

# Animal Source Foods to Improve Micronutrient Nutrition and Human Function in Developing Countries

## Experiences of a Community-Based Dietary Intervention to Enhance Micronutrient Adequacy of Diets Low in Animal Source Foods and High in Phytate: A Case Study in Rural Malawian Children<sup>1</sup>

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**ABSTRACT** A community-based dietary diversification/modification intervention, employing a quasiexperimental design with a nonequivalent control group, was conducted in two intervention and two control villages in rural Southern Malawi. The aim was to enhance the content and bioavailability of micronutrients in maize-based diets of stunted children ages 30–90 mo. Efficacy was evaluated through a comparison of the changes in knowledge and practices, anthropometry, malaria screening, hemoglobin and hair zinc after 12 mo, common infections monthly postintervention and nutrient adequacy postintervention via 24-h recalls. Intervention diets were more diverse and of higher quality than the control diets, supplying significantly more animal source foods, especially soft-boned fish, but less phytic acid ( $p < 0.01$ ). Median intakes of energy, protein, calcium, available zinc, heme iron and vitamin B-12 were greater ( $p < 0.05$ ) in intervention compared to the control groups; some spread of knowledge and practices to the control groups occurred. Intervention enhanced Z-scores for mid-upper-arm circumference and arm muscle area ( $p < 0.001$ ), but had no impact on weight or height gain. After controlling for baseline variables, mean hemoglobin was higher (107 vs. 102 g/L;  $p < 0.01$ ) postintervention, whereas incidence of anemia and common infections were lower in the intervention groups compared to the control groups, with no change in malaria or hair zinc status. Dietary strategies reduced the prevalence of inadequate intakes of protein, calcium, zinc and vitamin B-12, but not iron, because fish was the major source of animal food consumed. More efforts to raise small animals and promote their consumption are needed to enhance dietary quality and ensure optimal growth, health and cognitive development in young Malawian children. *J. Nutr.* 133: 3992S–3999S, 2003.

**KEY WORDS:** • animal source foods • phytic acid • dietary quality • Malawi • micronutrients

Chronic malnutrition arising from the interaction between inadequate dietary intakes and morbidity has a major impact on growth and development of African children. In rural Malawi, inadequate intakes of several nutrients are widespread because staple diets are predominantly maize based, and intakes of animal source foods are low (1, 2). Consequently, the content

and bioavailability of iron, zinc, preformed vitamin A, vitamin B-12 and calcium in these rural diets are often low. Because most of these nutrients have a critical role in immune competence and/or linear growth, it is not surprising that deficiencies of these nutrients have been associated in part with the high prevalence of morbidity and stunting reported in Malawian children (3).

A dietary intervention strategy that simultaneously enhances the content and bioavailability of iron, zinc, preformed vitamin A, vitamin B-12 and calcium in rural Malawian diets is required urgently. We developed a unique food-based intervention for use in countries in Southern Africa where staple diets are low in animal source foods and based on unfermented maize. The intervention was based on dietary diversification and modification to certain household methods for preparing and processing indigenous foods. Specifically, these dietary strategies involved: *i*) increasing production and consumption of micronutrient dense foods including animal source foods, especially whole dried fish with bones, and orange-red fruits

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such as papayas and mangoes (fresh and dried); *ii*) incorporating absorption enhancers of iron, zinc, provitamin A carotenoids and vitamin A in household diets; and *iii*) germination, fermentation and soaking to reduce the phytate content of maize and/or legumes by enzyme-induced hydrolysis of phytate and/or passive diffusion of water soluble phytate. A participatory approach was used to implement the strategies to enhance their awareness, feasibility and acceptability to caregivers in the local community. Ongoing nutrition education and community mobilization techniques were utilized to enhance the adoption of the dietary strategies and to empower the community to sustain them. Details of these dietary strategies and their implementation were published earlier (4). A team of specialists in agriculture, foods and nutrition, home economics, psychology, adult education and rural extension, as well as community health, were involved in this multidisciplinary project.

We assessed the efficacy of our dietary diversification/modification on Malawian children ages 30–90 mo using a quasiexperimental design. This was achieved by comparing: 1) content and bioavailability of micronutrients in their diets; 2) growth and body composition via anthropometric indices; 3) incidence of malaria and common infections; 4) anemia; and 5) hair zinc concentrations of two groups of children. One group received their habitual rural maize-based Malawian diet modified by our dietary intervention for 6 mo, and a second group consumed an unmodified diet. Nutrition knowledge and practices related to the preparation and consumption of micronutrient-rich foods were also assessed in the two groups. Control communities received the intervention at the end of the postintervention data-collection period.

## METHODS

The study population consisted of households with children born between 1989 and 1993 in two intervention and two control villages in Mangochi District in Southern Malawi. Intervention and control communities were located ~40 min walking distance apart, and were comparable with respect to distance to the lakeshore and socioeconomic variables. The heads of households were primarily involved in subsistence agriculture. Villages were selected by an advisory committee, in keeping with the participatory approach, based on perceived need and similar socioeconomic and food production characteristics (primarily access to fish). Staff working in the communities from the Ministries of Agriculture, Health, and Community Services and Social Welfare were among the advisory committee members. No attempt was made to select a random sample of children for this project because this approach was not acceptable to the advisory committee. The community group leaders chose the project name "Tulimbe," which means "let us be strong" because it embodies the idea of empowerment or self-reliance. Children ages 30–90 mo were chosen as the target group because they are no longer being breastfed in rural Malawi (3), and they consume a high phytate diet in which >50% of energy is from maize (1). Therefore, our phytate reduction strategies had the potential to enhance the bioavailability of iron, zinc and calcium in these rural children's diets. Children's ages were validated, where possible, by comparing stated birth dates to documented birth dates, or by agreement of birth dates across survey periods.

Details of this study were published elsewhere (5, 6). Briefly, the study was approved by the Health Sciences Research Committee, University of Malawi and the University of Otago Human Ethics Committee, New Zealand. All children were treated with antihelminthic medication (albendazole) at baseline and, when necessary, at monthly clinics beginning in December, 1996. Baseline comparability between the intervention and the control groups was assessed via sociodemographic status and knowledge and practices questionnaires, anthropometry, hemoglobin and hair zinc status; no significant differences were apparent. All these measurements were repeated after 12 mo, with the exception of the questionnaire on

sociodemographic status. The pretested knowledge and practices (KP)<sup>5</sup> questionnaire was administered in the households and developed from earlier questionnaires (7), as well as interviews and focus groups conducted in the study communities.

Standing height (to nearest mm), weight (to nearest 0.1 pound), mid-upper-arm circumference (MUAC) and triceps skinfolds were taken in triplicate using calibrated equipment and standardized techniques (8), with children wearing light clothing and no shoes. Each measurement was taken by the same trained anthropometrist to eliminate interexaminer error. Z-scores for height-for-age (HAZ) and weight-for-height (WHZ), skinfolds, arm muscle area (AMA) and arm fat area (AFA) were calculated as described earlier (6).

Hemoglobin was assayed via finger-prick blood samples using a portable hemoglobinometer (Hemocue AB). Hair samples were collected from the occipital portion of the scalp, and checked for nits and lice before washing by a standardized method (9). Analysis for zinc was carried out by flame atomic absorption spectrophotometry (10) after acid digestion.

Morbidity assessment was undertaken via malaria screening using thick blood smears and morbidity picture calendars completed by mothers monthly from December, 1996. Mothers tracked the incidence of common illnesses (fever, diarrhea, respiratory tract infections) over a 1-mo period. Completed calendars were reviewed by a clinical officer at monthly morbidity clinics.

Dietary data were collected from one child per household at baseline and postintervention using a validated interactive 24-h recall method (11). Due to unforeseen circumstances, only the postintervention dietary data were available for analyses. Postintervention, one recall was conducted during the postharvest food-plenty season (June, 1997), and a second during the preharvest food-shortage season (November, 1997). Average daily intakes and major food sources ( $n=10$ ) of energy, selected nutrients and antinutrients were calculated using our Malawian food composition data base (5) and the WorldFood Dietary Assessment System (12). A food diversity score was also calculated based on the average number of unique food items eaten, with the exception of water, discretionary sugar and tea.

Adjustments were made to the intakes of phytate from maize-based staples for children in those households that reported using phytate-reducing practices in the postintervention knowledge and practices survey. The adjustments were based on a 50% reduction in the average hexa- and penta-inositol phosphate content of nsima (stiff maize-based porridge) and phalas (thin maize-based porridge) prepared from soaked and/or fermented unrefined white maize flour (13). The predicted prevalence of inadequate intakes was calculated for selected nutrients based on the probability approach (14).

## RESULTS

Baseline height, weight (as height-for-age and weight-for-height Z-scores), hemoglobin and hair zinc concentrations were compared between those who participated in the 1-y measurements and dropouts. There were no significant differences between participants and dropouts, suggesting that participation was random and not linked to initial differences in anthropometric or biochemical status.

Baseline results revealed no significant differences between the intervention and control groups for household size (5.1 vs. 5.4), number of children (3.0 vs. 3.2), maternal height (154.9 vs. 153.7 cm), sex of children, sex of head of household (HOH), house quality, household food production characteristics and knowledge or practices. Households in both groups were involved primarily in subsistence farming, although in the intervention households, more HOH and mothers had

<sup>5</sup> Abbreviations used: AFA, arm fat area; AFA Z-score, arm fat area Z-score; AMA, arm muscle area; AMA Z-score, arm muscle area Z-score; HAZ, height-for-age Z-score; HOH, head of household; KP, knowledge and practices; MUAC, mid-upper-arm-circumference; MUAC Z-score, mid-upper-arm-circumference Z-score; NCRSP, Nutrition and Human Function Collaborative Research Support Program; WHZ, weight-for-height Z-score.

no formal education and were Muslims compared to the control households ( $p < 0.01$ ) (Fig. 1). Intervention children were significantly younger (68 vs. 74 mo;  $p < 0.05$ ) than the control children. After adjusting for age and sex, and controlling for differences in sociodemographic variables, there were no significant differences between the intervention and control groups at baseline for median anthropometry Z-scores (HAZ: 1.76 vs. -1.91; WHZ: 0.04 vs. 0.03), hair zinc (182.5 vs. 177.5  $\mu\text{mol/g}$ ), and hemoglobin (109 vs. 109 g/L) concentrations, or prevalence of anemia (hemoglobin  $< 110\text{g/L}$ ; 59 vs. 62%).

### Comparison of reported knowledge and practices

After 1 y, intervention parents had a greater knowledge of iron (60 vs 8%,  $p < 0.001$ ) and vitamin A (84 vs 74%,  $p < 0.05$ ), and food sources of iron (50 vs 36%,  $p < 0.05$ ) than those of the control parents, whereas the only significant difference in practices after 1 y was a greater use of fermented maize flour by the intervention households (56 vs. 1%,  $p < 0.001$ ) (data not shown). The lack of differences in most of the practices at 1 y was explained by a significant increase in both knowledge and practices within both the intervention and control groups.

### Comparison of food consumption patterns and adequacy of dietary intakes

Figure 3 compares the median intakes (g/d) of animal source foods postintervention. Note that the intervention group had a significantly greater intake of fish than the control group (62 vs. 48 g/d). Their intake of meat and insects also tended to be larger (15 vs. 3 g/d) ( $p = 0.06$ ), but there was no difference in their intake of dairy products and eggs (26 vs. 32 g/d). Intakes of other major food groups (i.e., maize-based staples, other starchy staples, nuts and legumes, sugars and sweets) were also similar with the exception of beverages. Of the latter, the intake of thobwa, a fermented maize drink, was significantly higher in the intervention compared to the control group (578 v. 492 g/d;  $p < 0.001$ ). Some notable differences in food sources of iron and zinc also existed ( $p < 0.05$ ). Fish contributed more iron and zinc (13 vs. 10% for iron; 26 vs. 21% for zinc), whereas maize-based staples contributed less (46 vs. 50% iron; 46 vs. 55% zinc) in the intervention compared to the control group (data not shown).

Table 1 presents the median daily intakes (1st and 3rd quartiles) of energy, nutrients and antinutrients. Intakes of protein, fat and the percentage of energy from fat were higher, whereas intakes of phytate were lower in the intervention children compared to control children ( $p < 0.01$ ). A similar pattern was shown for the intakes of vitamin B-12, calcium and zinc (primarily from small fish eaten whole with bones),

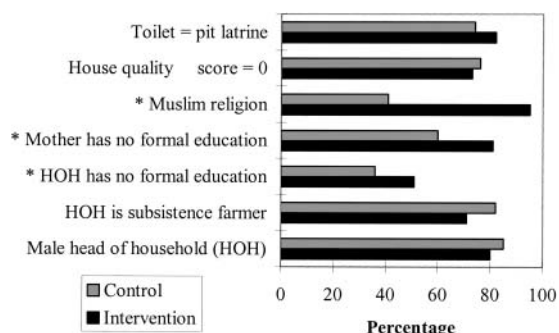


FIGURE 1 Baseline sociodemographic, house quality and sanitation data for the intervention and control groups. \* $p < 0.05$ .

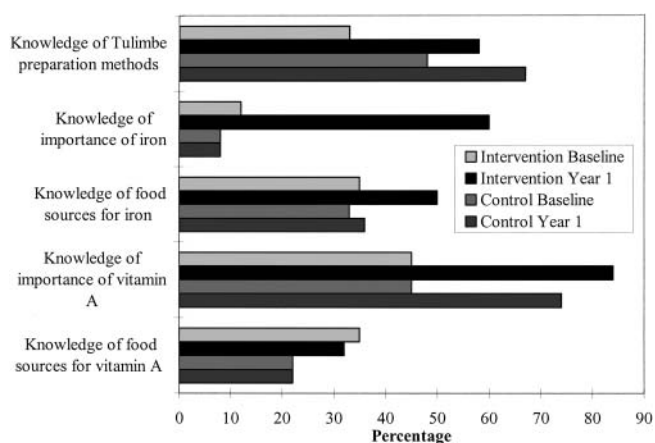


FIGURE 2 Comparison of baseline and 1-y knowledge scores within group.

whereas no differences for intakes of total iron, vitamin A, vitamin C, folate and dietary fiber were noted. In contrast, intervention children had a markedly lower median phytate intake than the control children.

Dietary quality indicators are shown in Table 2. The intervention children consumed more diverse diets, with a higher proportion of their total energy (as %) and protein (g/d) intake contributed by animal sources compared to the control children. As a consequence, densities for protein and vitamin B-12, but not zinc, iron or vitamin A, were also higher for the intervention diets. Intakes of available zinc but not available iron followed a similar trend. In contrast, the intervention children had diets with lower median phytate:zinc and phytate:iron molar ratios, and fewer intervention children consumed diets with phytate:zinc molar ratios above 15 than the control children ( $p < 0.05$ ).

The intervention children had a lower risk of inadequate intakes of all the nutrients examined, with the differences being significant for protein, folate, vitamin B-12, calcium and zinc

TABLE 1

Median (1<sup>st</sup> and 3<sup>rd</sup> quartile) daily intakes of energy, nutrients and antinutrients of the intervention and control groups post intervention

Nutrient	Intervention (n = 200)	Control (n = 81)	$p^1$
Energy (KJ)	5071 (4092, 6075)	4686 (3356, 5665)	0.002
Protein (g)	33 (27, 42)	28 (22, 37)	<0.001
<sup>2</sup> Utilizable protein (g)	26 (20, 32)	20 (14, 28)	<0.001
Fat (g)	14 (10, 22)	9 (7, 14)	<0.001
% energy from fat	11 (8, 15)	9 (7, 11)	<0.001
Vitamin A (RE)	916 (454, 1461)	914 (398, 1602)	0.869
Folate ( $\mu\text{g}$ )	172 (139, 231)	173 (132, 234)	0.433
Vitamin B-12 ( $\mu\text{g}$ )	1.2 (0.6, 1.9)	0.8 (0.3, 1.3)	<0.001
Vitamin C (mg)	118 (78, 176)	129 (66, 171)	0.856
Calcium (mg)	421 (289, 579)	318 (221, 469)	0.002
Iron (mg)	9.4 (6.8, 12.0)	9.6 (6.5, 12.2)	0.889
Zinc (mg)	6.6 (5.1, 8.7)	6.0 (4.5, 7.3)	0.042
Dietary fiber (g)	24 (19, 30)	24 (18, 31)	0.732
<sup>3</sup> Phytate (mg)	638 (466, 870)	812 (502, 1170)	0.007

<sup>1</sup> Probability of the differences being significant (Kruskal Wallis).

<sup>2</sup> Adjusted for amino acid composition and digestibility.

<sup>3</sup> For subjects adopting phytate reduction practices, phytate intakes were based on an estimated 50% reduction of phytate in maize-based staples.

TABLE 2

Median (1<sup>st</sup> and 3<sup>rd</sup> quartile) daily dietary quality indices for the intervention and control groups, postintervention

Indicator	Intervention (n=200)	Control (n=81)	p <sup>1</sup>
No. of different foods per day	6 (5, 7)	5 (4, 7)	<0.001
% energy from animal sources	5 (3, 8)	3 (2, 5)	<0.001
Animal protein (g)	8.9 (5.1, 12.7)	5.1 (2.9, 9.0)	<0.001
Protein (g/MJ)	6.9 (6.2, 7.4)	6.5 (6.0, 6.9)	0.008
Vitamin A (RE/MJ)	179 (94, 275)	198 (101, 318)	0.381
Vitamin B-12 (μg/MJ)	0.24 (0.12, 0.36)	0.17 (0.07, 0.31)	0.002
Calcium (mg/MJ)	83 (62, 108)	78 (55, 97)	0.170
Iron (mg/MJ)	1.82 (1.53, 2.20)	2.06 (1.77, 2.39)	0.002
Zinc (mg/MJ)	1.31 (1.05, 1.65)	1.36 (1.10, 1.63)	0.445
Heme iron (mg)	0.51 (0.46, 0.56)	0.33 (0.26, 0.41)	<0.001
Available iron (mg)	1.0 (0.6, 1.3)	0.9 (0.7, 1.2)	0.777
<sup>2</sup> Available zinc (basal) (mg)	3.2 (2.3, 4.1)	2.3 (1.7, 3.0)	<0.001
<sup>2</sup> Available zinc (normative) (mg)	2.8 (2.1, 3.6)	2.1 (1.6, 2.7)	<0.001
<sup>2</sup> Phytate:iron molar ratio	9 (8, 12)	11 (9, 14)	0.006
<sup>2</sup> Phytate:zinc molar ratio	16 (12, 20)	20 (15, 23)	<0.001
Phytate:zinc molar ratio <15	23% (47)	12% (10)	0.045 <sup>3</sup>

<sup>1</sup> Probability of the differences being significant (Kruskal Wallis).

<sup>2</sup> Available iron and zinc have been calculated taking into account enhancing and inhibiting dietary factors consumed during the same meal (see text).

<sup>3</sup> Probability of the differences being significant (Pearson  $\chi$ -square).

(Fig. 4). For energy, fewer intervention children than control children had energy intakes less than two-thirds of the FAO/WHO/UNU average requirement (14 vs 36%,  $p < 0.01$ ; data not shown) (15).

### Comparison of anthropometric indicators

There were no significant differences in growth indices between the two groups after 12 mo, after adjusting for age, sex and baseline values (Fig. 5). At the end of the study, the overall prevalence of stunting in the intervention group had not changed compared to the baseline (i.e. 50 vs. 52%) and was similar to that in the control group (i.e. 49%;  $n = 62$ ), whereas

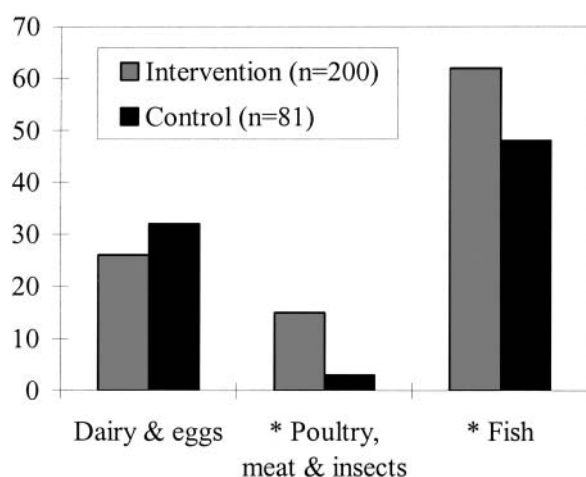


FIGURE 3 Comparison of median intakes of animal source foods (g/d) of the intervention and control groups postintervention.

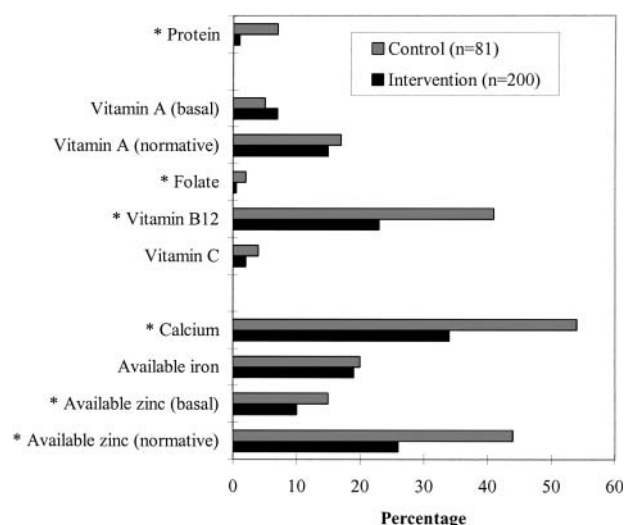


FIGURE 4 Predicted prevalence of inadequate intake of selected nutrients in the intervention and control groups postintervention. \*Probability of the differences being significant (Pearson  $\chi$ -square)  $< 0.01$ .

that of wasting was still very low in both groups (2% intervention; 1% control). In contrast, mean Z-scores for MUAC and AMA were significantly greater in the intervention children compared to the control children ( $p < 0.001$ ) at the end of the study, after adjusting for age, sex and baseline values.

### Comparison of biochemical and morbidity indicators

There were no significant differences in hemoglobin concentrations between the two groups at baseline. After adjusting for age and sex, hemoglobin (g/L) had fallen by 7 g/L in the control group at 1 y, (i.e., 109.6 at baseline; 102.2 after 1 y;  $p < 0.05$ ), with no change in the intervention group (i.e., 107.8 at baseline; 107.1). In fact, there was an increase in the incidence of anemia in the control group at 1 y compared to baseline values (80 vs. 59%;  $p < 0.001$ ) but no change in the incidence in the intervention group (62% at baseline; 62% at 1 y). In contrast, no significant differences in initial or final hair zinc concentrations existed between the groups.

At baseline, the prevalence of malaria was lower in the intervention children compared to control children (26% vs 48%,  $p < 0.001$ ), but at 1 y no significant difference in malaria incidence existed between the groups, after controlling for baseline status (data not reported). 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 preharvest hungry season. In the intervention children, the median illness scores were

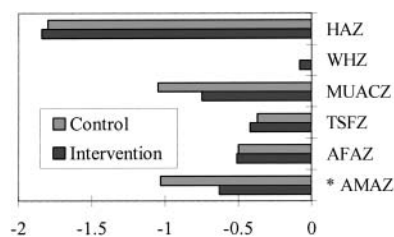


FIGURE 5 Mean growth and body composition indices after 1 y by group adjusted for age, sex and baseline values. HAZ, height-for-age Z-score; WHZ, weight-for-height Z-score; MUACZ, mid-upper arm circumference Z-score; TSFZ, triceps skinfold Z-score; AFAZ, arm fat area Z-score; AMAZ, arm muscle area Z-score. Differences for MUACZ and AMAZ are significant ( $p < 0.001$ ).

significantly higher in February, March and May ( $p < 0.05$ ) but significantly lower in June and August compared to the control children ( $p < 0.001$ ).

### *Interrelationships among anthropometric, biochemical and morbidity variables*

The effect of treatment group, hair zinc and hemoglobin concentrations and morbidity on final anthropometric outcomes were examined using general linear models, all of which included age, sex and baseline values. As expected, a significant proportion of the variation in all anthropometric outcomes was explained by baseline anthropometric values. 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.

With respect to potential explanatory variables, treatment group was a significant predictor of Z-scores for both MUAC and AMA. Final hemoglobin was a significant predictor of height and HAZ, and final hair zinc approached significance in predicting MUACZ ( $p = 0.06$ ). Neither average illness score nor occurrence of malaria was significant in any of the models examined (6).

## DISCUSSION

To our knowledge, this is the first community-based trial that has incorporated both dietary diversification and modification strategies to enhance simultaneously the iron, zinc and vitamin A nutriture of African children. Consequently, we have compared our results with those of supplementation trials, based mainly on single micronutrients, and conducted on children with the same pattern of growth (i.e., stunted but not wasted), as reported here. 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. Furthermore, we recognize that these results must be interpreted in light of the limitations of the quasiexperimental design utilized here. However, both the nature of our dietary interventions and the participatory approach we employed precluded the use of a double-blind randomized controlled design.

### *Comparison of nutrient adequacy*

In this study we compared the nutrient adequacy of the diets of the children from the intervention and control households postintervention based on selected indices of dietary quality and risk of inadequate intakes. Our results showed that, at the end of the study, diets of the intervention children were more diverse and of higher quality compared to those of the control children. Specifically, intervention children had a higher intake of animal protein (g/d), and animal protein as a percentage of total energy (Table 2), reflecting the adoption of our recommendation to increase intakes of animal source foods. The importance of animal source foods in the diet was emphasized by the results of the Human Nutrition Collaborative Research Support Program (NCRSP). In the latter, animal source food was the single most important factor positively associated with early childhood growth (16,17) and cognitive development (18).

In this study, the animal source food consumed most frequently was soft-boned fish from Lake Malawi, rather than meat or poultry (Fig. 3). Consumption of the latter was constrained by availability and cost and hence was very low, con-

tributing only ~2% of the iron in both the intervention and the control groups. The soft-boned fish eaten whole with bones, was generally dried and powdered when used to enrich the thin maize-based porridges (phalas) for young child feeding, or fresh when eaten as a relish with the stiff maize porridge (nsima). Soft-boned fish provides a good source of readily absorbable calcium, zinc and some heme iron (19), while simultaneously enhancing zinc and non-heme iron absorption (20,21).

In contrast, the traditional Malawian maize-based staples, specifically nsima, contributed less energy to the diets of the intervention children compared with the control children, although intakes in grams per day were comparable. This suggests that animal source foods did not displace maize, or indeed any other food, but instead provided much needed additional sources of vitamin B-12, calcium, zinc, heme-iron, retinol and fat for the intervention group (Table 1).

Several of our dietary strategies focused on reducing the level of hexa- and penta- inositol phosphates, strong antagonists of non-heme iron and zinc absorption (22,23). Of these, the strategies that were most widely practiced, and probably the most effective, involved soaking and, to a lesser extent, fermenting maize before preparing it as nsima or phala. Our laboratory work showed that >50% of the hexa- and penta- inositol phosphate content can be removed after soaking unrefined white Malawian maize flour for 1 h and decanting off the excess water (13). Natural lactic fermentation of maize flour slurries or porridges with or without the addition of germinated flour can also reduce their hexa- and penta- inositol phosphate content by enzymatic hydrolysis to lower inositol phosphates. The magnitude of this hydrolysis depends on the conditions used, and ranges from 30% to >60% in maize. The lower inositol phosphates have a much less potent adverse effect on iron, and no inhibitory effect on zinc absorption (22,23).

In this study, adoption of our phytate reduction strategies by the intervention group led to markedly lower intakes of phytate (Table 1), a lower median phytate-to-zinc molar ratio, and thus a greater proportion of diets with phytate-to-zinc molar ratios below 15, a level above which zinc absorption is enhanced (Table 2). In a Mexican study, phytate-to-zinc molar ratios of diets of both toddlers and school-aged children correlated negatively with height (16,17), a relationship that was not apparent here, possibly because of the uniformly stunted nature of our Malawian children (height =  $105.4 \pm 9.6$  cm) (Fig. 5).

The lower intakes of dietary phytate were reflected in turn by the significantly higher intakes of available zinc of the intervention children compared to the control children. We did not observe a similar finding for available iron intakes (Table 2). This discrepancy arose, in part, because the model used to calculate available iron intakes (12), unlike that for available zinc, does not take into account the negative impact of inositol phosphates on non-heme iron absorption.

The postintervention children had a lower risk of inadequate intakes of protein, calcium, zinc and vitamin B-12 than the control children (Fig. 4), arising from a marked improvement in the quality of their diets, attributed to our dietary strategies, as discussed above. In contrast, risk of inadequate intakes of iron did not differ between the two groups, in part because, as noted above, our estimates of available iron did not take into account the impact of our phytate reduction strategies, and fish, rather than meat and poultry, was the major source of heme iron in the diets of these Malawian children.

In this study, risk of inadequate intakes of protein in both the control and intervention groups was very low, despite their very low intakes of animal protein (Table 2), a trend consistent

with earlier studies of Malawian (1) and Kenyan (24) children. Likewise, risk of inadequate intakes of folate and vitamin C was low in both groups, as noted for the Kenyan toddlers (25), with no differences between the groups for vitamin A or vitamin C.

The diets of the Malawian children studied here were inadequate in more than one nutrient, the most common deficits being calcium + iron + zinc, and vitamins A and B-12. These findings are consistent with those of the NCRSP study (26). Nevertheless, fewer intervention children than control children had diets at risk of low intakes of more than one nutrient (23 vs. 39%;  $p < 0.05$ ), based on a cutoff of at least an 85% probability of inadequacy. The existence of such multiple deficits may explain in part why several single micronutrient supplementation trials have failed to demonstrate any positive growth response (27–29).

### Comparison of indices of growth and body composition

Our dietary intervention had a significant effect on the lean body mass of these stunted rural Malawian children after 12 mo, but had no impact on their height or weight gain. Of the few single-micronutrient supplementation trials that have assessed changes in body composition as well as growth, several involving zinc (but not iron or vitamin A) have reported comparable improvements in anthropometric indices of lean body mass in children, with no effect on linear growth (27,30,31). Zinc, unlike vitamin A and iron, has a direct role in lean tissue deposition, through a regulatory role in protein synthesis, reportedly mediated via its interaction with insulin activity and changes in serum IGF-I levels (32,33). Therefore, the significantly greater increases in MUAC and AMA Z-scores reported in our intervention group may have arisen because of their significantly higher intakes of available zinc compared to those of the control children.

Zinc, 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. The intervention children of this study had a significantly lower incidence of common infectious illnesses than the control children after the rainy season, despite a higher incidence earlier, perhaps associated in part with the enhanced bioavailability of zinc and vitamin A (through increased intake of dietary fat), and possibly iron in their diets. It was unlikely that these morbidity findings were due to unequal access to medical services because a clinical officer visited both the intervention and control communities on a monthly basis.

In several zinc supplementation studies of children, improvements in height and/or weight gain have been reported in those receiving the zinc supplement compared to the control group (34–36). Less consistent positive responses on growth have been observed in vitamin A or iron supplementation studies (29,37,38). In this study, no significant improvement in linear or ponderal growth was apparent in the intervention group (Fig. 5), despite the apparent enhanced bioavailability of zinc, iron and vitamin A in their diets compared to those of the control group. Several factors may have been responsible for the lack of any positive growth response. For instance, in an earlier three-year Guatemalan study, a positive growth response to a nutritional supplement was only reported in those children aged  $< 3$  y, when linear growth is very fast (i.e.,  $\sim 20$  cm/y), but

not in the older children with slower growth rates (i.e., 7 cm/y) who were comparable in age to those studied here (i.e., 30–90 mo) (39). As well, we evaluated the changes in growth over 12 mo, which was probably too short to detect the small improvement in linear growth anticipated for this age group. More sensitive techniques such as knee-height measurements might have helped detect any subtle improvement, as noted by Sandstead et al. (40) in a study of Chinese children aged 6–9 y. Finally, in rural Malawi where the prevalence of stunting among rural adults is high (2,3), any growth response is likely to be constrained by a combination of intrauterine growth retardation and intergenerational effects of malnutrition. Indeed, mothers of the stunted children of this study were significantly shorter than those of nonstunted children (153.6 vs. 155.7 cm;  $p < 0.001$ ), despite no differences in socioeconomic status.

### Comparison of biochemical and morbidity indicators

The significantly higher hemoglobin concentrations presented here resulted in a lower incidence of anemia in the intervention group than in the control group, postintervention (62 vs. 80%;  $p < 0.001$ ). Our dietary intervention was not designed to identify which etiological factor(s) were responsible for these higher hemoglobin values in the intervention group. Nevertheless, three critical factors may have played a role: malaria, other infections and nutritional status. In general, the incidence of malaria postintervention was higher compared to the previous year (45% vs. 33%), probably as a result of increased rainfall (41). We suspect that the decline in hemoglobin concentrations in the control group was a function of this increased incidence of malaria. Certainly, at the end of 12 mo, the prevalence of anemia was higher in malaria-positive children. However, because the prevalence of malaria was similar in both groups at 12 mo, malaria was probably not implicated in the differences in anemia prevalence that distinguished the two groups at the end of the study.

Low hemoglobin concentrations may also result from a variety of other parasitic infections including hookworm. All the children in this study, however, were treated initially with albendazole and then monthly thereafter, where necessary, to minimize this potential confounder, even though hookworm is not a major cause of anemia in rural Malawian children (42). Schistosomiasis may also reduce hemoglobin concentrations (43). Although we did not test the children studied here for schistosomiasis because of the reluctance by the communities to provide urine samples, its prevalence in the control and intervention villages was likely similar because all the children lived in close proximity to Lake Malombe.

Deficiencies of iron, vitamin B-12, folate, riboflavin and vitamin A (via its interaction with iron) (44) have been implicated in the etiology of anemia in Malawi (45). Indeed, our Malawian control children consuming their habitual maize-based diets were at high risk for inadequate intakes of vitamin B-12 (Fig. 3). Therefore, the reduction in the risk for inadequate intakes of vitamin B-12 in the intervention group could have contributed in part to their lower incidence of anemia at 1 y. As well, some increase in iron bioavailability in the diets of the intervention children induced by alterations in the content of non-heme iron absorption modifiers, particularly phytate and cellular animal protein, may have also played a role.

No significant differences in hair zinc concentrations were apparent between the two groups postintervention (data not shown), despite the significantly higher intakes of available zinc in the intervention group. A similar finding has been reported in several zinc supplementation trials; hair zinc values have not

consistently increased in response to a daily zinc supplement (35,46). This finding has been reported even in those children with low hair zinc values at baseline and who subsequently showed a positive response in linear growth to a zinc supplement compared to their counterparts receiving a placebo (35). None of the children in this study at baseline, or at 12 mo had low hair zinc concentrations indicative of suboptimal zinc status, based on conventional cut-off values, even though they had low mean height-for-age Z-scores. For children with impaired linear growth, however, zinc may not necessarily be the first limiting nutrient (47). Linear growth faltering may arise from multiple causes including the coexistence of multiple micronutrient inadequacies in the diet, especially when habitual diets are maize based, the effects of chronic infections, and prenatal and intergenerational effects of maternal malnutrition. Indeed, deficits in iron, riboflavin, vitamin A, iodine and/or zinc have all been implicated in linear growth faltering (48). 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. Indeed, 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 (49). This hypothesis, if correct, would explain the apparently normal hair zinc concentrations of these stunted Malawian children.

In summary, our results emphasize that our dietary diversification and modification strategies, implemented using a participatory approach, significantly increased intakes of animal source foods, while simultaneously reducing intakes of phytic acid. Nevertheless, intakes of animal source foods, especially meat and poultry, remained very low, despite our intensive nutrition education and community mobilization efforts to enhance their consumption. Therefore, more efforts to assist households in raising small animals and promoting their consumption are required in rural subsistence settings to ensure optimal growth, health and cognitive development of young children.

### Lessons learned

Our community-based dietary strategies reduced the prevalence of inadequate intakes of protein, calcium, zinc and vitamin B-12 in children from rural Malawian households with very limited resources. However, we did not succeed in reducing the prevalence of inadequate intakes of iron, despite significantly larger intakes of animal source foods in the intervention compared to the control group. This arose in part because fish was the major animal source food in these children's diets, rather than meat and poultry, consumption of which was constrained by availability and cost. Therefore, our findings highlight the importance of practical low cost strategies to enhance the production and consumption of meat and poultry in the diets of Malawian children to ensure adequate intakes of readily available iron. As well, in these stunted Malawian children, our dietary strategies were not associated with any improvements in linear growth, despite a significant reduction in the phytate-to-zinc molar ratio of the diets of the intervention children compared to that of the control children. We did, however, observe a significant and positive effect on lean body mass, together with reductions in the incidence of anemia and common infectious illnesses in the intervention group compared to the control group. Longer dietary diversification/modification interventions that succeed in promoting the production and consumption of small animal livestock are probably needed to show an impact on growth for children ages 30–90 mo.

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