Evaluation of a new mineral and trace metal supplement for use with synthetic diets

MARGARET S. LAWSON, BARBARA E. CLAYTON, H. T. DELVES, AND J. D. MITCHELL

From the Institute of Child Health, and The Hospital for Sick Children, London

SUMMARY In an earlier study (Alexander *et al.*, 1974) it was shown that the mineral mixture used in association with a synthetic diet was deficient in zinc, copper, iron, and manganese. A new mixture containing appropriately increased amounts of these trace metals has now been evaluated by means of metabolic balance studies carried out on children with phenylketonuria. This new mixture was shown to be satisfactory for zinc, copper, and iron, but minor changes have been recommended for manganese.

Synthetic diets are used in infants and children for the treatment of inherited and acquired metabolic disorders. In a previous study (Alexander *et al.*, 1974) using diets in which natural protein was replaced largely by amino acids, trace metals were provided from some natural foods and from a trace metal mixture, M1 (either Aminogran Mineral Mixture: Allen & Hanbury Ltd., or Metabolic Mineral Mixture: Scientific Hospital Supplies Ltd., both of which had a similar composition). The results obtained from conventional metabolic balances indicated that these mixtures required the following modifications: a fourfold increase in the content of Zn, a twofold increase in Cu, and a 25% increase in Fe. In addition, it was proposed that manganese (Mn) should be removed from the mixture and provided separately at a dose of $1.1 \mu\text{mol}$ ($60 \mu\text{g}$)/kg per day. Adjustments were not recommended for any other components of the mixture. The object of the present study was to evaluate a new mineral mixture (M2) based on the above recommendations,

using conventional balance techniques in patients with phenylketonuria (PKU).

Patients

Balances were performed on 7 children (Table 1) aged 23 days to 8.25 years receiving treatment for PKU with a controlled low phenylalanine diet in which a mixture of synthetic L-amino acids provided most of the protein requirements (Aminogran: Allen & Hanbury Ltd.; for details see Bentovim *et al.*, 1970; Smith *et al.*, 1973). 5 of the patients were changed from M1 to M2 at least 4 weeks before the balances were performed, but Cases 1 and 2 had received M2 for only 9 and 8 days respectively before the studies began.

Materials and methods

The composition of the new mineral mixture M2 is given in Table 2 together with that of M1 for

Table 1 *Details of the patients*

Case no.	Sex	Age (yr)	Body weight (kg)	Period on M2 before study (w)	Other clinical details
1	F	23 d	3.8	9 d	Detection of PKU at 10 d
2	M	0.25	6.0	8 d	Detection of PKU (atypical) at 19 d
3 (a)*	M	2.08	11.5	6	Detection of PKU at 8 d; developing satisfactorily
3 (b)*	M	3.33	13.0	6	Detection of PKU at 5 d; developing satisfactorily
3 (c)*	M	4.92	17.5	11	Detection of PKU at 8 w; developing satisfactorily
4	M	8.0	23.2	4	Diagnosis of PKU at 2 y; IQ < 60
5	F	8.25	19.6	4	Diagnosis of PKU at 1.17 y; IQ 83

*Sibs; balances carried out at home. All other balances performed in hospital.

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comparison. The amount included in the diet was 1.5 g/kg up to a maximum of 8 g daily. Manganese was given in the form of one tablet per kg body

weight daily. Unfortunately, analysis of the tablets showed that each contained 1.4 μmol (75 μg) Mn and not the 1.1 μmol (60 μg) that was recommended.

The collection of diets, faeces, and urine, preparation of samples for analysis, and analytical procedures used were as previously described (Alexander and Delves, 1972; Alexander *et al.*, 1974). Analyses for N, P, and Ca were performed in addition to those for Zn, Cu, Fe, and Mn.

Table 2 Composition of mineral and trace metal mixtures

	Original formula (M1) (per kg)	Formula (M2) (per kg)
Calcium	2.05 mol (82 g)	2.05 mol (82 g)
Potassium	2.12 mol (83 g)	2.12 mol (83 g)
Sodium	1.74 mol (40 g)	1.74 mol (40 g)
Magnesium	399 mmol (9.7 g)	399 mmol (9.7 g)
Iron	9.00 mmol (550 mg)	11.2 mmol (628 mg)
Copper	0.99 mmol (63 mg)	2.00 mmol (127 mg)
Zinc	1.84 mmol (120 mg)	7.34 mmol (480 mg)
Manganese	1.04 mmol (57 mg)	—*
Iodine	60.3 μmol (7650 μg)	60.3 μmol (7650 μg)
Aluminium	10.6 μmol (285 μg)	10.6 μmol (285 μg)
Cobalt	17.8 μmol (1048 μg)	17.8 μmol (1048 μg)
Molybdenum	20.7 μmol (1981 μg)	20.7 μmol (1981 μg)
Phosphorus	1.92 mol (60 g)	1.92 mol (60 g)

*Manganese administered in tablets, each containing 1.4 μmol (75 μg).

Note: The above mixture was prepared from simple salts of the metals shown, i.e. chloride, sulphate and phosphate together with lactate as diluent.

Results

Figs. 1, 3, 5, 7 show the total daily intake plotted against body weight, and Figs. 2, 4, 6, 8 show the daily intake per kg body weight plotted against daily excretion per kg body weight for the trace metals Zn, Cu, Fe, and Mn.

The mean values for daily intake, excretion, and absorption are given in Table 3, and in Table 4, the percentages of the daily intakes of Zn, Cu, Fe, and Mn absorbed and retained by the patients are compared with results seen in healthy children on normal diets.

Table 3 Mean uptake and excretion of nutrients (units/kg per day $\pm$ SD)

Element	Study*	Intake	Excretion			Absorbed	Retained
			Total	Urine	Faeces		
Zn (μmol)	A	2.57 $\pm$ 1.30	2.71 $\pm$ 1.58	0.70 $\pm$ 0.87	2.01 $\pm$ 0.96	0.56 $\pm$ 0.81	-0.14 $\pm$ 0.58
	B	4.64 $\pm$ 1.62	3.96 $\pm$ 1.59	0.32 $\pm$ 0.23	3.64 $\pm$ 1.64	1.00 $\pm$ 1.45	0.68 $\pm$ 1.25
	C	6.23 $\pm$ 3.72	5.02 $\pm$ 1.99	0.18 $\pm$ 0.15	4.84 $\pm$ 1.87	1.39 $\pm$ 1.62	1.21 $\pm$ 1.84
Cu (μmol)	A	0.86 $\pm$ 0.37	1.21 $\pm$ 0.76	0.12 $\pm$ 0.24	1.09 $\pm$ 0.56	-0.23 $\pm$ 0.34	-0.35 $\pm$ 0.50
	B	0.55 $\pm$ 0.35	0.48 $\pm$ 0.27	0.05 $\pm$ 0.06	0.43 $\pm$ 0.29	0.12 $\pm$ 0.21	0.07 $\pm$ 0.19
	C	1.52 $\pm$ 0.68	1.16 $\pm$ 0.47	0.05 $\pm$ 0.06	1.11 $\pm$ 0.41	0.41 $\pm$ 0.32	0.36 $\pm$ 0.27
Fe (μmol)	A	6.80 $\pm$ 2.15	6.97 $\pm$ 1.88	0.02 $\pm$ 0.04	6.95 $\pm$ 1.88	-0.15 $\pm$ 1.95	-0.17 $\pm$ 1.97
	B	7.22 $\pm$ 5.32	6.03 $\pm$ 4.87	0.27 $\pm$ 0.20	5.76 $\pm$ 4.73	1.46 $\pm$ 0.95	1.19 $\pm$ 0.88
	C	11.6 $\pm$ 5.77	9.11 $\pm$ 2.74	0.28 $\pm$ 0.43	8.84 $\pm$ 2.52	2.79 $\pm$ 3.89	2.52 $\pm$ 3.90
Mn (μmol)	A	0.98 $\pm$ 0.27	0.95 $\pm$ 0.19	0.02 $\pm$ 0.02	0.93 $\pm$ 0.17	0.05 $\pm$ 0.20	0.03 $\pm$ 0.19
	B	1.05 $\pm$ 0.49	0.89 $\pm$ 0.52	0.02 $\pm$ 0.01	0.87 $\pm$ 0.52	0.18 $\pm$ 0.30	0.16 $\pm$ 0.30
	C	2.22 $\pm$ 0.86	1.78 $\pm$ 0.86	0.01 $\pm$ 0.02	1.77 $\pm$ 0.86	0.45 $\pm$ 0.36	0.44 $\pm$ 0.37
Ca (mmol)	A	1.65 $\pm$ 1.10	1.27 $\pm$ 0.86	0.06 $\pm$ 0.04	1.21 $\pm$ 0.83	0.44 $\pm$ 0.36	0.38 $\pm$ 0.34
	B	1.35 $\pm$ 0.82	0.67 $\pm$ 0.37	0.05 $\pm$ 0.02	0.62 $\pm$ 0.32	0.73 $\pm$ 0.85	0.68 $\pm$ 0.83
	C	2.32 $\pm$ 1.37	1.38 $\pm$ 0.75	0.12 $\pm$ 0.07	1.26 $\pm$ 0.56	1.06 $\pm$ 0.99	0.93 $\pm$ 0.93
Mg (mmol)	A	0.52 $\pm$ 0.24	0.36 $\pm$ 0.15	0.09 $\pm$ 0.02	0.27 $\pm$ 0.15	0.25 $\pm$ 0.11	0.16 $\pm$ 0.11
	B	0.35 $\pm$ 0.09	0.26 $\pm$ 0.09	0.10 $\pm$ 0.29	0.16 $\pm$ 0.08	0.19 $\pm$ 0.07	0.09 $\pm$ 0.07
	C	0.48 $\pm$ 0.19	0.36 $\pm$ 0.10	0.16 $\pm$ 0.03	0.20 $\pm$ 0.09	0.28 $\pm$ 0.12	0.12 $\pm$ 0.10
P (mmol)	A	1.84 $\pm$ 1.00	1.08 $\pm$ 0.39	0.62 $\pm$ 0.22	0.46 $\pm$ 0.18	1.38 $\pm$ 0.90	0.76 $\pm$ 0.82
	B	1.90 $\pm$ 0.61	1.26 $\pm$ 0.47	0.78 $\pm$ 0.33	0.48 $\pm$ 0.20	1.42 $\pm$ 0.51	0.64 $\pm$ 0.34
	C	2.19 $\pm$ 1.24	1.39 $\pm$ 0.61	0.85 $\pm$ 0.37	0.54 $\pm$ 0.26	1.65 $\pm$ 1.03	0.80 $\pm$ 0.73
N (mmol)	A	25.8 $\pm$ 6.28	15.8 $\pm$ 4.71	13.9 $\pm$ 4.57	1.9 $\pm$ 0.51	23.9 $\pm$ 6.00	10.0 $\pm$ 9.35
	B	25.1 $\pm$ 8.78	18.6 $\pm$ 4.71	16.3 $\pm$ 6.28	2.3 $\pm$ 0.84	22.8 $\pm$ 8.35	6.5 $\pm$ 7.78
	C	32.1 $\pm$ 8.85	19.0 $\pm$ 7.85	16.5 $\pm$ 7.50	2.5 $\pm$ 0.62	29.6 $\pm$ 8.57	13.1 $\pm$ 8.06

*A, Patients receiving synthetic diets
B, Healthy children receiving normal diets
C, Patients—this study. } (Alexander *et al.*, 1974).

Conversion: SI to traditional units—Zn: 1 μmol $\approx$ 65.4 μg . Cu: 1 μmol $\approx$ 63.5 μg . Fe: 1 μmol $\approx$ 55.8 μg . Mn: 1 μmol $\approx$ 54.9 μg . Ca: 1 mmol $\approx$ 40.1 mg. Mg: 1 mmol $\approx$ 24.3 mg. P: 1 mmol $\approx$ 31 mg. N: 1 mmol $\approx$ 14 mg.

Table 4 Effect of increasing trace metal content of a synthetic diet on absorption and retention of the trace metals

Study*	Zinc		Iron		Copper		Manganese	
	%Abs	%Ret	%Abs	%Ret	%Abs	%Ret	%Abs	%Ret
A	15	-7	-6	-6	-30	-40	3	1
B	18	11	23	20	21	13	20	18
C	13	11	18	16	27	24	24	22

*A, Patients receiving synthetic diets and M1
B, Healthy children receiving normal diets
C, Patients—this study. } (Alexander *et al.*, 1974).

%Abs, mean values for percentage absorption;

%Ret, mean values for percentage retention.

Discussion

These patients consumed a greater quantity of freely allowed natural foods, mainly fruit and vegetables, than those in the earlier study (Alexander *et al.*, 1974; for details of diets in PKU, see Francis, 1975). Their daily intakes of P and Ca were therefore higher (Table 3) even though the amounts of these macro-constituents in M2 were not increased over those in M1.

The mean daily intakes of Zn and Cu were approximately those expected from the larger amounts of these metals in M2, but for Fe and Mn, the observed increases were greater than expected. This may be explained by the increased consumption of freely allowed foods, particularly by the older patients, which would have relatively little effect on

the total daily intakes of Zn and Cu because of their large increases, fourfold and twofold respectively, in M2. The consumption of larger quantities of natural foods would have a greater effect on the total intake of Fe, which was increased by only 25% in M2, and of Mn which was administered separately in tablet form at only the slightly higher dosage of 1.4 μmol (75 μg)/kg per day compared with the previous intake of 0.98 μmol (54 μg)/kg per day (Alexander *et al.*, 1974) provided by M1.

Zinc. The daily intake of Zn by the patients who received M2 closely matched that observed for the control children in the earlier study (Fig. 1) and ranged from 46 to 75 μmol (3.0 to 4.9 mg)/day which is almost exactly the recommended intake for children of 46 to 77 μmol (3.0 to 5.0 mg)/day (National Research Council, 1973). 6 of the 7 patients were in positive balance (Fig. 2) compared with only 3 out of 6 in the earlier study and the

Fig. 1 Daily zinc intake v. weight. (1) Patients and (2) healthy children, from Alexander *et al.* (1974); (3) patients from this study. Conversion: SI to traditional units—Zinc: 1 $\mu\text{mol/kg} \approx 65.4 \mu\text{g/kg}$.

Fig. 2 Daily zinc balance.

Fig. 3 Daily copper intake v. weight. (1) Patients and (2) healthy children, from Alexander et al. (1974); (3) patients from this study. Conversion: SI to traditional units—Copper: $1 \mu\text{mol/kg} \approx 63.5 \mu\text{g/kg}$.

percentages of the daily intake which were absorbed and retained by the patients were closer to those of the control children (Table 4).

Copper. The mean daily intake of copper by the patients receiving M2 was $1.5 \mu\text{mol}$ ($96 \mu\text{g}$)/kg per day which is slightly in excess of the 0.6 – $1.2 \mu\text{mol}$ (40 – $80 \mu\text{g}$)/kg per day recommended for children (WHO, 1973; National Research Council, 1973). All the patients were in positive balance (Fig. 4) com-

pared with only 2 out of 6 in the earlier study and the percentages of dietary copper absorbed and retained were similar to those previously observed in control children (Table 4).

Iron. The daily intake of iron by the patients receiving M2 ranged from 78 to $167 \mu\text{mol}$ (4.38 – 9.35 mg). Though this is lower than the 179 to $269 \mu\text{mol}$ (10 – 15 mg) daily recommended for children (National Research Council, 1973), it encom-

Fig. 5 Daily iron intake v. weight (1) Patients and (2) healthy children, from Alexander et al. (1974); (3) patients from this study. Conversion: SI to traditional units—Iron: $1 \mu\text{mol/kg} \approx 55.8 \mu\text{g/kg}$.

Fig. 4 Daily copper balance.

Fig. 6 Daily iron balance.

passes the normal intake of $143 \mu\text{mol/day}$ (8 mg/day) quoted by Macy (1942). 5 of the 7 patients receiving M2 were in positive balance for Fe (Fig. 6) compared with only 2 of the 6 patients who received M1 in the earlier study. The percentages of the intake absorbed and retained by the patients receiving M2 were closer to those observed in the control children than in the previous study (Table 4).

Manganese. Alexander *et al.* (1974) had recommended that the patients should receive $1.1 \mu\text{mol}$ ($60 \mu\text{g}$)/kg per day of Mn from tablets each containing $1.1 \mu\text{mol}$. Owing to a manufacturing error each tablet contained $1.4 \mu\text{mol}$ ($75 \mu\text{g}$) so that the patients received $1.4 \mu\text{mol/kg}$ per day. The 2 infants (Cases 1 and 2) who received only milk and Aminogran had dietary intakes of $1.37 \mu\text{mol}$

Fig. 7 Daily manganese intake v. weight. (1) Patients and (2) healthy children, from Alexander *et al.* (1974); (3) patients from this study; (4) $1.4 \mu\text{mol/kg}$ per day. Conversion: SI to traditional units—Manganese: $1 \mu\text{mol/kg} \approx 54.9 \mu\text{g/kg}$.

($75.2 \mu\text{g}$)/kg per day and $1.41 \mu\text{mol}$ ($77.4 \mu\text{g}$)/kg per day (Fig. 7), which were close to the theoretical intakes from the tablets. The older children, however, had significantly greater intakes of $1.9\text{--}3.4 \mu\text{mol}$ ($102\text{--}185 \mu\text{g}$)/kg per day due to freely allowed natural foods, though the absolute amounts received by these patients of $5.5\text{--}58 \mu\text{mol}$ ($0.3\text{--}3.2 \text{ mg}$)/24 hours is comparable with that observed for preadolescent girls of $38\text{--}87 \mu\text{mol}$ ($2.1\text{--}4.8 \text{ mg}$)/24 hours by Engel *et al.* (1967).

Six out of the 7 patients were in positive balance (Fig. 8) compared with only 3 out of 6 who received M1, and Table 4 shows that the percentages of the intake absorbed and retained by the patients receiving M2 were closer to those of the control children.

The recommended increases in the contents of Zn, Fe, Cu, and Mn in the new trace metal supplement (M2) have therefore resulted in an overall improvement of the balance status of the patients for these metals. 6 out of 7 of the patients receiving M2 were in positive balance for all four metals (Figs. 2, 4, 6, 8) whereas in the earlier study of 6 patients who received M1, only 2 were in positive balance for Fe and Cu, and only 3 for Zn and Mn.

Although the percentages of the dietary intakes of Zn, Cu, Fe, and Mn that were absorbed by the patients receiving M2 are now much closer to those previously observed for the healthy children, the absolute amounts absorbed and retained are higher due to the higher absolute intakes. The intakes were increased in order to produce positive retentions of these metals by the patients and this has been accomplished. There is therefore no indication that any further change should be made for Zn, Cu, and Fe, though the long-term effects, if any, of greater than normal retentions of these metals will need to be kept under review.

In the case of Mn the intakes were much higher than expected and do not compare with our previous findings. This is due to the greater intake of natural foods by the present group of patients. It is

Fig. 8 Daily manganese balance.

therefore recommended that Mn should be supplied in tablet form in an amount of $1.1 \mu\text{mol}$ ($60 \mu\text{g}$)/kg body weight daily up to a maximum of $6.6 \mu\text{mol}$ ($360 \mu\text{g}$). Alternatively, in order to avoid making the supplement more complex, this intake of Mn could be achieved by adding the Mn to the supplement at a concentration of 0.82 mmol (45 mg)/kg of M2.

In view of this study and our earlier data (Alexander *et al.*, 1974), it is likely that our patients being treated with the diet containing M1 were receiving deficient intakes of Cu, Mn, and Fe, and especially of Zn.

In a recent paper Lombeck *et al.* (1975) have shown reduced concentrations of serum selenium (Se) in patients receiving synthetic diets for the treatment of PKU and maple syrup urine disease. Though Se is essential for animals, the requirement for this element in man is not known, but patients with kwashiorkor who had low serum Se levels had an increased weight gain when Se supplementation was given (Levine and Olson, 1970; Schwarz, 1965). Lombeck *et al.* (1975) emphasize the risk of deficiencies with synthetic diets and give this as an example.

In a collaborative study in the USA it was found that children with PKU were developing normally on treatment (Koch *et al.*, 1973) and it was encouraging to see how closely the intellectual assessment approximated to that of the general population of 3 and 4 year olds. The patients did, however, appear to be slightly lower in intelligence than their normal sibs. There are many reasons why this should be, and nutritional deficiency is one possible contributory factor. In an early series of patients, Smith *et al.* (1973) observed a negative correlation between IQ and overt symptoms and signs of nutritional deficiency occurring in the first year of life. It is important that the intakes of mineral and trace metals in addition to phenylalanine and vitamins should be adequate. Until recently only limited information has been available on requirements for trace metals, and it is likely that their intake in synthetic diets has not been optimal.

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References

- Alexander, F. W., and Delves, H. T. (1972). The preparation of a 'metal-free' nappy and its application to metabolic balances in children. *Journal of Clinical Pathology*, **25**, 927-932.
- Alexander, F. W., Clayton, B. E., and Delves, H. T. (1974). Mineral and trace-metal balances in children receiving normal and synthetic diets. *Quarterly Journal of Medicine*, **43**, 89-111.
- Bentovim, A., Clayton, B. E., Francis, D. E. M., Shepherd, J., and Wolff, O. H. (1970). Use of an amino acid mixture in treatment of phenylketonuria. *Archives of Disease in Childhood*, **45**, 640-650.
- Engel, R. W., Price, N. O., and Miller, R. F. (1967). Copper, manganese, cobalt and molybdenum balance in pre-adolescent girls. *Journal of Nutrition*, **92**, 197-204.
- Francis, D. (1975). *Diets for Sick Children*, 3rd ed., p. 280. Blackwell, Oxford.
- Koch, R., Dobson, J. C., Blaskovics, M., Williamson, M. L., Ernest, A. E., Friedman, E. G., and Parker, C. E. (1973). Collaborative study of children treated for phenylketonuria. *Treatment of Inborn Errors of Metabolism*, p. 3. Ed. by J. W. T. Seakins, R. A. Saunders, and C. Toothill. Churchill Livingstone, Edinburgh and London.
- Levine, R. J., and Olson, R. E. (1970). Blood selenium in Thai children with protein-calorie malnutrition. *Proceedings of the Society for Experimental Biology and Medicine*, **134**, 1030-1034.
- Lombeck, I., Kasperek, K., Feinendegen, L. E., and Bremer, H. J. (1975). Serum-selenium concentrations in patients with maple-syrup-urine disease and phenylketonuria under dieto-therapy. *Clinica Chimica Acta*, **64**, 57-61.
- Macy, I. G. (1942). *Nutrition and Chemical Growth in Childhood*. Thomas, Springfield, Illinois.
- Mills, C. F. (1974). The detection of trace element deficiency and excess in man and farm animals. *Proceedings of the Nutrition Society*, **33**, 267-274.
- National Research Council Food and Nutrition Board. (1973). *Recommended Dietary Allowances*, 8th revised ed. National Academy of Sciences, Washington.
- Schwarz, K. (1965). Selenium and kwashiorkor. *Lancet*, **1**, 1335-1336.
- Smith, I., Lobascher, M., and Wolff, O. H. (1973). Factors influencing outcome in early treated phenylketonuria. *Treatment of Inborn Errors of Metabolism*, p. 41. Ed. by J. W. T. Seakins, R. A. Saunders, and C. Toothill. Churchill Livingstone, London.
- World Health Organization (1973). *Trace Elements in Human Nutrition*. UN/WHO Technical Report Series, 532.

Correspondence to Prof. B. E. Clayton, Department of Chemical Pathology, The Hospital for Sick Children, Great Ormond Street, London WC1N 1EH