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Hair zinc and copper in Indonesian infants

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The objective of this study was to determine the zinc and copper status in hair of a group of Indonesian infants aged 0–5 months, a period when growth faltering in this population is known to occur, and to determine the daily zinc and copper availability in the habitual diet. A mixed cross-sectional longitudinal design was used. Infants 0–3 months of age were recruited in two villages on the south coast of the island of Madura, Indonesia and followed up to the age of 5 months. All newborns during the study period were included. Hair samples were collected between the ages of 0 and 5 months at monthly intervals. Zinc and copper concentrations were determined by flame atomic absorption spectrophotometry and compared with a sample of Belgian controls, recruited cross-sectionally. Zinc and copper content of the habitual diet was calculated on the results of a food intake study previously performed in the same community. For the 42 Madurese infants recruited, 107 hair zinc and 96 hair copper concentrations were determined. Belgian infants (15 boys, 15 girls) served as controls. One Madurese infant died during the study and six moved from the area. Hair zinc concentrations were found to decrease with age in both populations, while the zinc and copper values did not differ from the Belgian controls. Hair zinc values were not correlated with growth performance. The boys had lower hair zinc values than did the girls. Copper values among the Indonesian infants did not show a trend over time; however, the Belgian children showed an increase towards the age of 12 months, although this was not significant. The mean daily zinc and copper availability in the habitual diet was less than half of the recommended daily allowance for adult women. The situation was much worse for lactating women given that the availability of these elements increased very little. The hair zinc and copper values indicate that they are not responsible for the early onset of linear growth retardation. The lower zinc values in boys might be an indication of a marginal deficiency. The very low zinc content of the diet consumed in this population could be an indication of a zinc and copper deficit in the Madurese population, although this needs to be confirmed.

Key words: Madura, Indonesia, Belgium, hair zinc, hair copper, infant.

Introduction

Short stature is perhaps the most prevalent nutritional problem today.[1] World-wide, country prevalences vary widely. South-East Asia, with prevalences of 62% of the under 5-month-olds being too short for their age as compared with the National Centre for Health Statistics (NCHS) reference, is the worst region.[2] On the Indonesian island of Madura, the infant population is characterized by a very early onset of linear growth retardation. Compared with the NCHS reference, the mean height-for-age (H/A) Z-score at the age of 1 month, is –0.61 for boys and –0.54 for girls. The deficit increases rapidly. By the age of 12 months the average H/A Z-score is –2.39 for boys and – 1.9 for girls. This accumulation in linear deficit takes place at a time when the weight-for-height (W/H) indices are well above the NCHS reference value and actually increase to reach a value of 0.5 Z-scores for both sexes by the age of 6 months.[3] The effect of morbidity on growth was analyzed in the same population. Before the age of 6 months, a clinical morbidity period had no significant short- or long-term effect on either weight or height increments. After the age of 6 months, illness affected weight increments over a short period. There was, however, no long-term effect. Illness had no significant consequences for linear growth during the first year of life.[4] The total energy intake per kilogram of body weight of the Madurese infants was higher for the first 6 months than has been observed previously in North American infants.[5,6]

The very early onset of linear growth retardation, and the fact that neither morbidity nor energy intake explained the growth variability, could indicate a micronutrient deficiency and/or intra-uterine factors as being responsible for affecting linear growth during infancy in this particular population. Zinc and copper have on numerous occasions been proposed as growth limiting factors.[7–22] Although hair analysis has been criticized as a tool for identifying individuals in a state of zinc or copper deficiency, it is considered adequate for the study of populations.[23–25] Hair concentrations also have the characteristic of reflecting the history of availability rather than the situation at the time of analysis.[26]

The objectives of this study were: (i) to determine the zinc and copper status in hair of a group of Indonesian infants in the first 6 months of life, a period when growth faltering in this population is known to occur; (ii) to determine the

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trend over time of the hair concentrations; (iii) to determine whether any difference could be observed with a Belgian control group; and (iv) to determine the zinc and copper availability in the habitual diet.

Subjects and methods

Hair zinc and copper

The study took place in the regency of Sampang on the south coast of the island of Madura, Indonesia. The entire population is Muslim. Agriculture and both sea and inland farming are the main occupations of males. Women farm, sell fish and repair fishing nets. The main staple foods are rice and maize, complemented by fish and to a lesser degree, pulses. All infants are breastfed as well as being force-fed with a mashed rice-banana mixture from as early as the first week of life.

From 26 September 1993 until 8 October 1993, infants below 3 months of age were recruited for the study from two villages. Newborns entered the study up to 27 December 1993. The infants were followed at monthly intervals for up to 5 months. The study ended on 1 January 1994. The purpose of the study was explained to each family and consent obtained. At each visit the weight and the height of the children were measured and a hair sample was collected. Hair of the occipital area was cut with a pair of scissors near the scalp and kept in a sealed plastic bag. Hair samples were analyzed in Belgium.

A group of 30 Belgian children (15 boys, 15 girls) who visited the outpatient clinic of the Children's University Hospital 'Reine Fabiola' in Brussels, served as controls. The children came from middle-class families and had no history of severe illness (chronic or acute). They all visited the hospital for minor illnesses. The age of the control infants ranged from 3 to 12 months. Younger infants were omitted due to the difficulty of obtaining sufficiently large hair samples.

Hair samples were washed with a non-ionic detergent (Triton 1%) for 30 minutes and rinsed five times with de-ionized water. After overnight drying in the oven, hair samples were weighed (± 5 mg) and transferred to plastic tubes. Hairs were digested by adding 0.1 mL of HNO_3 (6N) and keeping the samples overnight in the oven at 80°C. Samples were diluted with de-ionized water, and the zinc and copper content determined by flame atomic absorption spectrophotometry. Results were expressed as microgram per gram dry weight.

Zinc and copper content of habitual diet

Accurate food consumption data are very time-consuming to obtain and, therefore, a very expensive exercise. In order to calculate the zinc and copper content of the habitual diet in the study population, we decided to use available information on dietary intake of the same village population. During the East Java Pregnancy Study Phase II, which ran from January 1987 to December 1989 in the same two villages, food intake data were gathered.[6,27] Field workers stayed in the homes of pregnant and lactating women for 48 hours while they were cooking and eating. All edible raw food to be cooked for the family was weighed on food scales (with 5 kg capacity and 50 g divisions, or 1 kg capacity and 10 g divisions, depending on the amounts to be weighed). The staple food and the side dishes were weighed again after cooking. The conversion factors of cooked raw foods were calculated from these two figures. The food eaten by the women was weighed every mealtime.[28] Variance ratio analyses for energy and protein intake within and between individuals confirmed that the customary mean intake could be measured with relatively high precision with two replicates for each women.[29]

The zinc and copper content of the foods have been calculated using food composition tables.[30–32] The concentration of dried fish was calculated by assuming that the entire water content would be eliminated with no loss in minerals. These values have also been used for fermented fish.

Statistical analysis

Data were entered and checked in Data-Ease (DataEase International Inc., Essex, UK). Anthropometric indices were calculated with Epi6 (Centre for Disease Control (CDC), USA). Preliminary data management and statistical analyses were performed with the SAS system Release 6.09 for Unix (SAS Institute Inc, Cary, NC, USA).

Age was rounded to the nearest half-month and the derived variable was used in all subsequent analyses. The distributions of raw data within age-groups for zinc and, less markedly, for copper showed an asymmetry in a preliminary analysis (quantile plots). Logarithmic concentrations, ln[Zn] and ln[Cu], gave a more symmetrical distribution and were subsequently used as response variables in all of the analyses.

Because the same children were followed over consecutive months, the repeated measurements structure of the data had to be accounted for. The results of a fixed-effects general linear model are only valid under the hypothesis of independence between observations. To take the auto-correlations between measurements made on the same subject at different ages into account, the data are analysed with a general linear mixed model.[33] Several models for the within-subject covariance matrix were tested: diagonal (independence hypothesis), compound symmetry, first-order autoregressive and other types of time-dependent structures. The choice of the auto-correlation structure was assessed by likelihood-based statistics such as the likelihood ratio test, and Akaike and Schwartz criteria.[34]

All of the mixed models fitted to assess the ln[Zn]–age relationship and the ln[Cu]–age relationship included a categorical child random effect together with age. Models with age, sex and interaction age*sex were also fitted to assess the modifying effect of sex on the linear part of the relationship.

In order to compare the slope of the linear part of the ln[Zn]–age relationship observed among Indonesian children to that of the Belgian children, we fitted a mixed model with age, country and interaction age*country as fixed effects, and child as a random effect. As above, different models for the auto-covariance matrix were fitted.

In all of the models, the fixed effects (polynomial of age, sex, sex*age interaction, country*age interaction) were assessed by F-test, taking into account the covariance structure chosen. These models were implemented with the Proc Mixed procedure of the SAS system using the REML algorithm and the repeated statement to estimate covariance parameters.[35]

Hair concentrations for zinc and copper at birth were correlated with weight and length velocities 1 month later. Pearson's correlation coefficients were used.

The study was approved by the ethical committee of the Institute of Tropical Medicine Antwerp.

Results

For the 42 recruited infants there were 193 observations. On 27 occasions either no hair sample could be obtained or the parents refused. One Madurese infant died and six relocated during the study. Of the remaining hair samples, 32 were too small to be weighed. A total of 27 of the zinc readings and 38 of the copper readings were discarded for analysis. They showed zero absorbency or excessively high readings. Upon checking, however, the calibration standards read normal. Newborns have very little hair and what they do have is thin, such that the manipulation of 5 mL, which is only a few strands of hair, can give variations in concentration. Below 3 months of age the samples often were too small to give a confident analysis. Therefore, only readings that gave a confident result were used. This resulted in 107 data values for zinc and 96 for copper. Table 1 shows the number of measurements per child. Hair zinc and copper values are shown in Table 2.

Hair zinc and copper concentration and relationship with age

The first model fitted to the data, ln[Zn] and ln[Cu] respectively, was a mixed model including a third degree polynomial of age, taking into account the repeated measurements. From the eight different types of covariance structures that were fitted to the data, the first-order autoregressive one was retained as it provided a good overall fit for the models used throughout the analysis process. Table 3 gives the results for the third degree polynomials fitted on the ln[Zn] and ln[Cu] data: the F-tests are sequential and assess the supplementary information taking into account the preceding effects. (The estimates of the coefficients of the variables in the model are given for information: the F *does not* test the hypothesis that these coefficients are zero in the population.)

The results clearly show that the main trend for the ln[Zn]–age relationship is a decreasing linear trend and that there is no obvious link between age and ln[Cu] (Table 3). It is to be noted that overall, these results were observed, with minor differences for all of the alternative choices of covariance structures. The ln[Zn]–age and ln[Cu]–age relationships were thus modelled as a linear function of age, with a first-order autoregressive error-structure. The results are given in Fig. 1a,b.

Effect of sex on hair concentrations and the link with age

A model was fitted to see whether the decreasing linear trend in hair zinc differed between boys and girls.

The model featured sex, age, the interaction sex*age, and ln[Zn] and ln[Cu] as dependent variables (still with the autoregressive error-structure). The hypothesis of equality of slopes could not be rejected for ln[Zn] ($T(103) = -0.15$, $P = 0.88$) nor for ln[Cu] ($T(92) = -1.18$, $P = 0.24$), suggesting that the linear part of the ln[Zn]–age and ln[Cu]–relationship does not depend on sex. Since there was no sex*age interaction, a simpler model could be fitted to compare hair zinc and copper values between boys and girls adjusted for age (Table 4). Overall log hair concentrations of Zn are lower for boys. This is not the case for Cu. It is to be noted that due to the similar age means in each sex, age-adjusted means are very close to the raw means (particularly for ln[Cu] as it was shown above not to depend on age). The age distribution is relatively similar among boys and girls.

Comparison of Indonesian and Belgian children

The comparison of the ln[Zn]–age and ln[Cu]–age relationship between the two child populations focused on a comparison of the slopes of the regression lines and the age-adjusted means. The age range of the sampled Belgian and Indonesian infants is quite different (Table 5). Interpreting the results of the analyses needs, therefore, to be done with extra caution. For the same reason, comparing raw mean values between countries did not make much sense.

The results of the mixed models, including age, country, age*country as dependent variables and as featuring an autoregressive error-structure, are given in Table 6 and Fig. 1c,d.

For ln[Zn] as well as ln[Cu] the observed data did not challenge the hypothesis of equality of slopes, which suggests that the two populations exhibit the same trend over time even if they are estimated for different age ranges.

Table 1. Number of sample results by age group

	Copper		Zinc	
No. of times child is present	No. children	Data values	No. children	Data values
1	11	11	11	11
2	14	28	12	24
3	6	18	9	27
4	6	24	5	20
5	3	15	5	25
Total	40	96	42	107

Table 2. Hair concentration for zinc and copper by age groups. Concentrations are in micrograms per gram dry weight

Age in months	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Zinc													
Mean	220	229.6	258.9	239.5	200.1	273.0	202.1	172.4	275.5	288.2	190.0	180.9	107.7
SD	18.6	68.8	46.2	62.4	62.3	73.8	55.9	32.0	138.8	41.1	107.0	—	19.4
n	8	7	14	8	16	5	14	8	11	3	9	1	3
Copper													
Mean	11.2	9.9	12.4	11.8	10.1	14.6	12.0	12.9	12.3	11.8	10.3	8.6	9.1
SD	6.4	5.9	8.1	5.5	5.3	11.7	5.9	7.9	8.6	—	7.1	—	4.5
n	8	6	14	8	15	4	13	8	8	1	8	1	2

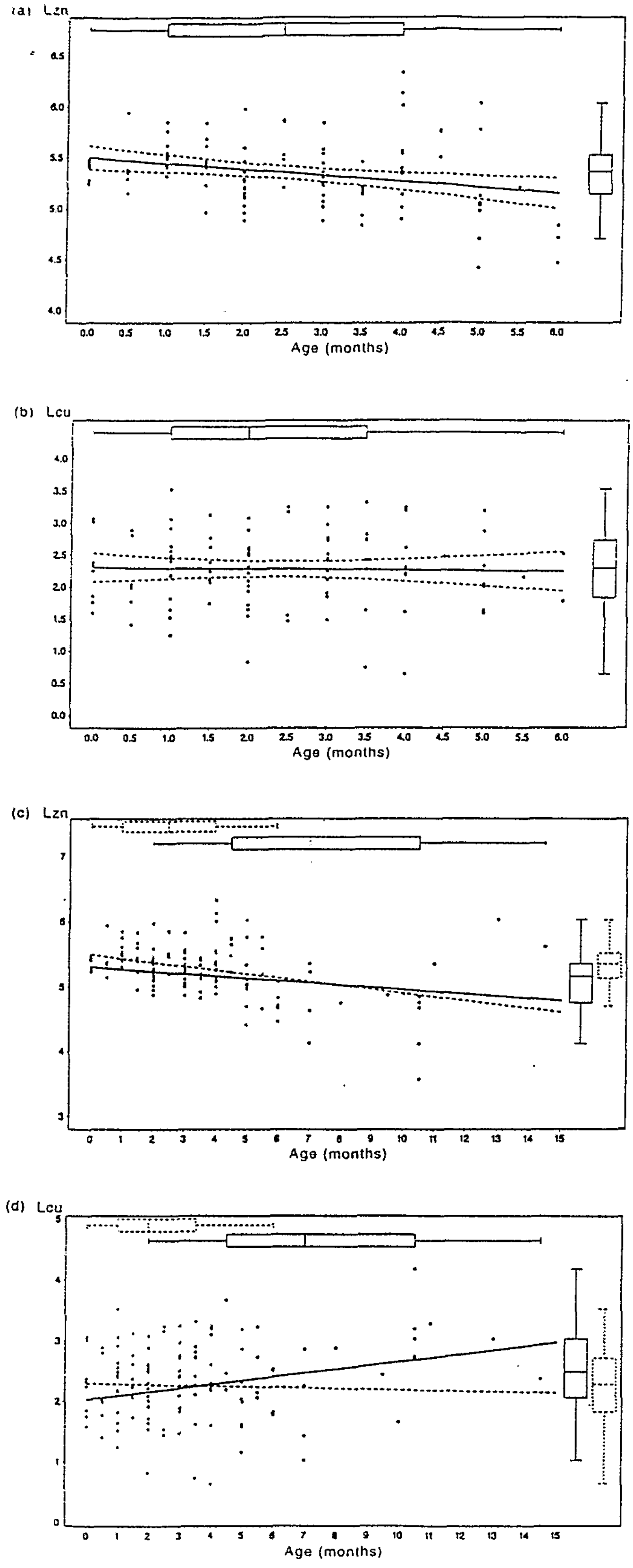

Figure 1. (a) Lnzinc. Age relationship in Madurese infants: first degree polynomial (n = 107: Lzn = 5.487–0.061*age). (b) Lncopper. Age relationship in Madurese infants: first degree polynomial (n = 96; Lcu = 2.291–0.0054*age). (c) Lnzinc. Age relationship by country. (#). Indonesia. Lzn = 5.48–0.061*age: (●), Belgium, Lzn = 5.30–0.035*age. (d) Lncopper. Age relationship by country. (#), Indonesia, Lcu = 2.29–0.054*age: (●). Belgium. Lcu = 2.03–0.063*age. (a,c) Top: box plots of age: right: box plots of lnzinc: Lnzn: natural logarithm of zinc: (b,d) top: box plots of age: right: box plots of lncopper: Lncu: natural logarithm of copper.

Table 3. Results of a third degree polynomial fitted on hair ln[Zn] and ln[Cu] values in relation to age, age,^{2} and age^3 (cubic model)

	Cubic model for ln[Zn]			Cubic model for ln[Cu]		
Param.	Estimate	$F_{1,103}$	Pr > F	Estimate	$F_{1,9}$	Pr > F
Age	–0.121	10.27	0.0018	0.017	0.02	0.89
Age^2	0.049	1.89	0.17	0.0070	0.25	0.62
Age^3	–0.0077	1.36	0.25	–0.0022	0.03	0.87
ar(1)part	r = 0.16		P = 0.01	r = 0.14		P = 0.03

Given this result, it was appropriate to fit a model featuring a common slope for the two countries (Table 7). For the Indonesian population alone, there was a significant trend for ln[Zn] but not for ln[Cu]. In this latter model the test of equality of intercepts (null hypothesis of equality of the age-adjusted means between the two countries) was made: with all due caution it suggested no difference of overall ln[Zn] and ln[Cu] levels, and that the decreasing trend in zinc is a physiologic phenomenon.

Hair copper and zinc and growth

The linear growth of the infants showed a marked decrease from the first months onwards with a decrease in the length-for-age Z-score of 0.3 standard deviation (SD) per month. At the age of 5 months, children have lost 1.5 SD for length-for-age. Weight-for-height concomitantly increases with 0.8 SD from the first month to 4–5 months of age. The zinc and copper values were correlated with Z-scores for weight-for-height and length-for-age 1, 2 and 3 months later. For zinc, the correlations with length-for-age were 0.003, –0.13 and –1.16 after 1, 2 and 3 months, respectively, and –0.17, 0.06 and –0.16 for weight-for-height over the same intervals. Hair copper concentrations correlated with length-for-age with 0.07, –0.15 and –0.16 at one, two and three months, and with weight-for-height the respective values were 0.05, –0.06 and –0.01. At no time were the correlations significant.

Zinc, copper and food intake

Tables 8 and 9 show the calculated concentrations of zinc and copper in the habitual diet. The National Research Council's recommended dietary allowances for zinc amount to 12 mg per day for adult women, 15 mg per day in pregnancy.[36] During lactation, an extra 7 mg per day is suggested. For copper, the recommendations are 1.5–3 mg per day.

Even with a large margin of error, the average daily intake in Madura is well below the recommendations. The zinc availability is less than half of the recommended dietary allowance (RDA). The availability of copper is between 65 and 33% of the RDA according to the cut-off value used.

Discussion

Hair analysis as an indicator of mineral status has great potential for the obvious reason that a sample can be taken without trauma to the child. A number of elements can, however, affect the results of the hair analyses.[17,25,37] Bleaching and dyeing, environmental contamination, the place of sampling, and seasonal variation can all affect the mineral concentration. The values are also age and sex specific.

Table 4. Mean log hair zinc and copper concentrations adjusted for sex

Sex	n	ln[Zn] adj. mean	ln[Zn] mean	Age mean	Age SD	n	ln[Cu] adj. mean	ln[Cu] mean	Age mean	Age SD
Boys	63	5.27	5.29	2.45	1.60	53	2.36	2.36	2.35	1.55
Girls	44	5.44	5.42	2.63	1.61	43	2.17	2.18	2.45	1.60
Boys–Girls		–0.17		2.52	1.60		0.19		2.40	1.57
	T(103) = –2.91					T(93) = 1.47				
	Pr > ITI = 0.0045					Pr > ITI = 0.14				

ln[Zn], log zinc; ln[Cu] = log copper.

Table 5. Hair zinc and copper concentrations of Madurese and Belgian infants

		Zinc		ln[Zn]		Age (months)			
Country	n	Mean	SD	Mean	SD	Mean	SD	Min	Max
Indonesia	107	222.3	79.2	5.35	0.34	2.52	1.60	0	6
Belgium	30	176.7	82.5	5.06	0.52	7.23	3.14	2	14.5
Total	137	212.3	81.9	5.28	0.40	3.56	2.81	0	14.5
		Copper		ln[Cu]		Age (months)			
Country	n	Mean	SD	Mean	SD	Mean	SD	Min	Max
Indonesia	96	11.50	6.65	2.27	0.60	2.40	1.57	0	6
Belgium	30	15.57	12.63	2.48	0.74	7.23	3.14	2	14.5
Total	126	12.47	8.58	2.33	0.64	3.55	2.90	0	14.5

Table 6. Results of the mixed model analysis with age, country age* country as dependent variables

Country	Parameter	n	ln[Zn] Estimate		n	ln[Cu] Estimate	
Indonesia	Intercept	107	5.48	T(133) = –0.94	96	2.29	T(122) = –0.81
Belgium		30	5.30	Pr > ITI = 0.35	30	2.03	Pr > ITI = 0.42
Indonesia	Slope	107	–0.061	T(133) = 0.84	96	–0.0054	T(122) = 1.19
Belgium		30	–0.035	Pr > ITI = 0.40	30	0.063	Pr > ITI = 0.24

Table 7. Linear model fitting to hair zinc and copper concentrations using a common slope for the two populations

Country	Parameter	n	ln[Zn] Estimate		n	ln[Cu] Estimate	
Indonesia	Intercept	107	5.45	T(134) = 0.45	96	2.20	T(123) = 0.23
Belgium		30	5.40	Pr > ITI = 0.65	30	2.24	Pr > ITI = 0.82
Both	Slope	137	–0.047	T(134) = –3.0	126	0.034	T(123) = 1.20
				Pr > ITI = 0.003			Pr > ITI = 0.23

Bleaching and dyeing of hair could not have affected our results since it is not practised in young children. The place of sampling was controlled in the study; all samples came from the occipital area. Analytical procedures can give variations in results. We have, therefore, chosen to limit our comparison to our own control group. All the samples were analysed in the same laboratory and by the same person. Detergents, although they can affect hair mineral concentrations, do not affect copper and zinc concentrations.[25] Environmental contamination can affect hair copper concentration. This was demonstrated by analysing hair samples at different distances on the scalp. All the Belgian infants, however, had short hair and only the first centimetres were used. Seasonal variations have also been reported, but the findings of different studies are not consistent.[10]

An additional problem of this study was the specimen size itself. It was indeed difficult to obtain a hair sample large enough to give a confident result and which was also acceptable for the parents of the younger infants. It was for this reason that the Belgian controls, taken at a later date, were from infants 3 months of age and older.

The hair zinc analysis indicates that the trend with age is identical in Madura and in Belgium. The concentrations were slightly higher for girls. The comparison of the regression lines of the Belgian and the Madurese children show that there is no inherent difference in the two populations. The decreasing hair zinc concentrations would appear to be a normal physiological phenomenon at this age. Friel *et al.*, who reported a comparable downward trend in hair zinc concentration in full-term newborns during the first year of life, suggested that this decline, because it was not seen in breastfed children, was associated with low intakes of readily available zinc.[38,39] Although comparison with other studies are difficult because they report concentrations on different age groups and with different analyses,[10,14,23,24,37,40–43] the majority describe a downward trend at young ages, indicating a

Table 8. Average food consumption and intake of zinc and copper of 301 adult women of Madura

		Average consumption per day		Concentration in 100 g product	
	Grams	Zn mg	Cu mg	Zn mg	Cu mg
Rice	350.8	1.75	0.53	0.5	0.15
Maize	108.2	2.70	0.17	2.5	0.16
Coco oil	4.3	0.00	0.00	trace	trace
Fresh fish	20.6	0.10	0.07	0.5	0.32
Dried fish	9.5	0.16	0.10	1.7	1.09
Eggs	3.9	0.05	0.00	1.35	0.1
Fresh cassava	30.0	0.48	0.12	1.6	0.4
Coco milk	1.3	0.01	0.00	0.1	0.04
Shrimps	1.9	0.04	0.00	2.31	0.24
Spinach	0.9	0.00	0.00	0.5	0.12
Sugar	0.7	0.00	0.00	0.0	0.0
Shell Fish	1.5	0.03	0.01	2.0	1.0
Gourd	1.2	0.00	0.00	0.28	0.09
Total		5.32	1.00		

Table 9. Average food consumption and intake of zinc and copper of 375 lactating women of Madura

		Average consumption per day		Concentration in 100 g product	
	Grams	Zn mg	Cu mg	Zn mg	Cu mg
Rice	297.0	1.49	0.45	0.5	0.15
Maize	123.2	3.08	0.20	2.5	0.16
Coco oil	4.5	0.00	0.00	trace	trace
Dried fish	16.2	0.28	0.18	1.7	1.09
Fresh fish	15.2	0.06	0.05	0.5	0.32
Coco milk	4.5	0.02	0.01	0.1	0.04
Fresh cassava	31.1	0.50	0.12	1.6	0.40
Fermented fish	3.1	0.05	0.03	1.7	0.09
Sugar	3.0	0.00	0.00	0.0	0.0
Tahu	2.1	0.01	0.00	0.7	0.2
Gourd	42	0.01	0.00	0.28	0.09
Eggs	1.7	0.02	0.00	1.35	0.1
Glutinous rice	9.2	0.05	0.01	0.5	0.15
Total		5.57	1.05		

normal physiological observation. Zinc values also did not correlate with linear growth performance, an indication that zinc is probably not relevant in explaining the early onset of linear growth retardation. Boys in Madura were found to have lower zinc values than girls. Zinc supplementation studies found that boys have a greater response in linear growth than do girls, or that they are the only ones to respond to a zinc supplement.[7–22,44] Previous growth studies in the same population also showed that the linear deficit in boys in relatively more important than in girls, compared with the NCHS reference.[3] Thus it appears that, although an overall zinc deficit responsible for the early onset of linear growth retardation is unlikely, the possibility of a marginal zinc deficit in boys remains.

The interpretation of the copper results are less straightforward. The data suggest that there is an increase in hair copper concentrations in Belgian children, whereas no trend with age was observed in the Madurese infants. Nevertheless, the test of equality of slopes suggests that the linear trend of the ln[Cu]–age relationship is the same in the two populations. The small sample size may account for this result. Because the slopes are estimated for different age ranges of Belgian and Indonesian infants, this difference in mean age is also likely to influence the results.

The test of equality of intercepts is that of equality of age-adjusted ln[Cu] means, based on a common slope estimate. Thus, although the raw mean hair copper concentration of the Madurese infants is lower than that of the Belgian infants and there is no evolution over time, the mixed model cannot demonstrate a significant difference. The results are therefore not entirely conclusive. The possibility remains that Belgian children are increasing their hair copper concentration, whereas Madurese infants are not. Data on hair zinc and copper status of Belgian infants is not available.

Trace element metabolism can be influenced by a quantitatively insufficient intake, reduced bio-availability, increased losses, increased requirements, a disturbed handling of the element by organisms, or possibly by separate metabolic pools at the cellular or sub-cellular level.[45–51]

Increased losses and requirements are unlikely to have been causes in our population, because they relate to pathological situations such as urinary protein loss exudation of skin, etc. All infants received breastmilk. Minerals in breastmilk have a high bio-availability.[51] Hair mineral content could, however, also reflect fetal availability and hence, intake of the mother. This in turn can also be reflected in mineral content of breastmilk.[52,53] No direct information, however, is available on these parameters.

The intake of minerals is dependent upon the content of these elements in foods, which in turn are dependent up to a degree on the soil content of the element. The island of Madura is a limestone area which contains only minute concentrations of zinc and copper. Other factors can also influence trace element concentrations in food. These are humidity, texture of the soil, pH, and the presence and content of organic material which determine the geo-biological transport of trace elements.[54] The soil content of minerals is, therefore, only indicative of mineral status in animals.[55]

The dietary analysis must be regarded as a means to gather additional information on intake of micronutrients in the population. Because these are only rough estimates, with the problems of such studies as enumerated below, they should be looked at with due caution. As shown in Tables 7 and 8, the type of diet consumed in Madura is based predominantly on non-animal food sources. The main staple foods are rice and maize. Although fish is available, it is consumed in limited amounts. The total amount of zinc and copper available in the habitual diet is exceedingly poor. The diet is also rich in fibres and phytates, two potent inhibitors of bio-availability of minerals.[51,56,57] The biological availability will be even less in this condition. In Nepal, the traditional diet is rich in fiber and phytate, and contains equal or higher zinc and copper amounts than the American diet.[58] Indices of zinc and copper were, however, found to indicate a deficiency. The type of soil in Madura, the low zinc and copper content of the diet, and the likely presence of inhibitory factors make it very likely that there is an important deficit of both zinc and copper in this community. Although the food intake data are of an earlier date, the observations made of the study area from that time up until the present indicate that the diet has not changed. Even if minor changes had occurred, a deficit is still very likely given its magnitude.

Although the trace element content of the Madurese diet is surprisingly low, the results should only be taken as indicative. Local food composition tables for minerals were not available and those from other regions were used. As mentioned earlier, mineral food content can vary widely from region to region. On the other hand, RDA also allow for a large margin of safety. The differences, therefore, do not automatically entail physiological effects. The results do warrant, however, further investigations on micronutrient intake in this and other populations. Re-analysing existing food intake data might even provide an insight into the magnitude of micronutrient deficiencies.

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Hair zinc and copper in Indonesian infants

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印度尼西亞嬰兒頭髮的鋅和銅含量

摘　要

目的：測定印度尼西亞嬰兒（0—5 個月）頭髮鋅和銅含量，祈求找出其與嬰兒早期生長減慢的關係，同時測定了每日膳食鋅和銅的供應。

設計：作者在印度尼西亞 Madura 島南岸兩個村莊選擇了 0—3 個月的嬰兒爲對象，在嬰兒 0—5 個月研究期間，每月收集頭髮樣本，用火焰原子吸收分光光度法測定鋅和銅濃度並與對照組比利時嬰兒相比較，預先在相同地區分析食物，計算膳食中鋅和銅含量。

結果：測定了 42 名 Madura 嬰兒 107 次髮鋅和 96 次髮銅濃度，測定了比利時嬰兒（15 名男嬰，15 名女嬰）的髮鋅與髮銅作對照。試驗期間一名 Madura 嬰兒死亡，6 名離開了研究。兩組髮鋅濃度隨年齡的增加而下降，兩組髮鋅和髮銅值沒有不同，髮鋅值與生長無關，男嬰髮鋅較女嬰低，雖然比利時嬰兒 12 個月大時，顯示髮銅增加，但無顯著性。當地成年婦女平均每日鋅和銅的攝取較每日供給量（RDA）少一半，如果這些微量元素給予很少，授乳婦女的情況更壞。

結論：髮鋅與髮銅值並非早期生長減緩的一個可靠性指標，男嬰髮鋅值較低可能是臨界缺乏。該地膳食鋅含量極低可能是 Madura 人群鋅和銅的短缺，但仍需進一步證實。

References

1. Waterlow JC. Causes and mechanisms of linear growth retardation (stunting). Eur J Clin Nutr 1994; 48 (Suppl. 1): 1–4.
2. UNICEF. Child malnutrition. Progress toward the world summit for children goal. New York: UNICEF, 1993.
3. Kolsteren PW, Kusin JA, Sri Kardjati. Growth faltering in Madura, Indonesia. A comparison with the NCHS reference and data from Kasongo, Zaire. Ann Trop Paediatr 1996; 16: 233–242.
4. Kolsteren PW, Kusin JA, Sri Kardjati. Morbidity and growth performance in Madura, Indonesia. Ann Trop Paediatr 1997; in press.
5. van Steenbergen WM, Kusin JA, Sri Kardjati, Renqvist UH. Nutritional transition during infancy in East Java, Indonesia: 1. A longitudinal study of feeding pattern, breast milk intake and the consumption of additional foods. Eur J Clin Nutr 1991; 45: 67–75.
6. Kusin JA, Sri Kardjati, van Steenbergen WM, Renqvist UH. Nutritional transition during infancy in East Java, Indonesia: 2. A longitudinal study of growth in relation to the intake of breast milk and additional foods. Eur J Clin Nutr 1991; 45: 77–84.
7. Ronaghy HA, Spivex Fox MR, Garn SM *et al.* Controlled zinc supplementation for malnourished school boys: a pilot experiment. Am J Clin Nutr 1969; 22: 1279–1289.
8. Halsted JA, Ronaghy HA, Abadi P *et al.* Zinc deficiency in man. The Shiraz experiment. Am J Med 1972; 53: 277–284.
9. Ronaghy HA, Reinhold JG, Mahloudji DM, Ghavami P, Spivex Fox MR, Halsted JA. Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects. Am J Clin Nutr 1974; 27: 112–121.
10. Walravens PA, Hambidge KM. Growth of infants fed a zinc supplemented formula. Am J Clin Nutr 1976; 29: 1114–1121.
11. Hambidge KM, Walravens PA, Brown RM *et al.* Zinc nutrition of preschool children in the Denver Head Start program. Am J Clin Nutr 1976; 29: 734–738.
12. Golden MHN, Golden BE. Effect of zinc supplementation on the dietary intake, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition. Am J Clin Nutr 1981; 34: 900–908.
13. Castillo-Duran C, Heresi G, Fisberg M, Uauy R. Controlled trial of zinc supplementation during recovery from malnutrition: effects on growth and immune function. Am J Clin Nutr 1987; 45: 602–608.
14. van Wouwe JP, van Gelderen HH, Bos JH. Subacute zinc deficiency in children with upper respiratory tract infection. Eur J Pediatr 1987; 146: 293–295.
15. Golden MHN. The role of individual nutrient deficiencies in growth retardation of children as exemplified by zinc and protein. In: Waterlow JC, ed. Linear growth retardation in less developed countries. Nestlé Nutrition Workshop Series, Vol. 14. New York: Raven Press, 1988: 143–163.
16. van Wouwe JP, van Gelderen HH, Enschede FAJ, van de Velde EA. Acute diarrhea, non-responsive to dietary restriction, zinc deficiency and subclinical growth retardation in pre-school children. Trace elements in medicine 1988; 5: 90–92.
17. Walravens PA, Hambidge KM, Koepfer DM. Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double blind, controlled study. Pediatr 1989; 83: 532–538.
18. Gibson RS, Vanderkooy PDS, MacDonald AC, Goldman A, Ryan BA, Berry M. A growth-limiting, mild zinc-deficiency syndrome in some Southern Ontario boys with low height percentiles. Am J Clin Nutr 1989; 49: 1266–1273.
19. Goyens P, Brasseur D. Cuivre et malnutrition protéino-énergétique. In: Ingenbleek Y, Hennart P, eds. Alimentation et nutrition dans les pays en développement. 4es Jounées Internationales du GERM. Paris: Karthala, 1991: 562–573 (in French).
20. Sandstead HH. Zinc deficiency. A public health problem? Am J Dis Child 1991; 145: 853–859.
21. Walravens PA, Chakar A, Mokni R, Denise J, Lemonnier D. Zinc supplements in breastfed infants. Lancet 1992; 340: 683–685.
22. Ahmed F, Barua S, Mohiduzzaman M, Shaheen N, Bhuyan MAH. Interactions between growth and nutrient status in school-age children of urban Bangladesh. Am J Clin Nutr 1993; 58: 334–338.
23. Dorea JG, Paine PA. Hair zinc in children: its uses, limitations and relationship to plasma zinc and anthropemetry. Hum Nutr: Clin Nutr 1985; 39C: 389–398.
24. Saner G. Hair trace element concentrations in patients with protein-energy malnutrition. Nutr Rep Intl 1985; 32: 263–268.
25. Hambidge KM. Hair analyses: worthless for vitamins, limited for minerals. Am J Clin Nutr 1982; 36: 943–949.
26. van Wouwe JP. Clinical and laboratory assessment of zinc deficiency in Dutch children: a review. Biol Trace Elem Res 1995; 49: 211–225.
27. Garn SM, LaVelle M. Interaction between maternal size and birth size and subsequent weight gain. Am J Clin Nutr 1984; 40: 1120–1121.
28. Kusin JA, Sri Kardjati. Maternal and child nutrition in Madura, Indonesia. Amsterdam: Royal Tropical Institute, 1994.
29. Launer LJ, Sri Kardjati, Kusin JA, Reed GF. Patterns of variability in the nutrient intake of nutritionally vulnerable pregnant women. Eur J Clin Nutr 1991; 45: 131–138.
30. Sauci SW, Fachmann W, Kraut H. Food composition and nutrition tables 1986/87. Stuttgart: Wissenschaftlige Verlagsgesellschaft mbH, 1986.
31. Holland B, Unwin ID, Buss DH. Fruits and Nuts. The first supplement to McCance & Widdowson's. The Composition of Foods (5th edn). Cambridge: Royal Society of Chemistry, 1992.
32. McCance, Widdowson. The composition of foods, 5th edn. Cambridge: Royal Society of Chemistry, 1991.
33. Searle SR, Casella G, McCullough CE. Variance components. New York: John Wiley & Sons, 1992.
34. Jenrich RI, Schluchter MD. Unbalanced repeated measures models with structured covariance matrices. Biometrics 1986; 42: 805–820.
35. SAS Institute Inc. SAS/STAT Software: Changes and enhancements through release 6.11. Cary, NC: SAS Institute Inc. 1996.
36. National research council. Recommended dietary allowances, 10th edn. Washington DC: National Academy Press, 1989.
37. Lamand M, Favier A, Pineau A. Interet de l'analyse pilaire pour estimer le niveau nutritionnel en oligo-elements. Méd et Nut 1990; 26: 352–361.
38. Friel JK, Andrews WL, Matthew JD *et al.* Zinc supplementation in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr. 1993; 17: 97–104.
39. Friel JK, Gibson RS, Balassa R, Watts JL. A comparison of the zinc, copper and manganese status of very low birth weight pre-term and full-term infants during the first twelve months. Acta Pediatr Scand 1984; 73: 596–601.
40. Rodriguez A, Soto G, Torres S, Venegas G, Castillo-Duran C. Zinc and copper in hair of children with chronic diarrhea. Acta Pediatr Scand 1985; 74: 770–774.
41. Weber CW, Nelson GW, Vasquez de Vaquera M, Pearson PB. Trace elements in the hair of healthy and malnourished children. J Trop Pediatr 1990; 36: 230–234.
42. Gentile PS, Trentalange MJ, Coleman M. The relationship of hair zinc concentrations to height, weight, age and sex in the normal population. Pediatr Res 1981; 15: 123–127.
43. Bates CJ, Evans PH, Dardenne M *et al.* A trial of zinc supplementation in young rural Gambian children. Br J Nutr 1993; 69: 243–255.
44. Walravens PA, Krebs NF, Hambidge KM. Linear growth of low income preschool children receiving a zinc supplement. Am J Clin Nutr 1983; 38: 195–201.
45. Costa M, Allegrini M, Casadei E, Gallorini M, Lanzola E, Panatta GB. Bénin: considerations d'ordre nutritionnel sur les oligo-éléments dans le régime alimentaire. Alimentation et Nutrition 1976; 2: 8–18 (in French).
46. Mbofung CMF, Atimo T. Dietary zinc intake pattern of some Nigerian villagers. Nigerian J Nutr Sci 1980; 1: 9–14.
47. Davies NT. Anti nutrient factors affecting mineral utilization. Proc Nutr Soc 1979; 38: 121–128.
48. Fitzgerald SL, Gibson RS, Quan de Serrano J *et al.* Trace element intakes and dietary phytate/Zn and Ca × phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. Am J Clin Nutr 1993; 57: 195–201.
49. Favier A, Favier M. Conséquences des déficits en zinc durant la grossesse pour la mère et le nouveau-né. Rev Fr Gynecol Obstet 1990; 85: 13–27 (in French).
50. Bremner I, Mills CF. Absorption, transport and tissue storage of essential trace elements. Philos Trans R Soc Lond B Biol Sci 1981; 294: 75–90.

51. Solomons NW. Factors affecting the bioavailability of zinc. J Am Diet Assoc 1982; 80: 115–121.
52. Krebs NF, Hambidge KM, Jacobs MA, Rasbach JO. The effect of a dietary zinc supplementation during lactation on longitudinal changes in maternal zinc status and milk concentrations. Am J Clin Nutr 1985; 41: 560–570.
53. Lönnerdal B, Keen CL, Hendrickx AG, Golub MS, Gershwin ME. Influence of dietary zinc and iron on zinc retention in pregnant rhesus monkeys and their infants. Obstet Gynecol 1990; 75: 369–374.
54. Aswathanarayana U. Geochemical pathways of trace elements and toxicity. Nutr Res 1985; 5 (Suppl 1): 592–600.
55. Khalili M, Lindgren E, Varvikko T. A survey of mineral status of soil, feeds and cattle in the Selale Ethiopian highlands. Trop Anim Health Prod 1993; 25: 193–201.
56. Lonnerdal B. Dietary factors affecting trace element absorption in infants. Acta Paediatrica Scandinavica 1989; Supplement; 351: 109–113.
57. Aggett PJ. Aspects of neonatal metabolism of trace metals. Acta Paediatrica, Supplement 1994; 83: 75–82.
58. Moser PB, Reynolds RD, Acharya S, Howard MP, Andon MB, Lewis SA. Copper, iron, zinc, and selenium dietary intake and status of Nepalese lactating women and their breast-fed infants. Am J Clin Nutr 1988; 47: 729–734.