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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

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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 lengthfor-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

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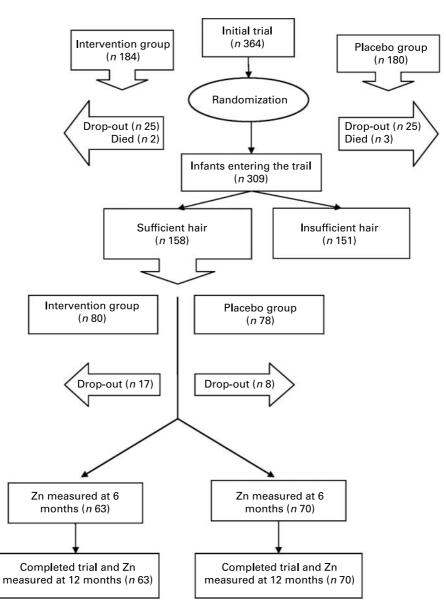


Fig. 1. Study design. Number of infants from the initial trial receiving processed (intervention group) or unprocessed complementary food (placebo group) and those providing hair samples for zinc analysis.

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(NO₃)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 $14.8 \,\mu g$ 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 (sp 20) mg/100 g DM for PCF and 1150 (sp 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: $2.53 \, \text{mg}$ $Zn/100 \, \text{g} \times 104 \, \text{g}$ $CF/d \times 6.24 \, \%$ soluble $Zn = 0.164 \, \text{mg}$ Zn/d for PCF and $2.4 \, \text{mg}$ $Zn/100 \, \text{g}$ $CF \times 104 \text{ g}$ $CF/d \times 2.74 \%$ 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) μ g/g for the control group and 253·4 (sD 100·0) μ g/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) μ g/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

PCF (n 63)		At basell	At baseline (6 months)	nths)				At end	At end (12 months)	inths)		_	Overall c	lifference k	oetween	Overall difference between baseline and end	
	(2)	UPCF (n 67)	٦ (29			PCF (n 63)	(63)	UPCF (n 67)	n 67)			PCF (n 63)	7 63)	UPCF (n 67)	n 67)		
Mean	SD	Mean	SD	Mean difference	Д	Mean	SD	Mean	SD	Mean difference	Д	Mean	SD	Mean	SD	Mean difference	Д
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	1.00*	*69.0	1.00*	-0.19	0.10	-1.56	1-1	-1.26	1.06	-0.30	0.12	-0.60	0.70	-0.62	0.83	0.02	0.88
- 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	92.0	-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)

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

^{*}Twelve random samples of production batches.

At baseline, 7.9% of the infants receiving the PCF had hair Zn concentrations $<110\,\mu\text{g/g}$ 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 (sp 127·8) $\mu g/g$ while the infants with hair Zn levels higher than the cut-off showed an average decrease of 31·0 (sp 103·1) $\mu g/g$. The difference of mean changes in hair Zn levels between these groups was highly significant ($P \!<\! 0 \!\cdot\! 001$).

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).

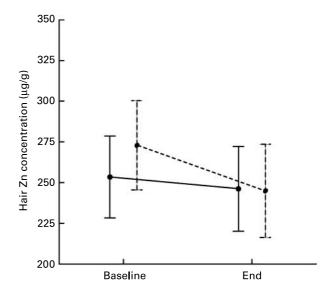


Fig. 2. Hair zinc concentration (μ g/g) at baseline and end of the trial for infants who received processed complementary food (PCF; ——) or unprocessed complementary food (UPCF; ----). Values are means with their 95 % CI shown by vertical bars for sixty-three subjects in the PCF group and seventy subjects 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 0-5sp 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 growthlimiting nutrient in the infants (Gibson, 2000) and the trial used 10 mg Zn as ZnSO₄ 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 cerealbased 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 homebased 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 advan180 C. K. Lachat et al.

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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References

- Agte VV, Gokhale MK & Chiplonkar SA (1997) Effect of natural fermentation on *in vitro* zinc bioavailability in cereal–legume mixtures. *Int J Food Sci Technol* **32**, 29–32.
- Bates CJ, Evans PH, Dardenne M et al. (1993) A trial of zinc supplementation in young rural Gambian children. Br J Nutr 69, 243–255.
- Brown K, Dewey K & Allen L (eds) (1998) Complementary Feeding of Young Children in Developing Countries: A Review of Current Scientific Knowledge. Geneva: World Health Organization.
- Davidsson L (2003) Approaches to improve iron bioavailability from complementary foods. J Nutr 133, Suppl., 1560S-1562S.
- Dombovari J & Papp L (1998) Comparison of sample preparation methods for elemental analysis of human hair. *Microchem J* 59, 187–193.
- Erdfelder E, Faul F & Buchner A (1996) Gpower. A general power analysis program. *Behav Res Methods Instrum Comput* **28**, 1–11.
- Ferguson E, Gibson R, Opare-obisaw C, Ounpuu S, Thompson LU & Lehrfeld J (1993) The zinc nutriture of preschool children living in two African countries. *J Nutr* **123**, 1487–1496.
- Food and Agriculture Organization (1984) Cereals and grain products. In *Food Composition Table for Use in Africa*, pp. 98–101 [US Department of Health Education and Welfare, editor]. Rome: FAO.
- Food and Agriculture Organization/World Health Organization (2002) Human Vitamin and Mineral Requirements. Report of a Joint WHO/ FAO Expert Consultation, Bangkok, Thailand. Rome: FAO.
- Friis H, Ndhlovu P, Mdluza T, Kaondera K, Sandstrom B, Michaelsen K, Vennervald B & Christensen N (1997) The impact of zinc supplementation on growth and body composition: a randomized, controlled trial among rural Zimbabwean schoolchildren. *Eur J Clin Nutr* **51**, 38–45.
- Gibson RS (2000) Zinc supplementation for infants. *Lancet* **355**, 2008–2009.
- Gibson RS (2004) *Principles of Nutritional Assessment*. New York: Oxford University Press.
- Gibson RS & Ferguson EL (1998) Nutrition intervention strategies to combat zinc deficiency in developing countries. *Nutr Res Rev* 11, 115–131.
- Gibson RS, Ferguson EF, Vanderkooy PDS & MacDonald AC (1989) Seasonal variations in hair zinc concentrations in Canadian and African children. Sci Total Environ 84, 291–298.
- Gibson RS, Ferguson EL & Lehrfeld J (1998) Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. Eur J Clin Nutr 52, 764–770.
- Gibson RS, Heath A-LM & Ferguson EL (2002) Risk of suboptimal iron and zinc nutriture among adolescent girls in Australia and New Zealand: causes, consequences, and solutions. *Asia Pac J Clin Nutr* 11, Suppl., S543–S552.
- Harrison W, Yuracheck J & Benson C (1969) The determination of trace elements in human hair by atomic absorption spectroscopy. *Clin Chim Acta* 23, 83–91.
- Hautvast JLA, Tolboom JJM, Kafwembe EM, Musonda RM, Mwanakasale V, van Staveren WA, van't Hof MA, Sauerwein RW, Willems JL & Monnens LAH (2000) Severe linear growth retardation in rural Zambian children: the influence of biological variables. *Am J Clin Nutr* **71**, 550–559.

- Hotz C & Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. International Zinc Nutrition Consultative Group. *Food Nutr Bull* 25, S91–S204.
- Hotz C, Gibson R & Temple T (2001) A home-based method to reduce phytate content and increase zinc bioavailability in maize-based complementary diets. *Int J Food Sci Nutr* **52**, 133–142.
- Hotz C, Lowe NM, Araya M & Brown KH (2003) Assessment of the trace element status of individuals and populations: the example of zinc and copper. *J Nutr* **133**, Suppl., 1563S–1568S.
- Kimanya ME, Mamiro PRS, Van Camp J, Devlieghere F, Opsomer A, Kolsteren P & Debevere J (2003) Growth of *Staphylococcus aureus* and *Bacillus cereus* during germination and drying of finger millet and kidney beans. *Int J Food Sci Technol* **38**, 119–125.
- King JC, Shames DM & Woodhouse LR (2000) Zinc homeostasis in humans. *J Nutr* **130**, Suppl., 1360S–1366S.
- Kolsteren PW, Kardjati S, Traissac P & Goyens P (1998) Hair zinc and copper in Indonesian infants. *Asia Pac J Clin Nutr* 7, 151–159.
- Kumar A & Chauhan BM (1993) Effects of phytic acid on protein digestibility (*in-vitro*) and HCl-extractability of minerals in pearl-millet sprouts. *Cereal Chem* **70**, 504–506.
- Lartey A, Manu A, Brown KH, Peerson JM & Dewey KG (1999) A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micronutrient status of Ghanaian infants from 6 to 12 mo of age. *Am J Clin Nutr* **70**, 391–404.
- Lee DY, Prasad AS, Hydrickadair C, Brewer G & Johnson PE (1993) Homeostasis of zinc in marginal human zinc-deficiency role of absorption and endogenous excretion of zinc. *J Lab Clin Med* **122**, 549–556.
- Lind T, Lonnerdal B, Stenlund H, Ismail D, Seswandhana R, Ekstrom EC & Persson LA (2003) A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nutr 77, 883–890.
- Mamiro PS, Kolsteren PW, Van Camp J, Roberfroid DA, Tatala S & Opsomer AS (2004) Processed complementary food does not improve growth or hemoglobin status of rural Tanzanian infants from 6–12 months of age in Kilosa district, Tanzania. J Nutr 134, 1084–1090.
- Manary MJ, Hotz C, Krebs NF, Gibson RS, Westcott JE, Arnold T, Broadhead RL & Hambidge KM (2000) Dietary phytate reduction improves zinc absorption in Malawian children recovering from tuberculosis but not in well children. *J Nutr* **130**, 2959–2964.
- Mbithi-Mwikya S, Van Camp J, Mamiro P, Ooghe W, Kolsteren PW & Huyghebaert A (2002) Evaluation of the nutritional characteristics of a finger millet based complementary food. *J Agric Food Chem* 50, 3030–3036.
- Meeks Garner J, Witter M & Ramdath D (1998) Zinc supplementation: effects on growth and morbidity of undernourished Jamaican children. *Eur J Clin Nutr* **52**, 34–39.
- Michaelsen K & Friis H (1998) Complementary feeding: a global perspective. *Nutrition* **14**, 763–766.
- Michaelsen KF & Jorgensen MH (1995) Dietary-fat content and energy density during infancy and childhood the effect on energy-intake and growth. *Eur J Clin Nutr* **49**, 467–483.
- Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Weill R & Boccio JR (2002) The role of zinc in the growth and development of children. *Nutrition* **18**, 510–519.
- Umeta M, West CE, Haidar J, Deurenberg P & Hautvast JGAJ (2000)Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. *Lancet* 355, 2021–2026.
- Wade MS & Sinclair RD (2002) Disorders of hair in infants and children other than alopecia. *Clin Dermatol* **20**, 16–28.
- Wood RJ (2000) Assessment of marginal zinc status in humans. J Nutr 130, Suppl., 1350S-1354.
- World Health Organization (1996) Trace Elements in Human Nutrition and Health. Geneva: WHO.
- World Health Organization (2002) The World Health Report: Reducing Risks, Promoting Healthy Life. Geneva: WHO.