

## ORIGINAL COMMUNICATION

# Efficacy of a multi-micronutrient dietary intervention based on haemoglobin, hair zinc concentrations, and selected functional outcomes in rural Malawian children

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**Objective:** To investigate the efficacy of enhancing the content and bioavailability of micronutrients in diets of stunted rural Malawian children on their growth and body composition, morbidity, anaemia and hair zinc concentrations.

**Design:** A quasi-experimental design with non-equivalent control group involving 410 intervention and 220 control children aged 30–90 months.

**Setting and subjects:** Children from two intervention and two control villages in Mangochi District, Southern Malawi participated in a 6 month dietary intervention. Anthropometry, malarial screening, haemoglobin, and hair zinc were measured at baseline and after 12 months, as well as socio-economic status at baseline, and common infections monthly post-intervention.

**Results:** Groups were comparable at baseline. Post-intervention children had greater Z-scores for lean body mass (mid-upper arm circumference  $-0.75$  vs  $-1.05$ ; arm muscle area:  $0.63$  vs  $-1.03$ ,  $P < 0.001$ ) than controls but Z-scores for weight-for-height and height-for-age were similar. After controlling for baseline variables, mean haemoglobin was higher ( $107$  vs  $102$  g/l,  $P < 0.01$ ), whereas the incidence of both anaemia ( $62$  vs  $80\%$ ) and common infections (based on a median overall illness score for fever, diarrhoea, upper and lower respiratory infections) were lower in intervention compared to controls, with no change in hair zinc concentrations or malaria status post-intervention.

**Conclusion:** Improvements in the micronutrient adequacy of diets of post-intervention children were associated with a favourable increase in indices of lean body mass and reductions in the incidence of anaemia and common infections in these rural Malawian stunted children.

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## Introduction

Chronic malnutrition arising from the interaction between morbidity and inadequate dietary intakes has a major impact on growth and development of African children. In rural Malawi, inadequate intakes of several micronutrients are widespread because staple diets are predominantly maize-based, and intakes of flesh foods and dairy products are low (Gibson & Hotz, 2001a). As a result, the content and bioavailability of iron, zinc and preformed vitamin A is often low in these rural diets. These three micronutrients have a critical role in immune competence (Beard, 2001; Beaton *et al*, 1993;

Shankar & Prasad, 1998) and linear growth (Angeles *et al*, 1993; Bahl *et al*, 1997; Brown *et al*, 1998). Hence, it is not surprising that deficiencies of these micronutrients have been associated, in part, with the high prevalence of morbidity and stunting reported in Malawian children (National Statistical Office, 1997). Clearly, a dietary intervention strategy that simultaneously enhances the content and bioavailability of these micronutrients in rural Malawian diets is urgently required. We devised such a strategy based on our detailed knowledge of local food consumption patterns and food preparation and processing methods in Southern Malawi. Our strategies included dietary diversification via the introduction of new crops to increase the fat and provitamin A content of the diets, as well as dietary modification. The latter focused on strategies to reduce the high phytate content of Malawian maize-based diet via soaking, fermentation and germination: phytate is a strong antagonist of iron and zinc absorption. At the same time efforts were made to increase the content of enhancers of zinc and/or non-haem iron absorption (eg cellular animal protein and/or ascorbic acid). Details of these strategies and their impact on the micronutrient adequacy of the diets have been published elsewhere (Gibson *et al*, 1998; Yeudall *et al*, 2002).

In this report we compare the changes in anthropometric indices, incidence of malaria, common infections, and anaemia, as well as haemoglobin and hair zinc concentrations over 12 months in two groups of rural Malawian children aged 30–90 months, one group receiving their habitual rural maize-based Malawian diet modified by our dietary intervention for 6 months, and a second group consuming an unmodified diet. We selected this age group rather than younger children to maximize the impact of our dietary strategies, especially the phytate reduction strategies, on their intakes and bioavailability of micronutrients. Children of this age are known to receive more than 50% of their energy from maize (Ferguson *et al*, 1993) and hence have high phytate intakes. In addition, this age group will not be breastfeeding (National Statistical Office, 1994), so that we could quantify their dietary intakes more readily, and thus assess their exposure to our dietary interventions.

We assessed body composition changes as well as growth because alterations in the relative proportions of fat to lean tissue have been related to changes in zinc status in earlier studies of severely malnourished (Golden & Golden, 1981; Golden & Golden, 1992) and stunted rural African children (Bates *et al*, 1993; Friis *et al*, 1997; Kikafunda *et al*, 1998).

## Subjects and study design

Details of the subjects and sample selection have been described elsewhere (Yeudall *et al*, 2002). Briefly, baseline data were collected on a self-selected sample of 630 children (410 intervention; 220 control) from 412 households born between 1989 and 1993 (30–90 months at baseline) in two intervention and two control villages in Mangochi District in Southern Malawi during the post-harvest food plenty

season. Village selection was based on similar socio-economic and food production characteristics as well as perceived need determined by a consultative committee, in keeping with the participatory research approach of the project (Gibson *et al*, 1998). Children's ages were validated, where possible, by comparing stated birthdates to documented birthdates, or by agreement of birthdates across survey periods. Children lacking accurate birthdate information ( $n=42$ ) were excluded from analysis. Verbal consent was obtained from the traditional authorities in each village, and from the parents or guardians of the children after the nature of the study had been fully explained to them. The study was approved by the Health Sciences Research Committee, University of Malawi and the University of Otago Human Ethics Committee, Dunedin, New Zealand.

A quasi-experimental design with non-equivalent control group was utilized. Control communities were provided with the intervention at the end of the post-intervention data collection period. Following recruitment, households were interviewed in their homes by trained research assistants using a pretested structured questionnaire and observations to obtain data on general health, demographics, and socio-economic status (SES), over a 6 week period at baseline (April and May, 1996), after which anthropometric measurements, hair samples, and finger-prick blood samples were taken. The dietary intervention was implemented from June until November 1996. Post-intervention anthropometry, hair and finger-prick blood collections were performed 12 months after the baseline collection in the same sequence, followed by dietary assessment. Data on common infectious illnesses were recorded daily by the parents or guardians and collected at monthly intervals from December 1996.

## Methods

### Anthropometric assessment

Anthropometric assessment was performed on 450 children at baseline, and 306 children after 12 months (participation rates 71 and 48%, respectively). The following measurements were taken in triplicate using calibrated equipment and standardized techniques (Lohman *et al*, 1988) with children wearing light clothing and no shoes: standing height, weight, mid upper arm circumference (MUAC) and tricep skinfolds (TSF). Each measurement was taken by the same trained anthropometrist to eliminate inter-examiner error. Height was measured to the nearest millimeter using a portable stadiometer (Physical Education Workshop, University of Otago). Weight was measured to the nearest 0.1 pound using a portable battery operated scale (OH, USA). The mid-upper arm circumference and the tricep skinfold thickness were measured on the right side with a fibre glass insertion tape (Ross Laboratory, Columbus, OH, USA) and precision calipers (Lange, Cambridge Scientific Industries Inc, Cambridge, MA, USA), respectively.

Z-scores for height-for-age (HAZ) and weight-for-height (WHZ) were calculated from the National Center for Health

Statistics (NCHS) reference data using EPIINFO (version 6.0 USD Inc., Stone Mountain, GA, USA). Arm fat area (AFA) and arm muscle area (AMA) were calculated using standard equations (Frisancho, 1990). Z-scores for skinfolds and AMA and AFA were calculated from the U.S. NHANES I and II data for African Americans compiled by Frisancho (1990). The LMS method was used to correct for skewed body composition indices in Z-score calculations where appropriate (Davies *et al*, 1993).

### Biochemical assessment

Biochemical assessment included on-site haemoglobin assays on finger-prick blood samples using a portable haemoglobinometer and manufacturer's controls (Hemocue AB, Sweden) from 389 children at baseline and 325 children at 1 y (participation rates 62 and 52%, respectively). Hair samples were collected with stainless steel scissors from 414 children at baseline and 96 at 1 y (participation rates 66 and 15%, respectively) from the occipital portion of the scalp. The lower participation rate at 1 y arose because of the reluctance of the participants to provide hair samples at this time. Indeed, some children's heads were shaved immediately prior to the scheduled date for hair sample collection. Consequently, caution must be exercised in the interpretation of these results. Hair samples were checked for nits and lice, washed with non-ionic detergent (1% Actinox) using a standard procedure (Gibson & DeWolfe, 1979), dried, and then analysed for zinc by flame atomic absorption spectrophotometry (Smith *et al*, 1979) following acid digestion with ultra pure nitric acid (70%, Aristair BDH Laboratory Supplies). Twenty-four aliquots of a certified reference material for human hair (Commission Bureau of Reference, Reference Material no. 125) were analysed giving a mean value of 3.05  $\mu\text{mol/g}$  (s.d. = 0.11, CV = 3.5%) compared to the certified value of  $3.04 \pm 0.08 \mu\text{mol/g}$ . The CV for zinc in aliquots ( $n = 7$ ) of a pooled powdered hair sample was 2.5%.

### Morbidity assessment

Morbidity assessment was undertaken via malaria screening using thick blood smears stained with 4% Giemsa stain and examined for malaria by an experienced technician. Parasite densities were determined as the ratio of parasites to white blood cells. Anthelmintic medication (albendazole 400 mg) was distributed to all children at baseline and was available at monthly clinics from December 1996. Tests for other parasitic infections were not conducted because of reluctance by community members to provide biological samples, as shown by the significant loss to follow-up with respect to hair samples compared to anthropometry undertaken at the same time.

Morbidity picture calendars were distributed to all mothers monthly from December 1996 to track the incidence of common illnesses over one month. Mothers were taught to mark daily whether their child had experienced

fever, diarrhoea (loose watery stools), upper (cold) or lower (cough) respiratory tract infections (URTI and LRTI) over a 1 month period. Completed calendars were reviewed by a clinical officer at monthly morbidity clinics. A score of 1 was assigned if the child experienced the illness at any point during the month. Duration and severity of illness were not assessed because the measurements were based on maternal reports. An overall score of infection was calculated for each child, with one point for each of the illnesses monitored for a maximum score of four per month.

### Statistical analysis

SPSS version 8.0 was used. All continuous variables were examined for normality and transformed as necessary and are reported as mean (95% CI). Differences between groups for normally distributed or transformed variables with homogeneity of variance were assessed using a general linear model, controlling for age and sex, and ensuring that the assumptions of the model were met. Illness scores were compared using the Kruskal–Wallis test. Examination of potential impact of confounding and explanatory variables on anthropometric outcomes was conducted using the general linear model. Statistical significance of differences in proportions between intervention and control groups was assessed by Pearson  $\chi^2$  test or Fisher's exact test for categorical data. Statistical significance was set at a  $P$ -value  $< 0.05$ .

### Results

Baseline height, weight, haemoglobin and hair zinc concentrations were compared between those who also participated in the 1 y measurements and drop-outs. There were no significant differences between participants and drop-outs, suggesting that participation was random and not linked to initial differences in anthropometric or biochemical status.

### Growth and body composition data

For the intervention and control groups at baseline, when adjusted for age and sex, growth and body composition data did not differ significantly (Table 1). There were also no significant differences in the prevalence of stunting or wasting between intervention and control children at baseline, nor in any of the other growth or body composition indices examined, after controlling for those socio-economic variables that differed at baseline (ie religion, parental education and water source).

Growth and body composition data at one year, again adjusted for age and sex as well as baseline variables, are presented in Table 2. The children increased in weight and height from baseline to 12 months, gaining on average approximately 6 cm in height and 2 kg in weight. There was a small positive change in the mean Z-scores for height-for-age and weight-for-height in both groups over the 12 month period (Figure 1). However, at the end of the

**Table 1** Mean (95% CI) baseline growth and body composition variables adjusted for age and sex by group

	n	Intervention	Control	P treatment	P sex	P age
Height (cm)	434	99.5 (98.7, 100.2)	99.7 (98.7, 100.8)	0.310	0.316	< 0.001
Weight (kg)	450	15.6 (15.3, 15.8)	15.6 (15.3, 16.0)	0.534	0.04	< 0.001
HAZ	434	-1.89 (-2.06, -1.73)	-1.87 (-2.10, -1.64)	0.626	0.608	0.203
WHZ	430	-0.09 (-0.20, 0.02)	-0.06 (-0.21, 0.09)	0.693	0.256	0.179
MUACZ	433	-0.97 (-1.05, -0.89)	-1.00 (-1.12, -0.89)	0.942	0.016	0.003
TSFZ	425	-0.02 (-0.12, 0.07)	-0.21 (-0.33, -0.09)	0.078	0.003	< 0.001
AFAZ	425	-0.28 (-0.35, -0.21)	-0.42 (-0.51, 0.32)	0.175	0.028	< 0.001
AMAZ	425	-1.15 (-1.23, -1.06)	-1.13 (-1.24, -1.01)	0.807	0.002	0.236

HAZ = height-for-age Z-score; WHZ = weight-for-height Z-score; ZMUAC = mid-upper arm circumference Z-score; ZFAA = arm fat area Z-score; ZAMA = arm muscle area Z-score.

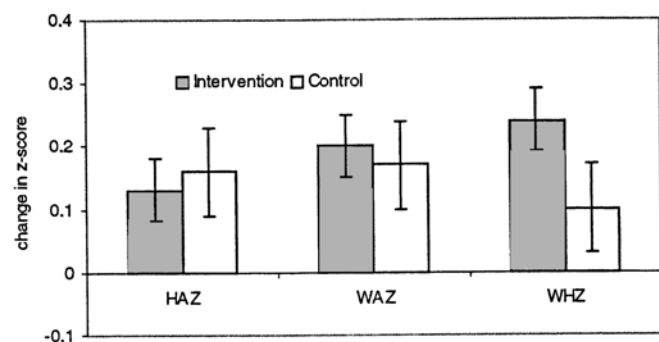
**Table 2** Mean (95% CI) 1 y growth and body composition variables adjusted for age, sex and baseline value by group

	n	Intervention	Control	P treatment	P sex	P age
Height (cm)	281	105.8 (105.3, 106.2)	105.3 (104.7, 106.0)	0.300	0.702	0.057
Weight (kg)	305	17.5 (17.3, 17.7)	17.3 (17.1, 17.6)	0.254	0.467	0.247
HAZ	281	-1.84 (-1.93, -1.74)	-1.80 (-1.94, -1.67)	0.662	0.551	< 0.001
WHZ	276	-0.08 (-0.008, 0.17)	-0.001 (-0.12, 0.12)	0.293	0.009	< 0.001
MUAC	293	-0.75 (-0.83, -0.68)	-1.05 (-1.15, -0.94)	< 0.001	0.254	< 0.001
TSFZ	274	-0.42 (-0.49, -0.36)	-0.37 (-0.46, -0.28)	0.403	0.003	0.064
AFAZ	274	-0.51 (-0.55, -0.46)	-0.50 (-0.56, -0.44)	0.902	0.011	0.061
AMAZ	287	-0.63 (-0.74, -0.52)	-1.03 (-1.19, -0.88)	< 0.001	0.020	< 0.001

HAZ = height-for-age Z-score; WHZ = weight-for-height Z-score; ZMUAC = mid-upper arm circumference Z-score; ZTSF = triceps skinfold Z-score; ZFAA = arm fat area Z-score; ZAMA = arm muscle area Z-score.

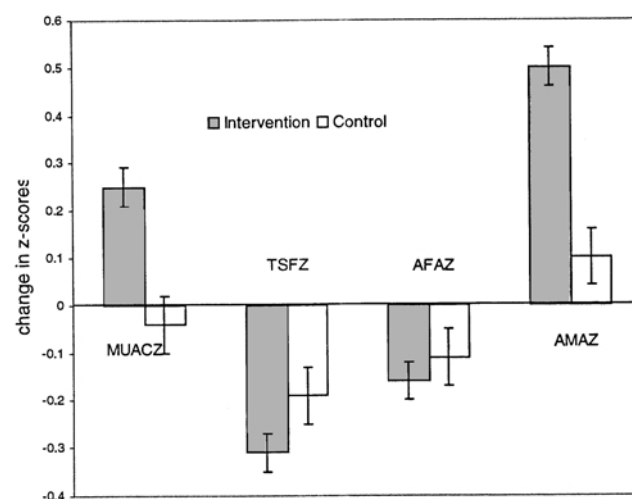
study, the overall prevalence of stunting (ie HAZ < -2 s.d.) in the intervention group had not changed (50%; *n* = 115) compared to baseline (ie 52%) and was similar to that of the control group (ie 49%; *n* = 62), whereas that of wasting (WHZ < -2 s.d.) was still very low in both groups and comparable to initial baseline values (ie 2% intervention; 1% control, NS).

Figure 1 presents the adjusted mean change in growth Z-scores by group. After controlling for age and sex, there were no significant differences between the two groups in any of the growth variables examined. Figure 2 presents the corre-



**Figure 1** Mean change in growth Z-scores by group, adjusted for age and sex.

sponding age- and sex-adjusted mean change in body composition Z-scores. On average, MUACZ and AMAZ increased in both groups over the year whereas TSFZ and AFAZ decreased. In contrast to growth, significant differences in the mean changes in Z-scores for body composition indices existed between the two groups. Mean Z-scores for MUAC



**Figure 2** Mean change in body composition Z-scores by group, adjusted for age and sex.

and AMA increased significantly more in the intervention compared to the control children after 12 months ( $P < 0.001$ ), whereas the mean Z-score for TSF tended to decrease more in the intervention than control group ( $P = 0.05$ ).

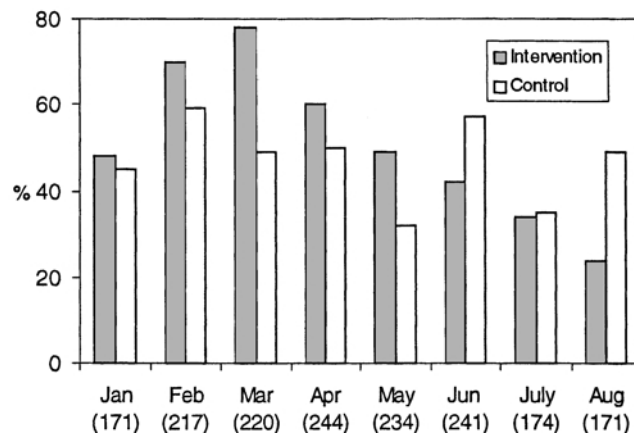
### Biochemical results

Biochemical results at baseline and the end of the study are summarized in Tables 3 and 4. All children with anaemia (ie haemoglobin  $< 110$  g/l for  $\leq 60$  months; 115 g/l for  $> 60$  months; Stoltzfus & Dreyfuss, 1998), were referred to the hospital for treatment. No significant differences in haemoglobin concentrations between the two groups existed at baseline (Table 3), but after 1 y, haemoglobin had fallen by 7 g/l on average in the control group with no change in the intervention group (Table 3;  $P < 0.05$ ). Likewise, the prevalence of anaemia did not differ significantly between groups at baseline (59 vs 62% in control and intervention group, respectively). However, at 1 y the incidence of anaemia in control children had increased to 80%, but remained unchanged (ie 62%) in the intervention children ( $P < 0.001$ ).

In contrast to haemoglobin, there were no significant differences in initial or final hair zinc concentration between groups, although concentrations increased in control children from baseline values at 12 months (2.72 vs 2.91  $\mu\text{mol/g}$ ,  $P < 0.05$ ).

### Morbidity

The prevalence of malaria in the subjects overall increased from 33% ( $n = 156$ ) at baseline to 45% ( $n = 195$ ) at 1 y. At baseline, prevalence of malaria was lower in the intervention compared to control children (26 vs 48%,  $P < 0.001$ ), but at 1 y no significant difference in malaria incidence existed between the intervention and control groups, after controlling for baseline status (data not reported). Children testing positive for malaria at the time of the one year blood test had a lower mean haemoglobin concentration (104 vs 107 g/l,  $P < 0.01$ ), and a higher prevalence of anaemia (74 vs 26%,  $\chi^2 < 0.01$ ) than those with a negative test, after controlling for age and sex.



**Figure 3** Percentage reporting at least one illness by group per month (total  $n$ ). The rainy season extends from January to April.

In both groups, the incidence of all individual illnesses reported by mothers followed a similar pattern, being highest in January through March, corresponding to the height of the rainy, pre-harvest hungry season. In the intervention children, the median illness scores were significantly higher in February, March and May compared to controls, a trend that was reversed in June and August when the median illness scores were significantly lower for the intervention compared to the control group (Figure 3).

### Inter-relationships among anthropometric, biochemical and morbidity variables

A series of general linear models were examined to determine the effect of treatment group, hair zinc and haemoglobin concentrations and morbidity on final anthropometric outcomes. Age, sex and baseline values were included in each model for consistency. As expected, baseline anthropometric values explained a significant proportion of the variation in all anthropometric outcomes. Age was significant for all Z-scores, with the exception of AFAZ, whereas sex explained a significant proportion of the variation in WHZ, AMAZ and AFAZ.

**Table 3** Mean (95% CI) baseline and one year haemoglobin concentrations (g/l) adjusted for age and sex by group

n	Intervention	Control	P treatment	P sex	P age
Baseline ( $n = 389$ )	107.8 (105.8, 109.8)	109.6 (106.8, 112.4)	0.300	0.333	$< 0.001$
One year ( $n = 325$ )	107.1 (105.2, 109.0)	102.2 (99.6, 104.7)	0.003	0.289	$< 0.001$

**Table 4** Mean (95% CI) baseline and one year hair zinc concentrations ( $\mu\text{mol/g}$ ) adjusted for age and sex by group

n	Intervention	Control	P treatment	P sex	P age
Baseline ( $n = 414$ )	2.74 (2.68, 2.80)	2.68 (2.59, 2.76)	0.239	0.002	0.002
One year ( $n = 96$ )	2.72 (2.58, 2.86)	2.91 (2.73, 3.08)	0.103	0.013	0.420

With respect to potential explanatory variables, treatment group was a significant predictor of MUACZ ( $\beta=0.298$ ,  $P<0.001$ ) and AMAZ ( $\beta=0.650$ ,  $P<0.001$ ). Final haemoglobin concentration was a significant predictor of height ( $\beta=0.368$ ,  $P<0.05$ ) and HAZ ( $\beta=0.070$ ,  $P<0.05$ ). Final hair zinc concentration approached significance in predicting MUACZ ( $\beta=-0.175$ ,  $P=0.06$ ). Neither average illness score nor occurrence of malaria was significant in any of the models examined. However, there was a consistent trend for children with higher illness scores to have lower values for all anthropometric outcomes, with the exception of WHZ, which tended to be higher. These trends persisted, even after controlling for differences in SES variables at baseline.

## Discussion

To our knowledge, no dietary diversification/modification trials designed to improve simultaneously the iron, zinc and vitamin A nutrition of children in Africa or elsewhere, have been published. Consequently, we have compared our results with those of supplementation trials, based mainly on single micronutrients, and conducted on children whose growth status was similar to that of the children reported here (ie stunted but not wasted). However, when comparing our results with these supplementation trials, a much smaller effect of the dietary strategies is to be expected: the increased intake of the micronutrients is very much lower in a dietary strategy relative to a supplementation trial.

### Inter-relationship between anthropometric indices, morbidity and the treatment

Our 6 month micronutrient dietary intervention demonstrated a significant effect on the lean body mass of these stunted rural Malawian children after 12 months, but no impact on their height or weight gain. Specifically, changes in Z-scores for MUAC and AMA were significantly greater in the intervention than the control group after 12 months (Figure 1); no comparable improvement in HAZ and WHZ scores was observed.

Only a few double-blind supplementation trials with zinc (Bates *et al*, 1993; Cavan *et al*, 1993; Friis *et al*, 1997; Kikafunda *et al*, 1998; Ruz *et al*, 1997; Rivera *et al*, 1998; Umeta *et al*, 2000), iron (Latham *et al*, 1990), or vitamin A (West *et al*, 1988, 1997) have assessed anthropometric indices of body composition as well as growth. Of these, several zinc (but not iron or vitamin A) supplementation studies have noted improvements in indices of lean body mass, comparable to those reported here, with no effect on linear growth (Bates *et al*, 1993; Friis *et al*, 1997; Kikafunda *et al*, 1998). This is not surprising because zinc has a much more clearly defined role in lean tissue deposition than vitamin A or iron, through a regulatory role in protein synthesis, reportedly mediated via its interaction with insulin activity and changes in serum IGF-I levels (Begin-Heick *et al*, 1985; Clausen & Dorup, 1998). For example, in severely malnour-

ished Jamaican children, accretion of lean tissue markedly improved in response to a zinc supplement (Golden & Golden, 1981), consistent with findings observed in adults (Prasad, 1991) and in animal studies (Giugliano & Millward, 1984, 1987; Morgan *et al*, 1988). Therefore, it is possible that the significantly greater increases in MUAC and AMA Z-scores reported in the intervention children studied here may have arisen because of their significantly higher intakes of available dietary zinc compared to those of the control children (Yeudall *et al*, 2002). Such improvements in available zinc in the diets of the intervention compared to the control children (ie 3.2 vs 2.3 mg/day;  $P<0.001$ ) were attributed, in part, to their lower intakes of phytate (638 vs 812 mg/day;  $P<0.01$ ) and lower phytate: Zn molar ratios (16 vs 20;  $P<0.001$ ), together with their higher intakes of animal foods, especially fish (62 vs 48 g;  $P<0.010$ ), a rich source of readily available zinc.

Zinc, together with iron and vitamin A, may also have indirect effects on body composition via their role in immune competence. Any reduction in diarrheal and respiratory infections and associated symptoms such as vomiting, fever and anorexia, induced by improved zinc, iron and/or vitamin A nutrition, is likely to enhance absorption and utilization of energy and nutrients, leading to a greater production of cellular energy and accelerated protein anabolism. In the present study, the intervention children had a significantly lower incidence of common infectious illnesses than the controls after the rainy season, despite a higher incidence earlier. Such trends, may have been associated in part with the enhanced bioavailability of zinc, vitamin A, and possibly iron, noted in the diets of the intervention children compared to their corresponding controls. Certainly, at the end of the study, animal sources (5 vs 3%,  $P<0.001$ ) and fat (11 vs 9%,  $P<0.001$ ) contributed significantly more energy in the diets of the intervention children, whereas their phytate intakes were lower (638 vs 812 mg/day,  $P<0.01$ ) than those of the controls (Yeudall *et al*, 2002). Unequal access to medical services was unlikely to be responsible for these morbidity findings because in the present study a clinical officer visited both the intervention and control communities on a monthly basis.

In several zinc supplementation studies (Walravens & Hambidge, 1976; Walravens *et al*, 1983; 1989; Gibson *et al*, 1989; Castillo-Duran *et al*, 1994) in which growth but not body composition was measured, positive responses in terms of height and/or weight gain have been reported in the supplemented compared to the placebo children. Less consistent positive effects on growth have been observed in vitamin A or iron supplementation studies (Angeles *et al*, 1993; Lawless *et al*, 1994; Bahl *et al*, 1997). Our dietary intervention did not produce any significant improvement in linear or ponderal growth, despite the marked reduction in phytate intakes (638 vs 812 mg,  $P<0.01$ ) and thus greater intakes of available dietary zinc (3.2 vs 2.3 mg,  $P<0.001$ ) in the intervention compared to the control children (Yeudall *et al*, 2002). There are at least three possible explanations for

our failure to observe a positive response of our dietary intervention on growth; these are itemized below:

- In most nutrition supplementation studies, the greatest effects on linear growth have occurred during the first year of life. Linear growth is very fast at this time (ie approximately 20 cm/y), compared to the expected 7 cm/y on average for the 3–7 y children studied here (Baumgartner *et al*, 1986). In a 3 y study in Guatemala, for example, the nutritional supplement had the greatest impact on the linear growth of children aged less than three years but no impact on children aged 3–7 y (Ruel *et al*, 1995). Most of the children studied here were more than three years of age, their ages ranging from 30 to 90 months. We selected this age for our target group, despite their slower growth rate, because our intervention involved strategies to modify habitual Malawian maize-based diets rather than supplying a nutritional supplement. Children of this age in Malawi are known to receive more than 50% of their energy from maize (Ferguson *et al*, 1993) and hence have high phytate intakes. In addition, they are not breastfeeding (National Statistical Office, 1997), so that we could readily quantify their dietary intakes.
- The 12 month time frame over which the change in our anthropometric indices was evaluated, was probably too short to detect even a small improvement in linear growth anticipated for this age group, especially because catch-up growth in height tends to occur after weight gain, even in marginally malnourished children (Brown *et al*, 1982). (Walker & Golden 1988). More sensitive techniques such as knee-height measurements might have helped detect any subtle improvement (Himes *et al*, 1991). Certainly, Penland *et al*, (1997) reported a significant increase in knee-height after only 10 weeks, in Chinese children aged 6–9 y of age receiving a daily supplement containing 20 mg Zn plus micronutrients compared to those receiving a daily supplement of 20 mg Zn alone.
- In rural Malawi where the prevalence of stunting among rural adults is high (Gibson & Huddle, 1998), any growth response is likely to be constrained by a combination of intrauterine growth retardation and inter-generational effects of malnutrition. Indeed, mothers of the stunted children of this study were significantly shorter than those of non-stunted children (153.6 vs 155.7 cm;  $P < 0.001$ ), despite no differences in SES.

#### **Inter-relationship between haemoglobin concentrations, morbidity and the treatment**

Haemoglobin concentrations presented here (Table 3) were significantly higher in the intervention compared to the control group at 12 months, after adjusting for age, sex and baseline variables. As a result, the incidence of anaemia was lower in the intervention than in the control group post-intervention (62 vs 80%;  $P < 0.001$ ). Although our dietary intervention was not designed to identify which etiological

factor(s) were responsible for the higher haemoglobin values in the intervention group, three critical factors are worthy of discussion in relation to the haemoglobin results: malaria, other infections, and nutritional status.

The incidence of malaria post-intervention was in general higher compared to the previous year (45% vs 33%), probably as a result of increased rainfall (Famine Early Warning System, 1997). We suspect that the decline in haemoglobin concentrations in the control group was a function of this increased incidence of malaria. Certainly, at the end of 12 months, the prevalence of anaemia was higher in malaria-positive children. However, the prevalence of malaria was similar in the control and intervention groups at 12 months, suggesting that malaria was not implicated in the differences in anaemia prevalence that distinguish the two groups at the end of the study.

Low haemoglobin concentrations may also result from a variety of other parasitic infections including hookworm. To minimize this potential confounder, all the children studied were treated initially with albendazole and then monthly thereafter, where necessary, even though hookworm is not a major cause of anaemia in rural Malawian children (Williams *et al*, 2000). Schistosomiasis may also reduce haemoglobin concentrations (Stoltzfus *et al*, 1997). We were not able to test the children for schistosomiasis because the communities were very reluctant to provide urine samples. However, the prevalence of schistosomiasis in the control and intervention villages was likely to be similar because all the children lived in close proximity to Lake Malombe.

Several nutrients are known to have a role in erythropoiesis, and deficiencies of iron, vitamin B<sub>12</sub>, folate, riboflavin, and vitamin A (via its interaction with iron; Bloem *et al*, 1989) have been implicated in the etiology of anaemia reported earlier in Malawi (van den Brock & Letsky, 2000). Indeed, our Malawian control children consuming their habitual maize-based diets were at high risk for inadequate intakes of iron and vitamin B<sub>12</sub> (Yeudall *et al*, 2002). Implementation of our dietary strategies significantly decreased the prevalence of inadequate intakes of vitamin B<sub>12</sub> in the intervention compared to the control group (ie 23 vs 41%;  $P = 0.001$ ), as a result of their increased intake of animal products, mainly fish. Hence, enhancing the vitamin B<sub>12</sub> nutriture of the intervention group could have contributed to the lower incidence of anaemia at 1 y in the intervention compared to the control group. Also, an increase in the bioavailability of iron induced by alterations in the content of non-haem iron absorption modifiers, particularly phytate and cellular animal protein in the diets of the intervention children, may have also played a role.

#### **Inter-relationship between hair zinc concentrations and the treatment**

Unlike haemoglobin, no significant differences in hair zinc concentrations were apparent between the two groups post-intervention (Table 4), even though calculated intakes of

available zinc were significantly higher in the intervention compared to the control group (Yeudall *et al*, 2002). A similar finding has been reported in several zinc supplementation trials; hair zinc values have not increased in response to a daily zinc supplement, even after twelve months (Gibson *et al*, 1989; Cavan *et al*, 1993).

It is noteworthy that the hair zinc concentrations for both females and males presented here are consistently higher at baseline and after 12 months than those reported earlier for rural Malawian children aged 4–6 y studied at the same season of the year (Ferguson *et al*, 1993), as well as those from some earlier studies in other developing countries (Cavan *et al*, 1993; Gibson *et al*, 1991). This apparent discrepancy with earlier findings is unlikely to be attributed to differences in the hair zinc analyses because the accuracy and precision of the methods were established both in the present and earlier studies by use of certified reference materials and pooled hair samples, respectively. As well, the girls in the present study had consistently higher hair zinc values than boys at baseline and after 12 months, a pattern consistent with earlier reports (Hambidge *et al*, 1972; Buzina *et al*, 1980; Smit-Vanderkooy & Gibson, 1987; Gibson *et al*, 1991; Cavan *et al*, 1993).

None of the children in this study at baseline, or at 12 months had low hair zinc concentrations indicative of sub-optimal zinc status, based on conventional cut-off values, even though they had low mean height-for-age Z-scores. This is in marked contrast to our earlier small study ( $n=57$ ) of younger ( $62 \pm 10$  months) rural Malawian preschool children of whom 27% had hair zinc values below a cut-off value of  $1.07 \mu\text{mol/g}$ . For children with impaired linear growth, zinc may not necessarily be the first limiting nutrient (Hauvast *et al*, 2000). Linear growth faltering may arise from multiple causes including the effects of chronic infections, prenatal and inter-generational effects of maternal malnutrition, as well as the co-existence of multiple micronutrient inadequacies in the diet, especially when habitual diets are maize-based.

Of the micronutrients, deficits in iron, riboflavin, vitamin A, iodine, and/or zinc have all been implicated in linear growth faltering (Gibson & Hotz, 2001b). Thus it is perhaps not surprising that when zinc is not the first growth-limiting nutrient, some children with impaired growth may have apparently normal hair zinc concentrations. In these circumstances, children are unlikely to show any improvement in linear growth in response to a zinc-supplement (Gibson *et al*, 1989; Bates *et al*, 1993; Friis *et al*, 1997), despite being stunted. In the present study, even the stunted Malawian children in the control group had an intake of bioavailable zinc (calculated to take into account the inhibiting effect of phytate on zinc absorption) that was above the estimated normative physiological requirement (ie  $1.2 \text{ mg/day}$ ) set by WHO (1996). Moreover, these Malawian children probably had a reduced demand for zinc compared to healthy children growing normally, because of their smaller body size, and possibly greater intestinal conservation of endogenous

zinc (Lee *et al*, 1993). The latter may have arisen as a result of an adaptation to the low content of absorbable zinc in their habitual high phytate maize-based diets. This hypothesis, if correct, would explain the apparently normal hair zinc concentrations of these stunted Malawian children.

These results emphasize that only in circumstances when zinc is the first limiting nutrient can significant relationships between hair zinc concentrations and physiological functional indices of zinc status such as linear growth and taste acuity be expected, as observed in some earlier studies (Hambidge *et al*, 1972; Gibson *et al*, 1989; Buzina *et al*, 1980; Xue-Cun *et al*, 1985; Smit-Vanderkooy & Gibson, 1987; Cavan *et al*, 1993, Umeta *et al*, 2000). Several other investigators have emphasized that the co-existence of multiple micronutrient deficiencies limits the response of children to a zinc supplement, especially in developing countries, where the quality of their diets is often poor (Rosado *et al*, 1997; Rivera *et al*, 2001). The dietary results of this present study have confirmed this suggestion, and have highlighted the inadequate intakes of iron, zinc, vitamin B<sub>12</sub>, calcium and vitamin A in Malawian children consuming habitual maize-based diets. For example, the predicted prevalence of inadequate intakes in our control group were 20% for available iron, 44% for available zinc (assuming a normative requirement estimate), 41% for vitamin B<sub>12</sub>, 54% for calcium and 17% for vitamin A (assuming a normative requirement estimate). Moreover, of these inadequacies, the most common combinations of deficits were those of calcium + iron + zinc and the vitamins A and B<sub>12</sub> (Yeudall

*et al*, 2002). Hence, deficits in intakes of all these nutrients, with the exception of vitamin B<sub>12</sub>, could potentially be limiting linear growth in these Malawian children. Only after overcoming deficits in all these growth-limiting nutrients, can a significant growth response be expected.

## Conclusions

In conclusion, in these stunted Malawian children, our dietary strategies had a favourable effect on lean body mass but not growth, and were associated with reductions in the incidence of anaemia and common infectious illnesses. Longer dietary diversification/modification interventions of this type are needed to show an impact on growth for children aged 30–90 months of age.

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## References

- Angeles I, Schultink W, Matulesi P & Gross R (1993): Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. *Am. J. Clin. Nutr.* **58**, 339–342.
- Bahl R, Bhandari N, Taneja S & Bhan M (1997): The impact of vitamin A supplementation on physical growth of children is dependent on season. *Eur. J. Clin. Nutr.* **51**, 26–29.
- Bates C, Evans P, Dardenne M, Prentice A, Lunn PG, Northrop-Clewes CA, Hoare S, Cole TJ, Horan SJ, Longman SC, Stirling D & Aggett PJ (1993): A trial of zinc supplementation in young rural Gambian children. *Br. J. Nutr.* **69**, 243–255.
- Baumgartner RN, Roche AF & Himes JH (1986): Incremental growth tables: supplementary to previously published charts. *Am. J. Clin. Nutr.* **43**, 711–722.
- Beard JL (2001): Iron biology in immune function, muscle metabolism and neuronal functioning. *J. Nutr.* **131**, 568S–580S.
- Beaton GH, Martorell R, Aronson KJ, Edmonston G, McCabe G, Ross AC & Harvey B (1993): *Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries*. International Nutrition Program, Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, ACC/SCN State-of-the Art Series Nutrition Policy Discussion Paper no. 13. New York: United Nations.
- Begin-Heick N, Dalpe-Scott M, Rowe J & Heick HMC (1985): Zinc supplementation attenuates insulin secretory activity in pancreatic islets of the ob/ob mice. *Diabetes* **34**, 179–184.
- Bloem MW, Wedel M, Egger RJ, Speek AJ, Schrijver J, Saowakontha S & Schreurs WH (1989): Iron metabolism and vitamin A deficiency in children in northeast Thailand. *Am. J. Clin. Nutr.* **50**, 332–338.
- Brown KH, Black RE & Becker S (1982): Seasonal changes in nutritional status and the prevalence of malnutrition in a longitudinal study of young children in rural Bangladesh. *Am. J. Clin. Nutr.* **36**, 303–313.
- Brown K, Peerson J & Allen L (1998): Effect of zinc supplementation on children's growth: a meta-analysis of intervention trials. *Bibl. Nutr. Dieta.* **54**, 76–83.
- Buzina R, Jusic M, Sapunar J & Milanovic N (1980): Zinc nutrition and taste acuity in school children with impaired growth. *Am. J. Clin. Nutr.* **33**, 2262–2267.
- Castillo-Duran C, Garcia H, Venegas P, Torrealba I, Panteon E, Conche N & Perez P (1994): Zinc supplementation increases growth velocity of male children and adolescents with short children. *Acta Paediatr. Scand.* **83**, 833–837.
- Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M & Solomons NW (1993): Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. *Am. J. Clin. Nutr.* **57**, 344–352.
- Clausen T & Dorup I (1998): Micronutrients, minerals and growth control. *Bibl. Nutr. Dieta* **54**, 84–92.
- Davies P, Day J & Cole T (1993): Converting Tanner-Whitehouse reference triceps and subscapular skinfold measurements to standard deviation scores. *Eur. J. Clin. Nutr.* **47**, 559–566.
- Famine Early Warning System (1997): *Special Report 97-2, Southern Africa Food Security Update* [Online], 26 March 1997 (Cited 12 February 2001). Available from URL: [www.fews.org](http://www.fews.org)
- Ferguson EL, Gibson RS, 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.
- Friis H, Ndhlovu P, Mduluzi T, Kaondera K, Sandström B, Michaelsen KF, Vennervald BJ & Christensen NO (1997): The impact of zinc supplementation on growth and body composition: a randomized controlled trial among rural Zimbabwean school children. *Eur. J. Clin. Nutr.* **51**, 38–45.
- Frisancho A (1990): *Anthropometric standards for the assessment of growth and nutritional status*. Ann Arbor, ME University of Michigan Press.
- Gibson R & DeWolfe M (1979): The zinc, copper, manganese, vanadium and iodine content of hair from 38 Canadian neonates. *Pediatr. Res.* **13**, 959–962.
- Gibson RS & Hotz C (2001a): Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *Br. J. Nutr.* **85**(Suppl 2), S159–S166.
- Gibson RS & Hotz C (2001b): Nutritional causes of linear growth faltering in infants during the complementary feeding period. In: *Nutrition and Growth*, ed. R Martorells F Haschke. 47th Nestle Nutrition Workshop Series, Pediatric program. Philadelphia, PA: Lippincott, Williams & Wilkins, pp 159–196.
- Gibson RS & Huddle J-M (1998): Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. *Am. J. Clin. Nutr.* **67**, 702–709.
- Gibson RS, Smit-Vanderkooy PD, MacDonald AC, Goldman A, Ryan B & Berry M (1989): A growth limiting mild zinc deficiency syndrome in some Southern Ontario boys with low growth percentiles. *Am. J. Clin. Nutr.* **49**, 1266–1273.
- Gibson RS, Heywood A, Yaman C, Thompson LU & Heywood P (1991): Food consumption patterns and trace element intakes of children from the Wosera, Papua New Guinea. *Ecol. Food. Nutr.* **25**, 69–77.
- Gibson RS, Yeudall F, Drost N, Mitimuni B & Cullinan T (1998): Dietary interventions to prevent zinc deficiency. *Am. J. Clin. Nutr.* **68**, 484S–487S.
- Golden M & Golden B (1981): Plasma zinc, rate of weight gain, and energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet. *Am. J. Clin. Nutr.* **34**, 892–899.
- Golden B & Golden M (1992): Effect of zinc on lean tissue synthesis during recovery from malnutrition. *Eur. J. Clin. Nutr.* **46**, 697–706.
- Giugliano R & Millward DJ (1984): Growth and zinc homeostasis in the severely Zn-deficient rats. *Br. J. Nutr.* **52**, 545–560.
- Giugliano R & Millward DI (1987): The effects of severe zinc deficiency on protein turnover in muscle and thymus. *Br. J. Nutr.* **57**, 139–155.
- Hambidge KM, Hambidge C, Jacobs M & Baum JD (1972): Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. *Pediatr. Res.* **6**, 868–874.
- Hauvast JLA, Tolboom JJM, Kafwembe EM, Musonda RM, Mwanakale 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.
- Himes JH, Carson C & Pheley A (1991): Measurement errors for a portable device measuring short-term growth. *Am. J. Hum. Biol.* **3**, 325–329.
- Kikafunda JK, Walker AF, Allan EF & Tumwine JK (1998): Effect of zinc supplementation on growth and body composition of Ugandan preschool children: a randomized, controlled, intervention trial. *Am. J. Clin. Nutr.* **68**, 1261–1266.
- Latham MC, Stephenson LS, Kinoti SN, Zaman MS & Kurz KM (1990): Improvements in growth following iron supplementation in young Kenyan school children. *Nutrition* **6**, 159–165.
- Lawless J, Latham M, Stephenson L, Kinoti S & Pertet A (1994): Iron supplementation improves appetite and growth in anemic Kenyan primary school children. *J. Nutr.* **124**, 645–654.
- Lee DY, Prasad AS, Hydrick-Adair 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.
- Lohman T, Roche A & Martorell R (1988): *Anthropometric Standardization Reference Manual*. Champagne IL: Human Kinetics Books.
- Morgan N, Keen CL & Lönnerdal B (1988): Effect of varying dietary zinc intake of weanling mouse pups during recovery from early undernutrition on growth, body composition and composition of gain. *J. Nutr.* **118**, 690–698.

- National Statistical Office (1997): *Malawi demographic and health survey*. Zomba: National Statistical Office.
- Penland JG, Sandstead HH, Alcock NW, Dayal HH, Chen XC, Li JS, Zgao F & Yang JJ (1997): A preliminary report: effects of zinc and micronutrient repletion on growth and neuropsychological function of urban Chinese children. *J. Am. Coll. Nutr.* **16**, 268–272.
- Prasad AS (1991): Discovery of human zinc deficiency and studies in an experimental human model. *Am. J. Clin. Nutr.* **53**, 403–412.
- Rivera JA, Ruel MT, Santizo MC, Lönnerdal B & Brown KH (1998): Zinc supplementation improves the growth of stunted rural Guatemalan infants. *J. Nutr.* **128**, 556–562.
- Rivera JA, González-Cossio T, Flores M, Romero M, Rivera M, Téllez-Rojo MM, Rosado JL & Brown KH (2001): Multiple micronutrient supplementation increases the growth of Mexican infants. *Am. J. Clin. Nutr.* **74**, 657–663.
- Rosado JL, Lopez P, Munoz E, Martinez H & Allen LH (1997): Zinc supplementation reduces morbidity, but neither zinc nor iron supplementation affects growth or body composition of Mexican preschoolers. *Am. J. Clin. Nutr.* **65**, 13–29.
- Ruel M, Rivera J, Habicht J & Martorell R (1995): Differential response to early nutrition supplementation: long term effects on height at adolescence. *Int. J. Epidemiol.* **24**, 404–412.
- Ruz M, Castillo-Duran C, Lara X, Codoceo J, Rebollo A & Atalah E (1997): A 14-mo zinc supplementation trial in apparently healthy Chilean preschool children. *Am. J. Clin. Nutr.* **66**, 1406–1413.
- Shankar AH & Prasad AS (1998): Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr.* **68**(Suppl), 447S–463S.
- Smith JC Jr, Butronovitz GP & Purdy WC (1979): Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clin. Chem.* **25**, 1487–1491.
- Smit-Vanderkooy PD & Gibson RS (1987): Food consumption patterns of Canadian children in relation to zinc and growth status. *Am. J. Clin. Nutr.* **45**, 609–616.
- Stoltzfus RJ & Dreyfuss ML (1998): International Nutritional Anemia Consultative Group/WHO/UNICEF. *Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia*. Washington, DC: ILSI Press.
- Stoltzfus R, Chwaya H, Albobnico M, Schulze K, Tielsch J & Savioli L (1997): Epidemiology of iron deficiency and anemia in Zanzibari school children. *Am. J. Clin. Nutr.* **65**, 153–159.
- Umata L, West CE, Haidar J, Deurenberg P & Hautvast JG (2000): Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. *Lancet* **355**, 2021–2026.
- van den Broek NR & Letsky EA (2000): Etiology of anemia in pregnancy in south Malawi. *Am. J. Clin. Nutr.* **72**(Suppl), 247S–256S.
- Walker SP & Golden M (1988): Growth in length of children recovering from severe malnutrition. *Eur. J. Clin. Nutr.* **42**, 395–404.
- Walravens PA & Hambidge KA (1976): Growth of infants fed a zinc supplemented formula. *Am. J. Clin. Nutr.* **29**, 1114–1121.
- Walravens PA, Krebs NF & Hambidge KM (1983): Linear growth of low income preschool children receiving a zinc supplement. *Am. J. Clin. Nutr.* **38**, 195–201.
- Walravens PA, Hambidge KM & Koepfer MA (1989): Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind controlled study. *Pediatrics* **83**, 532–538.
- West K, Djunaedi E, Pandji A, Kusidiono, Tarwotjo I & Sommer A (1988): Vitamin A supplementation and growth: a randomized community trial. *Am. J. Clin. Nutr.* **48**, 1257–1264.
- West KP, LeClerq SC, Shreshtha SR, Wu LS-F, Pradhan EK, Khatri SK, Katz J, Adhikari R & Sommer A (1997): Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. *Am. J. Clin. Nutr.* **127**, 1957–1965.
- WHO (1996) Zinc. In: *Trace Elements in Human Nutrition and Health*, pp. 72–104. Geneva: World Health Organization.
- Williams T, Young M, Ramakrishnan U & Schroeder D (2000): *The relationship of hookworm infection to anemia among pre-school children in rural Malawi*. San Diego, CA FASEB, A510 (abstract).
- Xue-Cun C, Tai-An Y, Jin-Sheng H, Qiu-Yan M, Zhi-Min H, & Li-Xiang (1985): Low levels of zinc in hair and blood, pica, anorexia, and poor growth in Chinese preschool children. *Am. J. Clin. Nutr.* **43**, 694–700.
- Yeudall Y, Gibson RS & Mtimuni B (2002): Impact of a community-based dietary intervention on micronutrient adequacy of high phytate maize-based diets of rural Malawian children. *Pub. Hlth Nutr.* (in press).