pre-albumin, retinol-binding protein and quantitative determination of immunglobulins. Length and weight were measured regularly and recorded on two growth charts (Sundal, 1957; Karlberg *et al.* 1976). At the last visit an X-ray of the wrist was taken. A detailed description of procedures has been given in an earlier publication (Kindt *et al.* 1983).

The biochemical control of the disease was determined by frequent analyses of plasma phenylalanine. During the initial period, determinations were made three times weekly until the plasma phenylalanine concentration fell within the target range of between 180 and 667 μmol/l, usually within 2 weeks. Later determinations were made weekly during the first year of life, every 2 weeks during the second year of life, and later every 1 or 2 months. The weight of all children was calculated as the mean weight for each month, from the start of the dietary treatment and throughout the study.

Two subgroups with infants of similar weights at the start of the dietary treatment were established. The RDA subgroup consisted of five infants with weights between 3400 and 3820 g (mean 3620 g). The FAO subgroup consisted of four infants with weights between 3500 and 3860 g (mean 3640 g; see Table 1, p. 438). The weight gains during the first 76 d on the diet were calculated for the two groups of infants.

Diet

The diet was based on Albumaid XP (Scientific Hospitals Supplies Ltd, Liverpool). The amino acids in this formula were derived from the acid-hydrolysis of ox serum from which phenylalanine had been removed. The protein content of Albumaid XP was calculated assuming that 1 g protein yields 1·2 g amino acids during hydrolysis (Milupa PKU 1 and PKU 2, 1980). Intact protein, and hence phenylalanine, was supplied from cow's milk during the first 3–5 months of life, except for some of the infants who received human milk for

a few days (Lønnerdal *et al.* 1976). Throughout the study the amount of milk supplement was individually regulated to maintain the plasma level of phenylalanine within the target range.

When necessary an increase or decrease of only 5–10 ml milk, representing 9 or 18 mg phenylalanine, was sufficient to alter the plasma level of phenylalanine. Small amounts of protein from vegetables and fruit were added to the diet of both groups from 3 to 5 months of age, and the protein and phenylalanine intakes were calculated using standard sources (FAO, 1970; Statens Ernaeringsråd, 1977).

The RDA group received protein from the protein hydrolysate, milk, vegetables and fruit at the level of intake recommended by the (US) Food and Nutrition Board (1974, 1980). The FAO group received a level of protein intake recommended by the Joint FAO/WHO *ad hoc* Expert Committee (1973) as safe levels of intake of egg or milk protein, assuming that the protein from hydrolysate, milk, vegetables and fruit at the concentrations used in the present study was equivalent to egg or milk protein.

The amount of phenylalanine (mg/kg body-weight per d) and the total amount of protein (g/kg body-weight per d) was calculated for each child as the mean intake for each month divided by the mean weight for each month. For infants with similar initial weights the total intake of protein from hydrolysate and from milk was calculated as g/76 d.

RESULTS

All children grew and developed showing normal skeletal maturation. Their electroencephalograms were normal. Haematological and chemical analyses were within the normal ranges and no difference was found between the two groups. However, a fall in linear growth velocity occurred in one child from the FAO group during infancy, in two from the FAO group from the age of 12 months and throughout the study and in one from the FAO group during the greater part of the second year of life. All children in the RDA group maintained a normal growth velocity. X-ray of the hand showed possible osteoporosis in five children from the FAO group, three of whom had a decrease in linear growth velocity, whereas no signs of osteoporosis were present in the RDA group.

Plasma phenylalanine

During the first 76 d of dietary treatment, the number of analyses (median and range) were 15 (13–22) and 15 (13–29) for the RDA and FAO subgroups respectively. One infant, no. 5 in the RDA subgroup, had rather high plasma phenylalanine values throughout the first 76 d of dietary treatment, the median value being 1454 μmol/l. The median values for the other four infants in the RDA subgroup were from 376 to 836 μmol/l, and in the FAO subgroup from 381 to 776 μmol/l. From 5 to 15 months of age the number of analyses (median and range) were 23 (18–39) and 20 (17–42) for the RDA and FAO groups respectively. The ranges of median values during this period were from 352 to 690 and from 412 to 703 μmol/l for the RDA and FAO groups respectively. For both groups, the percentage of values below 121 μmol/l was 2 and the percentage above 967 μmol/l was 6.

Weight

The weights of the children at the start of the dietary treatment are shown in Table 1. At 5 and 15 months of age the weights were similar in both groups, the values (kg; mean and range) for the RDA group were 7·12 (6·18–8·32) and 10·7 (9·0–12·4) for 5 and 15 months respectively, and for the FAO group 7·28 (6·18–9·0) and 10·7 (9·2–12·0) respectively. The children in the RDA and FAO subgroups with similar birth weights had similar weight gains during the first 76 d on the diet.

Table 1. *Details of the infants at the start of the dietary treatment*

Group	Subject no.	Sex	Age (d)	Wt (g)	Plasma phenylalanine (μmol/l)
RDA	1	♀	18	3840*	3030
	2	♂	15	3600*	1000
	3	♂	—	—	—
	4	♀	16	3300*	2182
	5	♀	15	3700*	1176
	6	♀	16	3470*	1818
	7	♀	16	4860	1393
	8	♂	16	2175	1030
FAO	9	♀	17	2820	2061
	10	♂	15	3470*	2212
	11	♀	15	3770*	2000
	12	♂	15	4200	2103
	13	♀	16	3860*	3006
	14	♂	16	4100	1909
	15	♀	16	3495*	2139
	16	♂	17	3000	1030

RDA, group receiving protein at a level based on the recommendations of (US) Food and Nutrition Board (1974, 1980); FAO, group receiving protein at the level of intake corresponding to the Joint FAO/WHO *ad hoc* Expert Committee (1973) safe levels.

* Individuals comprising RDA and FAO subgroups.

Protein and phenylalanine intakes

Table 2 shows the total protein intake (g/kg per d; median and range) for both groups. From the age of 4 months the RDA children received more protein than the FAO children. There was a broader range of protein intake in the RDA group than in the FAO group. Table 2 also shows the phenylalanine intake (mg/kg per d; median and range) and again there was a broader range of phenylalanine intake in the RDA group than in the FAO group. Except for the first 15 d on the diet the RDA group required more phenylalanine than the FAO group to maintain the plasma phenylalanine level at the required concentration. This difference was statistically significant from 5 to 15 months of age using a one-sided Wilcoxon range test. The protein intake (g/76 d) for all infants in the RDA subgroup was greater than that for the FAO subgroup, both from hydrolysate alone and from hydrolysate and milk (see Table 3). The phenylalanine intake (g/76 d) was also greater for the RDA subgroup than for the FAO subgroup, although there was some overlap between the two subgroups.

Fig. 1 shows the correlation between weight gain and phenylalanine intake, within each subgroup, during the first 76 d on the diet. Using a least-square approximation, there was good correlation for the FAO subgroup (r 0·99) but for the RDA subgroup there was a lower extent of correlation (r 0·72).

DISCUSSION

In PKU there is a metabolic block in the conversion of phenylalanine to tyrosine. The absorbed phenylalanine is used for protein synthesis and excess phenylalanine will accumulate in the body fluids and the disorder, if untreated, leads to permanent brain damage. The therapy is to give just as much phenylalanine as is required for protein synthesis, the phenylalanine intake being regulated by the plasma phenylalanine level. To cover the requirement for protein, a protein hydrolysate devoid of phenylalanine is given.

Table 2. *The total intakes of protein and phenylalanine by the RDA and FAO groups*

(Phenylalanine intake in the RDA and FAO groups was that required to maintain the plasma phenylalanine within the required range)

Age (months)	Protein intake (g/kg body-wt per d)				Phenylalanine intake (mg/kg body-wt per d)			
	RDA group		FAO group		RDA group		FAO group	
	Median	Range	Median	Range	Median	Range	Median	Range
0·5–1	2·98	2·62–3·37	2·45	2·21–2·77	43	35–67	44	21–57
1–2	2·90	2·51–3·50	2·40	2·21–2·80	59	35–84	53	37–60
2–3	2·68	2·46–3·40	2·10	2·00–2·51	46	39–65	45	41–52
3–4	2·70	2·21–2·85	1·88	1·75–2·49	43	30–50	39	36–42
4–5	2·65	2·05–2·85	1·81	1·77–1·93	39	28–46	37	34–40
5–6	2·40	2·10–2·75	1·70	1·64–1·74	39	31–43	34***	27–36
6–7	2·35	2·20–2·70	1·64	1·58–1·69	37	29–45	32**	26–33
7–8	2·30	2·10–2·58	1·57	1·49–1·73	35	28–60	31*	26–32
8–9	2·15	2·00–2·60	1·58	1·43–1·71	33	27–49	29***	27–33
9–10	2·20	2·00–2·85	1·54	1·51–1·69	33	27–54	28***	25–34
10–11	2·05	1·90–2·70	1·50	1·44–1·64	32	25–48	27***	24–32
11–12	2·00	1·85–2·65	1·46	1·38–1·57	31	26–47	26*	23–30
12–13	1·95	1·85–2·60	1·42	1·32–1·63	29	26–45	26*	23–29
13–14	2·05	1·80–2·65	1·47	1·29–1·61	29	25–43	26**	22–28
14–15	2·10	1·75–2·65	1·33	1·27–1·48	29	24–41	25**	22–27
15–16	2·05	1·75–2·45	1·32	1·25–1·51	27	21–40	24***	20–26
16–17	2·00	1·70–2·45	1·29	1·26–1·50	25	21–38	23	19–27
17–18	1·90	1·65–2·45	1·31	1·26–1·48	25	20–37	22	19–28
18–19	1·88	1·61–2·40	1·29	1·21–1·46	25	20–36	22	19–27
19–20	1·89	1·61–2·36	1·29	1·20–1·45	25	20–34	22	19–27
20–21	1·89	1·65–2·29	1·30	1·24–1·43	24	20–34	22	19–27
21–22	1·90	1·60–2·34	1·30	1·25–1·43	24	20–34	21	18–27
22–23	1·90	1·57–2·41	1·29	1·23–1·40	23	19–33	22	18–26
23–24	1·97	1·59–2·35	1·28	1·21–1·40	23	18–33	21	18–26

RDA, group receiving protein at a level based on the recommendations of (US) Food and Nutrition Board (1974, 1980); FAO, group receiving protein at the level of intake corresponding to the Joint FAO/WHO *ad hoc* Expert Committee (1973) safe levels.

Median values for the two groups were significantly different: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}005$.

Table 3. *Weight gain, protein and phenylalanine intakes during the first 76 d on the diet in the RDA and FAO subgroups*

Group	*n*	Initial values (g)		Wt gain (g)		Protein intake (g)				Total phenylalanine intake (g)	
						Albumaid XP*		Milk			
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
RDA subgroup†	5	3620	3400–3820	1880	1600–2330	663	599–754	359	334–407	19·53	18·12–22·17
FAO subgroup†	4	3640	3500–3860	1940	1400–2370	513	464–577	296	259–341	15·78	13·12–18·63

RDA, group receiving protein at a level based on the recommendations of (US) Food and Nutrition Board (1974, 1980); FAO, group receiving protein at the levels of intake corresponding to the Joint FAO/WHO *ad hoc*. Expert Committee (1973) safe levels.

* Scientific Hospitals Supplies Ltd, Liverpool.

† For details, see p. 436 and Table 1.

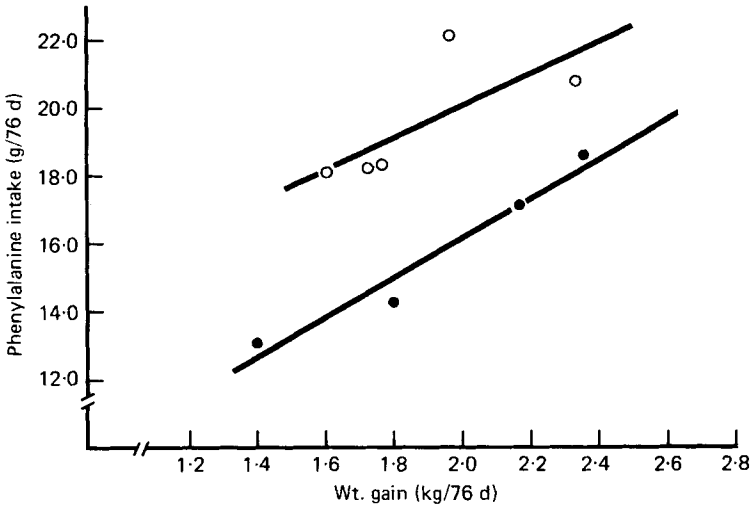

Fig. 1. The correlation between weight gain (kg/76 d) and phenylalanine intake (g/76 d) during the first 76 d on the diet for (○) RDA subgroup (receiving protein at a level based on the recommendations of the (US) Food and Nutrition Board (1974, 1980)) and (●) FAO subgroup (receiving protein at the level of intake corresponding to the Joint FAO/WHO *ad hoc* Expert Committee (1973) safe levels).

In the present study one group of children was given a protein intake based on the Recommended Daily Allowances of the (US) Food and Nutrition Board (1974, 1980), and that of the other group of children followed the recommendations of the Joint FAO/WHO *ad hoc* Expert Committee (1973). For both groups the protein was derived from a protein hydrolysate, milk, vegetables and fruit. The phenylalanine intake necessary to maintain the plasma phenylalanine at the required concentration was greater in the RDA group than in the FAO group. In the RDA group the intakes during the first and second year of life were 39 and 26 mg/kg per d respectively. In the FAO group the corresponding intakes were 34 and 23 mg/kg per d. The broader range of phenylalanine intake in the RDA group compared with the FAO group was probably caused by the corresponding broader range of protein intake. During the first 4 months of age the difference was not statistically significant, probably because some of the FAO children actually received more protein than the RDA children. From 5 to 15 months of age the difference was statistically significant and the weight gain was the same in both groups during this period.

A more detailed examination of phenylalanine intake in the first months of life in relation to protein intake was made by comparing infants weighing approximately the same at the start of dietary treatment. In these subgroups there was no overlap in protein intake between the two groups either in terms of protein hydrolysate alone or as protein hydrolysate and milk protein. The period of 76 d was chosen because during this period the only intact protein given to all the infants was cow's milk protein. More phenylanine was required in the RDA subgroup than in the FAO subgroup and Fig. 1 shows that with the same weight gain more phenylalanine was required in the RDA subgroup than in the FAO subgroup. Fig. 1 also shows that within each subgroup there was a correlation between weight gain and phenylalanine intake. The correlation was very good for the FAO subgroup but not as good for the RDA subgroup, probably because the FAO subgroup had been followed more closely, with more frequent and smaller increases in protein hydrolysate.

We believe that the greater phenylalanine intake in the RDA group compared with the

FAO group reflects a greater N retention in the former group. This increased retention cannot be explained by a greater weight gain in the RDA group as the mean weight gain at 5 and 15 months of age was the same for the two groups.

There are, however, possible alternative explanations. The higher intake of phenylalanine in the RDA group could be accounted for by more phenylalanine hydroxylase (phenylalanine 4-monooxygenase; *EC* 1.14.16.1) activity remaining in the RDA children compared with the FAO children. However, it seems unlikely that all such children should be in the RDA group.

Phenylalanine is also lost in the urine and faeces. The difference in phenylalanine intake cannot be accounted for by a difference in phenylalanine excretion in the urine, since the loss of phenylalanine in the urine is very small and is influenced only by the plasma level of phenylalanine which had been equally-well regulated in both groups (except for child no. 5 in the RDA subgroup who had high plasma phenylalanine values throughout the first 76 d with dietary treatment).

Phenylalanine in the morning urine of fasted subjects has been analysed in ten normal children from 3 to 5 years of age, ten samples from the RDA children from 2·8 to 5·8 years of age and in seven samples from the FAO children from 0·9 to 3·8 years of age. The values (μmol/l; median and range) were 0·08 (0·04–0·14), 0·31 (0·06–1·02) and 0·31 (0·07–1·16) respectively. Faeces N is derived from two sources, one being the N in the diet. In the present study the difference in N was almost exclusively the N derived from Albumaid XP which contains no phenylalanine and only a small amount of milk protein. Thus the difference in phenylalanine intake could not be explained by faecal losses. The other source of N in faeces is endogenous N. This N represents N from intact protein containing phenylalanine. At least for rats the amount of endogenous N in faeces is dependent on the amount of food eaten and not on the percentage of protein in the diet. This N has been estimated to be 20 mg/kg per d for infants (Joint FAO/WHO, 1973). It thus seems unlikely that the difference in phenylalanine intake could be accounted for by a difference in this endogenous N, particularly as faeces N losses are small when only amino acids are given. A similar conclusion was reported by Snyderman *et al.* (1964) who gave infants amino acids equivalent to 3 g protein/kg per d. Mean faecal N losses were 32 mg/kg per d.

The retention of N, or rather body composition, is influenced by the N content of the diet during infancy and until chemical maturation is accomplished. Our results indicate that only a small increase in protein intake of about 0·6 g/kg per d is enough to increase the retention of N. A very rough estimate of the difference in retained N can be made from 5 to 15 months of age. If the difference in phenylalanine intake during this period were 4·2 mg/kg per d, and the mean weight were 9·0 kg, then $4{\cdot}2 \times 9{\cdot}0 \times 330 = 12472$ mg more phenylalanine was retained in the RDA children than in the FAO children. Assuming 5% of the protein was phenylalanine, this represents 39·8 g more N retained or 0·44% higher concentration of N in the body in the RDA group than in the FAO group. The results of our earlier study suggested that the FAO group had received a marginal protein intake, and the results of the present study suggest that a protein intake slightly higher than the Joint FAO/WHO (1973) safe level is desirable. However, the present study is based on few children and several assumptions, and the results need to be confirmed by other studies using other criteria for an adequate dietary protein intake.

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