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Increased food intake after the addition of amylase-rich flour to supplementary food for malnourished children in rural communities of Bangladesh

M. Iqbal Hossain, M. A. Wahed, and Shaheen Ahmed

Abstract

Background. *In Bangladesh, as in other developing countries, protein–energy malnutrition is most prevalent among children during weaning. After weaning, children are often fed cereal-based diluted low-calorie porridge, resulting in growth-faltering.*

Objective. *To assess the effect on food intake of adding amylase-rich flour (ARF) from germinated wheat to supplementary food among children in nine rural Community Nutrition Centers under the Bangladesh Integrated Nutrition Project (BINP).*

Methods. *A total of 166 malnourished children of either sex, aged 6 to 24 months, received one of three diets randomly allocated to the Community Nutrition Centers. The composition of the diets was the same; however, the consistency and calorie density were altered by adding either ARF or water. Thirty-five children received the standard supplementary food of the BINP (S-SF), 65 received supplementary food with added ARF (ARF-SF), and 66 received supplementary food with added water (W-SF). The children were studied for six weeks.*

Results. *The mean ± SD intake of supplementary food from a single meal by children completing six weeks on the diets was higher for children receiving ARF-SF (33.91 ± 8.25 g) than for those receiving S-SF (25.66 ± 6.73 g) or W-SF (30.26 ± 8.39 g) ($p < .05$ for both comparisons). The weight of vomited food was significantly higher for children receiving W-SF than for children in the other two groups. Weight gain and increments in length and weight-for-height were higher for children who received ARF-SF than for children in the other two groups, but the differences were not statistically significant. The acceptability of ARF-SF was higher than that of the two other diets. The additional cost of adding 2 g of ARF to the diet was about Taka 0.25 (US$1 = Taka 48).*

Conclusions. *Addition of ARF to existing standard supplementary food, as used under the BINP program, is a simple and effective means to increase the intake of food by changing its consistency, thus making it easier for malnourished children to ingest.*

Key words: Supplementary food, amylase-rich flour, food intake, malnourished child

Background

Protein–energy malnutrition in young children is the most prevalent form of malnutrition in developing countries [1]. Although the pathogenesis of malnutrition is complex, inadequate dietary intake is a major contributor. The usual complementary foods in Bangladesh and other low-income countries are based on cereals and are of lower energy density than what is recommended by the World Health Organization (WHO). Thicker porridge is more energy dense but is highly viscous, and young children cannot ingest it in adequate amounts even when they are in good health. Thus, these complementary foods are often diluted by adding water before feeding them to infants and young children [2]. The low energy density of such foods, along with the limited stomach capacity of young children, results in low intake of calories and protein by infants and young children aged 6 to 24 months, which in turn leads to growth-faltering [3]. The Bangladesh Integrated Nutrition Project (BINP) was initiated with the goal of reducing malnutrition in Bangladeshi children through community-based nutrition interventions.

Provision of supplementary food has been one of the major interventions of BINP. A low-cost supple-

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mental food, consisting of 20 g of roasted rice powder, 10 g of roasted lentil powder, 5 g of molasses, and 3 g of soybean oil in each packet (150 kcal/packet with 11% protein:energy ratio), was formulated and given to children with severe malnutrition, growth-faltering (defined as a weight gain during the previous 2 months of < 500 g for infants and < 300 g for children aged 13 to 24 months), or both at the Community Nutrition Centers [4]. Community Nutrition Centers are the primary village-level units of the BINP administration, serving two to four nearby villages (combined population of approximately 1500 people). A woman recruited from the community designated as a Community Nutrition Promoter supervised each local Community Nutrition Center. With the help of other group members and volunteers, the Community Nutrition Promoter monitored the growth of children through anthropometry at monthly growth-monitoring sessions, in a fixed location within the catchment area of each Community Nutrition Center, and screened eligible children for the supplementary food program.

Children selected for the program (severely malnourished, i.e., weight for age < 60% of the National Center for Health Statistics median, or faltering in growth, i.e., weight gain during the previous 2 months < 500 g for infants and < 300 g for children 13 to 24 months) were brought to the Community Nutrition Centers six days per week in the morning to eat the supplementary food. It was observed that if supplementary food was dissolved in a small volume of water, it became a thick gruel that was difficult for young children to eat; and if it was dissolved in a larger volume of water, its nutrient density was substantially reduced.

The problem of high viscosity may be effectively overcome by liquefying the energy-dense thick porridge with amylase-rich flour (ARF) from germinated wheat [5]. Earlier studies showed that energy-dense porridge, liquefied with ARF, was well-accepted by malnourished young children recovering from diarrhea and dysentery and that the mixture with ARF delivered a higher amount of energy [6–8]. We evaluated the effect of addition of ARF to existing supplementary food in selected BINP-operated Community Nutrition Centers to determine whether this would improve the food supplementation program by reducing viscosity and changing the consistency of the supplementary food, thus making it easier for young children to eat, and increasing the energy intake of these children.

Methods

Study protocol

Our study was carried out in nine BINP Community Nutrition Centers in the Sadar *thana* (subdistrict) of the Narshingdi District of Bangladesh from December 1998 through April 1999. The centers were selected randomly from all Community Nutrition Centers in the district, and were randomized in equal numbers to three different diets: the standard supplementary food (S-SF), supplementary food with added ARF (ARF-SF), and supplementary food with added water (W-SF) (**table 1**). Both girls and boys aged 6 to 24 months with severe protein–energy malnutrition (weight-for-age < 60% of the National Center for Health Statistics [NCHS] median) and/or growth-faltering, but without clinical evidence of any acute infectious disease or other disease or morbid condition that could interfere with the intake of study diets, were considered eligible. Children fulfilling the enrollment criteria received the assigned diet allocated to the particular Community Nutrition Center six days per week. Details of the study diets and the study procedure were discussed with the mothers or caretakers, and their oral consent was obtained for enrollment of their children into the study. The Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) approved the study.

Sociodemographic data were obtained and anthropometric (weight and length) measurements of the participating children were performed at baseline. Children (in light clothing) were weighed by a Salter scale (Industrial Measurments Ltd., Derby, UK) with a precision of 100 g, and recumbent length was measured by using a locally made, two-track length board with a precision of 1 mm. All anthropometric measurements were performed in triplicate, and the average of the nearest two measures was recorded.

The children reported to their respective Com-

TABLE 1. Composition of a packet of supplementary food[a]

Ingredient	Standard SF (S-SF)	ARF-added SF (ARF-SF)	Water-added SF (W-SF)
Roasted and powdered rice (g)	20	20	20
Roasted and powdered lentil (g)	10	10	10
Molasses (g)	5	5	5
Soybean oil (g)	3	3	3
ARF (g)	—	2	—
Added water (ml)	On demand	50[b]	70[c]

ARF, amylase-rich flour; SF, supplementary food.

a. The cost of 2 g of ARF was Taka ~ 0.25 (US$1 = Taka 48). Each packet provides ~150 kcal with a protein:energy ratio of 11%. ARF-SF contained some simpler carbohydrates because of partial hydrolysis of the flour.

b. The consistency after the addition of 50 ml of water to the ARF-SF diet was found suitable for ingestion by the young malnourished children.

c. 70 ml of water was required to make the consistency of W-SF similar to that of ARF-SF.

munity Nutrition Centers in the early morning. The mothers and caretakers were advised not to offer any food, including breastmilk or water, to the children for half an hour before offering the allocated diets. The meal was freshly prepared each time at the Community Nutrition Center. The mothers and caretakers accompanying the children were instructed on the feeding protocol before starting the study and were asked to feed their children the study diet using a spoon and cup over a half-hour period. It was previously determined that the osmolality of the study diet with ARF substantially increased 30 minutes after its preparation, and that the point of maximum intake by the children occurred within the first 15 to 20 minutes of offering the food [8]. During this half-hour period, no other food, including breastmilk, was allowed. Research assistants observed the process of feeding and performed all measurements related to food intake daily and anthropometry weekly. The amount actually ingested was calculated by subtracting the leftover supplementary food from the offered amount. A preweighed towel and bowl were used to collect vomit, if any, and the weight of vomit was subtracted from the amount of food given. The weight of foods was measured by a kitchen scale (Model Number: HCK-T, Shine [HK] Development Limited, Shenzhen, China) with a precision of 1 g. To determine acceptability of the diets, the mother or caretaker was asked whether her child liked the diet.

Preparation of ARF

ARF was prepared from germinated wheat, as described earlier [5]. Briefly, cleaned and picked grains of wheat (*Triticum aestivum*) were steeped in an equal volume of water at room temperature (23°–25°C) for 12 hours. The grain was drained, wrapped in moist black cloth, and allowed to germinate for 48 hours at room temperature. Water was occasionally added to keep the environment moist. The well-germinated grains (with shoots approximately 2–5 cm long) were spread on paper to remove surface moisture and air-dried for 12 hours under a ceiling fan until they were dry to the touch, then oven-dried at 50°C for 12 hours. The dried grain (both seeds and shoots) was ground to a fine powder with a kitchen blender. This constituted the ARF.

Initially the production was carried out at the Nutritional Biochemistry Laboratory of ICDDR,B, and subsequently small production units were established at the field sites. Drying ovens for the wheat grain were not available at field sites so the drying temperature could not be held at 50°C. This problem was overcome by drying the grain intermittently in a pan at low heat. The efficacy of the ARF produced at the ICDDR,B laboratory and in the field was tested and found comparable.

Quality control measures

Weighing machines and length boards were checked and calibrated daily with a known weight and measuring stick, respectively. The investigators made random checks (~ 10%) on anthropometry and supplementary food intake. Inter- and intraobserver variations were monitored, and on no occasion was the coefficient of variation for any measurements more than 1.5%. Investigators visited the field sites and Community Nutrition Centers every week on average, without prior notification to the research assistants and field workers.

Sample size estimation

We expected a 35% increase in daily supplementary food intake in the experimental (ARF-SF) group. This estimate was based on food-intake data from a similar diet by children aged 6 to 23 months [9]. In that study, the mean ± SD intake of ARF-treated porridge was 44 ± 13 g and that of untreated porridge was 28 ± 15 g. The sample size required to detect the difference at a 5% level of significance and with 80% power was 37 in each group. In order to adjust for the cluster effect, the derived sample size was multiplied by a factor of 1.5, and thus a sample size of 56 for each group was determined. On the assumption of a 20% dropout rate, the final sample size was determined as 70 in each group, for a total sample of 210 subjects.

Data analysis

Data were entered and analysis was carried out by SPSS for Windows (version 10.0; SPSS, Chicago, Ill., USA) software package. Analysis of variance (ANOVA) was used to compare the means and the Scheffé test to compare the groups. Variables not normally distributed were compared by the Kruskal-Wallis test to detect differences between the groups, and if these were significant at the 5% level, the Mann-Whitney U test was applied to identify the groups that were different. Categorical variables were compared by the chi-square test, and Fisher's exact test was applied if the expected number in any cell was 5 or less.

Results

A total of 210 children were initially enrolled; 44 dropped out (35 in the S-SF group, 5 in the ARF-SF group, and 4 in the W-SF group) within a few days. The mothers or caretakers of these children did not visit the Community Nutrition Centers regularly, and after one to two weeks they stopped coming. The reasons for dropout included living far away from the respective Community Nutrition Centers, the need to travel a long distance on foot to reach the Community

Nutrition Center every morning, and disruption of household activities by study-related activities. Incidentally, one of the Community Nutrition Centers allocated to S-SF (CNC no. 72) had a large catchment area with the highest number of eligible children. The problem of dropout was greatest at this Community Nutrition Center. The study was of a “quick result” type, and the time constraints of the study limited our scope to address the higher dropout rate in one of the study groups. Finally, 166 children continued with the feeding program for at least six weeks; of these children, 35, 65, and 66 received S-SF, ARF-SF, and W-SF, respectively, and analyses were performed on data from these children.

The baseline characteristics of the children before enrollment were comparable among the three groups (**table 2**), including their socioeconomic background, sanitation facilities at home, breastfeeding and immunization status, and other characteristics (e.g., age, sex, body weight, length, weight-for-length z score, gestational age, birth weight, etc.). Baseline characteristics of the children who dropped out were also comparable (data not shown). The intake of supplementary food (grams per child, excluding any water component) and energy (kilocalories per child) from a single meal each day was significantly higher in the ARF-SF group than in the other two groups (**table 3**). The amount of vomiting was significantly higher in the W-SF group than in the other two groups. Weight gain and increments in length and weight-for-length z scores were slightly higher in children receiving ARF-SF, although the differences were not significant. Children accepted ARF-SF more readily than the two other diets (**table 3**), and no adverse events (e.g., diarrhea) were associated with ARF-SF.

TABLE 2. Baseline characteristics of the study children[a]

Characteristic	Standard SF (S-SF) $n = 35$	ARF-added SF (ARF-SF) $n = 65$	Water-added SF (W-SF) $n = 66$
Age (mo)	13.4 ± 4.6	13.6 ± 4.3	14.5 ± 4.6
Male/female (*n*)	13/22	30/35	22/44
Weight (kg)	7.17 ± 1.0	7.21 ± 1.1	7.44 ± 1.3
Length (cm)	68.7 ± 4.3	69.2 ± 9.9	70.5 ± 4.5
Weight-for-length z score	–1.11 ± 1.03	–1.59 ± 1.02	–1.34 ± 0.78
Gestational age (mo)	9.5 ± 0.7	9.5 ± 0.5	9.4 ± 0.5
Place of birth—no. (%)			
Hospital/clinic	0 (0)	3 (4.6)	2 (3)
Home with TBA	31 (88.6)	53 (81.5)	57 (86.4)
Home without TBA	4 (11.4)	9 (13.8)	7 (10.6)
Birth weight (A/B)[b] (no.)	26/9	43/22	45/21
Birth order	2.7 ± 1.6	2.7 ± 1.9	2.9 ± 1.7
Breastfeeding continuing—no. (%)	32 (91.4)	59 (90.8)	65 (98.5)
Vaccine received—no. (%)			
DPT/polio (3rd dose)	30 (86)	40 (62)	49 (74)
DPT/polio (at least 1 dose)	35 (100)	65 (100)	66 (100)
Measles (among children > 9 mo old)	25 (86)	50 (86)	51 (93)
BCG	35 (100)	59 (91)	63 (96)
No. of family members	5.8 ± 1.9	5.2 ± 2.0	5.8 ± 2.0
No. of siblings < 5 yr old	1.5 ± 0.6	1.5 ± 0.6	1.3 ± 0.6
Type of family (N/J)[c] (*n*)	19/16	48/17	43/23
Age of mother or caretaker age (yr)	26.8 ± 7.4	26.5 ± 5.7	27.0 ± 7.1
Monthly family income (Taka)[d]	2774 ± 1807	2882 ± 1138	2742 ± 1054

ARF, amylase-rich flour; TBA, trained birth attendant

a. Plus-minus values are means ± SD and are not significantly different among the groups.

b. Birth weight: A = average or apparently normal birth weight, B= below average or low birth weight (exact weight was not known, categorized according to mother’s assessment).

c. Type of family: N = nuclear, J = joint.

d. US$1 = Taka 48.

Discussion

Addition of ARF to appropriate diets has been shown to be an effective means of increasing dietary intakes by healthy young children [3, 10, 11] and also by malnourished children during and after recovery from diarrhea in a health-care setting in Bangladesh [6–9]. We aimed to determine the efficacy of ARF in improving food intake by children who were severely malnourished (weight-for-age < 60% of NCHS median) [12] or were faltering in growth [4] in community settings. In our study, the significantly higher intake of supplementary food at a single meal by children in the ARF-SF group is attributable to the addition of ARF to supplementary food. ARF contains amylase, an amylolytic enzyme that hydrolyzes starch granules in a thick porridge into maltodextrins and simple sugars with lower water-holding capacity, which results in liquefaction of the porridge. The converted simple sugars in the ARF porridge also make it sweeter and more palatable compared to thick porridge without ARF. The observed higher amount of vomiting by children receiving porridge with water added (W-SF) than by children consuming the other two diets was probably due to a consequent increase in volume and less sweetness. Water was added to the usual supplementary food to make its consistency similar to that of ARF-SF, which resulted in higher intake but also made it less energy-dense. Therefore, the advantage and disadvantage of this diet and possible consequences were discussed with mothers or caretakers before they enrolled their children in the study.

The ARF-added diet (ARF-SF) was well accepted by the mothers and caretakers, and most (97%) of them felt that ARF-SF was suitable for their children. However, comparative acceptability could not be tested, because each child was allocated to a single diet over the study period. Some mothers and caretakers resisted taking weight and length measurements of the children on the first occasion due to the belief that these measurements could adversely affect their children's growth; however, the situation was resolved through proper counseling.

The frequent absences and ultimate dropout of 44 children after initial enrollment was beyond our control. These dropouts included 35 in the S-SF group, and consequently the final number of children in this group was the lowest, which might have affected the statistical power. We believe the high dropout rate may not be due to the assigned food group, because S-SF was being used in Community Nutrition Centers across the country and compliance was reported to be satisfactory.

Although weight gain and increments in length and weight-for-height were higher in the children receiving the ARF-SF diet than in the two other diet groups, the differences did not achieve statistical significance. In assessing the effect of the ARF-SF diet on growth, our study had some limitations. The children were offered only one meal in the morning, six days per week, and thus its impact was limited. We could not supplement food at other meals for these children from poor families. The average weight of the children on recruitment was approximately 7 kg, and if we consider the lowest range of energy (i.e., 150 kcal/kg) required for optimal growth and to catch up in growth, each child needed about 1,050 kcal/day. The amount of supplemented energy was only about one-seventh of this actual need.

Adding ARF increases the osmolality of porridge and thus may have the potential for causing osmotic diarrhea; however, none of the children receiving the diet experienced this or any other undesirable effects. The usual complementary foods in Bangladesh are based on milk and cereal (rice or wheat). Many children in Bangladesh are exposed to wheat, and thus it would be unlikely that about 2 g of ARF from germinated wheat would increase the risk of gluten intolerance.

TABLE 3. Supplementary food intake, vomiting, and effect on growth of the study children[a]

Variable	Standard SF (S-SF) *n* = 35	ARF-added SF (ARF-SF) *n* = 65	Water-added SF (W-SF) *n* = 66
SF intake (excluding water component) per single meal (g/child)*	25.66 ± 6.73	33.91 ± 8.25	30.26 ± 8.39
Calorie intake from SF per single meal (kcal/child)*	101 ± 27	134 ± 33	119 ±33
Vomiting (g/child/day)**	0 ± 0	0.07 ± 0.41	0.40 ± 0.73
Weight gain (kg)	0.4 ± 0.47	0.50 ± 0.34	0.45 ± 0.38
Length increment (cm)	0.57 ± 0.58	0.82 ± 0.98	0.76 ± 0.80
Weight-for-length z score increment	0.33 ± 0.63	0.49 ± 0.48	0.33 ± 0.49
Diet acceptable—no. (%)***	32 (91)	63 (97)	40 (61)

ARF, amylase-rich flour; SF, supplementary food.

a. Plus-minus values are means ± SD.

*$p < .05$ for ARF-SF vs. S-SF, S-SF vs. W-SF, ARF-SF vs. W-SF (ANOVA); **$p < .05$ for ARF-SF vs. W-SF, S-SF vs. W-SF (nonparametric test); ***$p < .001$ for ARF-SF vs. W-SF, S-SF vs. W-SF (chi-square test).

Mitra et al. [9] and Moursi et al. [13] observed that breastmilk consumption by young children was not affected by adding ARF to their diet. The latter group [13] also found that consumption of amylase-treated gruel was associated with increased energy intake, and our findings are in agreement.

The technology for the preparation of ARF could be successfully transferred to local Community Nutrition Centers, indicating that it could be sustained at the community level if ARF-enriched supplementary food is recommended. The cost of 2 g of ARF in our study was only about Taka 0.25 (US$1 = Taka 48). Gopaldas and colleagues [14] reported successful transfer of ARF technology from their laboratory to urban slums, whereby nearly every mother was able to easily grasp and execute all steps in making ARF and gruel. The stomach capacity is relatively smaller in severely malnourished children, so energy-dense ARF-mixed food could have an advantage in meeting increased energy requirements during the rehabilitation of severely malnourished children.

The addition of ARF to existing supplementary food for malnourished and growth-faltering young children is a simple and effective means to change the consistency of the food to facilitate its ingestion by young children and thus optimize their dietary (and energy) intake.

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This is a collaborative study among the College of Home Economics, the BINP, and the ICDDR,B, which prepared ARF for the study and transferred the technology of producing amylase-rich flour to others for its sustained production and supply in the study communities, and was also responsible for project implementation of BINP and reporting. The College of Home Economics provided training to field-level staff. BINP provided financial and field support to conduct the study at the selected sites.

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Effects of a food supplementation program on the nutritional status of pregnant women in Bangladesh

M. Mahmud Khan, Shakil Ahmed, Ali Ehsan Protik, Badal Chandra Dhar, and S. K. Roy

Abstract

Background. *The Government of Bangladesh implemented a comprehensive nutrition intervention in 1997 to reduce the rates of malnutrition among women and children. The pilot program, the Bangladesh Integrated Nutrition Program (BINP), adopted a multisectoral approach targeting women and children through food supplementation, home gardening, and health and nutrition education.*

Objective. *This paper estimates the effectiveness of BINP's food supplementation and nutrition education on the nutritional status of pregnant women.*

Methods. *Three effectiveness measures were considered: target efficiency, improvements in the nutritional status of beneficiaries, and the persistence of nutritional effects. To isolate the effects of the intervention, the nutritional status of participants and nonparticipants was compared after controlling for various demographic and socioeconomic characteristics. Data were collected in 2000 from a random sample of 3,262 households in a BINP intervention area.*

Results. *Thirty-nine percent of pregnant women were correctly targeted by the program's food supplementation activities. The nutrition program reduced the prevalence of thinness among participant pregnant women by about 3 percentage points per month of enrollment. The prevalence of thinness among program graduates was 62%, which was much higher than that of the matched (nonparticipant) group (35%). This finding is perplexing but it may simply imply that those who enrolled at the initial phase of the project were severely underweight and they fell back to their original status within a short period of time.*

Conclusions. *The nutrition program was intended to improve the nutritional status of women in the longer run through the provision of nutrition education during the food supplementation phase. The prevalence of thinness or severe underweight in women who exited the program after completion of the enrollment period was found to be much higher than in women of similar age and socioeconomic status in the community. This apparent lack of persistence of program benefits requires careful re-evaluation of alternative mechanisms for improving the long-term nutritional status of women.*

Key words: Bangladesh Integrated Nutrition Program (BINP), evaluation, food supplementation, maternal nutrition

Introduction

Although food supplementation is a popular nutrition intervention strategy, most studies find that supplementation programs have very little impact on the nutritional status of the participants [1–6]. The Bangladesh Integrated Nutrition Program (BINP), currently known as the National Nutrition Program (NNP), combines food supplementation with a number of other nutrition interventions. The stated purpose of BINP is to address the nutritional problems of Bangladesh in a comprehensive manner [7]. The program design started from the premise that improving the nutritional status of populations requires a comprehensive strategy with multisectoral initiatives, such as food supplementation, home gardening, and health and nutrition education.

At the community level, BINP focuses on growth-monitoring of children, dissemination of nutrition-related information, and supervised supplementary

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feeding of women and children through the Community Nutrition Centers. The program's interventions are based on the provision of services to three demographic groups: pregnant women, lactating mothers, and children below two years of age. Individuals from these groups who satisfy a number of eligibility criteria are brought into the food supplementation component of the program. For example, the program identifies and enrolls pregnant women with a body-mass index (BMI) < 18.5 during the third month of pregnancy. Enrollment in BINP was based strictly on nutritional status, irrespective of the household's socioeconomic status. Once the women are enrolled, BINP provides food supplementation and nutrition education to them throughout pregnancy and up to six months after delivery.

The purpose of this study was to quantitatively measure the effect of the BINP food supplementation and nutrition education components on the nutritional status of pregnant women participating in the program. More specifically, the paper deals with three aspects of program effectiveness. The first examines the effectiveness of targeting and the degree of mistargeting. The second examines the improvements in the nutritional status of participants (measured by the prevalence of thinness) with increasing duration in the program. The final aspect seeks to evaluate whether the benefits of the program persist after graduation.

Methods

Various designs have been proposed to evaluate the effectiveness of nutritional intervention activities [8, 9]. The most direct approach to measuring the impact of an intervention would be to monitor the nutritional status of enrollees from the time of enrollment to exit from the program. The Community Nutrition Centers enroll women into the program through Growth Monitoring and Promotion (GMP) sessions. These sessions are conducted once a month in each of the Community Nutrition Centers, with the exact date of a session determined locally. Since the number of enrollments per month is relatively low in a Community Nutrition Center, this approach would require data collection from many Community Nutrition Centers for at least two to three months to ensure a statistically valid sample size. Coordinating data collection from Community Nutrition Centers on the Growth Monitoring and Promotion session days was determined to be extremely complex logistically. For this reason, our study conducted the evaluation using a survey of households at a specific point in time. We have compared a cross-section of program participants with nonparticipants to measure the difference in nutritional status rather than observing the changes in nutritional status of women over time.

We noted that participants and nonparticipants may vary significantly in terms of socioeconomic status and demographic situation, and we determined that the control group should include a subset of nonparticipants who were similar to the participants with respect to relevant control variables such as age, gestational period, and socioeconomic status. To enable matching of participants and nonparticipants by various socioeconomic and demographic groups, a relatively large cross-sectional survey—more than 3,000 households—was carried out in the project area.

To measure the effectiveness of BINP in improving the nutritional status of pregnant women in rural Bangladesh, we followed the following three steps. The first estimated the extent of mistargeting in BINP, that is, the number of women enrolled in the program who did not fulfill the enrollment criterion (BMI < 18.5) or the number of women not in the program who did fulfill the criterion. The second involved examining the effect of the intervention on the nutritional status of participants. This was done by examining the nutritional status of current participants with varying periods of participation in the program with a matched group of nonparticipants.

To match participants with nonparticipants, women were divided into three age groups: 15–25, 26–30, and > 30 years. Socioeconomic status was defined by per capita monthly household total expenditure tertiles. Based on the distribution of women in these nine cells (age group and socioeconomic status), women in the program were matched with women not in the program. For comparing women at different stages of pregnancy, month of pregnancy was used as an additional control variable.

In the third and final step, we examined the persistence of intervention effects in the longer term. A comparison between the nutritional status of pregnant women who exited after completing the entire program and a matched group of nonparticipants was carried out for this purpose. Again, the matching was carried out by considering the socioeconomic status and age of women, as outlined above.

The study proposal and the questionnaires were reviewed and approved by the Institutional Review Board of the International Center for Population and Health Research (ICDDR,B), Bangladesh.

Study area and data collection

The BINP was implemented in Bangladesh in phases. The first phase of the program introduced the nutrition activities in six rural subdistricts in 1997. For this study, one of the six first-phase BINP subdistricts was specifically selected.

The selected subdistrict (Shahrasti) is located in the Chandpur District of Bangladesh. There are nine unions (fourth-level administrative units) in the

Shahrasti Subdistrict, and one of the unions (Uttar Suchipara) was randomly selected for the survey. This union has 14 villages with a combined population of 16,539; nutritional services are provided to the population through 13 Community Nutrition Centers. A household survey was carried out during May and June 2000 to collect information from all 3,262 households in the area.

A research team of eight enumerators used a structured questionnaire to collect data from the households. All of the enumerators had at least a bachelor's degree and three to four years of experience in data collection, including taking anthropometric measurements at the field level. The enumerators were given intensive training on administering the questionnaire, anthropometric measurement techniques, and other socioeconomic data collection methodology.

Anthropometric data collection

Uni-scales with a precision of 100 g were used to measure weights, and wooden height scales were used to measure heights. The weight and height measurements were converted into the body-mass index (BMI), calculated as weight (kg)/height $(m)^2$. Women with BMI < 18.5 were considered eligible for participation in food supplementation activities. Although using BMI for determining eligibility of pregnant women is problematic, this study used the same measure the program itself used. The BMI values of pregnant women increase as the gestation period increases, and therefore the indicator will overestimate the effectiveness of a nutrition intervention. To correct for this bias, the BMI values of participants and nonparticipants were compared after controlling for duration of pregnancy and socioeconomic status.

Data quality

The research team was divided into two groups with a supervisor accompanying each of the groups to ensure the quality of data collected. At the end of each day's survey, the questionnaires were checked for accuracy and consistency by the field supervisors. If any inconsistencies or incomplete information was discovered, the supervisor was instructed to visit the household to clarify and complete the questionnaires the following day. Variations among the enumerators in the measurements of weight and height were regularly checked and minimized by repeated measures. To reduce the day-to-day variability of the uni-scale readings, the scales were checked and adjusted by measuring a constant weight every day.

Data processing and analysis

Data were coded at the field project office by the supervisors. Data entry, cleaning, and consistency checks were done under the supervision and guidance of the Data Management Officer at the research center. Data analyses were carried out by the statistical software packages SPSS and Epi Info.

Results

Demographic and socioeconomic characteristics of surveyed households

The average number of household members in the survey area was five, similar to the Bangladeshi average. The survey collected information from 3,262 households and identified all ever-married women aged 15 to 49 years and children less than 6 years of age. The total number of women and children in the survey area in these categories were 3,189 and 2,513, respectively. For this study, information collected from 3,189 ever-married women 15 to 49 years was used. Of these women, about 9% were pregnant, 27% were lactating, and 1% were both pregnant and lactating. More than half (about 51%) of the women participating in the program were in the age group from 25 to 34 years, and about a third (34%) were in the group from 15 to 24 years. For nonparticipants, these percentages were 37% and 23%, respectively. The survey indicated that at least one member from each of the 218 households was participating in the nutrition intervention program. The probability of participation in the nutrition intervention program was negatively associated with household's monthly per capita expenditures. As expected, the average per capita household expenditures of participating households were lower than those of nonparticipating households. More than 51% of all current and former beneficiaries of food supplementation activities were in the two lowest expenditure groups, compared with about 38% of nonparticipants. Similarly, only 28% of the participants belonged to the top two per capita expenditure groups, compared with 42% of the nonparticipants.

Degree of mistargeting

In the survey area, 24% of pregnant women and 9% of lactating mothers were participating in the program. BINP does not enroll lactating mothers directly; rather, pregnant women continue in the program as lactating mothers after delivery.

Table 1 shows that at the time of the survey 25% of the pregnant women in the program fulfilled the BMI cutoff criterion of < 18.5. Among the nonparticipant pregnant women, 21% were found to be thin (BMI < 18.5) as well. Empirical studies often use this information to estimate the degree of mistargeting. Using the data from **table 1**, effectiveness indicators

TABLE 1. Number of malnourished (BMI < 18.5) pregnant women in the survey area according to program participation status

Group	Malnourished (BMI < 18.5)	Not malnourished (BMI ≥ 18.5)	Total
Participants	17 (25%)	51 (75%)	68
Nonparticipants	46 (21%)	169 (79%)	215

[10] were calculated and reported (**box 1**). Note that 73% of women eligible for the program were not enrolled in it.

However, these numbers overestimate the extent of mistargeting due to the lumping of new entrants with participants who entered the program earlier. Those who are in the program for some time are likely to be better off in terms of nutritional status than those who have entered recently. Thus, some of the women may have entered the program with a BMI < 18.5, although their BMI at the time of the survey may have been > 18.5.

In order to reduce this bias, pregnant women who entered the program within the previous two months were identified. Among the nonparticipants, pregnant women with similar socioeconomic status and duration of pregnancy were identified as the matched group. The matching was carried out by defining three socioeconomic-status groups and three duration-of-pregnancy groups. Socioeconomic status was defined by per capita consumption levels (including consumption of goods or services from one's own production), and the duration of pregnancy was calculated by obtaining information on the onset of the last menstrual period. Although this method of estimating the duration of pregnancy may not be particularly reliable in a poor developing country, it provides some information to control for pregnancy duration. Although two months of enrollment may still have some impact on the nutritional status of pregnant women, the average duration in the program for this group was found to be only one month. Therefore, we used a duration of less than two months as a rough cutoff to evaluate the degree of mistargeting. **Table 2** compares the nutritional status or thinness of pregnant women participating in the program for less than two months with the status of the matched group of nonparticipants.

Table 2 shows that about 39% of the participating pregnant women had BMI < 18.5, while 61% were not underweight. Among the nonparticipants, the corresponding figures were about 19% and 81%. When these numbers are used (**box 2**), we find that 67% of women eligible for enrollment in the program were not enrolled. The proportion of enrollees who fulfilled the stated criterion was 38.7%, much higher than the percentage reported in **box 1.**

Effects of the program on nutritional status of participants

The participants were divided into two groups based on duration of program enrollment: less than two months and two months or more.

Table 3 shows that the nutritional status of the pregnant women improved with increasing duration of enrollment. The percentage of women with BMI < 18.5 decreased from 38.7% to 15.6% when the duration of enrollment increased from less than two months to more than two months. Given the average duration of enrollment in the program for these two groups and assuming a linear decline over the period, the rate of decline in the percentage of underweight pregnant women becomes 7.7% per month. The result appears to support the hypothesis that women participating in the BINP have better anthropometric measures than

BOX 1. Degree of mistargeting based on observed BMI of participants during the survey

Using data from **table 1**, the following indicators of degree of mistargeting or effectiveness of the program were calculated:

Proportion of total enrollees who fulfilled the stated criterion = 17/68 (25%)

Proportion of total enrollees who did not fulfill criterion = 51/68 (75%)

Proportion enrolled among those who do not fulfill criterion = 51/220 (23%)

Proportion who fulfilled criterion but not enrolled in the program = 46/63 (73%)

Proportion of total nonenrollees who were eligible for enrollment = 46/215 (21%)

BOX 2. Degree of mistargeting based on estimated BMI of participants at entry

Using data from **table 2**, the indicators were recalculated. These numbers should be considered more accurate measures of mistargeting.

Proportion of total enrollees who fulfilled the stated criterion = 12/31 (38.7%)

Proportion of total enrollees who did not fulfill criterion = 19/31 (61.3%)

Proportion enrolled among those who do not fulfill criterion = 19/148 (12.8%)

Proportion who fulfilled criteria but not enrolled in the program = 24/36 (66.6%)

Proportion of total nonenrollees who were eligible for enrollment = 24/153 (15.68%)

TABLE 2. Malnutrition status (BMI < 18.5) of currently enrolled pregnant women with less than two months' participation in the program and the matched group of nonparticipating pregnant women

Group	Expenditures[a]	Malnourished[b] (BMI < 18.5)	Not malnourished (BMI ≥ 18.5)	Total
Participants	Taka 5880.00 (US$117)	12 (38.7%)	19 (61.3%)	31
Nonparticipants	Taka 5793.75 (US$115)	12 (19.4%)	50 (80.6%)	62[c]

a. Median household monthly expenditures.

b. $p < .05$ for the difference between participants and nonparticipants in the percentage of malnourished subjects (χ^2 test).

c. Matched nonparticipants were drawn from a sample of 153 pregnant women, of whom 24 were malnourished.

nonparticipants after socioeconomic status and age have been controlled for.

However, the information in **table 3** should be viewed with caution. BMI values tend to increase with longer duration of pregnancy, irrespective of a woman's participation in the nutrition program. Therefore, the program effects must be separated from the effects of pregnancy duration. One indirect approach would be to compare the BMI values of participants with those of a matched group of nonparticipants. Nonparticipating pregnant women with similar socioeconomic status (based on per capita household expenditures and duration of pregnancy) were identified as controls. For matched nonparticipants, the percentage of malnourished women decreased from 19.4% to 5.4%, indicating a monthly rate of decline of underweight or thinness of 4.7%. In the absence of an intervention, this may be considered the expected rate of decline in thinness. Therefore, among the participants, the percentage of underweight women (as defined by the program) would have decreased to 24.7% from about 39% even in the absence of the program. Since the prevalence of malnutrition among the participants was reduced to 15.6% after an average duration of three months in the program, the net effect of the intervention is a 9% decline in the prevalence of underweight, from 24.7% to 15.6% over a period of three months, implying a 3% rate of decline per month.

From comparison of the participants with the matched group, it is clear that the BMI is improved more among participants in the program than among nonparticipants of similar socioeconomic status and stage of pregnancy. From this we concluded that increasing the time a pregnant woman participates in the program has a positive effect on her nutritional status.

Persistence of program effects

Nonpregnant women who were not enrolled at the time of the survey but had been enrolled in the nutritional intervention program in the past were identified.

TABLE 3. Comparison of malnutrition status (BMI < 18.5) of participant and nonparticipant pregnant women according to duration of enrollment in the program

		Duration of enrollment[b]	
		< 2 mo	≥ 2 mo
Group	Expenditures[a]	no. (%)	
Participants	Taka 6149.50 (US$122)		
Malnourished		12 (38.7)	5 (15.6)
Not malnourished		19 (61.3)	32 (84.4)
Total		31 (100)	37 (100)
Nonparticipants[c]	Taka 6440.00 (US$128)		
Malnourished		12 (19.4)	4 (5.4)
Not malnourished		50 (80.6)	70 (94.6)
Total		62 (100)	74 (100)

a. Median household monthly expenditures.

b. Duration of enrollment is defined by the length of time the participant is in the program from enrollment until exit. $p < .05$ for all differences between subjects with < 2 mo and ≥ 2 mo enrollment in the percentage of malnourished subjects (χ^2 test).

c. Nonparticipants were matched with participants using characteristics of the participants only (age and household expenditure) in the two categories of duration of enrollment. Nonparticipants have never participated in the program and so, by definition, have no duration data.

This group was also divided into two categories based on the elapsed time since exiting the program following the food supplementation phase. The first group consisted of those who exited within six months of the survey date, and the second group consisted of those who exited more than six months prior to the survey date. All former enrollees were matched with nonpregnant women who had not been in the program in the past and were not enrolled at the time of the survey. The matching of former participants with nonparticipants was carried out according to age and socioeconomic status.

It is surprising that the prevalence of thinness or underweight, measured by BMI, among the two categories of former participants was found to be very high. About 62% of women who exited the program within six months of the survey had BMI < 18.5, and 61.7% of those who exited more than six months ago had BMI < 18.5. The chi-square test indicated that these two groups were not significantly different in nutritional status. The striking result is that the percentage of malnourished women (measured by BMI < 18.5) among the matched group—i.e., nonparticipants—was about 35%.

Discussion

The survey of households in one area of Bangladesh served by BINP indicates that a significant proportion of malnourished or underweight women (67%) were not enrolled in the food supplementation program, and about 61% of those who were enrolled were not malnourished (BMI < 18.5). Although many of these thin women were enrolled, the extent of mistargeting was high. BINP should carefully evaluate its management and implementation strategy to enroll more women who are eligible for the program. Mistargeting increases the cost by diverting resources to those who are not eligible and reduces the overall effectiveness of the program, ultimately threatening its sustainability in the longer term.

When the nutritional status of the enrolled women is compared with matched non-participants it becomes clear that the program had sufficient positive effects. The prevalence of thinness (BMI < 18.5) among the participants showed a consistent decline with increasing length of participation in the program. None of the pregnant women enrolled in the program for more than four months were found to be malnourished (BMI < 18.5). Although the program used the fixed cutoff point of BMI < 18.5 to define malnutrition among pregnant women, the use of BMI may actually be a misleading indicator. Pregnant women are expected to gain weight with increasing length of gestation, and so it is not surprising that many in our study may appear to have improved in terms of BMI. Therefore, to obtain a more meaningful estimate of the effect of the program on underweight rates measured by BMI, it is important to correct the observed improvements in BMI for the improvements expected during pregnancy. In this study, a correction factor was derived by examining the nutritional status of nonparticipating pregnant women after controlling for the number of months of gestation and socioeconomic status. The correction factor was found to be smaller than the actual rate of decline in the malnutrition rates observed for enrollees. From this we conclude that as time enrolled in the program increases, the nutritional status of participants improves.

On the basis of the observed improvements in nutritional status among participants and with application of a linear improvement function (assuming that prevalence of low BMI declines in a linear fashion), the proportion of about 46% malnourished (BMI < 18.5) participants at enrollment should decline to zero after six months of enrollment. If we factor out the nonprogram effects (from matched data), the effect of BINP alone should have reduced the malnutrition rates among participants from 46% to 28% over the six months of enrollment.

To test the persistence of the program effects, we selected two groups of former participants: women who exited the program within six months prior to the survey and women who exited more than six months prior. If the program effects persist in the long term, both groups should show lower rates of malnutrition or underweight (BMI < 18.5) than the control group (i.e., nonparticipants). If the persistence of program effects is stronger in the short term but tends to decay gradually over the longer term, the first group of former participants should show better nutritional status than the second group. However, the results we obtained were surprising. The prevalence of low BMI in these two former participant groups was found to be identical and much higher than the malnutrition rates for the matched nonparticipant group (**table 4**).

There are a number of reasons that may help explain the apparent inconsistency between the BMI values of former program participants and the matched group. First, the BINP supplied about 160,000 kcal [7] of energy to the pregnant women over the last two trimesters and up to six months after delivery to close the energy gap of 600 kcal/day. The FAO/WHO/UNU [11] recommendation for the energy requirements of the last two-thirds of pregnancy and the lactation period is also about 170,000 kcal. Therefore, the extra energy requirements of pregnancy and lactation were met almost entirely by the energy supplements obtained from the program, leaving no extra nutrients available for weight gain. Second, the matching of the former participants with nonpregnant women of similar age and socioeconomic status may itself be a problem. A significant proportion of women among the highest

TABLE 4. Comparison of nutritional status (BMI < 18.5) of former participants and matched nonparticipants

Group	Expenditures[a]	Malnourished[b] (BMI < 18.5)	Not malnourished (BMI ≥ 18.5)	Total
		no. (%)		
Former participants[c]	Taka 5,580 (US$ 111)			
Within < 6 mo		28 (62.2)	17 (37.8)	45 (100)
Within ≥ 6 mo		366 (61.7)	227 (38.3)	593 (100)
Nonparticipants[d]	Taka 5,618 (US$ 112)	223 (35.0)	415 (65.0)	638 (100)

a. Median household monthly expenditures.
b. Differences between subjects leaving the program < 6 and ≥ 6 months previously in the percentage of malnourished subjects are not significant ($p > .05$, χ^2 test).
c. Former participants were women who left the program; none of them left more than three years prior to the survey.
d. Nonparticipants are women who were never in the program (matched group).

per capita consumption group are also below 18.5 BMI, indicating that economic status was not the best matching factor. Moreover, all the former BINP participants had been pregnant in the recent past, but in identifying the matched group we could not control for the women's pregnancy histories (i.e., whether they had been pregnant during the previous two years).

It is also possible that the program enrolled only the very low BMI women during the first few years of the program and that many of these severe cases fell back to their original state of malnutrition or low BMI due to other social and living-standard factors not controlled for in defining the matched sample. The matching did control for the general socioeconomic status and age of the women. Therefore, the underlying factors that created high prevalence of low BMI among the former participants must be something beyond these general socioeconomic and demographic variables. In any case, the apparent lack of persistence of the benefits of nutrition intervention is a serious concern and requires further analysis. If the program effects are very short-term and fail to protect the nutritional status of women after they exit the program, then the whole approach needs careful rethinking to identify alternative mechanisms of improving the nutritional status of women in Bangladesh.

The data for this study were obtained through a survey of participants and nonparticipants, and we have extrapolated potential longitudinal effects based on this cross-sectional approach. Although cross-sectional data are widely used to imply changes expected over time, they cannot control for individual socioeconomic status perfectly. This type of extrapolation requires comparison of the beneficiaries with a matched group. Although a number of relevant variables were used for matching, it is still possible that the control variables were not the best suited ones for this analysis and that some biases were introduced by the empirical approach itself. A longitudinal study will be able to indicate more clearly the trend of nutritional status and the reasons for apparent lack of persistence of the program effects.

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Effect of iron supplementation during pregnancy on birthweight: Evidence from Zimbabwe

Vinod Mishra, Shyam Thapa, Robert D. Retherford, and Xiaolei Dai

Abstract

Background. *Iron deficiency in pregnant women has been shown to reduce the oxygen supply to the fetus, cause intrauterine growth retardation, and increase the risk of premature delivery and reduced birthweight. Yet the effects of iron supplementation programs on pregnancy outcomes are not well documented for developing countries.*

Objective. *To examine the relation between iron supplementation of mothers during pregnancy and children's birthweight using data from a national population-based survey in Zimbabwe.*

Methods. *The analysis uses information on 3,559 births during the five years preceding the 1999 Zimbabwe Demographic and Health Survey. The effect of iron supplementation during pregnancy on birthweight was estimated by multiple regression, controlling for potential confounding effects of prenatal care, child's sex and birth order, mother's education and nutritional status (measured by body-mass index), household living standard, smoke exposure, and other variables.*

Results. *Babies born to mothers who received iron supplementation during pregnancy were 103 g heavier (95% confidence interval, 42–164;* p = *.001), on average, than babies born to mothers who did not receive iron supplementation during pregnancy. The difference was 64 g (95% confidence interval, 2–125;* p = *.043) for children whose birthweights were taken from health cards and 163 g (95% confidence interval, 44–281;* p = *.008) for children whose birthweights were reported by their mothers.*

Conclusions. *Iron supplementation during pregnancy is associated with significantly higher birthweight, independent of other pregnancy care factors, mother's nutritional status, smoke exposure, and a number of demographic and socioeconomic factors. Prenatal iron supplementation programs can improve pregnancy outcomes and promote child survival in developing countries.*

Key words: Birthweight, iron supplementation, micronutrients, pregnancy outcome, Zimbabwe

Introduction

Birthweight is strongly associated with infant mortality, impaired development in childhood, and, to a lesser degree, an increased risk of various diseases in adulthood, including diabetes, asthma, high blood pressure, and heart disease [1–6]. Although some questions have been raised about causality [7–9], birthweight remains one of the key predictors of infant mortality and a number of important health outcomes in later life.

Maternal nutrition and pregnancy care are among the most important determinants of fetal growth and birthweight [10–12]. Other causes include environmental factors, such as tobacco smoke and cooking smoke [13, 14], and infections, such as malaria [15]. Multiple micronutrient supplements are often prescribed during pregnancy to remedy any nutritional deficiencies of the mother and to meet the nutritional needs of the growing fetus.

Iron deficiency is the most widespread form of malnutrition in the world, affecting about a third of the world's population [16]. It is a particularly serious problem among women in many developing countries [17]. Iron deficiency reduces the amount of hemoglobin in the blood, causing anemia. Hemoglobin

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is necessary for transporting oxygen from the lungs to other body tissues. Thus, iron-deficiency anemia in pregnant women can reduce the oxygen supply to the fetus, cause intrauterine growth retardation, and increase the risk of premature delivery and reduced birthweight [11, 17–19].

Although a number of studies have associated prenatal iron deficiency and anemia with adverse pregnancy outcomes, including preterm delivery and reduced birthweight [20, 21], an extensive review of the literature on iron supplementation and birthweight, published in 2001, determined that the existing evidence was insufficient to conclude that iron supplementation during pregnancy lowers the risk of preterm delivery and increases birthweight [22]. On the other hand, a recent study involving controlled trials in rural Nepal found that iron supplementation during pregnancy can significantly reduce the risk of premature delivery and increase birthweight [23]. Another recent study in the United States found benefits of prophylactic iron supplementation during pregnancy for increasing birthweight, even in women who are not iron-deficient [24, 25]. A randomized, controlled trial in a semirural community in Mexico found that multiple vitamin and mineral supplements were not any more effective in improving birthweight than iron supplements alone [26, 27].

Prenatal multimicronutrient supplementation, which usually contains iron, has also been associated with increased birthweight in a number of recent randomized, controlled trials in sub-Saharan Africa. A randomized trial among prenatal care attendees in Harare, Zimbabwe, found that multimicronutrient supplementation during pregnancy increased gestational length and birthweight [28]. A trial in Tanzania found that prenatal multivitamin supplementation was associated with significantly increased birthweight for babies who were HIV-negative at birth but had no effect on those who were HIV-positive at birth [29]. Another recent trial in Tanzania found that pre- and post-delivery multivitamin supplementation of HIV-infected women had a significant positive effect on the attained weight of their children at 24 months of age [30].

This study examines the effect of iron supplementation during pregnancy on birthweight in Zimbabwe, using data from the 1999 Zimbabwe Demographic and Health Survey. The Zimbabwe data are uniquely suited for this study, because a large proportion (more than three-quarters) of births in Zimbabwe, unlike those in most developing countries, occurs in health facilities where newborns are weighed by trained health professionals [31].

Zimbabwe has a national iron supplementation program for pregnant women. The program was introduced in the early 1980s, when iron supplementation was included in the Essential Drug List. The Ministry of Health and Child Welfare distributes 200 mg ferrous sulfate tablets, containing 60 mg of elemental iron and 500 μg of folic acid, free of charge to all pregnant women attending its prenatal clinics. The supplementation is provided for five months starting at 12 weeks of pregnancy. For women starting prenatal care later in pregnancy, the recommendation is to continue iron supplementation until six weeks after delivery. There are no national iron-fortification programs in the country.*

Methods

The analysis uses data on 3,559 births to interviewed women during the five years immediately preceding the 1999 Zimbabwe Demographic and Health Survey (ZDHS). The ZDHS collected demographic, socioeconomic, and health information from a nationally representative probability sample of 6,369 households, representing all 10 provinces of the country. The sample was a two-stage cluster sample with an overall response rate of 97.8%. Basic data were collected on households, and more detailed data were collected on women of reproductive age within those households. Further details about sample design, survey management, and quality control are provided in the basic survey report [31].

The ZDHS asked a series of questions to all ever-married women aged 15 to 49 about prenatal care received for the pregnancy preceding the last birth in the five years before the survey. This included information about number and timing of prenatal care visits and services received during these visits, including whether the woman received iron supplementation during pregnancy and whether she received enough to last three or more months. The survey also collected information on delivery care and birthweight for children born in the five years before the survey.

The mother was also asked if the child was weighed at the time of birth. For those children who were weighed at birth, the mother was asked to show the health card on which the birthweight (in grams) was recorded. For mothers who did not have the card, the birthweight was recorded based on mother's recall. This reported birthweight (from the health card or mother's recall) is the response variable in our analysis.

Birthweights were recorded to the nearest 10 g, but in the ZDHS data, even for children whose birthweights were taken from health cards, there is considerable heaping on birthweights ending in 100 g, indicating that many health workers recorded birthweight to the nearest 100 g. As expected, such heaping is somewhat more common in the case of mother's recall. The ZDHS data do not allow assessment of the magni-

* Personal communication, Elizabeth Gonese, Ministry of Health and Child Welfare, Harare, Zimbabwe.

tude or direction of the effect of this heaping on our results. Multiple births (about 3% of the sample) were excluded from the analysis.

We used the SURVEYREG procedure in SAS with the Multiple Classification Analysis [32] to estimate the mean birthweight of children according to whether the mother received iron supplementation during pregnancy, after controlling for potential confounding effects of other variables. The ZDHS supplied all the variables included in the analysis. Separate analyses were conducted for children whose birthweight was obtained from health cards and for those whose birthweight was reported by the mother. Besides iron supplementation and birthweight, we also examined the effects of other aspects of pregnancy care, such as tetanus injections, malaria treatment, and place of delivery, as well as the effects of other risk factors, such as maternal nutritional status and smoke exposure from tobacco and household use of biomass fuels.

Other risk factors and potentially confounding variables included in our analysis were as follows: mother received two or more prenatal care visits (yes, no); number of tetanus toxoid injections during pregnancy (none, one, two or more); mother received malaria drugs during pregnancy (yes, no); sex of child (boy, girl); birth order of child (1, 2, 3, 4+); mother's age at child's birth (15–24, 25–34, 35–49); mother's body-mass index (BMI = weight kg/height m^2) classified as undernourished (BMI < 18.5 kg/m^2), normal (BMI = 18.5–24.9 kg/m^2), or overweight or obese (BMI ≥ 25.0 kg/m^2); mother's education in completed years (0–2, 3–5, 6+); mother works outside the home (yes, no); mother's religion (Christian, non-Christian); household living standard (low, medium, high); mother smokes tobacco (yes, no); type of cooking fuel [high-pollution fuel (wood, dung, or straw), medium-pollution fuel (coal, charcoal, or kerosene), low-pollution fuel (liquefied petroleum gas, natural gas, or electricity)]; residence (urban, rural); region (Bulawayo, Manicaland, Mashonaland Central, Mashonaland East, Mashonaland West, Masvingo, Matabeleland North, Matabeleland South, Midlands, Harare). The analysis also controlled for source of information on birthweight (health card or mother's recall). The prenatal care visits variable was not included in the final analysis, because most women (93%) received two or more prenatal care visits for their last birth in the five years preceding the ZDHS, so that there was not sufficient variability in this value. The urban/rural residence variable was also excluded, because it is highly correlated with type of cooking fuel, resulting in problems of multicollinearity. In a separate analysis (not shown), controlling for urban/rural residence instead of type of cooking fuel made no difference in the estimated effect of iron supplementation on birthweight.

In the ZDHS, certain categories of respondents were over-sampled. Our analysis uses weights to compensate for over-sampling. We also estimate robust standard errors that adjust for design effects due to clustering at the level of the primary sampling unit (villages or urban blocks). There is no clustering in our sample at the individual (mother) or household level, because the analysis is restricted to last births in the five years preceding the ZDHS. Regression results are presented in the form of predicted mean birthweights for categories of each predictor variable, with other variables in the model held constant at their mean values [32].

The analysis presented in this paper is based on secondary analysis of existing survey data with all identifying information removed. The survey was conducted after obtaining necessary ethical and institutional review board (IRB) approvals from the Zimbabwe Ministry of Health and Child Welfare, the United States Agency for International Development, the United Nations Children's Fund (UNICEF), and Macro International. The survey obtained informed consent from each respondent before she was interviewed (in this case, from mothers of children born during the five years preceding the ZDHS).

Results

Sample characteristics

Table 1 shows the sample distribution of last births in the five years preceding the survey according to whether the child was weighed at birth, whether the mother received iron supplementation during pregnancy, and other variables. Seventy-eight percent of the children born during the five years preceding the survey were weighed at birth. The mothers of three in five children received iron supplementation during pregnancy. Children who were weighed at birth were more likely to be born of mothers who received iron supplementation during pregnancy. Children who were not weighed (excluded from the analysis in this study) were about evenly distributed according to whether or not the mother received iron supplementation during pregnancy.

Children who were weighed were more likely to have mothers who received tetanus injections during pregnancy, to be delivered in health facilities, to be first births, to be born to younger mothers, and to have mothers who were Christian. As expected, children who were not weighed tended to be rural, from poorer households, and from households using high-pollution biomass fuels. The likelihood of being weighed at birth also varied considerably by region of residence. However, infants born to mothers with 0 to 2 years of education were about as likely not to be weighed (35%) as those to mothers with 6 or more years of education, indicating that the reason a child is not weighed at birth is not related to the mother's education.

TABLE 1. Sample (%) distributions of births in the five years preceding the ZDHS according to whether or not the infant was weighed at birth and source of information on birthweight (health card or mother's recall)[a]

Variable	% weighed at birth			% not weighed at birth	All children
	Card	Recall	Total		
Iron supplements during pregnancy[b]					
Yes	63.8	61.6	62.8	51.7	60.6
No	36.2	38.4	37.2	48.3	39.4
Malaria drug during pregnancy[b]					
Yes	23.2	22.1	22.7	23.6	22.9
No	76.8	77.9	77.3	76.4	77.1
No. of tetanus injections during pregnancy[b]					
None	12.0	13.9	12.8	37.4	17.7
One	34.7	34.9	34.8	30.7	34.0
Two or more	53.3	51.2	52.4	31.9	48.3
Child born in a healthcare facility					
Yes	94.8	91.4	93.2	3.5	75.2
No	5.2	8.6	6.8	96.5	24.8
Sex of child					
Boy	51.5	51.1	51.3	51.7	51.4
Girl	48.5	48.9	48.7	48.3	48.6
Birth order of child					
1	36.6	39.3	37.9	18.7	33.7
2	23.5	24.4	23.9	18.9	22.9
3	14.7	13.6	14.2	15.6	14.5
4 +	25.2	22.6	24.0	46.8	28.9
Mother's age at child's birth (yr)					
15–24	54.1	59.6	56.7	45.1	54.2
25–34	34.8	32.6	33.8	36.6	34.4
35–49	11.1	7.8	9.5	18.4	11.5
Mother's body-mass index (kg/m^2)					
< 18.5	4.3	3.9	4.1	5.1	4.3
18.5-24.9	69.1	65.9	67.6	75.7	69.3
≥ 25.0	26.6	30.3	28.3	19.2	26.4
Mother's education (yr)					
0–2	24.4	24.9	24.6	34.7	26.8
3–5	45.9	46.6	46.2	27.8	42.2
6 +	29.7	28.5	29.1	37.6	31.0
Mother works outside home					
Yes	57.1	57.0	57.1	56.6	57.0
No	42.9	43.0	42.9	43.4	43.0
Mother's religion					
Non-Christian	18.0	14.4	16.3	26.3	18.5
Christian	82.0	85.6	83.7	73.7	81.5
Standard of living index[c]					
Low	31.5	23.7	27.9	52.3	33.2
Medium	36.8	39.0	37.8	40.5	38.4
High	31.7	37.3	34.3	7.1	28.4
Mother smokes tobacco					
Yes	0.6	0.4	0.5	1.3	0.7
No	99.4	99.6	99.5	98.7	99.3

continued

TABLE 1. Sample (%) distributions of births in the five years preceding the ZDHS according to whether or not the infant was weighed at birth and source of information on birthweight (health card or mother's recall)[a]

Variable	% weighed at birth			% not weighed at birth	All children
	Card	Recall	Total		
Type of cooking fuel[d]					
High pollution	65.1	51.7	58.9	90.8	65.8
Medium pollution	8.9	15.3	11.8	5.7	10.5
Low pollution	26.0	33.0	29.3	3.5	23.7
Residence					
Urban	34.4	45.0	39.4	8.7	32.7
Rural	65.6	55.0	60.6	91.3	67.3
Region					
Manicaland	10.7	11.4	11.0	35.7	16.4
Mashonaland Central	10.7	7.1	9.0	11.2	9.5
Mashonaland East	8.6	9.9	9.2	7.7	8.9
Mashonaland West	8.9	9.9	9.4	10.5	9.6
Matabeleland North	6.5	1.9	4.3	7.7	5.1
Matabeleland South	8.7	4.1	6.6	4.3	6.1
Midlands	13.8	10.7	12.4	13.8	12.7
Masvingo	10.9	12.3	11.5	5.6	10.3
Harare	11.6	28.1	19.3	2.1	15.6
Bulawayo	9.6	4.5	7.2	1.3	5.9
No. of births[e]	1,390	1,220	2,610	721	3,331

ZDHS, 1999 Zimbabwe Demographic and Health Survey

a. 108 multiple births and 120 births with missing information on birthweight are excluded from the analysis.

b. Information available only for the last birth in the five years preceding the survey for each woman.

c. Standard of living is measured by an index calculated by adding the following scores: 3 each for a car or tractor; 2 each for a scooter or motorcycle, television, telephone, refrigerator, piped or public tap water, flush toilet, electricity, or wood/vinyl/asphalt/ceramic/cement/carpet for main floor material; and 1 each for a bicycle or radio. Index scores range from 0 to 2 for a low standard of living, 3 to 8 for a medium standard of living, and 9 to 21 for a high standard of living.

d. High-pollution fuels include firewood, dung, and straw; medium-pollution fuels include coal, charcoal, and kerosene; low-pollution fuels include liquefied petroleum gas (LPG), natural gas, and electricity.

e. The number of births varies slightly depending on the number of missing cases for each variable.

Health cards were available for 53% of the children who were weighed at birth. For the remaining 47%, the mother reported the birthweight. Children in rural households, poorer households, and households using biomass fuel were somewhat more likely to have health cards available at the time of the survey than children in urban households, richer households, and households using cleaner cooking fuel. The sample distributions of children according to the source of information on birthweight are quite similar for other background characteristics.

Among children who were weighed at birth, few (8%) were of low birthweight (< 2,500 g) (**fig. 1**). The mean birthweight of children born in the previous five years who were weighed at birth was 3,140 g (95% confidence interval, 3,118–3,163 g). A comparison of mean birthweight distribution shows that children whose birthweights were based on mother's recall (3,174 g) were significantly heavier than those whose birthweights were taken from a health card (3,111 g; $p = .004$).

The average height of Zimbabwe women is 159 cm, and fewer than 2% of women are shorter than 145 cm. Only 4% of the mothers recorded a BMI below 18.5 kg/m^2 [31]. According to WHO guidelines, adult women shorter than 145 cm and with a BMI below 18.5 kg/m^2 are considered undernourished and at a greater risk of having low-birthweight babies [33].

Effect of iron supplementation

Table 2 shows the observed and predicted mean birthweights according to whether the mother received iron supplementation during pregnancy, as well as the estimated mean birthweights according to other variables, from alternative multiple regression models. The table shows that children born to mothers who received iron supplementation during pregnancy were 93 g heavier, on average, than children born to mothers who did not receive iron supplementation during pregnancy ($p = .003$).

The effect of iron supplementation on birthweight remains virtually unchanged (92 g) when the source of information on birthweight and other pregnancy and

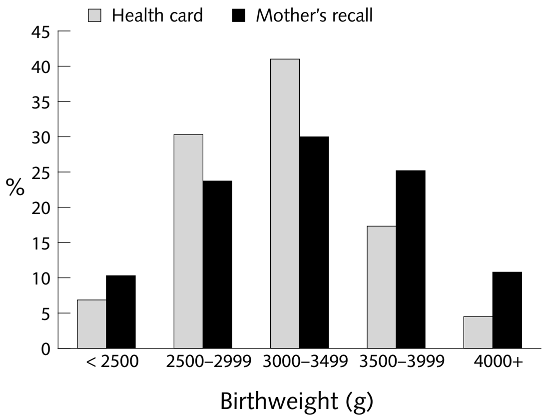

FIG. 1. Distribution of births in the five years preceding the 1999 Zimbabwe Demographic and Health Survey (ZDHS) according to birthweight (g) and source of information on birthweight

delivery care variables are controlled for statistically in Model 1 in **table 2**. The effect of iron supplementation on birthweight increases slightly (96 g) when the child's sex, the child's birth order, the mother's age at the child's birth, and the mother's BMI are also controlled for in Model 2. The effect of iron supplementation on birthweight is further increased in Model 3 (full model) when indicators of socioeconomic status, smoke exposure, and geographic region are additionally controlled for. In the full model, children born to mothers who received iron supplementation during pregnancy were 103 g heavier (95% confidence interval, 42–164 g; $p = .001$), on average, than children born to mothers who did not receive iron supplementation during pregnancy.

Effects of other risk factors and confounders

With the effect of iron supplementation and other factors controlled for in Model 3, children whose mothers received malaria drugs during pregnancy were 51 g heavier at birth ($p = .079$, marginally significant) than children whose mothers did not receive malaria drugs

TABLE 2. Observed and predicted mean birthweights of children born during the five years preceding the ZDHS according to whether or not the mother received iron supplementation during pregnancy[a]

Variable	Mean birthweight (g)			
	Observed	Predicted		
		Model 1	Model 2	Model 3
Iron supplements during pregnancy				
Yes	3,182**	3,178**	3,181**	3,184***
No[b]	3,089	3,087	3,085	3,080
Malaria drug during pregnancy				
Yes		3,182	3,186	3,184
No[b]		3,133	3,132	3,133
No. of tetanus injections during pregnancy				
None[b]		3,101	3,120	3,118
One		3,164	3,147	3,147
Two or more		3,142	3,149	3,150
Child birth in a health facility				
Yes		3,146	3,146	3,145
No[b]		3,124	3,125	3,142
Source of birthweight				
From health card		3,110**	3,109***	3,108**
From mother's recall[b]		3,189	3,191	3,191
Sex of child				
Boy[b]			3,213***	3,212***
Girl			3,073	3,073
Birth order of child				
1[b]			3,072	3,071
2			3,178**	3,181**
3			3,167	3,161
4 +			3,203*	3,205*

continued

TABLE 2. Observed and predicted mean birthweights of children born during the five years preceding the ZDHS according to whether or not the mother received iron supplementation during pregnancy[a]

Variable	Mean birthweight (g)			
	Observed	Predicted		
		Model 1	Model 2	Model 3
Mother's age at child's birth (yr)				
15–24[b]			3,125	3,125
25–34			3,149	3,146
35–49			3,237	3,243
Mother's body-mass index (kg/m^2)				
< 18.5[b]			3,080	3,042
18.5–24.9			3,103	3,110
≥ 25.0			3,254*	3,241**
Mother's education (yr)				
0–2[b]				3,132
3–5				3,142
6 +				3,160
Mother works outside home				
Yes				3,154
No[b]				3,132
Mother's religion				
Non-Christian				3,105
Christian[b]				3,153
Standard of living index				
Low[b]				3,157
Medium				3,156
High				3,122
Mother smokes tobacco				
Yes				2,951
No[b]				3,146
Type of cooking fuel				
High-pollution				3,098*
Medium-pollution				3,129
Low-pollution[b]				3,242
Region				
Manicaland				3,144
Mashonaland Central				3,123
Mashonaland East				3,249***
Mashonaland West				3,111
Matabeleland North				3,169
Matabeleland South				3,204*
Midlands				3,208*
Masvingo				3,183*
Harare[b]				3,074
Bulawayo				3,041
No. of births	2,104	1,977	1,890	1,860

ZDHS, 1999 Zimbabwe Demographic and Health Survey
* $p < .05$, ** $p < .01$, *** $p < .001$.
a. Analysis in this table is limited to last births only.
b. Reference category.

during pregnancy. Receiving one or more tetanus toxoid injections during pregnancy was also positively associated with birthweight, but the effect was not statistically significant. Place of delivery had no effect on birthweight. Independently of other factors, children whose birthweight was recorded from a health card were 83 g lighter at birth than children whose birthweight was based on mother's recall ($p = .001$).

Independently of other factors, girls were much lighter than boys at birth (138 g lighter), and first-born babies were lighter than babies of second- and higher-order births. Children born to older mothers (age 35 to 49) were heavier at birth than children born to younger mothers (age < 35). As expected, the mother's BMI had a strong positive relationship to the child's birthweight. Children of overweight or obese mothers (BMI ≥ 25) were 199 g heavier at birth, on average, than children of undernourished mothers (BMI < 18.5) ($p = .004$). Notably, none of the socioeconomic status characteristics—mother's education, mother's work status, mother's religion, or household living standard—had a significant effect on birthweight. As expected, children born to mothers who smoked tobacco were lighter at birth (195 g) than children born to mothers who did not smoke tobacco ($p = .078$). The large p-value in this case mainly reflects the small number of mothers who smoked (mothers of fewer than 1% of the children in the ZDHS sample reported smoking tobacco). Also as expected, children born to mothers who cooked with high-pollution biomass fuels (wood, dung, or straw) were lighter at birth (144 g) than children born to mothers who cooked with low-pollution fuels (electricity, liquefied petroleum gas, or biogas; $p = .022$). Children born in the two predominantly urban provinces of Bulawayo and Harare were significantly lighter at birth than those born in Mashonaland East, Masvingo, Matabeleland South, and Midlands provinces. The reasons for these regional differences in birthweight are not clear.

Separate analysis according to source of information on birthweight

The full model in **table 2** (Model 3) was repeated separately according to source of information on birthweight (results not shown). With other variables controlled for, children born to mothers who received iron supplementation during pregnancy were heavier at birth than those born to mothers who did not receive iron supplementation during pregnancy in both groups—64 g heavier in the group in which birthweight was recorded from a health card (95% confidence interval, 2–125; $p = .043$) and 163 g heavier in the group in which the mother reported the birthweight from recall (95% confidence interval, 44–281; $p = .008$). Among the control variables, only the child's sex had a statistically significant effect on birthweight in both groups. Birth order of child, mother's BMI, mother's tobacco smoking, type of cooking fuel, and region of residence had significant effects on birthweight in the group in which birthweight was recorded from a health card, whereas use of malaria drugs during pregnancy had a significant effect in the group in which birthweight was recorded from mother's recall.

Conclusions

Our analysis suggests that supplementation of mothers with iron during pregnancy had a strong positive effect on child's birthweight independently of other pregnancy care factors, mother's nutritional status, smoke exposure, and a number of demographic and socioeconomic factors. These results, based on a population-based national survey in Zimbabwe, are consistent with those of recent randomized, controlled trials in Nepal and the United States [23, 24], and they provide further evidence that iron supplementation during pregnancy can substantially increase birthweight and reduce the risk of adverse pregnancy outcomes in developing countries.

Our estimated effect of iron supplementation on birthweight is not much affected by the introduction of control variables in the various models we considered, which provides confidence in the findings. Our study has some limitations, however.

First, because the ZDHS did not collect information on tobacco smoking by other household members, we were unable to control for environmental tobacco smoke, which is a known risk factor for adverse pregnancy outcomes, including reduced birthweight. We were able to control only for maternal smoking, but very few women in Zimbabwe smoke.

Second, there may be some selection bias on the response variable (birthweight), because children who were not weighed may not have had the same average birthweight as the children who were weighed. Selection on the response variable can lead to biased estimates of the effect of iron supplementation on birthweight. In the ZDHS sample, children who were not weighed at birth were more likely to be from low-socioeconomic status households, whose mothers are less likely to receive iron supplementation during pregnancy. If all children were weighed at birth, our estimated effect of iron supplementation on birthweight would be even greater.

Third, we were unable to control directly for urban/rural residence owing to its collinearity with type of cooking fuel ($r = .95$), which is preferred in this analysis because it is a more direct risk factor for reduced birthweight [14]. In any case, when we replaced type of cooking fuel with urban/rural residence in the analysis (results not shown), the effect of iron supplementation on birthweight remained virtually unchanged (105 g).

Fourth, as mentioned earlier, information on iron supplementation is based on mothers' reports. Although most women who received iron tablets reported consuming them for at least 90 days, we cannot assess the exact intake of iron during pregnancy and its impact on birthweight. Moreover, our analysis is based on mothers' recall of receipt of iron tablets for their last pregnancy in the five years preceding the survey, which may have biased our results.

Fifth, because of lack of data, we were unable to control for gestational age. However, controlling for gestational age may be undesirable to the extent that iron supplementation during pregnancy affects the likelihood of having a preterm delivery [20, 21], which may, in turn, affect the birthweight of the child.

Sixth, given that Zimbabwe is a country with a high prevalence of HIV infection, the findings of our study could be biased if HIV-infected women were treated differently in terms of iron supplementation and other prenatal care than non-HIV-infected women. However, there are no official recommendations in Zimbabwe to treat HIV-infected women differently than noninfected women with regard to iron supplementation. Moreover, a recent randomized trial among prenatal care attendees in Harare, Zimbabwe, found no significant difference in the effect of multimicronutrient supplementation during pregnancy on birthweight between HIV-infected and -noninfected women [28]. Another randomized trial of vitamin supplements in relation to vertical transmission of HIV in Tanzania found that prenatal multivitamin supplementation did not affect the risk of vertical transmission of HIV [29].

Finally, it is possible that receipt of iron pills during pregnancy is a proxy for some other prenatal care intervention, which may improve birthweight. However, we did control for two other important aspects of prenatal care—receiving malaria prophylaxis and tetanus injections during pregnancy; and controlling for these other prenatal care interventions, as well as for other variables, makes virtually no difference in the estimated effect of iron supplementation on birthweight.

Many of these limitations could be addressed by undertaking carefully designed studies with better measures of iron intake during pregnancy and clinical measures of pregnancy outcomes, including birthweight. Such studies could provide further evidence for the effect of iron supplementation during pregnancy in improving pregnancy outcomes and promoting child survival in developing countries.

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Vitamin A deficiency and child survival in sub-Saharan Africa: A reappraisal of challenges and opportunities

Victor M. Aguayo and Shawn K. Baker

Abstract

Background. *Children with vitamin A deficiency have higher risk of morbidity and mortality than vitamin A–sufficient children. Estimates on the potential child survival benefits of vitamin A deficiency control are needed for policy and program advocacy.*

Objective. *To determine the current prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa in order to estimate the potential child-survival benefits of effective and sustained policies and programs for the control of vitamin A deficiency in this region.*

Methods. *Estimates of the prevalence of vitamin A deficiency generated in 1998, data from 11 nationally representative vitamin A deficiency surveys conducted in sub-Saharan Africa between 1997 and 2003, and the measured effects of vitamin A deficiency on child mortality were combined to estimate the prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa and the potential child-survival benefits of effective and sustained policies and programs for the control of vitamin A deficiency in this region.*

Results. *Our analysis shows that in the absence of effective and sustained policies and programs for the control of vitamin A deficiency, an estimated 42.4% of children 0 to 59 months of age in sub-Saharan Africa (43.2 million children) are at risk for vitamin A deficiency. Such effective and sustained policy and program action for the control of vitamin A deficiency can bring about a potential 25% reduction in mortality in children 0 to 59 months with respect to 1995 mortality levels (i.e., before the onset of large-scale vitamin A supplementation programs in sub-Saharan Africa).*

Conclusions. *Effective and sustained control of vitamin A deficiency has the potential to be among the most cost-effective and high-impact child-survival interventions in sub-Saharan Africa. A stronger political commitment and a more appropriate level of investment in the effective control of vitamin A deficiency could make a large contribution toward the attainment of the Millennium Development Goal for the reduction of child mortality rates by two-thirds between 1990 and 2015. Among the many challenges that Africa will need to face in the coming years, vitamin A deficiency is one that can be overcome. The need is urgent, and the solutions are known, effective, and affordable.*

Key words: Child mortality, child survival, sub-Saharan Africa, vitamin A deficiency

Introduction

For several decades, vitamin A deficiency has been recognized as the leading cause of preventable pediatric blindness in developing countries [1]. A better understanding of the public health importance of vitamin A deficiency began in the early 1980s, when community-based studies showed that the rates of morbidity and mortality from diarrhea and respiratory infections were higher in children with mild xerophthalmia than in children without any vitamin A deficiency–related eye signs [2, 3]. Between 1986 and 1993, eight population-based intervention trials, enrolling more than 165,000 children worldwide, assessed the contribution of vitamin A deficiency to child mortality [4–11]. In 1993, four independent meta-analyses based on these trials showed that, in areas where vitamin A deficiency is prevalent, child mortality is reduced by 23% to 34% after vitamin A intervention [12–15]. This significant reduction in childhood mortality is attributable largely to the reduction in mortality from measles [10, 16], severe diarrhea and dysentery [17], and possibly falciparum malaria [18].

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To bring the attention of policy makers to vitamin A deficiency in countries where country-level vitamin A deficiency survey data were not available, the Micronutrient Initiative, UNICEF, and Tulane University generated country-level estimates of the prevalence of vitamin A deficiency worldwide (referred to here as the MI/UNICEF/TU estimates) [19]. These estimates were developed by using interpolation models built on a data set that included 42 vitamin A deficiency surveys (3 national and 39 subnational surveys) conducted in 36 countries worldwide between 1987 and 1995. The models that maximized the concordance between the observed and predicted values for countries that actually had vitamin A deficiency survey data were selected to generate country-level estimates of the prevalence of vitamin A deficiency for countries where no survey data were available.

Although these estimates have been critical in advancing policies and programs for the control of vitamin A deficiency over the past five years, we hypothesize that they significantly underestimate the true prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa and thus underplay the contribution of vitamin A deficiency to child mortality in this region. The objectives of our analysis were to estimate the current prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa, to estimate the potential child-survival benefits of effective and sustained policies and programs aiming at controlling vitamin A deficiency in this region, and to outline the key components of an integrated regional strategy for the effective control of vitamin A deficiency among children under five years of age (0 to 59 months old).

Methods

To estimate the current prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa, we proceeded in five steps.

First, we identified the countries in sub-Saharan Africa in which a nationally representative vitamin A deficiency survey had been conducted between 1997 and 2003. For each of these countries, we estimated the number of children under five years of age with vitamin A deficiency, combining the observed prevalence of vitamin A deficiency and the population of children in this age group. To estimate the average observed prevalence of vitamin A deficiency in this set of countries, we calculated the proportion of the total population of children under five years of age who were observed to have vitamin A deficiency.

Second, for each of these countries we estimated the predicted number of children with vitamin A deficiency, combining the prevalence of vitamin A deficiency predicted by MI/UNICEF/TU and the population of children under five years of age. To estimate the average predicted prevalence of vitamin A deficiency in this set of countries, we calculated the proportion of the total population of children under five years of age who were predicted to have vitamin A deficiency.

Third, we divided the average observed prevalence of vitamin A deficiency in this set of countries by the average predicted prevalence of vitamin A deficiency to estimate the observed/predicted vitamin A deficiency underestimation factor.

Fourth, we applied this factor to the MI/UNICEF/TU average predicted prevalence of vitamin A deficiency to obtain a corrected estimate that would better reflect the true prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa.

Fifth, by combining the corrected prevalence of vitamin A deficiency and the measured effects of vitamin A deficiency on child mortality, we estimated the potential contribution of vitamin A deficiency to child mortality by the equation

$$PAR = PREV\ (RR–1)\ /\ 1+\ [PREV\ (RR–1)]$$

where PAR (population attributable risk) is the proportion of all-cause child mortality attributable to vitamin A deficiency, PREV is the corrected prevalence of children at risk for vitamin A deficiency, and RR is the increased risk of death in vitamin A–deficient children relative to vitamin A–sufficient children. On the basis of data from the eight population-based studies of vitamin A deficiency and child mortality [4–11], it has been estimated that the risk of death in vitamin A–deficient children is 1.75 times higher than that in vitamin A–sufficient children [20]. This relative risk (RR = 1.75) was used to estimate the contribution of vitamin A deficiency to child mortality in sub-Saharan Africa.

Results

Table 1 shows the prevalence of vitamin A deficiency predicted by MI/UNICEF/TU for the countries included in our analysis. Estimates for Cape Verde, Equatorial Guinea, Gambia, Guinea Bissau, and São Tomé and Príncipe in the West and Central Africa Region (WCAR) were not available. These countries account for 1.1% of the total population of the WCAR. Estimates for Comoros, Eritrea, Seychelles, and Swaziland in the East and Southern Africa Region (ESAR) were not available. These countries account for 1.7% of the total population of the ESAR.

The predicted prevalence of vitamin A deficiency for sub-Saharan Africa was estimated by combining the predicted prevalence of vitamin A deficiency and the population of children under five years of age in the 36 countries for which MI/UNICEF/TU estimates were available. This calculation yielded an average predicted prevalence of vitamin A deficiency

TABLE 1. Predicted prevalence of vitamin A deficiency (VAD) among children under five years of age (0–59 months) and predicted population of children in that age group with VAD in sub-Saharan African countries in 1995

West and Central Africa	Predicted VAD prevalence[a] (%)	Predicted population 0–59 mo with VAD[b]	East and Southern Africa	Predicted VAD prevalence[a] (%)	Predicted population 0–59 mo with VAD[b]
Benin	15.2	16,7200	Angola	23.6	519,200
Burkina Faso	26.8	509,200	Botswana	8.7	17,400
Cape Verde[c]	18.1	18,100	Burundi	20.0	240,000
Côte d'Ivoire	15.4	446,600	Comoros[c]	20.1	20,100
Gambia[c]	18.1	36,200	Eritrea[c]	20.1	120,600
Ghana	15.2	456,000	Ethiopia	26.9	2,824,500
Guinea	25.9	336,700	Kenya	11.6	614,800
Guinea Bissau[c]	18.1	36,200	Lesotho	13.4	40,200
Liberia	23.3	139,800	Madagascar	17.9	465,400
Mali	36.0	756,000	Malawi	31.3	657,300
Niger	25.1	476,900	Mauritius	6.5	6,500
Nigeria	16.0	3,296,000	Mozambique	32.9	954,100
Senegal	11.8	165,200	Namibia	11.1	22,200
Sierra Leone	40.3	322,400	Rwanda	21.9	306,600
Togo	15.3	122,400	Seychelles[c]	20.1	2,010
Cameroon	10.7	246,100	Somalia	24.1	433,800
Central African Republic	18.5	111,000	South Africa	9.2	524,400
Chad	24.3	267,300	Swaziland[c]	20.1	20,100
Congo (Republic of)	15.9	79,500	Tanzania	15.6	826,800
Congo (Democratic Republic of)	16.5	1,419,000	Uganda	22.2	976,800
Equatorial Guinea[c]	18.1	18,100	Zambia	20.0	340,000
Gabon	15.8	31,600	Zimbabwe	11.8	224,200
São Tomé and Principé[c]	18.1	1,810			
Total for West and Central Africa	18.1	9,459,310	Total for East and Southern Africa	20.1	10,157,010

a. Data are from MI/UNICEF/TU [19]. Data refer to 1995.
b. Predicted populations were calculated on the basis of 1995 child population data from UNICEF [21].
c. No estimate is available from MI/UNICEF/TU [19]. The regional (weighted) average was used as proxy.

in sub-Saharan Africa of 19.1% (18.1% in the WCAR and 20.1% in the ESAR).

The predicted number of children at risk for vitamin A deficiency in sub-Saharan Africa was estimated by combining the predicted prevalence of vitamin A deficiency and the population of children under five years of age in the 45 countries included in the analysis. In the absence of estimates of vitamin A deficiency for Cape Verde, Gambia, Guinea Bissau, Equatorial Guinea, and São Tomé and Príncipe, the average predicted prevalence in the WCAR (18.1%) was used as a proxy. Similarly, in the absence of estimates of vitamin A deficiency for Comoros, Eritrea, Seychelles, and Swaziland, the average predicted prevalence of vitamin A deficiency for the ESAR (20.1%) was used as a proxy. **Table 1** shows that according to the MI/UNICEF/TU estimates, in 1995 (before the onset of large-scale vitamin A supplementation programs) an estimated 19.6 million children under five years of age in sub-Saharan Africa were suffering from vitamin A deficiency (9.5 million in the WCAR and 10.1 million in the ESAR).

Between 1997 and 2003, 15 sub-Saharan African countries conducted nationally representative vitamin A deficiency surveys (**table 2**). Although the observed prevalence of vitamin A deficiency varies across countries, it is consistently and significantly higher in all 15 countries than that predicted by MI/UNICEF/TU.

The average observed prevalence of vitamin A deficiency in these 15 countries was calculated by combining the observed prevalence of vitamin A deficiency in children and the population of children under five years of age in each country. This calculation yielded an average observed prevalence of vitamin A deficiency (weighted) of 42.9% in this set of countries. Similarly, the average predicted prevalence of vitamin A deficiency was calculated by combining the prevalence

TABLE 2. Observed and predicted prevalence of vitamin A deficiency (VAD) among children under five years of age (0–59 months) and observed and predicted population of children in that age group with VAD in sub-Saharan African countries in which a national VAD survey was conducted between 1997 and 2003

Country	Year	Observed VAD prevalence[a] (%)	Observed population 0–59 mo with VAD[b]	Predicted VAD prevalence[a] (%)	Predicted population 0–59 mo with VAD[b]	Underestimation factor
Angola	1998	64.3	1,414,600	23.6	519,200	2.7
Benin	1999	70.2	772,200	15.2	167,200	4.6
Cameroon	2000	40.0	919,310	10.7	246,100	3.7
Central African Republic	1999	68.2	409,200	18.5	111,000	3.7
Gambia	1999	64.0	128,000	18.7	37,400	3.4
Democratic Republic of the Congo	1998	61.1	5,254,600	16.5	1,419,000	3.7
Kenya	1999	60.2	3,243,600	11.6	614,800	5.2
Liberia	1999	52.9	317,400	23.3	139,800	2.3
Madagascar	2000	41.8	1,086,800	17.9	465,400	2.3
Malawi	2001	59.2	1,243,200	31.3	657,300	1.9
Mozambique	2002	71.2	2,064,800	32.9	954,100	2.2
Nigeria	2001	26.8	5,520,800	16.0	3,296,000	1.7
Tanzania	1997	24.0	1,272,000	15.6	826,800	1.5
Uganda	2001	27.9	1,227,600	22.2	976,800	1.3
Zambia	1997	65.7	1,116,900	20.0	340,000	3.3
Average (weighted)		42.9	25,938,010	17.8	10,770,900	2.4

a. All 15 VAD surveys were based on a national-level representative sample of children. In all surveys, the sample was obtained by using a random, multistage, proportional-to-size, cluster sampling method. In all surveys (as in those included in the data set used for the MI/UNICEF/TU predictions), VAD in children was defined as a serum retinol level below 0.70 µmol/L. The surveys were conducted at the time of the year when the risk of VAD in children was expected to be the highest.

b. For comparison purposes, we used 1995 child population data as published in UNICEF [21].

c. Data are from MI/UNICEF/TU [19]. Data refer to 1995.

of vitamin A deficiency among children under five years of age predicted by MI/UNICEF/TU with the population in this age group for each country. This calculation yielded an average predicted prevalence of vitamin A deficiency (weighted) of 17.8%. This means that in the 15 countries with recent nationally representative vitamin A deficiency surveys (1997–2003), the prevalence of vitamin A deficiency predicted by MI/UNICEF/TU underestimated the true (observed) prevalence of vitamin A deficiency by a factor of 2.4. In other words, the prevalence of vitamin A deficiency observed was 2.4 times higher than that hypothesized (predicted).

Four of the nationally representative vitamin A deficiency surveys conducted between 1995 and 2003 (in the Central Africa Republic, Democratic Republic of the Congo, Liberia, and Malawi) did not include children 36 to 59 months old in their samples. Using data on the prevalence of vitamin A deficiency among children under three years of age as a proxy for its prevalence in children under five years of age could lead to an overestimation of the prevalence of vitamin A deficiency among children under five years of age. To avoid this potential bias, the average observed and predicted prevalence of vitamin A deficiency was calculated for the 11 countries with nationally representative data on the prevalence of vitamin A deficiency among children under five years of age.*

Each of the 11 vitamin A deficiency surveys used a national-level representative sample of children under five years of age. In all 11 surveys, the sample was obtained by using a random, multistage, proportional-to-size, cluster sampling method. In all 11 surveys (as in those included in the data set used for MI/UNICEF/TU predictions), vitamin A deficiency in children was defined as a serum retinol concentration below 0.70 µmol/L. As recommended, the surveys were conducted at the time of the year when children were expected to be at the highest risk of vitamin A deficiency. These 11 national vitamin A deficiency surveys provide us with a geographically balanced (47% and 53% of the total population in the WCAR and ESAR regions respectively) representative sample of 49.2 million children under five years of age (48% of the total under-five population in sub-Saharan Africa). In this set of 11 countries, the average child mortality rate and the predicted prevalence of vitamin A deficiency (174

* Reports of the 15 nationally representative vitamin A deficiency surveys are not referenced here but can be obtained from the Ministries of Health of the respective countries.

per 1,000 live births and 17.4%, respectively) were not significantly different from those in the rest of sub-Saharan Africa (177 per 1,000 live births and 19.1%).

In this set of 11 countries, the prevalence of vitamin A deficiency predicted by MI/UNICEF/TU (17.4%) underestimates the true (observed) prevalence of vitamin A deficiency (38.6%) by a factor of 2.2. In other words, the prevalence of children under five years of age with vitamin A deficiency is 2.2 times higher than that hypothesized (predicted).

The average prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa was estimated by applying a correction factor of 2.2 to the MI/UNICEF/TU prevalence estimates and combining this corrected prevalence with the under-five population. This yielded (**table 3**) an average prevalence of children 0 to 59 months at risk for vitamin A deficiency of 42.4% (40.2% in the WCAR and 44.8% in the ESAR) and says that in the absence of effective and sustained vitamin A deficiency-control policies and programs, an estimated 43.2 million children under five years of age in sub-Saharan Africa would be currently at risk for vitamin A deficiency.

The potential contribution of vitamin A deficiency to child mortality in sub-Saharan Africa was then estimated by combining the risk (RR) of death in children with vitamin A deficiency relative to that in children without vitamin A deficiency (RR = 1.75) and the corrected prevalence of vitamin A deficiency. Our analysis shows that in 1995, before the onset of large-scale vitamin A supplementation programs in many countries in sub-Saharan Africa, an estimated 25.1% of all-cause child mortality (more than 646,000 child deaths annually) was attributable to vitamin A deficiency.

Discussion

Our analysis used a systematic approach. However, three caveats must be acknowledged. In the absence of MI/UNICEF/TU estimates of the prevalence of vitamin A deficiency in Cape Verde, Comoros, Equatorial Guinea, Eritrea, Gambia, Guinea Bissau, São Tomé and Príncipe, Seychelles, and Swaziland (these countries represent 1.4% of the total population in sub-Saharan Africa), the average prevalence of vitamin A deficiency predicted for the corresponding region (WCAR or ESAR) was used as a proxy. Four of the 11 national-level vitamin A deficiency surveys conducted between 1995 and 2003 (Benin, Cameroon, Gambia, and Kenya) did not include infants 6 to 11 months old. For these countries, the prevalence of vitamin A deficiency among children 12 to 59 months old was used as a proxy for vitamin A deficiency among children 6 to 59 months old (without upward or downward adjustment). The rates of prevalence of vitamin A deficiency were not adjusted for potentially high infection rates in the surveyed populations. Errors generated from these three assumptions would affect our estimates.

However, despite these three limitations, our analysis provides a robust estimate of the potential current prevalence of children at risk for vitamin A deficiency in sub-Saharan Africa, and it shows that effective and sustained control of vitamin A deficiency may be among the most cost-effective and high-impact child survival interventions in sub-Saharan Africa. Improving the vitamin A status of children may reduce child mortality in sub-Saharan Africa by up to 25% from the 1995 levels. These results are in line with previous analyses showing that in developing countries, up to one-third of deaths in pre-school-age children could be averted by improving vitamin A nutriture [22]. Moreover, this should be considered a conservative estimate of the potential contribution of improved vitamin A nutriture to the survival of children in sub-Saharan Africa, for two reasons: first, our analysis did not account for the potential contribution of vitamin A deficiency to infant mortality in the first half of infancy (0 to 5 months), because the available data from which to derive estimates are inconclusive; and second, our analysis did not account for the contribution of vitamin A deficiency to the mortality of children in the sixth and seventh years of life, although trials have reported reductions in mortality in this age group following vitamin A repletion [1, 5].

It is possible that in areas with a high prevalence of both vitamin A deficiency and HIV infection, the reduc-

TABLE 3. Estimated prevalence of children at risk of vitamin A deficiency (VAD), child population at risk for VAD, and mortality attributable to VAD among children 0 to 59 months of age in sub-Saharan Africa

Region	Corrected current prevalence of VAD risk (%)	Population at risk for VAD (millions)	No. of deaths attributable to VAD in 1995[a]	% of deaths attributable to VAD in 1995
West and Central Africa	40.2	20.8	315,960	23.5
East and Southern Africa	44.8	22.4	330,295	26.9
Sub-Saharan Africa	42.4	43.2	646,255	25.1

a. Before the onset of large-scale vitamin A supplementation programs in numerous countries in sub-Saharan Africa. Calculations were made on the basis of infant and child mortality rates and population of children 0–59 months old as published in UNICEF [21].

tion in mortality following vitamin A repletion is lower than in regions with a high prevalence of vitamin A deficiency and a low prevalence of HIV infection. However, from the available evidence it appears that the impact of vitamin A repletion on disease progression and death depends largely upon the stage of HIV infection. In the early stages of HIV infection, vitamin A repletion could have a positive impact by slowing the progression of the disease and increasing survival rates in early childhood. In a trial in Tanzania, for example, vitamin A supplementation of a cohort of children 6 to 59 months old, in which 9% of the children were HIV-infected, resulted in a 49% reduction of all-cause mortality; among the HIV-infected children, all-cause mortality was reduced by 63% [23]. In the light of these findings, it has recently been concluded that vitamin A supplementation of HIV-infected children appears to be beneficial in reducing the incidence, severity, and mortality of diarrhea [24], one of the leading causes of child mortality in sub-Saharan Africa.

Implications

Our analysis shows that the implementation of effective and sustained policies and programs for the control of vitamin A deficiency can bring about a reduction of up to 25% in child mortality rates in sub-Saharan Africa, compared with the mortality rates in 1995, before the onset of large-scale vitamin A supplementation with National Immunization Days for polio eradication. In many countries in sub-Saharan Africa, one high-dose vitamin A capsule is given annually on National Immunization Days for polio eradication, thus ensuring a vitamin A reserve of four to six months to more than 80% of children 6 to 59 months old. This is a remarkable achievement. However, as National Immunization Days for polio eradication are phased out or scaled down, it is imperative to ensure that all children 6 to 59 months old receive preventive vitamin A supplementation twice yearly, as recommended by the World Health Organization (WHO) and UNICEF. Promising experiences such as those with National Micronutrient Days (Niger and Burkina Faso), Nutrition Weeks (Mali), Child Health Weeks (Zambia), Vitamin A Weeks (Ghana), or the integration of vitamin A supplementation into Community-Directed Treatment with Ivermectin (CDTI) for onchocerciasis control (Nigeria and Cameroon) demonstrate that countries in sub-Saharan Africa can deliver vitamin A supplements to children twice yearly. All these strategies have in common the periodic, active distribution of vitamin A supplements through existing permanent institutions [25]. In combination with the integration of vitamin A supplementation into the Expanded Program of Immunization (EPI) and the therapeutic dosing of children suffering from malnutrition, infectious diseases, or both, these strategies can effectively deliver vitamin A supplements to children. It is therefore crucial that this high-impact and cost-effective child survival intervention (human rights imperatives aside) be made available to all children in sub-Saharan Africa.

It is also important to ensure that vitamin A supplementation drives an integrated, effective, and sustained sub-Saharan Africa–wide assault on vitamin A deficiency that integrates improved infant and young child nutrition and improved vitamin A dietary intakes throughout the life-cycle. Mortality data from the eight population-based field trials included in the meta-analysis by Beaton et al. [12] show that the reductions in mortality among children 6 to 24 months old made up more than 70% of the number of lives saved as a result of improving the vitamin A status of children 6 to 59 months old. Optimal feeding practices in the first two years of life are therefore essential for optimal vitamin A nutrition. Breastmilk, in particular, is vital to keep infants adequately nourished with vitamin A in the first six months of life, and possibly throughout infancy [26]. Gambia and Ghana, among other countries in sub-Saharan Africa, have proved that well-designed community-based and/or facility-based behavioral change communications programs can bring about dramatic improvements in the rates of early initiation of breastfeeding and adoption of exclusive breastfeeding in the first six months of life.

Dietary improvement must be an integral part of a sustainable strategy to control vitamin A deficiency, particularly among women of reproductive age. Significant progress has been achieved in the past 10 years in the design and implementation of a new generation of approaches that integrate production, nutrition education, and behavioral change communication strategies [27]. Vitamin A fortification of locally available foods can be crucial in improving the vitamin A status of the population and that of women of reproductive age in particular. An increasing number of countries in sub-Saharan Africa have conducted national-level food-consumption surveys to identify potential food vehicles for vitamin A fortification programs, have assessed the capability of selected food industries to implement vitamin A fortification, and/or are implementing successful private–public partnerships for vitamin A fortification [28].

Conclusions

At the United Nations Millennium Summit in 2000, African and other world leaders made a commitment to reduce mortality rates in children by two-thirds between 1990 and 2015. A stronger political commitment and a more appropriate level of investment in the effective control of vitamin A deficiency have the promise to be among the most cost-effective and high-impact policy and program actions towards the achievement of the Millennium Development Goal for the reduction of child mortality in sub-Saharan Africa. Among the many challenges that Africa needs to face in the coming years, the control of vitamin A deficiency is one that can be accomplished. The need is urgent, and the solutions are known, effective, and affordable.

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Community assessment of availability, consumption, and cultural acceptability of food sources of (pro)vitamin A: Toward the development of a dietary intervention among preschool children in rural Burkina Faso

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Abstract

Background. *Vitamin A deficiency remains a public health problem in Burkina Faso and elsewhere in the developing world. Dietary diversification is a promising strategy that needs to be explored to strengthen the country's ongoing supplementation program.*

Objective. *The purpose of this study was to identify locally available and acceptable (pro)vitamin A–rich foods to be included in a dietary intervention addressing vitamin A deficiency in children aged six months to three years.*

Methods. *A food ethnographic study combining recall methods, observation, and focused group discussion was conducted in the dry and rainy seasons. Thirty-five mother–child pairs were randomly selected and included in the study.*

Results. *The dietary pattern of children was characterized by low diversity with extremely low energy and vitamin A intake in both seasons. The study identified the availability of numerous (pro)vitamin A–rich foods, but these foods are either not consumed or consumed by few in low amounts and/or in low frequencies. The main constraining factors identified are related to financial accessibility (for liver), seasonal availability (for egg, milk, mango, papaya, and green leafy vegetables), and beliefs related to consumption and preparation (for green leafy vegetables). However, the study also revealed that the study population associated all identified (pro)vitamin A–rich foods with positive attributes such as health, strength, and vitamin richness, which might offer an entry point for designing and implementing dietary interventions.*

Conclusions. *Based on the findings of this formative research, intervention strategies with mango and liver are proposed to improve the vitamin A intake and status of children in the rural areas of Burkina Faso.*

Key words: Children, constraint, (pro)vitamin A food availability, rural area, Burkina Faso

Introduction

Vitamin A deficiency is a public health problem of great importance in Burkina Faso. The National Plan of Action for Nutrition, on the basis of data from several local surveys, reported a prevalence of night-blindness of 1% to 5% among children under five years old [1]. Recently, serum retinol levels below 0.7 μmol/L were found in 85% of preschool children and 65% of their mothers in the department of Kaya [2]. The strategies at the national level to combat vitamin A deficiency mainly involve periodic distribution of high-dose vitamin A capsules twice a year to children six months to five years old, with strong external support. Unfortunately, less emphasis has been placed on dietary approaches to preventing and controlling vitamin A deficiency.

A small-scale qualitative study of factors influencing

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the choice of strategies for combating vitamin A deficiency in Burkina Faso revealed that lack of awareness of the importance of food diversification strategies on the part of decision makers, combined with lack of evidence of effectiveness of food-based interventions and the low interest of donors in food diversification, appeared to be the main limiting factors in the development of dietary diversification strategies [3]. However, food-based approaches have a real potential to prevent and alleviate vitamin A deficiency in a sustainable way [4]. Controlled trials using green leafy vegetables, fruits, and/or yellow sweet potatoes, with or without fat supplementation or deworming, were effective in improving the vitamin A status of children [5–10]. A pilot study conducted in north-central Burkina Faso on the promotion of red palm oil as a source of vitamin A for mothers and children was effective in improving the serum retinol status of the targeted group [2]. Moreover, the impact evaluation of this pilot study showed that it is possible and feasible to introduce a new food into existing dietary patterns through sufficiently persuasive approaches, taking into account the availability of the product and its affordability and acceptability to the population [11].

In Burkina Faso a wide variety of green leafy vegetables, fruits, and animal products rich in (pro)vitamin A are available. However, their utilization in child-feeding practices in rural areas is not well documented. A careful understanding of patterns of dietary nutrient intake and their determinants is essential [12]. Vitamin A intake is one of the most difficult values to estimate in a precise manner at the individual level, given the high day-to-day variability in the consumption of (pro)vitamin A–rich food [13]. However, at the group level, the use of 24-hour recall may provide a reliable estimate of dietary vitamin A intake in preschool children [14]. This study was designed to investigate the availability of (pro)vitamin A–rich foods and the patterns of consumption of these foods by preschool children in two seasons, and to identify factors that may facilitate or constrain their adequate incorporation into child-feeding practices.

Methods

Study design

The research protocol consisted of a focused food ethnographic study combining anthropological and nutritional methods [15]. The study was carried out in two survey rounds, one during the rainy season (July to August) and the other during the dry season (March to April). At each round, data were collected in three phases with both qualitative and quantitative tools. The first phase was focused on determining the availability of (pro)vitamin A–rich food in the study area. The second phase studied the food consumption of preschool children, with special emphasis on (pro)vitamin A–rich foods. The third phase investigated the constraints on consumption of (pro)vitamin A–rich food by preschool children.

Study area and subjects

The research was conducted in the department of Kokologho in rural west-central Burkina Faso. Sector 4, the smallest administrative unit (comparable to a village) in the setting, which was composed of three communities, was selected for this study because a previous food consumption study indicated that people in this sector were at risk for vitamin A deficiency due to a low consumption of vitamin A.* The study population included children aged six months to three years and their caretakers; this age group was targeted because this stage of development is considered to be the most critical with respect to mortality and nutrient deficiencies [16, 17]. At the beginning of the study, an exhaustive census of households from sector 4 was conducted by local fieldworkers. Thirty-five households with a child in the age range from six months to three years were randomly selected from the household census list. A total of 65 mother–child pairs (34 in the rainy season and 31 in the dry season) successfully completed the study. This sample size is considered adequate for generating valid socioethnographic data because of high concordance in food culture [18].

Food availability

The availability of (pro)vitamin A–rich food was assessed by key informant interviews, a market survey, and observation in fields and gardens in both seasons. The free listing technique [15] was used to gather information about food availability in the community. This is done by asking key informants to list all foods available in the community and their seasonal availability. The market survey provided information on the availability and stability of food in the local market and stores. Observations in fields and gardens were conducted with collection of herbarium specimens of wild green leafy vegetables for further identification by a botanist. This resulted in a list of key foods rich in (pro)vitamin A, which were selected on the basis of information from food-composition tables [19–23].

Anthropometric measurements

The age of the child was determined from the birth certificate or health record; in the absence of these, a calendar of local events was used to determine the age

* Bengaly MD, van Raaij JMA, Traoré AS, Kok FJ, unpublished data.

of the child as accurately as possible. Height and weight were measured by standard methods [24]. Height was measured to the nearest 0.1 cm with a conventional wooden height board. Weight was measured to the nearest 0.05 kg on a digital display scale (Soehnle, Murrhardt, Germany), which was calibrated with test weights before each weighing session. The subjects wore a minimum of clothing and no shoes. Weight and height measurements were compared with those of the National Center for Health Statistics (NCHS) reference population [25] and converted to age- and sex-standardized z scores with NutStat software. Wasting, stunting, and underweight are defined by z scores below –2 SD for weight-for-height (WHZ), height-for-age (HAZ), and weight-for-age (WAZ), respectively.

Nutrient intake

A repeated 24-hour recall [14] was used to estimate the nutrient intake of each child on one market day and two nonmarket days. The mothers were asked to report all foods and dishes consumed by their children on the previous day. They were also asked to report the exact amount of all foods and ingredients of dishes consumed by showing the bowl used and to indicate the volume of the ingredients by using water (which was weighed) to estimate the volume or by indicating the price of the amount consumed. For foods or dishes bought at the market, recipes were collected from the vendors, and the amounts of ingredients were estimated from the prices of the ingredients. The conversion factors from volume or price to weight were determined afterwards. The percentage of foods consumed by the child was calculated from the volume of the total dish and the portion consumed by the child (estimated by the water method). The amount of all foods consumed by the child was converted into energy, protein, and vitamin A intake on the basis of information from the food-composition table of Mali [19] completed with information from other sources [20–23]. The recent conversion factors recommended by the International Vitamin A Consultation Group (IVACG) [26] for retinol activity equivalent (RAE) were used. The weekly frequency of consumption of (pro)vitamin A–rich foods was assessed with a nonquantitative food frequency questionnaire developed by Helen Keller International [27].

Dietary diversity

A dietary diversity score was created based on data from the repeated 24-hour recall. The dietary diversity score was defined as the number of food groups consumed by each child during the registration period. Foods and food groups were regrouped and summed into a seven-point dietary diversity score [28] according to the following seven food groups: starchy staples (grains, roots, and tubers); legumes; dairy (milk other than breastmilk, cheese, and yogurt); meat, poultry, fish, and eggs; vitamin A–rich fruits and vegetables (pumpkin, red and yellow yams and squash, carrots and red sweet potatoes, green leafy vegetables, and fruits such as mango, papaw, or other local vitamin A–rich fruits); other fruits and vegetables (or fruit juice); and foods containing oil, fat, or butter.

Children received a score of 1 if they consumed a particular food group during the registration period. So, for example, if a child consumes rice, he or she would receive a score of 1 for the category "starchy staples." If the child consumes beans, he or she receives a score of 1 for "legumes," and so forth. Maximum score in this scheme is 7 (for all seven groups) and minimum score is 0 (for no foods consumed). On the basis of cutoff points defined by Arimond and Ruel [28], children who consumed foods from zero to two food groups were classified as having low-diversity diets; those who consumed foods from three or four groups were classified as having middle-diversity diets; and those who consumed foods from five to seven groups were classified as having high-diversity diets.

Food attributes and constraints on key food consumption

A semistructured questionnaire was used to collect information on food attributes. The key informants included mothers and caretakers, who were asked to describe the perceived attributes or characteristics associated with individual foods. The pile-sorting technique, using pictures of individual foods [15], was used to study the relationship among key foods. The respondents were asked to sort the foods that belonged together according to any criteria that made sense to them and to explain the reasons for the grouping. Social and cultural beliefs, as well as economic constraints on key food consumption, were explored in four focus group discussions, with each group composed of six to eight women.

Data analysis

The distribution of each variable was examined, and implausible values were identified and excluded from the analysis. In the absence of significant differences in nutrient intake between market and nonmarket days, the nutrient intake was calculated as the average intake on the three days recorded. The nutrient intake was compared with the estimated average requirement (EAR) from the World Health Organization/Food and Agriculture Organization (WHO/FAO) [29, 30], which assumes normal breastmilk intake for the study age group. The results are presented as means ± SD (weight-for-height, height-for-age, and weight-for-age z scores; nutrient intake; nutrient adequacy ratio;

dietary diversity score; number of meals consumed per day; consumption of key foods) or percentages (z score below –2 SD for weight-for-height, height-for-age, and weight-for-age; children with low, middle, and high dietary diversity score). The Mann–Whitney test was used to test differences between the two seasons in nutrient intake, and the independent *t*-test was used to compare differences in dietary diversity scores. The results from the semistructured interviews, pile-sorting test, and focus group discussions were sorted according to key food. For each key food, information from all three methods was combined and analyzed. All data analysis was performed with SPSS 11.0 for Windows, version 11.0 [31].

Results

Table 1 summarizes the characteristics of the study population. The average age of the children studied was about 20 months. Overall, the proportion of children classified as malnourished according to each of the three measures of malnutrition was high; 39%, 32%, and 50%, respectively, were classified as stunted, wasted, and underweight in the dry season, compared with 32%, 29%, and 39% in the rainy season.

Sixty-five percent of the children were still being breastfed. The daily frequency of meal consumption was 3.3 ± 0.1 in the dry season and 3.5 ± 0.1 in the rainy season (**table 2**). In the dry and rainy seasons, 95% and 98% of children, respectively, consumed breakfast, 91% and 95% consumed lunch, 88% and 92% consumed dinner, and 22.5% and 38.5%, consumed at least one snack. The main meals consisted of *tô* and gruel for breakfast, *tô* and rice for lunch, and *tô* for dinner. *Tô* is the staple dish in Burkina Faso, consisting of a cereal-based thick porridge consumed with various sauces. Most of the children consumed mango and shea fruit (*Vitellaria paradoxa*) as snacks, depending on the season. The mean dietary diversity score was higher in the rainy season (3.2 ± 0.6) than in the dry season (2.9 ± 0.6); most of the children were classified as having a medium dietary diversity score (73.7% in the dry season and 90.9% in the rainy season), and none had a high dietary diversity score. Daily energy intake (403 ± 227 and 565 ± 324 kcal in the dry and rainy seasons, respectively) and vitamin A intake (105.2 ± 151.8 and 72.2 ± 62.6 µg RAE, respectively) represented a nutritional adequacy ratio (the ratio of a subject's intake to the current recommended allowance for the subject's sex and age category) of 36% and 48%, respectively, for daily energy and 26% and 19%, respectively, for vitamin A intake, whereas protein intake was above the EAR in both seasons (**table 2**). Fat intake was 7.0 ± 6.5 g in the dry season and 9.8 ± 9.5 g in the rainy season.

(Pro)vitamin A–rich foods identified in the community included three animal products (liver, eggs, and cow's milk), two fruits (mango and papaya), one tuber (yellow-fleshed sweet potato), and nine green leafy vegetables (**table 3**). Few children consumed animal products; 12.5% and 21% consumed liver, 3% and 0% consumed egg, and 0% and 21% consumed cow's milk, in the dry and rainy seasons, respectively, and none of these was consumed more than twice a week. The mean portion size consumed was 10.8 g, 25 g, and 100 ml for liver, eggs, and cow's milk, respectively,

TABLE 1. Characteristics of the study population

Characteristic	Dry season	Rainy season
Age (mo)	19.7 ± 7.4	20.2 ± 8.1
Sex (% male)	45.6	50.9
Nutritional status		
WAZ	–1.94 ± 1.36	–1.71 ± 1.12
HAZ	–1.51 ± 1.53	–0.98 ± 1.23
WHZ	–1.36 ± 1.02	–1.11 ± 1.16
Prevalence of malnutrition (%)		
Underweight (WAZ < –2SD)	50	39
Stunting (HAZ < –2SD)	39	32
Wasting (WHZ < –2SD)	32	29

WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score. Plus–minus values are means ± SD.

TABLE 2. Dietary diversity, nutrient intake, and nutrient adequacy ratio of children six months to three years old in Kokologho, Burkina Faso

Variable	Dry season (mean ± SD)	Rainy season (mean ± SD)
No. of meals consumed per day	3.3 ± 0.1	3.5 ± 0.1
DDS	2.9 ± 0.6	3.2 ± 0.6[a]
DDS category [b]		
Low (%)	20.7	9.1
Middle (%)	73.7	90.9
High (%)	0	0
Energy intake (kcal)	403 ± 227	565 ± 324
Protein intake (g)	13.4 ± 8.0	19.9 ± 11.7[a]
Fat intake (g)	7.0 ± 6.5	9.8 ± 9.5
Vitamin A intake (µg)	105.2 ± 151.8	72.2 ± 62.6
NAR[c]		
Energy	0.36 ± 0.17	0.48 ± 0.25
Protein	1.26 ± 0.68	1.71 ± 0.99
Vitamin A	0.26 ± 0.38	0.19 ± 0.16

DDS, dietary diversity score; NAR, nutrient adequacy ratio

a. Rainy season values different from dry season values, $p < .05$.

b. Low DDS, foods from 0–2 food groups consumed; medium DDS, foods from 3 or 4 groups consumed; high DDS, foods from 5–7 groups consumed.

c. The NAR is the ratio of a subject's intake to the current recommended allowance for the subject's sex and age category.

and the contribution of the portion consumed to the EAR of vitamin A was 22%, 11%, and 7%, respectively. Mango was consumed by more children than papaya (72% and 6%, respectively), and none of the children consumed yellow-fleshed sweet potatoes. The weekly frequency of consumption was five for mango and one for papaya, with a mean portion size of 147.5 g and 125 g, respectively, and a contribution to the EAR of 56% and 8%, respectively. Amaranthus and arzentiga leaves were consumed by 24% and 27% of the children, respectively; bean and boulvaka leaves by 42% and 49%, respectively; and more than 50% of the children consumed boundo, sorrel, or baobab leaves. Okra was the most frequently consumed vegetable by all children. The weekly frequency of consumption was 2 to 3.8 for amaranthus, arzentiga, bean, boulvaka leaves, and boundo leaves, 4 to 5 for baobab and sorrel leaves, and 9 for okra.

The characteristics of the (pro)vitamin A–rich key foods can be divided into health-related perceptions, availability and accessibility issues, and beliefs related to consumption and preparation. All selected (pro)vitamin A–rich foods were believed to provide health and strength and were perceived as vitamin-rich foods. In addition, all animal foods, fruits, yellow-fleshed sweet potato, and okra were seen as "blood-rich" foods by the local population, which meant that these foods were believed to provide blood when consumed.

Liver and sun-dried leafy vegetables were available year-round, whereas eggs, milk, fruits, yellow-fleshed sweet potato, and fresh vegetables were seasonal. Eggs were mostly used for breeding or sold to generate cash, whereas liver, especially hare liver, was given to persons suffering from night-blindness. Amaranthus leaves were grown mainly for sale and were hardly consumed because of lack of knowledge of how to prepare them. Animal foods and papaya were considered costly foods, whereas mango and okra were perceived as cheap foods. Animal products and papaya were considered suitable as food for children. Liver, eggs, and yellow-fleshed sweet potatoes could be prepared with oil. In addition, yellow-fleshed sweet potatoes were consumed either raw or boiled, but according to local culture they should not be eaten every day by children because they were believed to cause stomach problems. Mango and bean leaves were considered to "fill the stomach." Boundo leaves were perceived as easy to digest because they are slippery; yellow sorrel flowers had a particular acid taste; and okra was considered easy to cook.

Discussion

The dietary pattern of our study population is characterized by poor diversity and low volume of food intake, resulting in inadequate intake of energy and vitamin A, which is reflected in the high level of malnutrition in children aged six months to three years in the study area. The Demographic and Health Survey in 2003 reported comparable high rates of wasting, stunting, and underweight for this age group [32]. The low quality of the diet in terms of both insufficient nutrient supply and low diversity is typical of the food-consumption patterns in West Africa [33]. Hence, for any intervention strategy aimed at reducing vitamin A deficiency in the study area, the intake of energy should be properly addressed at the same time [3]. The protein intake of the subjects of our study appeared to be higher than the EAR because of the children's consumption of beans and the utilization of a protein-rich condiment called "soumbala," which is obtained from fermentation of the seeds of *Parkia biglobosa* in the preparation of the sauce for *tô*; however, this protein comes mainly from vegetable sources, and it remains important to encourage the consumption of animal protein.

This study indicated that (pro)vitamin A–rich foods are available in the study community but are not consumed, or are consumed by few children in low amounts and/or at low frequencies. The main constraining factors identified are related to financial accessibility, seasonal availability, and beliefs about consumption and preparation. However, the study showed that the study population associated all identified (pro)vitamin A–rich foods with positive attributes such as health, strength, and vitamin richness. An important positive empirical practice is the use of liver to treat night-blindness. These positive associations may offer an entry point for interventions aimed at improving vitamin A intake in the community.

Evaluating the dietary practices of a group targeted for vitamin A intervention programs before promoting natural food sources rich in vitamin A is of great importance, given the significance of both intercultural and intracultural diversity with respect to dietary inclusions and exclusions [34]. Moreover, every cultural setting maintains concepts about the classification and attributes of foods, which in turn affect decision-making about food selection, preparation, serving, and consumption [35]; such attributes could affect the failure or success of dietary intervention.

On the basis of the findings of this study, the most suitable (pro)vitamin A–rich foods to be promoted in a dietary diversity intervention to increase vitamin A intake could be liver and mango because of their important contribution to vitamin A intake and perceived positive attributes such as health, strength, good source of vitamins and blood fortification. However, it appears that the financial issue is the main limiting factor for liver consumption and that seasonal availability affects the consumption of mango.

To be effective, nutrition education programs should be built on the basis of a good understanding of the

TABLE 3. Weekly consumption and attributes of key food items

Food item	% of children consuming		Frequency of consumption (no./wk)		Portion size[a]	RAE/ portion[a]	Key food attributes
	Dry season	Rainy season	Dry season	Rainy season			
Animal products							
Liver (ox)	12.5	21	1.25 ± 0.5	1.9 ± 0.4	10.8 ± 0.99 g[b]	1,800	Perceived as strength and health provider and as blood- and vitamin-rich food Available year-round; high cost; hare liver in particular is used to treat night-blindness Fried in oil, can be eaten by children
Egg	3	0	1.0 ± 0.0	NA	25 ± 0 g[c]	43.8	Perceived as strength and health provider and as blood- and vitamin-rich food Available 4–5 mo/yr; high cost; mostly used for breeding or sale Fried in oil, can be eaten by children
Cow's milk	0	21.2	NA	2.3 ± 0.75	100 ± 1.41 ml	27	Perceived as strength and health provider and as blood- and vitamin-rich food Available 3–4 mo/yr; high cost Can be eaten by children
Fruits and tubers							
Mango	71.9	0	5.1 ± 6.5	NA	147.5 ± 83.2 g	224.3	Perceived as strength and health provider and as blood- and vitamin-rich food Available 5–6 mo/yr; low cost Eaten as snack "to fill the stomach"
Papaya	6	0	1.0 ± 0.0	NA	125 g	31.3	Perceived as strength and health provider and as blood- and vitamin-rich food Available 1–2 mo/yr; high cost; mostly grown for sale Can be eaten by children
Yellow-fleshed sweet potato	0	0	NA	NA	NA	NA	Perceived as strength and health provider and as blood- and vitamin-rich food Available 1 mo/yr in small quantities Belief that it should not be consumed every day by children Fried in oil, boiled, or eaten raw as snack

continued

Vegetables							
Boundo leaves (*Ceratotheca sesamoides)*	0	66.7	NA	3.8 ± 1,68	102.5 ± 52.4 g[d]	13.3	Perceived as strength and health provider and as vitamin-rich food Fresh leaves available 2–3 mo/yr; sun-dried and kept year-round Perceived as easy to digest
Sorrel leaves (*Hibiscus sabdarifa)*	0	78.9	NA	5. 5 ± 2.69	134.5 ± 74.4 g	88.8	Perceived as strength and health provider and as vitamin-rich food Available 6–7 mo/yr; affordable in wet season and grown for sale in dry season
Boulvaka leaves (*Corchorus olitorius/ tridens)*	0	48.5	NA	3.6 ± 1.96	102.5 ± 52.4 g[d]	30.6	Perceived as strength and health provider and as vitamin-rich food Available 3–4 mo/yr Dried leaves not consumed
Arzentiga leaves (*Moringa oleifera)*	0	27.3	NA	3.2 ± 1.92	102.5 ± 52.4 g[d]	16.3	Perceived as strength and health provider and as vitamin-rich food Fresh leaves available 3–4 mo/yr Not consumed; no recipes for processing and cooking
Amaranthus leaves (*Amaranthus candilis)*	0	24.2	NA	2.0 ± 1.31	102.5 ± 52.4 g[d]	17.9	Perceived as strength and health provider and as vitamin-rich food Fresh leaves available available 3–4 mo/yr; mostly grown for sale Not consumed; no recipes for processing
Bean leaves *(Vigna unguiculata)*	0	42.4	NA	3.6 ± 1.55	102.5 ± 52.4 g[d]	9.3	Perceived as strength and health provider and as vitamin-rich food Fresh leaves available 3–4 mo/yr Perceived as "filling the stomach" Can be grown in family garden
Baobab leaves (*Adansonia digitata)*	0	69.7	NA	4.4 ± 2.7	102.5 ± 52.4 g[d]	2.8	Perceived as strength and health provider and as vitamin-rich food Fresh leaves available 2–3 mo/yr; sun-dried and kept year-round
Yellow sorrel (*Hibiscus sabdarifa)*	0	0	NA	NA	NA	NA	Perceived as strength and health provider and as vitamin-rich food Fresh flowers available 2 mo/yr; sun-dried and kept year-round Particular taste (acid)
Okra (*Hibiscus esculentus)*	100	0	8.9 ± 7.2	NA	102.5 ± 52.4 g	3.6	Perceived as strength and health provider and as vitamin-rich food Fresh okra available 3 mo/yr; always processed and kept dried for year-round consumption Easy to cook, low cost

NA, not applicable. Plus–minus values are means ± SD.
a. Values in this column indicate average consumption during all seasons (i.e., dry and rainy) or actual consumption during one season.
b. The average weight is given for a portion of liver bought in the market.
c. Only one child consumed eggs.
d. Portion sizes could not be determined for these vegetables because no child consumed them during the 24-hour recall. It is assumed that the portion sizes were equal to that of okra.

cultural context, feeding practices, and constraints [36]. Given that liver is the single best source of vitamin A, is available year-round, and is culturally acceptable for children in the study area, an intervention program might act to promote weekly consumption of one portion of liver by children in the community and place emphasis on increasing the number of children who consume liver, at least on a weekly basis.

A successful experience in behavioral change communication strategy in Niger reported an improvement in liver consumption [37]. Therefore, the current thinking that it is difficult to increase the consumption of animal-source food in developing countries could shift to one that allows better management of the situation [38] through, for example, a persuasive approach. A pilot project conducted in Burkina Faso on the promotion of red palm oil demonstrated that it is possible to introduce a new food, to raise awareness of its importance, and to increase its consumption if a sufficiently persuasive approach is used [2, 11, 39]. Mango is the second promising food for the reduction of vitamin A deficiency in the community. As an important source of (pro)vitamin A, mango could contribute effectively to improving the intake of vitamin A in the study area during the period of availability if adequate amounts were consumed. An intervention program should stress increasing the portion size and frequency of mango consumption by children. Promoting the consumption of mango, taking into account factors that enhance (pro)vitamin A absorption (e.g., consumption of fat and controlling parasite infection) [9], will be effective in controlling vitamin A deficiency in rural areas.

Future studies may consider the development of a simple technique at the community level, based on earlier studies [9, 40], to process mango and vegetables for year-round availability. In our study community, an intervention program focused on green leafy vegetables will be unlikely to reduce vitamin A deficiency, not only because of the low bioavailability of plant-based vitamin A [41], but also because many children already consume vegetables on a regular basis, and the portion sizes cannot readily be increased because of their bulk. However, it should be kept in mind that vegetables and fruits remain the main source of vitamin A in developing countries, and given the positive health effects of vegetables [42], their consumption should be encouraged. It is important to recognize that dietary approaches may need to be complemented by additional approaches to maximize their effectiveness [43], and that increased consumption of vitamin A–rich foods may make a significant contribution to existing programs. A study by Grubesic [44] showed that increasing the consumption of vitamin A–rich foods by children who were also receiving supplementary vitamin A might provide better protection from malnutrition and vitamin A deficiency. Therefore, implementation of dietary programs should go hand in hand with supplementation.

This study showed that dietary approaches need to be investigated in a more systematic way in Burkina Faso and in other developing countries, with the objective of identifying all possible limiting factors, because the selection and consumption of vitamin A–rich foods appear to be highly situation-specific [34]. We therefore present a methodologic flow chart in **table 4** that

TABLE 4. Methodologic flow chart for study of vitamin A deficiency and related factors

Phase 1: Availability of (pro)vitamin A rich foods
Is vitamin A deficiency related to production, availability, and seasonality of vitamin A–rich food? » Background information on ongoing programs in food and nutrition in the study area and identification of key informants » Interview of key informants » Market survey (at onset and end of the study, visit in garden and in the field) » Identification of key foods
Phase 2: Food consumption
Is vitamin A deficiency related to insufficient consumption of vitamin A–rich-food? » Frequency of consumption » Vitamin A intake
Phase 3: Constraints and favourable factors on consumption
What factors might influence consumption of vitamin A–rich food? » Food attributes » Pile sorting » In-depth focused discussion on factors constraining consumption of vitamin A–rich food What is the local wisdom about signs and treatment of night-blindness? » Identification of positive practices

outlines such an approach. The procedures in the protocol could be compared to that described by Kuhnlein [45], with some variants. Field visits are an important aspect of this approach, which allows the collection and identification of indigenous green leafy vegetables that might not be available in the market. In addition, the present protocol suggests in-depth exploration and identification of all possible constraints and barriers that limit the consumption of vitamin A–rich food by children. Finally, the investigation of local practices in the treatment of night-blindness might help to identify positive practices that could constitute an entry point for designing and implementing effective dietary interventions to combat vitamin A deficiency.

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Power from below: Enabling communities to ensure the provision of iodated salt in Kyrgyzstan

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Abstract

Background. *In Kyrgyzstan, as in many countries around the world, progress in universal salt iodization has been slow because of difficulties in enforcing existing national regulations.*

Objective. *To study the effects of community testing of the iodine content of salt in households, at local retailers, and at wholesale markets on the percentage of households using iodized salt in Naryn Oblast, a region of Kyrgyzstan.*

Methods. *In response to a stated community priority to address iodine deficiency in Naryn Oblast, volunteers from village health committees and personnel of Primary Health Care units living in the communities were trained in testing salt using test kits. A phased introduction of two testing components was conducted in 2002–2003 in two areas with a combined population of 160,000. The two components included testing of salt for iodine content by community members in as many households as possible (Component 1) and testing of retail salt for iodate content by community members and by retailers at wholesale markets (Component 2). Results from these two components provided the data for this study.*

Results. *For Component 1, salt testing reached 65% of households; coverage of iodized salt increased from 87.6% to 96.8% within 5 to 7 months (averages of the two areas;* $p < .001$*), mostly owing to a great decrease in the variation among settlements. For Component 2, in area 1, the percentage of households using iodated salt increased from 71.0% to 90.3% within 5 to 7 months, whereas the percentage of households using iodinated salt decreased from 18.6% to 5.6%. In area 2, the percentage of households using iodated salt increased from 65.2% to 76.2% within 5 to 7 months, with no change in the percentage of households using iodinated salt (21.7% and 20.8%). The differences between areas 1 and 2 are highly significant (*$p < .001$*). At 18 to 21 months, the percentage of households using iodated salt was 97.5% in area 1 and 90.2% in area 2. The intervention cost around US$1,500.*

Conclusions. *Testing salt in a large percentage of households is an effective, low-cost approach to increasing the percentage of households using iodized salt to satisfactory levels in a very short time. Empowering community members to check salt at retailers and retailers to check salt at wholesale markets with test kits for iodated salt can rapidly ensure almost exclusive consumption of iodated salt in households.*

Key words: Action Research, community empowerment, iodized salt, iodine test kits, salt retailers, universal salt iodization (USI)

Introduction

Iodine-deficiency disorders in Kyrgyzstan

As a result of the controlled production and sale of salt during the Soviet era, the prevalence of goiter in Kyrgyzstan decreased from 30% in the 1950s to about 5% in the 1970s, as determined by palpation in adults [1]. The prevalence of iodine-deficiency disorders increased greatly during the 1990s when the collapse of the Soviet Union in 1991 led to a deterioration of the health-care system and to a loss of control over the production and sale of iodized salt. (We refer to salt as *iodized* when iodine is added in any form, *iodated* when potassium iodate is added, *iodinated* when potassium iodide is added, and *noniodized* when no form of iodine is added.) Between 1995 and 1998, several studies in different regions of Kyrgyzstan among schoolchildren aged 10 to 12 years revealed severe iodine deficiency (prevalence of goiter determined by ultrasonography, 31% to 56%) and moderate deficiency in iodine

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nutrition (median urinary iodine excretion 30–50 μg/L) [2]. In 1994, 60.1% of 190 newborns in Bishkek and Osh had elevated thyrotropin levels (> 5 mIU/L) [3].

In countries where iodine-deficiency disorders are a public health concern, the World Health Organization (WHO) recommends that at least 90% of households consume adequately iodized salt. WHO promotes the use of potassium iodate (iodated salt) because it is more stable than potassium iodide (iodinated salt) [4]. A review of lessons learned [5] found that the following measures were crucial for combating iodine-deficiency disorders by salt iodization: raising public awareness of the disorders, ensuring easy access to iodated salt, promoting compliance with iodization standards by the salt industry, and monitoring and enforcement. All of these measures have been addressed in Kyrgyzstan to various degrees. International organizations have helped a core group of salt manufacturers to produce iodated salt for a number of years and have implemented educational campaigns through mass media and printed material, as well as by inserting messages in educational curricula. The Kyrgyz Government adopted universal salt iodization (USI) as a national strategy by decree in 1994 and by law in 2000. These regulations forbid the import and sale of noniodized salt for human and animal consumption but exempt salt used for industrial purposes. Using qualitative test kits and/or quantitative titration, the Sanitary-Epidemiological Service under the Ministry of Health has been monitoring salt iodization at the producer level by spot checks and at the retailer level by quarterly studies.

These measures have ensured sufficient production capacity for iodated salt in Kyrgyzstan* and wide access to iodated salt, which can now be found at virtually all retailers in the country [6]. Ninety-two percent of people in Kyrgyzstan have heard about iodized salt, and 88% know it prevents iodine-deficiency disorders [7]. However, consumption at the household level has so far failed to reach the WHO goal of at least 90% of households with adequately iodized salt. In a countrywide study in 2003, 79% of the salt samples brought to school by children were iodized, but only 55% were sufficiently iodized (≥ 15 ppm of iodine, measured with test kits) [7]. A recent study titrated the iodine content of packets collected from 242 retailers from around the country; all were marked as containing iodized salt and were still within the specified expiration date. Of 338 packets, 11.5% contained no iodine, 55% had adequate iodine levels (> 15 ppm), and 13% of the packets containing iodine contained iodinated salt. In addition, 7% of the retailers had unpacked, noniodized salt for sale [6]. Every year, government agencies measure several thousand salt samples from retailers by titration and publish the results according to whether the samples contained < 25 ppm or ≥ 25 ppm of iodine (only potassium iodate is measured). The results improved slowly between 1996 (53.5% of samples with ≥ 25 ppm of iodine) and 2003 (67% of samples with ≥ 25 ppm of iodine) [8].

The following factors seem to contribute to the continued presence of noniodated or insufficiently iodated salt at retailers and in households, even though proper legislation is in place. First, the core group of producers produce salt with inconsistent levels of iodine, even though they are provided with potassium iodate by international projects.* Second, a number of smaller companies are not supported with potassium iodate by outside projects. Some of them produce noniodized salt and label it as iodized, or they produce the cheaper but less stable iodinated salt. Last but not least, fraudulent businesses copy the packets of mainstream producers and fill them with noniodized or iodinated salt [6].

The sources of noniodized salt include illegal imports, leakage of salt from industrial sources, and natural salt deposits. The retail price of nonpacked, noniodized salt is about 20% to 30% lower than of iodized salt. However, this does not seem to influence purchasing decisions much: only 3% of households state that they usually buy noniodized salt, 80% state that they only buy salt marked as iodized, and 17% do not care [7].

Iodine-deficiency disorders in Naryn Oblast

Naryn Oblast, where the intervention took place, is in a mountainous region of Kyrgyzstan with a population of about 250,000. (An oblast is an administrative unit comprising several rayons, or districts.) Three-quarters of the population of Naryn Oblast live in remote, dispersed villages. In 2000 the median excretion rate of iodine in 208 children 9 to 11 years of age was 49.6 μg/L, and palpation of 4,887 children of the same age group found that 31% had goiter [9]. The percentage of retailers selling packets of iodated salt containing ≥ 25 ppm of iodine was 33% in 1996 and 44% in 2001 [8]. At a number of places in Naryn Oblast, rock salt is available and is collected at no cost.

Low enforcement of iodized salt legislation as a worldwide problem

One can conclude from the above that there is a gap in Kyrgyzstan between the legislation that has been enacted to control iodine-deficiency disorders and the ability to enforce it. A review of the recent literature indicates that Kyrgyzstan shares this problem with many countries and regions around the world. There are successes, such as Iran, where the percentage of

* Personal communication: Artur Buiuklianov, Coordinator, Asian Development Bank project JPFR 9005-Kyrgzystan. Improving nutrition for poor mothers and children for Asian countries in transition, financed by the Japan Poverty Reduction Fund, Bishkek 2003.

households using iodized salt increased from 50% to 90% within two years after a law for the mandatory production of iodized salt was passed [10]. In Zimbabwe, universal salt iodization led to the elimination of iodine-deficiency disorders within a few years, as measured by urinary iodine excretion and iodine content of salt at retailers [11].

The majority of reports, however, suggest that in most countries and regions the effect of introducing legal regulations is limited, even if they are coupled with awareness-raising to create demand. These countries seem to have a common difficulty in enforcing their own regulations with regard to the manufacture and sale of iodized salt. Jooste concluded that “geographical variation in the iodine concentration of salt, and in the availability or use of iodized salt, appears to be a world wide problem” [12]. Jooste analyzed the following contributing factors for South Africa: leakage of noniodized salt into the market, inadequate iodization of salt at the production level, loss of iodine during transport and storage, and coarseness of the salt. Latief et al., in their report from Indonesia, agreed that the lack of enforcement remained a major difficulty and that “the main constraint in achieving the Universal Salt Iodization is the infiltration of uniodized salt to the markets” [13]. Numerous further examples of countries with a high percentage of households using noniodized salt, despite universal salt iodization, can be found (**table 1**).

To address this problem, most authors point to raising awareness to increase demand [5, 14, 22] and/or call for better enforcement by governments [5, 17, 20, 22]. But in most of these countries enforcement cannot be easily improved, because poor enforcement is a symptom of inherent weaknesses of governance, porous borders, corruption, and lack of resources. Demanding better enforcement under such circumstances is often futile. As for raising awareness to increase the demand for iodized salt, Goh found no statistically significant correlation between public awareness of iodine-deficiency disorders and iodated salt coverage in data from Indonesia and China, and concluded that knowledge is a necessary but not sufficient condition to ensure consumption of iodated salt [5].

TABLE 1. Examples of countries and regions with high percentages of households using noniodized salt despite universal salt iodization (USI)

Country or region	Reference and year	Results
South Africa	Jooste [12], 2000	Mandatory iodization of table salt in 1995; more than 2 years later, 38% of household samples had < 15 ppm iodine and 24% had < 2 ppm
Indonesia	Latief et al. [13], 2000	USI adopted in 1990; in 1998 20% of households consumed noniodized salt
Bulgaria	Timtcheva and Zlatarov [14], 2000	Legislation introduced in 1994; 5 years later, 33% of households consumed salt with iodine content below the required standard (> 28 ppm)
Oman	Hussein [15], 2000	“Oman is an example of a country with strict USI legislation but badly suffering from noniodized salt infiltration to the country throughout the joint borders” with the United Arab Emirates
Armenia	Rossi and Branca [16], 2003	Combined measures in 1998–2000, consisting of increasing the supply of iodized salt and educational campaigns, led to 83% of households consuming iodized salt in 2000, up from 66% in 1997; however, some regions had significantly lower percentages; authors link this to illegal import of noniodized salt
Mongolia	Yamada et al. [17], 2000	USI introduced in 1996; 2 years later, great regional variations (between 3% and 82%) were found in the usage rates of properly iodized salt (> 20 ppm)
India (overall)	Vir et al. [18], 2002	USI introduced in 1992; noniodized salt was found in 28% of households in 1998/99; almost half of the salt samples had < 15 ppm iodine
Assam, India	Patowary et al. [19], 1995	Sale of noniodized salt banned in 1989; in 1992/93, 47% of salt samples still had < 15 ppm iodine, due to transport of raw salt from another state
Tripura, India	Chandra et al. [20], 2002	USI introduced in 1988/89; a decade later, there was no decline in the prevalence of goiter, and two-thirds of household salt samples had < 15 ppm iodine
Calcutta, India	Sinha et al. [21], 1999	Ban on noniodized salt in early 1990s; by end of the 1990s, the proportion of household salt samples with < 15 ppm iodine was 15.5% according to the test kit method and 39% according to titration, with a strong variation among parts of Calcutta (between 21% and 61% by the titration method)

Purpose of this study

This study reports an innovative, effective, low-cost approach to raise the percentage of households using iodated salt to satisfactory levels in a very short time, despite ineffective government enforcement. The approach is based on empowering community members and retailers to check the iodate content of salt.

Methods

Process

Rapid test kits have been used traditionally for on-the-spot qualitative or semiquantitative monitoring of the iodine content of salt [23]. A drop of the test solution turns the salt blue if iodine is present in the sample. Innovative use of such test kits by communities and retailers was the defining element of our intervention. The strategy had two components.

Component 1 consisted of Action Research (defined below) by community members, who went to as many households as they were able to cover, tested a sample of the salt in the household with a drop of the test kit, and documented the results. In addition, the community testers distributed information about iodine-deficiency disorders and their prevention, which promoted the exclusive use of iodized salt. The testing helped people learn which brands contained iodized salt and which did not, and that the rock salt used in some areas is not iodized.

Component 2 focused on salt retailers. Community members regularly tested salt at all local retailers with test kits for iodated salt, and educated the retailers about iodine-deficiency disorders and how they can be prevented by the exclusive use of iodized salt. Typically, one packet of salt was opened for testing about once a month for each retailer. The community testers also provided all retailers with test kits for iodated salt and asked the retailers to use them at the wholesale markets to be sure they purchased only iodated salt.

We used test kits from MBI Kits International (Chennai, India). These simple kits provide good qualitative indication of the presence of iodine [23]. There are two types of kit, one for iodated salt and one for iodinated salt. The initial Action Research and the follow-up at 5 to 7 months were performed using both types of test kit in order to determine the overall coverage of iodized salt and to follow the relative change in the use of iodinated and iodated salt in households. Differences between the two forms of iodine in changes in rate of usage would be the crucial means of discerning an effect of Component 2. The results of the tests conducted in the household were documented as iodated, iodinated, or noniodized salt. The cutoff point for a finding of noniodized salt was the lack of any color reaction, which meant that the tests were purely qualitative. Therefore, salt with less than 15 ppm of iodine was counted as iodized for the purpose of this study. This was done to ensure more consistent results among the large number of testers and between baseline and follow-up tests. The follow-up at 18 to 21 months was done only with test kits for iodated salt in order not to undermine the promotion of this preferred form of iodized salt.

Community participation in research is being increasingly practiced under various names, most of which seem to involve almost identical approaches. Two common terms are Action Research (also called Participatory Action Research) and Community-Based Participatory Research (CBPR). Definitions of Action Research usually include four basic themes: empowerment of participants, collaboration through participation, acquisition of knowledge, and social change [24]. CBPR has been defined as a systematic inquiry, with the participation of those affected by the issue being studied, for the purpose of education and taking action or effecting social change [25].

Health committee volunteers and Primary Health Care staff were trained in a one-day seminar on features of iodine-deficiency disorders, their prevention through iodized salt, use of the test kits, planning the Action Research in their village, and documenting the results.

Study area and timeline

The intervention was performed in all five rayons of Naryn Oblast. However, in one rayon and in Naryn City the first follow-up at 5 to 7 months was unintentionally carried out with test kits for iodated salt only. Data from these two regions were therefore not included in the analysis.

This report analyzes the data from the remaining four rayons, with a population of 160,000 people in 89 settlements. In one rayon (referred to as area 1), both components of the intervention were performed simultaneously. In the other three rayons (area 2), Component 1 was introduced at the same time as in area 1, but Component 2 was introduced more than a year later because of a delay in the delivery of test kits from abroad. This unintended phased introduction offered the opportunity to analyze the effects of the Action Research (Component 1) and the work with retailers (Component 2) separately. **Table 2** summarizes the sequence of events.

Area 1 was Jumgal Rayon, a remote, mountainous area of Naryn Oblast with 16 settlements and a population of about 40,000. In area 1, the participants in the campaign were volunteers from village health committees and personnel of Primary Health Care units living in the communities. The village health committees were formed to promote Community Action for

TABLE 2. Timetable of phased introduction of study components in areas 1 and 2

Period	Area 1	Area 2
Jun–Jul 2002	**Component 1** (initial Action Research) Household-level testing campaign for iodated and iodinated salt by village health committees and PHC staff living in the communities **Component 2** Distribution of test kits for iodated salt to all retailers by health committees and PHC staff. Regular checking of salt at retailers by health committees and PHC staff with kits for iodated salt	**Component 1** (initial Action Research) Household-level testing campaign for iodated and iodinated salt by PHC staff living in the communities
Oct 2002–Jan 2003	First follow-up at 5 to 7 months with kits for iodated and iodinated salt	First follow-up at 5 to 7 months with kits for iodated and iodinated salt
Sep 2003		**Component 2** Distribution of test kits for iodated salt to all retailers by PHC staff Regular checking of salt at retailers by PHC staff with kits for iodated salt
Dec 2003–Feb 2004	Second follow-up at 18 to 21 months with kits for iodated salt	Second follow-up at 18 to 21 months with kits for iodated salt

PHC, primary health care

Health [26], defined by WHO as "collective efforts by communities which are directed towards increasing community control over the determinants of health, and thereby improving health" [27]. The population of the intervention area had identified iodine-deficiency disorders as one of their health priorities in a participatory assessment that initiated the process of Community Action for Health. The intervention was developed as a response to this community priority.

Area 2 consisted of three other rayons of Naryn Oblast (Ak-Tala, At-Bashy, and Naryn) with 73 settlements and a population of 120,000. The population of the rayons ranged between 32,000 and 40,000 people. Area 2 was similar to area 1 in remoteness and settlement structure. In area 2, the participants in the campaign were Primary Health Care personnel living in their communities, since there were no village health committees in these rayons at the time of the study.

To check the accuracy of the reported data on coverage with household salt testing, members of 85 randomly selected households in 20 villages were interviewed after the initial round of testing and asked whether their salt had been tested. At the time of the second follow-up at 18 to 21 months, informal interviews with 68 retailers were conducted on their use of the test kits. The selection of retailers was not random but occurred by chance contact in 23 villages.

Organizations supporting this study approved it in accordance with their guidelines for research in human subjects. Please see Acknowledgments section for a list of these organizations.

Results

Coverage of salt testing in households

The Action Research reached about two-thirds of households at baseline (65%) and follow-up rounds (63%) in both areas combined (**tables 3 and 4**). The coverage of salt testing was higher in area 1 than in area 2. In the verification interviews, 57 of 85 households (67%) reported that testing had taken place in their houses, thus confirming the figures reported by the Action Research.

Effect of the Action Research testing campaign on the percentage of households with iodized salt (Component 1)

At baseline, the Action Research found a surprisingly high percentage of households with iodized salt (87.6% for both areas combined) but also considerable variation among settlements. Even though the baseline was high, the use of iodized salt in households rose considerably within 5 to 7 months post-intervention to 96.8% in both areas combined (**table 3**), mainly by sharply reducing variation among settlements. **Figure 1** demonstrates this reduction in variation as the change in the percentage of households with noniodized salt in individual settlements.

TABLE 3. Households with iodized salt at baseline and at follow-up at 5 to 7 months

Variable	Area 1 (8,726 households)		Area 2 (28,857 households)	
	Initial	5 to 7 months follow-up	Initial	5 to 7 months follow-up
No. (%) of settlements covered by testing	16 (100)	13 (81)	53 (73)	51 (70)
No. (%) of households covered by testing	6,643 (76)	5,751 (66)	17,869 (62)	18,057 (63)
No. (%) of households with iodized salt	5,950 (89.6)	5,548 (96.5)[a]	15,515 (86.8)	17,510 (97.0)[a]
No. (%) of settlements with ≥ 90% households having iodized salt	10 (63)	13 (100)[b]	26 (49)	47 (92)[a]
Lowest percentage of households with iodized salt in a settlement	59	93	61	85

a. $p < .001$ vs. baseline.
b. $p < .02$ vs. baseline.

TABLE 4. Households with iodated and iodinated salt at baseline and at follow-up at 5 to 7 months and 18 to 21 months

Variable	Area 1 (8,726 households)			Area 2 (28,857 households)		
	Initial campaign (6–7/02)	First follow-up (5 to 7 months)	Second follow-up (18 to 21 months)	Initial campaign (6–7/02)	First follow-up (5 to 7 months)	Second follow-up (18 to 21 months)
No. (%) of settlements covered by testing	16 (100)	13 (81)	13 (81)	53 (73)	51 (70)	47 (64)
No. (%) of households covered by testing	6,643 (76)	5,751 (66)	5622 (64)	17,869 (62)	18,056 (63)	16,086 (56)
No. (%) of households with iodated salt	4,716 (71.0)	5,194 (90.3)[a]	5497 (97.5)[b]	11,643 (65.2)	13,763 (76.2)[a]	145,05 (90.2)[b]
No. (%) of households with iodinated salt	1,234 (18.6)	354 (5.6)[a]	Not tested	3,872 (21.7)	3,747 (20.8)[a]	Not tested
Ratio iodated:iodinated salt	3.8: 1	14.7:1		3.0:1	3.7:1	

a. $p < .001$ vs. baseline.
b. $p < .001$ vs. first follow-up.

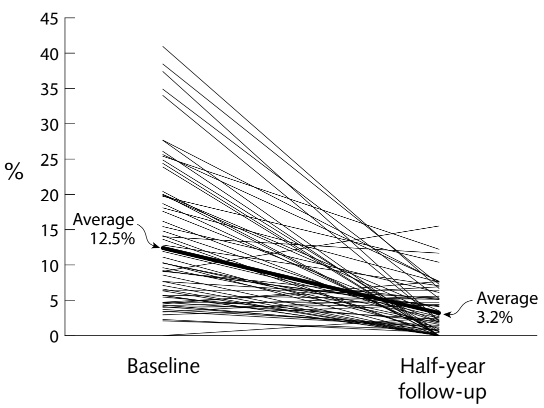

FIG. 1. Percentage of households with noniodized salt in individual settlements, at baseline and follow-up at 5 to 7 months in both study areas (each line represents one of 64 settlements.

Effects of checking salt at retailers by community members and providing retailers with test kits for iodated salt (Component 2)

At the first follow-up, performed at 5 to 7 months, area 1 saw a striking increase in the ratio of the usage of iodated to that of iodinated salt, whereas in area 2 the ratio remained almost unchanged. The difference between the areas is statistically significant ($p < .001$). However, at the second follow-up, performed at 18 to 21 months, the presence of iodated salt in households had increased greatly in area 2, three to five months after Component 2 was introduced (**table 4**).

In informal interviews with retailers, most reported using the kits to test a sample before purchasing salt at wholesale markets. Most gave two reasons for this: a responsibility to provide their communities with iodized salt, and the knowledge that noniodized salt is difficult to sell because community members will check their salt. In one instance, a retailer was threatened with banishment from the village if he continued to sell noniodized salt.

Costs

The total cost of this intervention was around US$1,500. Each kit costs US$0.30 and can be used to test an average of 350 samples; approximately 900 kits were used: 500 for the initial Action Research and for two follow-up rounds, and 400 for distribution to retailers. The cost of the 900 test kits was US$270. The cost of materials and transport for the training seminars and collection of data was US$1,200. There were no additional financial costs involved for the labor of testing, since testing was done as part of the working schedule of the Primary Health Care units for health promotion, and the village health committees worked as volunteers. For the total population involved in the study (160,000), the costs were US$0.009 per person or US$0.04 per household. At the second follow-up, 3,343 additional households had iodated salt in their kitchens. The costs for these households were US$0.1 per person or US$0.45 per household.

Discussion

Both components of our strategy proved to have significant independent effects. The increase in the percentage of households with iodized salt at 5 to 7 months (from 87.6% to 96.8%, both areas combined) can be attributed to the Action Research (Component 1), because both areas showed similar increases (**table 3**), with and without the additional work with retailers (Component 2). In addition, the effect of the Action Research was greatest in those settlements where the initial percentage of households with iodized salt was lowest, almost eliminating the variation among settlements (**fig. 1, table 3**). We believe that the powerful educational effect of the Action Research derived from the fact that people saw the salt in their kitchens turning blue or staying white and received an explanation of its significance. Given the high level of awareness of iodine-deficiency disorders and of the beneficial effects of iodized salt in Kyrgyzstan, this effect is probably due more to the use of existing knowledge for action than to the provision of new knowledge. It also appears that testing salt in two-thirds of the households within a short period of time results in the spread of this awareness to households beyond those actually covered by testing.

The effect of Component 2 is convincingly demonstrated by the highly significant differences at first follow-up in the changes in coverage of iodated and iodinated salt both between areas and within area 1. Retailers who had test kits for iodated salt and were checked regularly with such kits appeared to selectively purchase iodated salt at wholesale markets instead of noniodized or iodinated salt. This in turn led to a sharp increase in the coverage of iodated salt and to a decrease in the presence of noniodized and iodinated salt in households. Our informal interviews with retailers support this conclusion. The test kits have become very popular among the retailers and have made them allies in promoting the use of iodated salt. The kits in the hands of retailers effectively transmit the demand of communities to the wholesale markets. The further increase of the coverage of iodated salt to 97.5% in area 1 at 18 to 21 months demonstrates that continued provision of test kits to retailers combined with checks of these retailers by community members can sustain the observed effect over time.

An independent national study in Kyrgyzstan allows us to compare our results with data from other parts of Kyrgyzstan. The study, with a sample of approximately 3000 households, found almost no increase in the coverage of iodated salt between 2002 (69%) and 2003 (72%) [7]. During this period, our intervention achieved an increase of 38 percentage points (from 66.7% to 92.1% for both areas combined) in the proportion of households with iodated salt. An intervention consisting of an intensive information campaign on iodine-deficiency disorders that also took place during 2002–2003 in three other oblasts of Kyrgyzstan resulted in an increase in the coverage of iodated salt of 18 percentage points, from 65.6% in 2002 to 77.5% in 2003 [7]. This campaign included community-based awareness programs by nongovernmental organizations, printing and distribution of information, and television and radio advertisements. An educational campaign in the local mass media in Turkey led to a an increase of 18 percentage points (from 54.5% to 62.4% over three months) in the proportion of households using iodized salt [28].

The intervention as an answer to weak enforcement of legislation

Goh, in his review of lessons learned [5], concedes that involvement of communities may be helpful in ensuring universal consumption of iodated salt, but concludes that enforcement by the government is critical. The reason he gives is interesting in the context of our study: "Because *informed consumers cannot readily differentiate* between *iodated* and non-*iodated* salt, intervention by the government as a monitor and enforcer is necessary"(italics added). Component 2 of our approach aims precisely at overcoming this inability of consumers to differentiate between iodated and noniodated salt.

The findings of this study strongly suggest that control "from below," i.e., in the hands of the people, can very effectively complement the four elements of effective iodization cited above in the Introduction. In the context of our study, this control from below proved to be the missing fifth element that catalyzed, on the basis of the four other elements, a sudden rise in the percentage of households with iodated salt to more than 90%, about 20% higher than in other parts of the country. In the study areas this intervention effectively shut down smuggling and fraudulent operations that were leaking noniodized and iodinated salt into Kyrgyz markets. We therefore assume that if the test kits were in the hands of the vast majority of communities and retailers in the country, they could become the "teeth" that would make consumer demand bite at the level of producers and smugglers. The low costs of providing communities and retailers with test kits for continuing Component 2—estimated at US$0.005 per household per year or US$10,000 per year for the whole country—and the relatively small effort involved in doing so would allow the approach to be extended throughout the country and to be sustained as long as needed.

The costs of the approach described in this paper were very low, for the following reasons. First, the test kits are inexpensive. Second, in area 1 health committees worked voluntarily. And third, it was possible to take advantage of the Primary Health Care system in Kyrgyzstan, which has functioning units in most settlements, even in remote areas. There were no additional financial costs involved in using Primary Health Care staff for this work because it was integrated into their existing health promotion duties. The Ministry of Health of Kyrgyzstan supported the intervention because it was in line with the health reform objective of strengthening the role of the Primary Health Care system in health promotion. The greater coverage of testing in the Action Research in area 1 than in area 2 reflects the involvement of the volunteer village health committees. For Primary Health Care staff in area 2, testing without help from volunteers required considerable additional work and a greater motivational effort. In our experience, the intervention is much easier to implement with the help of voluntary organizations. However, our experience also shows that it can be implemented by a functioning Primary Health Care system with sufficient human resources.

The findings of this study suggest that in countries with difficulties in enforcing universal salt iodization regulations, it may be worthwhile to look beyond calling for better enforcement and awareness campaigns toward empowering communities and retailers to check the iodate content of salt as a means of achieving universal coverage.

Limitations of the approach

It should be noted that in this study the presence of any blue coloring in the salt after testing was interpreted to indicate iodated or iodinated salt, and that we therefore do not know the proportion of households with insufficient levels of iodine (< 15 ppm) in their salt at baseline and follow-up. Although the findings nonetheless indicate a considerable improvement over the course of the intervention, the high proportion (45%) of inadequately iodized salt found at retailers [6] remains an important challenge. The test kits we used offer a very crude semiquantitative measurement of iodine content below 15 ppm, but with mass distribution of the kits to thousands of people, only the qualitative result (i.e., absence or presence of iodine) can be used in a meaningful way. A quantitative rapid test kit has been developed [23], but it is too complicated to be used by communities and retailers. Therefore, although data exist to suggest that our strategy does considerably improve urinary iodine excretion [29], control from below cannot now sufficiently address the problem of inadequate iodine levels in iodated salt. Efforts are under way to use market pressure by publishing the iodate content of various brands at regular intervals.

Enabling communities to increase control over a determinant of their health

The Ottawa Charter defines health promotion as "the process of enabling people to increase control over the determinants of their health, to improve their health," and it declares that "people cannot achieve their fullest health potential unless they are able to take control of those things which determine their health" [30]. In this study, people used Action Research to investigate one reason for a major health problem that previously had been identified by them, they checked and supported retailers with test kits and monitored the outcome of the intervention. It is important to note that they did not do this to provide monitoring data for program managers, but that they used the data primarily for interaction with retailers and to give feedback to their fellow community members. They thus took control over a key determinant of their health—provision of iodated salt by retailers. The approach therefore is an example of the empowering dimension of health promotion that the Ottawa Charter calls for. The retailers themselves, most of whom were members of the community, were part of that empowering process.

Comparison with similar approaches

Traditionally, test kits are used for monitoring at the retail and household level by agencies promoting the use of iodized salt. Latief et al. pointed out the importance of test-kit monitoring for decision-making by program management in Indonesia [13]. Vir reported from India that the percentage of the population consuming iodized salt was much higher in states where test kits were used for systematic monitoring [18]. Both of these programs offer examples of the use of test kits as an educational tool for creating demand. In Indonesia, community members (teachers and nurses) monitor salt samples at the community level. The data are used by program management as well as for social mobilization and for increasing awareness at the community level. However, our literature review did not find any reports of the use of an Action Research testing campaign covering a large number of households for education and for the creation of demand. We also could not find any reports of the use of test kits as a tool to empower communities to control the salt sold to them by testing the salt at retailers. Among the reports reviewed, there are examples of the use of test kits for educational work with retailers, but we did not find an instance in which test kits were given to retailers in a systematic way to enable them to test salt at wholesale markets. Only Sinha et al. [21] discuss this as an approach that could possibly improve the iodine content of salt. Thus, in the published literature we could not find an intervention with the key elements described here: testing of salt in households by community members in a broad-based Action Research, empowering communities to test the salt at their retailers, and handing out test kits to all retailers for use at wholesale markets.

Conclusions

An Action Research based on testing salt in a majority of households is a very effective approach to increasing the use of iodized salt in households to recommended levels in a short time. Enabling communities to check the salt sold by retailers with test kits for iodated salt and providing all retailers with such test kits can rapidly ensure almost exclusive consumption of iodated salt in households, even in the absence of effective governmental enforcement of relevant legislation. The intervention is also very low cost.

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Development of fortified dried broken rice as a complementary food

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Abstract

Background. *Commercially produced dried broken rice is widely used to prepare complementary foods for Thai infants, and it is both convenient and acceptable to persons from all socioeconomic classes. However, inadequate levels of calcium, iron, thiamine, and folate are common in complementary foods for breastfed infants.*

Objective. *We developed dried broken rice fortified with these nutrients at levels recommended by the 2001 guidelines of the World Health Organization.*

Methods. *The fortification process involved predrying broken rice at 90°C for 1 hour, soaking in a nutrient solution (2:1 ratio of rice to solution), and drying at 70°C for 1 hour and 50 minutes. Calcium lactate or calcium lactate gluconate was the calcium source, and ferrous sulfate, ferrous lactate, or ferric sodium ethylenediaminetetraacetic acid (NaFeEDTA) was the iron source. The vitamin sources were thiamine hydrochloride and folic acid. The product contained 40 mg of calcium, 5.3 mg of iron, 0.08 mg of thiamine, and 11 µg of folate per 20-g serving.*

Results. *Approximately 5% and 10% of calcium and iron, respectively, were lost during processing, with a thiamine loss of approximately 13%, and a folate loss ranging from 17% to 23%. The thiamine loss during accelerated storage (42°C for three months) was not significant (*$p > .05$*).*

Conclusions. *NaFeEDTA was the most appropriate iron fortificant because it provided prolonged product stability and high in vitro dialyzability.*

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Key words: Complementary food, fortification, minerals, vitamins, iron dialyzability

Introduction

Complementary foods are playing an increasingly important role in contributing adequate nutrients for infants older than six months. In developing countries, the first year of life is the vulnerable period for the development of malnutrition, and it usually coincides with the introduction of complementary foods [1–3]. In Thailand up to 90% of mothers prepare complementary foods by themselves, and most of them use rice as the basic ingredient [2]. Usually, the preparation is based on a guideline developed by the Ministry of Public Health for complementary feeding [4].

Previous studies indicated that most home-prepared complementary foods do not provide adequate calcium and iron, particularly for breastfed infants aged 3 to 24 months, whereas the other nutrients, such as vitamin A, were found to be adequate [2, 5]. In addition, Thailand's Ministry of Public Health is concerned about deficiencies of vitamin B_1 and folate in infants [5].

In Thailand, imported instant complementary foods are normally available in powdered form and contain adequate levels of nutrients. However, these products may limit the educational development and acculturation of children due to inadequate experience in transitioning from liquid to solid foods [6]. In addition, Thai consumers do not readily accept the imported, ready-to-eat type of complementary food because it is considered an expensive, "Western-style" food.

Consequently, rice is the most suitable choice for fortification with important nutrients, since it is widely consumed by children of all socioeconomic classes. This study evaluated the feasibility of producing dried broken rice fortified with adequate levels of vitamins and minerals for use in preparing complementary foods that could be used to prevent malnutrition in infants aged 6 to 24 months in rice-consuming countries.

Materials and methods

Development of fortification and production processes in the laboratory

Four potential fortification and production processes were developed, keeping in mind their practicality at an industrial scale and their ability to retain the fortified nutrients (**table 1**). The first two processes required a long preparation time, especially for drying, and the nutrient solution was not easily prepared by the third process because of limited solubility of the minerals. Therefore, the fourth process was finally selected. This process involved cleaning the rice, drying it in a hot oven at 90°C for 1 hour, soaking it in a vitamin and mineral solution for 10 minutes at a ratio of 2:1 (dried rice:solution), and drying it again in a hot oven at 70°C for 1 hour and 50 minutes. After production, 10 samples were taken from each batch for analysis of iron content and calculation of the coefficient of variation (CV = SD*100/mean). The product was prepared in triplicate and kept frozen in plastic laminated aluminum foil bags until the quality evaluation and in vitro dialyzability tests were performed. The product was also used for a shelf-life study.

Types and levels of fortification

The vitamins were obtained from Roche, Bangkok, and included thiamine hydrochloride and folic acid. Calcium sources were obtained from Nutrition Ltd. Partnership, Bangkok, and included calcium lactate (13% calcium) and calcium lactogluconate (10% calcium). The iron sources used included dried ferrous sulfate (33% iron) from Ajax, Aubern, Australia, ferrous lactate (19% iron) from Vicky Consolidate Co., Bangkok, and ferric sodium ethylenediaminetetraacetic acid (NaFeEDTA) (13% iron) from Akzo Nobel Functional Chemicals, Arnhem, Netherlands. The serving size of the cooked rice was estimated as 180 g, which was prepared from 20 g of dried broken rice [2]. The fortification levels followed the new dietary reference intakes (DRIs) of the Institute of Medicine (IOM) [7–10], i.e., 40 mg of calcium, 5.30 mg of iron, 0.08 mg of thiamine, and 11.00 μg of folate per serving.

TABLE 1. Description of fortification processes

Process	Description
1	Broken rice → clean → cook in fortificant solution → dry → disintegrate
2	Broken rice → clean → cook in mineral solution → dry → spray with vitamin solution → dry → disintegrate
3	Broken rice → clean → cook in fortificant solution → dry → spray with vitamin and mineral solution → dry →disintegrate
4	Broken rice → clean → dry → soak in fortificant solution → dry

Quality analyses

Both dried and cooked fortified broken rice samples were tested for physical properties, including color, in terms of the Hunter Lab value (L = white represented by "100" – dark represented by "0", a = red represented by "+ VALUE"– green represented by "– VALUE", b = yellow represented by "+ VALUE" – blue represented by "– VALUE") by a Spectro Colorimeter Model JS 555 (Tokyo). They were tested for water activity by the water activity instrument Novasina MIK 3000 (Pfäffikon, Switzerland). In addition, a Eutech pH meter model Cyberscan 2500 (Singapore) was used to measure the pH of the cooked rice.

Nutritive value was analyzed in duplicate. Moisture content was analyzed by drying a sample in a hot oven at 110 ± 5°C until a constant weight was attained [11, 12]. Calcium content was determined by an atomic absorption spectrophotometer after the rice was incinerated in muffle at 550°C until complete ashing was obtained [13]. Total iron was analyzed by an atomic absorption spectrophotometer after wet digestion [13]. Thiamine content was determined by high-performance liquid chromatography after acid and enzymatic digestion [14, 15]. Folic acid was analyzed by microbial assay, based on the growth of *Lactobacillus casei* ATCC 7469 [16, 17].

Shelf-life study

Three product batches from the fourth fortification process were used in a shelf-life study. Approximately 70 g of sample was packed in two types of packaging, a nylon laminated polyethylene plastic bag normally used for commercial distribution, and a metallized plastic bag (oriented polypropylene, OPP/metallized cast polypropylene, MCCP) The heat-sealed bags were stored under fluorescent and luminescent lamps at 42°C for three months. The products were periodically sampled on the second week and at one, two, and three months to determine moisture content and water activity. Thiamine content was the indicator for nutrient loss. In addition, the products were evaluated for changes in their sensory quality.

Evaluation of iron bioavailability

The in vitro gastrointestinal digestion method was used to determine the bioavailability of iron in the cooked products. The determination was based on an analysis of iron dialyzability under simulated physiological conditions according to the in vitro method of Mahoney and Hendricks [18]. The method involved two digestion stages using the enzymes pepsin and pancreatin. Iron bioavailability was determined as the amount of iron that permeated the dialysis bag after digestion in the pancreatin enzyme. The enzymes, bile extract porcine (B8631), pancreatin (P1750), and pepsin from porcine stomach (P7125), were obtained from Sigma Chemical Co., St. Louis, Mo, USA. Iron dialyzability (%) was calculated as dialyzability (%) = [D / (W × A)] × 100, where D is the total amount of iron in the dialysate, W is the weight of sample used (g), and A is the concentration of iron in the sample (mg/g).

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows, version 9.0. Differences between the means of the results from chemical analyses were tested at a significance level of $p = .05$ by one-way analysis of variance (ANOVA) and Scheffé's multiple comparison test. The effect of packaging materials on the product's shelf-life was tested by the paired sample *t*-test.

Results and discussion

Fortification method

Food industry representatives noted that all developed fortification processes except the fourth one were impractical because of many limitations both in technology and in time required. The drying period of 12 hours in the first and second processes was too long, and the cooked rice was sticky and not easy to spread on the drying tray. The third process was totally impractical because of the very large volume of water needed to dissolve the large amount of calcium. The fourth process was modified from the process currently used in industry. It was thus the most practical and acceptable, since it required less than 3 hours of drying time and resulted in homogeneous fortified nutrients with a coefficient of variation (CV) of only 3.2%. The process resulted in a convenient product that needs only 8 to 9 minutes of cooking, as compared with regular rice, which needs at least 20 minutes.

Quality evaluation

Physical properties

The color of the product was determined as Lab. The color of the fortified products was different from that of the unfortified products (**table 2**). The L value indicated that the color of the fortified products was comparatively dull. Different types of fortificants also resulted in products with different tones of color. The product fortified with calcium lactate gluconate tended to be more yellow (higher b value) and brighter (higher L value) than the one fortified with calcium lactate. Most of the dried fortified rice had a greenish-yellow color (–a and +b values). Iron and calcium fortificants from different sources affected the sensory quality of the products differently, especially in terms of color. Usually, iron fortification is quite complicated, because it always causes color changes in the food vehicles. Iron fortification with ferrous sulfate, ferrous lactate, or NaFeEDTA often results in products that are yellowish to light-brownish in color. However, such an effect was not seen in the cooked product that was fortified with NaFeEDTA, which had a normal white color. This was different from the cooked products fortified with the other two iron fortificants, which had a slightly yellow color (**table 2**). In a preliminary study (V. Chavasit, unpublished data, March 2002), the mothers mentioned that the cooked products fortified with either type of iron at the study level were still appropriate for use in preparing complementary foods. Fortification with calcium lactate resulted in a product with a duller white color than that of the product fortified with calcium lactate gluconate. However, products fortified with calcium from either source became normal and

TABLE 2. Color and water activity of dried rice and color and pH of cooked broken rice fortified with different calcium and iron fortificants[a]

Sample	Dried rice			Cooked rice			pH of cooked rice	Water activity of dried rice
	L[b]	a[b]	b[b]	L[b]	a[b]	b[b]		
Control	92.75 ± 0.23	–2.11 ± 0.13	10.88 ± 0.21	68.33 ± 0.16	–3.23 ± 0.08	6.34 ± 0.06	6.27 ± 0.08	0.21 ±0.03
CLG + $FeSO_4$	91.16 ± 0.27	–2.91 ± 0.08	13.68 ± 0.27	70.41 ± 0.10	–5.18 ± 0.14	5.00 ± 0.03	5.25 ± 0.02	0.22 ± 0.03
CLG + NaFeEDTA	88.29 ± 0.61	–0.32 ± 0.12	11.44 ± 0.22	71.00 ± 0.62	–4.3 ± 1.33	3.17 ± 3.23	5.69 ± 0.02	0.26 ± 0.13
CLG + Fe lactate	90.73 ± 0.60	–2.98 ± 0.03	13.85 ± 0.50	72.93 ± 0.57	–3.56 ± 0.57	2.21 ± 3.54	5.33 ± 0.03	0.18 ± 0.05
CL+ $FeSO_4$	87.77 ± 0.51	–1.53 ± 0.12	14.86 ± 0.08	72.65 ± 0.86	–4.45 ± 0.79	6.72 ± 0.82	5.24 ± 0.01	0.20 ± 0.05
CL+ NaFeEDTA	87.82 ± 0.41	–0.22 ± 0.29	10.56 ± 0.46	71.20 ± 0.37	–3.11 ± 0.49	7.47 ± 0.10	5.72 ± 0.03	0.17 ± 0.02
CL+ Fe lactate	88.40 ± 01.20	–1.45 ± 0.11	14.03 ± 0.06	70.83 ± 1.13	–2.91 ± 0.37	6.99 ± 0.67	5.22 ± 0.03	0.21 ± 0.05

CLG, calcium lactate gluconate; CL, calcium lactate; NaFeEDTA, ferric sodium ethylenediaminetetraacetic acid

a. Values are means ± SD.

b. L = "0" = BLACK → "100" = WHITE; a = "+ " = RED → "–" = GREEN; b = "+" = YELLOW → "–" = BLUE.

were no different from each other after being cooked.

The combination of NaFeEDTA with either calcium source resulted in a cooked product with sensory characteristics closest to those of "normal" rice. The use of calcium lactate in combination with other iron sources, i.e., ferrous sulfate and ferrous lactate, resulted in dull yellow cooked product, whereas the use of calcium lactate gluconate with these iron sources resulted in a brighter yellow color.

pH values and water activity

The pH values of the cooked fortified products were slightly lower than that of the unfortified product (**table 2**). In a previous study (V. Chavasit, unpublished data, March 2002), the pH of water was affected by iron but not by calcium fortificants. Different sources of iron could affect the pH of water differently. With the same amount of iron obtained, the pH of water became 3.58, 4.89, and 5.49 for ferrous sulfate, NaFeEDTA, and ferrous lactate, respectively, while the pH values of calcium lactate and calcium lactate gluconate solutions were 6.56 and 7, respectively. Because of its very low water activity (0.2), the dried fortified product was very stable in terms of microbial quality. However, lipid oxidation is a problem that must be considered, since oxidative rancidity may be accelerated under low water activity conditions (lower than 0.3), especially with iron as a catalyst [19].

Nutrient composition and loss due to processing and cooking

Calcium and iron

The calcium content of the products was only about 6% lower than expected (inherited + fortified = 8 + 200 = 208 mg/100 g). There were no significant differences among products fortified with different kinds of fortificants in calcium content or percentage lost during processing and cooking ($p > .05$). Up to only 6% of calcium was lost during processing, and approximately 3% was lost during cooking (**table 3**). Calcium is a stable nutrient that normally is lost by leaching. In this fortification process, most of the fortificant solution was absorbed into the rice. Consequently, only a small amount of calcium remained in the unabsorbed solution. Loss of calcium during drying and cooking is not likely to occur, since calcium would not be destroyed by oxidation and heat during cooking [20]. Furthermore, the water used for cooking was not drained out.

Iron loss was higher than calcium loss in the dried product. The cause of this loss should be similar to the cause of calcium loss. On average, calcium and iron were lost during processing of dried broken rice, 5% and 10%, respectively (**table 3**). However, more iron might have been adsorbed on the surface of the plastic basin that was used for soaking the rice. In a previous study (V. Chavasit, unpublished data, March 2002), approximately 2 mg of iron was adsorbed on the surface of the plastic basin. During cooking in a stainless steel pot, the iron content of the product was increased. In our trial, we found that the dissolved iron fortificant could reduce the pH of water from 6.9 to 4.6. After such a solution had been boiled for 8 to 9 minutes in a stainless steel pot, the iron content was found to increase up to 1.7 mg per pot of 200 ml water (V. Chavasit, unpublished data, March 2002). Such evidence of iron dissolution, however, was not found, as the water (pH 6.9) was boiled in the same pot as the solution of iron fortificant. The acidic condition (pH 4.6) that resulted after the iron compound had been added could be the main reason for iron dissolution.

Vitamin content

There were no significant differences ($p > .05$) in thiamine content between fortified products (**table 3**). Thiamine losses due to processing were on average about 13% (based on the fortified dosage of 0.4 mg/100 g). The loss was due to heat, atmospheric oxygen, light, and moisture during soaking and drying. Therefore, the product should be fortified with thiamine by at least 20% more than the requirement in order to compensate for the loss during processing. Slight thiamine losses were found during cooking. Vandrasek and Warthesen mentioned that the thiamine loss in rice and pasta products during cooking was as high as 34% to 53%, mainly from leaching into the cooking liquid, which is not consumed [21].

The level of folic acid in the unfortified product was 18.3 µg/100 g. After fortification at a dosage of 55 µg/100 g, the folic acid content of the dried products ranged from 62 to 73 µg/100 g. High losses of folic acid occurred during processing (17%–23%). Sunlight, particularly ultraviolet radiation, seriously affects the stability of folic acid [22, 23]. During cooking, losses of folic acid (up to 34%) were still observed. Together with vitamin C, thiamine and folic acid are the most heat-sensitive nutrients [24]. The loss of folic acid in boiled eggs is only 10%, whereas other cooking methods involving exposure to heat and air may cause losses of up to 30%. A higher loss (50%) has been observed during the cooking of vegetables [23]. However, the cooked fortified dried broken rice still contained folic acid at the required levels (> 55 µg/100 g).

Nutritive value

Table 4 gives the nutritive values and nutrient adequacies of the dried broken rice that was fortified based on the revised guidelines for nutritive value per 100 kcal, as compared with current (WHO/UNICEF, 1998) [25] and new (IOM) [7–10] recommendations. The energy density was 0.7 kcal/g of the cooked product. The fortification nutrients were adequate for ≥ 50% of both

TABLE 3. Calcium, iron, thiamine, and folic acid losses due to processing and cooking[a,b]

Nutrient	Sample	Raw products[c]	Cooked products[c]	% processing loss[d]	% cooking loss[e]
Calcium (mg/100 g)	Control	8.00	—	—	—
	CLG + $FeSO_4$	196.44 ± 1.56	191.65 ± 9.97	5.5 ± 0.8	2.6 ± 2.1
	CLG + NaFeEDTA	193.59 ± 5.09	203.25 ± 9.71	5.9 ± 2.4	0.0 ± 0.0
	CLG + Fe lactate	194.41 ± 5.97	198.98 ± 17.08	6.5 ± 2.8	2.4 ± 2.1
	CL + $FeSO_4$	200.00 ± 1.29	200.67 ± 4.98	3.7 ± 0.6	0.6 ± 1.0
	CL + NaFeEDTA	202.07 ± 6.31	201.38 ± 14.4	2.6 ± 2.2	3.3 ± 5.7
	CL + Fe lactate	203.45 ± 6.21	200.54 ± 12.90	4.7 ± 2.5	3.2 ± 5.6
Iron (mg/100 g)	Control	0.034	—	—	—
	CLG + $FeSO_4$	23.29 ± 0.77	25.49 ± 1.21	9.0 ± 3.0	0
	CLG + NaFeEDTA	22.50 ± 0.46	25.48 ± 0.51	16.0 ± 1.8	0
	CLG + Fe lactate	24.29 ± 0.68	25.99 ± 1.39	5.1 ± 2.6	0
	CL + $FeSO_4$	22.29 ± 0.33	26.76 ± 0.15	12.9 ± 1.3	0
	CL + NaFeEDTA	23.44 ± 0.93	24.85 ± 0.34	8.4 ± 3.6	0
	CL + Fe lactate	24.13 ± 0.57	26.33 ± 0.45	5.8 ± 2.2	0
Thiamine (mg/100 g)	Control	ND	ND	ND	ND
	CLG + $FeSO_4$	0.354 ± 0.012	0.343 ± 0.001	11.5 ± 2.9	3.0 ± 2.6
	CLG + NaFeEDTA	0.358 ± 0.012	0.367 ± 0.008	10.5 ± 2.6	0.1 ± 0.2
	CLG + Fe lactate	0.316 ± 0.006	0.361 ± 0.010	20.9 ± 1.5	0.0 ± 0.0
	CL + $FeSO_4$	0.361 ± 0.012	0.350 ± 0.020	9.9 ± 5.2	3.4 ± 5.9
	CL + NaFeEDTA	0.356 ± 0.020	0.355 ± 0.010	16.6 ± 5.0	0.1±0.2
	CL + Fe lactate	0.354 ± 0.027	0.357 ± 0.007	11.5 ± 6.8	1.7 ± 1.5
Folic acid (mg/100 g)	Control	18.30	—	—	—
	CLG + $FeSO_4$	63.95 ± 14.80	60.70	21.27 ± 25.07	22.99
	CLG + NaFeEDTA	62.25 ± 13.91	54.70	23.06 ± 26.77	27.92
	CLG + Fe lactate	68.43 ± 20.74	59.10	20.23 ± 26.86	34.33
	CL + $FeSO_4$	66.07 ± 15.16	57.00	17.55 ± 27.66	27.04
	CL + NaFeEDTA	66.72 ± 15.58	59.70	17.04 ± 27.48	25.07
	CL + Fe lactate	73.16 ± 24.55	68.40	16.95 ± 28.50	30.20

CLG, calcium lactate gluconate; CL, calcium lactate; NaFeEDTA, ferric sodium ethylenediaminetetraacetic acid; ND, not detectable

a. Values are means ± SD of the samples obtained from three production batches.

b. Means without SD are from analysis of the sample from the mixture of three cooking batches.

c. There were no significant differences between columns in the values for the fortified products ($p > .05$).

d. Calculation is based on initial values of 208 mg/100 g, 26.5 mg/100 g, 0.4 mg/100 g, and 73.3 mg/100 g for calcium, iron, thiamine, and folic acid, respectively.

e. Calculation is based on difference in dry basis, nutrient content x 100/(100 – moisture content), between raw and cooked products.

recommendations, since the products were consumed during one to three meals per day by infants of different age groups. Moreover, although the energy density of the cooked fortified broken rice was low, this was not a problem. In the preparation of complementary foods, the product is normally used as a base for mixing with other foods, such as egg yolk, meat, liver, and vegetable oil, as recommended in the guidelines of Thailand's Ministry of Public Health, 1999 [4]. On the basis of the complementary food standard for infants aged six to eight months, complementary foods prepared by using the product as the basic ingredient, with the addition of about 25 to 30 kcal, could provide close to 100% of the nutrient requirements (40 mg of calcium, 5.3 mg of iron, 0.08 mg of thiamine, and 11 µg of folate per 100 kcal). **Table 5** shows that most of the fortified nutrients provided approximately 20% to 40% of the requirements of the Thai Dietary Reference Intake (DRI) for 2003 or of the WHO recommendations for 2002. Fortified broken rice on its own cannot fulfill the daily needs for certain nutrients, especially energy and protein, so other ingredients are required in order to fulfill the nutrient requirements.

If the product was fed to older infants as the sole food for three meals per day, it would provide too much iron: up to 250% of the recommended levels for complementary foods (**table 4**), based on the Thai DRI for 2003 and the WHO levels for 2002 (**table 5**). However, older infants usually do not rely solely on this complementary food for three meals, because they are able to eat a variety of foods.

TABLE 4. Nutrient contents of fortified dried broken rice and percent adequacy based on current (WHO/UNICEF, 1998)[25] and new (IOM) [7–10] daily recommendations of nutrients required from complementary food for children according to age group

Nutrient	Amount per 100 kcal	Amount per 20-g serving	% adequacy[a]					
			6–8 mo		9–11 mo		12–23 mo	
			WHO/ UNICEF 1998	New DRI (IOM)	WHO/ UNICEF 1998	New DRI (IOM)	WHO/ UNICEF 1998	New DRI (IOM)
Energy[b]	100	76.6	28	38	34	51	30	42
Carbohydrate (g)	22.7	17.40	NA	NA	NA	NA	NA	NA
Fat (g)	0.18	0.14	NA	NA	NA	NA	NA	NA
Protein (g)	1.9	1.44	77	72	91	96	84	87
Calcium (mg)	52	39.88	37	50	23	83	63	36
Iron (mg)	6.73	5.20	48	49	96	99	258	235
Thiamine (mg)	0.09	0.07	66	45	79	79	57	55
Folate (μg)	20.6	15.80	NA	72	NA	95	NA	45

DRI, dietary reference intake; NA, not applicable.

a. Perecent adequacy is based on one, two, and three meals per day for chi1dren 6–8, 9–11, and 12–23 months of age, respectively. The WHO/UNICEF (1998)[25] daily energy requirements for complementary foods are 269, 451, and 746 kcal for chi1dren 6–8 , 9–11, and 12–23 months of age, respectively. The IOM [7–10] daily energy requirements for complementary foods are 200, 300, and 550 kcal for chi1dren 6–8 , 9–11, and 12–23 months of age, respectively.

b. Energy density = 0.7 (kcal/g cooked product).

TABLE 5. Nutrient contents of fortified dried broken rice and percent adequacy based on 2003 Dietary Reference Intake for Thais (Thai DRI) [29], and 2002 FAO/WHO recommendations [30] for nutrients required per day for children according to age group

Nutrient	Amount per 20-g serving	% adequacy[a]					
		6–8 mo		9–11 mo		12–23 mo	
		Thai DRI 2003	WHO 2002	Thai DRI 2003	WHO 2002	Thai DRI 2003	WHO 2002
Energy (kcal)	76.60	10	NA	19	NA	45	NA
Carbohydrate (g)	17.40	NA	NA	NA	NA	NA	NA
Fat (g)	0.14	NA	NA	NA	NA	NA	NA
Protein (g)	1.44	9	NA	18	NA	45	NA
Calcium (mg)	39.88	15	10	29	20	24	24
Iron (mg)	5.20	55	55	111	111	267	267
Thiamine (mg)	0.07	24	24	47	47	42	42
Folate (μg)	15.8	20	20	39	39	33	30

NA, not applicable

a. Percent adequacy is based on one, two, and three meals per day for chi1dren 6–8, 9–11, and 12–23 months of age, respectively.

Shelf-life study

During accelerated storage conditions, only a slight increase in moisture content and water activity of the fortified dried rice products was observed for both types of packaging (polyethylene plastic and metallized bags). However, the final water activity was still only about 0.2 to 0.3, which was low enough to prevent microbial growth. Both types of packaging material are normally good barriers for moisture but not for oxygen. During the test, all forms of iron except NaFeEDTA catalyzed an oxidation reaction and caused product rancidity within two weeks. The product fortified with NaFeEDTA that was packed in metallized bags was the most stable in terms of oxidative rancidity. Thiamine was used as the indicator for nutrient loss during storage (**table 6**). No losses were found in fortified products packed in either type of packaging. Thiamine is not as sensitive to oxygen and light in the dry product as it is in the wet product [23].

Dialyzability of iron from various sources

Table 7 shows the percentage of in vitro dialyzable iron from different kinds of fortificants. The bioavailability of inherited iron in the dried broken rice (control) was very poor. Fortification of cooked dried broken rice with iron compounds significantly increased the iron content and iron bioavailability. However, the bioavailability of iron was different in cooked

TABLE 6. Thiamine content of fortified dried broken rice packaged in different materials during the shelf-life study[a]

Sample	Storage time	Thiamine (mg)	
		Metallized packaging	Plastic packaging
CLG + $FeSO_4$	0 days	0.354 ± 0.012	0.354 ± 0.012
	2 wk	0.344 ± 0.005	0.330 ± 0.028
	1 mo	0.329 ± 0.028	0.324 ± 0.012
	2 mo	0.347 ± 0.011	0.350 ± 0.009
	3 mo	0.345 ± 0.016	0.323 ± 0.028
CLG + NaFeEDTA	0 days	0.358 ± 0.010	0.358 ± 0.010
	2 wk	0.354 ± 0.010	0.332 ± 0.020
	1 mo	0.365 ± 0.023	0.350 ± 0.012
	2 mo	0.356 ± 0.015	0.351 ± 0.017
	3 mo	0.352 ± 0.016	0.358 ± 0.017
CLG + Fe lactate	0 days	0.364 ± 0.007	0.364 ± 0.007
	2 wk	0.331 ± 0.016	0.332 ± 0.027
	1 mo	0.331 ± 0.027	0.326 ± 0.038
	2 mo	0.356 ± 0.005	0.321 ± 0.032
	3 mo	0.359 ± 0.002	0.330 ± 0.021
CL + $FeSO_4$	0 days	0.360 ± 0.020	0.360 ± 0.020
	2 wk	0.338 ± 0.020	0.303 ± 0.018
	1 mo	0.337 ± 0.009	0.329 ± 0.002
	2 mo	0.351 ± 0.025	0.318 ± 0.013
	3 mo	0.348 ± 0.020	0.307 ± 0.005
CL + NaFeEDTA	0 days	0.356 ± 0.014	0.356 ± 0.014
	2 wk	0.326 ± 0.028	0.324 ± 0.027
	1 mo	0.333 ± 0.020	0.326 ± 0.017
	2 mo	0.339 ± 0.010	0.331 ± 0.012
	3 mo	0.338 ± 0.012	0.319 ± 0.007
CL + Fe lactate	0 days	0.354 ± 0.005	0.354 ±0.021
	2 wk	0.333 ± 0.020	0.293 ± 0.016
	1 mo	0.330 ± 0.016	0.323 ± 0.012
	2 mo	0.326 ± 0.007	0.317 ± 0.017
	3 mo	0.329 ± 0.012	0.303 ± 0.028

CLG, calcium lactate gluconate; CL, calcium lactate; NaFeEDTA, ferric sodium ethylenediaminetetraacetic acid

a. Values are means ± SD.

dried broken rice fortified with iron from different sources. NaFeEDTA was more effective than ferrous sulfate and ferrous lactate. The percentage of dialyzable iron from NaFeEDTA was significantly higher (approximately 20 times) than that from the other iron fortificants ($p > .05$).

NaFeEDTA was relatively soluble in different food matrices and was nonreactive, which could prevent iron binding with dietary inhibitors such as phytates and polyphenols. In cereal that has a high phytate concentration, the bioavailability of iron from NaFeEDTA is two to three times higher than that of iron from ferrous sulfate [26]. Trinidad et al. [27] investigated the bioavailability of iron from ferrous sulfate and NaFeEDTA in fortified rice by both in vitro and in vivo methods. The in vivo study showed that the percentage of iron absorption from NaFeEDTA was higher than that from ferrous sulfate, whereas no difference was found by the in vitro method. Pirapatdit [28] studied the bioavailability of various forms of iron in fortified fresh wheat noodles by the in vitro method and found that the percentage of iron dialyzability of the product fortified with NaFeEDTA was higher than that in products fortified with ferrous sulfate, ferrous fumarate, or encapsulated reduced elemental iron powder.

TABLE 7. Percentage of dialyzable iron from different sources in fortified dried broken rice

Sample	Dialyzable iron (%)[a]
Control	ND
CLG + $FeSO_4$	2.5 ± 0.4[d]
CLG + NaFeEDTA	61.1 ± 2.8[b]
CLG + Fe lactate	5.2 ± 0.7[d]
CL+ $FeSO_4$	1.6 ± 0.2[d]
CL + NaFeEDTA	56.4 ± 4.0[c]
CL+ Fe lactate	2.0 ± 0.1[d]

CLG, calcium lactate gluconate; CL, calcium lactate; NaFeEDTA, ferric sodium ethylenediaminetetraacetic acid; ND, not detectable

a. Results are the means of six determinations ± SD. Means followed by the same letter are not significantly different ($p > .05$).

The type of calcium fortificant was found to affect iron dialyzability. With the same type of iron source, the percentage of dialyzable iron in products fortified with calcium lactate gluconate was higher than it was in products fortified with calcium lactate. Calcium lactate gluconate has been reported to be an enhancer of iron absorption. Such enhancing effects were not significant ($p > .05$) in the products that were fortified with ferrous sulfate or ferrous lactate. The results of this study indicate only the trend of iron bioavailability, and not absolute absorption, which needs further study. In addition, an important factor to be considered is the cost of the fortificant: NaFeEDTA costs six times more than ferrous sulfate (US$0.0951 vs. US$0.0166 per 10 kg of product).

It is feasible to produce a dried broken rice product fortified with calcium, iron, thiamine, and folate at the levels recommended by the current (2001) FAO guidelines. NaFeEDTA is the most appropriate iron fortificant. For future studies, the in vivo bioavailability of nutrients under real consumption conditions should be investigated.

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Nutrient content of complementary foods based on processed and fermented sorghum, groundnut, spinach, and mango

Habiba Oumarou, Richard Ejoh, Robert Ndjouenkeu, and Agatha Tanya

Abstract

Background. *Many African mothers use gruels made of maize and sorghum as complementary foods for their infants because they cannot afford the cost of nutritionally superior commercial weaning foods.*

Objective. *To improve the nutritional quality of traditional sorghum gruels used as complementary foods for children between six months and five years in the northern part of Cameroon.*

Methods. *Sorghum grains were processed by dehulling, sprouting, dehulling and cooking, and sprouting and cooking, then fermented using* Saccharomyces cerevisae *and tested for nutritional quality. The processed samples were analyzed for their nutrient composition and the digestibility of proteins and carbohydrates.*

Results. *The resulting products showed a significant reduction in antinutritional factors (e.g., phenolic compounds and phytates). In addition, germination improved the* in vitro *digestibility of proteins and carbohydrates. These effects were enhanced by fermentation of the resulting sorghum flour, regardless of how grains were pretreated, although the protein and mineral content remained low. Use of a composite flour made from pretreated sorghum, groundnut, spinach, and mango improved the protein, mineral, and vitamin A and C content of the gruels. Fermentation of composite flours resulted in an improvement in the protein content.*

Conclusions. *This study, conducted under local conditions and using local technology, describes an opportunity for improving the quality of complementary foods using local ingredients.*

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Mention of the names of firms and commercial products does not imply endorsement by the United Nations University.

Key words: Boiling, composite flour, dehulling, digestibility, fermentation, sorghum, sprouting

Introduction

A variety of weaning flours are commercially available with high nutritive value, which are directly used for instant preparation of gruels. In many developing countries, these products are beyond the economic means of the majority of families, so mothers use traditional gruels—watery suspensions of maize or sorghum—as complementary foods for infants. These gruels usually have low energy density and poor protein, vitamin, and mineral contents [1, 2]. In many African countries, women improve the nutritional value of the gruel by addition of groundnut paste. In fact, the level of improvement, as well as the proportion of water to flour, is not standardized, so that children tend to receive gruels that are either too diluted or too concentrated. In addition, because of inappropriate processing technology, the gruels contain significant levels of contaminants and antinutritional factors, which further limit nutrient absorption by the young child [3]. These factors predispose children to protein–energy malnutrition [4, 5]. Apart from protein–energy malnutrition, micronutrient deficiencies are widespread among infants and young children and account for the high rates of child malnutrition. The most prevalent are iron deficiency, anemia, vitamin A deficiency, and iodine-deficiency disorders [6]. Because of the prevalence of these deficiencies, the gradual introduction of complementary foods to infant meals has become an issue of serious concern.

Many types of low-cost complementary foods have been developed from locally available, high-calorie cereals and legumes in tropical Africa [7, 8]. Such legumes, although rich in plant protein, are also rich in antinutritional factors such as tannins, trypsin inhibitor, and polyphenols [9]. Fermentation is known to increase digestibility and reduce the levels of antinutritional factors, thereby increasing the bulk density of reconstituted gruels [10].

The present study in Cameroon aimed to improve the nutritive quality of infant gruels by the use of local food materials and simple processing methods. The improvement techniques involved pretreatment of sorghum (dehulling, sprouting, and boiling), fermentation of flours, and the addition of groundnuts, spinach, and mango.

Materials and methods

Sample collection and preparation of materials

Sorghum grains (*Sorghum bicolor*) of the yellow variety and groundnuts (*Arachis hypogaea*) of the campala variety were purchased from a local market (Ngaoundéré, Cameroon). They were sorted to remove the moldy and broken grains. Local varieties of moderately ripe mango (*Mangifera indica*) and spinach (*Cnidoscolus chayamansa*) were obtained from local farms (Ngaoundéré, Cameroon). Ngaoundéré is a town of about 100,000 people situated in the lower part of the Sudano Sahelian region of Central Africa.

The sorghum sample was divided into portions and processed by dehulling, sprouting, dehulling and cooking, and sprouting and cooking. For dehulling, grains were wetted (2.5 g of grain/ml water) and hulled in a mortar. Hulled grains were washed and dried at 50°C for 24 hours in a prototype electric dryer (CKA200AUF) [11], built by the Department of Process Engineering at the University of Ngaoundéré. The sprouting involved

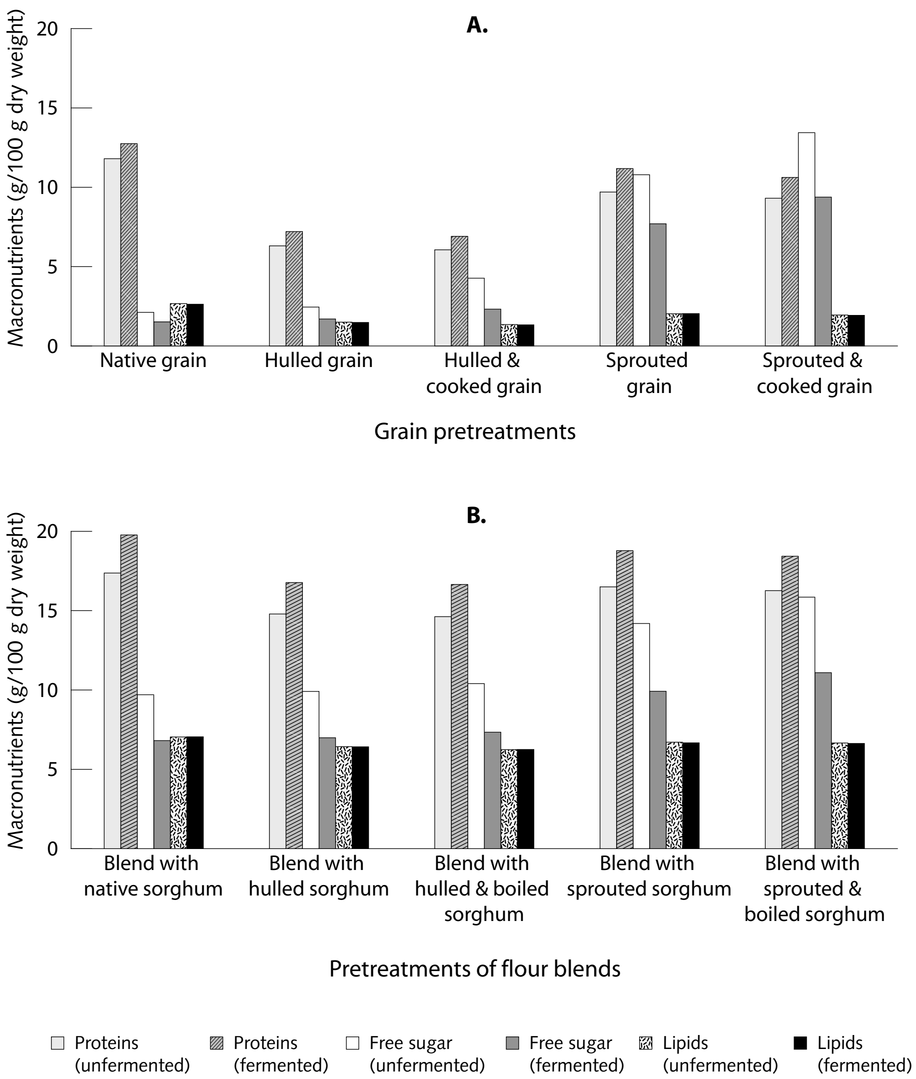

FIG. 1. Effects of pretreatment of grain and fermentation of flour on macronutrient contents of sorghum (A) and blends (B)

soaking [12], germination [13], and drying at 50°C for 24 hours in the same dryer. Cooking was done by steaming for 2 hours in a domestic steam pot. The cooked grains were dried at 50°C with the dryer. The pretreated grains were then ground to a fine powder with a domestic grinder and divided into two portions, one to be fermented and one to be left unfermented.

Groundnut was processed by roasting, which involved toasting the grains in a preheated aluminum pot on an electric stove for about 15 minutes, stirring vigorously with a wooden spatula, and then peeling the skin. The peeled grains were ground into a fine paste with a domestic pedal-crank grinder, which is a common appliance in study area households. Spinach flour was obtained by drying the fresh spinach leaves and young shoots at 50°C for 24 hours in the dryer and then grinding them to a fine powder. Mango powder was obtained by drying the fruit pulp at 50°C for 24 hours in the dryer, followed by immediate grinding.

Formulation of flour blends

Composite flour was prepared by mixing pretreated sorghum flour (65%), groundnut paste (15%), spinach flour (10%), and mango flour (10%) in a domestic mixer (Moulinex, France). The objective here was to maintain the nutrient composition of the composite flour close to the WHO/FAO standard values for complementary foods, i.e., 70% carbohydrate, 16% protein, 7% lipid, 2% ash, and 5% fiber [14]. The composite flour was divided into two portions, one to be fermented and one to be left unfermented.

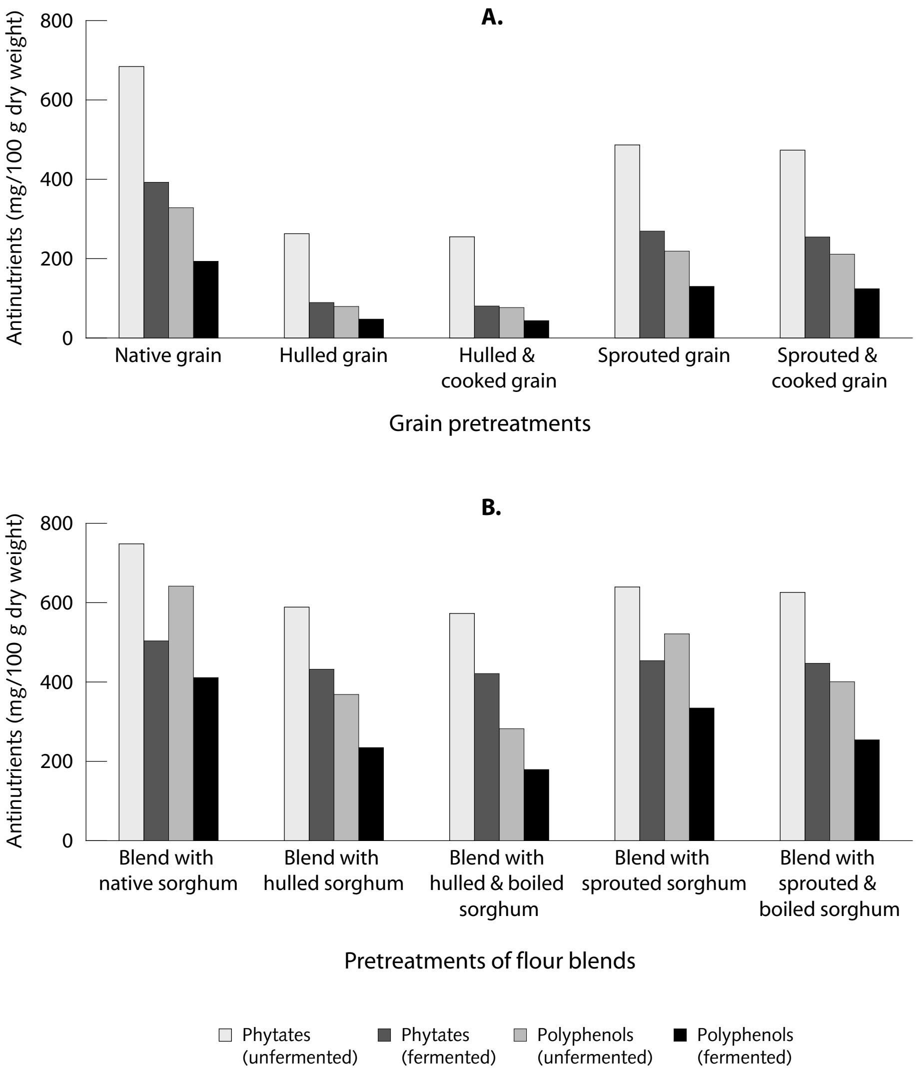

FIG. 2. Effects of pretreatment of grain and fermentation of flour on antinutrient levels of sorghum (A) and blends (B)

Fermentation of flours

Each of the pretreated sorghum and composite flours was made into a 20% (weight/volume) slurry [8]. One liter of each slurry was incubated with 1 ml of starter culture from *Saccharomyces cerevisiae* (250 mg/L), and fermentation was allowed to go on for 24 hours at 30°C and pH 4.5. Thereafter, the fermented flours were dried in the dryer at 50°C for 24 hours.

Chemical analysis

The flours (fermented and unfermented) were analyzed individually for protein [15], lipids [16], free sugar [17], fiber and ash [18], minerals (iron, calcium [19], phosphorus, and magnesium [20]), β-carotene [21], vitamin C [22], polyphenols [23], and phytates [24]. The in vitro digestibility of proteins and carbohydrates was determined for all flours by the methods of Gauthier et al. [25] and Mbofung et al. [26], respectively. For all chemical analyses, each sample was analyzed in triplicate and the values were averaged.

Results and discussion

Effect of pretreatment of grain and fermentation of flour on the nutrient content of sorghum

Changes in the nutrient content of sorghum as a result of pretreatment and fermentation are shown in **figs. 1A, 2A, 3A, 4A, and 5A**. Removal of the grain pericarp and germ by dehulling reduces the protein, lipid, and fiber levels, whereas the available sugar, which is present mainly in the endosperm, appears to be higher (**figs. 1A and 5A**). Most of the antinutrients are concentrated

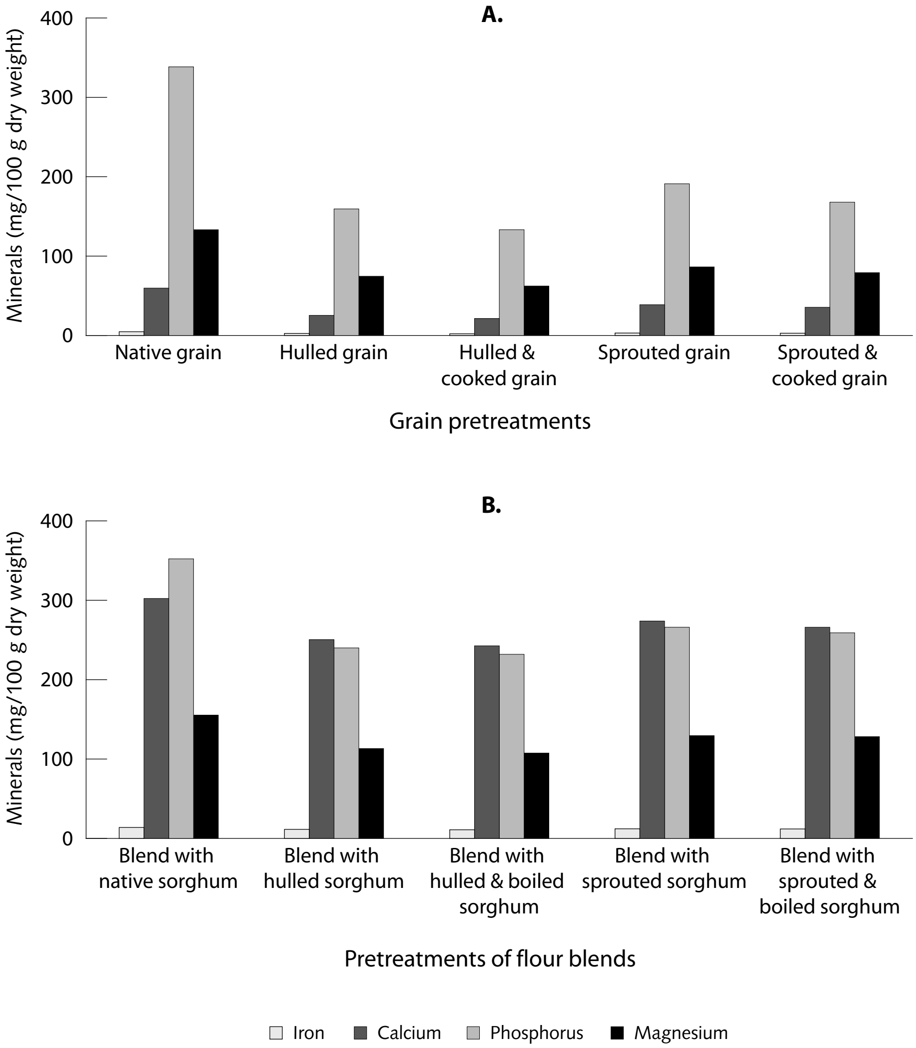

FIG. 3. Levels of minerals in pretreated sorghum (A) and blends (B)

in the grain pericarp [27], which is supposed to be removed by dehulling. But because the structure of sorghum grain does not allow easy dehulling, a significant amount of antinutrients remains in the hulled grains (**fig. 2A**). Boiling the hulled grains does not affect the residual proteins and lipids of the sorghum endosperm, but it contributes to the partial hydrolysis of starch, resulting in an increase in available free sugars (**fig. 1A**). The water-soluble character of phenolic compounds favors their solubility in boiling water and their leaching from the grain (**fig. 2A**). Germination also causes a reduction in protein, lipid, and antinutrient levels, though to a lesser extent than dehulling, because of the metabolic use of these nutrients during germination [27]. During germination, metabolism of these grains contributes to starch hydrolysis to produce free sugars. Phytates are also hydrolyzed by phytase produced during germination, allowing the plant to use its phosphorus during germination [28]. Boiling of sprouted grains does not significantly affect most nutrients, except to cause continuous leaching of residual polyphenols (**fig. 2A**).

Combination of the above pretreatments with fermentation contributes to a slight improvement in the protein level (**fig. 1A**) and a significant reduction in fiber and some other antinutrients (**figs. 2A and 5A**). The available sugar is used in the fermentation process, resulting in a reduction in its level (**fig. 1A**). The vitamin A and C levels in pretreated and fermented sorghum (**fig. 4A**) are less than 0.1 and 0.45 µg/100 g dry weight, respectively. Fermentation does not significantly affect these levels ($p < .05$), but grain pretreatment,

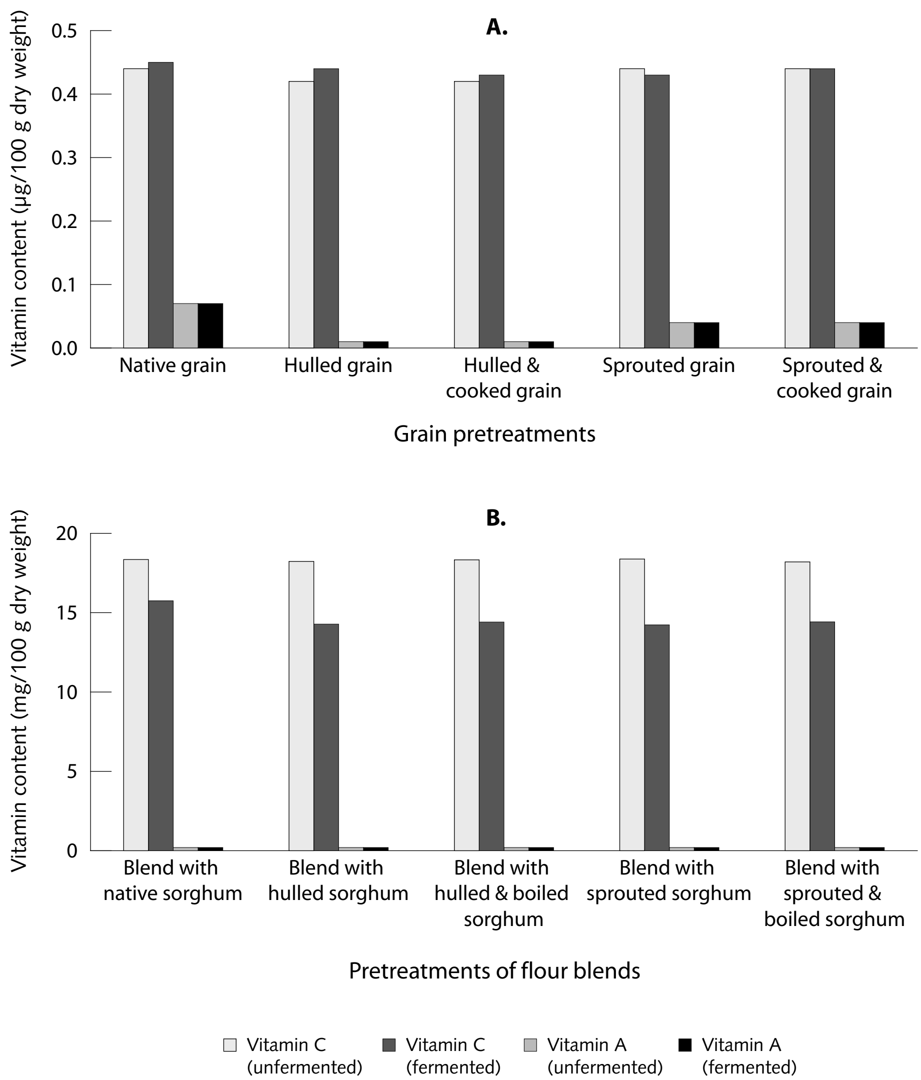

FIG. 4. Levels of vitamins A and C in pretreated sorghum (A) and blends (B)

particularly dehulling, reduces the vitamin A level, whereas the vitamin C level remains almost constant.

From the above results, it is evident that dehulling and sprouting, coupled with fermentation and boiling, contribute to the improvement in the nutrient content of sorghum. These treatments, particularly germination, make this positive contribution because the resulting flours produce energy-dense gruels with acceptable consistency [29]. In addition, most of the treatments have the advantage of being affordable at the household or village level. The processed sorghum gruel, although rich in carbohydrates, does not meet the minimum requirements for protein and vitamins for young children recommended by WHO/FAO/UNICEF [30]. These requirements mandate levels of 68% carbohydrates, 13% proteins, 7% lipids, 5% fiber, and 2% ash. The sorghum flour remains particularly poor in proteins and vitamins and could be improved by the addition of foods rich in these nutrients.

Effect of groundnut, spinach, and mango flour blends on the nutrient content of sorghum

Figures 1B, 2B, 3B, 4, and 5B show the effect of blending and fermentation on the processed sorghum as compared with unblended cereal.

Blending of processed sorghum with groundnut, spinach, and mango, with or without fermentation, improves the macronutrient (**fig. 1B**) and mineral (**fig. 3**) contents of the composite flours. The levels of macronutrients, phosphorus, and magnesium in these composite blends are higher than the recommended values for complementary foods [30], whereas the iron levels (**fig. 4**) fall short of the recommended level of 15

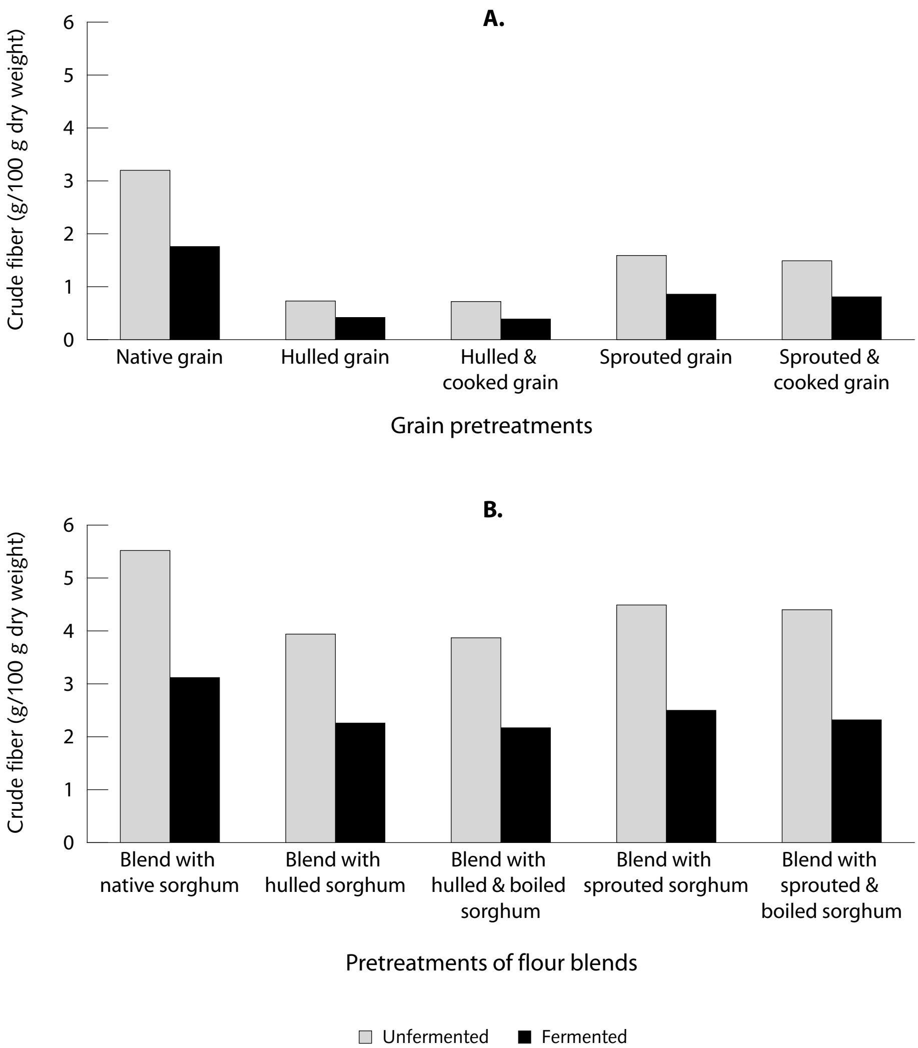

FIG. 5. Crude fiber contents of pretreated grains (A) and blends (B)

mg/100 g [31]. A similar trend is observed for calcium, with the highest levels found in the blend made with unprocessed sorghum flour (304 mg/100 g dry weight, instead of the recommended 468 mg/g). Vitamin A and C levels are generally higher than the recommended values. The principal source of vitamin C is mango (0.45 μg/100 g dry weight in sorghum alone, compared to 19 mg/100 g dry weight in blends); however, it should be noted that the levels of this vitamin are reduced during fermentation (**fig. 4**). This reduction was not observed during the fermentation of sorghum alone. The low level of vitamins in sorghum and the precision of the measurement technique used may be the reasons that a significant variation in sorghum was not observed.

Since the leafy vegetable (Indian spinach) and groundnut used in the blends contain some antinutritional factors, such as polyphenols and phytates, these antinutrients are present in higher levels in blends, though at lower levels in the processed and fermented samples (**fig. 2B**).

According to the results of in vitro digestibility studies (**table 1**), protein and carbohydrate digestibility significantly increased as a result of pretreatment of grain and fermentation of flour in both sorghum and flour blends, with the highest increase in sprouted and fermented samples, while the lowest digestibility was observed in nontreated sorghum flours. Carbohydrate digestibility improved more than protein digestibility; the maximum improvement was 57% and 29% respectively, in sprouted and cooked blends. In addition, fermented sorghum samples have digestibility comparable to their equivalent blended samples. This means that blending did not significantly improve the digestibility, because spinach flours were added without any pretreatment, and therefore their high antinutrient content might have reduced the digestibility of protein and carbohydrates.

Conclusions

Processing of sorghum grain, particularly dehulling and sprouting, contributes to improving the availability of nutrients in cereals. Pairing these processing techniques with fermentation improves the digestibility of the available nutrients, but this cereal remains poor in nutrients for growing children. Complementary foods developed with sorghum, groundnut, spinach, and mango have promising nutritional attributes. They contain reasonable quantities of most nutrients, significantly increased levels of protein, iron, calcium, and vitamins, in accordance with WHO/FAO/UNICEF standards. The processing techniques (sprouting and dehulling, blending, and fermentation) are affordable at the household level and can easily be performed by village women in our study area. However, for these techniques to be properly implemented, the village women must be educated in their use. The higher content of antinutritional factors in the leafy vegetables limits their nutritional quality, suggesting the necessity of treatment before use in order to improve the bioavailability of nutrients. In addition, the limited shelf-life of the flour blends, due to the susceptibility of lipids to oxidation, remains an issue of concern for the population.

TABLE 1. In vitro digestibility of proteins and carbohydrates as a function of grain pretreatments, flour blending, and fermentation*

	Initial protein content (%)		Initial carbohydrate content (%)	
Pretreatment	Unfermented	Fermented	Unfermented	Fermented
Native grain	74.33 ± 0.37^a	83.57 ± 0.11^a	62.60 ± 0.07^a	78.12 ± 0.07^a
Dehulled grain	76.54 ± 0.29^b	85.13 ± 0.19^b	68.30 ± 0.07^b	84.51 ± 0.07^b
Dehulled and cooked grain	80.31 ± 0.45^c	87.72 ± 0.20^c	87.36 ± 0.07^c	92.30 ± 0.07^c
Germinated grain	85.27 ± 0.14^d	92.51 ± 0.12^d	88.49 ± 0.07^d	95.21 ± 0.07^d
Germinated and cooked grains	88.92 ± 0.15^e	95.15 ± 0.13^e	91.67 ± 0.07^e	97.37 ± 0.07^e
Composite flours				
Blend with native sorghum	78.18 ± 0.16^f	85.146 ± 0.07^f	76.87 ± 0.08^f	83.49 ± 0.08^f
Blend with dehulled sorghum	79.45 ± 0.09^g	87.452 ± 0.08^g	78.24 ± 0.08^g	84.65 ± 0.08^g
Blend with dehulled and cooked sorghum	82.75 ± 0.09^h	90.091 ± 0.08^h	90.67 ± 0.08^h	96.15 ± 0.08^h
Blend with germinated sorghum	84.77 ± 0.08^i	93.110 ± 0.07^i	91.89 ± 0.08^i	97.19 ± 0.08^i
Blend with germinated and cooked sorghum	87.29 ± 0.08^j	95.869 ± 0.07^j	92.25 ± 0.08^j	98.14 ± 0.08^j

* For each column, means with the same superscript are not significantly different ($p < .05$). Values are means ± SD.

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Nutrition in emergencies

Food and nutrition concerns in Aceh after the tsunami

Patrick Webb

Editorial Comment

All too often, a natural disaster such as the December 2004 tsunami or complex emergencies that accompany conflict, expose the vulnerability of large segments of the world's population to shock and disruption. In a setting of drought, flood, and conflict, problems of borderline subsistence and food insecurity are transformed into hunger, starvation, and death. It will be our policy in the *Bulletin* to bring forward the nutritional challenges in these settings and the analysis of interventions directed toward their amelioration. Hurricane Katrina and the Kashmir earthquake in 2005 reinforced the importance of better understanding these issues. We invite manuscripts and policy documents in this category of nutritional emergency. The following is an account by the World Food Programme's former Chief of Nutrition of challenges posed to United Nations and other agencies by the December 2004 tsunami.

—*Irwin H. Rosenberg*

Introduction

The tsunami of December 26, 2004, was devastating. Its most immediate, and tragic, impact was in terms of lives lost. The estimated number of people either dead or missing in Northern Sumatra's mainland and northwest islands is around 250,000, mostly women and children [1]. That alone is a trauma of barely imaginable proportions. In addition, the wave devastated survivors' livelihoods. Almost 595,000 people were made homeless and forced to seek shelter in temporary camps or with relatives elsewhere in the province; roughly 514,000 of them were still in temporary housing in mid-2005. Even noncoastal families were cut off from regular sources of income as markets were destroyed, service jobs disappeared, and export cash crops (particularly rubber) could no longer reach outside buyers.

But did such devastation translate into a food and/or nutrition crisis? In the immediate aftermath, there were media reports of survivors cut off without food and water. Claims were made that more people could die of cholera and other epidemic diseases than were killed by the tsunami itself. Many claims, but few facts. Hence the urgency of mobilizing needs assessment teams to define the actual food, nutrition, health, and other needs of affected people.

However, it was not easy to organize the required assessments. Aceh was a "closed" province in more ways than one. It had been wracked by internal conflict and political dissent for many years, putting it out of bounds to most international agencies and media. The few maps available showed little detail of the mountains and valleys that were home to separatist forces, as well as to the so-called Mohajedin—radicals with whom even the nationalist separatists clashed periodically. In addition, the earthquake and wave knocked out most of the roads, bridges, culverts, ports, and warehouses along a vast coastline. The first relief teams to get into Aceh really didn't know what to expect. Would there be widespread pre-existing malnutrition, as in Darfur? Would there be large-scale epidemics, as in the Democratic Republic of the Congo?

This paper describes some of the food and nutrition concerns taken up by the first assessment teams in Aceh. In this space it is impossible to adequately describe the extent of the logistics and security problems faced, or to do justice to the creative solutions dreamed up by humanitarian professionals on the spot.

Patrick Webb was Chief of Nutrition for the United Nations World Food Programme during the tsunami response. He has since become Dean for Academic Affairs at the Friedman School of Nutrition Science and Policy, Tufts University, Boston. The opinions expressed here do not necessarily reflect the views or policies of the World Food Programme or of Tufts University.

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Mention of the names of firms or commercial products does not imply endorsement by the United Nations University.

The aim here is merely to encourage further analysis and dialogue on lessons that still need to be learned, and on emerging concerns.

Emergency needs assessment

Four major international needs assessments were in progress during January and February 2005, led by various UN agencies in collaboration with the Indonesian government. It has been argued that this represented duplication, but in reality such assessments were sectorally focused, each dealing with its own relief mandate and generating information needed to tailor relief appropriately.

By far the largest UN-led mission was the World Health Organization's (WHO) Inter-Agency Health Assessment, supported by the US military.* The mission was based on board the aircraft carrier USS *Lincoln,* making daily helicopter sorties to the mainland. The team members attempted to see what they could around dropoff zones, focusing mainly on health matters, but offering limited information on food needs or nutrition concerns [4].

The second major UN-led assessment was conducted by the World Food Programme in collaboration with numerous nongovernmental organizations (NGOs) and local authorities, which focused on the food needs of survivors and on the longer-term impact on food insecurity. Countless NGOs worked alongside the United Nations in conducting these main assessments, including Save the Children, Catholic Relief Services, CARE, Mercy Corps, World Vision, and others. Some also produced their own assessment results, such as Helen Keller International [5, 6]. The team did not conduct explicit nutrition surveys but considered dietary quality and quantity, breastfeeding practices, availability of clean water, sanitation arrangements, population displacements, availability of traditional and other coping mechanisms, income sources, and effects on market prices. The team provided considerable information on changes in consumption patterns, prices, and livelihood disruption, but little on nutrition outcomes [7].

The third was a joint UNICEF/CDC (US Centers for Disease Control and Prevention) nutrition survey based on a convenience sample of 19 camps in and around Banda Aceh/Aceh Besar. This was focused on the status of recently displaced people living in the often crowded and unsanitary temporary camps. It offered the most detailed nutrition and health information, but little information about food security or livelihood context [8].

The fourth was a rapid visual assessment of crop and livestock losses, farmland and plantation damage, and destruction of fishery assets led by the Food and Agriculture Organization (FAO) of the United Nations. This mission provided detail on agricultural impacts and reconstruction needs, but nothing on immediate nutritional implications [9].

None of these four assessments attempted to be statistically representative of the population of Aceh, or even of the directly affected population. There were significant security and logistical constraints that made household interviews, anthropometric measurements, and even visual assessments extremely hazardous in many locations. As a result, sample frames were established on the basis of broad geographic parameters (distance from the coast, main typologies of livelihood, distance from main urban centers, size of temporary camps, etc.), rather than on population-based sampling. The WHO teams visited around 30 locations (usually for a few hours each), the World Food Programme and its partners visited 18 sites (for up to an entire day each), and UNICEF/CDC visited 14 sites (for the hours needed to complete weights and measurements).

Assessment findings

Although the assessments focused principally on different domains, taken together they dovetailed to produce a mosaic of insights into the impact of the disaster on nutrition and food security for different population groups. At a macroeconomic level, it quickly emerged that the disaster would have far-reaching effects on livelihoods. For example, many tens of thousands of fishing families lost everything they had—their assets, as well as medium-term income streams. (Demand for fish plummeted for many months following the tsunami, as locals believed that fish were "unclean" because so many humans perished in the ocean.) Farming, formal business, and petty trading were badly affected in absolute terms, particularly in areas located within 5 km of the coast; these effects were exerted mainly through immediate loss of assets, including standing crops and animals, as well as the destruction of markets, roads, and warehouses. The loss of durable and productive

* At the height of the relief effort, around 16,000 US military personnel were deployed in the affected areas, based on two dozen US ships (including an aircraft carrier battle group, a Marine amphibious group, and the hospital ship USNS *Mercy*), and more than 100 aircraft—all at an estimated cost of some $5 million a day [2]. More than a dozen other countries also sent military capabilities to the regions affected. The inappropriateness of military support for humanitarian relief in complex emergencies has been widely agreed upon; however, similar consensus does not yet exist on the role of the military in support of relief in a natural disaster [3]. Although the military was generally praised for its role in the tsunami response, more discussion is needed on the political sensitivities involved, how much of its contribution represented value added (rather than substitution for activities of humanitarian agencies), and how cost-effective it was (an analysis demanded of relief agencies).

assets was extensive in all parts of Aceh—urban areas, the east coast, and the west coast. However, households depending on casual labor were less affected because of the upsurge in demand for labor for clearing and reconstruction.

The disruption of markets and trade had a marked effect on the prices of food and other commodities. Increases of 80% to 225% were recorded for various goods, depending on location (west coast communities saw much greater price increases than those on the east coast), demand for the commodity, and the size of pre-existing stocks [7]. For example, the cost of farm inputs (mainly fertilizer) rose by 85% in the month or so following the tsunami because of a curtailed supply and the need to replant paddy crops that had been destroyed. Most food prices rose even higher—85% for meat, 95% for cooking oil, and between 100% and 225% for pulses and staple cereals. Interestingly, the price of cleaning agents also rose sharply because of the need for disinfection; the price of soap rose by more than 80%, and the price of sanitary products (for personal hygiene) increased by 50% to 100%.

The leap in prices affected food consumption. On the basis of 24-hour recalls, it was found that 15% of the households interviewed were eating only one meal per day at the time of interview (almost one month after the shock), while another 30% were still eating only two meals per day. Moreover, dietary quality was suffering [7]. Most of the displaced people had an imbalanced and monotonous diet, especially if they were located on or near the west coast. For example, although most assessed households across all of Aceh were consuming at least one animal product per day (dried fish, eggs, or meat), around 80% of the households on the west coast had none of these items in their diet. Similarly, 42% of west coast dwellers were consuming no pulses at all, and almost 80% had no fats or oils. Their meals consisted of cereals, either rice or noodles, with little else other than fruits, which were plentiful despite the widespread destruction of coconut and papaya plantations.

The nutritional dangers posed by such widespread dietary deficiencies were obvious. It was decided to enhance food aid deliveries to ensure that they contained not only rice and micronutrient-fortified noodles (purchased in Indonesia), but also vitamin A–fortified vegetable oil (procured in the region), canned fish (from Japan), iodized salt, and multimicronutrient-fortified biscuits (from India). High importance was placed on ensuring not only adequate quantities of food for affected people, but also optimum micronutrient quality. Also obvious were the dangers posed by trauma, followed by physical displacement, followed by concentration of people in makeshift camps unequipped to provide clean water or appropriate sanitation. Initially it was feared that diarrhea, cholera, and malaria might greatly increase the death toll. Fortunately, efforts to anticipate such problems were relatively effective in controlling outbreaks of disease or micronutrient deficiencies. However, malnutrition was a concern.

The UNICEF/CDC nutrition survey of around 600 children younger than five years in selected camps in Banda Aceh found a level of global acute malnutrition (wasting) of 12.7%, with a prevalence of severe acute malnutrition of 1.5%. As is often the case in natural disasters, and as is the case throughout Indonesia, boys were worse off than girls [8]. Roughly 15% of adult nonpregnant women were found to be at risk for chronic energy deficiency, as manifested by body-mass index (BMI) levels under 18.5. These rates were thought to reflect worsening conditions in many camps, although the rate of pre-existing malnutrition was also high and played a role. Other nutrition surveys by NGOs found similar levels of wasting in and around Banda Aceh, while an additional, later survey by Helen Keller International on the islands of Nias and Simeuleu found higher levels of wasting among children 12 to 24 months (23%), but levels similar to those in Banda Aceh among children 6 to 59 months [6].

By the end of January, Indonesia's Ministry of Health invited UNICEF, WHO, the World Food Programme, and many other agencies to collaborate in setting up a province-wide, more representative baseline on nutrition and health to serve as a platform for regular surveillance. The first round of data found high anemia rates (59% among displaced preschoolers compared with 47% among the nondisplaced), but the prevalence of wasting among under-fives was generally less than 12%.

Assessment of lessons

So was there a food and nutrition crisis in Aceh post-tsunami? Most certainly, yes [3]. But it was not a crisis as defined by commonly used thresholds for child mortality and wasting. Although the prevalence of wasting was high and climbing, it was not generally above the widely used "trigger point" of 15% prevalence of –2 SD weight-for-height among preschoolers. Under-five mortality was of course high on December 26 itself, but it did not become elevated above background levels in the months that followed, thanks to rapid health, shelter, and food interventions that stabilized the situation.

Nevertheless, there *was* a danger of major loss of life in the absence of such interventions. That much was clear from the limited, diminished quality of the diet, abrupt changes in dietary patterns, a huge increase over pre-existing levels in the price of food and agricultural inputs, and compromised access to food markets due to destroyed infrastructure. Income flows were compromised for many hundreds of thousands of families, as they lost not only productive assets but their formal and nonformal livelihoods. Malnutrition was a concern, particularly among those temporarily settled in overcrowded and unsanitary camps where the threat of communicable disease, including diarrhea, was serious. And there was also a danger that precrisis levels of micronutrient deficiencies (which were already high, particularly on the west coast of Aceh) would evolve into outbreaks of specific life-threatening vitamin and mineral deficiency diseases. All in all, there was a post-shock humanitarian disaster in the making.

One conclusion is that a single, multisectoral assessment seeking to identify all of these problems among all population groups in all parts of the island simultaneously would arguably not have produced better information than was derived from four separate missions. A composite image of "needs" is important, partly for cross-validation and triangulation of information, and partly because of separate programmatic foci that allow each agency to delve into the operational issues pertaining to its particular mandate. Thus, recent calls for a single agency to conduct needs assessments, or for more centralized control of information-gathering, should be tempered by consideration of operational objectives. The process of data collection should be systematized rather than centralized; it is the process of interpreting data, with a view to optimal response, that needs better coordination. That is, predetermined agreement is needed on survey methods and sampling frames for such assessments, and building of local skills must of necessity pre-date the occurence of any emergencies. An important hurdle to rapid needs assessments is finding appropriately skilled nationals able to work as enumerators, supervisors, and data analysts. Such skill sets have to be built up over time, which requires investments in human capital specifically in countries most prone to disasters—which are often the places least likely to receive such investments.

A second conclusion is that information on nutrition should not be collected, interpreted, or even acted upon in isolation from a broader understanding of the nature of food insecurity, extent of livelihood collapse, water and sanitation needs, and likely dietary trends. Nutrition is not a sector; it is a multifaceted problem that cuts across conventional, narrowly focused sector coordination groups that are set up in most disaster situations. In other words, nutrition has to move from being a mere benchmark of the problem to become a key organizing principle for the solution across all sectors.

It has been argued that the relief response to the tsunami "is a story of aid done right" [10]. If that be true, it can be attributed to a rapid response on a massive scale, quick assessments of need that translated into fine-tuned interventions across multiple sectors, effective coordination among a great number of agencies, and a clear sense that preventing a deterioration in the food and nutrition situation was going to be central to efforts to move quickly from relief into reconstruction.

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Food, nutrients, and child growth: The role of specific foods and nutrients in child malnutrition and implications for food assistance programs

Conclusions and recommendations of a workshop held in Boston, Mass., USA, November 4–5, 2004

Compiled and summarized by Anna Herforth and Soha Moussa

Introduction

Recent research suggests a critical role for specific micronutrients in promoting the growth of nutritionally vulnerable individuals, particularly children. Other studies show that a varied diet, including fresh fruits and vegetables, milk, and animal-source foods may be the best way to promote child growth.

This workshop examined research focused on the growth-promoting roles of specific foods, including vegetables and animal-source foods (milk, fish, meat) in children's diets, the role of some nutrient-enriched foods (such as spreads, sprinkles, and blends), as well as implications of this knowledge for food assistance programs.

Some specific questions included the following:

» Can fortified foods substitute for a lack of dietary diversity in the food aid basket?

» Can micronutrient supplements offer a genuine alternative to food-sourced nutrients for food aid-dependent populations?

» If not, should food assistance programs make providing fresh foods a higher priority, and if so, which foods and how could that be done?

» What further research is needed to know which forms of food aid are most effective and efficacious?

» Is there a need to reformulate the macronutrient and/or micronutrient composition of food aid rations or baskets or of individual food aid commodities?

The overall goal of the workshop was to discuss whether current and emerging knowledge justifies a rethinking of the design of foods used in food assistance programs, to evaluate the feasibility of alternatives, and to identify research issues that would contribute to resolving key questions in coming years.

Tufts University's Friedman School of Nutrition Science and Policy organized the workshop on behalf of the United Nations World Food Programme to explore such questions. Nutrition researchers and food aid practitioners from around the world came together to discuss the current and emerging science and to evaluate the operational implications of new findings.

Presentations

Patrick Webb: Welcome. Why is the World Food Programme concerned with nutrients and child growth? *Tina van den Briel:* Novel foods and the evolving goals of WFP's food aid. *Lindsay Allen:* Foods versus nutrients: What is so special about food? *Marie Ruel:* Is dietary diversity important? Why? *Bruce Cogill*: Achieving nutrition objectives with US Food Aid: Options and operational challenges. *Reynaldo Martorell:* Comparison of effects on child growth of interventions that deliver food vs. those that deliver only micronutrients. *Kim Michaelsen:* Does cow's milk enhance linear growth? Evidence from developing and industrialized countries. *Montague Demment:* Meat. *Rosalind Gibson:* Use of fish to enhance the nutrient adequacy of diets of infants and young children. *Kirsten Simondon:* Consumption of animal-source and fresh foods among young children in Africa. *Martin Bloem:* The bioavailability of micronutrients from vegetable sources and its consequences in the context of poverty. *André Briend:* Can tablets, sprinkles, or spreads replace animal-source foods in children's diets? *Mark Manary:* Specially formulated therapeutic foods. *Francesco Branca:* Nutrient-dense foods reverse stunting in children under five.

Exploring value-added food aid options: Fortified blends, fresh foods, sprinkles, and spreads

Food aid agencies are increasing their efforts to meet micronutrient, as well as macronutrient, needs among beneficiaries by distributing fortified foods and appropriate supplements and by supporting fortification initiatives and policies. This involves paying careful attention to micronutrients in needs assessment and ration planning; delivering fortified foods to nutritionally vulnerable groups; promoting the use of locally produced and fortified commodities in low-income, food-deficit countries; and advocating fortification at the national and international policy levels.

The importance of these activities is becoming increasingly apparent as evidence accumulates for the pivotal role of micronutrient deficiencies not only in mortality, morbidity, and malnutrition, but also in a country's economic development potential. The World Food Programme has been distributing several fortified commodities (procured or donated) for many years, including oil and dried skimmed milk fortified with vitamin A, iodized salt, and fortified blended foods or biscuits. More recently, the World Food Programme has supported the processing of food commodities at a local level, including both the milling and the fortification of cereals and the production of fortified blended foods. Notwithstanding these efforts, scientific advances and lessons from best practice suggest that it is appropriate to revisit the types of foods used and their nutrient content.

In addition to micronutrient-fortified basic food aid commodities, such as bulk grains and flours, vegetable oil, and pulses, there may be circumstances in which other "value-added" food aid options may provide additional quality to the standard food aid rations. Possibilities in addition to fortified blends include fresh foods, sprinkles, and spreads.

Fortified blended foods

Blends such as corn-soy blend and wheat-soy blend are often used as food aid. They are popular because they are practical, highly palatable products for consumption by younger children that provide good nutritional value (macronutrients and micronutrients) at low cost. Fortified blended foods can be mixed with oil, sugar, or sometimes dried milk, depending on their intended end use and the nutritional needs of the beneficiaries. Many fortified blended foods, however, may not be fortified highly enough to meet the micronutrient requirements of the beneficiaries. They also usually contain phytates, which inhibit the absorption of iron.

Fresh foods

It is well established that dietary diversity is a main contributor to nutrient (including micronutrient) adequacy. Foods provide a unique blend of nutrients and other components that affect the nutrient absorption and health status of individuals by mechanisms not yet fully understood. Supplements cannot provide the nutritive and health benefits of a diverse diet.

Fruits and vegetables are particularly beneficial for health. They provide an array of micronutrients, minerals, and fiber and are associated with decreased risks of cancer, cardiovascular disease, diabetes, and stroke. Animal-source foods (milk, meat, and fish) are also excellent sources of macro- and micronutrients, often in forms that are more bioavailable than in plant sources. They are efficient at promoting growth, especially when they are provided to children with otherwise nutrient-deficient diets.

Because of their perishability, providing fresh foods in food aid contexts necessarily implies local or regional purchase. When that is possible, local food purchase stimulates local economies and supports existing food-related cultural habits.

Yet in a food aid context, fresh fruits and vegetables and animal-source foods are often not an option because of their low availability, high cost, and problems of storage, transportation, and safety in handling. Because of these difficulties, a varied diet based on fresh foods is not possible in many large-scale crises (such as Darfur) or in long-standing, but remote, refugee operations (such as the camps for Saharawi refugees in Algeria). Even in noncrisis situations where food aid is needed, there are challenges to routine quality control. Fresh foods present food safety risks unless standard quality-control measures can be implemented.

Although the design issues of food aid strategies that utilize local production are more complex, the workshop participants agreed that it is still worthwhile to creatively identify ways of making local purchasing and production a viable option. Indirect approaches to providing fresh foods may be particularly useful: income-generating activities can afford households more resources to purchase a broader range of foods, whereas home garden programs encourage consumption and barter or sale of cultivated foods, leading to a more diverse diet.

Fresh foods may be included in a diverse food aid basket through the following indirect or direct approaches:

» Local or regional procurement of fresh foods by a food aid agency;
» Improvement of resources at the household level so that enough resources are freed for food purchase;
» Improvement of beneficiaries' access to land, if they reside in camps, as well as to the agricultural inputs needed to grow some of their own foods.

Sprinkles

Sprinkles are composed of encapsulated micronutrients, similar to multivitamin tablets, but their form allows them to be conveniently sprinkled into food to increase micronutrient content. An advantage of sprinkles is that unlike blends, spreads, or other foods, there is low potential that they will be substituted for regularly consumed foods; they do not change food patterns. However, they require ample complementary education for caretakers to avoid misuse or overuse.

Spreads

Spreads, fortified paste formulations that are usually milk or peanut based, are increasingly used to treat acute severe malnutrition. The advantages of fortified spreads are that they are rich in micronutrients and energy, do not require cooking, can be safely stored without bacterial contamination, and are well accepted by children. That they are so well accepted raises some concern that children may eat too much of the spread and risk nutrient toxicity when high fortification levels are present. On the other hand, sharing with other children may dilute the intended effect. In order to counteract these potential problems, clear and convenient packaging (one jar per day per child) was used in trials to control dosage and prevent overuse. The possibility of using spreads in school feeding programs (as an "enhanced peanut butter and jelly sandwich") was also discussed. The technology to produce spreads is simple, and therefore they could be locally produced. Local production of spreads has been tried so far only on a small scale (in Malawi, Niger, and the Democratic Republic of the Congo) in pilot home-based therapy trials.

Strengths and limitations of different forms of food aid

Food	Strengths	Limitations
Fresh foods	Provide an array of nutrients and other food components that promote health	Delivery is difficult; political willingness may be low; not easily stored and transported
Fortified blended foods	Provide macronutrients and micronutrients; easy to standardize and deliver	Must be fortified to provide maximum benefit; if high in phytates, nutrient absorption is inhibited; desirable in supplementary feeding but not as much in therapeutic feeding
Spreads	High energy density; provide a balanced micronutrient mix; do not spoil; need no cooking prior to eating; very acceptable to children	High cost; potential for overuse toxicity if highly fortified; may be shared among other children
Sprinkles	Provide micronutrients; do not displace regularly consumed foods	Potential for misuse or overuse

Costs and cost-effectiveness of food aid options

The cost of tablets and sprinkles is 2 to 5 US cents/day/person; a spread costs about 2 to 3 US cents per 100 g; and corn-soy blend costs 3 US cents per 100 g. Adding dried milk to blends increases their cost by approximately 2 US cents per 100 g of fortified blended foods when it is added up to a level of 10%. The cost of fresh foods is much more variable, depending greatly on local capacity for production, storage, and transportation.

No study has compared the efficacy and effectiveness of fortified blended foods with that of fresh foods, tablets or sprinkles, and spreads in promoting growth and lowering morbidity. The cost and relevance of these different options will depend on the nutrient gap to be filled from locally available foods. For instance, fortified blended foods may be relevant when energy and protein are insufficient in the diet, whereas sprinkles and tablets may be more appropriate when one or two micronutrients, such as iron and zinc, are missing. Spreads provide energy and large quantities of minerals and vitamins, and they may be more relevant when these are lacking. The cost-effectiveness of different food aid options depends on the context and the desired outcome.

Considering context and objectives

The context in which aid is needed and the objectives of the intervention are the foundation upon which aid decisions should be based. Fortified blended foods may be most appropriate in the context of emergencies in which local food systems have ceased to function, whereas fresh foods and sprinkles may be better suited to development (nonemergency) situations. Spreads may best meet the objective of rehabilitating children with growth faltering, whereas if the objective is to reduce levels of iron-deficiency anemia, the food aid basket should include appropriate sources of available iron.

Different food basket compositions and levels of fortification or supplementation may be required, depending on whether the goal is promoting child growth, reducing acute malnutrition, or saving lives. Food aid composition could also be adapted to certain regions to better address widespread regional deficiencies, for example, by placing an emphasis on vitamin A in many parts of Asia.

Conclusions and recommendations

Providing nutritionally optimal foods in appropriate, cost-effective, and practical ways remains the focus of food aid. Existing research suggests that there is no single "best food" in terms of nutrient content, acceptability, and practicality of delivery. Food aid options should be considered complementary approaches rather than mutually exclusive.

Provision of fresh fruits and vegetables is often only possible through local purchases. The perishability and cost of animal-source foods prevent them from being used on a large scale in most cases. However, some dried forms of animal foods (such as dried fish and milk) may be used. Spreads and sprinkles warrant further development: they are a convenient source of micronutrients that can be targeted to specific populations, and there is a potential for local production. Fortified blended foods remain the "traditional" food aid commodity that combines practicality and low cost. Since blends often fail to meet micronutrient needs, however, they may need to be reformulated and more carefully targeted toward specific vulnerable groups, particularly young children, pregnant and lactating women, and persons with HIV/AIDS.

Special food needs among specific vulnerable groups
6- to 24-month-olds: The top-priority group

A strong recommendation was to focus attention on meeting the nutrient needs of children aged six months to two years. Early nutrition has a long-term effect on growth and metabolism, and 6 to 24 months is a critical time period for providing adequate nutrition so that normal growth can be achieved. Blanket-targeting of all children under two years of age in a region as a preventive intervention still needs to be explored, as does the efficacy of special blended foods. A main constraint on blanket-targeting is cost. Clinics were proposed as a venue for providing specially formulated foods for 6- to 24-month-olds, but the lack of clinics and infrastructure to reach clinics is a major constraint. A possible alternative is to provide a special food, or food package, for 6- to 24-month-olds to be included with a general ration. Such foods have in the past tended to be shared with all children (and adults) in a household, thereby curtailing their intended effects. Greater attention to nutrition education and social marketing of such items is essential.

Pregnant and lactating women

Another important nutritionally vulnerable group is pregnant and lactating women. Past programs (such as multimicronutrient supplementation of pregnant women by UNICEF) have shown that maternal supplementation does not always increase birthweight, even though it can improve the micronutrient status off the mother and child. Fortified blended foods could be reformulated to be more appropriate for pregnant and lactating women, which would mean increasing fortification levels, especially those of key nutrients such as iron. Again, appropriate social marketing would be needed.

In order to target this group and the 6- to 24-month-old group, three different formulations of food aid could be distributed concurrently: a general ration, a supplement for children, and a blend for pregnant and lactating women.

Persons living with HIV/AIDS

Looking at foods for persons with HIV/AIDS has become an important issue, especially in sub-Saharan Africa. Various kinds of food commodities should be explored to help improve compliance with antiretroviral therapy, satisfy the increased appetite linked to the drugs, and enhance the energy volume consumed (through improved palatability and digestibility). Addressing the needs of this subpopulation should be kept in mind when choosing commodities for inclusion in food aid programs.

Call for further research

The participants stressed the need for an implementation framework or decision tree, as well as programming tools, to help practitioners decide on the types of commodities most suited to their contexts, to program objectives, and to the intended target groups. For this to happen, however, more evaluations are needed.

Efficacy studies and outcome-based evaluations of specific foods and program options are the most pressing research need. Evaluations are essential for cost-effectiveness analyses and will inform programmers and donors on how to choose the best food aid strategies to better serve beneficiaries.

Other research questions were also identified (in no order of priority):

» What are the comparative advantages of blends, sprinkles, spreads, and single food items in terms of nutrient interactions, bioavailability, and nutrient adequacy?
» What are the consequences of diets with low variety or eating a monotonous diet for a long period of time?
» How can nutrient overexposure or underexposure be avoided in all subpopulations?
» What levels of fortification and types of fortificants best balance cost, palatability, and impact?
» How do spreads affect a child's developing palate?
» How does food aid affect long-term economic development? How will local procurement of food aid affect local economies?
» Should food aid be used differently for recuperation than for chronic malnutrition?
» Which foods would be most appropriate for people with HIV/AIDS?
» What "novel" formulations of foods (new blend

formulations or other nutrient value-added products) could be appropriate in food aid operations in different contexts?

Policy action

This workshop stressed that the international nutrition and aid communities need to have one voice in promoting nutrition in the context of food aid operations. National and international fortification efforts should be supported as low-cost ways of improving the micronutrient content of food and reducing deficiencies in populations. Local production and social marketing of "value-added" food aid commodities should also be encouraged where possible, to improve the sustainability of interventions. Evaluating food aid interventions is a priority not only for programmatic implications, but also for the potential to advocate for certain intervention options to stakeholders.

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Special Section

Proceedings of the Symposium and Workshop on Biotechnology-Derived Nutritious Foods: Challenges and Opportunities in Asia

Rodolfo F. Florentino, guest editor

Executive summary

Rodolfo F. Florentino

The Symposium and Workshop on Biotechnology-Derived Nutritious Foods held in Bali, Indonesia, from February 29 to March 1, 2004, provided an opportunity for more than 45 scientists from Asia and the United States to discuss the issues and challenges of modern biotechnology in improving the nutritional status of populations in Asia. This meeting, which was a follow-up to the first international workshop held in 2002 in Cancun, Mexico, was organized by the International Life Sciences Institute (ILSI), the ILSI Southeast Asia Region, the ILSI Human Nutrition Institute, the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), and Bogor Agricultural University.

In her opening address, Dr. Suzanne Harris, ILSI Global Executive Director, informed the participants of the long interest of ILSI in biotechnology, going back to the 1990s. In 2002, ILSI convened a workshop in Cancun, Mexico, much like this Symposium–Workshop, which looked at nutrition issues and the promise of biotechnology to improve the nutrition of people. This workshop is the follow-up to the Cancun meeting for this region of the world, in which we will try to look at nutrition issues and problems in Asia and how modern biotechnology, and perhaps other food-based interventions, could be of help. Dr. Harris hoped that this workshop would foster better communication and collaboration between the nutrition community and the plant-breeding community.

The first session of the symposium was chaired by Dr. Soekirman, Professor of Nutrition and Director of the Center for Food and Nutrition Policy Studies, Bogor Agricultural University, Indonesia. The session provided an overview of biotechnology and nutrition issues in Asia. Dr. Barbara Schneeman, Professor in the Department of Nutrition, University of California at Davis, USA, gave a summary of the first workshop on biotechnology-derived nutritious foods for developing countries, held in January 2001. A balanced group of nutritional, agricultural, and biotechnology scientists participated in the meeting. The group defined food and nutrition issues related to health, focusing on undernutrition and emphasizing the need to consider multiple nutrient inadequacies and the importance of selecting the most appropriate set of strategies among a portfolio of tools, including supplementation, fortification, dietary diversification, and biofortification. The agriculturists, on the other hand, emphasized the potential for improving food quality and availability based on the success of the Green Revolution, together with the challenges and barriers inherent in diversifying the food supply as a means of alleviating nutritional deficiencies. The meeting discussed the potential of biotechnology for improving the nutritional profile of crops through not only genetic engineering but also a range of other strategies, including tissue culture, the development of diagnostic techniques to improve crop production, and the development of markers for breeding technology. By the use of these tools, biotechnology can lead to the development of new crops, the adaptation of crops to new environments, and the nutritional enhancement of crops. The meeting discouraged overemphasis on one strategy, such as genetic engineering, but instead emphasized the importance of a food-systems approach in using biotechnology to improve nutritional status. Dr. Schneeman ended with a list of challenging issues for improving nutritional status: diversity of food systems versus improvement of single foods, focus on nutrients versus focus on poverty, energy needs versus micronutrient needs, economic factors in targeting strategies, sustaining multidisciplinary dialogue, and intellectual property issues.

Dr. Aman Wirakartakusumah of the Center for Food and Nutrition Study and the Department of Food Technology and Human Nutrition, Bogor Agricultural University, Indonesia, discussed the role of agricultural biotechnology in promoting food security in Asia. At

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the outset, he pointed out the major problems and challenges facing food security in the region, including the high rate of population growth, the high rate of conversion of agricultural to urban land, the lack of good-quality seeds, the low domestic production of animal feed, the limited development of fish culture, the high degree of dependence on rice for food, and the slow pace of food diversification. In addition, there is the limited role of the food industry in postharvest preservation, as well as the problem of food safety. Basically, therefore, there is a need to improve food availability, develop a reform agenda for agriculture, diversify food consumption, and improve food safety. Thus, Dr. Wirakartakusumah pointed out that agricultural biotechnology should be directed toward enhancing agricultural production efficiency, promoting sustainable agriculture, reducing environmental impact, increasing crop productivity, reducing crop damage and food loss, improving food safety, and enhancing orphan crops. Moreover, agricultural biotechnology should empower the rural sector by generating income and reducing economic inequity. However, Dr. Wirakartakusumah underscored the many constraints on the development of agricultural biotechnology, particularly in the areas of finance, technical capital, infrastructure, ambivalent policies, and trade issues such as biosafety regulation and intellectual property protection, not to mention social concerns, food safety, and environmental issues. He concluded that there is no simple solution to the food-security problem. Biotechnology is only one tool and can only work in conjunction with traditional approaches.

Dr. Emorn Wasantwisut, Director of the Institute of Nutrition, Mahidol University, Thailand, discussed the major nutrition issues and challenges in Asia. The high rate of childhood and maternal undernutrition, leading to increased maternal and child mortality in the region, stems from inadequate protein and energy intake as well as deficiencies of micronutrients, especially iodine, iron, vitamin A, and zinc. These deficiencies continue to have adverse consequences for the individual, such as retarded physical development, mental underdevelopment, and impaired resistance to infection, as well as reduced work capacity. Moreover, nutritional deficiencies have tremendous economic and social consequences in communities and nations, which translate into millions of lost years of healthy life. On the other hand, the rapid transition in diet and lifestyle in Asia has resulted in excessive consumption of foods high in saturated fats and simple sugars, leading to increasing prevalences of overweight and obesity, especially in the more developed countries in the region. In turn, the double burden of under- and overnutrition, seen from a life-cycle perspective, has led to increases in the prevalence of nutrition-related noncommunicable diseases, such as type 2 diabetes, ischemic heart disease, and certain cancers. These nutrition challenges call for a combination of cost-effective interventions, cooperation among development sectors beyond health, and strengthening capacity in research and program implementation.

The second session of the symposium on the potential of biotechnology for better staple foods was chaired by Dr. David R. Lineback of the Joint Institute for Food Safety and Applied Nutrition, University of Maryland, USA. Dr. William G. Padolina, the Deputy Director General for Partnerships of the International Rice Research Institute (IRRI), Philippines, discussed the improvements in the nutritional quality and agronomics of rice resulting from biotechnology. The newly established HarvestPlus consortium, composed of several Consultative Groups on International Agricultural Research (CGIAR) and other national and international organizations, is focusing on using traditional plant-breeding methods together with modern biotechnology for biofortification to enhance the micronutrient content of staple food crops. Rice, the major staple of more than half of the world's population, is an efficient micronutrient delivery system because it is compatible with consumer preferences, cost effective, readily stored and transported, and capable of reaching the poorest of the poor. A micronutrient database on rice, containing some 11,000 accessions, is now being assembled, which will soon be available to their collaborators. The description of the rice genome has been completed, and the task now is to identify the genes of interest. Dr. Padolina described recent initiatives to increase the levels of protein, iron, zinc, and provitamin A in the rice grain, while at the same time searching for robust varieties that are sustainable in both favorable and unfavorable environments. So far, breeding for high-iron rice has resulted in a progeny that is high-yielding, of excellent quality, disease resistant, and palatable. Lines with promising levels of iron (16.9 to 21.3 mg/kg) have been identified. Preliminary feeding trials have shown improvement in iron stores among the subjects tested. Genetic modification has produced IR64 Golden Rice with enhanced β-carotene content, while work is continuing to produce varieties with high insect resistance, fungal resistance, drought tolerance, low allergenicity, and high iron content. At the same time, work on postharvest technology is continuing. Dr. Padolina gave examples of how IRRI uses the integrated crop management approach, which includes a basket of integrated options to improve the agronomic properties of rice. He highlighted the international and collaborative nature of the work of the Asia Rice Biotechnology Network, including the International Rice Breeding Program, the Rice Bioinformatics Program, and the International Rice Functional Genomics Working Group. Dr. Padolina concluded that the goal of the IRRI road map is to have a positive impact on poor farmers.

Dr. Inez H. Slamet-Loedin of the Research Center for Biotechnology, Indonesian Institute of Sciences, described efforts to produce fungus- and drought-resistant rice by transgenic breeding with the goal of expanding rice cultivation in marginal dry land areas that are subject to drought and blast disease caused by the fungus *Pyricularia grisea*. One strategy is the transgenic expression of HD-Zip transcription factors involved in the drought response. On the basis of their past experience with Bt rice, they are working on the biologic mechanism of drought response with regulatory genes. The second strategy entails the up-regulation of salicylic acid levels by expression of microbial biosynthesis genes from *Pseudomonas* to enhance the defense response against blast infection. The results were quite positive, with increases in the rate of survival from blast disease as high as 80%.

Dr. Toshihiro Yoshihara of the Bio-Science Department, Central Research Institute Electric Power Industry, Japan, described progress in the development of "ferritin crops" (rice) in the fight against iron-deficiency anemia. Ferritin is an iron-storage protein that functions primarily as a reservoir of iron for the synthesis of iron-containing proteins. Ferritin rice, with a high iron content in the endosperm, would therefore be an excellent vehicle for enhancing the iron content of the diet to overcome the problem of iron-deficiency anemia. It is conceivable that three bowls of ferritin rice would satisfy the daily iron requirement. However, the development of ferritin crops still has some problems. The accumulation of iron is unstable, so that the iron concentration is not always sufficient. The problem is apparently one of iron supply within the transformant, especially in the translocation from leaves to grains during grain development. Another problem is possible contamination with undesirable metals such as cadmium. Moreover, additional factors of iron metabolism in the plant body must be considered in the development of ferritin rice. In addition, the translocation system from leaves to grains is very important in determining the accumulation of iron in the grain. The next target in the development of ferritin crops is the transport and translocation of iron from the roots to the grains via the leaves. Another issue that needs to be studied is the bioavailability of the iron in ferritin rice, as well as its safety.

The third session of the symposium dealt with the development of other biotechnologically enhanced crops and foods. The session was chaired by Dr. Sushila Chang, Director of the Centre for Life Sciences and Technology, Ngee Ann Polytechnic, Singapore. Dr. Ross M. Welch, Plant Physiologist and Lead Scientist with the USDA-ARS, US Plant, Soil and Nutrition Laboratory, New York, reviewed some opportunities for biotechnology to improve the micronutrient output of agricultural systems, as well as questions and concerns about the use of this technology to improve micronutrient status. Some of these issues and concerns include: the magnitude of the change in micronutrients that is possible within the genomes of staple crops, the effect of increasing micronutrient content on productivity, the interaction between environment and genotype, acceptance by farmers and consumers, the bioavailability of the micronutrients thus enhanced, sustainability, effects on processing and cooking, and the costs and benefits to society compared with other types of nutritional interventions. Dr. Welch pointed out that micronutrient-enriched seeds in fact have agronomic benefits, including better seed viability, greater seedling vigor, less soil erosion, lower seedling rates, increased resistance to disease, and better plant survival, all of which result in increased productivity. To improve the utilizable micronutrients, the breeding strategy and genetic modification are directed to increasing the total content of micronutrients in the plant portion eaten; decreasing the amount of antinutrients, such as phytic acid and tannin; and increasing the amount of promoter substances, such as ascorbic acid and hemoglobin. Dr. Welch concluded that changes in agricultural policies and systems must be made to ensure adequate supplies of essential micronutrients, while at the same time the nutrition and health sectors must turn to agricultural interventions as a primary tool in their efforts to eliminate malnutrition.

Dr. Suchirat Sakuanrungsirikul, Agricultural Research Scientist at the Khon Kaen Field Crop Research Center, Thailand, gave an update on the development of papaya resistant to papaya ringspot virus (PRSV) in their laboratory. The development of transgenic papaya began in 1995 at Cornell University through the joint efforts of the Department of Agriculture of Thailand and Cornell University. Subsequently, selection of the most PRSV-resistant lines with the desired horticultural characteristics was performed at Khon Kaen. Field trials were started in 1999 on 25 resistant lines, with outstanding results. Three years of field trials have conclusively shown that the transgenic papaya is able to control the virus under heavy disease conditions. Six experiments on environmental safety assessment have been successfully completed since 2001 to determine the possible effects that transgenic papaya might have on its ecological context. At the same time, preliminary experiments on food-safety assessment, including analysis of nutritional composition, molecular characterization of the gene insert, and expression of the coat protein product, have been performed, with positive results. Additional data from rat feeding experiments and tests on the stability of the gene inserts are currently being collected.

Dr. Jingjuan Yu, Associate Professor at the State Key Laboratory for Agricultural Biotechnology, College of Biology, China Agricultural University, People's Republic of China, described work in enhancing the protein content in maize seeds through modern biotechnology. The *sb401* gene from potato, encoding a pollen-specific protein with high lysine content, was successfully integrated into the genome of maize plants, and its expression was correlated with increased levels of lysine and total protein in maize seeds. The integration was confirmed by Southern blot analysis, and its expression was confirmed by Western blot analysis. Quantification of the lysine and protein content of the R1 seeds showed that the lysine content increased from 16.1% to 54.8% and the total protein content increased from 11.6% to 39.0%. The lysine content as a percent of total protein did not increase significantly. The levels of lysine and total protein remained high for six continuous generations, showing that the trait was heritable and stable. The levels of other amino acids were elevated in every generation. Dr. Yu concluded that the *sb401* gene, which encodes the natural protein Lrp, could be successfully employed in breeding programs aimed at improving the nutritional quality of maize.

Dr. Umi Kalsom Abu Bakar, Deputy Director of the Malaysia Agricultural Research and Development Institute (MARDI), Malaysia, shared Malaysian experiences in developing nutritionally enhanced biotechnology-derived crops. Most of the initial research on genetic modification was performed on rice, with the objective of increasing disease resistance. Work is now ongoing on papaya and pineapple to increase disease resistance against PRSV and fruit blackheart disorder, respectively, as well as on chili and passion fruit to enhance virus resistance. Research is also ongoing on citrus (pomelo) to improve fruit color. Dr. Abu Bakar described in more detail current projects at MARDI on papaya to increase shelf life and to increase disease resistance against PRSV. Thus, papaya will be the first genetically modified food to be commercially marketed in Malaysia. MARDI is also working in collaboration with Australian researchers to increase the resistance of pineapple to blackheart disorder. At the same time, a regulatory system has been set up to manage safety concerns. Biosafety approval and food safety approval start at the Institutional Biotechnology Committee, finally passing to GMAC Malaysia. On the other hand, the Malaysian Biotechnology Information Center (MABIC) is promoting public awareness and acceptance of genetically modified foods in cooperation with other institutions, such as the Institute of Islamic Understanding, as well as the research organizations themselves.

The fourth session of the symposium, “Balancing the Risk, Perception and Potential of Biotechnology,” was chaired by Dr. Corazon V. C. Barba, Director of the Food and Nutrition Research Institute (FNRI), Philippines. The first paper was given by Dr. Bruce Chassy, Executive Associate Director of the Biotechnology Center, University of Illinois, USA, who discussed safety assessment of nutritionally enhanced foods and feeds through biotechnology. The safety standard applied follows the general principle that a genetically modified food should be as safe as an appropriate counterpart that has a history of safe use. Absolute safety is not achievable. The questions that we need to answer are the safety of the introduced genetic material, the extent to which the genetic change affects the nature and amount of the expression of products and metabolites, and whether there are intended or unintended effects on the composition of the food. We should also be concerned with exposure, that is, how much of the food are people going to eat and how often. According to the concept of substantial equivalence, the key elements of safety assessment include molecular characterization, assessment of the safety of expressed proteins, comparative analysis of the macro- and micronutrient composition and contents of antinutrients and toxicants, determination of patterns of use and exposure, determination of toxicologic and nutritional characteristics, studies of safety in animals, and overall evaluation of safety. The concept of substantial equivalence is applied to three instances: crops that are more or less compositionally equivalent to an appropriate comparator, crops that are more or less compositionally equivalent but with some significant change made in their composition, and crops that have been significantly modified in their composition. If changes have been made, the focus is on their safety. Estimating the effects on health, however, is complex, because human diets vary greatly, there is complex interaction between diet and health, there are nutrient–nutrient interactions, and so on. It is therefore not enough to say that the food is beneficial to health simply because the content of a particular nutrient has been increased. Dr. Chassy concluded that all nutritionally improved novel foods should be evaluated for their potential impact on nutrition and health, regardless of the technology that has been used to develop them. He recommended that the primary focus of assessment be shifted from the composition of individual foods to the composition of diets, and that alterations in composition be evaluated to show that such alteration will not significantly and adversely affect the nutrient intake of a cross-section of the population. Finally, human dietary intake data and dietary intake forecast models need to be developed for all target populations to permit assessment of the safety of novel foods introduced into the market.

Ms. Georgina Cairns, Executive Director of the Asian Food Information Center (AFIC), based in Thailand, summarized the consumer perception surveys commissioned by AFIC in 1999 in seven ASEAN countries plus China. The street interviews found that consumers in the region were open-minded on the subject of food biotechnology. A large percentage (88%) of the respondents said they would definitely or probably be willing to try a food containing genetically modified ingredients. Focus group discussions in Manila, Shanghai, and India conducted in 2003 indicated that consumers were confident of the ability of scientists and regulators to manage the process of assessing the safety of genetically modified crops and food ingredients. The primary information sought by the respondents, preferably via the mass media and food labels, was on the potential benefits of biotechnology-derived foods. Among the potential benefits of biotechnology, the respondents valued improvement in the nutritional quality of foods, agricultural benefits, and improvement in agricultural productivity. On the other hand, although biotechnology ranked very low in the list of concerns of the respondents, most were highly concerned about food safety (e.g., microbiological contamination) and nutritional quality. Consumers did express a desire for further information on food biotechnology, particularly on its potential benefits. They believed, however, that assessment of the safety of such foods is best left to "experts."

Dr. Sakarindr Bhumiratana, Senior Advisor and Senior Specialist at the National Center for Genetic Engineering and Biotechnology (Biotech) in Thailand, discussed the future of biotechnology in nutrition and health improvement. He envisioned that systems biology, which is an integration of biology, mathematics, and engineering, would be able to integrate the vast amount of information on genes, proteins, cellular dynamics, and the responses of organisms and explain what makes whole organisms behave as they do. Systems biologists are relying heavily on mathematics and statistics to build models designed to integrate these data into more complex pictures and make predictions of how biologic networks function. Applying systems biology to nutritional science would allow us to focus on the effects of nutrients on the regulation of gene expression (nutrigenomics) and the effects of variation in gene structure on the response to nutrients (nutrigenetics). Dr. Bhumiratana concluded that the challenge for Asia is to use this new knowledge to develop new tools to enhance the use of traditional medicine and functional foods, while at the same time developing an adequate scientific infrastructure and promoting interactions among many scientific disciplines in the face of limited resources.

Opening address

Suzanne Harris

I want to welcome you all on behalf of the International Life Sciences Institute (ILSI), the ILSI Southeast Asia Region (ILSI SEAR), the ILSI Human Nutrition Institute, and the Joint Institute of Food Safety and Applied Nutrition (JIFSAN), and I wish to thank you all for your participation.

ILSI has had a long interest in biotechnology and in food-based strategies to address nutritional needs. This interest goes back to the 1990s, when we worked with the Food and Agriculture Organization (FAO) to develop a document outlining food-based strategies that could be used to deal with nutritional issues. The promise of biotechnology in this area is great, but it is largely a promise at this point. ILSI held a workshop in Cancun, Mexico, in January 2002, at which we brought together representatives of the nutrition and plant-breeding communities from Asia, Africa, and Latin America. One of the things we learned was that these individuals did not speak to each other often because they rarely had the opportunity. The Cancun workshop was therefore a learning experience for everybody. What we did was similar to what you are going to do here: look at the nutritional foods that are important in those regions of the world and at what we know about biotechnology. Our goal in that meeting was to better understand how scientists from Asia, Africa, and Latin America view their nutritional issues and what they know about the promise of modern biotechnology techniques. The meeting provided an opportunity to really look at the issues in a scientifically sound and constructive manner in order to say what was a good idea, what the problems were, and where gaps existed. A report of that meeting was published in December 2002 in the *Food and Nutrition Bulletin*.

The workshop that we are holding today and tomorrow is the first follow-on workshop to that in Cancun. We want to focus more closely on this particular region of the world. There are many exciting things going on here, and I am delighted to be here to learn from some of the things you are doing. Tomorrow's discussion will be a more in-depth exercise in getting to know each other better, looking at what the problems are, trying to solve the nutritional issues in this region, and learning to use biotechnology if it is appropriate. This is not an effort to say that biotechnology is the only tool that you should use; it may not be an appropriate technology for every problem. We want to know when it is not appropriate as well as when it is.

The objectives of this workshop are similar to those we had in Cancun—identifying nutritional problems and examining various food-based strategies (including modern biotechnology) as possible solutions. We hope that the workshop will foster better communication between the two communities of nutrition and plant breeding, with the goal of strengthening their interaction and collaboration. In this way, we can begin to address more fully the problems that exist.

I want to thank the organizers of this workshop, particularly the ILSI SEAR, JIFSAN, and Bogor Agricultural Institute, for all of their efforts to make this a successful workshop. We had a postponement because of severe acute respiratory syndrome (SARS) and other things, so we are delighted that this workshop has finally come to pass in this lovely location.

Thank you very much for all of the work you have done, and again I welcome all of you on behalf of ILSI and look forward to spending time here.

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Biotechnology-derived nutritious foods for developing countries: Needs, opportunities, and barriers—an overview. A summary of the first workshop report

Barbara O. Schneeman

The first workshop on biotechnology-derived nutritious foods, which was held in January 2002 in Mexico [1], was one of the first attempts to bring together a balanced group of nutrition, agriculture, and biotechnology scientists to engage in a multidisciplinary dialogue about the nature of nutrition problems in developing countries and potential strategies to address the challenges. The goal of the workshop was to ensure that the issues and the challenges were defined by the experts in the field so that the cross-disciplinary dialogue among participants would be meaningful to each area of expertise (i.e., nutrition, agriculture, and biotechnology). With regard to nutritional status, the groups focused on problems of undernutrition, and the nutrition experts cautioned against focusing on single nutrient problems and illustrated the need to consider the potential impact of multiple nutrient inadequacies. The workshop identified four primary strategies to improve nutritional status: supplementation, fortification, diversification of the food supply, and biofortification. The groups viewed these strategies as a portfolio of tools that are available and discouraged focusing on the comparative advantages of each approach. They instead emphasized the importance of selecting the most appropriate strategy for the prevailing conditions.

The agricultural experts illustrated the potential for improving food availability on the basis of the success of the Green Revolution in improving the yield and efficiency of agronomic crop production. Current research has demonstrated the natural variation in the nutrient content of crops, thus creating the opportunity to select for improved nutritional composition as a part of breeding strategies. The groups discussed the challenges inherent in diversifying the food supply as a means of providing foods that will alleviate nutritional deficiencies and illustrated the potential of biotechnology to improve the nutritional profile of certain crops.

The groups specifically focused on the potential of biotechnology to improve nutritional content. The groups emphasized that biotechnology is often viewed simply as genetic engineering of crops, but in fact a range of biological technologies is available. These technologies include tissue culture, the development of diagnostic techniques to improve crop production, and the development of markers for breeding technology as well as genetic engineering. By using these different tools, biotechnology can lead to a variety of strategies for improving nutritional status, including the development of new crops, adaptation of crops to new environments, enhancement of local strategies that improve nutritional status, and nutritional enhancement of crops. Just as there are several tools for addressing nutritional inadequacies, there are multiple ways to utilize biotechnology to improve nutrition. An overemphasis on one strategy, such as genetic engineering, may not be productive for addressing the core nutrition issues, and the group emphasized the importance of a food-systems approach as well as continued dialogue among scientists, farmers, technologists, consumers, and policy makers.

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Extended Abstract

Issues in food security and agricultural biotechnology

M. Aman Wirakartakusumah and Purwiyatno Hariyadi

Food security is defined as the situation in which all households have both physical and economic access to adequate and safe food for all of their members so that they can live a healthy and productive life. The factors of food security thus include food availability, stability, and accessibility.

Among the challenges facing food security in Indonesia—and in most developing countries—is the high growth rate of the population. Compounding this problem is the decreasing availability of productive land for agriculture because of very high conversion rates. In many Asian countries, the importation of food commodities such as rice, soybeans, corn, fruits, vegetables, and animal products has increased. The main obstacles to fulfilling the requirements of the food supply are the lack of good-quality seeds, lack of domestic production of animal feed materials, limited development of fish culture (especially marine culture), and high dependency on rice for food. Moreover, we still face the problems of malnutrition, especially vitamin A deficiency, iron-deficiency anemia, iodine deficiency, and calorie–protein malnutrition. There has been slow progress in food diversification and a limited role of the food industry in postharvest preservation, storage, and product development. At the same time, there are numerous problems of food safety, such as the use of non-food-grade additives and outbreaks of food poisoning and food-borne diseases, while there is a lack of awareness of the importance of food safety by consumers and producers. In addition, the economic crisis has resulted in reducing the rate of economic growth to less than 5%, thus contributing to the decline in rice production and the increase in rice importation and affecting the system of food distribution and marketing.

To improve food availability and security, the aim therefore is to stabilize national food availability, strengthen national food stocks, improve distribution systems, and eliminate food insecurity. This should be supported with an appropriate agricultural development strategy directed toward improving productivity and efficiency in the agricultural sector to enable it to compete globally. In addition, there is a need to diversify food consumption and improve food safety.

As one of the potential solutions of food security, the role of agricultural biotechnology cannot be overemphasized. Biotechnology can add value to agriculture through decreased use of pesticides, reduced agricultural losses from pests and diseases, improved nutrient efficiency, and improved productivity. The development of agricultural biotechnology should be directed toward improving food and nutritional security, enhancing production efficiency, promoting sustainable agriculture, reducing environmental impact, empowering the rural sector through income generation and reduction of economic inequity, increasing crop productivity, reducing crop damage and food loss, improving food safety, and enhancing orphan crops. However, many constraints on the development of agricultural biotechnology in developing countries also need to be considered, especially the limiting factors such as finance, technical capital, infrastructure, biosafety regulation, intellectual property protection, and public perception. Societal concerns, food-safety issues, and environmental impact must also be addressed. To capitalize on the potential of biotechnology, we must address consumer concerns by honest and open communication, building trust in technology with facts and information instead of promotion and advocacy, backed by a strong regulatory oversight.

Although biotechnology is not a panacea for the attainment of food security, it could be a very useful tool in conjunction with other traditional technologies. We should weigh all options and choose the most effective solution.

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Abstract

Nutrition issues and challenges in Asia

Emorn Wasantwisut

In September 2000, representatives of 189 countries attended the millennium summit to adopt the United Nations Millennium Development Goals as a framework for measuring progress in development by the year 2015. The first goal calls for the eradication of extreme poverty and hunger, including malnutrition in children. Another two out of eight goals specify reduction in child mortality and improvement in maternal health as benchmarks for achievement. Childhood and maternal undernutrition represent major health risk factors that stem from inadequate protein and energy intake as well as deficiencies of micronutrients, especially iodine, iron, vitamin A, and zinc. Asian countries represent a wide spectrum with respect to the magnitude and severity of the problems. The rate of child mortality in Southeast Asia is twice as high as the rate in the Western Pacific Region. In Southeast Asia, the prevalence of underweight children in countries with low child mortality is 26%, and the prevalence in countries with high child mortality is 46%. Even children with mild to moderate undernutrition are at increased risk of mortality from infectious diseases such as diarrhea, pneumonia, measles, and malaria. Vitamin A deficiency affects 30% to 48% of young children as well as a large proportion of pregnant women in parts of Asia with high maternal mortality rates. Iodine-deficiency disorders and iron deficiency continue to be significant problems in Southeast Asia. The prevalence of a low intake of bioavailable zinc, as an estimate of zinc deficiency, ranges from 4% to 73% across Asia. These micronutrient deficiencies continue to have adverse consequences, such as retarded physical and mental development, impaired resistance to infection, and reduced work capacity. It is estimated that the cost of iron deficiency alone is as much as 2% of the gross domestic product. When the disease burden is calculated according to disability-adjusted life years (DALYs), which can be thought of as lost years of healthy life, nutritional deficiencies account for 33 million DALYs per year worldwide, half of which are in Asia.

The rapid transition in diet and lifestyle in Asia has resulted in a double burden of malnutrition. Excessive consumption of foods high in saturated fat and free sugar, combined with reduced physical activity, results in an increase in the prevalence of overweight and obesity, especially in more developed countries in Asia. Values of body-mass index (BMI) above 21 kg/m^2 elevate the risk of noncommunicable diseases such as type 2 diabetes mellitus, ischemic heart disease, and certain cancers. Although the burden of these diseases is high in developed countries, recent data indicate that as much as 50% of the adult disease burden in countries with high mortality can be attributed to noncommunicable diseases. One possible explanation for this lies in the fetal origin of insulin resistance and diabetes, such as occurs, for example, when small Indian babies born with higher body fat and visceral fat undergo accelerated growth during childhood.

The challenges in Asia to addressing these problems include implementing cost-effective interventions, optimizing the use of science and technology, promoting cooperation among development sectors beyond the health sector, and strengthening capacity in research as well as in program counterparts.

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Extended Abstract

Advancement in improving nutritional quality and agronomics of rice through biotechnology

William G. Padolina

Food security and, in particular, household food security, continues to be of primary concern. Food security includes not only access to food for an active and healthy life by all people at all times, but a consideration of dietary quality. The question of hidden hunger generates some important issues that still persist, such as optimizing the level of each micronutrient in cereal grains; reducing the concentration of phytic acid and other antinutritional factors; optimizing the absorption of iron, zinc, and vitamin A/carotenoid from a cereal diet; and seeking a biotechnological solution for iodine deficiency. Some of these issues are being addressed by the Consortium of International Agricultural Research Institutes (CGIAR) in its micronutrient program, now known as Harvest Plus, whose goal is breeding crops for better nutrition.

Rice is the major staple food of nearly half of the world's population and is an important food crop in Asia. More than 90% of all rice is produced and consumed in Asia, and more than 2 billion people obtain around 30% of their calories from rice. In Asia rice provides an efficient micronutrient delivery system. It is compatible with consumer preferences and with agricultural and other social practices; it is cost effective and readily stored and transported; it is capable of reaching the poorest of the poor; and it is safe for human health and the environment.

The International Rice Research Institute (IRRI) recognizes that as the prospects of producing more rice continue to improve, new challenges continue to emerge. Thus, the IRRI research agenda addresses the need to enhance the nutritive content of the rice grain and to search for new rice varieties that can cope with environmental changes resulting from farm practices.

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The breeding techniques of IRRI include traditional breeding plus either phenotype or marker-aided selection, as well as genetic modification. With the aid of an extensive micronutrient database for rice from more than 11,000 germplasms that is now available, research efforts have focused on the search for rice varieties that contain higher levels of iron and provitamin A in the grain. Conventional breeding, while still important, has harnessed the tools of biotechnology to solve complex problems of enhancing nutritional value and enabling the plants to cope with biotic and abiotic stresses.

An example of the efforts of IRRI to produce nutritionally enhanced rice is breeding for high-iron rice. With traditional breeding and selection, varieties have been developed that are high in iron and at the same time high-yielding, of excellent quality, and disease resistant. Promising lines have been identified with grain iron content ranging from 16.9 to 23.2 mg/kg. DNA marker-aided selection helps in accelerating the breeding of the desirable progeny. High-iron traits have been found to be not only combinable with improved traits but also stable across various environments and retained after milling. Elite high-iron lines, such as IR68144-3B-3-3-2, are now undergoing advanced trials, and molecular tools will further accelerate the transfer of traits to locally adopted cultivars.

Genetic modification of rice requires a strong bioscience base, regulatory compliance, regulatory oversight, and intellectual property management. Among the traits enhanced by genetic modification that IRRI is working on, high β-carotene content has received much attention. An example that is now in the IRRI greenhouses is IR64, better known as Golden Rice.

Since these improved varieties have to be highly productive as well, the search for sustainable ways of producing these nutritionally enhanced varieties deserves priority attention. Using the integrated crop management approach, research activities designed to produce robust varieties and generate sustainable crop management technologies in both favorable and unfavorable environments are being pursued. Postharvest technology is also very important, since quality is affected by drying, storage, milling, and marketing.

IRRI uses various international networks to promote collaboration in biotechnology. The current networks include the Asian Rice Biotechnology Network, the International Rice Molecular Breeding Program, Rice Bioinformatics, and the International Rice Functional Genomics Consortium, all encompassing many countries in Asia and composed of multidisciplinary working groups.

The primary beneficiaries of all these efforts should be the poor farmers. Granted that numerous factors besides agriculture, including governance, affect farm productivity, it is unfortunate that support for agricultural research is declining. It is hoped that resources can again be mobilized to increase support for agricultural research at both the national and the international levels in order to advance science and technology for the benefit of poor farmers.

Abstract

Fungus- and drought-resistant rice: Impact on agronomics and food security

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The supply of and access to food and nutrition are crucial factors contributing to food security [1]. Rice production in Indonesia reached self-sufficiency in 1984, but Indonesia is now the biggest rice importer in the world. Decreasing areas of fertile wetland (lowland) available for rice farming due to population increase, urbanization, and industrialization, as well as adverse environmental conditions, pests, and disease, are the major causes of the declining rice production in Indonesia. To increase rice production in the coming decades, Indonesia will have to expand its rice cultivation to marginal dry land (upland) areas, where rice production is severely hampered by dehydration stress due to drought and the blast disease caused by the fungus *Pyricularia grisea* (also known as *Pyricularia oryzae* or *Magnaporthe grisea*). It has been estimated that the potential gain in rice production in such areas may amount to as much as 2 to 3 tons/ha if both drought-tolerant and blast-resistant varieties are cultivated. The objective of this project is to employ biotechnology to enhance the performance of rice under dry conditions. One strategy is the transgenic expression of *HD-Zip* transcription factors involved in the drought response in rice. A second strategy involves up-regulation of salicylic acid levels by the expression of microbial biosynthesis genes in order to enhance the defense response against blast infection. This approach was based on earlier studies showing that altered salicylic acid levels in transgenic tobacco plants resulted in increased resistance to pathogens [2]. The gene construct used is derived from a microbial salicylic acid biosynthetic pathway including two genes, *entC* and *pmsB* [2].

As a result of research on drought resistance through transgenesis, seven *HD-Zip* genes have been identified in rice. Northern blot analysis was performed to determine if the expression of these genes is affected by dehydration or by the plant hormone absisic acid (ABA), which often mediates dehydration responses. Screening of private and public sequence databases showed that 19 other related *HD-Zip* genes occur in the rice genome. One of the two dehydration-repressed *HD-Zip* genes was induced by four hours of flooding, a treatment opposite to the drought condition. This *HD-Zip* gene was selected for study in transgenic plants. The transgenic rice overexpressing this gene generally formed smaller leaves and exhibited reduced senescence and fertility compared with the controls. Experiments carried out in *Arabidopsis* found that overexpression of this particular *HD-Zip* gene can confer tolerance to drought.

The antifungal gene construct containing the microbial salicylic acid biosynthetic pathway genes was successfully introduced into embryogenic calli of IRAT 112 and Rojolele by using *Agrobacterium tumefaciens*. Molecular analysis showed that the genes were present in the independent transgenic lines of both varieties. The fungal blast bioassays in 40 transgenic Rojolele independent lines and 5 IRAT 112 independent lines showed improved resistance to the supervirulent blast fungus race 173.

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Improving rice nutrition: Challenges and practical approaches for iron fortification

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Ferritin is an iron storage protein that is ubiquitous in organisms. The molecular mass of ferritin generally consists of 24 homologous or heterologous subunits potentially storing a large amount of iron (up to 4,000 iron atoms per molecule) in the central cavity [1]. The role of ferritin in living cells is iron modulation [2]. The molecule works primarily as a reservoir providing iron for the synthesis of iron-containing proteins (e.g., ferredoxin) and subsequently acts as a protector against the damage caused by free radicals through the iron sequestration function. Ferritin would thus seem to be an attractive tool to develop high-iron-content crops to overcome the problem of iron-deficiency anemia [3]. Indeed, it is well known that exogenous ferritin expression induces a large accumulation of iron in the whole plant body or in specific tissues, such as the endosperm (**table 1**) [4–12].

The strategy used to develop ferritin crops is based on the hypothesis that a large vessel of iron in cells acts as an effective signal to stimulate iron uptake into the cells and sequesters the excess iron that subsequently flows into the cells (**fig. 1**) [3, 4, 11]. Although the strategy generally works well, the development of ferritin crops still has some problems to be resolved, and thus the practical use of ferritin crops as food to fight iron-deficiency anemia has not yet been achieved. One major problem is an unstable iron accumulation effect. The level of iron accumulation in the transformants is not always sufficient, and increasing the iron concentration in the ferritin transformants is also preferred for practical applications. That is, even within one homogeneous transgenic line, especially in the case of the ferritin rice lines grown in soil, the iron concentration varied with each experiment. Sometimes we found that the iron concentration was not high in the

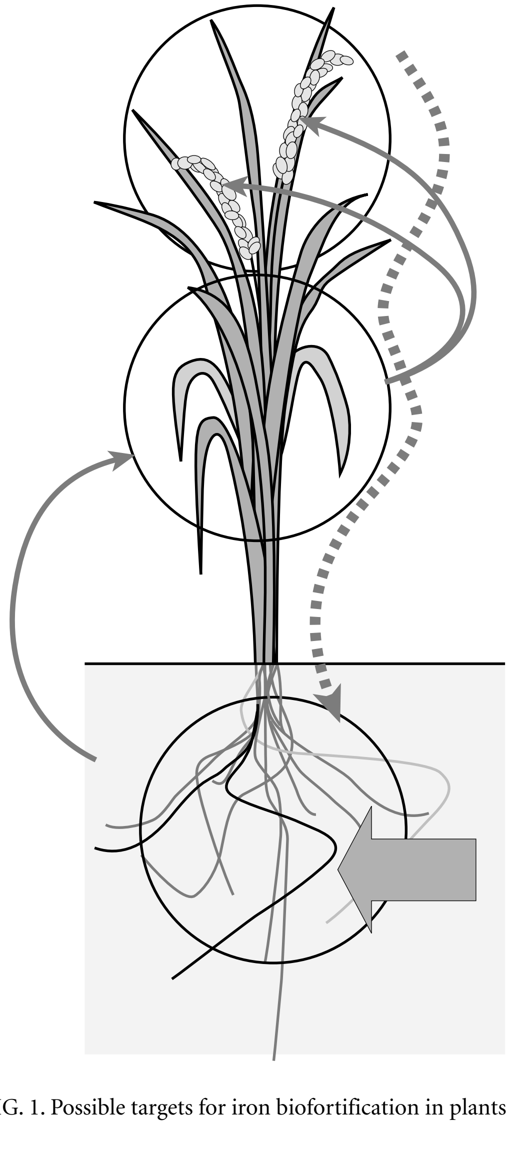

FIG. 1. Possible targets for iron biofortification in plants

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TABLE 1. Effect of exogenous ferritin expression on iron accumulation in plants

Origin of ferritin	Promoter regulating ferritin expression	Transformant	Tissues expressing ferritin	Iron accumulation[a]	Ref. no. (year)
Soybean[b] (*soyfer H-1*)	CaMV 35S	Tobacco	Constitutive	× 1.3 in leaves	4 (1998)
Soybean (*soyfer 1*)	CaMV 35S	Tobacco	Constitutive	× 2.0–3.0 in leaves	5 (1999)
Soybean[b] (*soyfer H-1*)	Rice GluB-1	Rice	Endosperm	× 2.7 in seeds	6 (1999)
Soybean[a] (*soyfer H-1*)	CaMV 35S	Lettuce	Constitutive	× 1.7 in leaves	7 (2000)
Soybean (*soyfer 1*)	Maize ubiquitin-1	Rice, wheat	Constitutive	NS in seeds (× 1.5–2.0 in leaves)	8 (2000)
Soybean (*soyfer 1*)	CaMV 35S	Tobacco	Constitutive	× 0.6–2.4 in leaves (dependent on phosphorus concentration)	9 (2000)
Phaseolus (*pfc*)	Rice Gtl	Rice	Endosperm	× 2.2 in seeds	10 (2001)
Soybean[b] (*soyfer H-1*)	CaMV 35S	Tobacco	Constitutive (chloroplast, apoplast)	× 2.3-2.8 in leaves (NS for contents of other metals)	11 (2003)
Soybean (*soyfer H-1*)	Rice GluB-1	Rice	Endosperm	× 2.2 in seeds (fieldwork)	12 (2003)

pfc, *Phaseolus vulgaris* ferritin gene

a. Maximum iron accumulation compared with that in nontransformant control.

b. The soybean ferritin gene, *soyfer H-1*, has been isolated and designated in our laboratory to emphasize the molecule type as the heavy-chain type of animal ferritin [13, 14], although it is the same gene as *soyfer 1*.

transformant, but was almost the same as the level in the nontransformant, even if the ferritin protein itself was synthesized at quite high levels. For example, we generated transformant rice lines in which two types of soybean ferritin, soyfer H-1 and H-2 [13, 14], were dually expressed under the regulation of two different seed-specific promoters, rice glutelin B-1 promoter (GluB-1p) and oat 12S globulin promoter (Glop). We expected these lines to have significantly increased iron concentrations; however, the results showed that the iron concentration in the dual ferritin lines was almost the same as that in the single ferritin lines, although it was higher than that in the nontransformant line. It should be noted that these dual ferritin lines expressed at least twice as much or more ferritin protein in the presumed tissues (i.e., the subaleurone layer and the inner tissue of the endosperm). This phenomenon is recognized as a problem of the iron supply within the transformant, in particular the translocation from leaves to grains during grain development. Interestingly, the transgenic rice and wheat in which ferritin gene expression was regulated by the constitutive promoter CaMV 35S (35Sp) had increased iron concentrations in the leaves but not in the grains [8]. This result suggests that exogenously produced ferritin in leaves would not release iron and that iron competition would occur between leaves and grains. It is also interesting that exogenously expressed human lactoferrin can induce about twice as much iron accumulation in the transgenic line as in the nontransformant control line [15]. Furthermore, the fact that lactoferrin also has iron-binding ability, although its capacity is only two ions per molecule, demonstrates that a huge iron capacity is not needed to promote the iron-uptake inducer, at least for this level of iron fortification. Another problem is possible contamination with undesirable metals such as cadmium. In fact, Vansuyt et al. [9] found that the accumulation of iron in the leaves of ferritin overexpressors depended on the soil in which the plants were grown, and that the accumulation of other divalent metals, including hazardous metals, is probable under specific soil conditions. However, other ferritin tobacco transformants grown under normal metal-balanced conditions demonstrated that divalent metals other than iron (calcium, copper, magnesium, manganese, and zinc) did not accumulate significantly in the leaves, whereas the contents of some of the non-ferrous metals were dramatically increased in the roots compared with those in the control line (manganese, × 1.9–10.4; zinc, × 1.6–2.3) [11]. Thus, even if the activation of iron uptake-related enzymes leads to the accumulation of nonspecific divalent metal ions in the roots, an iron loading/unloading system and/or an internal translocator in the xylem and phloem might specifically deliver iron to the upper parts of the plants.

We must consider additional factors of iron metabolism in the plant body when we develop ferritin rice. First, the iron-uptake system in gramineous plants, including rice, is different from that in dicotyledonous plants such as tobacco. Gramineous plants produce a phytosiderophore called mugineic acid and release it into the surroundings of the roots to chelate Fe^{3+}. The chelated Fe^{3+} is then taken into the cells by a membrane translocator [16, 17]. This may mean that the amount of contamination by undesirable metals itself would vary, at least in part, depending on the specificity to mugineic acid of the system in rice. In addition, the system of translocation from leaves to grains would play a very important role in the accumulation of iron specifically in grains. In the case of the soil-grown rice lines in our laboratory, the concentration of iron in the grains of transformants increased more than that in the control line (× 0.9–1.9), with little increase in the concentrations of copper, manganese, and zinc (× 0.8–1.6), whereas the concentration of iron in the leaves and stalks drastically decreased, without any changes in the concentrations of the other divalent metals. This led to the same conclusion as that mentioned above, that the iron supply within the transformant, especially translocation from the leaves to the grains during grain development, is essential. Although the clarification of the genomic or proteinaceous background for this step has not yet been completed [16], the next target in the development of the ferritin crop is iron transport/translocation from the roots to the grains via the leaves (**fig. 1**).

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Biotechnology, biofortification, and global health

Ross M. Welch

Abstract

Deficiencies of micronutrients such as iron, zinc, and vitamin A afflict over three billion people (more than 50% of the world's population), most of them women, infants, and children in resource-poor families in the developing world. This global crisis in nutritional health is the result of dysfunctional food systems that do not consistently supply enough of these essential nutrients to meet the nutritional requirements of high-risk groups. Deficiencies of micronutrients result in increased morbidity and mortality rates, lost worker productivity, stagnated national development, permanent impairment of cognitive development in infants and children, and large economic costs and suffering to those societies affected. Because agricultural systems are the primary source of all micronutrients for all people, changes in agricultural policies and systems must be made that will ensure consistent and adequate supplies of all essential nutrients to all people. Additionally, the nutrition and health sectors must turn to agricultural interventions as a primary tool in their efforts to eliminate malnutrition from the world if they want to ensure sustainability. Biotechnological advances show great promise for improving the output of bioavailable micronutrients from agricultural systems that feed the poor. This paper reviews some of these opportunities and discusses the questions and concerns that should be raised when these technologies are used to improve the micronutrient status of vast numbers of people who are dependent on staple food crops for their sustenance. Further, important issues surrounding micronutrient bioavailability and plant food factors that affect it are discussed.

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Key words: Antinutrients, bioavailability, biotechnology, food crops, food systems, iodine, iron, micronutrients, vitamin A, zinc

Introduction

Incredibly, micronutrient malnutrition (iron, iodine, selenium, zinc, vitamin A, folic acid, etc.) afflicts over 3 billion people worldwide (over half the world's population), most of them women, infants, and children in resource-poor families in the Global South [1]. The consequences to human health, felicity, livelihoods, and national development are staggering, resulting in increased mortality and morbidity rates, decreased worker productivity, poverty, and diminished cognitive ability in children with lower educational potential born to mothers with micronutrient deficiencies [2–4]. Micronutrient malnutrition continues to increase in many nations. For example, the global burden of iron deficiency has risen from about 35% of the world's population in 1960 to over 50% in 2000 [3], and iron deficiency among poor women is increasing at an alarming rate in many developing countries. Current intervention programs (food fortification and supplementation programs) to alleviate the problem have not proven to be effective or sustainable in many countries [5].

This global crisis in micronutrient malnutrition is the result of dysfunctional food systems that cannot deliver enough micronutrients to meet the nutritional requirements of all. Because agriculture is the primary source of all micronutrients for human consumption, agricultural systems must be contributing to this failure to meet nutritional needs [6]. How can agriculture be changed in ways that will result in enough micronutrient output from farming systems to ensure adequate nutrition for all? If agricultural technologies are directed at improving the nutritional quality of food crops, they must encompass a holistic food system perspective to ensure that such interventions will be sustainable and adopted by farmers and consumers [7].

Further, the agricultural sector must adopt a specific goal of improving human nutrition and health, and the nutrition and health sectors must adopt agricultural interventions as a primary tool to fight malnutrition.

Food systems, diet, and health

To live healthy and productive lives, humans require at least 50 known nutrients in adequate amounts and consistently. Unfortunately, global food systems are failing to provide adequate quantities of all of these essential nutrients to vast numbers of people in the developing world. Advances in crop production achieved during the Green Revolution were mostly dependent on improvements in cereal cropping systems and resulted in greatly increased food supplies for the world, thus preventing massive starvation. However, cereals, as normally processed (e.g., by milling and cooking) and eaten, supply carbohydrates needed for energy and a small amount of protein, but few other nutrients in the required amounts. This change in agricultural systems to more monoculture of cereals and away from more varied cropping systems contributes to micronutrient malnutrition by limiting food-crop diversity and micronutrient output of cropping systems [8]. This agricultural trend has had the unforeseen consequence of reducing available micronutrient supplies to the poor, who were formerly dependent on more diverse cropping systems that provided more traditional, micronutrient-rich food crops (e.g., pulses, fruits, and certain vegetables) that are now in low supply and are no longer affordable for this sector of society [6, 9]. Nutrition transitions are also resulting in increased rates of chronic diseases (cancer, heart disease, diabetes, obesity, osteoporosis, etc.) in many rapidly developing nations where people are switching from traditional diets to more calorie-rich diets adopted from the food systems of developed nations [10, 11].

Biofortification of staple food crops

Genetic modification of food crops to bring about significant increases in bioavailable micronutrients in their edible portions as they are eaten holds much promise as a sustainable solution to micronutrient malnutrition [12–14]. The newly established HarvestPlus consortium, which is composed of several Consultative Groups on International Agricultural Research (the CGIAR) Centers and other national and international organizations, is focusing on traditional plant-breeding methods in combination with modern genetic technologies to deliver micronutrient-enriched staple food crops (rice, wheat, maize, bean, sweet potato, and cassava) for distribution to farmers in developing nations globally. This type of agricultural micronutrient intervention is known as "biofortification."

During the initial development of the micronutrient biofortification program in 1993, various issues were of concern [15]. These included the following questions: What is the possible magnitude of change in micronutrients within the genomes of the major staple food crops? Would selecting for micronutrient-enriched staple food crops in breeding programs affect crop productivity? Is there a large environment–genotype interaction when micronutrient-dense staple food crops are selected for (large environmental effects increase the difficulty of breeding programs)? Would farmers grow them and consumers accept them, and would such enriched staple plant foods be safe to eat? Would the micronutrients in the enriched genotypes be bioavailable (i.e., absorbable from a typical diet and utilizable in the body)? Would breeding for micronutrient-enriched staples be sustainable? What would be the effects of processing and cooking on the amounts of bioavailable nutrients in the foods prepared from such enriched staples? What would be the costs and benefits to society compared with other types of nutritional interventions, such as food fortification and supplementation programs?

For the past 10 years, research has been conducted to address all of these questions. A full account of our present knowledge of the answers to these questions can be found in Graham et al. [16], but it is beyond the scope of this article to discuss them in detail. To ensure maximum impact and sustainability, such questions should always be addressed before attempting to apply genetic modifications to improve plant foods as sources of micronutrients for humans.

Agronomic benefits

The development of seeds enriched with micronutrient elements that are in low available supply in the soil of the fields where the seeds are grown has benefits for agriculture. Seeds enriched in micronutrient elements (such as zinc) that are sown in soils depleted of these micronutrient elements have better seed viability and greater seedling vigor, form denser stands (resulting in less soil erosion), require lower seeding rates (resulting in lower cost to farmers), exhibit faster root establishment (resulting in earlier use of soil water and nutrients), are more resistant to disease, and exhibit better plant survival. These benefits to seedlings ultimately result in increased productivity in comparison with seeds that have low micronutrient stores [17, 18]. Many soils in the developing world are deficient in various micronutrient elements [19], and developing micronutrient-dense seeds for these areas should be of great benefit to farmers there.

The bioavailability determinant

One of the most contentious points in convincing the nutrition sector that biofortification should be used as a primary tool to combat micronutrient malnutrition centers on the issue of bioavailability. Staple cereal grains and legume seeds contain factors (e.g., phytic acid, certain fibers, tannins, polyphenols, hemagglutinins, goitrogens, and heavy metals) that can inhibit the bioavailability of micronutrients from typical diets eaten in developing countries [20]. However, diets can contain other substances (promoters) that counteract the inhibitory effects of antinutrients on the bioavailability of micronutrients in foods (e.g., certain organic acids, such as ascorbic acid; hemoglobin; certain amino acids, especially cysteine; long-chain fatty acids; and indigestible carbohydrates) [8]. This leads to three direct breeding strategies coupled to genetic modifications that can be used either separately or in combination to improve the utilizable micronutrient content of staple food crops: increase their total content in the plant portion eaten, decrease the amounts of antinutrients present in the plant food, and increase the amounts of promoter substances in the plant food to counteract the negative effects of antinutrients. The following actions are recommended when genetic modifications are applied to improve the micronutrient levels in staple food crops:

» Caution should be used when attempting to reduce antinutrients.
» Promoter substances should be identified and stressed as ways to improve micronutrient bioavailability from staple food crops.
» Further research is required to understand micronutrient interactions with regard to bioavailability issues, especially in regions with multiple micronutrient deficiencies.
» The influence of dietary constituents on micronutrient bioavailability should be studied in much greater detail.
» The effects of hind-gut microorganisms on micronutrient bioavailability should be examined.
» More reliable bioavailability models are needed, and the basic mechanisms underlying micronutrient bioavailability should be further studied.

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Update on the development of virus-resistant papaya: Virus-resistant transgenic papaya for people in rural communities of Thailand

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Abstract

*Papaya (*Carica papaya *L.) is one of the most important and preferred crops in rural communities in Thailand. Papaya ringspot virus (PRSV) is a serious disease of papaya throughout Thailand. Efforts to control the virus by various methods either have not been successful or have not resulted in sustainable control. In 1995, collaborative research by the Department of Agriculture of Thailand and Cornell University to develop transgenic papaya resistant to PRSV was initiated. Two local Thai cultivars were transformed by microprojectile bombardment with the use of a nontranslatable coat protein gene of PRSV from Khon Kaen. Numerous kanamycin-resistant plants were regenerated and were inoculated with the PRSV Khon Kaen isolate for selection of resistant lines.*

Since 1997, promising R0 transgenic lines have been transferred to the research station at Thapra for subsequent screenhouse tests and selection of the most PRSV-resistant lines. In selection set 1, three R3 lines initially derived from Khaknuan papaya showed excellent resistance to PRSV (97% to 100%) and had a yield of fruit 70 times higher than nontransgenic Khaknuan papaya. In selection set 2, one R3 line initially derived from Khakdam papaya showed 100% resistance.

Safety assessments of these transgenic papayas have so far found no impact on the surrounding ecology. No natural crossing between transgenic and nonmodified papaya was observed beyond a distance of 10 m from the test plots. Analysis of the nutritional composition found no differences in nutrient levels in comparison with the nonmodified counterparts. Molecular characterization by Southern blotting revealed three copies of the transgene presented; however, no coat protein product was expressed. Data on additional topics, such as the effects of feeding the transgenic papaya to rats and the stability of the gene inserts, are currently being gathered.

Key words: Coat protein gene, microprojectile bombardment, papaya ringspot virus (PRSV), parasite-derived resistant, safety assessment, transgenic papaya, untranslatable

Introduction

Papaya (*Carica papaya* L.) is one of the most important and preferred crops in the rural communities of Thailand. It is particularly important as a subsistence crop in the Northeast, where the people consume large quantities of green papaya in the form of salad. Ripe papaya is also eaten when it is available and has become very popular in the last 15 years. In the Northeast, unlike other regions of the country, papaya is not grown on large plantations but instead is grown in the backyards of subsistence farmers for daily consumption. Thus, papaya traditionally has served as a very good source of vitamins in rural communities.

Papaya ringspot virus (PRSV) appeared in the Northeast in the 1970s and now severely affects papaya all over Thailand [1]. Trees infected with PRSV develop a range of symptoms. The leaves develop chlorosis, ringed spots appear on the fruit and the upper part of the trunk, and distortion of young leaves resembling mite damage occurs. More importantly, production becomes severely depressed and eventually the infected trees die [2, 3]. The impact on rural communities

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has been dramatic, because trees can no longer be grown without a high probability of being damaged by the virus.

Efforts to control PRSV were initiated by Mrs. Vilai Prasartsee of the Northeast Regional Office of Agriculture (NEROA) soon after the virus was discovered in the Northeast. The first efforts to eradicate the virus in the villages gave temporary relief until the incidence of the virus in the villages began to increase again [4]. Starting in 1986, Vilai Prasartsee collaborated with Dennis Gonsalves of Cornell University under the umbrella of the Northeast Rainfed Agricultural Development (NERAD) Project, which was supported by the United States Agency for International Development (USAID) to control the virus by using cross protection [5] and to develop papaya cultivars that are tolerant to the virus. The cross protection effort did not result in sustainable control, but tolerant cultivars have been developed and are now being distributed to people in the Northeast and in other regions of Thailand. A feature of the tolerant cultivar is that it becomes infected but still produces a good amount of fruit. In 1994, collaboration with Dr. Dennis Gonsalves was initiated to control PRSV by developing genetically engineered papaya that is resistant to the virus [6–8].

Approach to developing virus-resistant transgenic papaya

We have used the concept of "pathogen-derived resistance" to develop virus-resistant transgenic papaya [8]. According to this concept, transgenic plants that express a gene of the pathogen will be resistant to that particular pathogen. This is somewhat analogous to immunization. Very briefly, transgenic papayas are produced by isolating the coat protein gene from the virus, engineering the gene into a DNA vector, transforming papaya cells with the gene, regenerating the transformed cells into plants, and then identifying the plants that are resistant to inoculation with the virus. An important point of this approach is that the resistance is inherited and thus can be transferred through the seeds.

Development of transgenic PRSV-resistant papaya at Cornell

In 1994, a program to control PRSV by developing genetically engineered papaya that is resistant to virus was financially supported by the Thai Government. The work began in collaboration with Cornell University and the Department of Agriculture of Thailand. In September 1995, Dr. Nonglak Sarindu and Dr. Suchirat Sakuanrungsirikul of the Department of Agriculture went to work in the laboratory at Cornell under the supervision of Dr. Dennis Gonsalves to develop transgenic papaya specifically for Thailand. By July 1997, transgenic papayas of the Khakdam, Khaknuan, and Thapra varieties were transported to Thailand. They were transferred to the research station at Tha Pra, Khon-Kaen Province, and the process soon began to identify suitable lines resistant to PRSV that would be acceptable to Thai consumers [6].

Development and identification of PRSV-resistant transgenic lines in Thailand

In 1997, the transgenic papaya was transported to Tha Pra, and seeds from selfing and crossing among transgenic and nontransgenic lines were produced from the original plants (R0). The progeny (R1) were tested for virus resistance in the greenhouse. Selection included mechanical inoculation of PRSV isolated from five endemic areas in Thailand. The most resistant lines of each generation were subsequently evaluated under field conditions [9].

Field trials of transgenic lines

Field trials at Tha Pra Research Station were started in 1999 and have continued to the present time. A number of resistant lines, in particular Khakdam and Khaknuan, were identified. Twenty-five lines of Khaknuan and two of Khakdam in the R2 generation that showed various levels of resistance to virus when inoculated in the greenhouse were subsequently tested in a field that was severely infected with the virus. The results were outstanding. The transgenic lines showed excellent resistance under field conditions. The excellent lines were then carried on to the third generation. Among these, one excellent line of Khaknuan (R3 319-1KN-181) and one of Khakdam (R3 300KD-9) have been identified. The progeny from these lines exhibit 90% to 100% resistance to PRSV (**table 1**). These three years of field trials have conclusively shown that the transgenic papaya is able to control the virus under heavy disease conditions in the field [10–12].

Assessment of safety of transgenic papaya

In Thailand, all transgenic products are subject to regulation by various government agencies, including the Ministry of Agriculture and Cooperatives, the National Biosafety Committee, and the Food and Drug Administration. Papaya is the first transgenic product that has been being assessed in Thailand, and thus it is the model case. No set standardized protocols have been established yet. However, transgenic papaya has been successfully cultivated in Hawaii and has been thoroughly tested and sold commercially in the United

TABLE 1. PRSV resistance, fruit production, and CP gene reaction (by PCR) of R3 (set 1) transgenic papaya under field conditions

Papaya line	PRSV resistance (% of plants)	Average yield[a] (kg/rai)[b]	CP gene (no. of plants with gene/no. of plants tested)
R3 319-180	97	12,022 A	32/32
R3 319-181	97	11,865 A	32/32
R3 319-182	97	11,559 A	32/32
KN non-transgenic	0	169 B	NT

PRSV, papaya ringspot virus; CP, coat protein; PCR, polymerase chain reaction; KN, Khaknuan; NT, not tested

a. Means followed by the same letter are not significantly different at $p < .05$ by DMRT.

b. 1 rai = 0.16 ha.

States since 1998. No harmful effects on humans or the environment have been observed. The transgenic papaya for Thailand was created with the same vectors, except that the coat protein gene was from a PRSV isolate of Thailand.

To address the issue of safety of the transgenic papaya, a number of experiments and tests on both the environmental and the food safety aspects have been carried out since 1999. Safety precautions at the laboratory and field level were followed strictly according to the guidelines issued by the regulatory agencies to ensure safety in the handling of these genetically modified materials. Extensive studies are being carried out to assess biosafety and food safety.

Assessment of environmental safety

Six experiments have been performed since 2001 to assess biosafety. These studies were conducted to determine the possible effects that transgenic papaya might have on its ecological context, such as the soil microflora, beneficial insects, and other elements of the surroundings. These tests included the following.

Natural dispersal and possibility of crossing between transgenic and nontransgenic papaya

Our studies showed that the possibility of finding the coat protein (CP) gene in the population of nontransgenic papaya guard rows within 2 m of the transgenic test plots was approximately 20% to 22%. However, it was found that the fruits of female papaya trees 10 to 25 m from the plots did not produce seeds. It is therefore very likely that natural dispersal of the pollen is limited and the probability of crossing between transgenic and nonmodified papaya beyond a distance of 10 m is extremely low.

Possible impact of transgenic papaya on soil ecology

Rhizosphere soil samples from pots containing transgenic and nontransgenic papaya were examined to determine the total number of viable microorganisms and the number of strains, including bacteria and fungi. The results so far have shown no differences between the pots of transgenic and nontransgenic papaya in the populations of *Mycorrhiza* spores, *Rhizobium* bacteria, some heterotrophic bacteria, *Actinomyces* bacteria, and filamentous fungi.

Possible effects of transgenic pollen on pollinating insects in situ

An experiment was performed to check the survival and developmental stages of two- to three-day old larvae of honeybees (*Apis mellifera*) after feeding with the pollens of the selected transgenic lines. There were no significant differences in the survival rate of the larvae to capping and from capping to emergence. No abnormalities were observed in the population of the bees tested.

Possible effects of transgenic papaya on predatory mites and papaya pest mites

A study was performed to observe the survival rate and ovipositioning abnormality of the predatory mites *Amblyseius longispinosus* (Evans) after feeding them with papaya pest mites *Eutetranechus africanus* (African red mite) that had consumed transgenic and nontransgenic papaya. No abnormality was observed at any stage of development.

Possible effects of transgenic papaya on other crops replanted in the same soil

Six plant species (cucumber, peanut, Chinese spinach, radish, corn, and yambean) were replanted in the same soil and ecologic surroundings that were used for planting transgenic papaya. No abnormalities were found in the development and productivity of these plant species.

Possible cross infection by PRSV-w in transgenic papaya

PRSV is divided into two types: type P (PRSV-p) infects cucurbits and papaya, whereas type W (PRSV-w) infects cucurbits but not papaya [13]. Both types are serologically closely related; however, a number of observations suggests that papaya is a major source for the spread of PRSV-p [14]. A set of experiments was carried out to observe this possibility. Transgenic papaya plants and cucurbit were tested with PRSV-w and PRSV-p, and no cross-infection was observed.

Assessment of food safety

Food safety concerns product characterization that requires information in relation to the modified food crop, the introduced genetic material and its expression product, and acceptable levels of inherent plant toxicants and nutrients. In principle, assessment of food safety involves comparison of the characteristics of the transgenic plants with the characteristics of plants that are produced by traditional breeding. Preliminary experiments to assess the food safety of transgenic papaya products were performed following the recommendations of the Subcommittee on Food Safety of the National Biosafety Committee.

Analysis of nutritional composition

The analytical data obtained in this study included the determination of proximate composition (moisture, crude protein, crude fat, ash, carbohydrate, total dietary fiber, and energy), potassium level, -carotene, and vitamin C. Preliminary results on the analysis of Khaknuan transgenic lines found no significant differences between transgenic and nontransgenic papaya. Analysis of transgenic Khakdam is now being performed.

Molecular characterization of the gene insert

All characteristics of the gene insert must be known, including the size, number of insertions, flanking sequences, and stability of the gene inserted. This is to ensure that the introduced gene sequences do not encode harmful substances and are stably inserted within the plant genome to minimize any possibility of undesired genetic rearrangement. Our preliminary experiments using Southern blotting suggest that the two selected lines consist of three gene inserts. Investigation of the stability of the gene inserted is being planned.

Expression of coat protein product

It must be established that the introduced gene does not produce toxins or allergenic substances. In our case, the transgenic papaya was engineered by using the nontranslatable version of the coat protein gene of PRSV Thai-isolate. The resistance of the transgenic papaya to PRSV disease is mediated by RNA. The evaluation is therefore performed to demonstrate that no coat protein is produced in these transgenic papayas. Preliminary experiments were performed by dot-blot analysis of leaf and ripe fruit tissues and probing with PRSV antibody. The results showed that no coat protein was expressed in the transgenic line tested.

Toxicologic testing on animals

Experiments were being conducted at the beginning of 2004 in which male and female Norway rats (*Rattus norvegicus*) were fed transgenic papaya and observed for abnormalities of growth rate, fertility, blood cells, and gastrointestinal tissues. Analysis of the results is under way.

Intellectual property rights

The transgenic papaya was developed by direct collaboration between the Government of Thailand and Cornell University, without any grants from large companies. Thus this transgenic papaya is not connected with large multinational companies. However, since all transgenic products have components that are covered by intellectual property rights, we need to obtain licenses for these rights in order to freely distribute or sell the transgenic product. Fortunately, the Cornell Foundation, the intellectual property organization of Cornell University, has simplified and reduced the cost of this potentially complicated and expensive process. The Cornell Foundation is thus responsible for acquiring the intellectual rights to the Thai transgenic papaya. The Cornell Research Foundation and the Department of Agriculture of Thailand are now working on a memorandum of understanding on this subject.

Potential impact

One of the main reasons for developing transgenic papaya for Thailand is to help the poorer people in the rural communities, particularly in the Northeast. These efforts are worthy and sustainable because resistance to the virus is carried through generations in seeds. The involvement of the Department of Agriculture will ensure that the transgenic papaya will be made available to the communities. Most importantly, because PRSV has caused severe damage to papaya in Thailand, solving this problem will certainly improve the food security of rural communities by providing an inexpensive source of nutrition and vitamins. Furthermore, commercial farmers will not have to keep moving to new planting sites to escape the virus.

One attractive aspect of this transgenic project is the value of true collaboration between the two institutes. Challenging work has been successfully performed in a short period with the use of off-the-shelf technology. Papaya is the first transgenic product in Thailand to be at an advanced stage of evaluation. This project has provided a unique opportunity to pave the way by showing how transgenic products can be introduced and commercialized in Thailand.

Perspectives

The transgenic project was conceived and is being carried out because of the commitments of researchers and other personnel to help less-privileged people in Thailand. We have used the latest and most powerful technologies to address the PRSV problem in Thailand, and we have succeeded in developing transgenic papaya that is resistant to the virus. We have the expertise and the desire to carry the project to its completion in a timely manner. The success of the project will be judged according to whether the transgenic papaya is made available to the rural communities of Thailand, particularly in the Northeast. It is crucial to complete the project in the near future. If it is successful, transgenic papaya will show how biotechnology can be used to help less-privileged rural communities.

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Seed-specific expression of the lysine-rich protein gene *sb401* significantly increases both lysine and total protein content in maize seeds

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Abstract

The sb401 *gene from potato* (Solanum berthaultii) *encoding a pollen-specific protein with high lysine content was successfully integrated into the genome of maize plants, and its expression was correlated with increased levels of lysine and total protein content in maize seeds. A plasmid vector containing the* sb401 *gene under the control of a maize seed-specific expression storage protein promoter (P19z) was constructed and introduced into maize calli by microprojectile bombardment. The integration of the* sb401 *gene into the maize genome was confirmed by Southern blot analysis, and its expression was confirmed by Western blot analysis. Quantification of the lysine and protein contents in R1 maize seeds showed that, compared with the nontransgenic maize control, the lysine content increased by 16.1% to 54.8% and the total protein content increased by 11.6% to 39.0%. There were no visible morphological changes in the vegetative parts and seeds of the transgenic maize plants. Lysine and protein analysis of the transgenic maize grains showed that the levels of lysine and total protein remained high for six continuous generations, indicating that the elevated lysine and total protein levels were heritable. These results indicate that the* sb401 *gene could be successfully employed in breeding programs aimed at improving the nutritional value of maize.*

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Key words: Lysine content, lysine-rich protein *sb401* gene, microprojectile bombardment, protein content, transgenic plants, *Zea mays L.*

Introduction

Lysine is one of the eight essential amino acids in the metabolic pathways of humans and other monogastric animals. In major cereal crops, especially in maize, lysine is the most limited of these eight amino acids. Nutritional deficiencies in animals whose diets rely heavily on maize are often caused by a shortage of lysine. Efforts to increase the lysine content of maize seeds have been under way for a long time but have not succeeded in producing simultaneous increases in the levels of lysine and total protein. Conventional breeding approaches have targeted the maize *opaque-2* (*o2*) gene, which encodes a transcription factor for the α-zein (storage protein) family [1]. Mutations in the *o2* gene aimed at increasing lysine content disable its transcription factor product, causing a decrease in the production of two important α-zein proteins that together account for a significant percentage of the total protein in maize seeds [2]. At the Maize and Wheat Improvement Center (CIMMYT) in Mexico [3] and at the University of Natal [4], researchers have developed QPM (quality protein maize) materials through a complex process of backcrossing *o2* modifier genes into *o2* mutants. It took nearly 30 years to develop QPM with a nearly normal yield and protein content and with lysine and tryptophan levels comparable to those in the original soft *o2* mutants. The inverse relationship between lysine and total protein content, together with the long time requirements, limits the feasibility of conventional approaches aimed at increasing the lysine content in maize.

In recent years, attempts to improve the lysine content of maize seeds using plant genetic engineering approaches have been reported [5–9]. These approaches have been focused on artificially increasing the lysine content of a particular protein (such as zein, a common

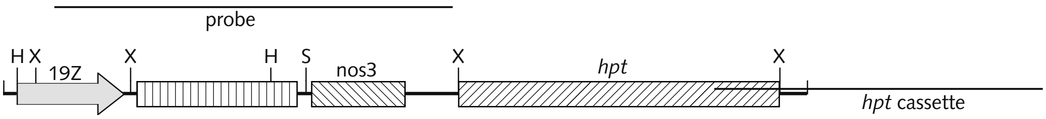

FIG. 1. Schematic diagram of plasmid p19ZKH. The plasmid consists of two gene expression cassettes: the *sb401* cassette, in which the *sb401* gene is regulated by the 19Z promoter and the *nos* 3 region; and the *hpt* cassette, in which the *hpt* gene is controlled by the CaMV 35S promoter and the *nos* 3 region. The probe used in Southern blot analysis is indicated above. H, *Hin*dIII; X, *Xba*I; S, *Sac*I

maize protein) by inserting more lysine codons or by changing some of its non-lysine codons to encode lysine. However, these modifications destabilized the protein and made it difficult to deposit in seeds.

In this study, transgenic methods were used to introduce a high-lysine-content protein gene (*sb401*) [10] from the diploid potato species *Solanum berthaultii* into maize lines to increase the lysine content of maize seeds. The *sb401* gene occurs naturally and encodes the lysine-rich protein (Lrp), a pollen-specific protein. The protein product of the *sb401* gene is 240 amino acids in length and contains 40 lysine residues, indicating a 16.7% (K/total amino acids) lysine content. Very little is known about the function of Lrp, although its sequence shows similarity with the microtubule-binding domain of mouse microtubule-associated protein (MAPIB), suggesting that it might be involved in cytoskeletal organization [11]. Our results show that seed-specific expression of Lrp using the maize zein promoter resulted in a significant increase in both total protein content and lysine content in mature maize seeds. Western blot analyses confirmed that the Lrp was stably deposited in maize grains. These results demonstrate that substantial increases in the lysine content of maize—and potentially other cereal crops—by expression of a natural, lysine-rich protein is feasible in practice.

Results

Plant transformation and molecular analysis

Plant transformation

Immature embryos 9 to 13 DAP (days after pollination) from hybrid (Z3XQ31) were cultured on N6 media containing 2,4-D (2.0 mg L^{-1}), and the calli were maintained in complete darkness at 27 jæ. All calli were bombarded at 25 inches Hg with the BioRad Biolistic PDS-1000/Helium vacuum system (Bio-Rad Company, Hercules, Calif, USA).

Construction of plasmids containing the *sb401* sequence is shown in **figure 1**. The transformation efficiency of the three independent experiments was 498 fertile plants out of 602 regenerated plants, the rest being sterile.

Stable integration of the transgene in transgenic maize plants

We performed Southern blot analyses on 260 plants with positive polymerase chain reaction (PCR) results out of 602 regenerated plants. The plant genomic DNA samples were digested with *Xho*I and hybridized with a probe for the *sb401* gene and 19Z promoter. A 1.6-kb band appeared, corresponding to the approximate size of the *sb401* gene and the 19Z promoter. Several hybridization bands of different sizes were detected (**fig. 2**). There is no *Xho*I site in the vector, so the results suggested that at least two copies of the transgene were present in the maize genome. No signal was detected when DNA extracted from an untransformed control plant was used. The unique banding pattern of each of the seven plants suggests the chimeric gene has been integrated at different sites in the genome.

Seed-specific expression of the lysine-rich protein (Lrp)

Western blot analysis performed with a rabbit polyclonal SB401 antiserum revealed the presence of Lrp protein in the seeds of 34 out of the 98 R1 progeny of the seven lines analyzed. A representative Western blot of the transgenic plants is shown in **figure 3**.

There is a contradiction between the deduced molecular mass of the *sb401*-encoded polypeptide (25 kD) and its apparent molecular mass of 50 kD after sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE). This contradiction was observed in previous reports [12]. The contradiction may be due to the fact that Lrp protein is very rich in glutamic acid and lysine. It may also be due to the S-S bond. The Lrp protein was not detectable in seed extracts of untransformed maize plants or in extracts from the leaves of transformed maize plants.

Significant increase of both lysine content and total protein content in transgenic maize seeds

The R0 transgenic and nontransgenic (control) seeds were planted in the field at the same place under the same environmental conditions. Lysine and protein contents were analyzed in transgenic and nontransgenic maize seeds taken from the R1 progeny. Thirty seeds from each of the self-fertilized R1 plants (about 6 g) were randomly selected, and the lysine and protein contents of the seeds were determined by the Cereal Quality Supervision and Testing Center, Ministry of

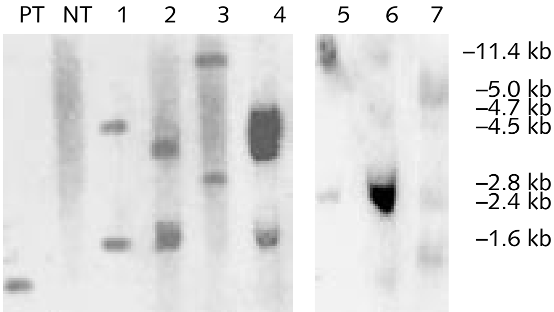

FIG. 2. Partial result of Southern blot analysis of genomic DNA for the seven transgenic plants selected for further experiments. DNA (20 μg) from each transgenic line containing p19ZKH and from the untransformed line was digested with *Xho*I, resolved on a 0.8% wt/vol agarose gel, blotted onto a nylon membrane, and hybridized with the α-P32-labeled 1.6-kb *Hin*dIII fragment containing the coding sequence of the *sb401* gene and 19Z promoter. Lanes 1–7 show DNA samples from BZ 8, BZ 15, BZ 29, BZ 32, BZ 37, BZ 47, and BZ 94 maize lines. NT, negative control (DNA isolation from untransformed maize line); PT, positive control (1.6-kb *Hin*dIII fragment)

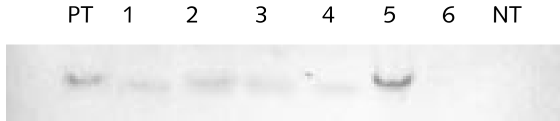

FIG. 3. Western blot analysis of R1 plants shows seed-specific accumulation of Lrp protein in transgenic maize plants. Total protein extracts were obtained from transgenic maize by homogenizing immature seeds in protein extract buffer (5 mmol/L potassium phosphate, pH 6.0; 2.55% sucrose; 0.15 % β-mercaptoethanol). Equal amounts (30 μg) of total soluble protein were loaded into each lane, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and electroblotted onto nitrocellulose membranes with a BioRad electrotransfer apparatus. Membranes were incubated with the *sb401* antiserum and then with mouse anti-rabbit alkaline phosphate-conjugated IgG as a secondary antibody (Promega). Protein bands were visualized in alkaline phosphatase substrate buffer (Promega). PT, positive control—Lrp protein in *Escherichia coli.* Lanes 1–5: protein extracted from transgenic plant seeds BZ 8, BZ 15, BZ 32, BZ 37, and BZ 47. Line 6: protein extracted from transgenic plant leaves. NT, protein extracted from nontransgenic plant seeds

Agriculture, People's Republic of China. The data are shown in **table 1**. The lysine and protein contents in the seeds were increased by various amounts in different transgenic maize lines. The increase in the lysine content of the seeds as a percentage of grain weight ranged from 16.1% to 54.8%, and the lysine content as a percentage of total protein scarcely increased at all. The increase in the protein content of the seeds ranged from 11.6% to 39.0% compared with that of nontransgenic maize.

To determine the heritability of the increase in lysine and protein contents, plants from the seven R1 lines were self-pollinated, and both the presence of the transgene and the lysine and protein contents were tracked after each selfing. Southern blot analysis confirmed the presence of the transgene in each of six subsequent generations. Further lysine and protein analysis performed on the seeds from each generation confirmed that the levels of lysine and protein did not decrease. These analyses also confirmed that the levels of other amino acids were elevated in every generation (data not shown here). The lysine content ranged from 0.38% to 0.46% (g/100 g dry seeds) for transgenic seeds, whereas the average value for the control seeds was 0.31%. The protein content of transgenic seeds ranged from 14.0% to 17.2% (g/100 g dry seeds), whereas the control

TABLE 1. Crude protein and lysine contents of R1 plant lines[a]

R1 plant line	Lysine				Crude protein		Weight of 100 seeds (g)
	g/100 g seed	Increase (%)	g/100 g crude protein	Increase (%)	g/100 g seed	Increase (%)	
BZ8-3	0.42	35.5	0.034	21.4	12.44	19.3	26.90
BZ15-6	0.48	54.8	0.032	14.2	15.04	39.0	20.90
BZ29-5	0.37	19.4	0.028	*	13.35	25.0	23.16
BZ32-2	0.38	22.6	0.031	10.7	12.08	11.6	24.52
BZ37-3	0.37	19.4	0.028	*	13.18	21.8	30.60
BZ47-8	0.38	22.6	0.030	7.14	12.58	16.3	21.29
BZ94-3	0.36	16.1	0.027	*	13.22	22.2	23.52
Control	0.31	*	0.028	*	10.82	*	19.36

* No increase compared to control

a. Protein and lysine contents were measured in R1 transgenic plant seeds selected from 7 R1 transgenic lines and compared with those of nontransgenic (control) seeds. Thirty seeds from each of the self-fertilized R1 plants (about 6 g) were randomly selected for analysis of lysine and crude protein contents. The content of crude protein is expressed as g/100 g seed, and the content of lysine is expressed as g/100 g seed and g/100 g crude protein. The percentage of increase of lysine and protein contents over those of the wild type is shown.

seeds averaged 10.8%. We have also produced several hybrid lines with high protein and lysine contents by crossing the transgenic inbred and the inbred used in production.

Discussion

In this study, the *sb401* gene was introduced into embryogenic calli derived from immature seeds of the maize hybrid Z31XQ31 to improve the nutritional quality of maize. Derived from the potato variety *Solanum berthaultii*, the *sb401* gene encodes a protein with a high proportion of lysine residues, making it a suitable target for transfer to improve the nutritional value of maize and other cereal crops.

Several studies have been conducted on the expression of a native amino acid-rich protein in a foreign species. For example, the seed albumin gene of *Amaranthus hypochondriacus* was successfully expressed in the potato tuber, resulting in an increase in the total protein content with an increase in most essential amino acids in the tuber [13]. The 2S Brazil nut albumin was successfully expressed in canola seeds, resulting in enhanced levels of seed protein with an increase in methionine [5]. However, no successful application of native plant genes to increase the lysine and protein contents in maize seeds by transgenic methods has been reported. Most previous studies have involved attempts to artificially increase the lysine content of a particular protein (such as zein) by inserting more lysine codons into the gene or by changing some of its non-lysine codons to encode lysine [14] in order to design a new gene encoding lysine-rich protein [15, 16]. These attempts were unsuccessful because artificial modifications destabilized the protein and made it difficult to deposit in seeds. Natural proteins do not share these limitations and possibly can be properly synthesized, folded, and accumulated in plants. In this study, transgenic techniques were used to introduce the *sb401* gene, which encodes the natural protein Lrp, to increase the levels of lysine and total protein in maize. The expression of this gene contributes to high levels of both lysine and protein in maize seeds.

As seen in **table 1**, there is no substantial difference between the control and the BZ94-3, BZ37-3, and BZ29-5 lines. This suggests that an increase in total protein rather than in the expression of *sb401* contributes most to the increase in lysine content, and that Lrp may play an indirect, rather than a direct, role in the increase of lysine and protein contents.

The results of an analysis of inbred lines obtained by six continuous generations of self-pollination showed that the expression of Lrp was hereditary, as were the levels of lysine and protein, with no visible morphological changes in vegetable parts and seeds. All the data suggested that this lysine-rich protein gene could be successfully applied in breeding programs aimed at improving the nutritional quality of maize.

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Sharing Malaysian experience with the development of biotechnology-derived food crops

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Abstract

Biotechnology-derived food crops are currently being developed in Malaysia mainly for disease resistance and improved postharvest quality. The modern biotechnology approach is adopted because of its potential to overcome constraints faced by conventional breeding techniques. Research on the development of biotechnology-derived papaya, pineapple, chili, passion fruit, and citrus is currently under way. Biotechnology-derived papaya developed for resistance to papaya ringspot virus (PRSV) and improved postharvest qualities is at the field evaluation stage. Pineapple developed for resistance to fruit black heart disorder is also being evaluated for proof-of-concept. Other biotechnology-derived food crops are at early stages of gene cloning and transformation. Activities and products involving biotechnology-derived crops will be fully regulated in the near future under the Malaysian Biosafety Law. At present they are governed only by guidelines formulated by the Genetic Modification Advisory Committee (GMAC), Malaysia. Commercialization of biotechnology-derived crops involves steps that require GMAC approval for all field evaluations and food-safety assessments before the products are placed on the market. Public acceptance of the biotechnology product is another important factor for successful commercialization. Understanding of biotechnology is generally low among Malaysians, which may lead to low acceptance of biotechnology-derived products. Initiatives are being taken by local organizations to improve public awareness and acceptance of biotechnology. Future research on plant biotechnology will focus on the development of nutritionally enhanced biotechnology-derived food crops that can provide more benefits to consumers.

Key words: Biotechnology, food crops, Malaysia

Introduction

The Malaysian National Agriculture Policy 3 (NAP3) and action plans have outlined important elements for agricultural transformation. The main goal is to enhance food security and wealth creation through increased food production. One of the strategies described in NAP3 is the utilization of high technologies, including biotechnology. Modern biotechnology is currently being applied for its potential to produce crops with higher yield and resistance to pests, disease, and adverse conditions, as well as improved quality [1]. This paper surveys the development of biotechnology-derived food crops in Malaysia and the process of their commercialization.

Development of biotechnology-derived food crops

Plant biotechnology has been practiced in Malaysia for the past 15 years. Plant tissue culture was the main research focus because of its vast application in plant breeding and in the production of elite planting materials. Tissue culture of fruit crops such as banana, pineapple, and papaya and anther culture of rice were actively pursued then. Tissue culture of banana has been commercialized and adopted by growers [2].

With the advent of modern biotechnology, including molecular techniques and recombinant DNA, local research organizations began to incorporate the new approaches as early as the mid-1980s. The potential of

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overcoming constraints faced by conventional breeding techniques has made modern biotechnology an attractive alternative to produce the new crop varieties constantly needed by growers.

Research and development on biotechnology-derived food crops

Biotechnology-derived food crops are crops that have been improved genetically by techniques of modern biotechnology, better known as genetic engineering or transgenic technology. Genetically engineered crops are commonly known as genetically modified (GM) crops. Food derived from genetically modified crops is known as GM food (GMF).

Biotechnology-derived crops are developed by transferring specific DNA sequences, known as genes, that encode desirable traits or characters. There are two major steps in the development: identification and isolation of the right gene for the traits of interest, and transfer of the gene of interest into the cells of the targeted crop plant. The gene-transfer process or transformation is achieved through particle bombardment or by the use of the bacterium *Agrobacterium tumefaciens*. Once the gene is incorporated into the genome of the plant, the cells are regenerated into plantlets that will later grow and produce as normal plants except for the characters encoded by the transferred gene.

In Malaysia, biotechnology research and development on food crops is conducted mainly at the Malaysian Agricultural Research and Development Institute (MARDI). The research complements MARDI's active breeding program of local food crops. Research is generally focused on overcoming major limitations in crop production, such as disease. However, traits to improve quality, such as increasing the shelf-life of fruit or delaying fruit softening, are also being incorporated.

The biotechnology-derived food crops being developed in Malaysia and their targeted traits have been described recently [3, 4]. The research is still ongoing, and to date no local biotechnology-derived food crops have been commercialized. The food crops that are being addressed at MARDI and the targeted traits are as follows:

» Papaya for resistance to papaya ringspot virus (PRSV)
» Papaya for delayed fruit ripening
» Papaya for delayed fruit softening
» Pineapple for resistance to fruit black heart
» Passion fruit for resistance to viruses
» Chili for resistance to viruses
» Citrus for the improvement of fruit color

Papaya and pineapple are at the most advanced stages of research, whereas passion fruit, chili, and citrus are at the level of gene identification and development of the transformation system.

Biotechnology-derived papaya for PRSV resistance

Papaya is an important industry in Malaysia, valued at $US7.89 million annually [3]. A PRSV-resistant papaya variety is needed to overcome the PRSV disease, which is currently a major threat to the industry. Research was initiated to develop PRSV-resistant papaya by a virus-mediated resistance strategy using coat protein gene isolated from the virus. Transgenic papaya plants containing the coat protein genes have been produced. Initial screening showed some putatively resistant plants that did not show disease symptoms when inoculated with the virus [5]. These plants are currently being grown in contained field trials to collect seeds for the next-generation field trial. Once the resistant plants are confirmed, they will be evaluated by a large-scale, multilocation field trial.

Biotechnology-derived papaya for quality improvement

Like other tropical fruits, papaya has a short shelf-life of about two weeks, which has reduced its export potential. Longer shelf-lives of fruits can help lower postharvest losses, since the products are able to reach the market and the consumers in good condition. Furthermore, consumers will enjoy the freshness of the fruits longer. Research was initiated to delay the ripening of Eksotika papaya fruit using the transgenic approach [6]. Transgenic papaya plants containing gene sequences that can suppress the formation of the ripening hormone ethylene were produced [7]. The lower level of ethylene produced in these fruits will result in a delay in the ripening process. Transgenic papaya plants are currently undergoing contained field evaluations for proof-of-concept [8]. When potential lines with delayed fruit ripening are obtained, they will undergo the same process as the transgenic PRSV-resistant papaya.

Another trait that is being incorporated in papaya is delayed fruit softening. The gene for the fruit cell-wall enzyme β-galactosidase, which is involved in the softening of papaya, has been isolated and transformed into papaya. Transgenic plants have been produced and will be evaluated in contained field trials for proof-of-concept.

Biotechnology-derived pineapple for resistance to black heart

Pineapple is also an important fruit crop for Malaysia. Malaysia has been a major producer of canned pineapple in the past. However, currently fresh pineapple production is gaining importance. Pineapple fruits of some commercial varieties are susceptible to browning of the inner core, known as black heart, if subjected to low temperature storage. The browning of fruit is unattractive to the consumer. An enzyme that causes fruit browning has been identified and the gene sequence has been used to develop transgenic pineapple resistant to black heart. Transgenic pineapple plants containing the gene have been developed and are currently being evaluated in the glasshouse for proof-of-concept.

Commercialization of biotechnology-derived food crops

The emergence of biotechnology-derived food crops and their subsequent release into the environment has raised concerns among the general public and highlighted issues regarding their safety for the environment and human health. A regulatory system has to be set up to oversee, assess, and manage the safety concerns of these biotechnology-derived food crops. In Malaysia, commercialization of any biotechnology-derived crops from the laboratory will involve steps requiring biosafety and food safety approvals. Biosafety approvals are required at various steps along the process in which field trials are involved, whereas food safety approval is required before placing the biotechnology-derived products on the market.

Biosafety

Biosafety is shorthand for the regulatory systems designed to ensure that applications of biotechnology are safe for human health, agriculture, and the environment. The Genetic Modification Advisory Committee (GMAC) of Malaysia was set up to formulate National Guidelines on the release of these crops into the environment and also to help draft the Biosafety Law of Malaysia. According to the guidelines and the proposed Biosafety Law, all activities involving biotechnology-derived food crops must receive approval from the National Biosafety Board (to be set up) or must notify them before any activities such as research, development, and marketing of biotechnology-derived crops and products are conducted. The Institutional Biosafety Committee (IBC) is set up at the institutional level to address persons conducting research, standards of contained glasshouses, and any field trials or open-field evaluations of all biotechnology-derived crops.

Food safety

According to the Organization for Economic Cooperation and Development:

> Food is generally considered to be safe if there is reasonable certainty that no harm will result from its consumption under normal anticipated conditions. Historically, food prepared and used in traditional ways is considered safe on the basis of long term experience, even though it may naturally contain harmful substances. In principle, food is presumed to be safe unless a significant hazard has been identified [9].

The purpose of Food Safety Assessment is to ensure that the consumer is not exposed to a food that will cause harm when prepared and consumed according to its intended use. The Biosafety Law requires, and consumers expect, that effective systems and procedures to assess the safety of a food or food component intended for consumption will be implemented. Communities and consumers have become more aware and more concerned (and in fact more misinformed) about food products derived from biotechnology.

A basic principle in the regulation of foods produced by biotechnology is a concept called “substantial equivalence.” According to this approach, evaluation is based on a comparison of the genetically modified crop plant with its conventionally bred counterpart or counterparts, assuming that these traditional foods and feeds have a long history of safe use. This principle provides equal or increased assurance of the safety of foods derived from genetically modified crops relative to the safety of foods derived from traditional counterparts [10].

Food safety assessments of Malaysian biotechnology-derived papaya have been proposed and will be taken up by member countries of the Papaya Biotechnology Network of Southeast Asia in the ISAAA Food Safety Workshop held in Bangkok in January 2000. The assessments data collected will be submitted to GMAC for approval before placing the product in the market. The assessment will be conducted by relevant regulatory bodies.*

Public awareness and acceptance

Public acceptance of the biotechnology product is another important factor for successful commercialization. A recent survey of key stakeholders in Malaysia conducted by the University of Illinois at Urbana-Champaign and the International Service for the Acquisition of Agri-biotech Applications (ISAAA) showed that various groups of stakeholders had a positive attitude toward biotechnology and believed that the technology would benefit smallholders [11]. A survey of 1,400 Malaysian Muslim respondents

* Current status remains unchanged since 2000; none of the member countries is ready with its GM product for commercialization.

around Kuala Lumpur conducted by the Institute of Islamic Understanding Malaysia (IKIM) found that 66.7% of respondents had heard of biotechnology but only 52.2% declared that they knew what it was about [12]. The survey also found that although about 67% of respondents could define genetically modified organisms (GMOs), genetic engineering, and biopharmaceuticals, only 40% knew what cloning is. These results indicate that the acceptance level of Malaysians is quite promising. However, to ensure full public acceptance, public awareness efforts have to be emphasized. Organizations like IKIM and the Malaysian Biotechnology Information Centre (MABIC) have been active in organizing seminars, workshops, and conferences on relevant areas of biotechnology for the public. Awareness of biotechnology by students is being coordinated by the National Biotechnology Directorate, Malaysia [1]. Government agencies conducting biotechnology research and development, such as MARDI, are very supportive of any activities to promote public awareness of biotechnology. Biotechnology scientists often participate in seminars and media interviews, hold exhibitions, and organize laboratory visits for the public.

Challenges and future prospects

To improve consumer acceptance, development of biotechnology-derived food crops should focus on characteristics that benefit consumers. With the know-how well established for the development and commercialization of existing biotechnology-derived crops, we plan to focus research efforts on developing nutritionally enhanced food crops that provide direct benefits to consumers. Efforts are already under way to look into the relevant pathways leading to enhancement of important metabolites through genomic approaches. Efforts are being made by the government to better coordinate biotechnology development in the country by strengthening biotechnology research and development, as well as the regulatory framework and public acceptance. With these, Malaysia hopes to be competitive in food production through biotechnology.

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Report of the ILSI International Food Biotechnology Committee

Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology

Task Force of the International Life Sciences Institute (ILSI) International Food Biotechnology Committee

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Executive summary

The global demand for food is increasing because of the growing world population. At the same time, availability of arable land is shrinking. Traditional plant breeding methods have made and will continue to make important contributions toward meeting the need for more food. In many areas of the world, however, the problem is food quality. There may be enough energy available from food, but the staple foods lack certain essential nutrients. In the developed world, demand for "functional foods" (i.e., foods that provide health benefits beyond basic nutrition) is increasing. Nutritional improvements in foods could help to meet both of these demands for improved food quality. Modern agricultural biotechnology, which involves the application of cellular and molecular techniques to transfer DNA that encodes a desired trait to food and feed crops, is proving to be a powerful complement to traditional methods to meet global food requirements. An important aspect of biotechnology is that it provides access to a broad array of traits that can help meet this need for nutritionally improved cultivars. The new varieties developed through modern biotechnology have been identified by a number of terms, including genetically modified (GM or GMO), genetically engineered (GE or GEO), transgenic, biotechnology, recombinant, and plants with novel traits (PNTs). For the present discussion, the term "GM" will be used because of its simplicity and broad public recognition.

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Background

The first genetically modified crops to be planted on a widespread basis consisted primarily of varieties with improved agronomic characteristics. These have been widely adopted and safely grown and used on a large scale in an increasing number of countries. A newly emerging class of genetically modified crops is being developed with a focus on improved human or animal nutrition. A number of these crops have reached the field trial stage and/or are advancing through regulatory approval processes toward commercialization. These nutritionally improved crops have the potential to help offset nutrient deficiencies; improve the nutritional value of foods and feeds; promote well-being through elevated levels of beneficial compounds; lower levels of natural toxins, toxic metabolites, or allergens; improve processing; and/or enhance taste. To keep this document to a manageable size, its scope was intentionally limited. The document does not discuss the safety or nutritional assessment processes for functional foods (i.e., foods that offer potential health benefits that go beyond satisfying basic nutritional needs), food or feed traits that are principally targeting a health or pharmacologic benefit, or crops that combine (i.e., stack) several improved nutrition traits into a single crop.

As long ago as 1263, the English Parliament decreed that nothing could be added to staple foods that were “not wholesome for a man’s body.” Consequently, a well-established history and process for assessing the safety of foods introduced into the marketplace exists that long precedes the introduction of genetically modified crops. The assessment of crops with improved nutritional properties, regardless of how those crops are developed, can follow these same well-established principles and processes to ensure that the intakes of essential nutrients in animal and/or human diets are not compromised. A key purpose of the assessment is to determine if adverse effects on health are likely to result from the intended compositional change. This kind of analysis has already been applied in several countries to crops with altered composition, and the principles of the evaluation are applicable to all novel foods. The scientific procedures for this kind of analysis require an integrated multidisciplinary approach, incorporating molecular biology, protein biochemistry, agronomy, plant breeding, food chemistry, nutritional sciences, immunology, and toxicology.

It is well recognized that absolute safety is not an achievable goal in any field of human endeavor, and this is particularly relevant with respect to ingestion of complex substances like foods and feeds. The safe use of a given food or feed has typically been established either through experience based on common use of the food or by experts who determine its safety based on established scientific procedures. Starting in the 1990s, the standard applied to novel, especially genetically modified, food and feed crops has been that they should be as safe as an appropriate counterpart that has a history of safe use. This comparative assessment process (also referred to as the concept of substantial equivalence) is a method of identifying similarities and differences between the newly developed food or feed crop and a conventional counterpart that has a history of safe use. The analysis assesses: (1) the agronomic/morphological characteristics of the plant, (2) macro- and micronutrient composition and content of important antinutrients and toxicants, (3) molecular characteristics and expression and safety of any proteins new to the crop, and (4) the toxicological and nutritional characteristics of the novel product compared to its conventional counterpart in appropriate animal models. The similarities noted between the new and traditional crops are not subject to further assessment since this provides evidence that those aspects of the newly developed crop are as safe as crops with a history of safe consumption. The identified differences are subjected to further scientific procedures, as needed, to clarify whether any safety issues or concerns exist. By following this process, the safety assessment strategies for genetically modified crops have proved, over the past 10 years, to be scientifically robust, providing a level of safety assurance that is comparable to, or in some cases higher than, that available for conventional crops. Approximately 30,000 field trials have been conducted with more than 50 genetically modified crops in 45 countries. As an endorsement to the robust nature of the comparative safety assessment process, more than 300 million cumulative hectares of genetically modified crops have been grown commercially over the past decade with no documented adverse effects to humans or animals.

Numerous independent evaluations of genetically modified crop assessment strategies by scientific organizations (e.g., WHO, FAO, OECD, EU Commission, French Medical Association, US National Academy of Sciences, Society of Toxicology) have concluded that current safety assessment processes for today’s genetically modified crops are adequate to determine whether significant risks to human or animal health exist. Indeed, a number of these reports suggest that the use of more precise technology for genetically modified crops may provide a higher level of safety assurance for these crops than for conventionally bred plants, which are usually untested. For example, the 2001 European Commission Report (EC-Sponsored Research on Safety of Genetically Modified Organisms; Fifth Framework Program—External Advisory Groups, “GMO research in perspective,” report of a workshop held by External Advisory Groups of the “Quality of Life and Management of Living Resources” Programme) summarized biosafety research of 400 scientific teams from all parts of Europe over 15 years. This study stated that research on genetically modified plants and their products following usual risk assessment procedures has not shown any new risks to human health or the environment beyond the usual uncertainties of conventional plant breeding. Another example is a 2002 position paper by the Society of Toxicology, "The Safety of Genetically Modified Foods Produced through Biotechnology," which corroborated this finding. It is, therefore, important to recognize that it is the food product itself, rather than the process through which it is made, that should be the focus of attention in assessing safety. This paper goes on to state that the Society of Toxicology supports the use of the substantial equivalence or comparative assessment concept as part of the safety assessment of foods derived from genetically modified crops.

The assessment process

The methods presently used to assess the safety of foods and feeds from genetically modified crops with improved agronomic traits are directly applicable to nutritionally improved crops. Molecular characterization studies that assess the sequence and stability of the introduced DNA and studies that assess the potential toxicity and allergenicity of any new proteins produced from the inserted DNA are as applicable to nutritionally improved crops as to other genetically modified products. Compositional analyses that quantify expected and unexpected changes in more than 50 key components (e.g., proximates, amino acids, fatty acids, vitamins, minerals, antinutrients) for agronomically improved genetically modified crops are also appropriate for nutritionally improved genetically modified crops. In 2001/2002, the OECD published lists of analytes for the compositional evaluation of specific crops, with the understanding that the need for analysis of specific compounds should be determined on a case-by-case basis. The compositional analyses provide information on the concentrations of macronutrients, micronutrients, antinutritive factors, and naturally occurring toxins. A database that contains detailed information on the composition of conventionally bred crops has been developed and made available by the International Life Science Institute (ILSI) at www.cropcomposition.org.

Any single safety assessment study has strengths and weaknesses, which leads to the conclusion that it is unlikely that any single study is sufficient to assess the safety of a food product whether developed through biotechnology or any other method. Therefore, consideration of the sum total of studies that comprise the safety and nutritional assessment of the crop is necessary to reach a conclusion that the food or feed products derived from a new genetically modified crop are as safe as the food or feed derived from the conventionally bred counterpart. The strength of the risk assessment depends not only on the sensitivity of any single method, but also on the aggregate sensitivity and robustness of the evidence provided by all methods combined.

Analysis of composition

The fundamental concepts used in food/feed assessments have been refined through extensive international dialogue and consensus building. The key concept is the need to determine whether changes other than the intended new trait have occurred in the new crop. It is recognized that statistically significant differences between the modified crop and its counterpart do not necessarily imply an outcome that might have an effect on human or animal health (i.e., the differences may not be biologically meaningful), but may indicate the need for follow-up assessment on a case-by-case basis. Also, the occurrence of unintended effects is not restricted to modifications introduced via biotechnology; unintended effects also occur frequently during conventional breeding. Therefore, the impact of the insertion of DNA into the plant genome as well as the potential of the introduced trait to alter plant metabolism in an unexpected manner must be evaluated in the context of natural variation present in conventionally bred plants.

A detailed agronomic assessment is one important way to help identify unintended effects. The agronomic assessment evaluates unintended effects at the whole-plant level (i.e., the morphological phenotype and agronomic performance data such as yield). Targeted analysis of composition focused on possible changes at the metabolic level (i.e., the biochemical phenotype) is also an important tool to evaluate unintended effects. Where crops have been modified with the specific intent to change nutritional characteristics, the analysis should include examination of metabolites relevant to the modified anabolic and/or catabolic pathways and the impact of such modifications on the metabolites in related pathways. In the case of nutritional improvements that do not directly modify specific metabolic pathways, special attention to the mechanism of action of the desired trait should be considered. Examples of such traits are crops expressing a protein with an amino acid composition that results in higher levels of specific essential amino acids or crops with other desirable functional or organoleptic properties.

Since the types of nutritionally improved crops anticipated are diverse, the safety and nutritional assessment of each new product should be approached on a case-by-case basis, building on the comparative assessment principles and methods applicable to any new food or feed. A significant change in the dietary intake of a nutrient is defined here as a change that meaningfully affects health, growth, or development.

In addition, the safety assessment of foods and feeds containing improved levels of nutrients will take into account the frequency and quantities in which the food or feed is consumed by humans or animals, as well as the existing knowledge concerning the safety of the nutrient in question. Conventional crops vary widely in composition, as indicated in the 2001/2002 OECD consensus documents and in the ILSI crop composition database (www.cropcomposition.org). Determining the most appropriate conventional comparator for a nutritionally improved crop needs careful consideration. In some cases, it may be appropriate to use the closest genetically related or near isogenic variety, considering simply the nutritional impact of the altered component when the modified crop is used as a direct replacement of the comparator. In other cases, where the nutrient composition is altered to an extent that no suitable comparator can be identified within the same crop, the comparator may be a specific food component derived from another food (e.g., a specific fatty acid profile). In these circumstances, the assessment should focus on the safety of the changed levels of the nutrient in the context of the proposed use and intake of the food or feed as well as the safety of the altered crop. It should also be noted that in cases where one part of the plant is eaten by humans (e.g., grain) and other parts are eaten by animals (e.g., forage) compositional analysis of both will need to be examined separately (e.g., seeds vs. seeds and forage vs. forage) and may lead to different results. Targeted compositional analyses using validated quantitative methods will continue to be the core method to assess whether unintended changes have occurred.

Nontargeted methods

Nontargeted "profiling" methods may supplement targeted analytical methods in the future for the detection of unintended effects in genetically modified crops. Examples of profiling methods include functional genomics, proteomics, and metabolomics for analysis of gene expression (for example, mRNA), proteins, and metabolites, respectively. These methods provide a broad view of complex metabolic networks without the need for specific prior knowledge of changes in individual plant constituents or pathways. These techniques have the potential to provide insight into metabolic pathways and interactions that may be influenced by both traditional breeding and modern biotechnology. A major challenge in the use of profiling methods for the detection of unintended effects is determining whether any observed differences are distinguishable from natural qualitative and quantitative variation due to varietal, developmental, soil, and/or environmental factors. In other words, it must be assessed whether the identified differences are biologically meaningful. Nontargeted profiling methods may thus provide additional opportunities to identify unintended effects, but they must be validated for the purpose, and the baseline range of natural variations must be clearly established and verified before they can be used in a regulatory framework. Profiling methods could, however, target specific metabolic pathways and identify expressed genes, proteins, or metabolites for which specific quantitative analytical methods could then be validated for the regulatory studies. These methods could also be used to assess whether there were changes in associated metabolic pathways. Hence, these methods may be useful during the developmental phase of a product because they can help to focus the safety assessment process by identifying the exact compounds that need to be measured in a specific nutritionally improved product.

The role of animal studies

Feeding studies in laboratory animals and targeted livestock species may be useful to assess the nutritional impact of the intended changes (e.g., the nutritional value of the introduced trait). Studies in laboratory animals may also serve a useful role in confirming observations from other components of the safety assessment, thereby providing added safety assurance.

The safety of the intended changes to a crop are normally tested using a tiered approach consisting of bioinformatic structure-activity relationship investigations for sequence homology with allergens and toxins, followed by in vitro determinations of the digestibility of newly expressed proteins and in vivo studies with appropriate animal species. The types of changes assessed in this manner include the newly expressed proteins, any new metabolites present in the improved nutritional quality of the crop, and substantially altered levels of metabolites preexisting in the crop. Because the type of modification to each new crop is unique, the specific scientific procedures for an assessment should be determined on a case-by-case basis. For this purpose, existing OECD toxicology test protocols may be applicable. In some cases, appropriately designed animal toxicity studies can provide an additional measure of safety assurance. In general, however, such studies in laboratory animals and targeted livestock species are unlikely to reveal unintended minor compositional changes that have gone undetected by targeted analysis because they lack adequate sensitivity.

Numerous animal feeding studies have been conducted with approved and commercialized genetically modified crops with improved agronomic traits. All published animal feeding studies have shown that performance of animals fed ingredients from genetically modified crops was comparable to that of animals fed the conventional counterpart. Thus, it has been concluded that routine feeding studies with multiple species generally add little to the nutritional and safety assessment of genetically modified crops that have no intended compositional changes.

Although animal feeding studies with crops (e.g., maize, soybeans, wheat) that are normal components of animal diets can be relevant and meaningful, animal testing of some food products (e.g., vegetables, fruits) presents additional challenges because animals may not normally consume these products (e.g., macadamia nuts can be eaten by humans with impunity, but cause transient paralysis when fed to dogs). In addition, some nutritionally improved crops create special challenges when choosing a comparator. Examples of these challenges include crops with increased nutrient content that enhances animal performance and crops from which an edible coproduct may remain after the desired nutritional ingredient has been extracted for other purposes. It is noteworthy that the most appropriate comparator may, in some cases, be a formulated diet that allows for comparison of the nutritionally improved crop to the conventional crop supplemented with a purified source of the enhanced nutrient (e.g., amino acid or fatty acid).

Animal studies also may play a role in testing the nutritional value of the introduced trait in a nutritionally improved crop. Analyses of nutrient composition provide a solid foundation for assessing the nutritional value of foods and feeds; however, they do not provide information on nutrient availability. Therefore, depending on the specific nutritional modification being introduced, it may be important to assess nutrient bioavailability in relevant animal studies. The intended changes in each nutritionally improved crop will determine which animal studies are most appropriate. Attention is drawn to guidelines being developed by an ILSI Task Force for animal study designs appropriate for nutritionally improved crops developed through biotechnology.

Postmarket monitoring

The premarket safety assessment of genetically modified foods and feeds provides a scientific basis for ensuring the safety of the food and generally eliminates the need for postmarket monitoring. The premarket safety assessment principles applied to foods derived from genetically modified crops are the same as those applied to other novel foods improved through other processes or methods. These scientific procedures and principles provide the basis for concluding that foods from genetically modified crops are as safe as foods with a history of safe use and consumption. Postmarket monitoring has not been a routine requirement in supporting the safety or regulatory approval of food products, except in a few unique instances where there has been a need to confirm premarket dietary intake estimates to ensure safety and/or nutritional impact. For example, in some cases regulators have used active postmarket monitoring for novel (albeit non-genetically modified) foods where such issues were identified in the premarket assessment of food ingredients (e.g., potential for digestive tract side effects of olestra or confirmation of consumer intake levels of aspartame and yellow fat spreads enriched with phytosterols).

Postmarket monitoring may be appropriate when there is a need to corroborate estimates of dietary intakes of a nutritionally improved food with expected beneficial effects on human health. Postmarket monitoring must be based on scientifically driven hypotheses relative to endpoints that potentially affect human safety or health. The investigation of adverse events or the potential for chronic health effects, the confirmation of premarket exposure estimates, or the identification of changes in dietary intake patterns represent examples where, in very specific instances, hypotheses may be appropriately tested through postmarket monitoring programs. In the absence of a valid hypothesis, postmarket monitoring for undefined hypothetical adverse effects from foods from a genetically modified (or non-genetically modified) crop is not feasible and adds nothing to the premarket testing results, while potentially undermining confidence in the overall safety assessment process.

The success of any postmarket monitoring strategy is dependent on the accurate estimation of exposure in targeted or affected population groups and the ability to measure a specific outcome of interest and associate it with exposure. There must be traceability from field to consumer and the ability to control confounding factors. Adequate data must be available, therefore, to assess the use, distribution, and destination of the product or commodity within the food supply. The safety and nutritional quality of nutritionally improved products can only be fully assessed in the context of their proposed uses and consequent human and animal exposure/intake. For example, exposure to enhanced levels of dietary components, such as fatty acids, in particular foods needs to be assessed in the context of total dietary exposure, which may be derived from multiple sources. Although this must be performed on a case-by-case basis, the analysis itself need not be complex. Methodologies for assessing human intake of nutrients and other dietary constituents range from per capita methods to methods that use available food consumption databases or direct consumer food consumption surveys. The analysis does not differ, in principle, from that applied to new food ingredients and food and feed additives. Another factor that may complicate the evaluation of nutritional exposure is the variability of the human diet and the global difference in diets and dietary consumption and, as a consequence, the resulting broad distribution of individual nutritional states. Unfortunately, reliable comprehensive dietary intake data are only available for a few countries.

Conclusions and recommendations

The crops being developed with a focus on improved human or animal nutrition hold great promise in helping to address global food security. The existing comprehensive safety and nutritional assessment processes used to assess the safety of genetically modified foods and feeds already introduced into the marketplace are fitting for nutritionally improved crops, although some additional studies may be needed to assess potential human health effects resulting from changed levels of the improved nutritional factor(s). The comparative assessment process provides a method of identifying similarities and differences between the new food or feed crop and a conventional counterpart with a history of safe exposure. The similarities noted through this process are not subject to further assessment since this provides evidence that those aspects of the new crop are as safe as crops with a history of safe consumption. The identified differences then become the focus of additional scientific studies and evaluation. The types of nutritionally improved products anticipated are diverse; therefore, the safety and nutritional assessment of each new product should be approached on a case-by-case basis. Many nutritionally improved crops have modified biosynthetic and/or catabolic pathways, and the impact of such modifications on metabolites in those and related pathways should be specifically and carefully examined. The use of profiling techniques to detect unintended effects is still limited by the difficulties in distinguishing possible product-specific changes from natural variation due to varietal, developmental, and/or environmental factors, and therefore, building databases containing information on natural variation is of high priority. These profiling methods may be useful as prescreens to help focus the safety assessment process by identifying the specific compounds that need to be measured in a particular nutritionally improved product. Depending on the nutritional modification being introduced, it may be important to assess nutrient bioavailability in relevant animal studies. Animal studies can play an important role in assessing the nutritional impact of the intended changes (e.g., the nutritional value of the introduced trait) and in confirming observations from other components of the safety assessment, thereby providing added safety assurance. Any postmarket monitoring that is deemed necessary must be based on scientifically driven hypotheses relative to endpoints that potentially affect human and animal safety or health. In the absence of an identified risk, postmarket monitoring for undefined adverse effects for foods from nutritionally improved (or any other) crop is virtually impossible to carry out, is unnecessary, and is inconsistent with, and may undermine confidence in, the premarket safety assessment process.

Recommendation 1. All nutritionally improved foods and feeds should be evaluated for their potential impact on human and animal nutrition and health regardless of the technology used to develop these foods and feeds.

Recommendation 2. The safety assessment of a nutritionally improved food or feed should begin with a comparative assessment of the new food or feed with an appropriate comparator that has a history of safe use.

Recommendation 3. The safety and nutritional assessment of any new nutritionally improved crop varieties should include compositional analysis. In cases where the nutrient composition is altered to an extent that no suitable comparator can be identified, the assessment should focus on the safety of the changed levels of nutrients in the context of the proposed use and intake of the food or feed.

Recommendation 4. To evaluate the safety and nutritional impact of nutritionally improved foods and feeds, it is necessary to develop data on a case-by-case basis in the context of the proposed use of the product in the diet and consequent dietary exposure.

Recommendation 5. Current approaches of targeted compositional analysis are recommended for the detection of alterations in the composition of the nutritionally improved crop. New profiling techniques might be applied to characterize complex metabolic pathways and their interconnectivities. These profiling techniques can also be used in a targeted fashion to generate information on specific nutrients or other metabolites. However, before using profiling methods, baseline data need to be collected and the methods must be validated and harmonized globally.

Recommendation 6. Studies in laboratory animals may serve a useful role in confirming observations from other components of the safety assessment, thereby providing added safety assurance. However, studies in laboratory animals and targeted livestock are unlikely to reveal unintended minor compositional changes that have gone undetected by targeted analysis because they lack adequate sensitivity.

Recommendation 7. Animal feeding studies should be conducted in target species to demonstrate the nutritional properties that might be expected from the use of the modified crop, crop component, or coproduct.

Recommendation 8. The premarket assessment will identify safety and nutritional issues before product launch. It is unlikely that any new product with scientifically valid adverse health concerns will be marketed. Postmarket monitoring of nutritionally improved food products may be useful to verify premarket exposure assessments or to identify changes in dietary intake patterns. Postmarket monitoring should only be conducted when a scientifically valid testable hypothesis exists, or to verify premarket exposure assessments.

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Food biotechnology and consumer perceptions in Asia

Georgina Cairns

Key words: Attitude survey, consumer research, food biotechnology, food safety assessment, genetically modified foods, public perceptions

Consumer perception surveys, based on street interviews commissioned by the Asian Food Information Center (AFIC) in 1999 (Singapore, Malaysia, Thailand, Philippines, and Indonesia; $n = 1,200$) and 2002 (China, Philippines, and Indonesia; $n = 600$), found that consumers in the Asia region are open-minded on the subject of food biotechnology. More than 60% of the 2002 survey respondents believed they had eaten foods that contained genetically modified ingredients, and almost all reported that they had taken no action to avoid such products. When asked if they were willing to try a food that contained genetically modified ingredients, 88% said they would definitely or would probably be willing to try, and less than 6% indicated they would probably not or definitely not be willing to try such a product.

In 2003, AFIC commissioned a qualitative study, using focus group discussion methods, to explore in more depth the consumer attitudes revealed by the earlier studies. Two focus groups were held in Makati, Philippines, and Shanghai, China, and three focus groups were held in Mumbai, India. Recruitment methods, screening criteria, and moderation of focus groups were in accordance with the Market Research Code of Conduct.

Analysis of focus group discussions indicates that consumers hold the scientific community and national regulatory bodies in high regard and are confident in the ability of scientists and regulators to manage the safety assessment process of genetically modified crops and food ingredients. Surprisingly, focus group participants demonstrated almost no desire to become better informed themselves on the safety assessment process. Indeed, it became very apparent that all information on safety assessment triggered alarm and confusion, and participants expressed a clear preference to leave scientific authorities and others to do this in their behalf.

The 2003 survey also found that those who were most knowledgeable about biotechnology-derived foods were the most positive about future potential benefits. Respondents in all surveys expressed a keen interest in receiving more information on the potential benefits.

The information sought by focus group participants was primarily on the potential benefits of biotechnology. The benefits most highly valued by respondents, and therefore those which they were most keen to receive information about, were improvements in the nutritional quality of foods and agricultural benefits, especially reduced use of pesticides and more efficient agricultural productivity.

The preferred information channels were overwhelmingly mass media and food packaging. Websites, schools, food companies, and government agencies were less preferred. Focus group participants generally expressed very high levels of confidence in the expertise of intergovernmental organizations such as WHO and FAO and their own national scientific communities (**table 1**). Focus group participants liked the terms "food biotechnology" and "genetically enhanced," but rejected the terms "genetically modified" and "engineered."

The respondents in all three surveys were questioned on food safety and quality concerns. All three surveys confirmed high levels of concern. The issues of greatest concern included zoonoses, microbiological contamination of food and drinks, pesticide residues, food additives and preservatives, and spoilage. Nutritional quality was also an issue of great concern to participants in the survey, who expressed some anxiety on this matter. In all three surveys, food biotechnology ranked very low on the list of concerns (**fig. 1**).

In summary, consumer demand for engagement in the issue is based on an assumption that biotechnology-derived foods are already established in the food

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chain, and that the safety assessment of such foodstuffs is best left to "experts." Consumers do express a desire for further information about food biotechnology, and the information perceived as relevant and in support of consumer choice is that which describes potential benefits to consumers. Information deemed "technical" is not popular; this includes information intended to provide a balanced overview of potential risks and benefits or of the safety assessment process. In other words, current stakeholder debates on the relative economic, political, and environmental benefits or risks of biotechnology do not appear to reflect the actual interests and concerns of consumers. The consumers participating in AFIC's three surveys consistently demonstrated an open-minded approach and were keen to consider and learn more about potential benefits.

TABLE 1. Preferred information channels according to 2003 AFIC survey

Channel	Philippines	China	India
Most effective method	Mass media, e.g., television, newspapers, magazines, and advertisements to reach out to a wider population		
Websites	Greater accessibility for younger population only		
Academics	To educate the young in schools	Suitable, but less outreach	To educate the young in schools
Food packaging	Yes		
NGOs	Yes, but don't know any	As nonprofit organizations, more acceptable than government	Yes, but don't know any
Government agencies	Department of Agriculture, Science, and Technology	Have more faith in scientists	Through consumer forums

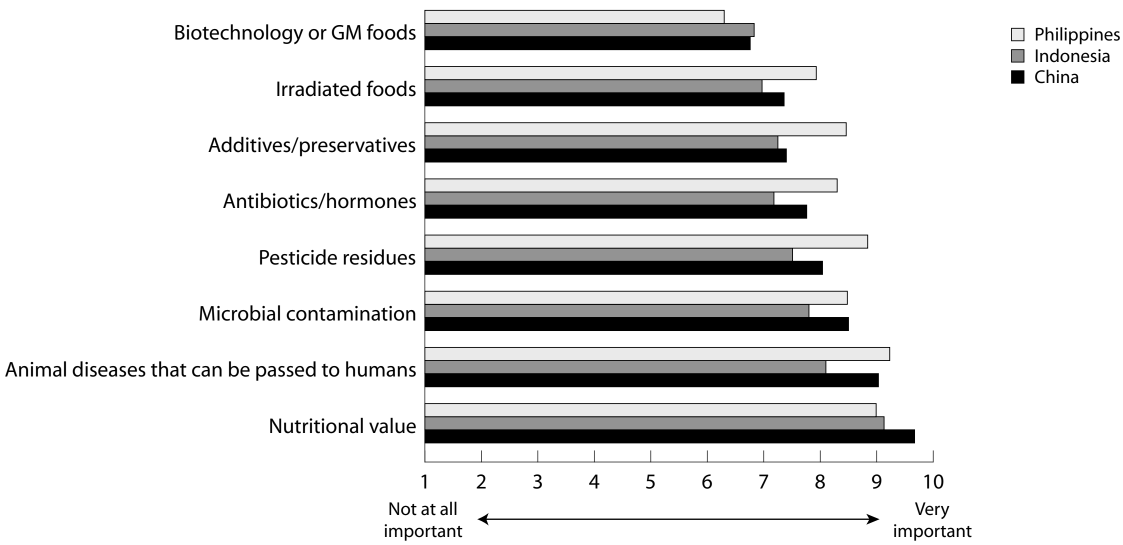

FIG. 1. Ranking of food safety concerns according to 2002 AFIC Survey. Upper bar represents the Philippines, the middle bar Indonesia, and the lowest China.

Abstract

The future of biotechnology for the improvement of nutrition and health: Realizing the potential for Asia

Sakarindr Bhumiratana, Asawin Meechai, and Supapon Cheevadhanarak

The genomics era, which has followed the success of the Human Genome Project, has drastically changed the approach we take to understanding living things. Traditionally, biologists have investigated biological systems by reductionism, providing us with much understanding of the living organisms; but we have yet to understand the underlying mechanisms of the complexity and robustness of the systems. It is quite clear that integrated/systematic approaches are needed if we will ever gain complete understanding of how living systems work, even at a single-cell level. Recently, systems biology, which is an integration of biology, mathematics, and engineering, has emerged and raised high hopes that systematic approaches will be able to integrate the gush of information on genes, proteins, cellular dynamics, and the responses of organisms to mutations and the environment and explain what makes whole organisms behave as they do.

Systems biologists are relying heavily on mathematics and statistics to integrate data into a more complete picture of how biological networks from cells to whole organisms function. Engineering sciences are used to build models and to make predictions about how biological systems will behave; the ultimate goal is to understand deep mysteries—such as how fungi choose their forms, how animals develop, how plant starch granules vary, and how humans and animals yield to pathogenic microbes. These models will allow us to get at fundamental theories on the complexity and robustness underlying each living cell, explaining how genes are expressed in the right order at the right time.

The pharmaceutical industry has already taken advantage of these powerful techniques to identify drug targets and has developed new drugs based on the understanding of mechanisms and regulatory networks at the molecular level. Likewise, the food industry has had an opportunity to position food and nutritional bioactives to promote health and prevent disease, based not only on the genetic constitution of the consumer, but also on the study of nutrient–gene interactions and molecular nutrition. Applying systems biology would allow us to focus on the effects of nutrients or food bioactives on the regulation of gene expression (nutrigenomics) or on the impact of variations in gene structure on responses to nutrients or food bioactives (nutrigenetics). The challenges for Asia are at least twofold. One is to combine this new knowledge to develop new tools to enhance the use and understanding of its rich traditional medicine and functional foods that rely on macro-integrative approaches. The other challenge is to develop, despite the weak infrastructure and limited resources of most Asian nations, systems biology, which requires adequate scientific infrastructure and active interactions among many scientific disciplines.

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Workshop and breakout sessions

The workshop that followed the symposium was chaired by Dr. David Lineback and was prefaced by two papers that expressed the speakers' viewpoints. The first paper, "Viewpoint: Nutritional Needs vs. Realities in the Scene of Biotechnology-derived Nutritious Foods," was given by Dr. Bhattiprolu Sivakumar, Director of the National Institute of Nutrition, India. Dr. Sivakumar focused on the potential of genetically modified foods to satisfy nutritional requirements, giving as an example Golden Rice, which was developed in an attempt to improve vitamin A nutrition by increasing the β-carotene content of rice. However, because of limitations in the bioavailability of β-carotene and its conversion to vitamin A in the body, it would be difficult to satisfy the vitamin A requirements of children with the level of β-carotene in Golden Rice (1.6 μg of carotene/g). Enriching canola oil with β-carotene (600 μg/g) would be better. The quantity and quality of protein in terms of its amino acid content is another case in point. In the genetically enriched transgenic potato (1.6 g protein/100 g), for example, the increase in the amount of protein over that in the nontransgenic potato is so small that it is not significant in terms of protein requirements. However, the improvement in the protein *quality* (about 6%) of transgenic vs. nontransgenic potato is significant. Thus, many technological considerations, in addition to sociological and safety considerations, need to be addressed. In fact, along with or even before studies on safety, careful nutritional evaluation should be considered.

The second paper, "Viewpoint: Opportunities for Biotechnology-Derived Nutritious Foods in Asia," was given by Dr. Soegiono Moeljopawiro, Director of the Center for Plant Variety Protection, Indonesia. The completion of the human genome project has opened up a better understanding of how genes work and to what extent they are affected by the environment, including the food we eat. The present socioeconomic condition in Asia has resulted in the rise of chronic degenerative diseases of overnutrition, such as obesity, diabetes, cardiovascular disease, and some forms of cancer, at the same time as deficiencies of vitamin A, iron, folic acid, calcium, and other nutrients continue to affect the poorer segments of the population. The application of nutrigenomics in the control of these conditions, as well as in the development of nutritious foods, functional foods, and dietary supplements, will be of great benefit to the population. To respond to the objections raised against biotechnology, there is a need for meaningful communication between scientists and the general public. The public will benefit from understanding how biotechnology has developed and how it influences society, while scientists and technologists will benefit from the understanding and appreciation of the nontechnical issues raised by the inquiring public. Dr. Moeljopawiro concluded that we have to balance between the need to protect the environment and the need to encourage scientific exploration.

In the breakout sessions that followed, the participants agreed that dietary energy deficiency, protein–energy malnutrition, and micronutrient deficiencies, including vitamin A deficiency, anemia, iodine deficiency disorders, and deficiencies of zinc and folate, constitute the nutritional priorities for the majority of the populations in Asia. At the same time, infections continue to affect the nutritional status of vulnerable groups. On the other hand, the problem of overnutrition and obesity and the resulting chronic noncommunicable diseases is growing among the more affluent sectors of society. Further discussion centered on the methodology of determining priorities. Several suggestions were made, such as prevalence surveys, health impact, and effect on socioeconomic conditions.

With regard to macronutrients, it was suggested that modern biotechnology could be applied to improving the protein quality and content of staples as well as nonstaples such as soybeans, sweet potatoes, pumpkin, papaya, and cassava. Raising the level of limiting amino acids (e.g., tryptophan, lysine, and sulfur-containing amino acids) would improve the protein quality of these foods. Research should also be directed to improving the fatty acid profile of fats and oils and animal foods, for example by increasing the level of omega-3 fatty acids or altering the proportion of saturated and unsaturated fatty acids; lowering the cholesterol content of animal foods; developing nutritious complementary foods; enhancing functional ingredients such as phytochemicals and dietary fiber; and improving the agronomics of agricultural crops to increase their yield, help in pest control, conserve water, and increase drought resistance, thus helping increase food supply and enhancing food security. Improving water safety was another area in which it was suggested that modern biotechnology could play a role.

With regard to micronutrients, the groups suggested that modern biotechnology could be applied to enhancing the micronutrient content of foods (e.g., β-carotene, iron, zinc); lowering the content of inhibitors, enhancing the content of promoters, or otherwise improving the bioavailability of micronutrients such as iron and zinc; reducing undesirable elements (e.g., oxalates, phytates, and toxic substances such as cyanide in cassava); promoting beneficial binders; and developing new products such as fortified complementary foods. However, there is still a need to understand the complexities of the bioavailability and metabolism of nutrients in order to avoid undesirable nutrient interactions. The participants noted that there has been no work on improving the iodine content of foods, since the gene has not been identified. On the other hand, it was agreed that there is no single or simple approach to improving the micronutrient quality of foods. It was suggested that it might be better to work on multiple micronutrients at the same time. However, in general, extreme care must be exercised in choosing the source of the gene in order to avoid problems of safety.

Opportunities are rising for implementing modern biotechnology in improving the nutrition of populations in Asia. The groups noted the rapid advances in the science and technology of genomics, particularly proteonomics, nutrigenomics, and bioinformatics, followed by the improving prestige of biotechnology research. The use of the systems approach has enabled the integration of biotechnology, mathematics, and engineering. There have been widening interdisciplinary partnerships among biotechnologists, nutritionists, and agriculturists, thus enhancing the feasibility of research collaboration through networking. Not only is the interest in genetically modified foods slowly increasing in the region, but the awareness of the benefits of modern biotechnology is improving. Thus opportunities exist for continuing the dialogue among nutritionists, biotechnologists, and fund raisers. It was suggested that perhaps ILSI could facilitate longer exchange of scientists in the region as a strategy for capacity building. Information technology could be harnessed in improving public perception of modern biotechnology. Finally, it was pointed out that there are other technologies that could go hand in hand with modern biotechnology in improving the nutrition of peoples.

There are, however, many barriers to implementing modern biotechnology for nutritional improvement. Apart from technical problems, the groups recognized that there is still equivocal acceptance by the public of modern biotechnology arising from ignorance of its benefits and safety, not to mention other ethical and sociocultural issues. There are regulatory issues, including nontariff trade barriers and intellectual property rights; added cost of novel products; limited resources and funding for research; limited investment in modern biotechnology; lack of scientific manpower; and low level of political commitment.

The groups then attempted to prioritize the areas where modern biotechnology should be applied, based on benefits, costs, proven efficacy, barriers, impacts, and penetration to the general population. Most of the participants agreed that increasing the food supply, improving the nutritional quality of foods, and ensuring food safety are the three priority areas for modern biotechnology.

Applying biotechnology to increasing the food supply was deemed to have the maximum benefit and impact on the population, as it would contribute to solving the problem of protein–energy malnutrition. As has been pointed out earlier, modern biotechnology has proven to be successful in improving the yield of agricultural crops and therefore helping achieve food security.

With regard to improving the nutritional quality of foods, the groups focused on increasing the iron content of staples such as rice and wheat, enhancing the provitamin A content of nonstaple crops, and improving the protein quality of agricultural products.

Improving the iron content of rice and other cereals has the benefits of wide reach, maximum impact, and easy acceptance by the population, while toxicity would not be an issue. There will, however, be a need to determine the bioavailability of the added iron and to show efficacy and effectiveness in improving iron status. Although a positive impact is likely, it might take a long time to demonstrate. Aside from increasing the iron content of foods, reducing absorption inhibitors, or enhancing absorption promoters, it might be possible for genetic engineering to incorporate heme iron or ferritin in plant foods to improve iron bioavailability.

Enhancement of the provitamin A content of plant foods has already been demonstrated. This has the benefit of easy consumer acceptance, because the host foods are widely consumed, gene sources are available, and toxicity is not an issue. However, there will be a need to demonstrate effectiveness because of the generally low fat consumption of the population. Incidentally, while working on β-carotene, it might be possible to look into the other carotenoids and other functional ingredients that might be of benefit to health.

Improving the protein quality of cereals and pulses would be of great benefit to the population in Asia, considering the high prevalence of protein–energy malnutrition. The challenge is in the selection of markers to increase the content of specific amino acids deemed lacking in the diet.

The groups also recognized that there are different technologies that could be used to improve nutrition, from conventional plant breeding, to DNA marker-assisted selection, and to transgenic technology. The advantages and disadvantages of these technologies, as well as opportunities for and barriers to their use, were discussed.

In the last part of the Workshop, the participants recommended several steps to pursue in order to implement the prioritized approaches. Apart from efforts to improve public funding and political commitment, there is a great need for capacity-building in research and development in modern biotechnology, not to mention the need to improve the expertise of the regulatory agencies in assessing risk and implementing the regulations on genetically modified foods. Training of scientists and leadership training in the area of modern biotechnology should be carried out in order to achieve a critical mass of scientists and technologists. Designation of Centers of Excellence within the region could be a first step. Networking of scientists within the country and within the region through working groups would promote scientific exchange and cooperation. Engaging existing and new stakeholders and reaching out to scientific organizations in regular dialogue would invite acceptance by technical groups, while effective public information, education, and communication in the areas of consumer concerns would promote general acceptance by the population. At the same time that a regulatory framework is put in place, the creation of a biosafety clearing house or a national committee on modern biotechnology in the country would build consumer confidence in genetically modified foods. Finally, the participants suggested that this ILSI symposium and workshop should be followed up by further meetings among agriculturists, nutritionists, and biotechnologists, perhaps together with social scientists and economists, to pursue discussions on the many issues of modern biotechnology, together with the recommendations of this workshop.

In summary, Dr. David Lineback expressed optimism about the future of modern biotechnology in this area of the world in light of the excellent presentations and fruitful discussions in this Symposium and Workshop, bolstered by his own observations on the scientific and technological advances that have been made in the past few years in the face of new challenges confronting Asia. Although there may have been a diversity of opinions and approaches, many similarities were expressed and many useful agreements were reached. At the same time that barriers to modern biotechnology were recognized, many opportunities for pursuing biotechnology were identified. Dr. Lineback hoped that dialogue among biotechnologists, nutritionists, agriculturists, and scientists from other disciplines would continue to advance the use of modern biotechnology in improving the condition of the peoples of Asia.

In closing, Mrs. Yeong Boon Yee, Executive Director of ILSI Southeast Asia Region, expressed her own pleasure on the outcome of the Symposium-Workshop and thanked everyone for making the effort to come and participate. On behalf of ILSI SEAR, she particularly thanked FAO and the other organizers for their support of and contribution to the success of the meeting.

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Letter to the Editor

Urinary iodine excretion levels among young adult women in a district with endemic iodine deficiency in Haryana State, India

To the Editor:

Iodine deficiency is a major public health problem in India. In Haryana State, India, nine districts were surveyed, and eight were found to have endemic iodine-deficiency disorders. In Faridabad District, the prevalence of goiter was reported as 20.5% [1]. Universal salt iodization has been implemented in the state since 1987, and a ban on the sale of noniodized salt for human consumption is in effect [2]. A reliable method of assessing iodine deficiency in a population is to estimate urinary iodine excretion (UIE) levels [3].

No study had previously assessed iodine deficiency among young adult women (most of them future mothers) in Haryana State. Therefore we conducted a community-based cross-sectional survey in six randomly selected villages of a rural community in Faridabad District, Haryana State, India, from November 2000 to October 2001. A total of 277 women (mean age, 20.3 ± 2.2 years) were enrolled in the study. The women were recruited by the authors, who visited the women in their homes. The Ethics Committee of the All India Institute of Medical Sciences, New Delhi, approved the study.

The objectives of the study were explained to the women and oral informed consent was obtained. A pretested questionnaire was administered to each woman to collect demographic and socioeconomic data [4]. The women were asked to provide "on the spot" casual urine samples, collected in plastic screw-capped bottles. The samples were refrigerated until the time of analysis. UIE levels were analyzed by the wet digestion method. Levels < 100 µg/L indicated iodine deficiency [5]. As an internal quality control measure, the UIE estimates of the pooled urine sample with known UIE levels were compared with the UIE estimates of the women's individual urine samples. If the result of the pooled urine (control) sample was outside the range, the whole batch was repeated.

Women were also asked to provide 20 g of the salt currently being consumed in their household in autoseal (automatically sealed) polyethylene pouches. The iodine content of the salt was estimated by the iodometric titration method [6]. The data collected were subjected to statistical tests with SPSS version 7.5 software.

Most of the women (78.1%) were of lower-middle-class or middle-class socioeconomic status; 23.3% were illiterate. UIE levels of < 20.0, 20.0–49.9, 50.0–99.9, and ≥ 100.0 µg/L were found in 1.8%, 2.5%, 5.8%, and 89.9% of the women, respectively. The median UIE level of the subjects was > 200 µg/L. Analysis of 277 salt samples revealed that all were iodized. In the present study, the median UIE level was more than 200 µg/L, and not more than 20% of the women had UIE levels less than 50 µg/L, indicating sufficient iodine nutriture among these women. The findings of the study may be a positive indicator of the success of the Universal Salt Iodization program in Faridabad District, Haryana State.

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Book reviews

The cultural politics of food and eating: A reader. Edited by James L. Watson and Melissa L. Caldwell. Blackwell Publishing, Malden, Mass., USA, 2005. (ISBN 0-631-23093-9) 320 pages, softcover. US$29.95.

For anyone interested in the globalization and international marketing of food products, this book is fascinating reading and difficult to put down. It is, however, difficult to review because its 19 case studies are so diverse. The six studies in Part I, titled "Food and globalization," include "How sushi went global," "Coca-Cola: a black sweet drink from Trinidad," and "China's Big Mac attack." Part II, with six more chapters, is titled "Gentrification, yuppification, and domesticating tastes," with six more case studies that include "The rise of yuppie coffees and the re-imagination of class in the United States," "Globalized childhood?: Kentucky Fried Chicken in Beijing," and "Domesticating the French fry: McDonalds and consumerism in Moscow." The final seven case studies, listed in Part III under "The political economy of foods," include "Industrial tortillas and folkloric Pepsi: The nutritional consequences of hybrid cuisines in Mexico," "The global food fight," and "The bakers of Bernburg and logics of communism and capitalism."

The case studies, for both undergraduate and graduate courses in food marketing or food policies, are revealing and educational, and the topics will make for stimulating discussions.

Diet and human immune function. Edited by David A. Hughes, L. Gail Darlington, and Adrianne Bendich. Humana Press, Totowa, N.J., USA, 2004. (ISBN 1-58829-206-1) 465 pages, hardcover. US$145.

No topic is more relevant to understanding the relationships among nutrition, health, and disease than the interactions of diet and immune function. This authoritative review documents the ability of diet to enhance immune function in health, in disease, and in response to stress. The influence of the immune system on key nutrients singly and in combination is given critical consideration. The book also examines how nutrition modulates immune function in various disease states and in three specific stressful situations: vigorous exercise, military conditions, and air pollution.

Another area looks at the nutritional consequences of drug–disease interactions and leads to nutritional interventions that can increase drug efficiency or decrease side effects. The authors try to provide data-driven answers to professional questions based on the totality of the evidence. This is a valuable text that provides timely, comprehensive, and well-referenced and -indexed information. Nutrition and health workers need access to this book. An appendix lists related books and websites.

Genomics and proteomics in nutrition. Edited by Carolyn Berdanier and Naima Moustaid-Moussa. Marcel Dekker, New York, 2004. (ISBN 0-8247-5430-1) 507 pages, hardcover. US$175.

Nutrition is entering a new era in which nutritionists must be familiar with the links between nutrients and gene expression that are responsible for metabolic variations among species and individuals. We are learning more and more about nutrients, gene expression, and organ, tissue, and cellular function. There are now two distinct but related approaches to molecular biology: genomics and proteomics. A third, metabolics, links these two approaches with changes in intracellular metabolites. The book explores the question of how genetic expression regulates the production of gene products. The 20 chapters have 47 authors, which gives some indication of the growing complexity of the subject. It has been preceded by other volumes in the series, including the third and most recent, *Nutrient–Gene Interactions in Health and Disease* (2001).

The first six chapters describe experience with the mouse model, the next seven deal with the use of large-scale microarrays for detecting gene expression, and the final seven focus on large-scale analysis of protein expression. The genome of an organism is relatively static, whereas its expression, the proteome, is a constantly changing mosaic of peptides and proteins of multiple functions and fates. The preface refers to the relationships of individual nutrients and gene expression with the signs and symptoms of acute and chronic nutrition-related diseases. A chapter entitled "Proteomics: Tools for nutrition research in the post-genomic era" gives examples from zinc and obesity research. The chapter "Phytochemicals and gene expression" is also overtly relevant to nutrition. However, nutritionists will be disappointed that, despite the title, there is little effort in this volume to link the relationships of genomics and proteomics to human and clinical nutrition.

Human diet: Its origin and evolution. Edited by Peter S. Ungar and Mark F. Teaford. Bergin & Garvey/Greenwood, Westport, Conn., USA, 2002. (ISBN 0-89789-736-6) 206 pages, hardcover. US$69.95.

Anthropologists, biologists, and nutritionists are in agreement that the human race evolved eating a diet with a very wide variety of vegetables, fruits, and fungi, as well as an extremely wide range of foods of animal origin, depending on seasonal and geographic availability. The latter included not only mammals, rodents, fish, and birds, but also many invertebrate species.

The book attempts to explain why an understanding of the food preferences of our distant ancestors can help us today. Early chapters describe links between recent changes in our diets and disease. In doing so, they describe the principal methods used to reconstruct the diets of our ancestors. In the section on diet and health, one chapter reviews the bioarcheological evidence from the bones and teeth of the fossil record for the effects of the transition to agriculture.

Evidence is presented that we began to evolve our unique ability to consume a broad spectrum of foods more than four million years ago. Modern hunter-gatherers have provided useful insights for reconstructing early hominid diets. One chapter notes that their children depend on plant foods, including tubers, gathered by their mothers and grandmothers. Several chapters suggest that many common health problems relate to discordance between what we eat today and what our bodies have evolved to eat. But if we attempt to return to our ancestral diet, there is no single dietary regime that characterized pre-agricultural peoples. They lived in a wide range of environments and made use of a broad range of resources. However, all were characterized by a wide variety of foods of plant and animal origin and by diets that were quite different from the low-fiber and high-fat, high-salt, and high-sugar diets of industrialized countries today.

In 1988, a book entitled *The Paleolithic Prescription: A Program of Diet and Exercise and a Design for Living* (Harper and Row, New York) deservedly attracted considerable attention. Its thesis is that drastic changes in the diet and physical activity for which we are genetically adapted are major factors in the epidemic of chronic disease associated with modern lifestyles. This new book on the origin and evolution of the human diet has the same thesis but is confined to the implications of the historical, archeological, and anthropological data and information on living aboriginals. Although this book does not offer specific dietary or behavioral guidelines, it well documents the problems associated with modern diets and lifestyles and reinforces the conclusions of *The Paleolithic Prescription*.

Both books conclude that our historical species-specific dietary adaptations involved mainly the consumption of many different foods. This ability was only in part biological adaptation; it also involved cultural innovations to procure, process, prepare, and store foods. Readers will enjoy this multidisciplinary exploration of the origin, evolution, and health correlates of human diets since the emergence of *Homo sapiens* and perhaps will be motivated to improve their own diets.

Phytochemicals: Mechanisms of action. Edited by Mark S. Meskin, Wayne R. Bidlack, Audra J. Davies, Douglas S. Lewis, and R. Keith Randolph. CRC Press/Taylor & Francis Group, Boca Raton, Fla., USA, 2004. (ISBN 0-8493-1672-3) 202 pages, hardcover. US$119.95.

To a considerable extent this book is complementary to *Human Diet: Its Origin and Evolution,* also reviewed in this *Bulletin*, because it offers explanations for why a variety of plant foods are desirable in the human diet and are more healthful than more restricted diets. An increasing number of phytochemicals are being discovered in plants that have antioxidant and other health benefits.

Chapters highlight recent developments in three areas: pharmacokinetics and bioavailability, mechanisms of action, and methods of evaluating effectiveness. Pharmacokinetics and bioavailability address how phytochemicals get into the body and how they are metabolized. Mechanisms of action explain how phytochemicals work at the cellular level. The third area considers possible biomarkers and methods for evaluating clinical effectiveness.

With only 202 pages, this book is less comprehensive than the one on the same subject reviewed in the June 2005 (Vol. 26, No. 2) *Bulletin*, *Phytochemicals in Health and Disease* (346 pages). Its emphasis on mechanisms of action gives it some supplementary value, but there is considerable overlap. Each book has chapters on polyphenols, flavonoids as cancer preventives, and the antioxidant resveratrol. Although found in many plants, the latter has received recent attention because of its high content in red grape skins, red grape juice, and red wine. Both books are useful references on the topic of phytochemicals and strengthen arguments for a variety of plant foods in human diets. Taken together, these two books make a convincing case for a variety of plant foods in the diet.

Public health nutrition. The Nutrition Society Textbook Series. Edited by Michael J. Gibney, Barrie M. Margetts, John M. Kearney, and Lenore Arab. Blackwell Publishing, Ames, Iowa, USA, 2004. (ISBN 0-632-05627-4) 378 pages, softcover. US$64.99.

Public health nutrition is now well established as a major discipline in its own right, although intimately related to both human nutrition and preventive medicine. This makes it an essential course in schools of public health and graduate schools of nutrition. Public health nutrition is advancing rapidly, and existing books for such a course have become outdated.

This book is part of the Human Nutrition Textbook Series from the (British) Nutrition Society and fills this need admirably. Appropriately, it focuses on the nutrition and health of populations and not individuals. After an overview of public health nutrition, it reviews the principles of epidemiology and the assessment of nutritional status in individuals and populations. It examines public health nutrition strategies for intervention at the ecological and individual level.

Other chapters deal with dietary guidelines, food choice, overnutrition, undernutrition, and the key micronutrient deficiencies of vitamin A, iodine, iron, folate, and related B vitamins. The coverage continues with chapters on nutrition and child development, infant feeding, cardiovascular disease, diabetes, cancer, and osteoporosis. The chapter entitled "Maternal nutrition, fetal programming and adult chronic disease" is written by a pioneer in this topic, David J. P. Barker, with Keith M. Godfrey. There are no references in the text, but at the beginning of each chapter there is a "Key Messages" box, plus "Further Reading" lists, and, where relevant, websites are listed at the end of each chapter.

Although an effort has been made to cover the public health problems of developing societies and those in transition, the examples are too limited for this purpose in some chapters. A serious deficiency is the scant treatment given nutrition and immunity and the synergistic interactions between nutrition and infection that are so critical for developing countries. It is intended as an introductory course and is highly recommended for this purpose; however, it should be recognized that this text alone is not expected to prepare health professionals for actual population intervention and field research. If used in a course for advanced students, it should be supplemented with additional material. The three-volume *Nutrition: A Comprehensive Treatise*, edited by George H. Beaton and Earle Willard McHenry (Academic Press; 1964) is still good for this purpose, provided it is extended with more recent research findings.

Reviews in food and nutrition toxicity. Volume 4. Edited by Victor R. Preedy and Ronald R. Watson. Taylor & Francis Group, Boca Raton, Fla., USA, 2005. (ISBN 0-8493-3519-1) 304 pages, hardcover. US$119.95.

Volume 4 of this series includes the latest reviews written by contributors from medicine, public health, and environmental sciences of the most recent issues related to the toxicology of foods. The topics discussed in this volume include the following:

» The extent to which postnatal exposure through breastfeeding can impair an infant's health
» Selenium bioavailability and metabolism, effects of selenium exposure, and mechanisms of selenium toxicity
» The fate of toxic and nontoxic arsenic compounds in the human body upon ingestion
» The biological role of sulfur and its metabolism, deficiencies, and toxicity
» The effects of fluoride on the teeth, bones, kidneys, arteries, hormones, brain, and reproductive system
» Food-borne disease outbreaks, microbial quality, and risk assessment of food-borne microorganisms in ready-to-eat foods
» Effect of T-2 toxin on DNA and chromosomes, circulatory system, skin, reproductive system, liver and spleen, gastrointestinal tract, brain and neurotransmitters, and more
» Investigation of the interference of AFB1 with molecular components of cell-cycle checkpoints
» Analysis of cycad consumption, its effects on neurological systems, and its manifestations; detailed description of biochemical changes and morphologic or pathologic outcomes over time
» Lectins in the human diet, toxicity and biological effects, immunomodulatory effects, and modulation of immune function by dietary lectins in disease

Most of these topics are of direct interest to nutrition and food scientists and are authoritatively presented. Anyone looking for a review of any of the above will find it useful to refer to this book.

—Nevin S. Scrimshaw

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The International Atomic Energy Agency (IAEA)
The United Nations Children's Fund (UNICEF)

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Nongovernmental organizations

International Life Sciences Institute (ILSI)

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Useful web sites and free materials

Access to Global Online Research in Agriculture (AGORA)	www.aginternetwork.org/en/about.php
Food and Agriculture Organization (FAO)	www.fao.org
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Abstract. An abstract of not more than 250 words should be included at the beginning of the manuscript, in the following format:

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—corporate author:

2. Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended method for the determination of gammaglutamyltransferase in blood. Scand J Clin Lab Invest 1976;36:119–25.

Book or other monograph reference

—personal author(s):

3. Brozek J. Malnutrition and human behavior: experimental, clinical and community studies. New York: Van Nostrand Reinhold, 1985.

—corporate author:

4. American Medical Association, Department of Drugs. AMA drug evaluations, 3rd ed. Littleton, Mass, USA: Publishing Sciences Group, 1977.

—editor, compiler, chairman as author:

5. Medioni J, Boesinger E, eds. Mécanismes éthologiques de l'évolution. Paris: Masson, 1977.

—chapter in book:

6. Barnett HG. Compatibility and compartmentalization in cultural change. In: Desai AR, ed. Essays on modernization of underdeveloped societies. Bombay: Thacker, 1971:20–35.

World Wide Web reference

7. WHO HIV infections page. WHO web site. Available at: http://www.who.int/topics/hiv_infections/en/. Accessed 12 October 2004.
8. Nielsen J, Palle V-B, Martins C, Cabral F, Aaby P. Malnourished children and supplementary feeding during the war emergency in Guinea-Bissau in 1998–1999. [serial online]. Am J Clin Nutr; 2004; 80:1036-42. Available at: http://www.ajcn.org/cgi/content/full/80/4/1036. Accessed 12 October 2004.

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