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Determination of trace elements in hair from Tanzanian children: Effect of dietary factors

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The objective of this study was to evaluate the concentrations of essential and toxic elements in hair of children in Tanzania in order to assess their nutritional status. 141 samples of hair from boys and girls living in Tanzania were analyzed using instrumental neutron activation analysis (INAA). The mean concentration levels of Zn and Cu were lower whilst those of other elements were in the same range as the hair elemental concentrations reported in the literature. The lower concentrations of Zn might be related to the diet of mainly cereals with low animal proteins consumed by most of the Tanzanian population.

Introduction

Trace elements in certain amounts are essential for childrens' health as they are present in tissues participating in metabolic reactions of organisms. Deficiency of essential elements may result in malnutrition, impaired body immunity and poor resistance to disease. These conditions might become more deleterious to health against a background of additional adverse environmental factors such as the presence of toxic elements. The determination of elemental concentrations in hair of children could be used to give information about the deficiency of essential elements and excess of toxic elements in their bodies which might be related to their diet.[1] In this study, hair samples from children (girls and boys) living in Dar es Salaam, Zanzibar and an orphanage/boarding school in Moshi the Kilimanjaro region have been analyzed for the detection of trace elements in relation to food consumption habits and environmental exposure. Dar es Salaam and Kilimanjaro are regions in the mainland Tanzania whilst Zanzibar is a Tanzanian island in the Indian Ocean.

Experimental

Sample collection

Samples were collected in Dar es Salaam, Zanzibar and in an orphanage/boarding school in the Moshi district in the Kilimanjaro region. The samples in Dar es Salaam and Zanzibar were collected from individual children's homes, under the permission of their parents following a discussion of the objectives of the study whilst those from the boarding school in Moshi by the permission of the school management. A total of 141 samples (60 girls and 81 boys) of hair from children of age between 5 and 15 years (mean 10±2 years) were collected; 50 samples were collected in Dar es Salaam, 45 samples in the boarding school and 46 samples in Zanzibar. About 70 to 500 mg of hair was cut using pre-cleaned stainless steel scissors as near as possible to the scalp from the left side of the nape. The hair samples were then cut into 1 cm long strands put into plastic bags and kept in desiccators.

Sample preparation

Prior to analysis, the hair samples were washed according to methods proposed by the IAEA[2] and adopted by the University of Surrey.[3] The samples were washed once with Analar grade acetone, three times in double distilled de-ionized water and once more with acetone. In each wash the samples were allowed to be in contact with the solvent for 10 minutes while stirring. The samples were dried for a minimum of 48 hours in air-dry at room temperature and then inserted into a PE disc and sent to the reactor centre for short and long irradiation.

Sample analysis

The irradiations were carried-out in the nuclear reactor LVR-15 of the Nuclear Research Institute Řež, operated at 9 MW. For short-lived isotopes, the irradiation time was 1 minute, waiting time was 10 minutes and 10 minutes counting time. For intermediate and long-lived irradiation, the samples were irradiated for 2 hours, followed by 4 days of initial decay and 30-minute counting time. Further 2 hours counting was carried out after 1-month decay.

After irradiation the samples were counted using well shielded n-type hyper pure germanium detector (HPGe) of relative efficiency 20.8% and resolution (FWHM) 1.75 keV for ^{60}Co 1332 keV line.

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The concentration levels of the elements were calculated by the comparative method of INAA, using multi-element standards (MES).[4] The quality control was carried out using certified Hair reference material GBW 07601 (National Research Centre for Certified Reference Materials, China), NIST Orchard Leaves (SRM 1571), Apple Leaves (SRM 1515) and Urban Particulate (SRM 1648) analyzed together and under the same conditions as the samples.

Results and discussion

A statistical summary of data in μg/g is presented in Table I showing the arithmetic mean±standard error of the mean (A.M.±SEM) and geometric mean ×/÷standard deviation (G.M.×/÷SD) for 22 elements in hair of children from three regions in Tanzania.

Since most of the elements in the overall data were not normally distributed non-parametric tests were applied for statistical analysis of the data. The Kruskel-Wallis test[5] was conducted to find the differences in the means of the elements in hair collected from the three regions (significant value was taken as $p<0.05$). Results from this test indicated that except for S and Cu, all the remaining elemental mean concentrations differ significantly among the three regions. Generally, S in hair has the narrowest range, which could be explained by the fact that this element occurs in keratin and is essential in the formation of hair.[6] The wide range interval of other elemental concentrations has also been reported in the literature.[7]

Samples from the boarding school in Moshi had significant and extremely higher mean values of Al than that in the other two regions. Al in these samples was 7 times and 12 times higher than Al in samples from Dar es Salaam and Zanzibar, respectively. The mean Al value from Moshi (639 $\mu g \cdot g^{-1}$) was also considerably higher than the Al in hair reported in children of the same age in Kazakhstan, Italy and Hong Kong.[8–10] Al in the body is non essential and toxic[8,11] hence, its exact source in the samples from this school should be analyzed further. The mean concentration value of Fe in the samples from this region was also very much higher than the levels of Fe in hair samples from Dar es Salaam (6 times) and Zanzibar (9 times). This value was also about 6 times higher than the values reported in Kazakhstan, Italy and Hong Kong.[8–10] Moshi district in the Kilimanjaro region (where the school is located) is home to Kilimanjaro – a volcanic mountain. Hence, the soil in this area might be characterized by high levels of Al and Fe.[12,13] Al and Fe from the soil might be easily absorbed and taken up by plants and vegetables grown locally which upon consumption enter the child's body.

Moreover, the children in this boarding school had significantly higher mean concentrations of Cr and V than children from the other two regions. Cr and V in the samples from Dar es Salaam and Zanzibar were similar while those from the boarding school were 2 times for Cr and 4 times for V, respectively, higher than those found in hair from Dar es Salaam and Zanzibar. The samples from the boarding school in Moshi had also significantly higher mean concentration of Co than the samples from Zanzibar and a similar mean concentration of Co with samples from Dar es Salaam. Several industries are located within the proximity of the school, these include leather, breweries and coffee industries. The mean concentration of V in the samples might be related to the operations of the industries, Co could be associated with the breweries industry whilst Cr could be associated with the tanning of leather.[14–16]

Samples from Zanzibar had significantly higher mean concentrations of As, Br, Ca and Zn than the other two regions. The mean concentration of As in Zanzibar was 3 times and 1.5 times higher than that obtained in the samples from the boarding school and Dar es Salaam, respectively. The main source of As is sea food and drinking water, but As in sea food exists mostly in organic compounds which are much less harmful to health because they are readily eliminated by the body as waste. Although the mean concentration of As in samples from Zanzibar were higher than that from the other two regions this value (0.32 $\mu g \cdot g^{-1}$) was in the range reported to be in the hair of normal children of the same age in Croatia.[17]

The samples from Zanzibar had significantly the lowest mean concentration of Fe (41 $\mu g \cdot g^{-1}$) of the three regions analyzed in this work. However, this value is similar to the value reported in Kazakhstan and Hong Kong and higher than the value reported in Italy.[8–10] The mean concentration of Zn in samples from Zanzibar is in the same range as the hair Zn value reported in healthy children of the same age (150 $\mu g \cdot g^{-1}$) in Italy and Hong Kong,[9,10] whilst the samples from the boarding school and Dar es Salaam were lower. HAMBIDGE et al.[18] reported Zn concentrations in the range of <105 $\mu g \cdot g^{-1}$ in the hair of low income children. They suggested further that these levels were the outcome of low income diets of these children because the concentrations of Zn in their hair increased by 71% after 3 month of being provided with rich in Zn meals. WEBER et al.[1] found the hair Zn mean concentration of <100 $\mu g \cdot g^{-1}$ in malnourished children. Out of which concentrations of ≤60 $\mu g \cdot g^{-1}$ were found in children with severe malnutrition. In this study 44% and 40% of the samples from the boarding school and Dar es Salaam, respectively, had Zn hair concentrations of <100 $\mu g \cdot g^{-1}$ in contrast to 4% of the samples from Zanzibar. 18% of the samples in children from the boarding school and 2% of the samples from Dar es Salaam had concentrations ≤60 $\mu g \cdot g^{-1}$ while no sample of this range of concentrations was found in samples from Zanzibar. The differences in mean concentrations

of Zn between the three regions demonstrate the variation in food consumption habits. Meat and fish which normally enhance the absorption of Zn in the stomach have been reported not to be consumed frequently by people in Tanzania mainland in contrast to the population of Zanzibar island who normally have fish in their diets on a regular basis.[19]

The samples from Dar es Salaam had significant higher mean values of I than from other regions. Mean concentration of I in these samples was approximately 4 times higher than the hair value in children from Zanzibar and the boarding school. Samples from Dar es Salaam had also significantly higher mean concentrations of Fe and Co than samples from Zanzibar.

The mean concentration of Cu in the samples from all three regions were similar, they were 10.4±1.0 $\mu g \cdot g^{-1}$ for Dar es Salaam, 9.6±0.6 $\mu g \cdot g^{-1}$ for the boarding school and 11.1±1.0 $\mu g \cdot g^{-1}$ for samples from Zanzibar. These values were lower than the values reported in hair of healthy children from Kazakhstan, Kenya, Hong Kong and Lahore city in Pakistan.[9,10,20,21] However, the values of hair Cu found in this study were comparable to the mean obtained in Islamabad, Pakistan (9.6±3 $\mu g \cdot g^{-1}$).[20] Most of the Cu values obtained in this study were within the range for normal concentration of hair Cu in healthy children (6–23 $\mu g \cdot g^{-1}$) reported by WEBER et al.[1]

Conclusions

The differences of elemental mean concentrations between the three regions showed variations in geographical locations, environmental pollution and the diet consumption habits of the children within a region. For instance, samples from a boarding school in Moshi district of the Kilimanjaro region showed higher concentrations of Al and Fe than the other two regions which are more than likely related to the type of soil. The samples from this area had also higher concentrations of V and Cr which were associated with industries located near the school. Samples from Zanzibar had higher concentrations of Zn, Ca and Br which were related to their diets and food consumption habits.

The mean concentrations of most of the elements in this study were comparable to the published elemental values of the healthy children of the same age group from Kazakhstan, Kenya, Italy and Hong Kong. However, accumulation of Al in the hair from the children in the boarding school in Moshi needs to be followed-up because Al is toxic.[9,10] The concentrations of Zn in some of the samples analyzed in this work were lower than the values reported in the literature.

Table 1. The concentration of elements in (in $\mu g \cdot g^{-1}$) showing arithmetic mean (A.M.±SEM) and geometric mean (G.M.×/÷SD) found in the hair of children in the three regions of Tanzania

Element	Dar es Salaam (n=50)		Moshi district (n=45)		Zanzibar (n=46)	
	A. M.	G. M.	A. M.	G. M.	A. M.	G. M.
Al	86 ± 9	72 ×/÷ 61	639 ± 71	497 ×/÷ 478	55 ± 5	46 ×/÷ 37
As	0.2 ± 0.02	0.1 ×/÷ 0.1	0.09 ± 0.01	0.1 ×/÷ 0.05	0.3 ± 0.01	0.3 ×/÷ 0.2
Au	0.1 ± 0.03	0.04 ×/÷ 0.2	0.04 ± 0.01	0.01 ×/÷ 0.09	0.02 ± 0.004	0.01 ×/÷ 0.02
Br	6 ± 1	4 ×/÷ 3	4 ± 0.4	4 ×/÷ 3	9 ± 1	7 ×/÷ 6
Ca	587 ± 61	491 ×/÷ 430	405 ± 46	259 ×/÷ 310	939 ± 99	580 ×/÷ 671
Cl	1158 ± 110	821 ×/÷ 780	1369 ± 148	985 ×/÷ 994	972 ± 102	711 ×/÷ 690
Co	0.1 ± 0.03	0.1 ×/÷ 0.2	0.2 ± 0.02	0.1 ×/÷ 0.1	0.04 ± 0.01	0.03 ×/÷ 0.05
Cr	0.4 ± 0.05	0.3 ×/÷ 0.3	0.8 ± 0.1	0.6 ×/÷ 0.7	0.4 ± 0.1	0.3 ×/÷ 0.4
Cu	10 ± 1	9 ×/÷ 7	10 ± 1	9 ×/÷ 4	11 ± 1	10 ×/÷ 6
Fe	60 ± 5	48 ×/÷ 39	381 ± 46	284 ×/÷ 311	41 ± 3	36 ×/÷ 23
I	4 ± 1	2 ×/÷ 4	0.9 ± 0.1	1 ×/÷ 0.8	0.7 ± 0.1	0.5 ×/÷ 0.5
K	38 ± 4	27 ×/÷ 30	77 ± 7	59 ×/÷ 47	17 ± 2	12 ×/÷ 14
Mg	146 ± 20	92 ×/÷ 144	110 ± 16	66 ×/÷ 104	166 ± 24	123 ×/÷ 161
Mn	6 ± 1	5 ×/÷ 5	14 ± 2	10 ×/÷ 12	3 ± 0.3	2 ×/÷ 2
Na	25 ± 3	18 ×/÷ 25	42 ± 6	32 ×/÷ 43	16 ± 2	11 ×/÷ 16
S%	4.7 ± 0.04	4.6 ×/÷ 0.2	4.6 ± 0.05	4.5 ×/÷ 0.3	4.6 ± 0.04	5 ×/÷ 0.2
Sb	0.4 ± 0.05	0.2 ×/÷ 0.4	0.23 ± 0.04	0.1 ×/÷ 0.3	0.06 ± 0.01	0.03 ×/÷ 0.05
Sc	0.012 ± 0.001	0.2 ×/÷ 0.01	0.08 ± 0.08	0.01 ×/÷ 0.06	0.01 ± 0.001	0.01 ×/÷ 0.01
Se	0.7 ± 0.2	0.5 ×/÷ 1	0.6 ± 0.01	0.5 ×/÷ 0.1	0.6 ± 0.02	0.6 ×/÷ 0.1
Sr	17 ± 3[a]	12 ×/÷ 11	20 ± 1[a]	5 ×/÷ 6	13 ± 1[b]	12 ×/÷ 7
V	0.2 ± 0.02	0.2 ×/÷ 0.1	0.8 ± 0.1	1 ×/÷ 0.7	0.1 ± 0.03	0.1 ×/÷ 0.2
Zn	127 ± 12	112 ×/÷ 85	105 ± 7	98 ×/÷ 45	154 ± 8	146 ×/÷ 54

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Processing of complementary food does not increase hair zinc levels and growth of infants in Kilosa district, rural Tanzania

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A community-based, randomized, placebo-controlled, double-blind trial was conducted from March 2001 to March 2002 in Kilosa, a rural district of Morogoro Region in Tanzania. One hundred and fifty-eight infants were selected randomly from lists of local Maternal and Child Health Care Centres and received either processed complementary food (PCF) or unprocessed complementary food (UPCF) from age 6 to 12 months. Processing increased Zn solubility and energy density of the porridge prepared from the complementary food (CF) as determined *in vitro*. Phytate:Zn molar ratio of the PCF and UPCF was 25·8 and 47·5, respectively. Under the study conditions, the processing of CF did not improve Zn status as measured by hair analysis. No significant correlations were found between hair Zn values and anthropometric measurements. Our findings suggest that processing alone of cereal-based CF may be insufficient to ensure an adequate supply of Zn to improve growth and Zn status of infants. Dietary modification to tackle Zn deficiencies in similar target groups may therefore only be successful when other Zn-rich foods such as meat and fish are included.

Complementary food: Zinc status: Infant growth: Tanzania

In developing countries, micronutrient deficiencies are widespread and manifest in the early stages of infant life. Zn deficiency is of particular importance (Salgueiro *et al.* 2002) and may result in retarded skeletal development and increased susceptibility to infections mediated via defects in the immune system (Food and Agriculture Organization/World Health Organization, 2002). The deficiency state of Zn is, however, difficult to diagnose because a reliable laboratory index to estimate Zn nutritional status is currently lacking (Wood, 2000; Hotz *et al.* 2003). Infants grow fairly well during the first months of life when they are exclusively breast-fed but, with the introduction of complementary foods (CF), a distinct plunge of mean weight-for-age or height-for-age is common. CF are typically cereal-based porridges with little vegetable or animal products (Michaelsen & Friis, 1998; Davidsson, 2003). Both the high degree of phytates (myo-inositol hexaphosphate) present in whole-grain cereals and legumes and the poor quality in terms of the presence of minerals and vitamins lead to micronutrient deficiencies (Food and Agriculture Organization/World Health Organization, 2002). Phytate:Zn molar ratios >15 are believed to reduce Zn absorption levels to 15 % (World Health Organization, 1996). Reduction of the phytate:Zn molar ratio is therefore a possible strategy to enhance Zn absorption (Manary *et al.* 2000). Hotz *et al.* (2001) demonstrated how household preparation techniques can enhance *in vitro* Zn bioavailability of locally prepared maize-based CF in Malawi.

Interventions that target Zn deficiencies in developing countries are commonly based on food supplementation or fortification (World Health Organization, 2002). However, a food-based approach is generally believed to be a feasible and sustainable strategy to address Zn deficiencies (Gibson & Ferguson, 1998).

The main hypothesis of the present trial was that an increased solubility of Zn through processing of CF would improve Zn status and growth of infants. The trial was part of a larger study that appraised the effects of locally prepared CF on growth and Fe status (Mamiro *et al.* 2004). Because low-cost, home-based strategies to alleviate Zn deficiencies are still not available in many developing countries, it was decided to document the effect of processing CF on Zn and growth in the present paper.

Materials and methods

Study area

The study was conducted in Kilosa district in Morogoro Region of the United Republic of Tanzania from March 2001 to March 2002. The region is located approximately

Abbreviations: CF, complementary food; LAZ, length-for-age Z-score; PCF, processed complementary food; UPCF, unprocessed complementary food; WAZ, weight-for-age Z-score; WLZ, weight-for-length Z-score.

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300 km west of Dar es Salaam and has a population of about 350 000 inhabitants. Kilosa was chosen for the present trial because of its high prevalence of nutritional Fe deficiency, which is believed to coincide with Zn deficiency (Gibson *et al.* 2002; Lind *et al.* 2003). Specific data on Zn deficiencies for Kilosa district were not found.

Study design

The study was part of a larger, double-blind, randomized controlled trial in which the main investigator and the mothers had no knowledge of the type of food given to the infants. A CF quite similar to local traditional CF in Tanzania was formulated by the authors and produced locally. The effect of a processed complementary food (PCF) was then measured against that of an unprocessed complementary food (UPCF) which served as control.

The trial was approved by the ethics committees of both the Tanzanian Food and Nutrition Centre and Ghent University, Belgium. Parents of 364 infants aged 0 to 6 years gave verbal consent to participate and were randomized. Only three parents who were contacted refused to participate. Prior to enrolment, the health status of the infants was assessed by a medical doctor. Infants who were too ill to participate in the study were excluded and received medical care. Infants were continuously enrolled and entered the study when they were 6 months old and followed until the age of 12 months. Because of logistical difficulties, some time elapsed between initial randomization and enrolment. This resulted in the drop-out of fifty-five participants. Allocation to the treatment or control group was determined using a block randomization technique. Individuals were randomized on the basis of pre-established census lists. The code was not broken to the main investigator before the end of the data collection. Of the 309 infants who participated in the trial, 133 infants had enough hair to provide samples (Fig. 1). We subsequently computed whether this sample size allowed detection of meaningful differences in hair Zn levels between infants from the control and intervention group. Given the apparent large inter-subject variation in hair Zn content of the samples, a meaningful difference between the groups was arbitrarily defined as half the SD from the hair Zn analysis. Taking into account a significance level of 0·05 and the SD obtained from the hair analysis, this sample size showed a power of 88 % to detect such difference and allows us to expose differences in change of mean weight-for-length Z-score (WLZ), weight-for-age Z-score (WAZ) and length-for-age Z-score (LAZ) of 0·54SD, 0·38SD and 0·37SD, respectively. This power was considered sufficient to proceed with further computations. Calculations were done with Gpower version 2.0 (Erdfelder *et al.* 1996).

Complementary food

The CF was a mixture of finger millet (*Eleusine corocana*), kidney beans (*Phaseolus vulgaris*), peanuts (*Arachis hypgoea*) and mango (*Mangifera indica*). Processing involved roasting of peanuts to improve protein digestibility and destroy pathogenic micro-organisms (Brown *et al.* 1998; Gibson *et al.* 1998) and germination of finger millet and beans to increase solubility of Zn and Fe (Mbithi-Mwikya *et al.* 2002). The finger millet and beans were sorted, cleaned and soaked in pre-boiled water for 2 and 7 h, respectively, and subsequently germinated for 48 h at 30°C. The batch was then autoclaved and solar-dried for about 6 h. Proliferation of pathogens such as *Staphylococcus aureus* and *Bacillus cereus* during germination was controlled with appropriate hazard analysis and critical control point procedures (Kimanya *et al.* 2003). Total phytate content of the CF was measured using colorimetric assays as described by Mbithi-Mwikya *et al.* (2002).

PCF and UPCF did not differ significantly in energy content ($P>0·05$), which was 1731 (SD 11) and 1731 (SD 18) kJ/100 g DM, respectively. The energy density of the porridge prepared with the PCF was 6100 kJ/l, compared with 1700 kJ/l for the UPCF. The amounts of CF were such to provide at least 1151 kJ/d for infants of 6–8 months and 1883 kJ/d for infants 9–11 months according to WHO guidelines (Brown *et al.* 1998) to meet the expected deficit in energy and protein. Since 30–45 % of daily energy intake from fat is recommended for children less than 2 years old (Michaelsen & Jorgensen, 1995), nurses advised to add 1–2 teaspoons (about 4 g) of sunflower seed oil for each portion of the CF. Every two weeks, 1 and 1·6 kg of CF were provided for infants 6–8 and 9–11 months of age, respectively. On a daily basis, each child was supplied with 69 and 113 g CF as DM. Mothers were instructed to prepare a fixed amount of this DM into porridge to give the child and to add 1–2 teaspoons of sunflower seed oil. A thorough description of the materials, methods and plan for hazard analysis and critical control point for the preparation of the CF has been given by the authors previously (Mamiro *et al.* 2004).

Compliance and assessment of dietary intake

The required amount of CF was distributed to the villages where it was stored in a securely closed cupboard to prevent spoilage. The mothers came to the health centre every two weeks to collect the CF. The nurses of the local health centre recorded every food collection using a list of the infants and demonstrated how to prepare the CF. In case of absence, the nurse ensured that a message was sent to the responsible mother or caregiver to collect her consignment on the same day. Nutrition officers from the health centres visited the mothers in their dwellings at least twice a week to verify that the CF was prepared and used correctly. They also made frequent surprise visits to observe compliance and solve any problems encountered. To estimate the amount of CF consumed by the infants, a 24 h dietary recall was carried out by a nutritionist assisted by a village health worker. Of the mothers of the 309 infants in the main trial, seventy-five were randomly selected in each intervention group, yielding seventy-one responses in the PCF and sixty-six in the UPCF group. The food consumed by the infants was estimated by the mothers and weighed using digital scales. FAO food composition tables were used to calculate macro- and micronutrient intakes (Food and Agriculture Organization, 1984).

Zinc analysis

Higher levels of soluble Zn in cereal–legume mixtures are associated with higher Zn uptake (Agte *et al.* 1997). *In vitro* solubility of Zn was therefore used in this study as a measure

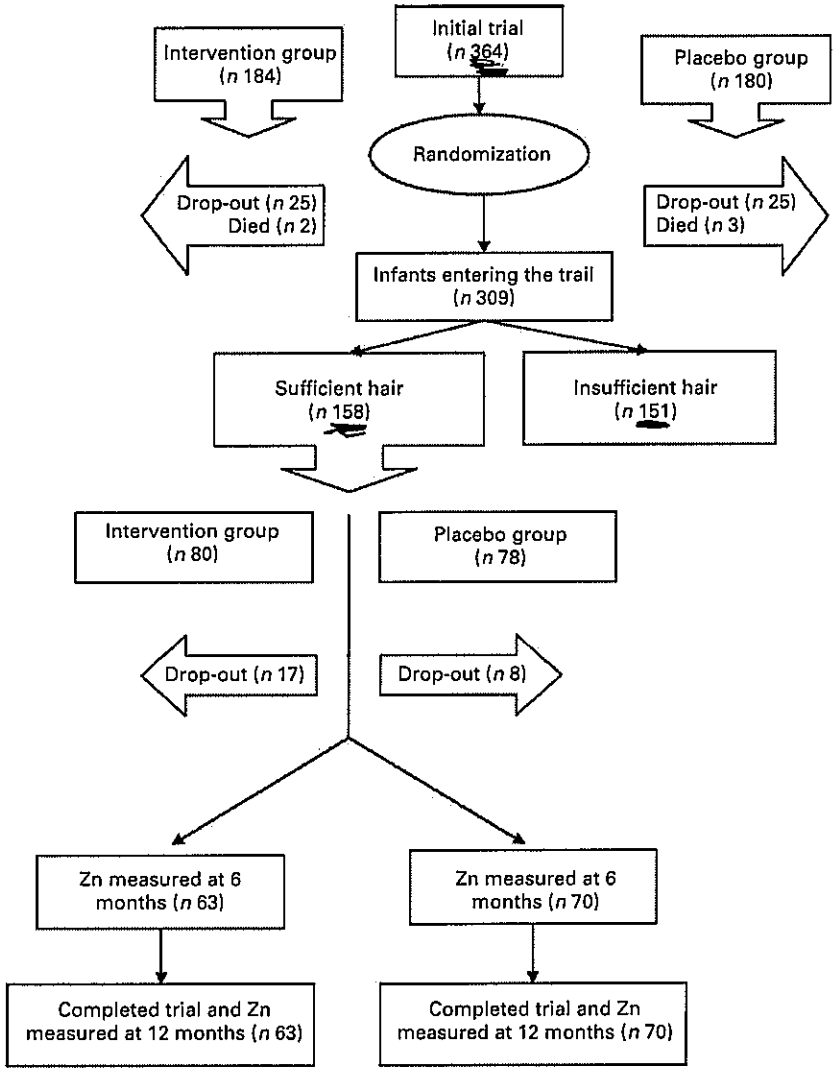

Fig. 1. Study design. Number of infants from the initial trial receiving processed (intervention group) or unprocessed complementary food (placebo group) and those providing hair samples for zinc analysis.

for Zn bioavailability in the CF. Extraction of Zn was carried out by wet-acid digestion using a nitric acid–perchloric acid mixture (5:1), 0·03-M HCl and atomic absorption spectrophotometry as described by Kumar & Chauhan (1993).

Zn status of infants was assessed using hair Zn. Because of the difficulty of drawing blood in our population (infants aged 6–12 months with high prevalence of anaemia), we wanted to limit the number of blood specimens and restrict those to finger pricks for Hb. Biochemical indices of Zn status, such as serum and erythrocyte Zn, or tests of immune competence were not attempted. Gibson *et al.* (1989) have proposed the cut-off value of hair Zn levels below 110 μg/g (1·68 μmol/g) as an indicator of sub-optimal Zn status. Scalp hair was collected at baseline (6 months of age) and at the end of the trial (12 months of age) and analysed for Zn content. All measures were taken to avoid external sources of adventitious contaminations such as nits and lice during collection and preparation of the hair samples. The hair was cut with stainless steel scissors from the occipital region of the head as close to the scalp as possible. The samples were collected in small, clean, sterile plastic envelopes with a self-adhesive mechanism. The envelopes were coded, stored in a securely closed plastic bag and transported by airfreight to a laboratory in Belgium for analysis. Only the proximal 1–2 cm of the hair shaft was used since this part reflects recent trace element uptake by the follicles. Hair grows on average 1 cm per month (Wade & Sinclair, 2002), so that the Zn content of hair 1–2 cm in length represents Zn uptake 2 to 3 months before sample collection (Dombovari & Papp, 1998). Hence, in the present study, Zn concentrations in hair samples probably represent Zn status at age approximately 4 and 10 months, and not 6 and 12 months, respectively.

Before hair of the infants was analysed, the sample extraction methodology was optimized using extra hair samples

from Belgian barber shops. Two methods of sample extraction were compared: dry ashing (Dombovari & Papp, 1998) and wet digesting (Harrison *et al.* 1969), with Zn concentration determined by atomic absorption spectrophotometry. All preliminary analysis was carried out in duplicate. Mean Zn with wet digestion was 114·8 (SD 3·6) μg/g dry hair and 108·3 (SD 5·9) μg/g dry hair for dry ashing (*n* 10). Recovery was assessed for addition of 100 ml and 200 ml 0.015 mmol/l $Zn(NO_3)_2$. Wet digestion showed a comparatively larger recovery for Zn compared with dry ashing (106·0 % and 91·2 %, respectively). Mean differences for preliminary analysis were 14·8 μg Zn/g dry hair for the wet digestion (*n* 12) method and 15·7 μg Zn/g dry hair for the dry ashing method (*n* 11). Repeatability SD was 14·0 and 17·6, respectively. The wet digestion method was subsequently adopted as the reference method for the determination of Zn in the hair samples from Tanzania. Hair of 6- and 12-month-old infants was analysed concurrently. Because of the limited amount of hair that could be obtained from the infants, Zn analysis could not be performed in duplicate.

Statistical analyses

Data were entered in EPI-INFO (version 6.04d, 1996; Centers for Disease Control and Prevention, Atlanta, GA, USA/WHO, Geneva, Switzerland) and analysis was done using the Stata package (version 8.0; Stata Corp., College Station, TX, USA). Anthropometric indicators were computed using EPINUT according to 1977 growth reference data from the National Center for Health Statistics (Hyattsville, MD, USA). Descriptive statistics were computed for each variable to identify outliers and assess the normal distribution of continuous variables. Outliers were defined from the box plot as values more extreme than three interquartile ranges of the box. In the presence of outliers, a new variable was created excluding these values. However, in case of doubt, the data set was cross-checked with original data in the rosters. All tests were done first with the original variable, and then redone with the new variable to assess the influence of such outliers. Normal distribution of continuous variables was appraised by the Kolmogorov–Smirnov test. In the case of departure from normality, the variables were log-transformed (lnskew0 command in Stata) to apply statistics. Geometric means are presented in the tables where appropriate.

The α error was set at 5 % and all tests were two-sided. A difference at 12 months between the two intervention groups was assessed for the primary outcome of mean Zn concentration in hair and anthropometric indicators, i.e. mean WLZ and mean LAZ. A standard *t* test was used for continuous variables. The general trend in main outcomes between the beginning and the end of the trial was assessed by applying a paired *t* test or a McNemar test for categorical variables.

Results

Subjects

In the main trial, the birth weight of the infants was 2·9 (SD 0·5) kg for the PCF group and 3·1 (SD 0·5) kg for the UPCF group and did not differ significantly between groups ($P=0·06$). Sex ratio was 1:1. The prevalence of wasting was significantly different between groups, while stunting, weight and length were equal in both groups. At baseline, infants with insufficient hair for analysis did not differ significantly in mean birth weight ($P=0·15$) and WLZ ($P=0·80$), WAZ ($P=0·95$) and LAZ ($P=0·80$) from those who provided hair samples. Of the infants who provided hair samples, no differences in mean WLZ, WAZ and HAZ were found at baseline between infants receiving PCF and UPCF (Table 1).

Complementary food

Phytate content was reduced significantly by processing ($P=0·04$) and was 660 (SD 20) mg/100 g DM for PCF and 1150 (SD 30) mg/100 g DM for UPCF. The processing of CF decreased the phytate:Zn molar ratio which indicates a successful improvement of absorption potential for Zn from the PCF compared with the UPCF. Table 2 describes the Zn content of the CF as obtained from analysis of twelve samples taken randomly every month from each CF production unit.

Average dietary zinc supply

Food consumption data from the 24 h recall showed no significant differences in daily intake of energy (1752 *v.* 1679 kJ, $P=0·99$) and protein (17·9 *v.* 18·3 g/d, $P=0·68$) from the CF between UPCF and PCF groups. The addition of oil increased the intake of energy from CF to 1943 and 1922 kJ ($P=0·47$) and for lipids to 31·3 *v.* 29·9 g/d ($P=0·24$) for UPCF and PCF, respectively. The CF alone contributed >50 % of the total daily energy intake and exceeded the age-specific WHO recommendations. The total number of meals of CF given to the child differed considerably between the groups, with 1–2 meals in the processed group *v.* 5–6 meals in the non-processed group. The overall average consumption of CF for both groups was 104 g DM/d. Using soluble Zn as proxy for bioavailability and the total Zn content of the CF, the total amount of available Zn received by the infants was thus: 2·53 mg Zn/100 g × 104 g CF/d × 6·24 % soluble Zn = 0·164 mg Zn/d for PCF and 2·4 mg Zn/100 g CF × 104 g CF/d × 2·74 % soluble Zn = 0·0684 mg Zn/d for UPCF. Taking into account the Zn losses and allowing for growth, the Zn requirements for infants aged 6 to 12 months are estimated to be 2·8 mg/d (Brown *et al.* 1998). Our PCF met these requirements for 5·8 % ((0·164/2·8) × 100) and UPCF for 2·4 % ((0·068/2·8) × 100).

Hair zinc levels

Mean Zn hair concentration at baseline was 272·9 (SD 115·0) μg/g for the control group and 253·4 (SD 100·0) μg/g for the intervention group. At the end of the trial, these levels were 244·9 (SD 120·0) and 246·2 (SD 103·5) μg/g, respectively (Fig. 2). There was no significant difference ($P=0·25$) in mean hair Zn concentrations at baseline and at 12 months ($P=0·75$) between infants receiving PCF and those receiving UPCF. The intervention did not produce a significant effect in both PCF ($P=0·33$) and UPCF ($P=0·06$) groups in terms of mean hair Zn concentration. Additionally, change in hair Zn at 6 and 12 months between PCF and UPCF was not significantly different ($P=0·30$).

Table 1. Comparison of mean weight-for-length Z-score (WLZ), weight-for-age Z-score (WAZ) and length-for-age Z-score (LAZ) of infants fed processed complementary food (PCF) and unprocessed complementary food (UPCF) at 6 and 12 months of age
(Mean values and standard deviations)

	At baseline (6 months)						At end (12 months)						Overall difference between baseline and end					
	PCF (*n* 63)		UPCF (*n* 67)				PCF (*n* 63)		UPCF (*n* 67)				PCF (*n* 63)		UPCF (*n* 67)			
	Mean	SD	Mean	SD	Mean difference	*P*	Mean	SD	Mean	SD	Mean difference	*P*	Mean	SD	Mean	SD	Mean difference	*P*
WLZ	0·37	1·26	0·75	1·25	−0·38	0·08	−0·18	1·02	0·10	1·08	−0·28	0·13	−0·54	1·07	−0·59	1·09	0·05	0·80
WAZ	−0·88*	1·00*	−0·69*	1·00*	−0·19	0·10	−1·56	1·11	−1·26	1·06	−0·30	0·12	−0·60	0·70	−0·62	0·83	0·02	0·88
LAZ	−1·57	1·20	−1·47	1·04	−0·10	0·62	−1·98	1·01	−1·86	0·91	−0·12	0·49	−0·41	0·76	−0·38	0·71	−0·03	0·82

*Geometric means and standard deviations.

Table 2. Zinc content of the complementary foods
(Mean values and standard deviations)

	Complementary food				
	Processed (*n* 12)*		Unprocessed (*n* 12)		
Variable	Mean	SD	Mean	SD	*P*
Zn (mg/100 g DM)	2·53	0·09	2·40	0·08	0·01
Soluble Zn (%)	6·24	2·47	2·74	1·49	0·003
Phytate:Zn molar ratio	25·8		47·5		

*Twelve random samples of production batches.

At baseline, 7·9 % of the infants receiving the PCF had hair Zn concentrations $<110\,\mu g/g$ compared with 5·7 % in the UPCF group. After the intervention, 6·3 % of the infants receiving PCF and 7·1 % receiving UPCF had hair Zn concentrations below the cut-off value. The percentages below the cut-off at baseline and end were not significantly different ($P=0·60$).

For all infants combined, infants with a hair Zn level below the cut-off value at baseline had an average increase in hair Zn of 159·0 (SD 127·8) μg/g while the infants with hair Zn levels higher than the cut-off showed an average decrease of 31·0 (SD 103·1) μg/g. The difference of mean changes in hair Zn levels between these groups was highly significant ($P<0·001$).

Effect of complementary food on growth

At 12 months, WLZ, WAZ and LAZ were not significantly different between PCF and UPCF groups. Furthermore, the change in mean WLZ, WAZ and LAZ from 6 to 12 months was not significant (Table 1).

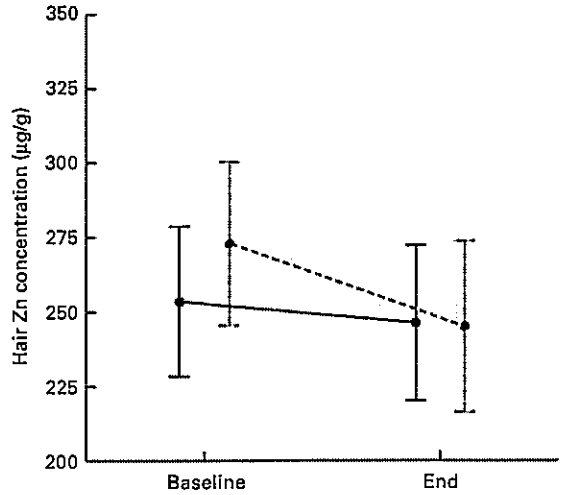

Fig. 2. Hair zinc concentration (μg/g) at baseline and end of the trial for infants who received processed complementary food (PCF; ——) or unprocessed complementary food (UPCF; ----). Values are means with their 95 % CI shown by vertical bars for sixty-three subjects in the PCF group and seventy subjects in the UPCF group.

Discussion

The present paper presents a secondary analysis of a trial investigating the effect of an improved processed CF on Hb status and growth of infants of aged 6 to 12 months. The results of the trial were reanalysed for their impact on Zn status and growth. Of the 309 infants in the main trial, 158 had sufficient hair for Zn analysis. Ex-post calculations showed that adequate detection power was obtained to show differences of at least 0·5SD in mean hair Zn concentration and WLZ, WAZ and LAZ between those receiving PCF and UPCF. No significant differences were found for the main indicators of nutritional status between infants with and without sufficient hair at baseline, suggesting that the analysis of a subgroup of the original sample was not a source of bias.

Although processing of a cereal-based CF resulted in higher energy density, higher levels of soluble Zn and lower phytate:Zn molar ratio, no significant expression of improved growth or Zn status was observed in the infants under the study conditions. Analysis of hair Zn levels showed no significant differences in hair Zn concentrations between the two groups post intervention, even though calculated intakes of the Zn were higher in the intervention group compared with the control group. Furthermore, regardless of food, infants in the present study with initial Zn deficiency as determined by cut-off levels of hair Zn managed to increase the Zn levels in hair while a decrease was observed for infants who were classified as not Zn-deficient at baseline. This could be due to gastrointestinal regulatory mechanisms for Zn homeostasis as has been observed earlier in human subjects with prolonged Zn deficiency (Lee *et al.* 1993; King *et al.* 2000).

It is evident that the interpretation of the study results is hampered by the lack of a reliable index for Zn status in man and reference data for the study population. It is noteworthy that the hair Zn values in our study exhibited large variations. Similar values, in terms of absolute levels and variability, were also observed in Indonesian infants aged 5 months (Kolsteren *et al.* 1998) and Jamaican children aged 6–24 months (Meeks Garner *et al.* 1998). The large variations in the present study are unlikely to be attributable to the laboratory technique since the precision of the analyses as assessed by the recovery experiments was satisfactory and may be physiological. This study did not address seasonal influences, which may have a marked effect on hair Zn concentrations as described by Gibson *et al.* (1989). Infants in the present study were enrolled over a relatively long period of one year from March 2001 and March 2002, which arguably has levelled out seasonal variations.

Few infants had initial hair Zn levels below cut-off, which is consistent with Meeks Garner *et al.* (1998). In that Zn supplementation trial of stunted children aged 6 to 24 months, 13 % of the Zn-supplemented group and 19 % of the control group had hair Zn levels lower than 70 μg/g. None of the infants in our study had hair Zn levels lower than this cut-off. Hair Zn content, however, may lack validity in cases of severe Zn deficiency (Gibson, 2004). The causes of stunting are complex and remain poorly understood. For children with impaired linear growth, Zn may not necessarily be the first limiting nutrient (Hautvast *et al.* 2000). Linear growth faltering may arise from multiple causes including the effects of chronic infections and prenatal and inter-generational effects of multiple micronutrient inadequacies in the diet, especially when the habitual diet is cereal-based. In these circumstances children are unlikely to show any improvement in linear growth in response to a Zn supplement unless Zn is the first limiting nutrient (Bates *et al.* 1993; Ferguson *et al.* 1993; Friis *et al.* 1997). Additionally, children in rural Tanzania are subjected to a multitude of infections. Environmental factors such as parasitic infections may have interfered with the effects of the intervention in terms of growth response. Mamiro *et al.* (2004) showed how nutritional status as measured by WLZ and LAZ deteriorated significantly during the intervention period for both the PCF and the UPCF groups. The elevated level of stunting amongst the infants in our study reflects an array of underlying deficiencies which may have masked the effect of the improved CF.

For ethical considerations, the mothers were asked to prepare a fixed amount of CF, similar in both groups, every day, which resulted in equal amounts of energy intake between the groups. Energy density of the UPCF was more than three times lower than the PCF. Mothers had to administer the UPCF more than five times per day, compared to two times per day for the PCF, to obtain similar energy intakes. This may have introduced a bias in the study. Under less intense follow-up, the effect of the energy-dense CF may have been more pronounced (Mamiro *et al.* 2004).

Higher Zn content of the CF failed to improve growth significantly in terms of WLZ, LAZ and WAZ. This is in contrast to the results of a study by Umeta *et al.* (2000), who showed a significant improvement of linear growth, weight, WLZ, WAZ and LAZ in stunted Ethiopian infants. Hair Zn concentration was positively correlated with increased growth in the supplemented children. In Ethiopia, however, Zn was the primary growth-limiting nutrient in the infants (Gibson, 2000) and the trial used 10 mg Zn as $ZnSO_4$ daily for 6 d per week during 6 months, which is considerably higher than in the present trial using dietary modification. Our findings are in line with the results of a study in Ghana, in which four groups of infants aged 6–12 months were fed for 6 months with different types of improved centrally processed CF. The study found no significant difference in Zn status measured by plasma Zn, WAZ and LAZ between 6 and 12 months in the infants who received the different CF (Lartey *et al.* 1999). Presumably, the levels of dietary intake in the present study are too low to produce any measurable effect in infants who are likely to be deficient in a number of nutrients.

Although processing decreased the phytate:Zn molar ratio considerably, the phytate content of the PCF still remained high. Even when Zn would have been 100 %, our cereal-based CF was unable to provide enough Zn. Bearing in mind that refined diets low in cereals and rich in animal foods rarely surpass absorption levels of 50 % (Hotz & Brown, 2004), it is obvious that processing will remain inadequate under the study conditions. Surprisingly, the present study showed a decrease (not significant) in mean hair Zn levels after 6 months of intervention. This trend was also found in the control group. This observation seems to support our conclusions that the CF was unable to provide enough Zn, regardless of the processing, and therefore suggests that home-based processing of cereal-based CF will not be able to improve growth and Zn status. Fortification of CF or Zn supplementation may be an alternative in this respect. Given the intricate relationship between micronutrient status and growth, however, a food-based approach has the considerable advan-

tages of supplying a vast array of additional dietary compounds which are naturally present. Adding supplementary sources of Zn-rich foods, such as meat and fish, to CF seems to be indispensable to ensure an adequate and sustainable supply of sufficient micronutrients.

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