Metabolic and Hormonal Responses to Oral Amino Acids in Infantile Malnutrition

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Milner, R. D. G. (1971). *Archives of Disease in Childhood*, 46, 301. **Metabolic and hormonal responses to oral amino acids in infantile malnutrition.** A mixture of 10 essential amino acids was given by gavage to 8 children with infantile malnutrition and 7 children who had clinically recovered from malnutrition. Blood samples taken before and at different times after the amino acid load were analysed for glucose, free fatty acid, α -amino nitrogen, insulin, and growth hormone. In both the malnourished and the recovered children the amino acid load caused a rise in plasma α -amino nitrogen and glucose, and a fall in growth hormone levels, but no change in plasma free fatty acid or insulin levels.

Malnutrition in man causes disturbances of endocrine function (see review by Milner, 1970). In particular plasma growth hormone (GH) levels are raised in infantile malnutrition (Pimstone *et al.*, 1966, 1967, 1968a) while insulin levels are low (Baig and Edozien, 1965; Hadden, 1967). Glucagon causes a further rise in GH levels (Milner, 1971) while hyperglycaemia results in a sluggish fall (Pimstone *et al.*, 1967). There is a subnormal insulin response to a glucose challenge in the malnourished child, both initially (Baig and Edozien, 1965; Hadden, 1967) and after clinical recovery (James and Coore, 1970; Milner, 1971). Glucose is not the only metabolite to influence plasma levels of these two hormones however. Recent studies have shown that oral protein or intravenous amino acids are potent stimuli of insulin and growth hormone secretion (Floyd *et al.*, 1966a, b; Knopf *et al.*, 1965). It has been suggested that the sequential rise of insulin and growth hormone after oral protein leads to synergism of the anabolic roles of these hormones (Rabinowitz *et al.*, 1966). This form of stimulus was therefore chosen to investigate further the interrelationships of insulin, growth hormone, and various metabolites in the malnourished infant on admission to hospital, and after clinical recovery.

Patients and Methods

The children studied were patients admitted to the Unit for the investigation and treatment of infantile

malnutrition. The nature of the investigation was explained carefully to the parent or guardian at the time of admission and written consent was obtained. The classification of clinical malnutrition was based on two objective criteria (Lancet, 1970): weight and oedema. Of the 15 patients studied, 4 had oedema and were less than 60% of the 50th centile for weight (Nelson, 1959); they may be described as having *marasmic-kwashiorkor*, and form the commonest type of malnourished child admitted to the Unit; 5 had *marasmus*, i.e. they were less than 60% of their expected weight, but had no oedema; 4 had oedema, and were more than 60% of their expected weight: they had *kwashiorkor*. 2 were 61 and 66% of their expected weight but had no oedema; they are described as *undernourished*. Clinical details of the 8 patients studied shortly after admission and the 7 studied after clinical recovery are given in Table I. As previous studies (Alleyne and Scullard, 1970) had not revealed characteristic metabolic differences between the clinical groups seen in Jamaica, no distinction was made on the basis of clinical classification. Each child was treated with milk feeds of increasing strength and for most of his stay received approximately 150 calories and 3 g protein/kg body weight per day. Folic acid, ferrous sulphate, and vitamin supplements were given routinely; infection was treated when present.

Blood samples were collected by venepuncture after an overnight fast (8–9 hr) at various times. A nasogastric tube was passed approximately one hour before the first blood sample was withdrawn. After collection of the initial sample, a mixture (0.5 g/kg body weight) of the 10 essential amino acids (Sigma Chemical Co., St. Louis, Mo., U.S.A.) prepared as described by Floyd *et al.* (1966b), was given via the tube. Further blood samples were withdrawn 15, 30, 60, 120, and 180 minutes afterwards.

Received 7 December 1970.

TABLE I
Clinical Features of 15 Children with Infantile Malnutrition

Case No. and Sex	On Admission			Diagnosis	EWA* (%)	Days From Admission to Time of Study
	Age (mth)	Weight (kg)	Oedema			
<i>Malnourished:</i>						
1 M	10	4.27	+	Marasmic-kwashiorkor	43	15
2 F	9	6.31	++	Kwashiorkor	72	21
3 F	10	5.52	—	Undernourished	61	11
4 M	9	3.94	—	Marasmus	43	12
5 M	22	6.20	++	Marasmic-kwashiorkor	51	17
6 F	12	6.83	++	Kwashiorkor	70	1
7 M	13	7.30	++	Kwashiorkor	73	1
8 F	8	5.37	—	Undernourished	66	4
<i>Recovered:</i>						
9 M	9	4.25	—	Marasmus	47	75
10 F	16	6.76	+	Kwashiorkor	64	63
11 F	9	3.86	—	Marasmus	44	80
12 M	13	4.46	+	Marasmic-kwashiorkor	43	80
13 M	11	3.86	—	Marasmic	40	72
14 M	12	5.75	++	Marasmic-kwashiorkor	57	83
15 M	9	3.36	—	Marasmus	37	98

* EWA = Expected weight for age, by comparison with the 50th centile (Nelson, 1959).

All blood samples were collected into bottles containing heparin and fluoride and were centrifuged within 30 minutes. Plasma glucose and free fatty acid (FFA) levels were estimated the same day. Aliquots of plasma were stored at -20°C until α -amino nitrogen, insulin, and GH levels were measured. 300 μl plasma sufficed for duplicate determinations of glucose, FFA, α -amino nitrogen, insulin, and GH, thus making feasible repeated blood samples on one infant on one day. Plasma glucose was determined by a glucose oxidase assay (Huggett and Nixon, 1957), FFA by a modification (Shelley and Thalme, 1970) of the method of Novák (1965), and α -amino nitrogen by the method of Stein and Moore (1954). Plasma insulin was measured by immunoassay (Hales and Randle, 1963) using an ox insulin standard and an antibody which did not discriminate between ox and human insulin (kindly given by Dr. B. A. L. Hurn of the Wellcome Laboratories, Beckenham, England). Plasma GH was measured by immunoassay using pre-precipitated antibody, filtration for the separation of 'free' and 'bound' hormone and a human GH standard (M.R.C. new preparation R4 kindly given by Dr. A. Stockell-Hartree, Department of Biochemistry, University of Cambridge). Standard GH solutions and plasma diluted in parallel in the assay. Statistical analyses were done by Students *t* test.

Results

The results of the investigations are presented in Table II and the mean values for plasma α -amino nitrogen glucose, insulin, and GH are plotted against time in the Fig. The fasting levels of glucose, FFA, and α -amino nitrogen were similar in the two groups. The fasting plasma insulin level was significantly lower in the malnourished

group. The GH level was higher but the difference between the malnourished and recovered groups was not significant because of the wide range of individual values. The administration of amino acids via a stomach tube caused a similar rise in α -amino nitrogen in both the malnourished and recovered infants, which was maximum at 60 minutes. This was associated with a modest rise in plasma glucose levels, the difference between the 60- and 0-minute value being significant in both the malnourished and recovered babies. No significant change occurred in FFA or insulin levels after the amino acid load. There was no significant difference between the plasma GH levels in the malnourished and recovered groups at any time though the mean levels in the recovered group were approximately half those in the malnourished group throughout the test. In both groups there was a significant and similar fall in plasma GH after the ingestion of amino acids. The fall occurred in 30 minutes and thereafter the plasma GH level remained steady.

Discussion

Oral amino acids given to malnourished children shortly after admission to hospital or after clinical recovery caused no change in plasma insulin levels, and a fall in plasma growth hormone levels. The importance of these findings lies in the fact that they are qualitatively different from those predicted on the basis of studies in the normal adult.

TABLE II

Plasma Glucose, FFA, α -Amino Nitrogen, Insulin, and GH Responses to Intragastric Amino Acids in Malnourished Children Before and After Recovery

Metabolite or Hormone	Mean $\pm$ 1SD Plasma Concentration at Various Times (min) Before or After α -amino Acid Load*					
	0	15	30	60	120	180
Glucose (mg/100 ml):						
Malnourished	72 $\pm$ 19 (8)	76 $\pm$ 21 (8)	87 $\pm$ 16 (8)	89 $\pm$ 15 (8)	83 $\pm$ 17 (8)	79 $\pm$ 17 (8)
Recovered	80 $\pm$ 7 (7)	83 $\pm$ 14 (7)	85 $\pm$ 14 (7)	91 $\pm$ 14 (7)	82 $\pm$ 20 (7)	70 $\pm$ 20 (7)
Statistical analysis	NS	NS	NS	NS	NS	NS
FFA (μM):						
Malnourished	340 $\pm$ 156 (8)	361 $\pm$ 201 (8)	325 $\pm$ 146 (8)	316 $\pm$ 166 (8)	350 $\pm$ 168 (8)	555 $\pm$ 370 (8)
Recovered	387 $\pm$ 177 (7)	353 $\pm$ 135 (7)	339 $\pm$ 120 (7)	246 $\pm$ 99 (7)	326 $\pm$ 146 (7)	406 $\pm$ 190 (7)
Statistical analysis	NS	NS	NS	NS	NS	NS
α-amino nitrogen (mMol):						
Malnourished	4.7 $\pm$ 1.5 (8)	5.4 $\pm$ 1.3 (8)	6.4 $\pm$ 1.5 (8)	6.9 $\pm$ 2.1 (8)	5.5 $\pm$ 0.9 (8)	5.1 $\pm$ 1.2 (8)
Recovered	4.2 $\pm$ 1.0 (7)	5.5 $\pm$ 1.8 (7)	6.5 $\pm$ 1.7 (7)	7.7 $\pm$ 2.2 (7)	6.1 $\pm$ 2.3 (7)	4.7 $\pm$ 1.2 (7)
Statistical analysis	NS	NS	NS	NS	NS	NS
Insulin (μU/ml):						
Malnourished	9 $\pm$ 2 (8)	11 $\pm$ 5 (8)	12 $\pm$ 5 (8)	12 $\pm$ 8 (8)	10 $\pm$ 1 (8)	12 $\pm$ 5 (8)
Recovered	16 $\pm$ 4 (7)	15 $\pm$ 5 (7)	15 $\pm$ 3 (7)	16 $\pm$ 7 (7)	17 $\pm$ 8 (7)	13 $\pm$ 4 (7)
Statistical analysis	P < 0.005	NS	NS	NS	P < 0.05	NS
GH (ng/ml):						
Malnourished	22.0 $\pm$ 14.6 (8)	18.7 $\pm$ 11.1 (8)	14.9 $\pm$ 11.4 (8)	15.3 $\pm$ 9.4 (8)	12.4 $\pm$ 9.2 (8)	11.9 $\pm$ 9.6 (8)
Recovered	14.9 $\pm$ 5.6 (7)	8.5 $\pm$ 2.8 (7)	7.7 $\pm$ 2.0 (7)	7.3 $\pm$ 2.3 (6)	8.6 $\pm$ 3.8 (7)	7.7 $\pm$ 3.7 (7)
Statistical analysis	NS	NS	NS	NS	NS	NS

* Number of observations in parentheses.

FIG.—Mean values of plasma α -amino nitrogen, glucose, insulin, and GH before (0 min) and at various times after the administration of a mixture of the 10 essential amino acids via a nasogastric tube to malnourished infants shortly after admission to hospital (closed circles) or after clinical recovery (open circles).

The amino acid load was given by gavage as a compromise between giving oral protein or intravenous amino acids, since the digestion and absorption of foodstuffs is known to be impaired in infantile malnutrition and it was not known if the intravenous administration of amino acids to small infants would have adverse side effects. The compromise was shown to be valid by the rise in plasma α -amino nitrogen levels. The qualitative similarity of the response of the sick and recovered group to the amino acid load may reflect the fact that though the sick group was physically malnourished, their metabolic responses might reflect more the current food intake than the chronic nutritional deprivation. For example, the lack of difference in the fasting plasma FFA values in the two groups is probably due to the fact that the malnourished group was studied up to 21 days after admission to hospital. Some of these infants had very high FFA values during the first three days of admission (Milner, 1971) but these fell rapidly to normal on resumption of a high caloric intake.

The failure of the amino acid challenge to cause a rise in plasma insulin levels is in keeping with the recent observations that β cell function remains impaired after clinical recovery from malnutrition (James and Coore, 1970; Milner, 1971). Insulin release is influenced by enteric hormones (Dupré *et al.*, 1969), and disordered gastrointestinal function which occurs in malnutrition may be a contributory factor to subnormal insulin secretion.

Caution must be exercised in making a causal relation between the rise in plasma α -amino nitrogen and the fall in plasma GH levels. It is possible that the passage of a nasogastric tube some 60 minutes before the start of the test was a sufficient non-specific stress to cause a spurious increase in the fasting GH values. Against this idea is the fact that fasting GH values in similar malnourished infants who did not have a nasogastric tube were similar to those in the present study (Milner, 1971). Also the stress of repeated venepunctures in such infants is known to cause a rise in plasma GH of about 10 ng/ml on the third venepuncture at 10 minutes, which disappears by 30 minutes (Milner, 1971). Thus if passing the nasogastric tube had been stressful, it was anticipated that the GH response would have faded by the time sample collection started. If the venepunctures themselves caused a rise in plasma GH this would lessen the true fall in plasma GH occurring in association with the amino acid load.

The reason for the raised plasma GH levels in infantile malnutrition is not clear. Pimstone *et al.* (1968a) suggested that the fall in GH with

recovery was causally associated with the rise in plasma albumin concentration, but were unable to influence plasma GH levels in the malnourished child by albumin infusions (Pimstone *et al.*, 1968b). If a direct relation between plasma GH levels and protein metabolism does exist, it may be between GH and an amino acid or acids rather than between GH and a protein. The results of the present study would be compatible with a scheme whereby amino acids have a direct feedback control on GH release analogous to that postulated for glucose in the adult. The results provide no clue, however, as to whether the changes in plasma GH after an oral amino acid load are a characteristic of malnutrition, of infancy, or more particularly of nutrition in infants.

I wish to thank Professor J. C. Waterlow, Director of the MRC Tropical Metabolism Research Unit, for his encouragement throughout these investigations and Miss M. Ceballos for technical assistance.

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