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Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley

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1. Zinc metabolism has been studied in a group of undernourished, slum-dwelling, lactating women in Manaus, Brazil, by means of modified metabolic balance techniques and the stable isotope [67]Zn.

2. The subjects were found to be consuming a diet which provided an average of 34 % of the recommended dietary allowance for lactating women, but six of the seven appeared to achieve Zn balance. In five of the subjects use of [67]Zn in a stable isotope dilution manner demonstrated that they were absorbing a high proportion of the dietary Zn (proportional absorption ranged from 0·59 to 0·84), suggesting an adaptation to the chronically low intake.

3. Two subjects had marginally low plasma Zn concentrations, although hair, urine and milk Zn contents were all within accepted normal values.

4. Preliminary findings on the rate of plasma Zn turnover and the size of the exchangeable body Zn pool obtained using [67]Zn suggest that these subjects may have a reduction in both.

Acute Zn deficiency in man is a well-recognized and characterized problem which is known to occur in a variety of different circumstances (Moynahan, 1974; Kay *et al.* 1976; Weismann *et al.* 1978). Zn deficiency may also occur as a complicating factor in protein–energy malnutrition (Golden & Golden, 1981) and has been proposed to be present in some poor urban communities (Prasad *et al.* 1961, 1963). Shrimpton (1980, 1984) and Amorosa & Shrimpton (1984) have studied nutritional intakes in the poor urban population of Manaus in the Amazon region of Brazil and found that several nutrients were quantitatively deficient compared with internationally recommended intakes; these were Zn, vitamin A, calcium, thiamin and riboflavin. Furthermore, Zn was found to be the most limiting nutrient when compared with international standards (Shrimpton, 1984). This survey work, together with the finding that 35 % of this population, in the lowest socio-economic group, have low serum Zn concentrations, has led to the suggestion that this urban population may be Zn-deficient (Shrimpton, 1980; Shrimpton *et al.* 1983, 1985). The (US) National Academy of Sciences/National Research Council (1980) recommend a daily intake of 15 mg Zn for adults, increasing by 5 mg in pregnant women and by 10 mg during lactation, but it appears that the daily intake of people in the low socio-economic group in Manaus may be as low as 6 mg (Shrimpton, 1980). It is unknown if these subjects have the ability to maintain Zn balance on such a low dietary intake and how the homeostatic mechanisms of the body respond to this intake.

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Stable-isotope techniques are now available which allow the study of whole-body Zn metabolism in normal human subjects (Janghorbani & Young, 1980; Janghorbani *et al.* 1981; Turnlund *et al.* 1982; Jackson *et al.* 1984), but these techniques have primarily been used to assess Zn absorption rates and other aspects of Zn metabolism in subjects maintained in specialist metabolic facilities and consuming relatively artificial diets. We have attempted to use these techniques to obtain information on Zn metabolism in chronically undernourished subjects in Manaus, living in their own surroundings and consuming their normal diet. These studies have examined Zn balance and the rates of Zn absorption and gastrointestinal secretion in a small group of poor lactating women while consuming their normal dietary intake. In addition preliminary findings relating to the rates of Zn turnover in these subjects have been obtained from the stable isotope studies.

METHODS

Seven lactating mothers were studied in detail. All mothers were feeding infants under 3 months of age. The breast-milk intake of the infants was supplemented with a cassava (*Manihot esculenta*) pap and some bottled formula milk. The mothers were visited by a nutritionist and by the authors several times each day for ten consecutive days during which time information concerning the socio-economic conditions of the family and dietary intake of the mother was obtained.

Zn balance studies

Subjects undertook a modified conventional metabolic balance study at home in which dietary intake of Zn was calculated and the Zn content of all output was measured by atomic absorption spectrometry.

Dietary intake

All subjects were provided with utensils of standard and known size in order to quantify intake. Each recorded the quantity of food eaten at every meal and the dietary intake was calculated each day by a nutritionist using 24 h recall techniques. The major foods consumed were cassava flour, white bread and fish. The intake each day was found to be very similar since the diet varied little, consisting mainly of these three ingredients. Nutrient composition of the diet was then calculated from tables of Amazonian foods previously produced by Rebelo & Shrimpton (1984). Drinking-water samples were taken each day and analysed for Zn content.

Faecal output

The 10 d of study were divided into two 5 d balance periods. Subjects were given 500 mg carmine markers at the beginning of the study and at 5 and 10 d later. All faecal specimens passed from the beginning of the study until the appearance of the last marker were collected in polyethylene-lined Zn-free containers. The carmine markers were used to decide which stool specimens derived from each dietary period. Stools from each subject for each period were then pooled, homogenized, and analysed for Zn by atomic absorption spectrometry as previously described (Jackson, 1977).

Urinary Zn output

Sequential 24 h urine specimens were collected into polyethylene-lined containers for the 10 d of the study. The volumes of these were measured, and portions were taken and frozen at $-20°$ until analysed for Zn by atomic absorption spectrometry.

Milk Zn output

Regular breast-milk samples from the subjects were collected during the 10 d of the study. The Zn content of these was not found to vary with the time of day at which the sample was provided or whether it was taken pre- or post-feed. The volume of milk supplied was calculated by weighing the baby before and after milk feeds. Weighing of babies was undertaken by trained personnel on balances accurate to ± 0.5 g. This gave a range of values for volume of milk per feed from each subject. It was assumed that the maximum volume was delivered at each feed and total possible milk output was calculated from this volume and the number of times nursed. The subjects were instructed to feed their infants on either breast milk or other foods at individual feeds and not to mix feeds. The maximum possible amount of Zn excreted via this route was used for all calculations.

Stable isotope studies

[67]Zn was used in an isotope dilution manner as previously described (Jackson *et al*. 1984). Each subject received a single intravenous injection of 4 mg 93·11 % enriched [67]Zn in sterile saline (9 g sodium chloride/l), and 2 h later received the first carmine faecal marker at which time urine and faecal collections were commenced. Blood (10 ml) was taken for [67]Zn analysis and further blood samples were taken at 3, 6 and 9 d. Analyses of [67]Zn enrichment of plasma and pooled faecal samples were undertaken by thermal ionization mass spectrometry as previously described (Jackson *et al*. 1984). A logarithmic plot of the plasma enrichment of [67]Zn *v*. time after the Zn infusion was plotted and from this the plasma enrichment at the mid-point of each balance period was derived. Comparison of this value with the [67]Zn enrichment of the pooled faeces from that period allowed the proportion of the total faecal Zn derived from gastrointestinal secretion to be calculated. Since the total faecal output (F) is equal to the dietary intake (D) minus the Zn absorbed (A) plus that secreted into the gut (S) (i.e. $F = D - A + S$) a knowledge of the gastrointestinal Zn secretion, the total faecal Zn and the dietary intake allowed the Zn absorbed to be calculated (i.e. $A = D - F + S$) (Jackson *et al*. 1984; Evans *et al*. 1979; Weigand & Kirchgessner, 1978).

Assessment of Zn status

Plasma and hair Zn contents were analysed at the beginning of the study using atomic absorption spectrometry. These studies were approved by the human studies ethical committee of the University of Amazonas, Brazil, and all subjects gave their informed consent.

RESULTS

The socio-economic status of the subjects together with their average intake of energy, protein and Zn at the time of the study is given in Table 1. All subjects were consuming less than the World Health Organization's (1973) recommendations for energy, but had adequate protein intake. Zn intake, as expected, was well below the (US) National Academy of Sciences/National Research Council (1980) recommendations. Financial income was extremely low and in two families no monetary income was declared.

Zn status

The plasma and hair Zn content is shown in Table 2 together with control values obtained in the UK using the same analytical techniques. On this basis two subjects had marginally low plasma Zn concentrations, but all had normal hair Zn content.

Table 1. *Socio-economic status and nutritional intakes of the slum-dwelling lactating Amazonian women*

Subject	Age (years)	No. in household	Family income (US$/month)	Energy intake (% of recommended intake*)	Protein intake (% of recommended intake*)	Zinc intake (mg/d)
A	18	7	8·3	65·2	176·1	9·1
B	25	9	9·7	47·0	136·9	7·2
C	18	9	18·8	71·3	167·4	7·3
D	29	5	14·1	75·5	175·2	9·6
E	24	5	—	80·0	207·1	11·1
F	22	8	—	43·5	87·5	5·8
G	20	4	37·7	71·2	195·6	8·6
Mean	—	—	17·7	64·8	163·7	8·4

* Recommended daily intakes are taken from World Health Organization (1973) and were: energy 11500 kJ (2750 kcal), protein 46 g.

Table 2. *Plasma and hair zinc content of seven slum-dwelling lactating Amazonian women*

Subject	Plasma Zn concentration (mg/l)	Hair Zn content (µg/g)
A	1·04	157
B	0·73	209
C	0·91	111
D	0·86	124
E	0·86	242
F	0·69	921
G	1·01	124
Normal values*	0·75–1·15	72–248

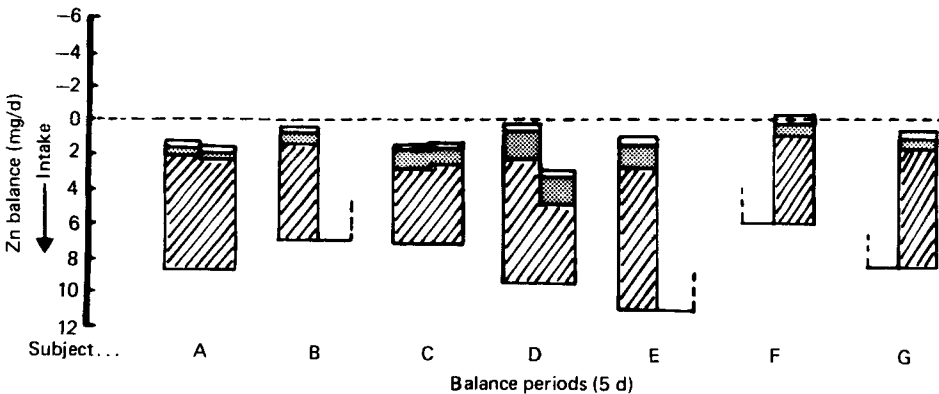* Derived from studies on normal subjects in England using the same methodology.

Fig. 1. Results of metabolic balances for zinc in subjects A–G (for details of subjects, see Table 1). When plotting balance results, dietary intake is plotted downward from the zero horizontal line. From this baseline, output of faecal (▨), milk (▥) and urinary (□) Zn is plotted above. If cumulative output is above the zero line then the subject is in negative balance: if output falls below the zero line, the balance is positive. All subjects were studied for two consecutive 5 d periods; where one period of balance is shown, incomplete loss of faecal material had occurred.

Table 3. *Total zinc content of diets and faeces, isotopic composition of plasma and faeces and calculated rates of gastrointestinal (GI) excretion and absorption of Zn of slum-dwelling lactating Amazonian women*

Subject	Experimental period	Dietary Zn (mg/d)	Faecal Zn (mg/d)	Plasma ^{67}Zn abundance*	Faecal ^{67}Zn abundance*	GI excretion (mg/d)	GI absorption (mg/d)	Proportion
A	1	9·1	6·7	0·02090	0·00948	3·0	5·4	0·593
	2	9·1	6·4	0·00478	0·00338	4·5	7·2	0·791
B	1	7·2	5·4	0·00871	0·00637	3·9	5·7	0·791
C	1	7·3	4·1	0·01348	0·00382	1·2	4·4	0·603
	2	7·3	4·1	0·00380	0·00148	1·6	4·8	0·657
D	1	9·6	7·8	0·00645	0·00444	5·4	7·2	0·750
	2	9·6	7·7	0·00302	0·00206	3·2	8·1	0·844
E	1	11·1	7·9	0·00708	0·00302	3·4	6·6	0·594

* Isotopic abundance is expressed as the spike: normal ratio which is the amount of spike (i.e. mainly ^{67}Zn): total Zn in the sample, and has been corrected for fractionation during mass spectrometry using the ratio, 66:64 and the ratio, 68:64.

Table 4. *Plasma ^{67}Zn turnover, exchangeable Zn pool size and whole-body Zn turnover in five slum-dwelling lactating Amazonian women*

Subject	Plasma Zn turnover, $t_{\frac{1}{2}}$ (d)	Exchangeable Zn pool (mg)	Whole body turnover*, $t_{\frac{1}{2}}$ (d)
A	2·2	86	39
B	2·5	235	77
C	2·8	159	105
D	4·4	418	96
E	2·9	324	107
Mean	2·8	244	85

$t_{\frac{1}{2}}$, Half-life.
* Whole-body Zn turnover (i.e. the apparent $t_{\frac{1}{2}}$ of the exchangeable Zn pool) was calculated from the exchangeable Zn pool size and the rate of Zn excretion during the first 5 d balance period.

Zn balance

The Zn balance results from the seven subjects studied are presented in Fig. 1. These results are presented in the manner previously described (Jackson, 1977). Complete dietary intake values were obtained in all seven subjects, as were urine and breast-milk collections. The mean 24 h urinary Zn for each 5 d period and the maximum possible excretion of Zn in the breast milk were used in all calculations. The completeness of faecal collections was difficult to ascertain in the present study. The frequent presence of the investigators in the homes of the subjects ensured the constant reitteration of the need for complete collections, but even so incomplete faecal collections were obtained on four occasions. In the remaining periods the faecal Zn output was found to be a relatively constant proportion of the dietary Zn intake with a mean of 72 % (range 56–81 %). If substantial and random variations in the completeness of these collections had occurred this would not have been expected.

Full balance values were therefore obtained in a total of ten of fourteen possible 5 d periods. In eight of the ten complete periods a positive balance result was obtained and in one, zero balance was found. The remaining balance gave a slightly negative result.

Rate of gastrointestinal secretion and absorption of Zn

^{67}Zn values were obtained in five subjects (A, B, C, D and E). In other subjects ^{67}Zn values could not be analysed because of technical problems. Table 3 shows the enrichment of the plasma ^{67}Zn at the mid-point of each period together with the enrichment of the pooled faecal samples for that period. The enrichment of the plasma with ^{67}Zn at the mid-point of each period was obtained by plotting the ratio, ^{67}Zn spike: normal (S:N) on a logarithmic scale *v.* time-interval following injection (Jackson *et al.* 1984). The 'best fit' straight line was drawn through these points and from this the enrichment at 2·5 d and at 7·5 d was derived. Gastrointestinal secretion and absorption of Zn were then calculated as previously described (Jackson *et al.* 1984) and are also shown in Table 3, together with the dietary and faecal Zn levels and the rates of gastrointestinal absorption expressed as a proportion of the dietary intake. The proportion of Zn absorbed in these subjects was generally high, ranging from 0·593 to 0·844.

Turnover rates of body Zn and body pools of exchangeable Zn

The half life of the plasma [67]Zn in the five subjects was derived from the plot log S:N *v.* time-interval after injection and is shown in Table 4. This ranged between 2·25 and 4·4 d. Extrapolation of the plasma [67]Zn enrichment back to the time of injection allowed an approximation of the size of the pool of Zn with which the [67]Zn had exchanged to be calculated. This is shown in Table 4 and ranges from 86 to 418 mg Zn. Whole-body Zn turnover (i.e. the apparent half life of the exchangeable pool) was also calculated from the exchangeable Zn pool size and the total rate of Zn excretion (i.e. the sum of the rates of gastrointestinal, milk and urinary Zn excretions) during the first 5 d balance period (Jackson *et al.* 1984). This gave a range of values between 39 and 107 d (Table 4).

DISCUSSION

Acute severe Zn deficiency is now a well-recognized and characterized problem which is known to occur in a variety of different circumstances (Moynahan, 1974; Kay *et al.* 1976; Weismann *et al.* 1978), but the effects of chronically low dietary intakes of Zn are much less well documented and indeed the minimum dietary intake of Zn which is sufficient for body requirements is unknown. The (US) National Academy of Sciences/National Research Council (1980) recommend a dietary intake of 15 mg for adults increasing to 20 mg for pregnant women, and 25 mg for lactating women. Several studies have suggested that many population groups do not achieve this, even in relatively affluent developed countries (e.g. Lyon *et al.* 1979). Overt Zn deficiency may occur as a complicating factor in acute protein–energy malnutrition (Golden & Golden, 1981) but the consequence of chronic long-term marginal Zn intakes on the ability of the body to maintain Zn balance has not been extensively studied.

We have attempted to study Zn metabolism in poor lactating women while they continued to consume their normal daily diet. The subjects studied were of poor socio-economic status with their normal dietary intake only providing an average 62·4% of the recommended energy intake (Table 1).

The daily dietary Zn intake of the subjects ranged from 5·8 to 11·1 mg, with a mean of 8·4 mg or 33·6% of the recommended dietary allowance for lactating women. Nevertheless, in eight of the ten balance periods for which full sets of values were obtained, a positive balance was calculated and the mean balance for the seven subjects (Fig. 1) was 1·3 mg positive. The findings therefore suggest that lactating women consuming much less than the recommended dietary allowance have the ability to maintain Zn balance. Excretion of Zn in sweat was not measured in these studies. There is some disagreement concerning the likely losses of Zn in sweat. Prasad *et al.* (1963) reported that losses of Zn in sweat are of the order of 1·1 mg/l, while Jacob *et al.* (1981) felt that surface losses of Zn were much less important, only increasing the apparent dietary requirements by 5%. The large losses of Zn in the sweat were found in studies where sweat was collected and analysed but, in everyday life, evaporation of water is likely to deposit Zn salts on the skin. Absorption of Zn via the skin has been demonstrated (Hallmans & Lasek, 1985) and it is therefore unknown what is the extent of the net loss via this route in normal circumstances. We therefore feel that our results provide good evidence of the ability of these lactating women to at least achieve Zn balance on their normal diet.

In animal experiments it has been found that the homeostatic mechanisms regulating body Zn have a considerable capacity to adapt in order to compensate for decreased dietary intake. Rats fed on a diet of low-Zn content increase the proportion of Zn absorbed such

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that greater than 0·90 of a test dose of Zn may be absorbed (Becker & Hoskstra, 1971; Jackson et al. 1981) and variation in the gastrointestinal excretion of the element also occurs in an attempt to maintain normal tissue levels. Similar mechanisms appear to exist in man; Jackson et al. (1984) have demonstrated changes in the rates of both absorption and gastrointestinal excretion of Zn with variations in Zn intake, while others have demonstrated that the proportion of a tracer dose of Zn absorbed increased during acute dietary Zn depletion studies in normal subjects (Istfan et al. 1983). We have utilized the isotope dilution technique to examine both the rate of gastrointestinal absorption and excretion of Zn while the subjects consumed their normal diet. This has revealed a proportional absorption of Zn between 0·593 and 0·844 % of the dietary intake (Table 3). Solomons (1982) reviewed reports from several laboratories where proportional absorption of Zn had been measured using isotopic techniques and concluded that when Zn was given orally in an aqueous solution the mean absorption of tracer ranged from 0·43 to 0·69 whereas when the isotope was consumed in the presence of assorted beverages, food or meals, proportional absorption ranged from 0·14 to 0·41. The findings obtained in the present study therefore suggest that the lactating Brazilian women were absorbing a higher proportion of the ingested Zn from their entire diet than is usually the case for tracer doses of Zn given with foodstuffs. The rates of gastrointestinal excretion of Zn in the subjects studied here range from 1·2 to 5·4 mg/d. The lower values obtained (Table 3) are similar to the obligatory endogenous losses in faeces and urine found by Baer & King (1984) in a study of acute experimental Zn depletion in young men and by Hess et al. (1977) in young women. It therefore appears that in the subjects studied here both the rates of gastrointestinal absorption and gastrointestinal Zn excretion are adapted to the chronically-low Zn intake by absorption of a high proportion from the diet and by reduction of the losses of Zn into the gut. This demonstration of a relatively high fractional rate of gastrointestinal absorption of Zn also indicates that the availability of Zn in this particular diet must be relatively high. This is somewhat surprising, but the cassava flour and white bread consumed in Manaus contain little fibre or phytate since the white bread is highly refined and the cassava flour is fermented. The diet is also high in fish protein, a combination of factors which favour Zn availability.

The kinetics of Zn turnover between different body pools is extremely complex and it has recently been calculated that there are at least fourteen 'pools' or compartments contributing to the kinetics of plasma Zn turnover (Wastney et al. 1986). Analysis of the kinetics of human Zn with this degree of refinement are unlikely to be possible given the sensitivity of current stable isotope techniques. In addition the frequency of blood sampling and the length of study required are not likely to be possible under 'field' conditions such as those in the present study. However, we have utilized the plasma ^{67}Zn enrichment data from the present study to obtain preliminary data on Zn turnover in these subjects. An examination of the activity of radioactive Zn in plasma following intravenous injection in normal subjects obtained by Wastney et al. (1986) and other workers (Aamodt et al. 1979; Foster et al. 1979; Prasad et al. 1963) reveals that the disappearance curve of isotopic plasma Zn could be resolved into a series of phases, the rapid parts of which were completed in a matter of hours. In the present study the initial plasma samples were not taken until 2 h after injection of the isotope in order to allow this equilibration and dilution of the isotope with endogenous Zn to have occurred.

In studies over a similar time-course to those described here Jackson et al. (1984) found that the half-life of plasma ^{67}Zn turnover in one normal subject was 12·5 d. In addition, Prasad et al. (1963) also found that in phase IV of their studies (10 h to 10 d post-intravenous ^{65}Zn injection), the half life of the plasma Zn was 4·9–5·9 d. It therefore appears that the lactating Amazonian women studied here may have a more rapid turnover of plasma Zn (mean half-life 2·8 d) than expected.

The increased plasma Zn turnover found in the subjects may also have introduced a slight negative bias in the calculations of gastrointestinal Zn secretion and hence in the absorption rates. In these calculations the overall faecal ^{67}Zn enrichment is compared with the plasma enrichment at the mid-point of each period, the periods being timed from ingestion of the carmine marker. However, there is a time lag between the marker entering the mouth until it passes into the part of the gastrointestinal tract where the bulk of Zn secretion occurs. Our subjects were generally found to have gut transit times of about 24 h and since animal data suggests that gastrointestinal Zn secretion occurs in the small intestine it is likely that the time-lag between the marker entering the mouth and the area of Zn secretion is relatively short (< 6 h). In a normal subject, such as that previously described (Jackson *et al.* 1984) in whom the half-life of the plasma Zn was 12·5 d, this lag would have little effect on the value for ^{67}Zn enrichment used in the calculations, but in the subjects described here with increased plasma Zn turnover rates the effect is more marked. For example, the calculated plasma Zn half-life for subject A was 2·2 d and if it is assumed that the true value of plasma ^{67}Zn enrichment which should be used in calculations was that 6 h after the mid-point, then this would mean the calculated gastrointestinal secretion rate was underestimated by about 10 % resulting in an underestimate of the proportional absorptional rate by about 0·035. This argument is not taken further because of a lack of knowledge of the actual sites of Zn secretion into the gut, but it should be noted that the values for gastrointestinal secretion and absorption rates and proportional absorption quoted in Table 3 may be slight underestimates.

The exchangeable pool with which the ^{67}Zn rapidly equilibrated in these subjects was found to be variable with a mean of 244 mg, which is well below the 650 mg found in the study of one normal male subject by Jackson *et al.* (1984). No other comparable values for this measurement appear to have been presented. Total body turnover of Zn (i.e. the half life of the exchangeable Zn pool) was found to be very variable in the lactating women studied, the average half-time was 85 d which is of the same order as the estimates made by Jackson *et al.* (1984) and Aamodt *et al.* (1979) in studies of comparable duration in normal subjects (i.e. 138 d and 87 d respectively).

The values obtained for both exchangeable pool size and whole-body Zn turnover were very variable. This variation did not appear to correlate with height, weight, Zn intake or Zn status of the subjects. While this may indicate that the simplified model of Zn kinetics used here is inapplicable in the subjects studied, it is also true that equivalent levels of variability were found in the rates of gastrointestinal Zn excretion (Table 3) and that variations of up to 250 % were found in more-commonly measured variables such as the hair Zn content (Table 2). Large variability between subjects may therefore be a feature of Zn metabolism in this population. It can therefore be argued that, although inconclusive, these results suggest that in the subjects studied here a chronically low dietary Zn intake may have reduced the average size of the exchangeable Zn pool in the body and increased the rate of plasma Zn turnover without affecting the average rate of whole-body turnover of Zn.

The question remains as to whether these subjects consuming a relatively-low dietary intake of Zn were in fact Zn-deficient. In this situation conventional indicators of body Zn status provide ambiguous results. The subjects studied showed similar levels of plasma Zn to the larger group examined by Shrimpton (1980), in that two of the seven had plasma Zn concentrations less than our accepted lower limit of normal (0·75 mg/l). However, the validity of a marginally low plasma Zn in the diagnosis of Zn deficiency has been the subject of considerable discussion since decreased plasma Zn concentrations can occur in various circumstances unassociated with Zn deficiency (Solomons, 1979). Hair Zn levels were normal in all subjects, but the use of this index of Zn status has also been questioned (Solomons, 1979; Hambidge, 1982; Dores & Paine, 1985). Urine Zn levels were within our

accepted normal range (100–500 μg/24 h) and the breast milk of the subjects contained a relatively normal concentration of Zn when compared with other population groups (Vuori & Kuitunen, 1979; Dorea *et al.* 1985). However, Shrimpton *et al.* (1983, 1985) provided evidence of a relative lack of Zn in these subjects by undertaking a supplementation study in other lactating women from the same population. They found that Zn supplements prevented a significant decrease in breast milk Zn content from 30 to 120 d of lactation, maintained the milk retinol content at a higher level throughout lactation and reduced the incidence of diarrhoea in the offspring. In addition, the results presented here suggest that the lactating women may have a low exchangeable pool of Zn and a rapid turnover of plasma Zn.

Taken together, these studies suggest that a chronically low Zn intake may lead to a sub-optimal state of Zn nutrition which conventional indicators of Zn deficiency (i.e. plasma, urine or hair Zn levels) may not reliably detect and in which the subjects are in Zn balance with homeostatic mechanisms maximally adapted to conserve Zn. However, supplementation studies suggest that this apparent 'status quo' may not be without 'cost' to the subject in terms of other biological functions. If indeed such a state does exist in various chronically undernourished populations then, of the commonly used techniques for assessing Zn status, only a trial of Zn supplements while monitoring a specific indicator of nutritional status (i.e. growth rates in children or milk quality in lactating women), or perhaps detailed and time-consuming measurements of the exchangeable Zn pool sizes and plasma turnover rate will reliably detect the situation.

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