

Contents

Fortification

- Thirty years of a ban on the sale of noniodized salt: Impact on iodine nutrition in children in Himachal Pradesh, India —U. Kapil, T. D. Sharma, P. Singh, S. N. Dwivedi, and S. Kaur 255
- Effect of fortification of drinking water with iron plus ascorbic acid or with ascorbic acid alone on hemoglobin values and anthropometric indicators in preschool children in day-care centers in Southeast Brazil —C. A. Nogueira de Almeida, J. E. Dutra-de-Oliveira, G. C. Crott, A. Cantolini, R. G. Ricco, L. A. Del Ciampo, and M. E. Costa Baptista 259
- Nutrient addition to corn masa flour: Effect on corn flour stability, nutrient loss, and acceptability of fortified corn tortillas —J. L. Rosado, L. Cassís, L. Solano, and M. A. Duarte-Vázquez 266

Micronutrient supplementation

- Effect of prenatal multiple micronutrient supplements on maternal weight and skinfold changes: A randomized double-blind clinical trial in Mexico —U. Ramakrishnan, T. González-Cossío, L. M. Neufeld, J. Rivera, and R. Martorell 273
- School-based iron and folic acid supplementation for adolescent girls: Findings from Manica Province, Mozambique —P. Horjus, V. M. Aguayo, J. A. Roley, M. C. Pene, and S. P. Meershoek 281

Agriculture research and technology

- Assessment of the nutritional impact of agricultural research: The case of mungbean in Pakistan —K. Weinberger 287
- Commentary on “Assessment of the nutritional impact of agricultural research: The case of mungbean in Pakistan” —B. L. Rogers 295
- Women’s access to food-processing technology at the household level is associated with improved diets at the pre-harvest lean season in The Gambia —I. Silva-Barbeau, S. G. Hull, M. S. Prehm, and W. E. Barbeau 297

Brief Communication

- Serum copper levels among a tribal population in Jharkhand State, India: A pilot survey —U. Kapil and P. Singh 309

The Des Moines Declaration 312

Book reviews 315

News and notes 317

Correction 318

Food and Nutrition Bulletin

Editor: Dr. Irwin H. Rosenberg, Friedman School of Nutrition Science
and Policy, Tufts University, Boston, Mass., USA

Senior Associate Editor: Dr. Nevin S. Scrimshaw

Associate Editor—Food Policy and Agriculture:

Dr. Suresh Babu, International Food Policy Research Institute (IFPRI),
Washington, DC, USA

Associate Editor—Food Science and Technology: Dr. V. Prakash, Central Food
Technological Research Institute (CFTRI), Mysore, India

Statistical Advisor—Dr. William M. Rand, Tufts University School of
Medicine, Boston, Mass., USA

Managing Editor: Ms. Susan Karcz

Manuscripts Editor: Ms. Jill Shuman

Copyeditor: Ms. Ellen Duff

Editorial Assistant: Ms. Shauna Sadowski

Editorial Board:

Dr. Ricardo Bressani, Institute de Investigaciones, Universidad del Valle
de Guatemala, Guatemala City, Guatemala

Dr. Hernán Delgado, Director, Institute of Nutrition of Central America
and Panama (INCAP), Guatemala City, Guatemala

Dr. Cutberto Garza, Professor, Division of Nutritional Sciences, Cornell
University, Ithaca, N.Y., USA

Dr. Joseph Hautvast, Secretary General, International Union of Nutritional
Sciences (IUNS), Department of Human Nutrition, Agricultural University,
Wageningen, Netherlands

Dr. Peter Pellett, Professor, Department of Food Science and Nutrition,
University of Massachusetts, Amherst, Mass., USA

Dr. Zewdie Wolde-Gabreil, Director, Ethiopian Nutrition Institute, Addis
Ababa, Ethiopia

Dr. Aree Valyasevi, Professor and Institute Consultant, Mahidol University,
Bangkok, Thailand

Food and Nutrition Bulletin, vol. 26, no. 3

© The United Nations University, 2005

United Nations University Press

Published by the International Nutrition Foundation for The United Nations University

53-70 Jingumae 5-chome, Shibuya-ku, Tokyo 150-8925, Japan

Tel.: (03) 3499-2811 Fax: (03) 3406-7345

E-mail: mbox@hq.unu.edu

ISSN 0379-5721

Design and production by Digital Design Group, Newton, MA USA

Printed on acid-free paper by Webcom Ltd., Toronto, ON Canada

Thirty years of a ban on the sale of noniodized salt: Impact on iodine nutrition in children in Himachal Pradesh, India

Umesh Kapil, Thakur Dutt Sharma, Preeti Singh, Sada Nand Dwivedi, and Supreet Kaur

Abstract

Background. A survey conducted by the central iodine-deficiency disorders team in Himachal Pradesh, a state in the goiter-endemic belt of India, revealed that 10 of its 12 districts have an endemic prevalence of goiter. The survey was conducted to provide health program managers data to determine whether it would be necessary to initiate intervention measures.

Objective. To assess the status of urinary iodine excretion and household salt iodization levels after three decades of a complete ban on the sale of noniodized salt in this goiter-endemic state in India as measured by assessment of urinary iodine excretion levels and iodine content of salt at the household level.

Methods. The guidelines recommended by WHO/UNICEF/ICCIDD for a rapid assessment of salt iodization were adopted. In each of the 12 studied districts, all senior secondary schools were enlisted and one school was selected by using a random sampling procedure. Two hundred fifty children 11 to 18 years of age were included in the study. Urine samples were collected from a minimum of 170 children and analyzed using the wet digestion method. Salt samples were also collected from a minimum of 170 children and analyzed using the spot testing kit.

Results. All districts had a median urinary iodine excretion level $> 200 \mu\text{g/L}$ and 82% of the families were consuming salt with an iodine content of 15 ppm or higher.

Conclusions. The results of the present study highlight the successful implementation of the salt iodization

program in the state of Himachal Pradesh. This positive impact may be due to the comprehensive strategy adopted by the state government to improve the quality of salt, development of an effective monitoring information system and effective information, education, and communication activities.

Key words: Iodine deficiency disorders, salt iodization, urinary iodine excretion, India

Introduction

Himachal Pradesh, a state in the northwest region of India, is in the sub-Himalayan range. Iodine deficiency disorders (IDD) continue to cause adverse health consequences that affect economic productivity and socio-economic development of populations. It is estimated that more than 200 million people live in IDD-endemic areas in India. Surveys conducted in 34 states and 4 union territories (i.e., an area under direct control of the central government of India) have revealed that out of 312 districts surveyed, 254 are IDD-endemic [1].

Surveys conducted by the central IDD survey team of the Directorate General of Health Services, Government of India, have revealed that 10 out of 12 districts in Himachal Pradesh have an endemic prevalence of goiter—20.9% to 41.6% of the entire population [2].

A survey conducted in 1956 reported a prevalence of goiter of 55% [3]. To ensure adequate availability and use of iodized salt, the government of Himachal Pradesh issued a ban on the sale of noniodized salt for human consumption in 1962 [4]. In 1973, a follow-up survey revealed that the salt iodization program had reduced the goiter prevalence to between 8.5% and 9.1% in the different regions of the state [3]. In the 1990s, an action plan for the prevention and control of IDD in the state was also introduced. It included state and district-level training of functionaries in various departments about the health consequences of IDD. Intensive information, education, and communica-

Umesh Kapil, Preeti Singh, Sada Nand Dwivedi, and Supreet Kaur are affiliated with the All India Institute of Medical Sciences. Thakur Dutt Sharma is affiliated with the Health and Family Welfare Training Center, Kangra.

Please direct queries to the corresponding author: Umesh Kapil, Department of Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, 1-110029 India; e-mail: kapilumesh@hotmail.com.

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

tion (IEC) material was provided by UNICEF to create demand and supply for iodized salt. A monitoring information system (MIS) was also developed to assess the quality and quantity of salt at different points of distribution and consumption. The present study was conducted in 2003 to assess the population levels of urinary iodine excretion (UIE) and levels of household salt iodization under the current iodization program after three decades of implementing a complete ban on the sale of noniodized salt in the state. The goal was to provide data on current iodine nutrition to the health program managers to initiate interventional measures required, if any.

Methods

The rapid survey was undertaken in 2003 in all 12 districts of Himachal Pradesh. The guidelines recommended by WHO/UNICEF/ICCIDD (World Health Organization/UNICEF/International Council for Control of Iodine Deficiency Disorders) for a rapid assessment of salt iodization in a district were adopted [5]. The total population of Himachal Pradesh is 6,077,248; children 11 to 18 years of age account for approximately 546,952 of the total number of residents. Assuming that 75% of the subjects would have adequate iodine nutrition, defined as UIE levels $> 100 \mu\text{g/L}$, at a confidence interval of 95%, relative precision of 15%, and with a design effect of 3, a sample size of 171 children was calculated for each district. The total sample size for the entire study was 2052.

A two-stage sampling methodology was adopted. In each district, all the blocks were enlisted and one

block was selected using purposive sampling to keep the various constraints to data collection in mind (e.g., operational feasibility, mode of communication, and accessibility). In the selected block, all the senior secondary schools were enlisted and one school was selected using random sampling with the help of random number tables for the detailed study. Two hundred fifty students between the ages of 11 and 18 were included in the study and were briefed about the objectives of the study during their morning assembly. Children attending senior secondary schools generally come from 20 to 25 villages within 10 to 15 km of the school, with about 8–10 children from each village. We enrolled more students in the target age group to ensure a sample yielding a wide range of iodine content in the salt samples. The Ethical Committee of the All-India Institute of Medical Sciences, New Delhi, approved this study. Written consent was obtained from the parents of the children included in the study.

A minimum of 170 children from each district were requested to provide “on the spot” urine samples. Plastic bottles with screw caps were used to collect the urine samples, which were stored in a refrigerator at the central laboratory, where a lab technician monitored the samples for purity until they were analyzed. UIE levels were analyzed using the wet digestion method [6]. An internal reference sample with a concentration range (± 2 SD of the known value) of iodine content was run with every batch of test samples. If the results of the internal quality control sample were within the range, then the test was deemed in control. If the results were outside the range, then the whole batch was repeated.

TABLE 1. Urinary iodine excretion levels in the study subjects by district ($n = 2574$)^a

District	<i>n</i>	UIE level ($\mu\text{g/L}$) ^b			
		< 20.0 (%)	20.0–49.9 (%)	50.0–99.9 (%)	≥ 100.0 (%)
Bilaspur	209	0 (0)	0 (0)	4 (1.9)	205 (98.1)
Chamba	218	0 (0)	3 (1.4)	35 (15.9)	180 (81.8)
Hamirpur	238	0 (0)	0 (0)	5 (2.1)	233 (97.9)
Kangra	225	0 (0)	7 (3.1)	33 (14.7)	185 (82.2)
Kinnaur	215	0 (0)	1 (0.5)	7 (3.3)	207 (96.2)
Kullu	208	0 (0)	6 (2.9)	22 (10.6)	180 (86.5)
Lahul & Spiti	211	0 (0)	1 (0.5)	3 (1.4)	207 (98.1)
Mandi	214	0 (0.0)	0 (0.0)	4 (1.9)	210 (98.1)
Shimla	187	4 (2.1)	2 (1.1)	7 (3.7)	174 (93.0)
Sirmaur	220	2 (0.9)	3 (1.4)	35 (15.9)	180 (81.8)
Solan	223	0 (0)	3 (1.3)	15 (6.7)	205 (91.9)
Una	206	0 (0)	0 (0)	2 (1.0)	204 (99.0)
Total	2,574	6 (0.2)	26 (1.0)	172 (6.7)	2,370 (92.1)

a. Sample sizes in tables 1 and 2 are different because table 1 represents the UIE levels and table 2 the iodine content of salt samples collected. Attempts were made to collect at least 170 salt and 170 urine samples from each district, but the exact number could not be collected from each district; hence, the difference in sample sizes in the tables and in text (2052).

b. Median UIE level for all districts $> 200 \mu\text{g/L}$.

Children were also provided with autoseal polyethylene pouches with an identification slip. They were asked to bring four teaspoons of salt (about 20 g) from their family kitchen. A minimum of 170 salt samples were collected from each district using uniform sampling for subject selection and collection of salt samples. The iodine content of salt samples was analyzed using the spot testing kit [7–9].

Results

Table 1 shows the distribution of UIE levels by district in the sample children. We found that 0.2%, 1.0%, 6.7%, and 92.1% of the children had urinary excretion levels of < 20.0, 20.0–49.9, 50.0–99.9, and ≥ 100.0 $\mu\text{g/L}$, respectively. All districts had median UIE levels > 200 $\mu\text{g/L}$, indicating sufficient iodine nutriture in the population studied. This finding was further substantiated by the fact that in all the districts, more than 70% of the families were consuming iodized salt with more than 15 parts per million (ppm) iodine, the standard acceptable level for household consumption as mandated by the Indian Ministry of Health.

A total of 2,553 salt samples were collected from 12 districts of Himachal Pradesh. The distribution of iodine content of salt by district is shown in **table 2**. Salt with no detectable iodine present was consumed by only 3.3% of the study participants. About 82% of the families were consuming salt with an iodine content of 15 ppm or more.

Discussion

The present study was a rapid assessment of iodine nutrition in Himachal Pradesh. According to WHO/UNICEF/ICCIDD recommendations, a population in whom the median UIE is 100 $\mu\text{g/L}$, defined as more than 50% of the urine samples having UIE levels of 100 $\mu\text{g/L}$ and more than 20% of the samples having levels of 50 $\mu\text{g/L}$, does not have iodine deficiency. In the present study, the median UIE was > 200 $\mu\text{g/L}$ and only 1.2% of children had levels less than 50 $\mu\text{g/L}$. These findings indicate that there was no biochemical deficiency of iodine in the subjects studied. This evidence of adequate iodine nutrition could possibly be due to the continued efforts of ensuring a supply of iodized salt to the population in the state. The ban on the sale of noniodized salt is strictly enforced, and the quality of salt transported by railway is vigilantly monitored by

TABLE 2. Iodine content of salt samples collected from study participants ($n = 2553$)^a

District	<i>n</i>	Iodine content (ppm)		
		Nil ^b (%)	< 15 (%)	≥ 15 (%)
Bilaspur	207	2 (1)	17 (8.2)	188 (90.8)
Chamba	214	6 (2.8)	54 (25.2)	154 (72)
Hamirpur	217	6 (2.8)	40 (18.4)	171 (78.8)
Kangra	241	8 (3.3)	22 (9.1)	211 (87.6)
Kinnaur	203	0 (0)	9 (4.4)	194 (95.6)
Kullu	214	9 (4.2)	20 (9.3)	185 (86.4)
Lahul & Spiti	201	5 (2.5)	56 (27.9)	140 (69.7)
Mandi	191	16 (8.4)	24 (12.6)	151 (79.1)
Shimla	179	1 (0.6)	21 (11.7)	157 (87.7)
Sirmaur	193	19 (9)	22 (11.4)	152 (78.8)
Solan	220	7 (3.2)	22 (10.0)	191 (86.8)
Una	245	6 (2.4)	64 (26.1)	175 (71.4)
Total	2,553	8 (3.3)	377 (14.8)	2091 (81.9)

a. Sample sizes in tables 1 and 2 are different because table 1 represents the UIE levels and table 2 the iodine content of salt samples collected. Attempts were made to collect at least 170 salt and 170 urine samples from each district, but the exact number could not be collected from each district; hence, the difference in sample sizes in the tables and in text (2052).

b. No detectable iodine levels present.

the inspectors of the Salt Department of the Ministry of Health before it is loaded on the train. Adequate iodine nutriture has been reported by earlier studies conducted in different districts of Himachal Pradesh, with the median value of UIE at 150 $\mu\text{g/L}$ (Kangra), 140 $\mu\text{g/L}$ (Hamirpur), 195 $\mu\text{g/L}$ (Kinnaur), and 150 $\mu\text{g/L}$ (Solan) [10–13].

Only 3.3% of the salt samples had no detectable levels of iodine, indicating that nearly all the samples available to the study participants was iodized. Approximately 82% of the families were consuming salt with iodine content of 15 ppm or more. The National Family Health Survey (NFHS-2) conducted in 1998–1999 also revealed that almost 90.5% of the families in Himachal Pradesh were consuming salt with the required level of iodine [14]. Studies with larger sample sizes using the 30 cluster sampling methodology need to be conducted for the entire state of Himachal Pradesh.

The results of the present study highlight adequate iodine nutrition as measured by median UIE levels as well as the availability of salt with adequate iodine content to the population in the state. This positive impact may be due to the comprehensive strategy adopted by the state government to improve the quality of salt, development of an effective MIS system, and effective IEC activities.

References

1. Policy guidelines on the National Iodine Deficiency Disorders Control Programme. IDD and Nutrition Cell, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi, India 2003.
2. Tiwari BK, Ray I, Malhotra RL. Policy guidelines on the National Iodine Deficiency Disorders Control Programme. IDD and Nutrition Cell, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, New Delhi, India 1998.
3. Sook SS, Deo MG, Karmarkar MG, Kochupillai N, Ramachandran K, Ramalingaswamy V. Prevention of endemic goiter with iodised salt. *Bull WHO* 1993; 49:307–12.
4. Vir S. Universal iodisation of salt: a mid decade goal. In: Sachdev HPS, Choudhury P, eds. *Nutrition in Children: Developing country concerns*. 1st ed. New Delhi: 1991.
5. WHO/UNICEF/IDD. Indicators for assessing iodine deficiency disorders and their control through salt iodisation. Geneva, World Health Organization, 1994.
6. Dunn JT, Crutchfield HE, Gutekunst R, Dunn D. Methods for measuring iodine in urine. A joint publication of WHO/UNICEF/ICCIDD. Geneva, World Health Organization, 1993.
7. Kapil U, Bhanti T, Saxena N, Nayar D, Dwivedi SN. Comparison of spot testing kit with iodometric titration method in the estimation of iodine content of salt. *Indian J Physiol Pharmacol* 1996; 40: 279–80.
8. Bhasin SK, Dubey KK. Validity of spot testing kit for estimation of iodine content of salt under field conditions in National Capital Territory of Delhi. *Indian J Matern Child Health*. 1997;8:42–3.
9. Kapil U, Dwivedi SN, Seshadri S, Swami SS, Beena, Mathur BP, Sharma TD, Khanna K, Raghuvanshi RN, Tandon M, Pathak P, Pradhan R. Validation of spot testing kit in the assessment of iodine content of salt: a multi-centric study. *Indian Pediatr* 2000;37:182–6.
10. Kapil U, Sohal KS, Sharma TD, Tandon M, Pathak P. Assessment of iodine deficiency disorders in district Kangra, Himachal Pradesh utilizing 30 cluster technique. *J Trop Pediatr* 2000;46:264–6.
11. Sohal KS, Kapil U, Kapil U, Tandon M. Assessment of iodine deficiency disorders in district Hamirpur, Himachal Pradesh. *Indian Pediatr* 1998;35:1008–11.
12. Kapil Umesh, Sharma NC, Ramachandran S, Nayar D, Vashisht M. Assessment of iodine deficiency in district Kinnaur, Himachal Pradesh—a pilot study. *Indian J Pediatr* 1998;65:451–3.
13. Sohal KS, Sharma TD, Kapil U, Tandon M. Assessment of impact of salt iodisation programme on iodine deficiency disorders in district Solan, Himachal Pradesh. *Indian Pediatr* 1999;36:1253–5.
14. International Institute for Population Sciences, Mumbai, India. NFHS (2000) India 1998–1999. National Family Health Survey-2 (NFHS-2). Nutrition and the prevalence of anemia, 2000.

Effect of fortification of drinking water with iron plus ascorbic acid or with ascorbic acid alone on hemoglobin values and anthropometric indicators in preschool children in day-care centers in Southeast Brazil

Carlos Alberto Nogueira de Almeida, José Eduardo Dutra-de-Oliveira, Gerson Claudio Crott, Alessandro Cantolini, Rubens Garcia Ricco, Luiz Antonio Del Ciampo, and Marina Elisa Costa Baptista

Abstract

Background. Iron-deficiency anemia currently is the most frequently occurring nutritional disorder worldwide. Previous Brazilian studies have demonstrated that drinking water fortified with iron and ascorbic acid is an adequate vehicle for improving the iron supply for children frequenting day-care centers.

Objective. The objective of this study was to clarify the role of ascorbic acid as a vehicle for improving iron intake in children in day-care centers in Brazil.

Methods. A six-month study was conducted on 150 children frequenting six day-care centers divided into two groups of three day-care centers by drawing lots: the iron-C group (3 day-care centers, $n = 74$), which used water fortified with 10 mg elemental iron and 100 mg ascorbic acid per liter, and the comparison group (3 day-care centers, $n = 76$), which used water containing only 100 mg ascorbic acid per liter. Anthropometric measurements and determinations of capillary hemoglobin were performed at the beginning of the study and after six months of intervention. The food offered at the day-care centers was also analyzed.

Results. The food offered at the day-care center was found to be deficient in ascorbic acid, poor in heme iron, and adequate in non-heme iron. Supplementation with fortified drinking water resulted in a decrease in the prevalence of anemia and an increase in mean hemoglobin

levels associated with height gain in both groups.

Conclusions. Fortification of drinking water with iron has previously demonstrated effectiveness in increasing iron supplies. This simple strategy was confirmed in the present study. The present study also demonstrated that for populations receiving an abundant supply of non-heme iron, it is possible to control anemia in a simple, safe, and inexpensive manner by adding ascorbic acid to drinking water.

Key words: Anemia, iron deficiency, drinking water, ascorbic acid, growth, dietary supplements

Introduction

Iron-deficiency anemia (IDA) is currently the most frequently occurring nutritional disorder worldwide, affecting an estimated 3.5 billion people of all ages to different extents [1]. Some countries, such as the United States, have been able to reduce or minimize the prevalence of anemia by means of different preventive measures, among them food fortification [2]. In Brazil, regional studies have shown that the prevalence of IDA is substantially increasing, even among people of higher socioeconomic status [3].

The urgent search for ways to prevent and control IDA is justified by the adverse effects of this condition on child development and by the increase in morbidity and mortality induced by this disorder [4]. The current trend is to adopt differentiated strategies based on the age range to which the intervention is targeted and according to the cultural characteristics of the population [5]. Different foods have been fortified in developed countries for preschool children, including milk, cereals, sugar, orange juice, and water [6–8]. Based on studies published by Ferreira, Dutra-de-Oliveira, and others, drinking water in Brazil has been considered an appropriate vehicle for this purpose, especially in institutions such as day-care centers and schools, where it is possible to control fortification in a more effective

Carlos Alberto Nogueira de Almeida, Gerson Claudio Crott, and Marina Elisa Costa Baptista are affiliated with the School of Medicine of the University of Ribeirão Preto. José Eduardo Dutra-de-Oliveira, Rubens Garcia Ricco, and Luiz Antonio Del Ciampo are affiliated with the Faculty of Medicine of Ribeirão Preto, University of São Paulo. Alessandro Cantolini is affiliated with the Department of Pharmaceutical Sciences of Araraquara, University of the State of São Paulo.

Please direct queries to the corresponding author: Prof. Dr. Carlos Alberto Nogueira de Almeida, Rua Tamoios, 262, apt. 154, 14020-700 Ribeirão Preto, SP, Brazil; e-mail: carlosnogueira@directnet.com.br.

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

and safe manner [9–13]. These studies used ascorbic acid mainly to keep iron in solution for a longer time, thus preserving the organoleptic properties of water [13]. The fact that ascorbic acid acts by facilitating the absorption of both iron added to water and non-heme iron from other ingested foods [14–17] has led investigators to consider the possibility that the good results obtained in other studies may be related not only to iron, but also to ascorbic acid.

The objective of the present study was to compare the efficacy of using drinking water fortified with ascorbic acid alone or with iron plus ascorbic acid for the control of iron deficiency in a population of preschool children in Southeast Brazil.

Methods

The study was conducted at six day-care centers in the municipality of Monte Alto, a town of approximately 35,000 inhabitants located in Southeast Brazil at an altitude of 735 meters. This town has a socioeconomic and cultural profile characteristic of all towns located in the interior of the country. The study consisted of a program of drinking water fortification offered at these six institutions. Children were evaluated at two points designated as time zero (before the intervention) and time one (after 6 months of intervention). All 335 children enrolled were initially considered for the study. We then excluded children younger than 12 months or older than 75 months, children who abandoned the institution during the study period and children receiving any type of oral supplementation with iron or vitamins. We also excluded children who had hemoglobin concentrations less than 7 g/dL at time zero; they were referred for individual treatment.

The large number of children who abandoned the institutions did so due to causes not related to our study. This is a frequent practice in day-care centers in Brazil, which are primarily used to shelter the children of working parents. When the parents are unemployed or are on vacation, they usually remove their children from the day-care centers. The working team did not detect any case in which the abandonment of the institutions was related to the intervention. Thus, 150 children from the six day-care centers investigated remained in the study and were divided into two groups: iron-C group (3 day-care centers, 74 children) and a comparison group (3 day-care centers, 76 children). The day-care centers assigned to the control and treatment groups were selected by drawing lots using a random number table. The parents of each child signed an authorization form to participate in the study, which was approved by the Ethics Committee of the University of Ribeirão Preto.

Identical drinking fountains were provided for the six day-care centers studied, consisting of a base with

a faucet and a 20-liter container. The fountains were cleaned every morning by the employees of the institutions and filled with filtered water. A premix prepared at the pharmacy of the local city hall was added to each container. The mix for the day-care centers of the iron-C group consisted of 20 mL of filtered water containing 1000 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 2000 mg ascorbic acid, which provided water with 10 mg elemental iron and 100 mg ascorbic acid per liter. The mix for the day-care centers of the comparison group consisted of 20 mL of filtered water containing 2000 mg ascorbic acid, which provided iron-free water containing 100 mg ascorbic acid per liter. The flasks containing the premix were identical for all day-care centers throughout the study. Only the pharmacist responsible for preparing the flasks and the investigators knew which day-care centers belonged to the iron-C group and which belonged to the comparison group.

The employees of the day-care centers were trained to ensure that only the fortified water would be used. In addition, any faucets that might be used by the children as an alternative source of drinking water were sealed. The employees, who did not know which premix the flasks contained, started to use the fortified water for their personal consumption and for food preparation. Other than iron fortification of the water, no other intervention was implemented at the day-care centers that might influence iron nutritional status. The study was of six months' duration and took place from July 1, 2002 through December 31, 2002.

At time zero, immediately before the fortified water was offered, the weight and height of each child were measured according to internationally accepted standard techniques [18], and always by the same examiner. The material used for data collection included a pediatric scale with 10-g increments used to weigh children who could not stand up unsupported, an anthropometric scale with 100-g increments used to weigh children who were able stand up unsupported, a horizontal height-measuring instrument with 5-mm increments manufactured in the workshop of the university hospital of Ribeirão Preto to measure the length of children up to two years of age, a vertical height-measuring instrument with 1-cm increments, also manufactured in the workshop of the university hospital to measure the height of children older than two years. Repeat measurements were not used. Personal data (date of birth and sex) were also obtained and capillary hemoglobin measured from a blood sample using the HemoCue apparatus previously calibrated and checked after every 10 measurements [19]. Anemia was defined as $\text{Hb} < 11$ mg/dL. The weight-for-age z score, weight-for-height z score, and height-for-age z score were calculated with the aid of the Epi-Info 3.2 software according to the National Center for Health Statistics standards [20]. During the six months of the study, the children were clinically monitored on a

daily basis in order to detect any possible occurrence of problems due to the use of fortified water or of diseases that might interfere with the results. After six months (at time one) the children's anthropometric measurements and blood samples were again taken.

The children's diet was analyzed by considering the standard menu offered by the city to all day-care centers. The menu consisted of four meals (breakfast, lunch, snack, and dinner) prepared in a kitchen that supplied the meals for all day-care centers. Despite small daily variations due to the seasonal nature of some of the foods offered, for calculation purposes we considered the most frequent menu, i.e., rice, beans, meat, and vegetables and derived a mean intake of approximately 1,430 calories per child per day. Nutritional information on ingredients and products was provided by a local nutritionist.

The experimental data concerning the continuous quantitative variables are reported as means \pm SD. After testing the normality of the distribution of the experimental errors around the mean by the one-sample Kolmogorov-Smirnov test, Student's *t*-test for paired samples was used to compare the hemoglobin levels and anthropometric indicators for each experimental group at the beginning and end of the study. Possible differences between the experimental groups

were determined by Student's *t*-test for independent samples with the Welch correction. Proportions were compared by the chi-square test with Yates correction. The level of significance was set at $p < .05$ in all analyses. The GraphPad Instat software (version 3.01, 32 bit for Win95/NT, GraphPad Software Inc., San Diego, CA, USA) and the Microcal Origin software (version 6.0, Microcal Inc., Northampton, Mass, USA) were used to analyze the data.

Results

The amount of water consumed was monitored at all day-care centers and corresponded to 534 ± 152 mL per day for the comparison group and 526 ± 159 mL per day for the intervention group, with no significant difference between groups ($p = .89$). There was no statistically significant difference between the iron-C and comparison groups regarding the proportion of boys (58% and 56%, respectively) and girls (42% and 44%, respectively; $p = .92$). Mean values for age, hemoglobin, and z scores for weight-for-age, height-for-age, and weight-for-height of the children examined at time zero are shown in **table 1**. At time zero, the groups were similar in terms of age ($p = .47$), hemoglobin levels (p

TABLE 1. Hemoglobin concentration and anthropometric indicators for study subjects at time zero and time one ($n = 150$)

Variables	Iron-C group ($n = 74$)		Comparison (ascorbic acid only) group ($n = 76$)		Between-group p
	Mean \pm SD	Paired t -test p	Mean \pm SD	Paired t -test p	
Age at time zero (m)	40.66 \pm 18.54	—	42.66 \pm 15.22	—	.47 ^b
Hb (g/dL)					
Time zero	10.98 \pm 1.66		11.13 \pm 1.28		.53 ^b
Time one	11.54 \pm 1.35		11.95 \pm 1.22		.0547
Difference ^a	0.56 \pm 1.68	.005	0.82 \pm 1.56	< .0001	.34
WAZ					
Time zero	-0.34 \pm 1.43		-0.01 \pm 0.91		.09 ^b
Time one	-0.30 \pm 1.46		0.17 \pm 1.04		.02 ^b
Difference	0.04 \pm 0.61	.62	0.18 \pm 0.42	.0004	.10 ^b
HAZ					
Time zero	-0.36 \pm 1.16		0.12 \pm 0.89		.005 ^b
Time one	-0.13 \pm 1.21		0.36 \pm 0.99		.008 ^b
Difference	0.23 \pm 0.35	< .0001	0.24 \pm 0.46	< .0001	.89 ^b
WHZ					
Time zero	-0.02 \pm 1.26		-0.04 \pm 1.03		.89 ^b
Time one	-0.22 \pm 1.35		0.03 \pm 0.97		.19 ^b
Difference	-0.20 \pm 0.73	.02	0.07 \pm 0.52	.20	.008 ^b

Hb, hemoglobin; WAZ, weight-for-age z score; HAZ, height-for-age z score; WHZ, weight-for-height z score

a. Mean change between the beginning and the end of the study.

b. Unpaired *t*-test with Welch correction.

= .53), weight-for-age z score ($p = .09$) and weight-for-height z score ($p = .89$) but the height of children in the iron-C group was lower than that of children in the comparison group ($p = .005$). The prevalence of anemia (hemoglobin < 11 mg/dL) at time zero was 45.9% for the iron-C group and 31.6% for the comparison group.

After the six months of intervention, there was a significant and similar increase in hemoglobin levels in both groups. Weight-for-age z score was significantly increased only for the comparison group ($p = .0004$), causing the occurrence of a difference between groups at time one. A significant and similar height gain was observed in both groups, with persistence of the difference observed at time zero. There was a significant reduction in the weight-for-height z score only for the iron-C group, but this reduction was not sufficient to create a statistically significant difference between groups at time one.

At time one, the prevalence of anemia had been reduced to 31.1% in the iron-C group and to 17.1% in the comparison group. Analysis of the children's diet, summarized in **table 2**, showed that the total iron supply (11.5 mg) was adequate according to dietary reference intakes (DRI) [21], although only 2.25 mg (19.5%) consisted of heme iron. The diet offered 11.86 mg ascorbic acid, corresponding to only 27.9% of the DRI. Daily clinical observation at the day-care centers showed no side effects or intolerance of the intervention.

Discussion

In a previous study by our group [13], water fortified with 10 mg iron and 100 mg ascorbic acid per liter proved to be efficient in increasing the hemoglobin concentration in children. In that study, the mean hemoglobin value (g/dL) fell from 11.3 ± 1.3 to 10.9 ± 1.2 for the group that received unfortified water, and increased from 10.9 ± 1.1 to 11.7 ± 1.1 ($p < .01$) for the group that received fortified water. However, there were doubts about the role of ascorbic acid in those results because the comparison groups in that study received only unfortified water. The results obtained in the previous study might have been due to the sum of the increased iron and ascorbic acid ingestion, since the role of ascorbic acid as a facilitator of non-heme iron absorption is well recognized [14–17].

It is also possible that a population receiving a satisfactory supply of non-heme iron may benefit from a supply of adequate amounts of ascorbic acid in their usual diet or from fortified foods. In the population studied here, this situation did not occur at the beginning of the study because the content of ascorbic acid in the diet was low (**table 2**).

The design of the intervention used in the present study permitted us to precisely evaluate the role of ascorbic acid as a vehicle for improving iron intake in children because one of the groups received only ascorbic acid while receiving its usual diet, which was rich in non-heme iron. This intervention could be compared with that of the comparison group, which received a

TABLE 2. Analysis of energy, iron, and ascorbic acid content of food provided at day-care centers in the study

Food	Energy value (kcal)	Heme iron (mg)	Non-heme iron (mg)	Ascorbic acid (mg)
Breakfast				
Milk (250 g)	124	0	0.13	2.38
Chocolate powder (15 g)	79	0	0.21	0
Bread (50 g)	145	0	0.35	0
Margarine (7.5 g)	54	0	0	0.01
Lunch				
Rice (290 g)	316	0	2.61	0
Beans (60 g)	70	0	1.63	0
Carrots (13 g)	5.9	0	0.08	0.3
Meat (30 g)	58.3	1.13	0	0
Lettuce (36 g)	6.5	0	0.5	6.48
Snack				
Sweet rice (300 g)	374	0	2.63	0
Supper				
Minestrone soup (300 g)	204	1.12	1.12	2.7
Total	1,436.7	2.25	9.26	11.86

combination of iron and ascorbic acid.

The results showed a significant and similar increase in hemoglobin in both groups, with a tendency toward a greater increase in the comparison group. Thus, we conclude that the addition of iron to the water containing ascorbic acid did not influence the increase in hemoglobin values observed in the two groups. A limitation of our study is that although the results suggest that the change in hemoglobin is similar, or even better, for those in the ascorbic acid only group compared with the combination iron and ascorbic acid group, the absence of a real control group (i.e., no fortification at all) make it unclear whether the change in hemoglobin levels seen in the ascorbic acid only group would have occurred in the absence of any water fortification. The only conclusion we can make from the evidence in this study is that the addition of iron to water fortified with ascorbic acid made no difference in the hemoglobin levels of study subjects over the six months of observation.

When these results are analyzed with evaluation of the diet provided at the day-care centers (table 2), it may be that in this population, the simple stimulation of non-heme iron absorption was sufficient to promote an increase in mean hemoglobin values and a fall in the prevalence of IDA. The total dietary intake of iron could be estimated at 11.51 mg/child/day on average. Although the total iron supply was adequate according to the DRI, only 19.5% consisted of heme iron. Unfortunately, the design of this study does not permit us to discuss whether ascorbic acid has an effect only when dietary iron—heme or non-heme—reaches a certain threshold, because we did not calculate the individual iron intakes. With the use of ascorbic acid, the program becomes less expensive, there are no risks of iron intoxication, and the organoleptic properties of the water are preserved.

In studies conducted in a rural area of Mexico, Garcia et al. [22] and Diaz et al. [23] did not observe an increase in hemoglobin values when they offered lemonade containing 25 mg of ascorbic acid twice a day to women with iron deficiency. Even though they observed an increase in iron absorption, women were still considered to have iron deficiency because of the low ferritin values observed. The women's diets were poor in heme iron and rich in non-heme iron and phytates. In addition to the difference in age range studied by Garcia et al. [22], an important difference in the present study is the fact that the children had low hemoglobin levels at time zero. The women, however, had low ferritin values but satisfactory hemoglobin concentrations. The currently accepted model regarding IDA proposes that recovery from iron deficiency starts with an increase in hemoglobin values, so that children with overt anemia must show more avidity for iron absorption in the intestinal cells [24]. This fact may explain the differences among the results obtained.

Indeed, many studies conducted in the 1970s and 1980s had already demonstrated the possibility of improving iron nutriture by adding sources of ascorbic acid such as fruit [14], fruit juices [14, 17], fortified drinks [15], and vegetables [16] to the usual diet. However, the advantage of the present study was the use of drinking water as a vehicle. Since all children consume water, full coverage of the population is guaranteed.

Regarding anthropometry, the comparison group showed an increase in both weight and height, with a consequent lack of change in the weight-for-height z score indicator between time zero and time one. In contrast, the iron-C group showed only an increase in height z scores, with a consequent fall in weight-for-height z score at time one. The increase in height, observed in both groups, may be considered an expected event when iron nutriture is improved in populations with iron deficiency, since iron is a fundamental element for cell multiplication [25] and the expression of the genetic potential may be limited in the presence of iron deficiency [26]. Most studies have demonstrated this tendency [27–32], although no consensus has been reached. Other investigators have obtained different results, including maintenance [8, 33–35] or even a reduction in the height-for-age z score [36]. The study by Majumdar et al. [32] showed that children with IDA showed an increase in height when they received therapeutic iron supplementation, in contrast to children with adequate iron status, who actually showed a reduction in the same measurement.

With improved hemoglobin concentration, appetite recovery is expected to occur along with increased food ingestion and increased weight-for-height z score [29]. In the present study, this effect was observed only in the comparison group, possibly because children in this group were not exposed to iron. There may have been an accompanying reduction of the potential interference of fortified water with the normal eating processes of the children and with the absorption of the ingested nutrients. In contrast, the children in the iron-C group may have experienced some degree of gastroduodenal discomfort, changes in palatability, or a metallic taste in the mouth, which, although not reported, may have led to decreased food ingestion. The studies published thus far about foods fortified with iron have reported increased [31–33, 37], maintained [8, 33, 34, 38], or reduced [36] weight-for-height z score. The physical growth of a child is a complex process that involves not only dietary factors but also social and psychological factors. It is difficult to control for all of these variables, which almost always leads to conflicting results among studies. In addition, the way iron is offered in each study may also be different, a fact that must influence the results obtained by each investigator. In the present study, we believe that the increase in z scores, especially for the height-for-age z score—which is less prone to acute variations—is indeed relevant since the vehicle

for fortification was water, which is habitually ingested by all children. Thus, fortification of drinking water may be considered the most likely intervention capable of explaining the response observed.

The indiscriminate use of iron salts associated with ascorbic acid is currently being questioned because of the potential increase in oxidative stress leading to ulcerations of the gastrointestinal tract in healthy individuals [39]. This fact has led to an intensified effort in properly selecting the populations that are candidates to receive foods fortified with these two nutrients. On the other hand, there is no doubt about the urgent need to look for efficient and locally adaptable ways of increasing the iron supply for populations with an elevated prevalence of IDA. In this group, access to foods rich in heme iron, mainly meat, is almost always difficult due to the high costs involved. Providing iron via drinking water is a simple strategy whose efficacy has been previously demonstrated [9–13] and confirmed in the present study, and is mainly indicated for popula-

tions whose habitual diet is deficient in both heme and non-heme iron. When the supply of non-heme iron is not abundant, a frequent condition in developing countries, one of the most frequently recommended strategies is to try to increase the bioavailability of the iron naturally present in foods habitually consumed by these populations, such as cereals, vegetables, and legumes [40]. The present study demonstrates that it is possible to reach this objective in a simple, safe, and inexpensive manner by adding ascorbic acid to drinking water, thus guaranteeing the ingestion of this vitamin at safe levels by all children of the institutions included in the study.

Acknowledgments

The authors wish to thank the University of Ribeirão Preto and the Monte Alto City Hall for supporting this study.

References

1. The Micronutrient Initiative and the International Nutrition Foundation. Preventing iron deficiency in women and children: Technical Consensus on Key Issues. New York: International Nutrition Foundation, 1999.
2. Sherry B, Mei Z. Continuation of the decline in prevalence of anemia in low-income infants and children in five states. *Pediatrics* 2001;107:677–82.
3. Monteiro CA, Szarfarc SC, Mondini L. Tendência secular da anemia na infância na cidade de São Paulo (1984–1996). *Rev Saúde Pública* 2000;34(6 Suppl):62–72.
4. Stoltzfus RJ. Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. Summary: implications for research and programs. *J Nutr* 2001;131(2S-2):697S–700S.
5. International Life Sciences Institute. Forging effective strategies to combat iron deficiency. Atlanta, Ga, USA. May 1, 2001.
6. de Paula RA, Fisberg M. The use of sugar fortified with iron tris-glycinate chelate in the prevention of iron deficiency anemia in preschool children. *Arch Latinoam Nutr* 2001;51(1 Suppl 1): 54–9.
7. Fomon S. Infant feeding in the 20th century: formula and beikost. *J Nutr* 2001;131(2):409S–20S.
8. De Almeida CAN, Crott GC, Ricco RG, Del Ciampo LA, Dutra-de-Oliveira JE, Cantolini A. Control of iron deficiency anemia in Brazilian preschool children using fortified orange juice. *Nutr Res* 2003;23:27–33.
9. Ferreira JF, Aranda RA, Bianchi MLP, Desai ID, Dutra-de-Oliveira JE. Utilização da água potável como veículo de nutrientes: estudos experimentais com ferro. *Arch Latinoam Nutr* 1991;41:400–8.
10. Dutra-de-Oliveira JE, Ferreira JF, Vasconcellos VP, Marchini JS. Drinking water as an iron carrier to control anemia in preschool children in a day-care-center. *J Am Coll Nutr* 1994;13:198–202.
11. Dutra-de-Oliveira JE, Marchini JS, Desai ID. Fortification of water with iron: a new strategy for combating iron deficiency in Brazil. *Am J Clin Nutr* 1996;63: 612–3.
12. Beinler MA. Fortification of drinking water with iron and ascorbic acid in eight municipal day-care centers in Brazil. Doctoral dissertation, University of Brasília, Distrito Federal, Brazil, 2002.
13. Dutra-de-Oliveira JE, de Almeida CAN. Domestic drinking water—an effective way to prevent anemia among low socioeconomic families in Brazil. *Food Nutr Bull* 2002;23:213–6.
14. Ballot D, Baynes RD, Bothwell TH, Gillooly M, MacFarlane BJ, MacPhail AP, Gillooly M, Bothwell JE, Bezwoda WR, Mayet F. The effects of fruit juices and fruits on the absorption of iron from a rice meal. *Br J Nutr* 1987; 57:331–43.
15. Hallberg L, Rossander L. Effect of different drinks on the absorption of non-heme iron from composite meals. *Hum Nutr Appl Nutr* 1982;36:116–23.
16. Rossander L, Hallberg L, Bjorn-Rasmussen E. Absorption of iron from breakfast meals. *Am J Clin Nutr* 1979;32:2484–89.
17. Maisterrena JA, Murphy CA, Tovar ZE. [Iron absorption in Mexico: effect of orange juice (authors' transl)]. *Rev Invest Clin* 1977;29:277–82.
18. Cameron N. The measurement of human growth. London: Croom-Helm, 1984.
19. Vanzetti G. An azide-methemoglobin method for hemoglobin determination in blood. *J Lab Clin Med* 1966;67:116–26.
20. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal K, Guo S, Mei Z, Curtin L, Roche A, Johnson C. CDC growth charts: United States. Advance data from vital and health statistics. No. 314. Hyattsville, Md, USA: National Center for Health Statistics. 2000.

21. Food and Nutrition Board. Institute of Medicine. Dietary reference intakes: recommended intakes for individuals. Washington, DC, USA: National Academy Press, 2002.
22. Garcia OP, Diaz M, Rosado JL, Allen L. Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. *Am J Clin Nutr* 2003;78:267–73.
23. Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP. The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* 2003;78:436–40.
24. Paiva AA, Rondó PHC, Guerra-Shinohara EM. Parameters for the assessment of iron status. *Rev Saude Publica* 2000;34:421–6.
25. Rivera JA, Hotz C, Gonzalez-Cossio T, Neufeld L, Garcia-Guerra A. The effect of micronutrient deficiencies on child growth: a review of results from community-based supplementation trials. *J Nutr* 2003;133 (11 Suppl 2): 4010S–20S.
26. Singh M. Role of micronutrients for physical growth and mental development. *Indian J Pediatr* 2004;71:59–62.
27. Chwang LC, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. *Am J Clin Nutr* 1988;47:496–501.
28. Briend A, Hoque BA, Aziz KM. Iron in tubewell water and linear growth in rural Bangladesh. *Arch Dis Child* 1990;65:224–5.
29. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. *J Nutr* 1994;124:645–54.
30. Giorgini E, Fisberg M, De Paula RAC, Ferreira AMA, Valle J, Braga JAP. The use of sweet rolls fortified with iron bis-glycinate chelate in the prevention of iron deficiency anemia in preschool children. *Arch Latinoam Nutr* 2001;51:48–53.
31. Bandhu R, Shankar N, Tandon OP. Effect of iron on growth in iron deficient anemic school going children. *Indian J Physiol Pharmacol* 2003;47:59–66.
32. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. *J Trop Pediatr* 2003;49:84–8.
33. Rahman MM, Akramuzzaman SM, Mitra AK, Fuchs GJ, Mahalanabis D. Long-term supplementation with iron does not enhance growth in malnourished Bangladeshi children. *J Nutr* 1999;129:1319–22.
34. Dossa RA, Ategbro EA, de Koning FL, van Raaij JM, Hautvast JG. Impact of iron supplementation and deworming on growth performance in preschool Beninese children. *Eur J Clin Nutr* 2001;55:223–8.
35. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. *J Nutr* 2003;133:1075–80.
36. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. *J Nutr* 2001;131:2860–5.
37. Bhatia D, Seshadri S. Growth performance in anemia and following iron supplementation. *Indian Pediatr* 1993;30:195–200.
38. Stoltzfus RJ, Chway HM, Montresor A, Tielsch JM, Jape JK, Albonico M, Savioli L. Low dose daily iron supplementation improves iron status and appetite but not anemia, whereas quarterly anthelmintic treatment improves growth, appetite and anemia in Zanzibari preschool children. *J Nutr* 2004;134:348–56.
39. Fisher AE, Naughton DP. Iron supplements: the quick fix with long-term consequences. *Nutr J* 2004;3:2.
40. Davidsson L. Approaches to improve iron bioavailability from complementary foods. *J Nutr* 2003;133 (5 Suppl 1): 1560S–62S.

Nutrient addition to corn masa flour: Effect on corn flour stability, nutrient loss, and acceptability of fortified corn tortillas

Jorge L. Rosado, Lorena Cassís, Lourdes Solano, and Miguel A. Duarte-Vázquez

Abstract

Background. Iron, zinc, and vitamin B complex are among the most prevalent nutritional deficiencies in Mexico, with iron deficiency being the leading cause of anemia. Mexico has the highest per capita consumption of corn in the world, consumed mainly as tortilla. Thus, corn flour for making tortillas has been suggested as an effective strategy to overcome malnutrition in developing countries such as Mexico where corn is a staple food. The stability of micronutrients added to food is an important factor for the success of fortification programs.

Objective. The aim of this study was to evaluate the stability of corn flour fortified with micronutrients, and to measure the effect of micronutrient fortification on the sensory quality and stability of the fortificants in fresh and stored tortilla.

Methods. A commercially homogenized nonfortified corn flour (NFCF) produced from degermed white corn was fortified with a premix containing iron, zinc, thiamin, and riboflavin. Changes in thiamin, riboflavin, iron, and zinc content in fortified corn flour (FCF) and nonfortified corn flour (NFCF) during storage were investigated. Vitamin B₁ and B₂ content was determined by fluorescence spectroscopy while iron and zinc content was analyzed by atomic absorption.

Results. Thiamin content in FCF and NFCF showed a significant ($p < .05$) decrease (24% and 37%, respectively) after 90 days of storage. Riboflavin losses of 18% and 22% were observed for FCF and NFCF, respectively. FCF retained over 90% of iron, while zinc content

remained constant. Losses of thiamin (27 to 39%) and riboflavin (37%) were produced during the process to convert corn masa flour into tortillas.

Conclusions. Storage time slightly affected the stability of riboflavin and thiamin in FCF while the cooking process produced considerable losses of both vitamins. Tortillas made from FCF were well accepted by Mexican adults. We conclude that the addition of vitamins and minerals in the forms and quantities used in this study do not modify the shelf-life of corn flour, and neither do they cause sensorial changes in tortillas made from FCF.

Key words: Corn flour, micronutrient fortification, micronutrient stability, corn-tortillas, sensorial stability

Introduction

Mineral and vitamin deficiency resulting from inadequate diets affect nearly two thirds of the world's population. The consequences of such deficiencies are sometimes not clinically evident, however they are important in the ability of individuals to function. Vitamin and mineral deficiencies may manifest themselves in delay of growth, a greater susceptibility to illnesses, and decreases in the cognitive capacity [1]. Iron, vitamin A, and iodine have gained the most attention, but deficiencies of other micronutrients are also relevant.

Interventions with micronutrient supplementation demonstrate the existence of such deficiencies and their functional consequences. A multiple micronutrient supplementation trial in Mexican infants that included vitamin B₁, B₂, iron and zinc increased their rate of growth [2]. A similar study with iron and zinc supplementation promoted motor and developmental exploratory behavior among Bangladeshi infants [3]. In 1993, Venezuela started fortifying pre-cooked yellow and white corn flour, as well as wheat flour, with vitamins B₁ and B₂, niacin and iron; a subsequent

Jorge Rosado is affiliated with Universidad Autónoma de Querétaro, Querétaro, México. Lorena Cassis and Lourdes Solano are affiliated with the Instituto Nacional de Ciencias Médicas y Nutrición. Ciudad de México, México. Miguel A. Duarte-Vázquez is affiliated with the Nucitec S.A. de C.V. Nutrición Ciencia y Tecnología, Querétaro, México.

Please direct queries to the corresponding author: Jorge Rosado, Universidad Autónoma de Querétaro, Apartado Postal No 31, Desarrollo San Pablo, Querétaro, 76160 Querétaro, México; e-mail: jrosado@avantel.net.

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

survey of 397 Venezuelan children showed a reduction in iron deficiency and prevalence of anemia [4]. In a prospective, double-blind randomized, controlled trial, Zlotkin et al. [5] found that anemic infants from rural Ghana receiving combined daily iron and zinc supplementation in the form of sprinkles showed concurrent improvement in iron and zinc status compared with anemic infants receiving iron sprinkles alone.

Currently, the most widely used strategies for reducing micronutrient deficiencies are supplementation, food diversification, and food fortification [6]. Food fortification is the most cost-effective intervention for reducing micronutrient malnutrition [7]. The process involves increasing the nutrient content of the food product through restoration or addition of micronutrients that have been lost or are normally absent. Effective fortification programs require foods that are commonly consumed by the target population in relatively constant amounts. In most industrialized countries, many cereal and grain products are fortified with micronutrients, and this practice has been introduced in some developing countries, as well. Poleti et al. [8] recently reviewed the progress in the field during last two years.

Corn flour can be considered in fortification programs because it is staple food in many parts of the world. Mexico has the highest per capita consumption of corn in the world, consumed mainly as tortillas. Tortillas are prepared by cooking lime-treated flour mixed with water, thus introducing possible losses of the added micronutrients through leaching. Considering that marginal micronutrient deficiency is widespread in Mexico, the Ministry of Health has promoted a program to add micronutrients to corn flour. The stability of the food itself and the micronutrients added to the food are important factors in predicting the success of a fortification program.

In this study we evaluated the stability of some vitamins and minerals added to corn flour and stored at room temperature for a 3-months period, losses of the added micronutrients during preparation of tortillas, and the acceptability of tortillas made with fortified flour.

Materials and methods

A commercially homogenized nonfortified corn flour (NFCF) produced from degermed white corn provided by MINSA (MINSA de Mexico S.A. de C.V.) was used for the study. The micronutrient mixture source consisted of a dry powder comprising vitamin B₁ (thiamine mononitrate), vitamin B₂ (riboflavin hydrochloride), zinc (zinc oxide) and iron (elemental reduced iron), and was supplied by Roche (Roche Vitamins Inc., Parsippany, NJ, USA). The composition of the premix was iron 30 mg/kg; zinc 30 mg/kg; thiamin 8 mg/kg;

and riboflavin 4 mg/kg.

Fortified corn flour was prepared as follows: 10 kg of corn flour was added to 0.15 kg of micronutrient mixture. The micronutrient mixture was added to corn flour to yield enriched flour conforming to the required standard [9]. To obtain a good distribution of the vitamin and mineral premix, this was blended first as a 1:4 w/w (weight/weight ratio) mixture premix with a portion of the corn flour and this blend was then added to the corn flour for a required final concentration. This process was carried out in a mixer at the pilot plant. Mixing time was the time it took to achieve completely homogeneous micronutrient concentration in the flour. To test homogeneity, samples of the flour from different sections of the mixer were taken at different times and assayed for zinc concentration. Homogeneous flour was achieved when zinc concentration was the same in the different sections of the mixer.

Paper bags of a type used in Mexico for storing corn flour were filled with 500 g (the normal packaging size) of fortified corn flour (FCF) as well as nonfortified corn flour (NFCF) and were manually sealed. Samples of FCF and NFCF (28 bags per each mixture) were stored for 3 months—the maximum shelf life of commercial corn flour products in Mexico—at a temperature of $22 \pm 2^\circ\text{C}$ and $39 \pm 2\%$ relative humidity. Four bags from each sample were removed from storage every 15 days (included time zero), and aliquots taken from each sample were analyzed for moisture content, water activity, peroxide index, vitamin B₁ and B₂ content, iron and zinc content, and microbiologic analysis. Another aliquot from the four bags at each time point (0, 15, 30, 45, 60, 75, and 90 days) was used to prepare tortillas. Sensory evaluation and analysis of vitamin B₁, B₂, iron and zinc was performed in order to determine the effect of flour storage and cooking process on micronutrient content and acceptability of corn tortilla.

Tortilla preparation

Fortified tortillas (FT) were prepared from each sample of FCF; those prepared from NFCF are nonfortified tortillas (NFT). Tortillas were prepared by first mixing the corn flour (700 g) and enough water (1200 mL) to provide masa (dough) with a moisture content around 45 percent. Masa was flattened into thin disks of 12.5 cm diameter and 1.2 mm thickness (30 g) using a commercial tortilla roller machine. The tortillas were baked for a total of 80 seconds as follows: 20 seconds on one side, 45 seconds on the other side, and 15 seconds on the first side on a griddle heated to $250 \pm 15^\circ\text{C}$.

Analytical methods

Moisture content was determined by drying samples (1.5 g) at 110°C until their weight became constant [10]. Water activity (*A_w*) was determined using a

Novasina DAL-20 Aw center (Novasina AG, Pfäffikon, Switzerland). Total iron and zinc were analyzed as follows: 15 mL of a 3:2:1 mixture of concentrated nitric, perchloric, and sulfuric acids, respectively, were combined with 5 g of the sample in a digestion tube. Tubes were heated for 45 minutes at 90°C, then for 40 minutes at 170°C and finally at 300°C for 40 minutes or until 2 or 3 mL remained at the tube bottom. The tubes were allowed to cool and the contents brought to 100 mL with 1 N nitric acid. Analysis of iron and zinc were performed using a Perkin-Elmer model 2380 atomic absorption spectrophotometer (Perkin-Elmer Co., Norwalk, Conn, USA) with an air-acetylene flame.

Vitamin B₁ was determined according to the fluorometric thiochrome method [11]. The spectrofluorometer was operated with the excitation wavelength set at 365 nm and the fluorescent wavelength at 417 nm. Vitamin B₂ was determined according to the fluorometric method [11]. Excitation and emission wavelength was set at 450 and 525 nm, respectively. Peroxide value (PV) of each sample was determined using the American Oil Chemists Society official method [12]. Five grams of each sample were placed into a 250 mL flask. 30 mL of acetic acid-chloroform (3:2, V/V) reagent was added, and the flask was shaken vigorously for 1 minute. One half mL of saturated potassium iodide was added to the flask, mixed for 1 minute and 30 mL of deionized water added and mixed again for 1 minute. After incubating the flask for 5 minutes, 10 mL of the upper aqueous solution from each flask was dispensed into a 16 mm × 120 mm borosilicate glass assay tube. The procedure continued with the addition of 0.5 mL 1% starch indicator and mixing for 5 seconds. The absorbance of samples was immediately measured at 563 nm.

Microbiology assays

Ten-gram samples were taken from the corn flour using aseptic techniques. They were placed in sterile Stomacher bags (Fisher Scientific, Pittsburgh, Pa, USA) with 90 mL of 0.1% peptone water (Fisher Scientific), and blended in a Stomacher (Tekmar, Cincinnati, Ohio, USA) for 2 minutes to achieve an initial 10⁻¹ suspension. Specific volumes of 10⁻¹ and further dilutions of the test sample were surface inoculated on the plate count agar (Difco Laboratory, Detroit, Mich, USA), then incubated at 32°C for 24 hours and enumerated for total plate count. Samples were also pour-plated in potato dextrose agar (Difco Laboratory), then incubated at 25°C for 24 hours and enumerated for yeast and molds. Counts were reported as colony-forming units per gram (CFUs/g) of the sample [13].

Sensory testing

A panel of 30 “tasters” was used for the evaluation. Panelists represented a corn tortilla-eating population,

which is the target population for fortified tortillas. Ethical approval was obtained from the institutional review board of the Instituto Nacional de Ciencias Médicas y Nutrición, Salvadore Zubirán. Many of these tasters were employees of the Institute and they provided their informed and signed consent on an Institutional Review Board approved form. Three 30-minute sessions were scheduled with all tasters participating. Panelists tested and rated the two types of tortillas made from FCF and NCF; the order of presentation was randomized across subjects to avoid position effects. Panelists were given one tortilla at a time to taste. Each panelist evaluated all products presented in a session and scored the degree of liking (DOL) for flavor, color, and odor using a 7-point hedonic scale (from 1 = disliked extremely to 7 = liked extremely) [14]. Distilled water (22°C) was provided between samples for cleansing the palate. Evaluations were made in individual sensory booths under controlled environmental conditions (22°C, incandescent lighting). Data analysis was performed using statistical analysis software (SAS for Windows, v 7.0z). Paired *t*-tests were performed on hedonic ratings to determine differences in DOL of flavor, odor, and color between FT and NFT tortillas. Comparisons were made at the *p* < .05 significance level.

Results and discussion

Physicochemical and microbiological characteristics of FCF

Moisture content

Initial moisture content was 13% (± 0.70%) for FCF and 12.9% (± 0.55%) for NCF and remained constant during storage. The combination of moisture content, temperature, and time determine the storage risk of cereal products. Carrillo-Pérez et al. [15] and Serna-Saldivar et al. [16] have indicated that the main factor affecting shelf life of corn products is moisture content. High moisture content will expose the flour to insect attack, mold, and bacteria. Cereal products with 13.5% moisture content or less can be stored without damage.

Water activity

Initial water activity value (*A_w*) obtained for NCF and FCF was 0.506 and 0.548, respectively. *A_w* values obtained for NCF and FCF are in agreement with those reported for grain flours ranging from 0.450 to 0.600 [17]. There were no changes in *A_w* of both formulations during storage (data not shown). The stability of water-soluble vitamins in foods has been shown to be strongly influenced by water activity. In the absence of oxidizing lipids, water-soluble vitamins generally exhibited little degradation even at *A_w* less than

0.2–0.3 [18]; thus, the probability that vitamin losses took place in our products during storage is unlikely.

Because water activity and moisture content are strongly influenced by storage conditions such as relative humidity and temperature, the correct selection of packing materials is an important factor to consider. However, the use of sophisticated containers could have a great impact in the cost of a fortified product, making it less accessible for fortification programs in developing countries.

Lipid oxidation

The storage stability of corn flour after micronutrient fortification did not show lipid oxidation (measured as peroxide index) even after 90 days' storage at 22°C; the same results were found with NFCF. This can be attributed to the low content of polyunsaturated fatty acids (PUFAs) in corn flour, which is produced from degermed corn, thus eliminating the main source of PUFAs. Development of oxidative rancidity, which results from lipid oxidation, is a critical factor affecting storage stability of iron-fortified foods, including corn and corn products [19]. In whole corn, PUFAs account for 60% of the total fatty acids [20], which makes these products prone to the development of rancid off-flavors [21].

Microbial analysis

The total plate counts for the FCF and NFCF at day 0 were 8.7×10^4 and 12×10^4 CFU/g, respectively, while no mold or yeasts were detected in the initial samples. Total plate count did not show any significant changes and no molds or yeasts were detected over 90 days of storage, indicating that the product was microbiologically stable during storage. This is mainly due to the low values of A_w and the moisture content of the corn flour during storage. Deteriorative microorganisms generally grow well between A_w values 0.995–0.980 and most microbes cease growth at $A_w \leq 0.900$.

Micronutrient stability of FCF and NFCF during storage

The content of thiamin and riboflavin in the FCF was 1.1 mg/100 g and 0.6 mg/100 g, respectively, almost three times as much as that in the NFCF—0.3 mg/100 g and 0.25 mg/100 g, respectively. The addition of these vitamins to the NFCF and FCF is in agreement with the recommended levels for the addition of these vitamins to corn flour in Mexico [9]. The recommended levels are 0.6 mg/100 g of thiamin as thiamin mononitrate and 0.3 mg/100 g of riboflavin as riboflavin hydrochloride.

Figure 1 shows the changes for thiamin in FCF and NFCF during storage at room temperature (22°C). Thiamin content in FCF did not show any significant change within the first 15 days of storage, but a sig-

nificant decrease of 13% was observed in NFCF during this period ($p < .05$). When storage was prolonged to 90 days, decreases in thiamin content of 24% for FCF and 35% for NFCF were observed, corresponding to a final content of 0.83 mg/100 g and 0.19 mg/100 g for FCF and NFCF, respectively. Thiamin is one of the most unstable B vitamins and baking, pasteurization, storage, or boiling can reduce its content by up to 50% [18]. The loss of vitamin B₁ occurred not only in FCF but also in NFCF, where losses after 90 days of storage were relatively higher than in FCF. These findings suggest a higher stability of mononitrate and hydrochloride forms of synthetic thiamin than the naturally occurring form, thiamin pyrophosphate, which has been previously documented by Gregory [18].

Vitamin B₂ (riboflavin) losses of 10% were observed in both samples within the first 30 days of storage at 22°C (**fig. 2**). After 90 days of storage, loss of vitamin B₂ in both samples was very similar, reaching values of

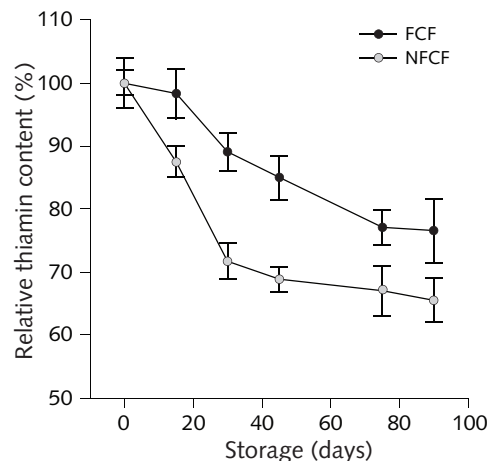


FIG. 1. Relative thiamin content in fortified (FCF) and non-fortified corn flour (NFCF) during 90 days of storage

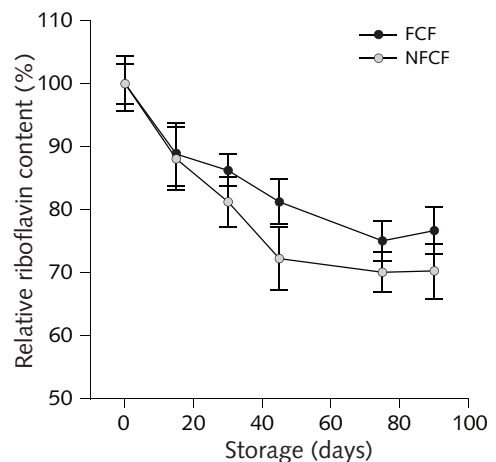


FIG. 2. Relative riboflavin content in fortified (FCF) and non-fortified corn flour (NFCF) during 90 days of storage

18% for FCF and 22% for NCF. The final content of riboflavin at the end of the storage period in FCF and NCF was 0.49 mg/100 g and 0.19 mg/100 g, respectively. Riboflavin was more stable than thiamin during storage of corn flour. Some studies have shown that storage of various types of food at room temperature and even under refrigeration can cause a loss of B-complex vitamins [22, 23]. Frias and Vidal-Valverde [24] reported that thiamin content in an enteral feeding formula suffered a drastic reduction within the first three months of storage at room temperature. Rubin et al. [25] showed that yellow corn flour retained about 95% of vitamins B₁ and B₂ after 6 months of storage at room temperature. The amount of vitamins lost during storage of corn masa flour should be considered for its fortification. One possibility is that fortification be carried out with an excess of vitamins added to the flour.

The iron content in FCF and NCF was 4.5 mg/100 g and 2.8 mg/100 g respectively; while the zinc content was 2.0 mg/100 g for NCF and 4.1 mg/100 g for FCF (**table 1**). The recommended fortification levels are 3.0 mg/100 g of iron as extra-fine reduced iron and 2.0 mg/100 g of zinc as zinc oxide [9].

In general, minerals are more resistant to storage deterioration than vitamins. The stability of iron and zinc in fortified corn flour stored at room temperature was good. Our results showed that FCF retained more than 95% (4.25 mg/100 g) of iron after 90 days of storage at room temperature (**table 1**). NCF lost 32% of iron during the same period ($p < .05$). Elemental reduced iron used for fortification in this study is insoluble in water and poorly soluble in diluted acid solutions. Because of their low reactivity and higher stability, these compounds are used to fortify cereals

TABLE 1. Mean (\pm SD) iron and zinc content of fortified corn flour (FCF) and nonfortified corn flour (NCF) at zero days and after 45 and 90 days of storage (mg/100 g of dry flour)*

	Iron	Zinc
FCF		
0 days	4.50 \pm 0.065 ^a	4.10 \pm 0.049 ^a
45 days	4.40 \pm 0.070 ^a	4.20 \pm 0.080 ^a
90 days	4.25 \pm 0.050 ^a	4.10 \pm 0.063 ^a
NCF		
0 days	2.80 \pm 0.039 ^a	2.00 \pm 0.046 ^a
45 days	1.80 \pm 0.060 ^b	1.90 \pm 0.055 ^a
90 days	1.90 \pm 0.075 ^b	2.00 \pm 0.038 ^a

* Means in both columns for each formulation that are not followed by the same letter are significantly different ($p < .05$).

and flours in combination with vitamins B₁, B₂, B₆, and niacin [26]. In contrast, iron in NCF was more susceptible to losses, suggesting that the organic form of iron in the cereal is less stable. Zinc content remained constant during the storage period in both samples (FCF and NCF) (**table 1**). Zinc oxide was used as the source of supplementary zinc in corn flour. It is the cheapest and most stable source and has been widely used for fortification of wheat flour [27]. Because of its lower cost and higher stability, zinc oxide is used about 10 times more than any other zinc compound by food manufacturers in the United States [28].

Effect of corn flour storage and cooking process on micronutrient content of tortilla

Moisture content increased to 22.5% (\pm 1.0%) in FT and 25% (\pm 1.2%) in NFT; the micronutrient content was corrected by humidity, as shown in **table 2** (mg/100 g of

TABLE 2. Mean (\pm SD) micronutrient content of tortillas made from fortified corn flour and nonfortified corn flour stored for different times (mg/100 g of dry tortilla)*

Time of flour storage	Iron	Zinc	B ₁	B ₂
FT				
0 days	4.2 \pm 0.28 ^a	3.6 \pm 0.39 ^a	0.81 \pm 0.039 ^a	0.41 \pm 0.017 ^a
15 days	3.7 \pm 0.29 ^a	3.1 \pm 0.42 ^a	0.59 \pm 0.023 ^b	0.32 \pm 0.030 ^b
30 days	3.7 \pm 0.32 ^a	3.1 \pm 0.38 ^a	0.59 \pm 0.28 ^b	0.30 \pm 0.022 ^b
45 days	3.6 \pm 0.33 ^a	3.5 \pm 0.26 ^a	0.60 \pm 0.033 ^b	0.32 \pm 0.023 ^b
60 days	3.6 \pm 0.39 ^a	3.6 \pm 0.32 ^a	0.59 \pm 0.028 ^b	0.33 \pm 0.031 ^b
75 days	3.7 \pm 0.30 ^a	3.5 \pm 0.25 ^a	0.49 \pm 0.032 ^b	0.33 \pm 0.010 ^b
90 days	3.7 \pm 0.29 ^a	3.7 \pm 0.29 ^a	0.51 \pm 0.027 ^b	0.31 \pm 0.012 ^c
NFT				
0 days	2.8 \pm 0.30 ^a	2.0 \pm 0.30 ^a	0.21 \pm 0.005 ^a	0.22 \pm 0.002 ^a
15 days	2.3 \pm 0.17 ^a	1.8 \pm 0.29 ^a	0.17 \pm 0.006 ^b	0.13 \pm 0.010 ^b
30 days	2.2 \pm 0.38 ^a	1.7 \pm 0.45 ^a	0.18 \pm 0.002 ^b	0.10 \pm 0.002 ^b
45 days	2.5 \pm 0.37 ^a	1.9 \pm 0.25 ^a	0.20 \pm 0.010 ^b	0.14 \pm 0.009 ^b
60 days	2.4 \pm 0.40 ^a	1.9 \pm 0.17 ^a	0.13 \pm 0.009 ^c	0.17 \pm 0.011 ^b
75 days	2.5 \pm 0.32 ^a	1.9 \pm 0.20 ^a	0.06 \pm 0.002 ^d	0.16 \pm 0.010 ^b
90 days	2.5 \pm 0.26 ^a	1.9 \pm 0.15 ^a	0.08 \pm 0.008 ^d	0.06 \pm 0.012 ^c

FT, fortified tortilla; NFT, nonfortified tortilla

* Means in both columns for each formulation that are not followed by the same letter are significantly different ($p < .05$).

dry tortilla). Tortillas made from FCF and not stored had vitamin B₁ and B₂ content of 0.81 mg/100 g and 0.41 mg/100 g, respectively (table 2). In addition to the losses of micronutrients occurring during the storage of corn flour, the cooking process affected the thiamin content in both tortillas (table 2). The micronutrient content of both types of tortilla as reported in table 2 reflects the net effect of flour storage and the cooking process. The total thiamin content decreased greatly in tortillas made with FCF previously stored. The thiamin content in tortillas made from FCF stored for 90 days was 0.51 mg/100 g.

The riboflavin content in tortillas also decreased with respect to the storage time of the corn flour used to make them. Tortillas made with FCF stored for 90 days had a riboflavin content of 0.30 mg/100 g. Overall, cooking produced FCF losses of about 35% of thiamin content and 37% of riboflavin in addition to the losses produced by the storage.

The effect of cooking on vitamin stability was more noticeable in the conversion of NFCF stored for 90 days into NFT. Overall losses ranging from 58% to 69% for vitamin B₁ and B₂ were observed. The thiamin content in tortillas made from NFCF stored for 90 days was 0.08 mg/100 g, whereas the riboflavin content in the same type of tortillas was 0.06 mg/100 g. These results suggest that vitamins in the corn masa flour are highly susceptible to heat degradation, making fortification an important procedure.

Table 2 also shows the mineral content of tortillas made from stored NFCF and FCF. Tortillas made from FCF without storage had iron and zinc content of 4.25 mg/100 g and 3.7 mg/100 g, respectively. FCF used to prepare these tortillas had iron content of 4.5 mg/100 g, and 4.1 mg/100 g of zinc. Thus, after correction for humidity the mineral content in tortillas did not show any significant difference ($p < .05$) compared with the mineral content in FCF.

Tortillas and related products are the foods most widely consumed by Mexicans. In some areas of Mexico, tortillas provide more than 75% of the total caloric and protein intake; thus, tortillas may be considered a more universally accepted vehicle for the incorporation of iron, zinc, and other micronutrients into the Mexican diet, over wheat flour or breakfast cereals.

Sensory evaluation of tortillas made from stored corn flour

The mean (\pm SD) hedonic ratings of the tortillas made with corn flour at time zero and after storage are shown by type of rating and type of tortilla (fortified and nonfortified) in table 3. FT received favorable flavor and odor evaluations, with a DOL of 5.3 and 5.6, respectively. The color of both samples of tortilla was less acceptable (3.6 to 4.0). There were no differences

TABLE 3. Mean (\pm SD) of the hedonic ratings of the tortilla made from corn flour at time zero and after 3 months of storage shown by type of rating and type of tortilla*

	DOL color	DOL flavor	DOL odor
FT time zero	3.8 \pm 0.7 ^a	5.3 \pm 0.6 ^b	5.6 \pm 0.7 ^c
FT after storage	4.0 \pm 0.8 ^a	5.5 \pm 0.7 ^b	5.8 \pm 0.8 ^c
NFT time zero	3.6 \pm 0.6 ^a	4.1 \pm 0.5 ^b	4.5 \pm 0.5 ^c
NFT after storage	4.0 \pm 0.7 ^a	5.6 \pm 0.8 ^b	5.5 \pm 0.5 ^c

DOL, degree of liking; FT, fortified tortilla; NFT, nonfortified tortilla

* Means sharing the same superscript within the same column are not significantly different ($p < .05$).

in DOL in either sample of tortillas made with corn flour previously stored for 90 days as compared with samples taken at time zero; thus flour storage did not affect the acceptability of tortillas. Sensory evaluation is an important consideration in developing a successful food fortification program. The hedonic scale used in this study is a measure of like/dislike or degree of liking, which in turn can be used to predict acceptability. The results of this study indicate that the tortillas made from FCF were accepted by Mexican adults and that fortification was not detected.

There are a few reports of the keeping qualities of tortilla made from stored corn flour. Paredes-López and Mora-Escobedo [29] studied the stability of corn flour stored for 60 days under accelerated storage conditions, observing that sensory qualities such as flavor, color, and aroma of tortilla prepared from stored corn flour decreased with longer storage time and higher relative humidity (RH), and was no longer acceptable at 60 days of storage. Since this study was done under accelerated storage conditions, it may be that in ambient conditions it would take more than 90 days of flour storage before tortilla acceptance would be affected.

Few studies have examined the sensory qualities or acceptability of cereal products fortified with iron and zinc. Sometimes the sensory properties of a given food can be affected by the addition of a determined fortificant. If iron is a component of the fortificant premix, the development of off-flavors and off-colors due to reactions with other components of the food material is critical when fortifying light-colored foods. In our study, the organoleptic properties of fortified tortillas did not change during the storage period, and were accepted by the panelists even after 90 days of storage.

Conclusions

Storage time slightly affected the stability of riboflavin and thiamin in FCF, which was acceptable during the first 3 months. FCF had losses of 24% for thiamin and 18% for riboflavin. No significant losses in iron and

zinc content were observed during the period of storage. The cooking process produced losses of thiamin ranging from 43% to 52% and riboflavin of 54%; these nutrient losses should be considered when attempting to fortify corn masa flour. Sensory evaluation indicated

that the FCF tortillas were well accepted by Mexican adults. Fortification of corn tortillas with iron, zinc, thiamin, and riboflavin represents a good alternative to providing micronutrients that are deficient in a substantial proportion of the Mexican population.

References

- Whittaker P. Iron and zinc interactions in humans. *Am J Clin Nutr* 1998;68(2 Suppl): 442S–446S.
- Rivera J, González-Cossio T, Flores M, Romero M, Rivera M, Téllez-Rojo MM, Rosado JL, Brown K. Multiple micronutrient supplementation increases the growth of Mexican infants. *Am J Clin Nutr* 2001;74:657–63.
- Black MM, Baqui AH, Zaman K, Persson LA, El Arifen S, Le K, McNary SW, Parveen M, Hamadani JD, Black R. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladesh infants. *Am J Clin Nutr* 2004;80:903–10.
- Layrisse M, Chaves JF, Mendez-Castellano H, Bosch V, Tropper E, Bastardo B, González E. Early response to the effect of iron fortification in the Venezuelan population. *Am J Clin Nutr* 1996;64:903–7.
- Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. *J Nutr* 2003;133:1075–80.
- Latham MC, Ash D, Ndossi G, Mehansho H, Tatala S. Micronutrient dietary supplements—A new forth approach. *Arch Latinoam Nutr* 2001;51:37–41.
- Lofti M, Mannar MG, Merx RJHM, Naber-van den Heuvel P. Micronutrient Fortification of Foods. Current Practices, Research and Opportunities. Ontario, Canada: The Micronutrient Initiative, c/o International Research Centre (IDRC)/ International Agriculture Centre (IAC), 1996.
- Poleti S, Wilhem G, Sautter C. The nutritional fortification of cereals. *Curr Opin Biotech* 2004;15:162–5.
- Rosado JL, Camacho-Solís R, Bourges H. Adición de vitaminas y minerales a harinas de maíz y de trigo en México. *Salud Pública de México* 1999;41:130–7.
- AOAC. Official Methods of Analysis, 15th ed. Arlington, Va, USA: Association of Official Analytical Chemists, 1995.
- AOAC. Official Methods of Analysis, 16th ed. Arlington, Va, USA: Association of Official Analytical Chemists, 1998.
- AOCS. American Oil Chemist's Society Official Methods. Champaign, Ill, USA: American Oil Chemist's Society, 1988.
- AMPH. Compendium of Methods for the Microbiological Examination of Foods. Washington, DC: American Public Health Association, 1992.
- Carpenter RP, Lyon DH, Hasdell TA. Guidelines for Sensory Analysis in Food Product Development and Quality Control. Frederick, Md, USA: Aspen Publishers, 2000.
- Carrillo-Pérez E, Serna-Saldivar SO, Rourk-Sánchez O. Effect of storage conditions and packaging materials on the physico-chemical, microbiological and sensory properties of corn dry masa flour. *J Food Proc Preserv* 1989; 13:335–53.
- Serna-Saldivar SO, Gomez MH, Rooney LW. The chemistry, technology and nutritional value of alkaline-cooked corn products. In: Pomeranz YD, ed. *Advances in Cereal Science and Technology*. St Paul, Minn, USA: American Association of Cereal Chemists, 1990.
- Beuchat LR. Microbial stability as affected by water activity. *Cereal Foods World* 1981; 26: 345–349.
- Gregory JF. Vitamins. In: Fennema OR, ed. *Food Chemistry*. New York: Dekker, 1996.
- Frankel EN. Lipid oxidation. *Prog Lipid Res* 1980;19: 1–22.
- Galliard T. Rancidity in cereal products. In: Allen JC, Hamilton RJ, eds. *Rancidity in Foods*, 2nd ed. New York: Elsevier Science Publishing Co, 1989.
- St Angelo AJ. Lipid oxidation in foods. *Crit Rev Food Sci Nutr* 1996;36:175–224.
- Montero CG, Vilchez T, Cantabrana F, Atienza M. Thiamine stability in parenteral nutrition. *Nutr Hosp* 1990; 5:32–35.
- Baumgartner TG, Henderson GN, Fox J, Gondi U. Stability of ranitidine and thiamine in parenteral nutrition solutions. *Nutrition* 1997; 13:547–553.
- Frias J, Vidal-Valverde C. Stability of thiamin and vitamins E and A during storage of enteral feeding formula. *J Agric Food Chem* 2001;49:2313–7.
- Rubin SH, Emodi A, Scialpi L. Micronutrient addition to cereal grain products. *Cereal Chem* 1977;54:895–903.
- Chen Z, Oldewage-Theron W. Food fortification to prevent and control iron deficiency. *Afr J Food Nutr Sci*; 2002;2:1–13.
- Ranhotra GS, Loewe LJ, Puyat LB. Bioavailability and functionality (breadmaking) of zinc in various organic and inorganic sources. *Cereal Chem* 1977;54:496–502.
- National Academy of Sciences. Pounding and technical effects update of substances added to food. Springfield, Va, USA: Department of Commerce: National Technical Information Service, 1989.
- Paredes-López O, Mora-Escobedo E. Influence of storage on the quality of maize meal for tortilla making. *J Food Technol* 1983;18:53–60.

Effect of prenatal multiple micronutrient supplements on maternal weight and skinfold changes: A randomized double-blind clinical trial in Mexico

Usha Ramakrishnan, Teresa González-Cossío, Lynnette Marie Neufeld, Juan Rivera, and Reynaldo Martorell

Abstract

Background. Recent trials of prenatal multivitamin-mineral supplements have yielded mixed findings for outcomes such as birth size, but the benefits of prenatal multivitamin-mineral supplements for maternal outcomes are unknown.

Objective. The main objective of this study was to examine the effect of prenatal multiple micronutrient supplements (MM) compared to iron only (FE) supplements on changes in maternal weight and body composition during pregnancy and the early postpartum period.

Methods. A randomized double-blind clinical trial was conducted in semi-rural Mexico. Women received either MM or FE supplements, 6 days per week from early pregnancy to delivery. Anthropometric measurements were obtained at recruitment, 26 and 37 weeks pregnancy, and 1 month postpartum. Women in both groups were similar at recruitment except that body-mass index (BMI) was greater in the FE group.

Results. Mean weight gain during pregnancy was significantly greater (~0.6 kg) in the MM group (n = 283) compared to the FE group (n = 287), but not after adjusting for maternal BMI at recruitment. Overweight women in the MM group gained 0.53 kg between recruitment and 1 month postpartum, whereas those in the FE group lost 0.63 kg; there were no differences between

experimental groups among non-overweight women ($p = .06$ for interaction).

Conclusions. Compared to iron supplements, MM supplements did not increase weight gain during pregnancy after adjusting for baseline differences in BMI but may lead to greater postpartum weight retention among overweight women.

Key words: Weight gain, pregnancy, iron, multivitamin-mineral supplements, body composition

Introduction

Poor maternal nutrition both before and during pregnancy is an important cause of poor pregnancy outcomes, especially in developing countries [1, 2]. Weight gain during pregnancy is widely used as an indicator of the adequacy of nutrition during pregnancy and has been associated with infant outcomes such as mortality, prematurity, and low birthweight [1, 3]. Studies have shown that while weight gain in healthy populations, such as the United States, averages around 12.5 kg, weight gain is only 7 to 8 kg in many developing countries [4]. Several intervention trials have been conducted to evaluate the benefits of increasing food intakes during pregnancy on maternal and infant outcomes, but the findings have varied either by the type of outcome examined or study setting [2]. Although improved birth outcomes such as higher birthweight are usually accompanied by increased maternal weight gain and/or body fat, this has not always been the case [5, 6]; further documentation of the impact of improved nutrition during pregnancy on maternal outcomes in addition to birth outcomes is necessary.

Improved micronutrient intakes during pregnancy may contribute to increased energy intakes by influencing appetite [7]. Although several intervention trials have been conducted using either food supplements or single nutrients such as vitamin A or zinc, few have examined the impact of prenatal multivitamin

Usha Ramakrishnan and Reynaldo Martorell are affiliated with the Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Ga, USA. Teresa González-Cossío, Lynnette Marie Neufeld, and Juan Rivera are affiliated with Centro de Investigaciones en Nutrición y Salud, Instituto Nacional de Salud Pública (INSP), Cuernavaca, Morelos, Mexico. Presented at the Annual Meeting of the American Public Health Association, Atlanta, Ga, October 2001.

Please direct queries to the corresponding author: Usha Ramakrishnan, Ph.D., Associate Professor, Department of Global Health, Rollins School of Public Health at Emory University, Atlanta, NE, GA, USA 30322; e-mail: uramakr@sph.emory.edu.

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

mineral supplements (MVMS), an approach that is being considered by many international agencies as a simpler strategy to improve pregnancy outcomes. Two observational studies in the US have shown that routine consumption of MVMS supplements was associated with increased weight gain during pregnancy and improved birth outcomes (low birthweight and preterm deliveries) even after controlling for differences in factors such as access to prenatal care and baseline nutritional status [8, 9]. In one intervention trial, multivitamin supplements reduced the incidence of prematurity and low birthweight by about 40% compared to routine iron-folate (FE-folate) supplements [10], and also improved maternal T-cell counts and weight gain during pregnancy in HIV-positive asymptomatic women [11].

The benefit of multiple micronutrient (MM) supplements for non-HIV infected women, however, is not known. In Nepal [12], MVMS did not improve birth outcomes when compared to FE-folate supplements (regarded as a standard of care), but the impact on maternal outcomes was not reported. We recently conducted a randomized, double-blind controlled trial in semi-rural Mexico to compare the efficacy of a multiple micronutrient (MM) supplement compared to an iron-only (FE) supplement during pregnancy in improving maternal and infant outcomes. In a previous publication, we reported that MM supplements did not improve birth weight or length compared to iron-only supplements [13]. Mean birth weight was 2.981 and 2.977 kg in the MM and FE groups, respectively. The specific outcomes examined in this paper are weight gain and changes in maternal anthropometry both during pregnancy and at 1 month postpartum.

Methods

Study setting and design

This study was a collaborative project between the Rollins School of Public Health, Emory University, Atlanta Ga, USA, and the Centro de Investigación en Nutrición y Salud, Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico. The study site was a semi-rural community, near the city of Cuernavaca, in Morelos, Mexico. We used a randomized double-blind design in which the treatment group received the MM supplement containing 1 to 1.5 RDA (Recommended Dietary Allowance) of key vitamins (A, D, E, B₁, B₂, B₃, B₆, B₁₂, C, and folic acid) and minerals (Zn, Mg), and 60 mg of iron. The control group received 60 mg iron, which was the standard practice of the Ministry of Health in Mexico at the time the study was conducted.

The study protocol was approved by the Human Investigations Committee at Emory University and at INSP, and written informed consent was obtained

from all eligible subjects at recruitment. We excluded women who were more than 13 weeks pregnant at recruitment or who reported the use of micronutrient supplements ($n = 8$). All eligible pregnancies were randomly allocated to either the MM or FE group using four color-coded groups (two per treatment) assigned *a priori* using a computer-generated list. Participants were visited at their homes six days a week by trained workers who administered and recorded the consumption of supplements until delivery. All study personnel and investigators were blinded to group assignment, the details of which were kept in sealed envelopes in both institutions and opened only after preliminary data analysis was completed. Additional details of the supplement composition and of recruitment and treatment allocation are provided elsewhere [13].

Data collection

At recruitment, which began in July 1997, all participants were provided with a prenatal examination that included a detailed obstetric history, physical examination, anthropometric and dietary assessments, and blood draw. All examinations and assessments were carried out by the study physician and a team of trained nurses at the study headquarters. Height, weight, mid upper arm circumference (MUAC), tricep skinfold (TSF), and subscapular skinfold (SSF) thicknesses were measured several times by workers trained in standard anthropometric techniques [14]. All subjects were asked to come to the study headquarters for routine prenatal care visits at 26, 32, and 37 weeks of pregnancy. An additional exam was conducted in each participant's home at one month postpartum, during which anthropometric measurements were taken again. Due to budgetary limitations, a modified protocol was implemented after September 1, 1999, from which point only weight was measured at the 32- and 37-week prenatal visits. Socioeconomic status (SES) was determined using a questionnaire that included details of education, ethnicity, water and sanitation, quality of housing, household size, occupation, and possessions such as a television set, radio, or bicycle. An index of economic status was derived from these data using factor analysis [15].

Data analysis

The main outcome variables were maternal weight gain and changes in MUAC, TSF, and SSF from recruitment to 37 weeks pregnancy and from recruitment to 1 month postpartum. We also examined early (recruitment to 26 weeks pregnancy) and late weight gain (26 weeks to 37 weeks pregnancy) and defined inadequate weight gain during pregnancy as weekly weight gain below 225 g/week.

Using an intent-to-treat design, all pregnancies

assigned to treatment between July 1997 and December 31, 1999 were included and the effectiveness of randomization was tested by comparing the two groups for selected sociodemographic, health, and nutrition characteristics of the women at recruitment. Comparisons between the final sample with information on maternal weight gain and changes in anthropometry and those participants lost to follow-up were also done for selected baseline characteristics and for measures of compliance (defined as the proportion of supplements consumed while in the study). All comparisons were done using Student's *t*-tests for normally distributed variables and chi-square tests of proportions for categorical variables.

The analysis was restricted to pregnancies that resulted in singleton term live births and had data available on the outcomes of interest. Following unadjusted comparisons of key outcomes by intervention group, adjusted analyses using multivariate techniques (general linear models) were done to control for maternal body-mass index (BMI) and marital status, both of which differed significantly between groups at recruitment. In addition, effect modification by characteristics selected *a priori* (maternal overweight at recruitment and economic status) was tested. Overweight was defined using the World Health Organization (WHO) definition of BMI ≥ 25 kg/m² [16]. All statistical analyses were conducted using SAS 8.2 (SAS Institute, Cary, NC, USA). Since some women contributed more than one pregnancy to the study, repeated measures analysis (SAS PROC MIXED) were used to compare the main outcomes of interest. Statistical significance was based

on the criterion of $p < .05$ for simple group differences and $p < 0.15$ for tests of interactions.

Results

A total of 921 pregnancies were identified and 873 were assigned to treatment—435 to the multiple micronutrient group and 438 to the iron group. Details of birth outcomes and reasons for loss to follow-up have been described elsewhere [13]. The comparison of selected maternal characteristics at recruitment (**table 1**) showed that the two groups were similar for the majority of characteristics, including maternal age at recruitment, number of weeks pregnant at entry, percent primiparous, years of schooling, economic status, and percent anemic. There were also no differences in reported energy and micronutrient intakes between intervention groups at baseline (results not shown). However, the proportion of single mothers was higher in the MM group compared to the FE group, although a majority of women in both groups had a partner. Additionally, although mean height was similar between the groups, women in the FE group were significantly heavier ($p < .05$) compared to those in the MM group as demonstrated by greater weight, BMI, and skinfold measurements; almost a third were overweight with a higher proportion in the FE group (38.7%), compared to the MM group (30.8%).

Out of the final sample of 602 pregnancies that resulted in singleton term live births, maternal weights were available at recruitment and 37 weeks' gestation

TABLE 1. Maternal characteristics at recruitment for all women assigned to receive multiple micronutrient (MM) or iron only (FE) supplements during pregnancy using intent-to-treat design

	Multiple micronutrients (N = 435)		Iron (N = 439)		<i>p</i> ^b
	N	Mean \pm SD ^a	N	Mean \pm SD ^a	
Maternal age (yr)	434	23.09 \pm 5.48	436	23.00 \pm 5.42	.80
Weeks pregnant (wk)	431	9.24 \pm 2.51	432	9.31 \pm 3.00	.73
Primiparous (%)	431	36.4	432	34.3	.51
Schooling (yr)	396	6.84 \pm 3.41	402	7.05 \pm 3.24	.36
Economic status	395	0.00 \pm 1.03	398	0.08 \pm 1.06	.27
Indigenous ethnicity (%)	396	32.3	402	29.1	.32
Single mother (%)	395	4.6	402	2.0	.04
Anemia (%)	400	13.3	403	10.2	.18
Height (cm)	432	148.66 \pm 4.95	439	148.54 \pm 4.70	.72
Weight (kg)	433	52.78 \pm 9.67	439	54.15 \pm 10.00	.04
Body-mass index (kg/m ²)	431	23.83 \pm 3.94	439	24.53 \pm 4.31	.01
MUAC	433	27.22 \pm 3.21	439	27.82 \pm 3.69	.01
TSF thickness	433	21.61 \pm 6.53	439	22.71 \pm 7.14	.02
SSF thickness	433	15.62 \pm 4.89	439	16.46 \pm 5.14	.01

MUAC, mid upper arm circumference; TSF, triceps skinfold; SSF, subscapular skinfold

a. Mean \pm SD unless indicated otherwise.

b. Using Student's *t*-test for comparison of means and chi-square tests for categorical variables.

for 570 pregnancies (502 women), and at recruitment and 1 month postpartum for 577 pregnancies (505 women). Approximately 14% of women contributed more than one pregnancy to the study. Mean birth weight was 3.1 kg with no group differences. The complete battery of anthropometric variables was available for a smaller subsample at 37 weeks' gestation ($n = 371$) and 1 month postpartum ($n = 456$), as only weight was measured after September 1999. Women for whom data on weight gain during pregnancy were available were younger ($p < .05$) compared to those without available data, and the subsamples with full anthropometry had a lower hemoglobin concentration ($p < .05$) compared to those without these data. However, there were no differences in anthropometric measurements at recruitment for all subsamples.

The mean values for the changes in weight and other anthropometric measurements from recruitment to 37 weeks' gestation and 1 month postpartum are presented by intervention group in **table 2**. Weight gain during pregnancy was lower than recommended in both groups [4], approximately 7-8 kg from recruitment to 37 weeks. Although mean weight gain was about 0.6 kg greater for the MM group, these differences were attenuated to 0.32 kg and ceased to be statistically significant ($p = .24$) after adjusting for baseline differences in BMI. The findings were similar for differences in weekly weight gain (270 g/week). The incidence of low weight gain was lower in the MM group (34.1%) compared to

the FE group (40.1%), but these differences (odds ratio: 0.75; 95% confidence interval [CI]: 0.54, 1.06) were also attenuated after adjusting for baseline differences in BMI (adjusted odds ratio: 0.89; 95% CI: 0.62, 1.28). Women retained about 2 kg from the time of recruitment to 1 month postpartum; although this was greater for the MM group (0.71 kg), these differences were also attenuated (0.33 kg) and not significantly different after adjusting for baseline differences in BMI. There were no significant differences in changes in MUAC, TSF, and SSF between the experimental groups. Restricting the analysis to women who contributed only one pregnancy did not alter these findings.

The comparison of the pattern of weight gain during pregnancy by intervention group (**table 3**) also did not reveal any significant differences after adjusting for baseline differences. Weight gain from recruitment to 26 weeks' pregnancy was higher in the MM group compared to the FE group in terms of actual weight gained and rate of weekly gain, but these differences were attenuated and not statistically significant after adjusting for baseline differences in BMI. In contrast, weight gain during late pregnancy i.e., from 26 weeks to 37 weeks of pregnancy was similar in both groups, suggesting that the differences seen in overall weight gain before adjustment occurred in the first half of pregnancy.

There were no interactions for changes in anthropometry and weight during pregnancy and from

TABLE 2. Changes in maternal anthropometry from recruitment to 37 weeks' gestation and to 1 month postpartum by intervention group

		Multiple micronutrients		Iron		p^a
		N	Mean \pm SD	N	Mean \pm SD	
Recruitment to 37 weeks' gestation						
Δ Weight (kg)	<i>unadjusted</i>	283	7.71 \pm 3.57	287	7.08 \pm 3.58	.04
	<i>adjusted^b</i>		7.57 \pm 3.30		7.25 \pm 3.30	.24
Δ MUAC (cm)	<i>unadjusted</i>	180	-0.13 \pm 1.32	191	-0.32 \pm 1.31	.16
	<i>adjusted^b</i>		-0.19 \pm 1.18		-0.25 \pm 1.18	.62
Δ TSF (mm)	<i>unadjusted</i>	180	-0.98 \pm 4.17	191	-1.04 \pm 4.17	.89
	<i>adjusted^b</i>		-1.13 \pm 3.97		-0.90 \pm 3.97	.60
Δ SSF (mm)	<i>unadjusted</i>	180	0.66 \pm 3.14	191	0.21 \pm 3.14	.16
	<i>adjusted^b</i>		0.54 \pm 2.95		0.31 \pm 2.96	.44
Recruitment to 1 mo post-partum						
Δ Weight (kg)	<i>unadjusted</i>	287	2.38 \pm 3.52	290	1.67 \pm 3.53	.02
	<i>adjusted^b</i>		2.18 \pm 3.05		1.85 \pm 3.05	.20
Δ MUAC (cm)	<i>unadjusted</i>	222	-0.42 \pm 1.41	234	-0.56 \pm 1.50	.32
	<i>adjusted^b</i>		-0.50 \pm 1.31		-0.47 \pm 1.27	.70
Δ TSF (mm)	<i>unadjusted</i>	222	-2.29 \pm 4.47	234	-2.59 \pm 4.50	.47
	<i>adjusted^b</i>		-2.48 \pm 4.17		-2.40 \pm 4.16	.85
Δ SSF (mm)	<i>unadjusted</i>	222	0.39 \pm 3.28	234	0.04 \pm 3.37	.26
	<i>adjusted^b</i>		0.28 \pm 3.13		0.20 \pm 3.06	.76

MUAC, mid upper arm circumference; TSF, triceps skinfold; SSF, subscapular skinfold

a. Using repeated measures analysis for comparison of means.

b. Adjusting for body-mass index at recruitment.

TABLE 3. Patterns of weight gain during pregnancy by intervention group

		Multiple micronutrients (N = 270)	Iron (N = 281)	p ^a
		Mean ± SD	Mean ± SD	
Recruitment to 26 weeks' gestation				
Δ Weight (kg)	<i>unadjusted</i>	4.20 ± 2.69	3.60 ± 2.71	.01
	<i>adjusted</i> ^b	4.05 ± 2.37	3.77 ± 2.37	.17
Rate of weight gain (kg/wk)	<i>unadjusted</i>	0.25 ± 0.17	0.22 ± 0.17	.01
	<i>adjusted</i> ^b	0.24 ± 0.17	0.23 ± 0.17	.23
26 – 37 weeks' gestation				
Δ Weight (kg)	<i>unadjusted</i>	3.53 ± 2.00	3.48 ± 1.98	.73
	<i>adjusted</i> ^b	3.52 ± 2.00	3.49 ± 1.98	.90
Weekly weight gain (kg/wk)	<i>unadjusted</i>	0.34 ± 0.17	0.33 ± 0.17	.79
	<i>adjusted</i> ^b	0.34 ± 0.17	0.33 ± 0.17	.96

a. Using repeated measures analysis for comparison of means.
 b. Adjusting for body-mass index at recruitment.

recruitment to 1 month post-partum by tertiles of economic status. In contrast, there is evidence of effect modification by maternal overweight at recruitment (tables 4 and 5). Compared to the FE group, overweight women in the MM group tended to gain more weight (p = .08) and have smaller reductions in MUAC (p = .05), TSF (p = .3) and SSF (p = .08) during pregnancy. There were no differences by treatment group (p > .3) among non-overweight women (table 4). Similar interactions by maternal overweight at recruitment (p < .1 for all interactions) were seen in mean changes

in weight and body composition from recruitment to 1 month postpartum (table 5). Overweight women retained more weight (0.53 kg) in the MM group compared to the FE group, who lost 0.63 kg (p < .01), in contrast to non-overweight women, who retained about 3 kg in both groups (p = .7). The reductions in MUAC, TSF, and SSF thickness from recruitment to 1 month postpartum among overweight women were less in the MM group when compared to the FE group (p = .06, p = .12, and p = .07, respectively), with no differences by treatment group among non-overweight women (p > .25). It should also be noted that these

TABLE 4. Effect of prenatal multiple micronutrient (MM) supplements on changes in maternal anthropometry from recruitment to 37 weeks' gestation stratified by maternal overweight at recruitment^a

	Multiple micronutrients		Iron		p ^b
	N	Mean ± SD	N	Mean ± SD	
Δ Weight (kg)					.320
	Normal	192 8.48 ± 3.34	177 8.22 ± 3.34		
Overweight	91 6.12 ± 3.34	110 5.27 ± 3.36			
Δ MUAC (cm)					.103
	Normal	123 0.13 ± 1.22	117 0.18 ± 1.18		
Overweight	57 -0.68 ± 1.21	74 -1.11 ± 0.94			
Δ TSF (mm)					.135
	Normal	123 -0.41 ± 3.99	117 0.16 ± 3.99		
Overweight	57 -2.22 ± 3.99	74 -2.95 ± 3.98			
Δ SSF (mm)					.149
	Normal	123 1.10 ± 3.00	117 1.10 ± 23.01		
Overweight	57 -0.29 ± 3.00	74 -1.24 ± 3.02			

MUAC, mid upper arm circumference; TSF, triceps skinfold; SSF, subscapular skinfold
 a. Normal: body-mass index < 25 kg/m²; overweight: body-mass index ≥ 25 kg/m².
 b. Using repeated measures analysis.

TABLE 5. Effect of prenatal multiple micronutrient (MM) supplements on changes in maternal anthropometry from recruitment to 1 month postpartum stratified by maternal overweight at recruitment^a

	Multiple micronutrients		Iron		p ^b
	N	Mean ± SD	N	Mean ± SD	
Δ Weight (kg)					.062
	Normal	195 3.24 ± 3.16	178 3.11 ± 3.15		
Overweight	92 0.53 ± 3.17	112 -0.63 ± 3.14			
Δ MUAC (cm)					.030
	Normal	155 -0.12 ± 1.36	145 0.04 ± 1.35		
Overweight	67 -1.12 ± 1.36	89 -1.53 ± 1.34			
Δ TSF (mm)					.071
	Normal	122 -1.54 ± 4.21	145 -1.08 ± 4.20		
Overweight	67 -3.99 ± 4.21	89 -5.05 ± 4.19			
Δ SSF (mm)					.075
	Normal	155 0.91 ± 3.13	145 1.10 ± 3.12		
Overweight	67 -0.76 ± 3.12	89 -1.68 ± 3.11			

MUAC, mid upper arm circumference; TSF, triceps skinfold; SSF, subscapular skinfold
 a. Normal: body-mass index < 25 kg/m²; overweight: body-mass index ≥ 25 kg/m².
 b. Using repeated measures analysis.

interactions remained significant ($p < .05$ for changes from recruitment to 1-month postpartum) when restricted to the sample of women who contributed only one pregnancy.

Discussion

The absence of an effect of the intervention on maternal weight gain and changes in anthropometry during pregnancy as well as from recruitment to 1-month postpartum following adjustments for baseline differences in BMI are consistent with our earlier findings of no improvements in birth size [13]. These results are not affected by inadequate sample size for most of the outcomes. Power calculations indicate that our study sample had at least 80% power to detect a difference of 0.6 kg between the two groups for both weight gain during pregnancy and changes from recruitment to 1 month postpartum, assuming a two-tailed test and significance level of .05 [17]. Similar calculations also revealed that we had at least 80% power to detect a difference of 0.5 cm and 1 mm for changes in MUAC and skinfold thickness during pregnancy ($n = 371$) and from recruitment to 1 month postpartum ($n = 456$) except for changes in TSF during pregnancy (power = 0.64 and 0.74 for a two- and one-tailed test, respectively). It should be noted that the above differences represent small to medium effect sizes (0.2–0.35 SD). The observed changes in body composition were also small and the mean values in both treatment groups fell within the normal distribution of values for Caucasian women of comparable age measured in the first and second National Health and Examination Surveys (NHANES) in the United States [18].

Furthermore, there appears to be no selection bias that may explain these findings. Baseline anthropometric measurements were similar in the final samples when compared to the original sample of all pregnancies recruited in the trial. The comparison of baseline characteristics by intervention group in the final subsamples (data not shown) was also similar to those presented for the original sample (table 1). Finally, although we had to exclude preterm births ($n = 43$) as they precluded measurements at 37 weeks, we found similar findings when we examined weight gain during early pregnancy for the larger sample ($n = 664$) and included preterm births in the analysis of outcomes from recruitment to 1-month postpartum (data not shown).

It is also important to note that the lack of effect occurred in the context of lower-than-recommended weight gains (mean = 12 kg) and cannot be attributed to a ceiling effect [4]. Although there is less information on changes in body composition [19], overall weight gain in this study is similar to those reported in populations from developing countries [1, 4, 20, 21]. Recent

findings from a longitudinal prospective study in Guatemala using similar protocols show mean weight gain during pregnancy of approximately 7 kg [22].

Although several observational studies and food supplementation trials have examined maternal outcomes such as weight gain during pregnancy [2, 23], there is very little information as to the role of micronutrients. Villamor et al. [11] reported that average weight gain (306 g/week) was significantly higher during the third trimester in the group of HIV-positive women who received prenatal multivitamins compared to those who received the placebo. These same researchers also reported that the risk of low weight gain (< 100 g/wk) was also significantly lower (~30%) in the intervention group [11], which is consistent with their previously reported results of reductions in the incidence of low birth weight [10]. However, the relevance of these findings to non-HIV infected populations is unclear. Among studies conducted in non-HIV infected populations, a prospective study of young African-American women by Scholl et al. [8] found that the proportion of women with inadequate weight gain (28.7%) was significantly higher among women who did not report consuming prenatal MVMS compared to those who did (22%). Similarly, in an earlier supplementation trial from Chile [24], women who consumed a fortified milk supplement had significantly higher weight gain (12.3 kg) compared to those who received the unfortified supplement (11.5 kg). Improved birth outcomes were reported in both these studies.

The change in weight from recruitment to 1 month postpartum is consistent with studies in well-nourished populations showing that postpartum weight retention ranges from none to 2 kg per pregnancy [25]. This is in contrast to overall weight loss, i.e., “maternal depletion syndrome” common in populations with moderate to severe chronic energy deficiency [26, 27]. Pregnancy-related weight retention is of increasing concern in populations such as the United States, where overweight and obesity are significant public health problems. Recent data from the National Nutrition Survey in Mexico also indicate that overweight and obesity are emerging public health problems that need to be addressed along with undernutrition [28]. Therefore, our finding that overweight women who received MM retained more weight compared to those who received only iron may be of concern. It is important to note that these women gained only about 6 kg during pregnancy, with little or no weight retention postpartum. These weight gains are comparable to the recommendations for overweight women in the United States [4] and it would therefore be important to determine if the observed differences are transient or not. Ongoing follow-up studies of these women will provide valuable information. Similarly, although the differences in MUAC and SSF measurements suggest that there may be increased retention of body fat

stores in the MM group, there were decreases in MUAC and SSF in overweight women in both supplement groups. It should also be noted that our findings were not affected by the exclusion of preterm births, as we had similar findings. Although few intervention trials have examined changes in maternal body composition, observational studies primarily from the United States have shown that higher gestational weight gain, African-American race, younger age and low socioeconomic status have been positively associated with increased pregnancy-related weight retention [25], whereas prepregnancy BMI was negatively associated with overall weight gain [4] as well as fat gain during pregnancy [29].

In summary, our findings indicate that compared to iron supplements, MM supplements do not affect weight gain during pregnancy, but may lead to greater postpartum weight retention and smaller decreases in MUAC and skinfold thickness among overweight

women. It should, however, be noted that while p values $< .15$ or even $< .20$ are recommended for tests of interactions, some may call for more stringent criteria, i.e., $p < .05$, and that there is a need to confirm these findings. Further work that examines whether these differences are explained by changes in dietary intakes during pregnancy will also be useful in understanding potential mechanisms, as well in determining if there are other benefits such as improved quality of breast milk in terms of micronutrient content and infant growth and development.

Acknowledgments

This study was supported by funds from the Thrasher Research Fund, UNICEF, New York, Conacyt and INSP, Mexico.

References

1. World Health Organization (WHO). Maternal Anthropometry and Pregnancy Outcomes: A WHO Collaborative Study. Bull WHO 1995; 73(suppl).
2. Kramer MS. Balanced protein/energy supplementation in pregnancy. Cochrane Pregnancy and Childbirth Group, Cochrane Database of Systematic Reviews. The Cochrane Library, Vol 1:2003.
3. Caulfield LE, Stoltzfus RJ, Witter FR. Implications of the Institute of Medicine weight gain recommendations for preventing adverse pregnancy outcomes in black and white women. Am J Public Health 1998;88:1168–74.
4. Total amount and pattern of weight gain: physiologic and maternal determinants. In: Nutrition during pregnancy. Washington, DC, USA: National Academy Press, 1990:96–120.
5. Kardjati S, Kusin JA, Scholfield WM and de With C. Energy supplementation in the last trimester of pregnancy in East Java, Indonesia—Effect of maternal anthropometry. Am J Clin Nutr 1990;52:987–94.
6. Winkvist A, Habicht JP, Rasmussen KM. Linking maternal and infant benefits of a nutritional supplement during pregnancy and lactation. Am J Clin Nutr 1998;68:656–61.
7. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. J Nutr 1994; 124:645–54.
8. Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, and Krueger PM. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. Am J Epidemiol 1997; 46:134–41.
9. Wu T, Buck G, Mendola P. Maternal cigarette smoking, regular use of multivitamin/mineral supplements, and risk of fetal death: The 1988 National Maternal and Infant Health Survey. Am J Epidemiol 1998;148:215–21.
10. Fawzi WW, Msamanga GI, Spiegelman D, Urassa EJN, McGrath N, Mwakagile D, Antelman G, Mbise R, Herrera G, Kapiga S, Willett W, Hunter DJ. Randomized trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. Lancet 1998;351:1477–82.
11. Villamor E, Msamanga G, Spiegelman D, Antelman G, Peterson KE, Hunter DJ and Fawzi WW. Effect of multivitamin and vitamin A supplements on weight gain during pregnancy among HIV-1-infected women. Am J Clin Nutr 2002;76:1082–90.
12. Christian P, Khatry SK, Katz J, Pradhan EK, LeClerq SC, Shrestha SR, Adhikari RK, Sommer A, West KP. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: double blind randomised community trial. BMJ 2003; 326:571–6.
13. Ramakrishnan U, González-Cossío T, Neufeld LM, Rivera J, Martorell R. Multiple micronutrient supplements during pregnancy do not increase birth size compared to iron-only supplements: a randomized controlled trial in a semi-rural community in Mexico. Am J Clin Nutr 2003; 77:720–5.
14. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, Ill, USA: Human Kinetics Publishers, 1988.
15. Rivera J, González-Cossío T, Flores M, Hernández M, Lezana MA, Sepúlveda J. Emaciación y déficit de talla en menores de cinco años en distintas regiones y estratos en México. Salud Pública de México 1995;37:95–107.
16. WHO Expert Committee. Physical status: the use and interpretation of anthropometry. WHO Technical Report Series 854, World Health Organization 1995, Geneva: World Health Organization:47.
17. Cohen J. Statistical power analysis for the behavioral sciences, revised edition. New York, USA: Academic Press, Inc, 1997.
18. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. Ann Arbor, Mich, USA: University of Michigan Press, 1990.

19. Soltani H, Fraser RB. A longitudinal study of maternal anthropometric changes in normal weight, overweight and obese women during pregnancy and postpartum. *Br J Nutr* 2000; 84:95–101.
20. Winkvist A, Stenlund H, Hakimi M, Nurdianti DS, Dibley MJ. Weight-gain patterns from prepregnancy until delivery among women in Central Java, Indonesia. *Am J Clin Nutr* 2002;75:1072–7.
21. Siega-Riz AM, Adair LS. Biological determinants of pregnancy weight gain in a Filipino population. *Am J Clin Nutr* 1993;57:365–72.
22. Ramakrishnan U, Martorell R, Hughes M, Melgar P. Weight gain during pregnancy and early childhood nutrition in Guatemala. International Union of Nutritional Sciences Meeting (IUNS97), Montreal, Canada, July 27–August 1, 1997.
23. Abrams B, Altman SL, Pickett KE. Pregnancy weight gain: still controversial. *Am J Clin Nutr* 2000;71 (suppl):1233S–41S.
24. Mardones-Santander F, Rosso P, Stekel A, Ahumada E, Llaguno S, Pizarro F, Salinas J, Vial I, Walter T. Effect of a milk-based food supplement on maternal nutritional status and fetal growth in underweight Chilean women. *Am J Clin Nutr* 1988;47:413–9.
25. Gunderson EP, Abrams B. Epidemiology of gestational weight gain and body weight changes after pregnancy. *Epidemiol Rev* 2000;22:261–74.
26. Martorell RM, Merchant K. Maternal nutritional depletion. *SCN News* 1994;11:30–2.
27. Winkvist A, Rasmussen KM, Lissner L. Associations between reproduction and maternal body weight: examining the component parts of a full reproductive cycle. *Eur J Clin Nutr* 2003;57:114–27.
28. Rivera DJ, Shamah LT, Villalpando HS, González de Cossío T, Hernández PB, Sepúlveda J. Encuesta Nacional de Nutrición 1999. Estado nutricional de niños y mujeres en México. Cuernavaca, Morelos, México: Instituto Nacional de Salud Pública, 2001.
29. Lederman SA, Paxton A, Heymsfield SB, Wang J, Thornton J, Pierson RN, Jr. Body fat and water changes during pregnancy in women with different body weight and weight gain. *Obstetr Gynecol* 1997;90:483–8.

School-based iron and folic acid supplementation for adolescent girls: Findings from Manica Province, Mozambique

Peter Horjus, Victor M. Aguayo, Julie A. Roley, Maurício C. Pene, and Stephan P. Meershoek

Abstract

Background. The 1997 Demographic and Health Survey in Mozambique shows that 47% of girls 15 to 19 years old living in Manica province (west-central Mozambique) are pregnant or have already had a child. A recent survey also shows that 45% of girls 10 to 18 years old attending school are anemic. Strategies are needed to build iron stores before pregnancy and to control seasonal and chronic iron deficiency and anemia in school-aged girls.

Objective. To assess the program effectiveness of two school-based weekly iron and folic acid (IFA) supplementation regimes (5-month supplementation vs. 8-month supplementation) in girls 10 to 18 years old attending school in Manica province.

Methods. Twelve schools were included in the study. Schools were ordered by descending mean hemoglobin concentration, and assigned alternately to study group 5 (six schools; 5-month supplementation) or study group 8 (six schools; 8-month supplementation). In both study groups, the weekly supplement contained 60 mg of elemental iron and 400 µg of folic acid. All girls received a single dose of mebendazol (500 mg) twice—once at the beginning of the study (T0) and once six months later (T6).

Supplementation was implemented and supervised by the teachers of the schools included in the study. Between T0 and T3, girls in study group 8 received IFA supplements weekly whereas girls in study group 5 did not. Between T3 and T8, all girls in both groups received weekly IFA supplements.

Results. At T0, mean hemoglobin concentration and anemia prevalence were comparable in study groups 8

and 5 (125.3 ± 12.6 g/L vs. 123.8 ± 12.8 g/L; 28% vs. 29%, respectively). At T3, the mean hemoglobin concentration in study group 8 was significantly higher (126.3 ± 14.3 g/L vs. 121.5 g/dL ± 11.9 g/L, $p < .001$) and the prevalence of anemia was lower (28% vs. 35%, $p = .076$) than in study group 5. At T8, after an additional 5-month supplementation period in both study groups, mean hemoglobin concentration and anemia prevalence in study groups 8 and 5 were not significantly different (126.5 ± 12.6 g/L vs. 124.9 ± 12.3 g/L; 23% vs. 27%, respectively).

Conclusion. In Manica Province, school-based weekly IFA supplementation is a feasible and effective intervention to prevent seasonal drops in hemoglobin concentration and increases in anemia prevalence. Short supplementation periods can have an important impact on girls' hematological status. However, the size of girls' hematological response in this study was significantly lower than that observed in studies with similar population groups, initial anemia prevalence, supplement dosing, and/or supplementation regime.

Key words: Anemia, iron, folic acid, supplementation, adolescent girls, schools, Mozambique

Introduction

Global estimates show that 42% of women of reproductive age and 52% of pregnant women are anemic [1]. Maternal anemia is associated with increased rates of maternal and infant disability and death, [2] as well as with significant losses in cognitive performance, productivity, and gross domestic product [3]. In Mozambique, maternal anemia is widespread, as an estimated 55% of Mozambican pregnant women are anemic. A recent four-province survey in Cabo Delgado, Gaza, Manica, and Maputo showed that the prevalence of anemia in pregnant women ranged between 50% and 68% [4]. In Mozambique, maternal anemia is a severe public health problem and a major constraint for

P. Horjus, J. A. Roley, M. C. Pene, and S. P. Meershoek are affiliated with Helen Keller International. V. M. Aguayo is affiliated with the UNICEF Regional Office for West and Central Africa.

Please direct queries to the corresponding author: Peter Horjus, Helen Keller International, Avenida Tomas Nduda 1489. CP 1854, Maputo, Mozambique; e-mail: phorjus@yahoo.com.

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

national human development and economic growth.

For most pregnant women, it is virtually impossible to meet the high iron requirements of pregnancy (> 4 mg of absorbed iron daily) from dietary sources alone. This is particularly true in Mozambique, where fertility levels are high, birth intervals are short, and adolescent pregnancies are frequent [5]. The national policy of the Ministry of Health in Mozambique recommends that pregnant women start taking iron and folic acid (IFA) supplements daily (60 mg of elemental iron and 400 µg of folic acid) at first prenatal contact and that they continue taking them until delivery. Prenatal IFA supplementation is intended to meet the increased iron requirements during pregnancy.

In Manica province (west-central Mozambique), 47% of adolescent girls 15 to 19 years old are or have been pregnant and 45% of adolescent girls 10 to 18 years old attending school are anemic [6]. The percentage of pregnant girls that continue to attend school is unknown. It may be that once girls are pregnant, they leave school and do not return. However, these pregnant girls are targeted to continue receiving supplementation through programs currently in place for pregnant women. The effectiveness of prenatal IFA supplementation when implemented as a large-scale program through the primary health care system is often suboptimal [7]. Additional strategies are needed to build iron stores before pregnancy and to control iron deficiency and anemia.

Schools have been used in some developing countries as an effective delivery point for health and nutrition services. In Mozambique, schools could be an effective pre-existing delivery channel for IFA supplementation programs for adolescent girls. School-based IFA supplementation programs have the potential to be particularly effective when they are implemented and supervised by teachers and/or health staff [8]. Such programs could help meet the increased iron requirements associated with rapid growth spurts and onset of menses in young women, while building adolescents' iron stores before pregnancy [9].

The objective of this study was to compare the effectiveness of two school-based weekly IFA supplementation regimes (weekly IFA supplementation for 5 months vs. weekly IFA supplementation for 8 months) in adolescent girls 10 to 18 years of age attending school in Manica province. Outcome measures were changes in blood hemoglobin concentration and changes in anemia prevalence in each comparison group.

Methods

The study was conducted in the districts of Macossa and Guro. All girls in a total of 12 schools, 6 per district, were included in the supplementation program. All girls between the ages of 10 and 18 were included

in the study. Schools were chosen in conjunction with local health and education officials, based on perceived need as well as consistent physical access throughout the year. Schools were ordered by descending initial mean hemoglobin and assigned alternatively to study group 5 (six schools; 5-month supplementation) or study group 8 (six schools; eight-month supplementation). This resulted in six schools randomly assigned to either study group 8 or 5. In both study groups, the weekly supplement contained 60 mg of elemental iron and 400 µg of folic acid. Twenty-six teachers from the 12 schools participated in a half-day workshop on the technical, logistical, and supervisory aspects of school-based weekly IFA supplementation programs. These 26 teachers trained and supervised the teachers in their respective schools. Official authorization to conduct this study was granted by the Ministry of Health after review by an ethics commission, and permission forms were also collected with signatures of each participant and one parent.

Girls in study group 8 started weekly supplementation in February 2002 (T1), immediately after the baseline survey in January 2002 (T0). Girls in study group 5 began weekly supplementation three months later, in May 2002 (T3), immediately after the mid-term survey. The study concluded in October 2002 (T8), when girls in study group 8 had completed eight months of weekly supplementation and girls in study group 5 had completed five months of weekly supplementation. To eliminate a potentially major source of variation among girls, anthelmintic therapy consisting of a single dose of mebendazol (500 mg) was given to all the girls participating in the program twice over the school year—once at the beginning of the study (T0) and once 6 months later (T6).

Hemoglobin concentration was measured in all girls present in the 12 schools (including girls younger than 10 years or older than 18 years, although they were not included in the data analysis) at T0, T3, and T8 using a portable hemoglobinometer HemoCue (Agelholm, Sweden). Epi Info 2002 was used for data entry and validation of internal consistency. SPSS 11.5 was used for data management and statistical analysis. SYSTAT 10.2 was used for power analysis.

Several types of statistical tests were used in the analysis. A *t*-test was used to compare means of hemoglobin levels between the groups of girls. A paired *t*-test was used to compare means of hemoglobin levels of repeated measures in the same girls. A chi-square test was used to compare prevalences of anemia between groups of girls. A McNemar test was used to compare prevalences of repeated measures of anemia in the same girls. Linear regression analysis was used to test for confounders when comparing hemoglobin levels between study groups at each measurement. All statistical tests were performed two-sided, and results were considered significant at $p < .05$ unless otherwise noted.

Results

A total of 991 girls were present at T0—493 in study group 8 and 498 in study group 5; 858 girls were present at T3—403 girls in group 8 and 455 girls in study group 5; 822 girls were present at T8—382 in study group 8 and 440 in study group 5. A total of 511 girls were present at T0, T3, and T8 surveys—258 girls in study group 8 and 253 in study group 5. **Table 1** shows that girls who were present at T0, T3, and T8 ($n = 511$) were not significantly different from those who were present at T0 but were absent at T3 and/or T8 ($n = 480$) with respect to age, hemoglobin concentration, prevalence of anemia, or severity of anemia at baseline (T0). The analysis presented here includes only the 511 girls who were present at T0, T3, and T8 unless otherwise indicated.

In girls 10 to 11.9 years of age, anemia was defined as hemoglobin concentration below 115 g/L; girls with a hemoglobin concentration between 114.9 and 105 g/L were classified as mildly anemic; girls with a hemoglobin concentration between 104.9 and 75 g/L as moderately anemic; and girls with a hemoglobin concentration below 75 g/L as severely anemic. In girls ≥ 12 years of age, anemia was defined as a hemoglobin concentration below 120 g/L; girls with a hemoglobin concentration between 119.9 and 110 g/L were classified as mildly anemic; girls with a hemoglobin concentration between 109.9 and 80 g/L as moderately anemic; and girls with a hemoglobin concentration below 80 g/L as severely anemic [10]. At T0, the prevalence of anemia among the girls included in the study was 29%; the prevalence of mild anemia was 21% and that of moderate anemia was 8%. No cases of severe anemia were observed. No significant differences were observed between study groups 8 and 5 with respect to age, mean hemoglobin concentration, anemia prevalence, or severity of anemia (**table 1**). Prevalence of pregnancy or menstrual status was not measured.

Figure 1 shows changes in the prevalence of anemia in study groups 8 and 5 over the course of the study. At T0, the prevalence of anemia in study group 8 was not significantly different from that in study group 5

(28% vs. 29%, respectively; $p = .812$). Study group 8 did not show any significant variation in the prevalence of anemia between T0 and T3, after a 3-month weekly supplementation period (28% both at T0 and T3, $p = .903$) whereas study group 5 showed a non-significant increase in the prevalence of anemia between T0 and T3 (29% vs. 35%; $p = .141$). At T3, the prevalence of anemia in study group 8 was lower, though not significantly so, than the prevalence of anemia in study group 5 (28% vs. 35%, respectively; $p = .076$). Sample size was based on survey resources and not on the sample required to achieve a certain power. However, a post-hoc test of power shows that the power (beta) at this sample size is 0.396. A total sample size of 1,380 would be necessary to achieve a power of 0.8, assuming the same prevalences.

Study group 8 showed a decrease in the prevalence of anemia between T3 and T8 after an additional 5-month weekly supplementation period (28% vs. 23%; $p = .162$). The power (beta) at this sample size is 0.149. A total sample size of 2382 would be necessary to achieve a power of 0.8, assuming the same prevalences. Study group 5 showed a significant decrease in the prevalence of anemia between T3 and T8 after a 5-month weekly supplementation period (35% vs. 27%; $p = .021$). At T8, the prevalence of anemia in

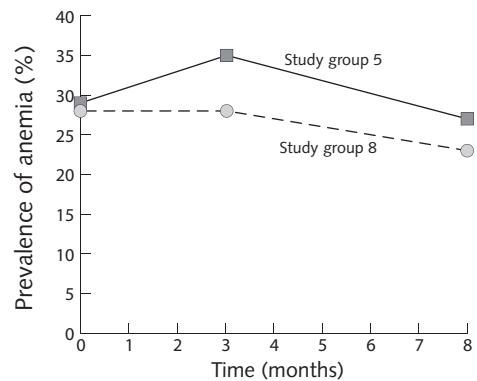


FIG. 1. Changes in the prevalence of anemia in study groups 8 and 5 over the course of the program

TABLE 1. Comparison of age, mean hemoglobin, and anemia prevalence as measured at T0 between study groups 8 and 5, and between girls present for all three surveys and those not present at T3 and/or T8

	Group 8 ($n = 258$) ^a	Group 5 ($n = 253$) ^a	p	Girls present T0, T3, and T8 ($n = 511$) ^a	Girls present T0, but missing T3 and/or T8 ($n = 480$) ^a	p
Age (yr \pm SD)	11.9 \pm 1.7	11.9 \pm 1.6	.966	11.92 \pm 1.7	11.8 \pm 1.8	.321
Mean (\pm SD) Hb (g/L)	125.3 \pm 12.6	123.8 \pm 12.8	.187	124.6 \pm 12.7	124.7 \pm 11.4	.899
Total anemia prevalence	28%	29%	.812	29%	25%	.158
Mild anemia prevalence	21%	19%	.584	20%	19%	.582
Moderate anemia prevalence	7%	10%	.237	8%	6%	.116

Hb, hemoglobin; T0, time zero; T3, 3 months; T5, 5 months; T8, 8 months

study group 8 was not significantly different from that of study group 5 (23% vs. 27%; $p = .294$). The prevalence of anemia at T0 and T8 were not significantly different in either study group 8 (28% vs. 23%; $p = .120$) or study group 5 (29% vs. 27%; $p = .585$).

Figure 2 illustrates the changes of mean hemoglobin concentration in groups 8 and 5 throughout the study. At T0, the mean hemoglobin concentration in study group 8 was not significantly different from that in study group 5 (125.3 ± 12.6 g/L vs. 123.8 ± 12.8 g/L, respectively; $p = .187$). Study group 8 did not show any significant variation in mean hemoglobin concentration between T0 and T3 after a 3-month weekly supplementation period (125.3 ± 12.6 g/L vs. 126.3 ± 14.3 g/L; $p = .219$), whereas study group 5 showed a significant decrease in mean hemoglobin concentration between T0 and T3 (123.8 ± 12.8 g/L vs. 121.5 ± 11.9 g/L; $p = .003$). At T3, the mean hemoglobin concentration in study group 8 was significantly higher than that in study group 5 (126.3 ± 14.3 g/L vs. 121.5 ± 11.9 g/L, respectively; $p < .001$).

Study group 8 did not show any significant variation in mean hemoglobin concentration between T3 and T8 after an additional 5-month weekly supplementation period (126.3 ± 14.3 g/L vs. 126.5 ± 12.6 g/L; $p = .746$). Study group 5 showed a significant increase in mean hemoglobin concentration between T3 and T8 after a 5-month weekly supplementation period (121.5 ± 11.9 g/L vs. 124.9 ± 12.3 g/L; $p < .001$).

At T8, the mean hemoglobin concentration in study group 8 was not significantly different from that in study group 5 (126.5 ± 12.6 g/L vs. 124.9 ± 12.3 g/L; $p = .150$). Mean hemoglobin concentration at T0 was not significantly different from that at T8 neither in study group 8 (125.3 ± 12.6 g/L vs. 126.5 ± 12.6 g/L; $p = .121$) nor in study group 5 (123.8 ± 12.8 g/L vs. 124.9 ± 12.3 g/L; $p = .205$).

The observed difference between study groups in mean hemoglobin concentration remains nonsignifi-

cant at T0 and T8, and significant at T3, when controlling for age and/or initial hemoglobin using linear regression analysis.

The average number of IFA supplements taken by girls in study group 8 was 21.4 ± 5.2 (maximum number of potential IFA supplements/girl, 35); average adherence to supplementation in study group 8 was 61%. The average number of IFA supplements taken by girls in study group 5 was 11.0 ± 3.0 (maximum number of potential IFA supplements/girl, 22); average adherence to supplementation in study group 5 was 50%. Adherence to supplementation was significantly higher in study group 8 than in study group 5 ($p < .001$).

Discussion

Iron requirements increase during adolescence and reach a maximum at peak growth. After menarche they remain almost as high because of the 15% additional iron requirements necessary to compensate for menstrual losses [11]. Requirements are even higher in developing countries because of the low bioavailability of dietary iron and the iron losses resulting from infectious diseases and parasitic infestations.

IFA supplementation can contribute to meeting the iron requirements during adolescence and can build iron stores before pregnancy [12]. Moreover, there is evidence that IFA supplementation enhances adolescent growth [13] and cognitive development [14]. IFA supplementation has been suggested as one of the most important nutrition interventions for adolescent girls for these and other benefits, such as decreases in the risk of maternal mortality, fetal growth retardation, and perinatal mortality, and increases in levels of concentration, physical activity, and productivity [15].

Recent analyses have shown that under supervised conditions, daily and weekly IFA supplementation regimes may have a similar impact on anemia prevalence in school-aged children and adolescents. It has therefore been suggested that weekly IFA supplementation be considered the supplementation regime of choice for adolescent girls in the context of school-based programs where there is strong assurance of supervision and adherence (i.e., teachers agree to supervise and encourage supplementation). However, there is no general "existing practice" with respect to the duration of school-based weekly IFA supplementation for adolescent girls.

In this study, anemia levels among adolescent girls attending school were high (29%), with about 75% of girls suffering from mild anemia and 25% from moderate anemia; no cases of severe anemia were observed. A high follow-up dropout rate (48%) was observed. This needs to be interpreted as a high rate of irregular

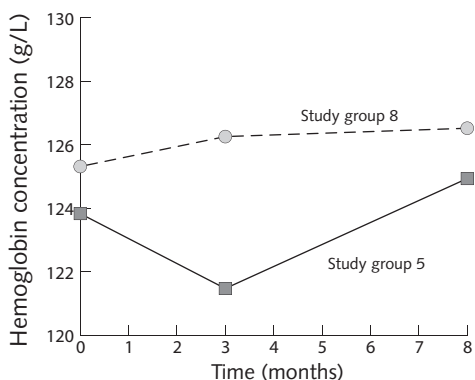


FIG. 2. Changes in mean hemoglobin concentration in study groups 8 and 5 over the course of the program

school attendance and/or school dropout rather than as high dropout from the supplementation program. In Manica Province, school officials report that adolescent girls' school attendance is suboptimal and often erratic. Although this dropout rate is high, it appears to be unrelated to treatment group, and where indicators are available, the girls who dropped out are not significantly different from the girls who were consistently measured, as presented in **table 1**.

A 3-month weekly supplementation period protected girls in study group 8 from a significant drop in hemoglobin concentration whereas lack of weekly supplementation translated into a significant drop in hemoglobin concentration in study group 5. At T3, the mean hemoglobin concentration in study group 8 was significantly higher than that in study group 5 and the prevalence of anemia in study group 8 was lower than that noted in study group 5. An additional 5-month weekly supplementation period did not translate into any significant improvement in mean hemoglobin concentration in study group 8, although a pattern of reduction in the prevalence of anemia was observed. However, a five-month supplementation period translated into a significant increase in mean hemoglobin concentration and a significant decrease in anemia prevalence in study group 5. At the end of the study (T8), mean hemoglobin concentration and prevalence of anemia in study groups 5 and 8 were not significantly different; similarly, mean hemoglobin concentration and prevalence of anemia at T0 and T8 were not significantly different in either study group.

The lack of significant difference (alpha, or *p* value) of the prevalence of anemia at T3 between group 8 and group 5, and the prevalence of anemia in group 8 between T3 and T8 is contrary to expected results. However, the low power (beta) of these results indicates a higher possibility of a type II error, failing to reject the null hypothesis when there is in fact a real difference that is not observed and may possibly be due to insufficient sample size.

The significant drop in mean hemoglobin concentration and subsequent increase in anemia prevalence observed in study group 5 between T0 and T3 (February–April 2002) could well reflect the impact of the food insecure season on the hematological/iron status of adolescent girls. In Manica, most residents are subsistence farmers and an estimated 69% of the population is food insecure [16]. The population faces an annual lean season between October and April. In this study, anthelmintic prophylaxis alone was not sufficient to prevent this seasonal drop in hemoglobin concentration and increase in anemia prevalence (as seen in study group 5). On the contrary, school-based weekly IFA supplementation provided a significant additional protection against this seasonal drop in hemoglobin concentration and increase in anemia prevalence (as

seen in study group 8) beyond any protection offered by single dose mebendazol administration.

At the end of the study (T8), the mean hemoglobin concentration in both study groups was comparable, as was the high prevalence of anemia observed in both groups. This suggests that an 8-month weekly supplementation period was not more effective than shorter supplementation periods in maintaining girls' hemoglobin concentration at seasonal heights and that neither of the two supplementation regimes appeared to improve girls' mean hemoglobin concentration above these heights. These findings suggest that in the girls included in the study, anemia has a multiple etiology that probably includes (but may not be limited to) intestinal parasite infestations, nutritional deficiencies (beyond iron and folic acid), and malaria infection [17]. In developing countries, malaria is a well-documented cause of anemia in women [18] and is likely to explain to a significant extent the high levels of anemia observed in adolescent girls in this study population.

Conclusions

In Manica region, school-based weekly IFA supplementation is a feasible and effective intervention to prevent seasonal drops in hemoglobin concentration and increases in anemia prevalence. However, less than ideal adherence to the supplementation regimen, particularly in areas where school attendance is irregular, needs to be addressed. Short supplementation periods can have an important impact on girls' hematological status. It is possible that improving adherence to the supplementation program would improve the results. Close supervision and comprehensive communication strategies have been shown to increase adolescent girls' compliance with weekly IFA supplementation programs. However, the extent of girls' hematological response in this study was significantly lower than that observed in studies with similar population groups, initial anemia prevalence, supplement dosing, and/or supplementation regime [19–24]. The contribution of malaria or other micronutrient deficiencies to anemia in the study population needs to be further explored and adequate measures for its control appropriately integrated into school-based anemia control programs for adolescent girls. The lessons learned from this study are being used to inform Manica's Regional Health Directorate in its policy and program efforts for the control of anemia in adolescents.

Acknowledgments

This paper is a product of Helen Keller International (HKI). It was developed with a grant from The Micro-

nutrient Initiative (MI), Ottawa, Canada, with financial assistance by the Canadian International Development Agency (CIDA). The opinions expressed in this paper

do not necessarily reflect those of MI or the Ministry of Health in Mozambique.

References

1. The Micronutrient Initiative. Iron improves life. Ottawa: The Micronutrient Initiative, 2003.
2. WHO. The World Health Report, 2002: Reducing risks, promoting healthy life. Geneva: World Health Organization, 2003.
3. Viteri FE. The consequences of iron deficiency and anemia in pregnancy. In: Allen L, King J, Lonnerdal B, eds. Nutrition regulation during pregnancy, lactation, and infant growth. New York: Plenum Press, 1994:121–33.
4. Ministério da Saúde and Helen Keller International. Avaliação da deficiência em micronutrientes a nível das províncias de Cabo Delgado, Manica, Gaza e Maputo. Ministério da Saúde, 1999. Maputo, Mozambique.
5. Instituto Nacional de Estatística and Demographic and Health Surveys. Moçambique: Inquérito Demográfico e de Saúde 1997. Caverton, Md, USA. Macro International Inc, 1998.
6. Meershoek S, Wetzler E, Roley J, and MacArthur C. Anemia in adolescent schoolgirls in rural Mozambique. A cross-sectional survey. In: Integrating programs to move iron deficiency and anemia control forward. Report of the 2003 International Nutritional Anemia Consultative Group (INACG) Symposium in Marrakech, Morocco. Washington DC, USA: ILSI Human Nutrition Institute, 2003.
7. Yip R. Iron supplementation during pregnancy: is it effective? *Am J Clin Nutr*, 1996;63:853–5.
8. Beaton G, McCabe G. Efficacy of intermittent iron supplementation in the control of iron deficiency anemia in developing countries. Ottawa, Canada. The Micronutrient Initiative, 1999.
9. Dallman PR. Changing iron needs from birth through adolescence. In: Fomon SJ and Zlotkin S, eds. Nutritional anemias. Nestlé Nutrition Workshop Series. New York: Vevey/Raven Press, 1992:29–38.
10. WHO. Iron deficiency: Indicators for assessment and strategies for prevention. Geneva: World Health Organization, 1997.
11. WHO. Physical status: the use and interpretation of anthropometry. Technical Report Series. Geneva: World Health Organization, 1995.
12. Lynch SR. The potential impact of iron supplementation during adolescence on iron status in pregnancy. *J Nutr* 2000;130(2S Suppl):452S–455S.
13. Kanani SJ and Poojara RH. Supplementation with iron and folic acid enhances growth in adolescent Indian girls. *J Nutr* 2000;130(2S Suppl):452S–455S.
14. Bruner BA, Joffe A, Duggan KA, Casella FJ, Brandt J. Randomized study of cognitive effects of iron supplementation in non-anemic iron-deficient adolescent girls. *Lancet* 1996;348:992–6.
15. Gillespie S. Major issues in controlling iron deficiency. Ottawa, Canada: Micronutrient Initiative, 1998.
16. International Food Policy Research Institute. Understanding poverty and well-being in Mozambique. First National Assessment 1996–97. Washington DC, USA: IFPRI, 1998.
17. Steketee RW. Pregnancy, nutrition and parasitic diseases. *J Nutr* 2003; 133 (5 Suppl 2):1661S–1667S.
18. Brabin L, Brabin B. Parasitic infections in women and their consequences. *Am J Clin Nutr* 1992;55:955–8.
19. Jayatissa R, Piyasena Ch. Adolescent schoolgirls: daily or weekly iron supplementation. *Food Nutr Bull* 1999;20:429–34.
20. Muro GS, Gross U, Gross R, Wahyuniar L. Increase in compliance with weekly iron supplementation of adolescent girls by an accompanying communication programme in secondary schools in Dar-es-Salam, Tanzania. *Food Nutr Bull* 1999;20:435–44.
21. Angeles-Agdeppa I, Schultink W, Sastroamidjojo S, Gross R, Karyadi D. Weekly micronutrient supplementation to build iron stores in female Indonesian adolescents. *Am J Clin Nutr* 1997;66:177–83.
22. Tee ES, Kandiah M, Awin N, Chong SM, Satgunasingam N, Kamarudin L, Milani S, Dugdale AE, Viteri F. School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls. *Am J Clin Nutr* 1999;69:1249–56.
23. Ministry of Health and Population/Health Insurance Organization. Egypt's adolescent anemia prevention program. A report on program development, pilot efforts, and lessons learned. Washington DC: John Snow Inc.–The Manoff Group, 2002.
24. Zavaleta N, Respicio G, Garcia T. Efficacy and acceptability of two iron supplementation schedules in adolescent school girls in Lima, Peru. *J Nutr* 2000; 130(2S Suppl):459S–461S.

Assessment of the nutritional impact of agricultural research: The case of mungbean in Pakistan

Katinka Weinberger

Abstract

Background. Evaluation of agricultural research often neglects consumption and nutrition aspects. Yet agricultural research can address micronutrient malnutrition by improving both quantity and quality of food intake.

Objective. To briefly review the conceptual linkages between agriculture and nutrition, to estimate the strength of the relationship between iron intake and productivity outcomes, and to estimate the nutritional benefit of improved mungbean varieties in terms of net present value. This paper presents a methodology for assessing the nutritional impact of mungbean, and summarizes current impact evidence on the path from mungbean research to consumption.

Methods. A consumption study was conducted among female piece-rate workers in Pakistan to analyze the impact of iron consumption on productivity, measured in wages. A two-stage least-squares analysis was used to estimate the elasticity of iron intake on wages. The results derived from this study were extrapolated to country level using secondary data sources.

Results. We found that anemia among women was widespread. Approximately two-thirds of women suffered from mild or severe anemia ($Hb < 12$ g/dL). We found the elasticity of bioavailable iron on productivity measured in wages was 0.056, and the marginal effect was 9.17 Pakistani rupees per additional mg of bioavailable iron consumed. Using the model results we estimated the impact of mungbean research on nutrition, in terms of productivity effects, and found it was substantial, ranging from US\$7.6 to 10.1 million cumulative present value (in 1995 US\$ at 5% discount rate).

Conclusions. Agriculture certainly plays an important role in the reduction of malnutrition. Agricultural research has greatly contributed to the reduction of hunger and starvation by providing millions of hungry people with access to low-cost staple foods. Now, as the challenge shifts to the reduction of micronutrient deficiencies, more efforts must be directed toward crops high in micronutrients, such as pulses and vegetables.

Key words: Asia, Pakistan, impact assessment, mungbean, agricultural research, nutrition

Introduction

The world has seen declining rates of hunger over the past three decades. The average per capita calorie consumption in developing countries has risen from 2054 in 1966 to 2803 in 1999, leading to a decline in the number of hungry people from approximately 900 million to approximately 700 million in 2000 [1]. Undoubtedly, much of this can be attributed to the successes of the “Green Revolution,” which contributed to productivity increases and a rise in the quantity of cereal crops available. Yet, despite these accomplishments, the number of those suffering from micronutrient malnutrition remains high, with an estimated 2 billion at risk of iron deficiencies, 1.5 billion at risk of iodine deficiencies, and 0.95 billion at risk of vitamin A deficiencies [2]. Micronutrient malnutrition—the “hidden hunger”—with its many health-related effects, has a number of adverse consequences on the development of poor countries. It reduces labor productivity, decreases educational achievement in children, reduces school enrollment and attendance rates, and leads to increased mortality and morbidity rates, thus increasing overall healthcare costs [3]. The consequence is seen in human and economic losses for economies, due both to current and future productivity losses.

Agricultural research can address micronutrient malnutrition by improving both quantity and quality

Katinka Weinberger is affiliated with AVRDC – The World Vegetable Center, Taiwan.

Please direct queries to the corresponding author: Dr. Katinka Weinberger, PO Box 42, Shanhua 741 Tainan, TAIWAN; e-mail: weinberg@avrdc.org.

An earlier version of this paper was presented at the Deutscher Tropentag, October 6–8, 2003 in Goettingen, Germany.

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

of food intake. Positive effects of agricultural research include declines in food prices, increased consumption, improved processing and preparation, and plant breeding [4]. Plant breeding is a direct form of contributing to the nutritional goal of agriculture by increasing the micronutrient concentration in the crop, decreasing the concentrations of absorption inhibitors, and increasing the concentration of promoter compounds [5]. The effects of agriculture on nutrition can pay off in terms of the positive impact on productivity and growth in the form of a workforce that will be stronger and healthier* over the long term by enhancing cognition in better-nourished schoolchildren.

One of the less well-researched crops is mungbean (*Vigna radiata*), common in many countries of Asia. Mungbean is a highly nutritious crop, regarded as a quality pulse for its rich protein content and excellent digestibility. Although mungbean primarily serves as a protein source, its high consumption rate and the improvement of its iron content and bioavailability render it an important contributor to iron consumption in the South Asian diet [8–10]. In a 1-year feeding trial conducted among schoolchildren aged 10 to 12 years in southern India, mungbean supplementation was shown to increase blood hemoglobin values. Children receiving supplementation with mungbean dishes high in bioavailable iron showed greater increases in hemoglobin (on average +8 g/L) compared to a group receiving supplementation with conventional mungbean dishes with low bioavailability (on average +3 g/L) supplementation and to a control group [11]. In Pakistan, production area under mungbean cultivation has increased from approximately 100,000 hectares (ha) to more than 200,000 ha between 1985 and 2000. In the same period, production increased sharply, increasing from 50,000 metric tons to more than 100,000 metric tons in 2000.

Earlier research has shown that the impact of mungbean research on consumer and producer surplus in Pakistan was approximately US\$19.7 million net present value (NPV) [12]—the discounted worth of research benefits (measured as economic consumer and producer surplus) less research costs. Since mungbean is important in diets of South Asians, it is worth asking what additional impact nutritional research on this crop may have had.

Methods

Impact assessment of agricultural research is usually confined to estimation of producer and consumer

surplus. Some studies have attempted to quantify the health benefits of agricultural research in terms of the number of “disability-adjusted life years” (DALYs) gained [13, 14]. However, the DALY approach, which is a method of combining information about mortality and morbidity within a single index, has been criticized on the grounds that it is an inequitable measure of aggregate ill health [15, 16]. Others have conducted cost-benefit analyses of nutrition interventions for anemia [17] or have quantified productivity losses in terms of gross domestic product (GDP) due to insufficient iron intake on a national and global scale by using microlevel studies and extrapolating results [18]. In this paper we attempt to estimate the size of the relationship between iron intake and productivity outcomes, and then extrapolate to country level using the results of our model as well as secondary data sources to estimate the nutritional benefit of improved mungbean varieties in terms of net present value.

Sample selection and characteristics

The survey was conducted in and around Lahore, Pakistan. The strata were based on industries employing female laborers on a piece-rate basis, since in such industries the productivity of workers has an observable wage outcome. Women working in those industries were then selected on a random basis. These industries included garments, embroidery, sewing, carpet making, kite making, pharmaceuticals, as well as cottage industries (such as quilt making, tape making, hair accessories, nut cracking, and salt crushing). Approximately 200 women participated in the survey from June 2001 through February 2002. Of these, complete data for 134 anemic women are available and used in the model estimation. The survey was repeated three times in order to account for seasonal variation in consumption and wages.

Food consumption

In each of the three survey rounds, food intakes were measured using the 7-day method for all meals consumed, i.e., breakfast, lunch, dinner, and snacks. Food intake was recorded for seven consecutive days before the survey by enumerators, who recorded dishes and quantity consumed by meal. The person primarily responsible for preparing the meal was questioned about dishes prepared and consumed, including the amount of each ingredient used; this information was then recorded. Women’s intake of nutrients at each meal was estimated using the food composition table for India [19].

* It is also important to note that when micronutrient intake is sufficient, people may have an enhanced capacity to overcome diseases such as HIV/ AIDS [6, 7].

Anthropometry and hematology

Height measurements were taken to the nearest 0.1 cm at the start of the study, using a wooden measuring board with the woman standing in upright position. Spring scales were used to measure the subjects' weight in light clothing, accurate to 0.25 kg. Hemoglobin status was measured in the three survey rounds using a fingertip sample of capillary blood obtained by a physician using system microlances. Blood was collected in a microcuvette and analyzed in a laboratory the same day. The study objectives and data-collection methods were described to all participants. All women provided written consent to be included, while those not willing to participate, either at baseline or at any time during the study period, were dropped from the sample. Permission to conduct this study was arranged by the Punjab Economic Research Institute in Lahore.

Statistical procedures

Productivity effects of iron intake

It is a relatively old idea that at low-income levels there is a relationship between nutrition and labor productivity; this hypothesis is known as the Efficiency Wage Hypothesis [20]. Others [21, 22] have argued that an increase in caloric intake enables workers to perform more demanding tasks, expressed in a greater marginal productivity as measured by wages. Iron is known to affect the productivity of individuals because it is a component of the mechanism that transports oxygen from the lung to the cells, and an increase in iron intake may also lead to an increase in productivity as measured by wages. If this is recognized by the market, i.e., if local labor markets operate relatively free and higher productivity is rewarded with higher wages, then better nutrition should result in higher market earnings, since workers would either be paid more for a given time unit of work or they would be able to work in particularly taxing and rewarding activities, or both [23].

To eliminate the potential problem of reverse causality, wages and iron intake are simultaneously predicted, employing a two-stage least-squares (2SLS) estimation procedure. The semi-log wage equation takes the following form (equation 1).

$$\ln W_{it} = \alpha_i + \gamma_t + \beta \hat{Fe}_{it} + \chi \hat{BMI}_{it} + \delta \hat{Hb}_{it} + \epsilon X_{it} + \varepsilon_{it} \quad (1)$$

Since the sample was collected over three rounds, a fixed effect model is estimated, where t indexes time and i indexes the group. In this model, α_i is the industry effect and γ_t is the time effect. Three variables are considered to be endogenous to the system: iron intake (FE), body-mass index (BMI), and serum hemoglobin (Hb); each variable can have an impact on productiv-

ity outcome. X is a vector of control variables (age, age squared, school years, and sick days reported) and ε is the error term. The instrumental variables that are used to estimate nutrient intake, BMI and blood hemoglobin level include a dummy each for current pregnancy and breastfeeding, the number of all children ever born, household size, per capita income and per capita replacement value of assets, and a price index each for cereals, pulses, vegetables, and animal products.

Iron intake is measured as intake of bioavailable iron (FeBIO), based on the method proposed by [24]. This is estimated based on total iron intake (FeTOT). Heme iron (FeMFP) is assumed to constitute 40% of iron from meat, fish and poultry. The enhancing factor (EF) for a meal is calculated as $EF = (M + F + P) + AA$ where M, F, and P are the edible quantities of meat, fish and poultry (in grams), respectively, and AA is the intake of ascorbic acid (in mg). If $EF > 75$, then EF is assumed to be 75. To take account of the inhibitory effects of phytates (PHY), a "correction term" (CT) ($0 < CT < 1$) is estimated that gives the proportion of FeBIO. For $PHY < 2.88$ mg, CT is defined as 1 (i.e., it is assumed that there are no inhibitory effects of phytate intake for such small values). For other values of PHY, CT is defined by $CT = 10^{[-0.2869 \log_{10}(PHY) + 0.1295]}$, where \log_{10} is logarithm to the base 10. Assuming that average body iron stores are 250 mg, the FeBIO can be calculated, respectively, from the following equation, \log_n being the natural logarithm [24].

$$Fe_{BIO} = 0.112 Fe_{MFP} + [4 + 14.296 \log_n \{(EF + 100) / 100\}] \times CT [Fe_{TOT} - 0.4Fe_{MFP}] / 100 \quad (2)$$

Bioavailable iron intake was estimated for each meal, and then added to obtain daily intakes. Daily intake was averaged across a week, since food intake was recorded by 7-day recall.

Present value of research

We quantify the impact of agricultural research on mungbean by estimating the effect of enhanced iron intake on overall productivity, and extrapolating impacts based on secondary production and consumption data of mungbean between 1985 and 1995. The year 1995 was chosen as the final year of the assessment in order to make the value comparable to the study by Ali et al. [12]. Wages were deflated to 1995 constant dollars, thus, the results include the total cumulative additional income achieved through productivity effects, and their value in 1995.

The present value (PV) of enhanced productivity (ΔW) due to the enhanced iron content of a modern variety (MV) of any crop, as opposed to iron consumption based on traditional varieties (TV) can be estimated based on equation (3). The change in overall bioavailable iron is given by the vector of bioavailable

iron consumption (FeBIO) based on the quantity (q) of modern and traditional varieties consumed in a given year and the difference in bioavailable iron from a particular crop in base year 0, and consumption of total bioavailable iron from all food sources in the base year, multiplied by the iron intake elasticity on wages η_{Fe} and wages W .

$$PV(\Delta W) = \sum_{n=1}^{t=n} \left(\frac{(\text{Fe}_{\text{BIO}}^{\text{MV}} \times q_t^{\text{MV}}) + (\text{Fe}_{\text{BIO}}^{\text{TV}} \times q_t^{\text{TV}}) - (\text{Fe}_{\text{BIO}}^{\text{TV}} \times q_0^{\text{TV}})}{\sum_{t=0}^{\text{Fe}_{\text{BIO}}} \right) \times \eta_{Fe} \times W_{t_0} \times (1+i)^t \tag{3}$$

Results

Food consumption

Pulses were found to be an important contributor to overall dietary iron intake, being the source of approximately 25% of all iron. The other two major sources were cereals (50%) and vegetables (20%). Four different varieties of pulses were consumed: chickpea, lentils, mungbean, and urdbean. Chickpea was most

frequently consumed (~3 kg per capita and annum), followed by mungbean and lentils (both at 1.2 kg per capita and annum), and urdbean (0.4 kg per capita and annum). This can be explained by the price of the different pulses, with chickpea being the least expensive at 37 Pakistani rupees (PKR)/kg, followed by 45 PKR/kg for mungbean and lentils, and 58 PKR/kg for urdbean.* Roughly one-third of all households had consumed mungbean the week preceding the survey. Total consumption of pulses was 5.8 kg per capita and annum.

Anthropometry and hematology

The data show that both energy malnutrition (measured by BMI) and micronutrient malnutrition are prevalent among the studied population. However, anemia is more prevalent than underweight. Approximately one-fifth of the sample suffers from underweight and one-quarter of the sample are obese (**table 1**). In contrast, two thirds of the sample suffers from mild or severe anemia (**table 2**). These results indicate that more attention should be directed toward quality, rather than quantity, of diet.

* The average exchange rate for Pakistani rupees (PKR) in June 2001 was PKR 63.5 for US\$1.

TABLE 1. Anthropometric measurements of study participants

	Survey round						Total	
	1		2		3			
	N	%	N	%	N	%	N	%
Severely underweight (BMI < 16.0 kg/m ²)	8	3.7	8	3.7	7	3.2	23	3.5
Moderately underweight (16.0–16.9 kg/m ²)	18	8.3	17	7.8	12	5.6	47	7.2
Mildly underweight (17.0–18.4 kg/m ²)	16	7.4	15	6.9	25	11.6	56	8.6
Low weight, normal (18.5–19.9 kg/m ²)	30	13.8	39	17.9	34	15.7	103	15.8
Normal weight (20.0–24.9 kg/m ²)	81	37.3	84	38.5	79	36.6	244	37.5
Overweight > 24.9 kg/m ²	64	29.6	55	25.2	59	27.4	178	27.4

BMI, body-mass index

TABLE 2. Hemoglobin values of study participants

	Survey round						Total	
	1		2		3			
	N	%	N	%	N	%	N	%
Severely anemic (Hb 7–9.9 g/dL)	30	13.6	31	14.1	22	10.1	83	12.6
Mildly anemic (Hb 10–11.9 g/dL)	131	59.5	107	48.6	119	54.6	357	54.3
Normal (Hb 12 g/dL and above)	59	26.8	82	37.3	77	35.3	218	33.1

Hb, hemoglobin

Relationship between iron intake and workers' productivity

A Lagrange multiplier test indicates that a fixed effect model is favorable to the classical regression model. The model is highly significant and the R square is 0.36. The results of a Hausman test indicate that the hypothesis—that women's iron intake and blood hemoglobin values are endogenously determined by women's wage level—cannot be dismissed. This does not hold true for the BMI. Additionally, the piece-rate wage of women is determined by their education and age, as well as by their health status (proxied by the days reported sick in the month preceding the survey). Current intake of bioavailable iron has a positive impact on current productivity, significant at $p < .10\%$ level. The BMI, a proxy for household health investments made earlier, does not show a significant impact on the productivity level of women. However, it does have a negative sign, indicating that obesity (shown to affect nearly one-third of the sample) has a negative impact on productivity when measured by piece-rate wages. See **table 3** for a summary of results.

The elasticity of bioavailable iron on productivity measured in wages is 0.056, the marginal effect is 9.17 PKR per additional mg of bioavailable iron consumed. The elasticity of blood hemoglobin level on productivity is higher at 2.347. Levin reports a similar output elasticity with respect to increases in hemoglobin levels between 1 and 2 (i.e., a 10% rise in blood hemoglobin levels would be associated with a rise in work output of 10% to 20%) [17]. In contrast to these high elasticities, at the sample mean one extra year of school education for women would result in only 0.7 and 0.8 PKR higher daily wages. This is not to say that education for women is not important, but our results also

show that without substantial improvements in health status and particularly as far as iron-deficiency anemia is concerned, increases in income and overall wealth of nations will be difficult to achieve.

Nutritional impact of mungbean research

Over the years, mungbean production in Pakistan has increased sharply, with an average annual growth rate of 5.8% between 1984 and 2000. This has resulted in an increase of annual per capita availability of domestic mungbean from 453 g to 739 g (total consumption increased from 1.08 kg in 1984/85 [12] to 1.42 kg per capita and annum in 1998 [25]). Apart from improved productivity characteristics, the modern mungbean varieties also have another, hidden, advantage. These varieties record 6.0 mg of iron per 100 grams dry matter, as compared to 3.5 mg of iron for traditional varieties [11].

Based on equation 3 we can now calculate the benefits of modern mungbean varieties for enhanced nutrition. In order to make the estimation of PV comparable to an earlier study on producer and consumer benefit by Ali et al. [12], the years 1984 (new mungbean varieties were released in 1985) to 1995 were used for this analysis. The production area under modern varieties has grown to 88% in 1995 (we assume linear annual increases of 8.8% per year). Total iron available from mungbean increased from 16.64 g/annum in 1985 to 36.97 g/annum in 1995, as compared to 14.95 mg in the base year. Compared to total iron intake, the increase in total bioavailable iron was 0.07% in 1985 and 1.1% in 1995.* Based on

* Based on a total iron intake of 6.7 mg daily for women in the base year [26] and the assumption that on average 5% of the iron intake in the diet are bioavailable.

TABLE 3. 2SLS with fixed effects: determinants of wage level ($n = 402$)^a

	Coefficient (<i>t</i> -value)	Marginal effect	Elasticity	Mean values
Hb level(g/dl) ^b	0.207*** (3.119)	6.69	2.218	10.7
BMI (kg/m ²) ^c	-0.002 (-0.402)	-0.06	-0.045	22.5
Iron intake (mg) ^b	0.281* (1.781)	9.09	0.056	0.2
School years	0.024*** (3.307)	0.78	0.034	1.4
Age	0.016 (1.578)	0.52	0.496	31.0
Age squared ($\times 10^{-3}$)	-0.299** (-2.169)	-9.67	-0.323	1.1
Days reported sick	-0.018*** (-3.224)	-0.58	-0.035	2.0
Constant	0.885 (1.144)			32.7
R ²	0.364			
F-value	23.09***			

2SLS, two-stage least-squares; Hb, hemoglobin; BMI, body-mass index

a. Dependent variable is log of daily wages.

b. Endogenous variables: include food prices, household income and assets, household size, number of all children born, and dummies for current pregnancy and breastfeeding.

c. Not treated as endogenous because of results of Hausman Test.

* $p < .10$; ** $p < .05$; *** $p < .01$.

TABLE 4. Quantification of nutritional impact of mungbean research in 1995 constant PKR and US\$

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
	MV	TV	MV	Bioavailable iron (mg)	Changes in bioavailable iron due to MV ^a	1995 constant PKR	Average productivity change	Female workforce	Economic benefit (50% anemic)	Economic benefit (60% anemic)	PV (50% anemic)	PV (60% anemic)
1984	0	453	0.0	MV	%	PKR	PKR	1000	1000 PKR (constant 1995)	1000 PKR (constant 1995)	1000 US\$ (constant 1995)	
1985	41	429	0.1	TV	0.0007	1,041	0.49	7,864.4	1,911	2,293	167	200
1986	88	413	0.3	0.7	0.0016	1,099	1.20	8,126.3	4,883	6,445	335	442
1987	146	407	0.4	0.7	0.0030	1,136	2.27	8,394.1	9,512	12,556	549	761
1988	149	273	0.4	0.5	0.0012	998	0.82	8,673.0	3,554	4,692	202	267
1989	172	219	0.5	0.4	0.0011	1,098	0.78	8,950.5	3,480	4,594	176	233
1990	279	249	0.8	0.4	0.0041	1,660	4.57	9,235.8	21,083	27,830	951	1,255
1991	315	196	0.9	0.3	0.0043	1,468	4.21	9,692.6	20,383	26,906	860	1,135
1992	316	133	0.9	0.2	0.0034	1,334	3.07	10,165.2	15,594	20,585	618	816
1993	422	111	1.3	0.2	0.0057	1,146	4.43	10,654.0	23,603	31,157	910	1,201
1994	511	70	1.5	0.1	0.0074	987	4.88	11,165.9	27,262	35,986	985	1,300
1995	573	78	1.7	0.1	0.0090	1,590	9.63	11,692.7	56,295	74,309	1,868	2,465
Σ											7,620	10,075
Source	[25]		(1) × 6 mg × 5%	(2) × 3.3 mg × 5%	(3) + (4) - (4) _{1984/122}	[30]	(5) × (6) × (1)	[27]	(7) × (8) × 50%	(7) × (8) × 60%	Exchange rate PKR/US\$	

PKR, Pakistani rupees; MV, modern varieties; TV, traditional varieties

a. Changes in bioavailable iron due to MV in column 5 are based on the assumption that total annual per capita bioavailable iron in diet during the base period was 122 mg (NIH 1985–1987 survey), and assuming a 5% bioavailability of iron in the diet; $\eta P = 0.056$ (see table 3).

the elasticity estimate of 0.056, per capita productivity increases due to enhanced consumption of bioavailable iron grew from 0.49 PKR per capita and annum in 1985 to 9.63 PKR per capita and annum in 1995.

The total female workforce in Pakistan increased from 7.8 million in 1985 to 11.7 million in 1995 [27]. Given that effects of increased iron intake on productivity can only be observed among anemic individuals, we considered only the share of the workforce that is anemic. Estimations are in the range of 50% to 60% [28, 29]. We use wage data provided by ILO for the textile industry, an important sector for women's work [30]. The data are adjusted by the GDP deflator [31] and multiplied by the annual additional income per woman. Discounted at a rate of 5% over the course of 10 years, the cumulative present value in 1995 of this additional income accrues to 7.6 to US\$10.1 million, depending on the number of anemic women in the workforce (see **table 4**).

This quantification does not include reductions in forfeited productivity due to deficient iron intake during childhood and youth, which could potentially be very large, nor productivity losses due to anemia among the male workforce. These benefits of mungbean research are in addition to the total consumer and producer benefit attributable to mungbean research that has been estimated at US\$19.7 million [12]. This analysis shows that the additional benefit of mungbean consumption in terms of enhanced human productivity is substantial and can be compared to direct research impacts.

Discussion

Quantifications of productivity losses due to iron deficiency range from 5% to 17% among agricultural laborers in India [32] to 17% losses in heavy labor and 5% losses in blue-collar work [18, 33]. Cognitive losses in children due to iron deficiency anemia have been estimated at US\$4 per capita [34]. For economies as a whole, overall losses have been estimated at between

0.9% and 1.25% of gross domestic product (GDP) [18, 33]. Combating micronutrient deficiencies is therefore not only a goal in itself, but is also an important means to decrease poverty in developing countries.

This study has shown that the nutritional impact of agricultural research, measured in productivity increases of population groups deficient in micronutrients, is substantial. In the case studied here, they amounted to between US\$7.6 and 10.1 million, approximately half of total consumer and producer surplus estimated for the crop. The approach presented here can be adapted for other crops to enable assessments at the macro level. For the assessment of the impact of agricultural research on the nutrition status at the micro level, the quality and reliability of data remains a question of major concern. The collection of anthropometrical data, the most reliable indicator for success, is expensive, as are food-intake recall variables. Some progress has been made in linking dietary diversity to overall food intake [35]. Another approach is to link attitude toward and knowledge about vegetables to their actual intake.

Agriculture certainly plays an important role in the reduction of malnutrition. Agricultural research has greatly contributed to the reduction of hunger and starvation by providing millions of hungry people with access to low-cost staple foods. Now, as the challenge becomes the reduction of micronutrient deficiencies, more efforts must be directed toward crops high in micronutrients, such as pulses and vegetables. Highlighting and measuring linkages between agriculture and nutrition will certainly become even more important in the future.

Acknowledgments

Financial support for this project by Deutsche Gesellschaft fuer Technische Zusammenarbeit (GTZ) and the Bundesministerium fuer Wirtschaftliche Zusammenarbeit (BMZ) is gratefully acknowledged.

References

1. Food and Agriculture Organization (FAO). World Agriculture: Towards 2015/30. An FAO perspective. Rome: FAO, 2003.
2. World Health Organization (WHO). Nutrition for health and development. A global agenda for combating malnutrition. Geneva: WHO, 2000.
3. Underwood B. Overcoming micronutrient deficiencies in developing countries: is there a role for agriculture? *Food Nutr Bull* 2000;21:356–73.
4. Haddad L. A conceptual framework for assessing agriculture–nutrition linkages. *Food Nutr Bull* 2000;21: 367–73.
5. Ruel M, Bouis H. Plant breeding: a long-term strategy for the control of zinc deficiency in vulnerable populations. *Am J Clin Nutr* 1998;68:488S–945S.
6. Friis H. The possible role of micronutrients in HIV infection. *SCN News* 1998;17:11–2.
7. Beisel W. Nutritionally acquired immune deficiency syndromes. In: Friis H, ed. *Micronutrients and HIV infection*. Boca Raton, Fla, USA: CRC Series in Modern Nutrition, 2002.
8. Yang RY, Tsou S. Mungbean as a potential iron source in South Asian diets. International Consultation Workshop on Mungbean: Proceedings of the Mungbean Workshop.

- New Delhi, India, 1998:152–58.
9. Asian Vegetable Research and Development Center (AVRDC): The World Vegetable Center. Mungbean as a better dietary iron source for South Asia. AVRDC Progress Report 1997:129–31.
 10. Asian Vegetable Research and Development Center (AVRDC): The World Vegetable Center. Promoting best practices for sustainable use of medicinal and indigenous food plants in developing countries. A case study on AVRDC's work in mungbean. Shanhua: AVRDC, 1999:13.
 11. Vijayalakshmi P, Amirthaveni S, Devadas RP, Weinberger K, Tsou S, Shanmugasundaram S. Enhanced bioavailability of iron of mungbeans and its effects on health and performance of school children. Technical Bulletin No. 30. Shanhua: AVRDC, 2003.
 12. Ali M, Malik I, Sabir H, and Ahmad B. The mungbean green revolution in Pakistan. Technical Bulletin No. 24. Shanhua: AVRDC, 1997.
 13. Zimmermann R, Qaim M. Potential health benefits of golden rice: a Philippine case study. *Food Policy* 2004;29:147–68.
 14. Stein A, Meenakshi JV, Qaim M, Nestel PH, Sachdev PS, Bhutta ZA. Analysing health benefits of biofortified staple crops by means of the DALY approach: a handbook focusing on iron and zinc. 2004. Washington, DC: HarvestPlus Technical Monograph Series. In press.
 15. Anand S, Hanson K. DALYs: efficiency versus equity. *World Develop* 1998;26:307–10.
 16. Arnesen T, Nord E. The value of DALY life: problems with ethics and validity of disability adjusted life years. *BMJ* 1999; 319:7222, 1423–5. <http://bmj.bmjournals.com/cgi/reprint/319/7222/142>. Accessed June 14, 2005.
 17. Levin H. A benefit-cost analysis of nutritional programs for anemia reduction. *World Bank Research Observer* 1986;1:219–45.
 18. Horton S, Ross J. The economics of iron deficiency. *Food Policy* 2003;28:51–75.
 19. Gopalan C, Rama Sastri BV, Balasubramanian SC. Nutritive Value of Indian Foods. Hyderabad, India: National Institute of Nutrition, 1999.
 20. Leibenstein H. Economic backwardness and economic growth. New York: Wiley, 1957.
 21. Mirlees J. A pure theory of underdeveloped countries. In: Reynolds L, ed. *Agriculture in development theory*. New Haven, Conn, USA: Yale University Press, 1975.
 22. Stiglitz J. The efficiency wage hypothesis, surplus labor and the distribution of income in LDCs. *Oxford Economic Papers, New Series* 1976;28:185–207.
 23. Strauss J. The impact of improved nutrition on labor productivity and human-resource development: an economic perspective. In: Pinstrip-Andersen P, ed. *The political economy of food and nutrition policies*. London, UK: The Johns Hopkins University Press, 1993.
 24. Bhargava A, Bouis HE, Scrimshaw NS. Dietary intakes and socioeconomic factors are associated with the hemoglobin concentration of Bangladeshi women. *J Nutr* 2001;131:758–64.
 25. NARC (National Agricultural Research Centre). Mungbean production figures for Pakistan 1997–2002. Islamabad: NARC, 2002.
 26. Pakistan National Institute of Health. National Nutrition Survey 1985–87. Government of Pakistan, 1988.
 27. World Bank. *World Development Indicators 2003*. Washington, DC: World Bank, 2003.
 28. Mason J, Lotfi M, Dalmiya N, Sethuraman K, Geibel S, Gillenwater K, Gilman A, Deitchler M, Mock N. The micronutrient report: current progress and trends in the control of vitamin A, iron, and iodine deficiencies. Ottawa: The Micronutrient Initiative/International Development Research Center, 2000.
 29. The Micronutrient Initiative. IDA Report Pakistan, 2002. <http://www.mn-net.org/idastat>. Accessed June 5, 2005.
 30. ILO (International Labor Organization). LABORSTA, 2003. <http://laborsta.ilo.org/>. Accessed June 5, 2005.
 31. Asian Development Bank (ADB) Key Indicators 2004. Manila: Asian Development Bank.
 32. Weinberger K. Micronutrient intake and labor productivity: evidence from a consumption and income survey among Indian agricultural laborers. *Outlook on Agriculture* 2004;33(2):255–60.
 33. Horton S. Opportunities for investments in nutrition in low income Asia. *Asian Develop Rev* 1999;17:246–73.
 34. Ross J, Horton S. Economic consequences of iron deficiency. Ottawa: The Micronutrient Initiative, 1998.
 35. Engle P, Menon P, Haddad L. Care and nutrition: concepts and measurement. *World Develop* 1999;27: 1309–37.

Commentary on “Assessment of the nutritional impact of agricultural research: The case of mungbean in Pakistan”

The article by Weinberger [1] makes a contribution to the literature on cost-benefit analysis of agricultural and nutrition projects, focusing on quantifying the possible impact of improving the iron content of a particular crop on iron consumption, iron nutriture, and thus, economic productivity. The value of this type of analysis is that it demonstrates the significant potential impact that a small change in the micronutrient content of the food supply may have, and it emphasizes the important point that biofortification as a focus of agricultural research can be interpreted not only as an agricultural, but also as a nutrition intervention.

The conclusions of this paper rest on a series of assumptions that are plausible, but should be recognized as such. The logical sequence is as follows. An improved variety of a staple crop (in this case, mungbean) is introduced; because it offers higher yields (the iron content was an unintended side effect of breeding for yield), farmers adopt this new variety. The new variety finds its way to the market, and it is purchased by households with members vulnerable to iron deficiency anemia. The food is consumed (at least in part) by those suffering from iron-deficiency anemia, and this raises their blood iron levels. Their higher iron results in greater physical and mental capacity, and this leads to greater economic productivity. Each of these links in a logical chain depends on certain assumptions: uptake by farmers, marketing to consumers, purchase and consumption by those suffering from iron deficiency, improvement in iron nutriture. For these improvements to be translated into economic contributions, in the present analysis, the (previously) anemic individuals must also participate in the paid labor force.

These assumptions are laid out in the present paper, and well justified with reference to the wider literature, but not clearly quantified in the Pakistan case. We don't see disaggregated purchase or consumption data at the household/individual level that would allow verification of the assumption that the households choosing to purchase mungbean are those in which there are anemic individuals who are in the labor force (or could be if they were healthier). Mungbean is more expensive than the least expensive pulse, chickpea, and might be less likely to be chosen by poorer, more price-sensitive households whose members may also be more vulnerable to anemia. In the survey reported here, one third of households had consumed mungbean the previous week, and two thirds had not. We don't know if those reporting consumption are the households with anemic individuals. Results of the analysis show that iron intake is weakly related to wage levels among anemic women, while hemoglobin is more strongly

related. (It is possible that the effect of iron intake is underestimated in this analysis because hemoglobin, presumably an outcome of iron intake, is included in the same equation—some of the effect of iron intake may be captured in the parameter for hemoglobin.) Mungbean is one likely contributor to iron intake and thus to improved hemoglobin, but not the only one. To gauge the contribution of improved mungbean to iron status and thus productivity (wages) directly, one would ideally want to know patterns of consumption in vulnerable households, and, equally important, patterns of substitution among foods that contribute iron to the diet. If mungbean substitutes for a food with lower iron content (including the traditional lower-iron mungbean variety), then it should raise total iron intake; if it substitutes for other good iron sources, the impact may be neutral (or even negative).

The use of instrumental variables to correct for endogeneity is commonly accepted in the econometric literature. Endogeneity exists when the causal relationships between the independent variables (iron intake, hemoglobin) and the dependent variable (wages) are bi-directional. Iron status determines wages (through its effect on productivity), but wages may determine iron status (by allowing the purchase of a better diet). In the present analysis, both iron intake and hemoglobin level are included in the key wage-determination regression as instrumented variables, that is, they are estimated based on a predictive equation, and it is the predicted, not the actual, values that are included in the regression on wages. It is helpful, in interpreting the results of analyses that correct for endogeneity in this way, to know the strength of the predicting equations, but that information is not provided here. Furthermore, it is not clear that all the variables included in the equations predicting the endogenous variables are uncorrelated with the error term in the wage equation (a key assumption underlying the instrumental variables approach). In particular, variables such as per capita income and household assets, included in the predicting equations, would seem on the face of it to be closely related to wages. If this is the case, then the parameters on the endogenous variables are both biased and inconsistent—that is to say, we cannot rely on the accuracy of these results. Of course, the use of instrumented variables for iron intake and hemoglobin also takes this analysis one step further away from the direct assessment of the contribution of iron specifically from mungbean to the iron status of the population studied.

The major contribution of a paper like Weinberger's is to provide a reasonable estimate of the potential

economic contribution of an intervention like the development of a biofortified food. The paper persuasively argues that the potential for an agricultural intervention to contribute to the economy may be realized not only through increased yields and farmer income, but also through its health and nutritional impacts, and it gives a plausible estimate of the possible magnitude of the contribution. In this sense, the paper is not so much a direct evaluation of the precise

quantitative contribution of improved mungbean to the economy of Pakistan as it is a contribution to the wider literature on the assessment of cost/benefit ratios for specific kinds of agricultural research.

*Beatrice L. Rogers
Friedman School of Nutrition Science and Policy
Tufts University
Boston, MA 02111*

Reference

1. Weinberger K. Assessing the nutritional impact of agricultural research: The case of mungbean in Pakistan. *Food Nutr Bull* 2005;26:287–94.

Women's access to food-processing technology at the household level is associated with improved diets at the pre-harvest lean season in The Gambia

Irma Silva-Barbeau, Stephen G. Hull, Marilyn S. Prehm, and William E. Barbeau

Abstract

Background. Women's access to food processing technology at the household level may have positive dietary benefits during the pre-harvest lean season when households are most stressed from food shortages and higher energy expenditures from agricultural work.

Objective. This study in rural Gambia was conducted to determine if women's access to small manually operated oil presses (ram) for sesame oil extraction had any significant effects on seasonal fluctuations of household oil supply and on dietary intakes of women and children.

Methods. Participants were 40 women and children with access to community-based motorized oil press expellers (Expeller-control), 37 women and children with access to village-based ram presses (Press-experiment), and 43 women and children with access to both ram press and motorized expeller (Combination). Dietary data were collected at baseline, at peak oil-pressing, at pre-harvest lean, and at the post-harvest seasons.

Results. Households in the Press-experiment and Combination groups consumed 37 and 51 percent more oil, respectively, than those in the Expeller-control group during the pre-harvest lean season. Women from the Press-experiment and Combination groups consumed more energy at the lean season than those in the Expeller-control group. Similarly, children from the Press-experiment

and Combination groups consumed more protein at peak oil-processing season than those from the Expeller-control group. At the pre-harvest season children from these two groups also consumed more protein, however, only the consumption of the Combination children was statistically significant compared with that of the Expeller-control group ($p < .05$). Press-experiment children consumed more nutrient-dense weaning foods during the pre-harvest lean season than Expeller-control children.

Conclusions. Women's access to appropriate technology can provide the means to "add value" to their agriculture product, which may serve as an economic stabilizer with potential to increase dietary intakes and incomes, especially during the pre-harvest lean season.

Key words: Women, ram press, food processing technology, economic stabilizer, pre-harvest lean season, dietary intakes, The Gambia

Introduction

Seasonal fluctuation in household food supply is well documented throughout West Africa [1–3], and The Gambia is no exception. The cyclic pre-harvest lean period occurs for many reasons, but is principally due to low household food supplies resulting from depleted food stores from the previous year's harvest and limited nonfarm employment opportunities, resulting in little income to purchase food. There is usually an increased incidence of malnutrition among children and adults during this period due to unmet dietary energy needs, coupled with the heavy labor demands of agricultural work and illness [3–10].

The National Nutrition Surveillance Program of the Gambian Ministry of Health estimates that in the dry and rainy seasons, respectively, 12% and 18% of Gambian children are malnourished. Children in rural parts of the country do not fare as well as their urban counterparts as indicated by a higher infant mortality rate, lower average daily energy consumption, a higher

Irma Silva-Barbeau is affiliated with Silva Associates, Blacksburg, VA, USA. Stephen G. Hull is affiliated with Westat, Rockville, MD, USA. Marilyn S. Prehm is affiliated with Concepts, Carmichael, CA, USA. William E. Barbeau is affiliated with the Department of Human Nutrition, Foods, and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

This work was conducted in partial fulfillment of Stephen Hull's M.S. degree in the Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

Please direct queries to the corresponding author: Dr. Irma Silva-Barbeau, Silva Associates, 1403 Locust Ave., Blacksburg, VA, USA 24060; e-mail: silvabarbeau@catholic.org.

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

percentage of kilocalories (kcal) from cereals, and a lower percentage of kcal from oil [11].

Catholic Relief Services (CRS) introduced sesame seed cultivation to women's groups in The Gambia in 1983 with the primary objective of increasing the household supply of cooking oil. Sesame production quickly became popular because it required minimal labor as compared with groundnuts, it was drought resistant, and could be planted after early millet and maize thus spreading out labor [12]. Sesame is primarily a woman's crop with production driven by an interest in processing the seed for oil that can be consumed or sold on the local market [13].

CRS installed 16 diesel-powered oil expellers at the community level between 1983 and 1989 [12] in hopes that sesame oil would provide a much-needed additional source of dietary energy. However, by 1993, 10 expellers were out of service because of mechanical failures. Gambian women reacted to this crisis by planting less sesame.

One promising solution to the problem was the introduction of locally manufactured ram presses, which were being promoted in the region by their developer, Enterprise Works Worldwide (formerly Appropriate Technology International, ATI). The ram press (**fig. 1**) is a relatively inexpensive* manual-powered device for extraction of edible oils that can be manufactured and repaired in rural workshops, and can be made available at the household level [14, 15]. The ram press was made available to the women sesame growers through the Sesame Growers Association (SGA) with the overall aim of helping to alleviate seasonal fluctuations in household food supply and increase and improve dietary intake of women and children in rural villages through stimulation of the sesame crop cultivation. The hypothesis was that a simple readily accessible press, under the women's control, would contribute to greater availability of edible oils for household consumption and for sale that would, in turn, stimulate greater sesame production.

To test the effects of the use of such a press on the dietary intake of women and children, the Small-Scale Sesame Oil Production Project was undertaken in 1994.

Methods

Ram press placement, training, and implementation procedures

The Small-Scale Sesame Oil Production Project was



FIG. 1. Gambian woman using a ram press to extract sesame oil

implemented in several phases. In December 1994, a CRS technician trained workers in four machine shops in The Gambia in manufacturing of the ram press. To test the potential use of the ram press for sesame, CRS provided 16 imported Tanzanian presses for 20 different user groups in 16 villages in The Gambia [16]. The participants in this initial field test were individuals and groups selected in conjunction with SGA leadership and CRS. ATI staff made several monitoring trips in February and April 1995 to oversee press operations and to evaluate overall functioning of the presses. In July 1995, 40 villages located in the Northern Bank Division of The Gambia were selected to receive presses.

In October 1995, after CRS and the Gambia Food and Nutrition Association (GAFNA) field visits, 80 women sesame growers with preschool children from these 40 villages were selected to participate in the study. The 40 presses were placed in the study villages in February 1996 and participants were trained in the use and maintenance of the ram presses.

Pilot study

A pilot study was conducted in May 1995. For the pilot study, the principal investigators, in collaboration with CRS and GAFNA staff, developed a preliminary questionnaire. Common household cooking and feeding utensils (including handfuls[†]) of women and children were standardized to gram weight measurements

* Estimated current price of a locally manufactured ram press is about \$300.00. The estimated current price of a motorized expeller is about \$4,000 (Enterprise Works Worldwide).

for the types of foods consumed. A list of *Mandinka* and *Wolof* food names with their English equivalents was developed to facilitate recording of dietary data. Standardized recipes were developed for all commonly consumed food mixtures [16]. A codebook containing weight measures was developed to facilitate coding of the dietary data in the nutritional analysis program, Nutritionist IV.

GAFNA conducted the pilot study in 10 villages to test and refine the survey instruments and to give the field staff the experience necessary to conduct the study. These villages were outside of the actual study area and were divided into two groups: press villages, those where the initial testing of the ram press took place, and non-press villages, those without sesame ram presses. A sample of 40 women with preschool children between the ages of 1 and 5 years participated in the pilot study. These women were selected for a socioeconomic status and ethnic background similar to those that would participate in the actual study. GAFNA analyzed the pilot study data, and the investigators used the results to refine the final questionnaires and procedures for the actual study [16].

Study design

A conceptual framework was developed that hypothesized the various pathways through which a readily accessible oil-pressing technology would increase sesame production and improve dietary intakes of women and children directly through oil consumption, and indirectly through incomes increased by the sale of oil and sesame cake. A detailed conceptual framework is discussed and presented elsewhere [17].

The study was conducted in 52 villages of the North Bank Division of The Gambia in an area of rice and peanut cultivation. These villages were chosen because of their similar agro-ecological characteristics (i.e., farming systems, farming practices, amount of rainfall, soil productivity, etc.). The study participants were women sesame growers with children between 1 and 5 years of age. The research design consisted of a control group (those with access to the motorized oil expeller (Expeller-control), and an experimental group (those with access to the ram press (Press-experiment.) Press-experiment subjects were recruited from 32 villages that had received ram presses in early 1995. The Expeller-control subjects were recruited from 20 villages from the same area as the Press-experiment villages. The Expeller-control subjects had access to functional motorized oil expellers but not to ram presses. The Virginia Polytechnic Institute and State University's Institutional Review Board (IRB) for Human Subject

Research reviewed and approved this study.

To ensure the possibility of finding statistically significant differences between groups, if indeed there were any, sample sizes were determined using power analysis. The results of a fortified weaning food study in Mali [3] were used to direct the power analysis. For this study, a control group of 40 women with preschool children (aged 1 to 5 years) and an experimental group of 80 women with children (aged 1 to 5 years) were selected.

Two groups of women were recruited: the Expeller-control group (domestic sesame growers with children from ages 1 to 5 years of age, members of SGA, and using a motorized expeller for processing oil) and the Press-experiment group (domestic sesame growers with children from ages 1 to 5 and members of SGA with no access to a motorized expeller). The final sample consisted of 40 women sesame growers with motorized expellers who made up the Expeller-control group, and 80 women sesame growers with ram presses who made up the Press-experiment group. All women participants in both groups were required to have the means to keep records and permission to participate in the study from their husbands or other influential members of their respective households. Often literate schoolchildren from the household kept the records for the women when they themselves could not. Since an individual household sometimes contained two or more eligible women, one woman was chosen randomly and was designated as the "target woman" and her preschool child was designated as the "target child." If she had more than one child aged 1 to 5 years, then one of them was randomly selected to be the "target child."

Monitoring and qualitative evaluation of oil pressing

All women in both groups were trained initially on how to keep records regarding the quantity of sesame seeds they pressed, and the amount of oil that was extracted. The administrative members of the SGA periodically monitored the record keeping. In addition to this, women were asked to participate in a post-pressing village-based qualitative evaluation of the ram press and benefits of the press versus the expeller. Areas of the evaluation included manufacturing and operation of the press, what women liked and disliked about the press (e.g., heaviness, limited capacity, speed and pre-heating time), and benefits and trade-offs (e.g., health benefits, use of sesame oil and cake, replacement of sesame oil for peanuts in different dishes, making of soap, residue for fertilizer, and animal feed). Areas of comparison regarding the press and expeller were the women's time, cost of fees to use the press, convenience, labor demands, and the like. The results of the evaluation of the manufacturing and operation of the press were fed back to the manufacturer to make the necessary ergonomic adjustments so the ram press would work better for women and be more specific for sesame

† In this culture, people customarily eat with their hands; therefore, it was necessary to standardize the amount of food in each handful.

seed. The full results of this evaluation are presented elsewhere [18].

Research instruments and data collection

Results from the pilot study were used to revise the questionnaire. A protocol was developed to guide data collection and ensure that data were collected in a precise manner. The questionnaire was designed to identify key direct and indirect benefits of the ram press suggested by the conceptual framework.

Research instruments were pre-tested in The Gambia during the pilot study. Enumerators were trained in November 1995 on the proper procedures for collecting the data. A Gambian Nutrition Specialist closely supervised all data collection. Baseline data were collected in December 1995 during the post-harvest season. After establishing a baseline, data were collected three times in the following year; in March, August, and December. These times corresponded to peak oil-pressing season (January to April with the month of March being the peak pressing month), the pre-harvest lean season (July and August), and the post-harvest season (December). There are no differences in the length of seasons among villages in the North Bank Division of The Gambia, where the study was conducted.

The questionnaire was composed of seven sections. Section A was distributed only at baseline and was designed to obtain basic descriptions of the households, women, and children. Section A also gathered further information on household profiles, including data on the number of households in the compound, the number of individuals in the household, the relative wealth of the households, and the source, quantity, and quality of the household water supply. A detailed description of each household member was also obtained, which included name, age in years, sex, and relationship to the household head. This profile of the household was updated at each subsequent data collection period. The households were ranked as to their relative wealth. The wealth rankings were obtained by convening a meeting of three key informants to determine the criteria for wealth ranking. The criteria established were based on: 1) the household's ability to produce enough food for their families throughout the year, 2) the household's access to labor and farm machinery, and 3) the household's involvement in non-farm activities, such as petty trading and salaried employment and the number and kind of livestock owned. The key informant in each village ranked each household participating in the study on a scale from 1 (poor) to 5 (rich) relative to other households in the village.

Information on target women included age, marital status, ethnic background, education, use of the nearest health center, and a description of her agricultural activities. Information on target children included date of birth from the infant birth card, birth weight,

and sex.

Section B of the questionnaire dealt with health-related issues of the child and mother. Information such as the child's weaning status, incidence of diarrhea in the last 2 weeks, incidence of illness in the last 7 days, the woman's enrollment in the health and nutrition supplement program was obtained, as well as information on her reproductive status. This section also gathered anthropometric data on women and children, and these results are presented elsewhere [19].

Sections C, D, E, and F of the questionnaire attempted to obtain detailed information on the food availability of the household, and the food consumption of the women and children.

Household oil consumption and food availability

Household oil consumption patterns in the previous seven days were obtained, as were data on per-annum household oil availability and purchasing patterns. Women responsible for household food preparation were asked to report all foods and amounts prepared for the household on the previous day.

24-hour recall

During the pilot study, methodology for collection of food recall was standardized as described in the pilot study. Women were asked to report the amounts of all food items that they consumed in the previous day. Children's consumption was always obtained in the same manner from the child's primary care giver. Two nonconsecutive 24-hour recalls were used to obtain food consumption data because it has been determined that a precision equal to that of a 1-day weighted food intake survey could be obtained with two 24-hour recalls at a much lower cost [20]. Ferguson et al. [21], and Villard and Bates [22] in The Gambia, reported similar results.

Food frequency questionnaire

A food frequency questionnaire was designed to ascertain each target child's pattern of consumption of oil and protein-rich foods. The food frequency questionnaire had been previously pre-tested in The Gambia [16] and used in Mali [3]. The target child's primary caregiver was asked to report the frequency of foods consumed by the child in the previous 4 weeks. The 27 food items included in the food frequency questionnaire were grouped into the following categories: high fat/high protein foods, nutrient-dense weaning foods, nutrient-dense table foods, low-calorie weaning foods, milk, and other snack foods.

Data analysis

Shortly after initiation of the study, we observed that some women who had been assigned to the Press-

experimental group were also using the motorized expellers. We addressed this in the data analysis by treating them as a separate group, and we refer to them as the Combination group. To ensure that the groups remained separate and distinct throughout the study, SGA monitors were vigilant in making sure that there was no additional crossover from the Press to the Combination group. This gave us a total of 40 mother-child pairs in the Expeller-control group, 37 in the Press-experiment group, and 43 in the Combination group.

Dietary data were analyzed using Nutritionist IV software to calculate amounts of macronutrients (total kcal, grams of protein, carbohydrate, and fat) and micronutrients (μg of β -carotene) in the diet. Gambian foods that were not in the Nutritionist IV database were added using macro and micronutrient values for these foods reported by the USDA [23] and FAO [24]. Results reported by Hudson et al. [25] and Hudson and Day [26] were used to estimate nutrient values for millet, sorghum, and maize prepared by three cooking methods commonly used in The Gambia. Values for β -carotene (expressed in retinol equivalents) content of foods were added using results obtained by the FAO [24], Villard and Bates [22], King and Burgess [27], and McCrae and Paul [28].

The household availability of all nutrients and oil was adjusted for age and sex using adult equivalents derived from the daily energy requirements reported in Energy and Protein Requirements (FAO/WHO) [29]. The adult equivalents used for this analysis were similar to the results obtained by Hudson [30] in The Gambia, who developed an algorithm based on body weight to estimate the distribution of food within a mixed sex and age group. Hudson concluded that this was likely to be appropriate in most circumstances. Adult equivalents have been used extensively to report household measures on a per-capita basis that is adjusted for age and sex [31–34].

In a similar manner, each child's intake of nutrients was adjusted for age and sex using consumption units based on the daily average energy requirements established by FAO/WHO [29].

The SAS software package was used to calculate means and SDs of dependent variables. Due to the seasonal nature of the data, statistical significance at $p < .05$ was determined by repeated measures analysis of variance (RMANOVA) when appropriate. Otherwise statistical significance was determined by the ANOVA method. Because of unequal sample sizes, significant differences between group means were determined using the Behrens-Fisher procedure and tables of the Studentized range.

Results

General characteristics of the study population

There were no statistically significant differences in major parameters regarding the households, women, and children at the baseline. Women had a mean age of 29 years, most were in a polygamous marriage, and were equally distributed among the Wolof, Mandinka, and Fulani groups and living in similar compounds and household sizes. The majority of the women had attended some type of school for at least three years (**table 1**). There were no differences in the types of crops that they grew, with the exception of white sesame. CRS had introduced white sesame in the early 1990s* as a cash crop for export and for the manufacture of confections. The black sesame was for local consumption as oil. More women in the Expeller-control group were growing white sesame than those in either the Press-experiment or the Combination group. Similarly, more women in the Expeller-control group were engaged in income-generating activities than those in the Press-experiment and Combination groups (**table 1**). These differences were statistically significant at $p < .05$.

Conversely, more women in the Press-experiment group were ranked "wealthier" than those in the Expeller-control or Combination groups. This difference was statistically significant ($p < .05$). The higher wealth ranking for the Press-experiment group is reflected in this group, which had a statistically significant ($p < .01$) greater number of heads of cattle in their households than the other two groups (**table 1**).

At baseline, the mean age of the children participating in the study was 24.2 months. The children were distributed almost equally between sexes with 57 boys and 63 girls. Over 55% of children had already been weaned, with the remainder consuming a mixed diet of breast milk and weaning foods. Women reported breastfeeding their children an average of 8.3 times per day. There were no significant differences between groups in any of these measures. Over half (55.8%) of the women reported that their child had been ill in the past seven days before the survey. Children in the Expeller-control group were reported to be ill more frequently than those children in the other two groups. This difference was statistically significant at $p < .05$. Fever and malaria were the most common illnesses reported by all three groups. At baseline, the children had a mean weight-for-height z score (WHZ) of -0.66 ± 1.60 ; weight-for-age z score (WAZ) of -1.48 ± 1.34 ; and height-for-age z score (HAZ) of -1.5 ± 1.5 with

* The introduction of white sesame was part of a package of strategies that CRS was experimenting with to respond to the decline in cultivation of black sesame because of problems with access to functional and dependable oil presses.

TABLE 1. General characteristics of the study population at baseline

Variable	Expeller-control	Press-experiment	Combination
Mother/child pairs (no.)	40 ^a	37	43
Households in a compound (no.)	2.10 ± 1.46 ^b	1.95 ± 1.35	1.65 ± 1.31
Household size (no. of persons)	14.25 ± 8.57	15.05 ± 9.21	14.42 ± 7.18
Household wealth rankings (1 to 5 scale)	2.28 ± 1.32	2.86 ± 0.82*	2.52 ± 0.91
Women growing black sesame (no.)	25 (20.8) ^c	24 (20.0)	31 (25.8)
Women growing white sesame (no.)	17 (14.2)*	8 (6.7)	4 (3.3)
Heads of cattle per household (no.)	1.53 ± 2.94	3.57 ± 5.03**	1.00 ± 2.81
Women involved in income generating activity (no.)	22 (18.3)***	8 (6.7)	9 (7.5)
Mothers' ages (yr)	29.68 ± 6.98	29.24 ± 6.64	27.65 ± 7.17
Mothers who attended some type of school (no.)	13 (10.8)	13 (10.8)	15 (12.5)
Mothers' schooling (yr)	3.46 ± 1.81	2.15 ± 0.69	3.33 ± 2.64
Mothers' enrollment in the GAFNA/CRS Food Program (mo)	19.61 ± 7.88	18.42 ± 6.49	18.71 ± 5.49

a. Sample size determined based on power analysis with similar anticipated effect size.

b. Mean ± SD.

c. Frequency (percentage of total reporting).

* $p < .05$; ** $p < .01$; *** $p < .001$.

no statistically significant differences among the three groups [19].

Household oil consumption and kcal availability

Press-experiment and Combination households reported that they had sesame oil available for a longer period after the peak oil-pressing season than did the Expeller-control households. These differences, however, were not statistically significant. At the pre-harvest lean season, the Press-experiment and Combination households consumed 37% and 51% more oil, respectively, than the Expeller-control households. Throughout the year, Press-experiment and Combination households consumed more oil than Expeller-control households ($p < .08$).

Mean adult equivalent availability of kcals at the household level was highest at baseline (post-harvest before intervention) and in the subsequent post-harvest period after intervention. It was lowest during the peak oil-pressing season in the month of March (table 2).

Macronutrient intake of target women

At baseline, there were no significant differences in energy consumption among the three groups (table 3). Although there was no group effect, the Press-experiment and Combination women consumed more kcals than the Expeller-control women at all other seasons and especially at pre-harvest, resulting in a highly significant seasonal effect ($p < .001$), and seasonal group interaction ($p < .0001$).

Similarly, there were no significant differences in the protein intake of women in the three groups at baseline

(table 3). After baseline, however, the Press-experiment and Combination women consumed more protein at other times of the year than the Expeller-control women with a significant difference at the peak oil-pressing season and pre-harvest ($p < .05$). At pre-harvest, the Press-experiment and Combination women consumed 35.0% and 31.4% more protein than the Expeller-control women.

Fat intake of women in all three groups fell dramatically at the pre-harvest lean season (table 3). The Press-experiment and Combination women consumed 35.2% and 47.8% more fat at the pre-harvest lean season than the Expeller-control women. The seasonal group interaction was highly significant ($p < .0001$).

Macronutrient intake of target children

At baseline, the Expeller-control children consumed more kcals than the Press-experiment and Combination children, but kcal differences were not statistically significant (table 4). Afterwards, the trend was reversed and the Combination and Press-experiment children consumed more kcals than the Expeller children at all other seasons. According to RMANOVA, there was a strong seasonal effect on the energy consumption of target children ($p < .0001$), but no significant group effect.

Children's protein intake followed a seasonal pattern similar to that of energy intake (table 4). At the pre-harvest lean season, the Press-experiment and Combination children consumed 24.1% and 32.8% more protein respectively than the Expeller-control children. The difference between the protein intakes of the Combination group children and the Expeller-control children were significant at $p < .05$. The Combination

TABLE 2. Kilocalories available to households (per adult equivalent) across seasons (mean \pm SD)^a

Group	Baseline	Peak oil-pressing	Pre-harvest	Post-harvest
Expeller-control (<i>n</i> = 39)	3,262 \pm 1,636	2,286 \pm 816	2,859 \pm 1,418	3,399 \pm 1,327
Press-experiment (<i>n</i> = 30)	3,725 \pm 2,172	2,402 \pm 967	2,971 \pm 1,673	3,619 \pm 1,520
Combination (<i>n</i> = 40)	4,119 \pm 1,758	2,504 \pm 1,077	3,402 \pm 1,851	4,214 \pm 1,887

a. Repeated measures analysis of variance (RMANOVA); significant seasonal effect $p < .001$; no statistically significant group or season*group interaction effect.

TABLE 3. Women's consumption of kilocalories, protein, and fat across seasons

Kilocalorie intake (mean \pm SD)*				
Group	Baseline	Peak oil-pressing	Pre-harvest	Post-harvest
Expeller-control (<i>n</i> = 38)	2,622 \pm 781	2,065 \pm 552	1,808 \pm 498	2,441 \pm 686
Press-experiment (<i>n</i> = 30)	2,560 \pm 1,120	2,556 \pm 641	2,276 \pm 603	2,598 \pm 535
Combination (<i>n</i> = 39)	2,155 \pm 747	2,398 \pm 596	2,312 \pm 935	2,469 \pm 544
Protein intake (g); mean \pm SD**				
Expeller-control (<i>n</i> = 38)	97.2 \pm 31.4 ^a	86.5 \pm 8.5 ^b	67.2 \pm 19.9 ^b	97.9 \pm 30.7 ^a
Press-experiment (<i>n</i> = 30)	98.3 \pm 50.1 ^a	122.2 \pm 37.5 ^a	90.7 \pm 24.9 ^a	107.6 \pm 23.8 ^a
Combination (<i>n</i> = 39)	84.1 \pm 30.9 ^a	101.6 \pm 34.4 ^a	88.3 \pm 34.0 ^a	98.2 \pm 25.1 ^a
Fat intake (g); mean \pm SD***				
Expeller-control (<i>n</i> = 38)	101.2 \pm 39.7	76.5 \pm 29.9	51.0 \pm 23.5	101.1 \pm 40.5
Press-experiment (<i>n</i> = 30)	94.0 \pm 45.2	97.9 \pm 37.7	68.9 \pm 25.3	99.8 \pm 27.9
Combination (<i>n</i> = 39)	82.8 \pm 31.1	100.4 \pm 34.6	75.4 \pm 39.8	95.3 \pm 31.3

* Group effect was not significant; seasonal effect was significant at $p < .001$; season*group interaction was significant at $p < .0001$.

** Group effect was significant at $p < 0.01$; seasonal effect was significant at $p < .0001$; season*group interaction was significant at $p < .001$. Means in the same column followed by different superscripts are significantly different at $p < .05$.

*** Group effect was not significant; seasonal effect was significant at $p < .0001$; season*group interaction was significant at $p < .001$.

TABLE 4. Children's consumption of kilocalories, protein, and fat across seasons

Kilocalorie intake (mean \pm SD)*				
Group	Baseline	Peak oil-pressing	Pre-harvest	Post-harvest
Expeller-control (<i>n</i> = 39)	1,322 \pm 588	978 \pm 405	978 \pm 441	1,239 \pm 393
Press-experiment (<i>n</i> = 30)	972 \pm 450	1,279 \pm 597	1,093 \pm 506	1,417 \pm 404
Combination (<i>n</i> = 40)	1,013 \pm 462	1,407 \pm 637	1,233 \pm 549	1,421 \pm 418
Protein intake (g); mean \pm SD**				
Expeller-control (<i>n</i> = 39)	42.9 \pm 19.1 ^a	33.6 \pm 16.4 ^b	32.3 \pm 15.7 ^b	44.1 \pm 16.5 ^a
Press-experiment (<i>n</i> = 30)	33.1 \pm 16.9 ^a	52.9 \pm 26.8 ^a	40.2 \pm 18.8 ^{ab}	53.2 \pm 15.1 ^a
Combination (<i>n</i> = 40)	35.8 \pm 17.2 ^a	52.5 \pm 27.8 ^a	43.0 \pm 17.8 ^a	50.7 \pm 14.8 ^a
Fat intake (g); mean \pm SD***				
Expeller-control (<i>n</i> = 39)	42.4 \pm 26.2 ^a	28.9 \pm 18.1 ^b	23.4 \pm 13.9 ^b	47.4 \pm 26.2 ^a
Press-experiment (<i>n</i> = 30)	31.8 \pm 18.5 ^a	48.9 \pm 31.8 ^a	29.2 \pm 17.4 ^{ab}	55.2 \pm 19.6 ^a
Combination (<i>n</i> = 40)	35.9 \pm 26.1 ^a	54.3 \pm 33.6 ^a	36.5 \pm 25.6 ^a	52.7 \pm 19.9 ^a

* Group effect was not significant; seasonal effect was significant at $p < .0001$; season*group interaction was significant at $p < .0001$.

** Group effect was significant at $p < 0.05$; seasonal effect was significant at $p < .0001$; season*group interaction was significant at $p < .0001$. Means in the same column followed by different superscripts are significantly different at $p < .05$.

*** Group effect was significant at $p < .05$; seasonal effect was significant at $p < .0001$; season* group interaction was significant at $p < .0001$. Means in the same column followed by different superscripts are significantly different at $p < .05$.

and Press-experiment children's fat intakes were significantly different from those of the Expeller-control children at the peak oil-pressing season ($p < .05$), however, at the pre-harvest lean season only the Combination children's fat intakes were significantly different from those of the Expeller-control ($p < .05$).

Vitamin A intake of women and children

Vitamin A intakes are shown in **tables 5 and 6** for women and children, respectively. Women from all three groups consumed the greatest amounts of vitamin A at baseline (post-harvest before intervention) and at the post-harvest season (after the intervention), and the least amounts at the pre-harvest lean season. This seasonal effect was highly significant at $p < .001$. After baseline, vitamin A consumption of the Expeller-control women fell and remained lower than the other two groups for the rest of the study period. This season group interaction was significant at $p < .01$.

Children's vitamin A consumption followed the same seasonal pattern as that of their mothers. At pre-harvest, Press-experiment and Combination children consumed 27% and 22.4% more vitamin A than the Expeller-control children. These differences were not significant; however, the season group interaction was significant at $p < .05$.

Children's consumption of foods rich in protein and fat

During the pre-harvest lean season, Press-experiment and Combination children consumed more frequently 22 out of the 27 foods included in the food frequency questionnaire than the Expeller-control children. The Press-experiment children consumed more frequently four of the high fat/high protein foods (eggs, meat, fish, and butter) than did the other two groups, while

the Combination children consumed more frequently two of the high fat/high protein foods (roasted groundnuts [peanuts] and *mafe jaro* [dried fish]). The Press-experiment children most frequently consumed all five nutrient-dense weaning foods with significant differences for *churah gerteh* (gruel of rice and groundnuts $p < .0001$), *mono* (millet flour porridge), and groundnut paste ($p < .0001$), and sesame paste ($p < .05$). The Combination children most frequently consumed five out of six nutrient-dense table foods with significant differences for *benachin* (rice with vegetables, fish, or meat, $p < .05$), *nyakatango* (boiled rice and groundnuts, $p < .04$), and palm oil fish stew ($p < .001$). The Expeller-control children least frequently consumed all high fat/high protein foods, nutrient-dense weaning foods, and nutrient-dense table foods. The Expeller-control children more frequently consumed (two out of five) high moisture/low-calorie weaning foods with the difference for *cherreh* (millet flour and water) significant at $p < .05$.

Discussion

The results of this study show that seasonal fluctuations in food consumption continue to exist in rural villages in The Gambia. These seasonal fluctuations followed the expected trends of highs at the post-harvest season, and lows during the pre-harvest lean season. The energy intakes of women in this study followed this seasonal trend; however, current intakes were considerably higher than those obtained by Prentice et al. [35]. This may have been due to the high enrollment of women in the Gambian Agricultural Food and Nutrition Association/Catholic Relief Services (GAFNA/CRS) supplementation project (**table 1**).

At the pre-harvest lean season, women and children in the Press-experiment and in the Combination

TABLE 5. Women's vitamin A (RE) intake across seasons (mean \pm SD)^a

Group	Baseline	Peak oil-pressing	Pre-harvest	Post-harvest
Expeller-control ($n = 38$)	880.4 \pm 1,743.9	240.1 \pm 115.5	205.9 \pm 151.0	303.5 \pm 606.3
Press-experiment ($n = 30$)	409.5 \pm 372.4	359.7 \pm 249.9	230.0 \pm 96.0	316.2 \pm 221.2
Combination ($n = 39$)	402.6 \pm 453.4	348.7 \pm 395.7	234.5 \pm 95.7	465.2 \pm 1,074.1

RE, retinol equivalent

a. Group effect was not significant; seasonal effect was significant at $p < .001$; season*group interaction was significant at $p < .01$.

TABLE 6. Children's intake of vitamin A (RE) across seasons adjusted for age and sex (mean \pm SD)^a

Group	Baseline	Peak oil-pressing	Pre-harvest	Post-harvest
Expeller-control ($n = 39$)	208.6 \pm 240.1	93.2 \pm 111.5	88.3 \pm 64.6	82.3 \pm 53.7
Press-experiment ($n = 30$)	146.3 \pm 109.8	147.1 \pm 138.6	112.2 \pm 65.0	167.5 \pm 149.1
Combination ($n = 40$)	174.9 \pm 433.7	211.0 \pm 327.8	108.0 \pm 38.0	257.3 \pm 620.9

RE, retinol equivalent

a. Group and seasonal effects were not significant; season*group interaction was significant at $p < .05$.

groups were consistently better off than their counterparts in the Expeller-control group in all measured parameters. This outcome is impressive and is important in terms of providing insight as to how we might best begin to address the decades-old (if not centuries-old) problem of the pre-harvest hungry period with its high morbidity and mortality rates. The synergy among all the parameters measured and the congruency of the behavior of these parameters with what has already been observed by numerous investigators give credence and validity to these data. As well, this synergy provides credibility to the direction of its overall conclusion that the manually operated press, with accessibility (i.e., locally manufactured and repaired and relatively affordable) and being under the control of women, has the potential to improve diets and to soften the impact of the pre-harvest lean period. The question then arises as to what extent these encouraging outcomes result from women's control over a simple oil extraction device such as the ram press.

There are several limitations to our study. First, although there was a great concern about the sample selection, the women who made up the final sample were those who met important selection criteria other than being sesame growers. They had to be available, with a desire to participate, able to follow instructions, and had to have full cooperation from their husbands or other influential members of the family—factors without which such a study could not have taken place. This lack of true random selection does not, however, diminish the significance of the results for the following reason. One of the conditions for the ram press to have the desired effect on women and children's diets was that it be under the woman's control. To be under the women's control requires that the women must enjoy a certain level of autonomy within their households, and are able to manage the press and make decisions on how their harvested sesame seeds are used. Most importantly, women must decide how the oil and its derivatives (e.g., sesame cake) are used. In this case, a random sample of women sesame growers perhaps would not have been the most appropriate. Therefore, the sample used in this study represents more truly the group of women who would use such a device and benefit from it [36].

Second, the women and households in all three groups, although comparable in all major characteristics, differed slightly in certain aspects (**table 1**). Households and women from the Press-experiment and Combination groups were more similar to each other, and consequently had similar outcomes. This was to be expected, given that the Combination group was an offshoot of the initial Press-experiment group who, early in the course of the study, also began to use the motorized expeller.

The reasons why the women from the Combination group felt the need to use both the motorized

expeller and the ram press are not clear. It is evident that the Combination group often had slightly higher consumption levels than the Press-experiment group, in spite of the fact that the Press-experiment women were ranked as being "wealthier" than the Combination and the Expeller-control groups.

Third, while we were cognizant of the role of income as a driving force affecting the outcome, we could not measure it directly. Income is usually categorized as "cash crop" and "off-farm" activities. In a broader sense, all household production—including food, cash crop and livestock production, off-farm activities, and trade—contribute to household income. This was very difficult to measure; therefore, we use wealth ranking and women's involvement in income generating activities, as well as cultivation of other crops that could potentially be cash crops (e.g., white sesame) as proxies. Wealth ranking was based, among other factors, on the number of cattle owned by the households. Cattle in rural Africa are traditionally the strongest indicator of wealth. However, their impact on family welfare, including elements such as education, housing, health, and nutrition are not clear. Cattle are not a liquid asset. They are rarely, if ever, liquidated to meet immediate family needs. Again, the "cattle factor" as a sign of "prestige," if not liquid wealth in modern economic terms, appears just as relevant in The Gambia as it always has been elsewhere in Africa.

The most salient difference among the three groups was that more women in the Expeller-control group were engaged in income-generating activities and were also growing white sesame. The growing of white sesame, which was strictly for sale and for making confections, indicates the role of these women as entrepreneurs—if not entirely by choice, certainly by circumstances. These women had access only to the expeller, the future of which was uncertain. In spite of their engagement in income-generating activities, the Expeller-control women had significantly lower intakes in peak oil-pressing season and also at pre-harvest lean season; they had sesame oil available for a shorter time period; and their children were reported to be more frequently ill and more wasted than the Press-experiment and Combination groups' children [19].

At the pre-harvest lean season, Press-experiment and Combination women consumed 96.9% and 98.4% of the FAO recommended kcals respectively, while Expeller-control women consumed only 76.9%. This difference in energy intakes of women at pre-harvest are reflected in higher household consumption of sesame oil and mean available adult equivalent kcals at the pre-harvest lean season by the Press-experiment and the Combination households than the Expeller-control households. Similarly, children from the Press-experiment and Combination groups at the pre-harvest lean season consumed 12% and 26% more kcals, respectively, than Expeller-control children.

Although engagement in income-generating activity is often associated with higher intakes and other desirable development indicators (such as education and health), women in the Expeller-control group were behaving more vulnerably (economically less stable) than the other two groups and perhaps spending more time outside the home and providing less quality care to their children. This situation is understandable, since they might have been feeling the uncertainty of their changing economic base, from growing black sesame for home consumption to white sesame for sale, with the market and commercial venues not fully developed or proven with any degree of certainty. Given this, some may argue that the differences in intake observed among these groups were not a result of the press itself but a result of the economic instability of the Expeller-control group. Indeed, in some respects, the Expeller-control households exhibited some aspects of the “feast and famine” syndrome, while those in the Press-experiment and Combination groups had less dramatic changes at the pre-harvest period. It is our thesis, however, that the press’ effect is precisely as an “economic stabilizer,” without which the Press-experiment and the Combination groups would be behaving the same way as Expeller-controls.

We hypothesized that the ram press would have an effect on diets and nutritional status of women and children by two main mechanisms: 1) directly through oil and cake consumption, and 2) indirectly through the sale of some of the oil and cake, thus generating extra cash to buy other foods and enable the women to participate more fully in health-related services. The observed improvements in diets of the Press-experiment and Combination groups in relation to the Expeller-control group could have been a result of either consumption of oil or purchase of other foods. The findings that women and children from the Press-experiment and Combination groups derived a greater percentage of their energy intake from protein and fat than those in the Expeller-control group seem to suggest that purchase of other foods may have been involved. This is partially supported by the greater dietary diversity of the children from the Press-experiment and Combination groups. In addition, these children consumed more fat/high protein foods, more nutrient-dense weaning foods, and nutrient-dense table foods their Expeller-control counterparts. Further, some studies have indicated that market-oriented technologies are significant determinants of income, which has a positive and significant influence on expenditures of food and non-food items [37].

The results of the participatory evaluation and monitoring indicated several advantages and disadvantages to the press. First, the ram press needed to be redesigned to fit women better, especially in terms of the physical force needed to press the sesame seeds to their optimal rate of oil extraction. Second, women

could press only small amounts of the seeds at one time. This could have been one of the factors that drove so many women from the Press-experiment group to seek the services of the motorized expellers. Although some of the women thought that the quantity issue was a disadvantage, others clearly saw it as an advantage. Many women thought that they could store the seeds more easily than oil. Therefore, it was viewed as a great advantage to press just the exact amount that they needed at one time, whenever they needed it. The extra savings in terms of the cake that was lost to the Expeller-control women was also seen as an advantage, not to mention the loss in time traveling to the expeller, waiting their turn to press the seeds, and the effort to transport the seed and oil to and from home. Another advantage of ram presses was that women could charge a small fee for using the press instead of paying a fee to use the motorized expellers.

The findings of this study indicate that women and children with access to a ram press (either alone or in combination with a motorized expeller) experienced much less seasonal fluctuation in food consumption than those without a ram press. For the women, access to the ram presses meant a 465- to 500-kcal advantage during the pre-harvest lean season over women without ram presses. Similarly, children of women with access to the ram press enjoyed a 115- to 255-kcal advantage during the pre-harvest lean season over children of women without access to the ram press.

Finally, women’s access to food processing technology can provide the means to “add value” to their raw agriculture product, which can be viewed as an “economic stabilizer” with potential to increase dietary intakes and incomes, especially during the pre-harvest lean season.

Acknowledgments

The authors wish to gratefully acknowledge the women’s groups and individual women and children in 32 villages in The Gambia whose dedication to improving the local food system made this study possible. The financial support of the Thrasher Foundation, Thrasher Award # 02903-4, and of the U.S. Agency for International Development, Bureau of Global Programs, Field Support and Research, Office of Health and Nutrition Monitoring Project (IMPACT), Contract No. DAN-5110-C-00-0013-00, TO 417 is also gratefully acknowledged. We wish also to thank the Department of Human Nutrition, Foods and Exercise at Virginia Tech, for its financial support of Mr. Stephen Hull, its cost sharing for Dr. Marilyn Prehm and for Ms. Sherry Saville’s time and technical assistance. We also acknowledge the excellent work of nutritionist Kinday Samba Ndure, and field monitors Kebba M. Jome, Alahaji Jawneh, and Ali Sey. Finally, we also acknowledge the

input and collaboration of our Gambian institutional partners, the Sesame Growers Association, the Gambian

Agricultural Food and Nutrition Association (GAFNA) and the Catholic Relief Services (CRS).

References

- Chambers R, Longhurst R, Pacey A. Seasonal dimensions to rural Poverty. London, UK: Frances Pinter Limited, 1981.
- Adams AM. Seasonal variation in nutritional risk among children in Mali. *Ecol Food Nutr* 1994;33:93–106.
- Silva-Barbeau I, Sissoko H, Berthe M, Haidara M, Barbeau WE, Caldwell JS. Addressing child feeding concerns of women farmers in Mali: composition and effects on child nutrition of a locally developed weaning. *Ecol Food Nutr* 1998; 37:1–19.
- Benefice E, Chevassus-Agnes S, Barral H. Nutritional situation and seasonal variations for pastoralist populations of the Sahel (Senegalese Ferlo). *Ecol Food Nutr* 1984;14:229–47.
- Von Braun J, Puetz D, Webb P. Irrigation technology and commercialization of rice in The Gambia: effects on income and nutrition. Washington, DC: International Food Policy Research Institute, Research Report No. 75, 1989.
- Wandell M, Holmboe-Ottesen G, Manu A. Seasonal work, energy intake and nutritional stress: a case study from Tanzania. *Nutr Res* 1992;12:1–16.
- The Gambia Ministry of Health, ICN focal point. The Gambia nutrition country paper for the international conference on nutrition. Republic of The Gambia, 1992.
- Jaffar S, Leach A, Greenwood A, Greenwood B. Season of birth is not associated with delayed childhood mortality in Upper River Division, The Gambia. *Trop Med Int Health* 2000;5:628–32.
- Hill AG, MacLeod WB, Sonko SST. Improvements in childhood mortality in The Gambia. *Lancet* 1998; 352:1909.
- Tompkins AM, Dunn DT, Hayes RJ, Bradley AK. Seasonal variation in the nutritional status of urban Gambian children. *Brit J Nutr* 1986;56:533–43.
- Harpham T. Urban health in The Gambia. *Health and Place* 1996;2:45–9.
- Galton-Fenzi JD. Project report to determine the potential of expanding sesame production in The Gambia. McLean, VA, USA: Unpublished report. Labat-Anderson Inc., 1992.
- ICN Focal Point. The Gambia Nutrition Country Paper for the International Conference on Nutrition. Republic of The Gambia, Ministry of Health, Nutrition Unit, 1992.
- Hyman EL. Production of edible oils for the masses by the masses: the impact of the ram press in Tanzania. *World Dev* 1993;21:429–44.
- Enterprise Works Worldwide (formerly Appropriate Technology (ATI) 1828 L Street, NW, Suite 1000, Washington, DC. www.enterpriseworks.org. Accessed June 5, 2005.
- Samba-Ndure K, Jawhneh A, Jome KM, Prehm MS, Silva-Barbeau I. Small Scale Sesame Oil Production: A Means of Child Nutrition Security in The Gambia. Semi-Annual Project Progress Report. Thrasher Research Fund. Salt Lake City, Utah, USA, 1995.
- Hull, GH. The stabilizing effects of sesame oil extraction technologies on seasonal fluctuations in food consumption and nutritional status of rural farming households in The Gambia. Master of Science Thesis, Department of Human Nutrition Food and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, Va, USA, 1999.
- Prehm M. Assessing an appropriate technology for improved nutrition security: A participatory learning appraisal with Gambian women. In: Small scale sesame oil production: a means to improved child nutrition security in The Gambia, Thrasher Research Fund, Semi-Annual Report. Salt Lake City, Utah, USA, March 1998.
- Silva-Barbeau I, Prehm MS, Samba-Ndure K, Jome K, Jawneh A., Hull, SG. The direct and indirect benefits of sesame oil production on the nutritional security of women and children: The experience with women-led monitoring and evaluation of a ram press technology in The Gambia. Paper presented at the 16th International Nutrition Congress, Montreal, Canada, 1997.
- Dop MC, Milan CH, Milan CL, N'Diaye AM. Use of the multiple-day weighed record for Senegalese children during the weaning period: a case of the 'instrument effect'. *Am J Clin. Nutr* 1994;59(suppl):266S–68S.
- Ferguson EL, Gibson RS, Opere-Obisaw C. The relative validity of the repeated 24-hour recall for estimating energy and selected nutrient intakes of rural Ghanaian children. *Eur J Clin Nutr* 1994;48:241–52.
- Villard L, Bates CJ. Dietary intake of vitamin A precursors by rural Gambian pregnant and lactating women. *Human Nutr Appl Nutr* 1987;41:135–45.
- United States Department Agriculture. USDA nutrient database for standard reference, release 11-1. Nutrient data laboratory home page <http://www.nal.usda.gov/fnic/foodcomp>, 1997. Accessed June 5, 2005.
- Food and Agriculture Organization (FAO). Food composition tables for use in Africa. Rome, Italy: Nutrition Division of FAO, 1968.
- Hudson GJ, John PMV, Paul AA. Variation in the composition of Gambian foods: the importance of water in relation to energy and protein content. *Ecol Food Nutr* 1980;10:9–17.
- Hudson GJ, Day KC. Water content of the rural Gambian diet. *Nutr Rep Inter* 1989;400:335–39.
- King FS, Burgess A. Nutrition for Developing Countries. Oxford, UK: Oxford University Press, 1993.
- McCrae JE, Paul AA. Foods of rural Gambia. Cambridge, UK. Medical Research Council, Dunn Nutrition Centre, 1996.
- World Health Organization. Energy and protein requirements. Report of a joint FAO/WHO Expert consultation. WHO technical report series 724. Geneva, Switzerland: WHO, 1985.
- Hudson GF. Food intake in a West African village: estimation of food intake from a shared bowl. *Br J Nutr*

- 1995;73:551–69.
31. Patore G, Branca F, Demissie T, Ferro-Luzzi A. Seasonal energy stress in an Ethiopian community: an analysis of the impact at the household level. *Eur J Clin Nutr* 1993;47:851–62.
 32. Von Braun J, Puetz D, Webb P. Irrigation technology and commercialization of rice in The Gambia: Effects on income and nutrition. Washington, DC: International Food Policy Research Institute, 1989.
 33. Kennedy E. The effects of sugarcane production on food security, health and nutrition in Kenya: a longitudinal analysis. Washington, DC: International Food Policy Research Institute, 1998.
 34. Kumar SK, Hotchkiss D. Consequences of deforestation for women's time allocation, agricultural production, and nutrition in hill areas of Nepal. Washington, DC: International Food Policy Research Institute, 1988.
 35. Prentice AM, Whitehead RG, Roberts SB, Paul AA. Long-term energy balance in child-bearing Gambian women. *Am J Clin Nutr* 1981;34:2790–9.
 36. Kurz KM, Johnson-Welch C. Enhancing women's contributions to improving family food consumption. *Food Nutr Bull* 2001;22:443–53.
 37. Ahmed A, Ehui S, Saleem M. Adoption of crossbred cow technologies and increased food security among smallholders dairy farmers in the East African Highland. In: Kataki PK, Babu, SC, ed. *Food Systems for Improved Human Nutrition*. New York: Food Products Press, 2002.

Serum copper levels among a tribal population in Jharkhand State, India: A pilot survey

Umesh Kapil and Preeti Singh

Abstract

Background. Copper is an essential trace element that plays a pivotal role in cell physiology. Dietary intake of copper by a population of low-income groups in India has been found to be low, and hence the possibility of dietary copper deficiency exists.

Objective. To determine serum copper levels among tribal populations in India, an area in which data are limited.

Methods. The study was conducted among tribal populations 18 to 75 years of age residing in Sahibganj, Jharkhand. Two blocks in the district were selected (from a total of eight) for the detailed study. A semistructured pretested questionnaire was used to collect demographic and socioeconomic information about subjects. A total of 995 subjects were enrolled for the present study. Blood was drawn from the antecubital vein and collected in previously labeled polypropylene tubes. Serum copper was determined by the atomic absorption spectrophotometry method and serum samples with copper levels less than 80 µg/dL were considered to have low serum copper levels.

Results. The mean serum copper concentration of the study subjects was 91.18 ± 35.48 µg/dL. Thirty-four percent of the study subjects had low serum copper levels (< 80 µg/dL). Nearly 35% of males and 34.4% of females had low serum copper levels.

Conclusions. This study documented a high prevalence of low serum copper levels among the studied tribal population. Further multicenter studies with larger sample sizes are needed to assess the biological implications of copper deficiency among the tribal populations in India.

Key words: Copper levels, tribal populations, nutrition, dietary intake

Introduction

Copper is an essential trace element that plays a pivotal role in cell physiology, particularly in its function as a core part of cuproenzyme [1]. Copper appears to have many important functional roles in the body that apparently relate to the maintenance of immune function, bone health, and hemostasis, among other functions [2]. Inadequate nutriture of copper alters immunocompetence in humans because it is required to maintain antioxidant defense [3]. Deficiency of copper leads to increased susceptibility to infectious illnesses [4].

India has one of the largest concentrations of tribal populations in the world, second only to Africa [5]. Dietary intake of copper by the population of low-income groups in India has been found to be low, and hence the possibility of dietary copper deficiency exists [6]. Limited data are available on serum copper levels among the tribal populations in India; hence the present study was conducted.

Methods

The study was conducted among members of a tribal population age 18 to 75 years in Sahibganj, Jharkhand. The tribal populations in India live in remote villages far from the urban population. All the tribal members have a similar dietary pattern. The staple diet is based on cereals and foods that are available in the forest where they live. To keep the study operation manageable, two blocks out of eight in the district were selected for the detailed study. Approval for conducting the study was obtained from the Ethics Committee of the All-India Institute of Medical Sciences, New Delhi. The subjects were informed of the study objectives and written informed consent was obtained.

Umesh Kapil and Preeti Singh are affiliated with the Department of Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India.

Please direct queries to the corresponding author: Umesh Kapil, Department of Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 1-110029 India; e-mail: kapilumesh@hotmail.com.

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

A semistructured pretested questionnaire was used to collect demographic and socioeconomic information about the subjects.

A total of 995 subjects were randomly selected for the present study. Subjects who were not suffering from any acute morbidity or chronic disease on the day of the examination were considered healthy and were included in the study. Only nonpregnant female subjects were included.

Out of 995 subjects, 98 refused to participate in the study, leaving a total of 897 collected blood samples. A total of 33 samples (3.3%) were hemolyzed during transportation, so the final number of blood samples analyzed was 864. Blood from the antecubital vein was drawn from the subjects and collected in previously labeled polypropylene tubes. Blood was transported in ice packs to the central laboratory and the serum was separated within six hours of collection. The samples were centrifuged at 3500 rpm at 4°C for 30 minutes for serum collection. Serum copper levels were determined by the atomic absorption spectrophotometry method (Spectra 250+, Varian, Palo Alto, Calif., USA). The estimations were done at the Industrial Toxicology and Research Center, Lucknow, India, which is the national facility for undertaking estimations of trace elements.

Quality control of the biochemical investigation was ensured using the following steps. Each serum sample underwent three estimations for serum copper levels and the mean of the three values were reported as the copper concentration of that particular sample. Seronorms procured from Randox Ltd. (with a known copper concentration) were run with each assay for internal quality assurance. When the serum copper level for the seronorm sample was over- or underestimated, the whole assay was repeated. The reference range for serum copper was 80 to 110 µg/dL; serum samples with copper levels < 80 µg/dL were considered to have low serum copper levels based on the standard cutoff for the atomic absorption spectrophotometry method described in Elmer and Conn [7].

Results

A total of 995 subjects were enrolled for the present study. The mean age of the subjects was 34 years. Sixty percent were female. Blood was collected from 864 subjects to assess serum copper levels. The socio-demographic characteristics of subjects who refused to participate in the study were similar to those who participated in the study.

The age and sex distribution of study subjects according to serum copper concentration is shown in **table 1**. The mean serum copper concentration of the study subjects was 91.18 ± 35.48 µg/dL. Thirty-four percent of the subjects had low serum copper levels (< 80 µg/dL). Nearly 35 percent of males and 34.4 percent of females had low serum copper levels. The prevalence of low serum copper levels was greatest in both men and women between the ages of 21 and 40.

Discussion

In the present study, 34.5% of the tribal population had low serum copper levels as revealed by serum tests. This was possibly because dietary intake of copper by the tribal population studied was marginal [6]. The absorption of copper is known to be inversely related to dietary intake [6, 8]. The main staple of the tribal population in Sahibganj is rice or maize, and consumption of animal foods is uncommon. It is well documented that a diet containing animal protein enhances the absorption of copper compared to a diet comprising mainly plant protein [9]. One limitation to our study is that we used purposive sampling, and therefore our sample may not be truly representative of the greater population. There is a need to undertake multicenter studies with larger sample sizes among tribal populations in different parts of India to assess the biological implications of copper deficiency in this population.

TABLE 1. Age and sex distribution of study subjects according to their serum copper concentration^a

Age group (yr)	Copper concentration (mean ± SD)			
	< 80 µg/dL		≥ 80 µg/dL	
	Males	Females	Males	Females
≤ 20	10 (3.0)	26 (4.9)	32 (9.6)	42 (7.9)
	59.50 ± 15.17	52.69 ± 16.38	108.91 ± 18.08	114.76 ± 39.62
21-40	66 (19.9)	120 (22.5)	124 (37.3)	214 (40.2)
	55.53 ± 15.47	56.00 ± 16.61	106.77 ± 27.69	111.29 ± 30.12
≥ 41	39 (11.7)	37 (6.9)	61 (18.4)	93 (17.5)
	57.31 ± 13.27	56.35 ± 14.03	110.16 ± 25.13	107.90 ± 22.62
Total	115 (34.6)	183 (34.4)	217 (65.4)	349 (65.6)
	56.47 ± 14.65	55.60 ± 16.05	108.04 ± 225.71	110.80 ± 29.66

a. Figures in parentheses denote percentage.

References

1. Camakaris J, Voskoboinik I, Mercer JF. Molecular mechanisms of copper homeostasis. *Biochem Biophys Res Commun* 1999;261:225–32.
2. Bonham M, O'Connor JM, Hannigan BM, Strain JJ. The immune system as a physiological indicator of marginal copper status? *Br J Nutr* 2002;87:393–403.
3. Strain JJ. Newer aspects of micronutrients in chronic disease: copper. *Proc Nutr Soc* 1994;53:583–98.
4. Sherman AR. Zinc, copper, and iron nutriture and immunity. *J Nutr* 1992;122:604–9.
5. Census of India. Provisional population totals. New Delhi, India: Gita Offset Printers, 1991.
6. Pathak P, Kapoor SK, Kapil U, Joshi YK, Dwivedi SN. Copper nutriture amongst pregnant women in a rural area of India. *Eastern J Med* 2003;8:15–17.
7. Elmer P, Conn N. Analytical methods for atomic absorption spectrophotometry. London: Oxford University Press, 1975:273–90.
8. Gopalan C, Ramashastry BV, Balasubramanian SC. Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, 2004. Hyderabad: National Institute of Nutrition Press, p. 21.
9. Turnlund JR, Swanson CA, King JC. Copper absorption and retention in pregnant women fed diets based on animal and plant proteins. *J Nutr* 1983;113:2346–52.

The Des Moines Declaration

A call for accelerated action in agriculture, food and nutrition to end poverty and hunger

Gathering in Iowa, the birthplace of Dr. Norman E. Borlaug, Nobel Peace Prize Laureate and Father of the Green Revolution, who is celebrating his 90th birthday in 2004; and

Assembled in Des Moines, on the occasion of the Presentation of the 2004 World Food Prize as we celebrate the International Year of Rice; and

Observing October 16 as United Nations World Food Day, and World Food Prize Day in America:

We the undersigned Laureates, Founders and Council Members of The World Food Prize do hereby address this joint declaration to the governments of the world, the leaders of major international organizations and to all involved in the struggle against hunger, poverty and disease in the world.

More attention to the Millennium Development Goals

We reiterate our support for the Millennium Development Goals resulting from the Summit Declaration adopted by the heads of government of the world assembled at the United Nations in September 2000 to reduce hunger, poverty and disease still afflicting half the population on our planet. Progress to move forward must be based on realistic plans and immediate action must be taken.

We recognize that adequate and available food and nutrition are essential to achieving most of these goals and important for all of them. We also recognize that improved nutrition is directly related to success in approaching and reaching population stabilization.

Hundreds of millions of poor people are left hungry in a world of food abundance because they lack access to adequate food and nutrition. It is essential that we close this gap between hunger and food availability.

More food-increased productivity to improve the lives of people and protect biodiversity in our environment

Agriculture is the main source of income for poor people living in rural areas. As such, a boost in agricultural productivity in the rural areas of developing

countries will greatly enhance earning potential as well as produce more food. However, agricultural production increases will not generate adequate gains in employment, and additional steps must also be taken to increase employment in agro based value added rural enterprises.

In addition, food productivity must be increased to improve the lives of people and protect biodiversity in our environment. With close to a billion people still suffering from hunger, malnutrition and food insecurity and with the population of our planet projected to grow by 50% by the middle of the 21st century, either we must produce more food on the land and in the water now available to us, or people will be forced to cut down precious forest areas and cultivate marginal lands to grow the food necessary to fuel our escalating demands. It is crucial that new agricultural innovations and technologies be developed.

More nutritious food—the best medicine

We emphasize that the nutritional quality as well as the quantity of food must be improved. Poverty, hunger, and ill-health are all interrelated. The hidden hunger of micronutrient deficiencies is particularly damaging to current and future generations. Nutrition needs to be improved to reduce the devastating effects of diseases that are keeping the hungry and malnourished from being productive members of society.

We call special attention to the fact that The World Food Prize Foundation is devoting its 2005 International Symposium to the issue of nutrition.

More available food—increased prosperity to counter terrorism

We believe that reducing poverty and hunger around the world is one of the most important means of reducing the terrorism that the world is confronting. Global political security can only be attained through an enhanced commitment to addressing global food security. Providing hope, progress and increased prosperity

in these same areas can be the single most effective means to achieve stability and tranquility.

More equitable income and land distribution needs to be achieved by inclusion of the poor and the disadvantaged in sustainable economic activities that ensure remunerative return for their material, labor and capital contributions. This will help to ensure purchasing power in the hands of the poor and the disadvantaged, making more affordable food available without compromising human dignity.

Role of The World Food Prize Laureates

Since its inception in 1986, The World Food Prize has been awarded to Laureates from a wide variety of disciplines and countries. Regardless of background or discipline, we have devoted our lives to striving for achievements that will eradicate hunger and uplift all humankind. Based on our experience, we believe that the steps below are crucial to the implementation of the Millennium Development Goals, and the abolishment of hunger in our world.

Accelerated action to defeat hunger in the world

Considering that in order to attain further breakthroughs in combating poverty, hunger, malnutrition and famine, we recommend that governments of the world and international organizations urgently undertake the following steps:

- » Significantly increase funding for pro-poor agricultural and nutritional research conducted by an integrated system of national, international and private sector research organizations.
- » Give higher priority to investment in public goods that would facilitate agricultural development, including those mentioned below.
- » Increase funding for the construction and improvement of roads, with a particular emphasis on rural roads.
- » Increase investment in rural infrastructure to facilitate the processing, storage, transport and marketing of agricultural products in low-income developing countries.
- » Encourage the provision of micro-credit facilities for farming communities.
- » Develop programs to ensure adequate nutrition.
- » Increase programs supporting education for all with special emphasis on women and girls.
- » Develop research and technologies for more sustainable use of natural resources and promote access to energy sources in rural areas, with emphasis on renewable energy.
- » Provide sufficient and clean water to support the human needs and develop and implement technologies for better water conservation and utilization in agriculture.

» Maintain and increase support for the delivery of humanitarian assistance to those facing hunger, starvation and death.

We transmit this statement to the leaders of the international community in order to emphasize crucial, life-sustaining issues.

The following World Food Prize Laureates, Founders, and Council of Advisors support the 2004 Des Moines Declaration:

Dr. Norman E. Borlaug
Nobel Peace Prize Laureate
Founder of the World Food Prize

Ms. Catherine Bertini
USA
2003 World Food Prize Laureate

Dr. Surinder K. Vasal
India
2000 World Food Prize Laureate

Dr. Perry L. Adkisson
USA
1997 World Food Prize Laureate

Dr. Hans R. Herren
Switzerland
1995 World Food Prize Laureate

Dr. John S. Niederhauser
USA
1990 World Food Prize Laureate

A.S. Clausi
USA
World Food Prize Founder

Michael G. Gartner
USA
Council of Advisors Member

Dean R. Kleckner
USA
Council of Advisors Member

Prof. Yuan Longping
China
2004 World Food Prize Laureate

Dr. Pedro A. Sanchez
USA/Cuba
2002 World Food Prize Laureate

Dr. Evangelina Villegas
Mexico
2000 World Food Prize Laureate

Dr. Henry M. Beachell
USA
1996 World Food Prize Laureate

Dr. Muhammad Yunus
Bangladesh
1994 World Food Prize Laureate

Dr. Verghese Kurien
India
1989 World Food Prize Laureate

Robert D. Havener
USA
World Food Prize Founder

Jonathan F. Taylor
Great Britain
Council of Advisors Member

Ambassador Kenneth Quinn
USA
President, World Food Prize

Dr. Monty P. Jones
Sierra Leone
2004 World Food Prize Laureate

Dr. Per Pinstrup-Andersen
Denmark
2001 World Food Prize Laureate

Badrinarayan R. Barwale
India
1998 World Food Prize Laureate

Dr. Gurdev S. Khush
India
1996 World Food Prize Laureate

Dr. Nevin S. Scrimshaw
USA
1991 World Food Prize Laureate

Dr. M.S. Swaminathan
India
1987 World Food Prize Laureate

Dr. Gregory Geoffroy
USA
Council of Advisors Member

Dr. Pekka Linko
Finland
Council of Advisors Member

The World Food Prize

Dr. Norman E. Borlaug conceived of the idea for a World Food Prize after receiving The Nobel Peace Prize.

The \$250,000 World Food Prize is awarded each October in Des Moines, Iowa, USA, to recognize the significant achievements of individuals who have reduced poverty, hunger, and malnutrition by improving the quality, quantity, or availability of food in the world.

Information about the World Food Prize Laureates and the annual programs conducted in conjunction with the Laureate Award Ceremony is available on

the web at www.worldfoodprize.org or by contacting Ambassador Kenneth Quinn at:

The World Food Prize Foundation
1700 Ruan Center, 666 Grand Avenue
Des Moines, Iowa USA 50309
Ph: 515-245-3783; Fax: 515-245-3785
E-mail: wfp@worldfoodprize.org

Book reviews

Evaluation of certain veterinary drug residues in food. Sixty-second Report of the Joint FAO/WHO Expert Committee on Food Additives. 2004 WHO Technical Report Series #925. Geneva, 2005. (ISBN 92-4-120925-9) 72 pages, softcover, US\$22.50.

This report presents the conclusions of a joint FAO/WHO (Food and Agriculture Organization/World Health Organization) Expert Committee convened to evaluate the safety of residues of certain veterinary drugs in food, and to recommend maximum levels for such residues in food.

The first part considers conclusions on specific toxicological end-points, lipid-soluble residues of veterinary drugs with maximum recommended levels (MRLs) in milk, statistical methods for the estimation of MRLs, and the Committee's review and comments on documents provided by Codex Committees. Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: five antibacterial agents (cefuroxime, chloramphenicol, flumequine, lincomycin, and pirlimycin); four insecticides (cyhalothrin, cypermethrin and α -cypermethrin, doramectin, and phoxim); and two production aids (melengestrol acetate and ractopamine). The Committee's comments on chloramphenicol found at low levels in animal products are also summarized.

The report also contains a summary of the Committee's recommendations on these drugs, including acceptable daily intakes and proposed maximum residue limits.

Preventive nutrition: The comprehensive guide for health professionals, 3rd Edition. Edited by Adrienne Bendich and Richard Deckelbaum. Humana Press, Totowa, NJ, 2005. (ISBN 1-588-29-445-5) 958 pages, hardcover, US\$145.00.

This is a major revision and expansion of a valuable reference book first published in 1997, with a second edition in 2001. It is by far the most comprehensive

compendium of the relationship between nutrition, health, strategies for the prevention of disease, and promotion of health through the application of nutrition knowledge. The 37 chapters, written by 80 experienced authors, provide a critical review of the newest research, and comprehensively document the benefits of good nutrition for health at all ages. Five chapters deal with cancer and cardiovascular disease, with emphasis on the role of nutrition in prevention. The role of prior diet in the prevalence of chronic disease in later life is well covered.

While no chapter specifically deals with nutrition and the elderly, the theme is integrated into a number of the chapters. Three chapters deal with diabetes, obesity, and bone diseases. The effects of improved nutrition on cataracts, respiratory disease, immunity, pregnancy, and blindness are covered in single chapters. Critical issues such as the use of alcohol, the impact of medication on nutritional status, and the problem of dubious health claims for foods and dietary supplements are covered in the final chapters, as well as preventive nutrition from a global perspective. The last chapter, which covers preventive nutrition throughout the life cycle was written by the editors and summarizes the book's message.

While the volume would seem too expensive for individual purchase, it should be available in the libraries of schools of public health, nutrition departments, and institutes in both industrialized and developing countries to be used as a reference in courses in preventive medicine and public health.

Dietary supplement labeling compliance review, 3rd edition. James L. Summers; Contributor, Elizabeth J. Campbell. Blackwell Publishing, Ames, Iowa, 2004. (ISBN 0-8138-0426-4) 232 pages, hardcover, US\$159.99; US\$189.99 with CD-ROM.

Dietary supplements are a growing focus of the food industry and their authorization, labeling, and compliance are of increasing importance in developing as well

as industrialized countries. This book, written from the perspective of a former consultant and dietary supplement labeling expert from the U.S. Food and Drug Administration, includes an overview of dietary supplement labeling, the need for specific regulations governing dietary supplements, compliance with dietary supplement levels, a compliance label review program, dietary supplement labeling issues, legislation, and regulations. It includes charts, graphics, illustrations, guidelines, a comprehensive index, and bibliography. This third edition will be useful for any public or private entity concerned with specifications, labeling, laws, and regulations relating to dietary supplements.

Why some like it hot: Food, genes, and cultural diversity. Gary Paul Nabhan. Island Press, Washington, DC, 2004. (ISBN 1-55963-466-9) 233 pages, hardcover, US\$24.00.

This is an entertaining and convincing attempt to discover the types of diets encoded in our genes. It rejects the dogmatic claims on which our presumptions of a single, genetically programmed "Paleolithic diet" are based, and explores the common features of the wide variety of primitive diets depending on differing physical and biological environments. The author, an ethnobotanist, points out that prehistorically or historically, some 30,000 wild plants were used for food. These plants are not merely a source of macro- and micronutrients, but are also an arsenal of secondary compounds that protect plants against environmental stresses and interspecific stresses such as competition, disease, predation, and herbivory. When consumed by humans, they have a variety of physiological and medicinal effects.

"Finding a Bean for your Genes" and "The Shaping and Shipping Away of Mediterranean Cuisines," chapters three and four, respectively, are as interesting as their titles. Chapter five, from which the book title is taken, explores the extraordinary variation among populations and genotypes in sensitivity to the hot taste of peppers and includes entertaining anecdotes. "Dealing with Migration Headaches" is subtitled "Should We Change Places, Diets or Genes?" and gives examples of how we consume potentially toxic foods along with practices that can counterbalance the most hazardous compounds. This chapter also includes a good analysis and refutation of the "thrifty gene hypothesis."

The final chapter describes the health problems of Native Hawaiians when they abandon the diet and ways of their ancestors. To some degree, the lessons apply to all of us. Rich information is now available

from nutrition, genetic, anthropological, and ethnobotany research, as well as from "an emerging set of biotechnologies, collectively known as *functional...or nutritional genomics*." Professors can use this book to enliven their nutrition lectures and students will find a broader approach to nutrition than in any conventional nutrition text. Although written in narrative and not textbook style, it has excellent references for each chapter.

Handbook of minerals as nutritional supplements. Robert A. DiSilvestro. CRC Press, Boca Raton, Fla., 2005. (ISBN 0-8493-1652-9) 254 pages, hardcover, US\$139.95.

This very convenient and helpful compendium of information imparts evidence for the role and effectiveness of mineral supplements for health maintenance and improvement. It summarizes and evaluates current research on supplement use in a wide range of disease states for various mineral supplements such as calcium, magnesium, potassium, iron, zinc, copper, selenium, manganese, and chromium. The potential toxicity of each and the interactions among minerals in a supplement are described. The book also includes a glossary and index.

The great brain debate: Nature or nurture? John E. Dowling. Joseph Henry Press, Washington, DC, 2004. (ISBN 0-309-09223-X) 189 pages, hardcover, US\$24.95. Available as read-only without cost at http://www.nap.edu/catalog.php?record_id=11004, Accessed June 6, 2005.

The focus of this book is "whether the development of our brain, our personality, our intelligence, and our behavior are more likely to be shaped and affected by our environment or our genetic coding." Dowling notes that the first three years are important for development, but does not mention nutrition as an important environmental factor. Disappointingly, he makes no reference to the extensive evidence that iodine deficiency in pregnancy and iron deficiency in infancy can cause permanent adverse effects on cognitive development. Evidence of the effects of vitamin deficiencies on behavior at any age is not mentioned, and nutrition as an important environmental factor is not mentioned at all in either the text or the index.

—Nevin S. Scrimshaw

**Food and Agriculture Organization (FAO),
2004**
**Globalization of food systems in
developing countries: impact on food
security and nutrition**

As a result of global economic and social change, food systems are being transformed at an unprecedented rate. Urbanization, foreign direct investment in the markets of developing countries and increasing incomes are prime economic facilitators for the observed changes, while social changes such as the increasing number of women in the workforce and rural to urban migration provide further stimulus. Changes are also driven by food production based on intensive agriculture, new food processing and storage technologies, longer product shelf life, the emergence of fast-food outlets and supermarkets, and the intensification of advertising and marketing of certain products. The sum of these changes has resulted in a diversity of food available throughout the year for those who can afford to purchase it, as well as a shift in home-prepared and home-based meals toward prepared and ready-to-eat meals, often consumed away from home.

These food system and lifestyle changes in turn impact the health and nutritional status of people in developing countries. There are indications of rapid increases in overweight and obesity, particularly among adults, accompanied by an increasing prevalence of diet-related noncommunicable diseases. At the same time, social inequalities are increasing.

The papers in this book were first presented at the workshop “Globalization of Food Systems: Impacts on Food Security and Nutrition” at FAO headquarters in Rome, October 8–10, 2003. Chapters are arranged in two parts. The first part consists of an overview chapter providing a synthesis of findings from 11 country case studies, followed by chapters on urban food insecurity, nutritional change in developing countries, and policy options to address these changes. The second part provides a detailed account of the changes in food systems and health and nutrition problems in 11 case-study countries from different regions of the world.

A hard copy of the publication is available by request from Gina Kennedy (gina.kennedy@fao.org) or Guy Nantel (guy.nantel@fao.org). An electronic copy is available at http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y5736e/y5736e00.htm

Correction to Vol. 26, No. 2, Supplement 2

“Proceedings of the International Workshop on Articulating the Impact of Nutritional Deficiencies on the Education for All Agenda,” Osman M. Galal, Charlotte G. Neumann, and Judie Hulett, guest editors

The following acknowledgments were omitted from the above-referenced supplement due to a production error in the *Food and Nutrition Bulletin's* editorial office. The editors regret this oversight.

Acknowledgments

The guest editors of this supplement express gratitude for the unique opportunity to coordinate this workshop and our sincere appreciation of the encouragement and interest from the people who made this effort possible.

Geoffrey Garrett
UCLA International Institute
Educating Global Citizens

Allen Roberts
US Department of Education Grant to the James S. Coleman African Studies Center, UCLA

Edmond Keller
Globalization Research Center – Africa, UCLA

Mark Wahlqvist
International Union of Nutritional Sciences
(IUNS)

Zoë Boutilier
The Micronutrient Initiative

Linda Rosenstock
Dean
UCLA School of Public Health

Montague W. Demment
Global Livestock CRSP, University of California,
Davis (USAID)

We are also indebted to our colleagues who made our visiting scholars feel at home and provided invaluable service to the workshop through their assistance and very hard work.

Susan Silah
Mary Vardazarian
Ritesh Mistry
Parisa Mirzadehgan

Food and Nutrition Bulletin Support for Subscriptions to Developing Countries

International agencies

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

Bilateral agencies

United States Agency for International Development (USAID)

Nongovernmental organizations

International Life Sciences Institute (ILSI)

Corporations

Akzo Nobel Chemicals
DSM Nutritional Products
Kraft Foods
Procter & Gamble Co.
Unilever

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
International Atomic Energy Agency (IAEA)	www.iaea.org
International Life Sciences Institute (ILSI)	www.ilsi.org
International Nutritional Anemia Consultative Group (INACG)	http://inacg.ilsi.org
International Nutrition Foundation (INF)	www.inffoundation.org
International Vitamin A Consultative Group (IVACG)	http://ivacg.ilsi.org
International Union of Nutritional Sciences (IUNS)	www.iuns.org
Iron Deficiency Project Advisory Service (IDPAS)	www.micronutrient.org/idpas
The Micronutrient Initiative	www.micronutrient.org
Pan American Health Organization (PAHO)	www.paho.org
Save the Children	www.savethechildren.org
Unilever	www.unilever.com
United Nations Children's Fund (UNICEF)	www.unicef.org
United Nations University (UNU)	www.unu.org
UN Standing Committee on Nutrition (SCN)	www.unsystem.org/scn
World Bank	www.worldbank.org
World Food Programme	www.wfp.org
World Health Organization (WHO)	www.who.int/en

Note for contributors to the *Food and Nutrition Bulletin*

The editors of the *Food and Nutrition Bulletin* welcome contributions of relevance to its concerns (see the statement of editorial policy). Submission of an article does not guarantee publication; acceptance depends on the judgment of the editors and reviewers as to its relevance and quality. All potentially acceptable manuscripts are peer-reviewed. Contributors should examine recent issues of the *Bulletin* for content and style.

Language. Contributions should be submitted in English.

Format. Manuscripts should be prepared on a computer, double-spaced, and with ample margins. **Authors are encouraged to submit manuscripts electronically.**

Abstract. An abstract of not more than 250 words should be included at the beginning of the manuscript, stating the purposes of the study or investigation, basic procedures (study subjects or experimental animals and observational and analytical methods), main findings (give specific data and their statistical significance, if possible), and the principal conclusions. Emphasize new and important aspects of the study or observations. Do *not* include any information that is not given in the body of the article. Do not cite references or use abbreviations or acronyms in the abstract.

Key words. Authors should provide a minimum of four key words for the article.

Tables and figures. Tables and figures should be placed on separate pages. Tables should be typed with data clearly organized. Footnotes should be keyed to the relevant data points by letters or symbols. Figures should be submitted electronically, as part of the manuscript file or as a separate electronic file. Please double-check your data for accuracy and consistency with the text.

Photographs. Photographs may be mailed or submitted electronically. Mailed photographs should be glossy black and white prints. However, we encourage electronic submission of all materials. Photographs will not be returned unless specifically requested.

Units of measure. All measurements should be expressed in metric units. If other units are used, their metric equivalent should be indicated.

Abbreviations. Please spell out all abbreviations used on the first reference.

References. References should be listed at the end of the article, also double-spaced. Unpublished papers should not be listed as references, nor should papers submitted for publication but not yet accepted. Please double-check that reference numbers correspond to the correct numbers in the text.

Number references consecutively in the order in which they are first mentioned in the text. Identify references in the text and tables and figure legends by arabic numerals enclosed in square brackets. References cited only in tables or figure legends should be numbered in accordance with the first mention of the relevant table or figure in the text. **Be sure references are complete and current.**

Reference citations should follow the format below.

Journal reference

—standard journal article (list all authors):

1. Alvarez MI, Mikasic D, Ottenberger A, Salazar ME. Características de familias urbanas con lactante desnutrido: un análisis crítico. *Arch Latinoam Nutr* 1979;29:220–30.

—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.

Identification. Please give the full name and highest degree of each author, the name of departments and institutions to which the work should be attributed, the name, address, fax number, and e-mail address of the author responsible for correspondence about the manuscript, and sources of support for the work. If the material in the article has been previously presented or is planned to be published elsewhere—in the same or modified form—a note should be included giving the details.

Page charges. Most peer-reviewed and indexed journals either have page charges for the publication of articles based on sponsored research or have very high subscription costs that limit their distribution. The *Food and Nutrition Bulletin* places major emphasis on reaching developing countries and has been fortunate to receive support from the United Nations University (UNU) (<http://www.unu.edu>) for most editorial costs and the equivalent of free subscriptions to more than 800 institutions and individuals in developing countries. To help meet editorial costs, the *Bulletin* has instituted page charges for all papers with major sponsors and the cost of publication should be incorporated into the cost of sponsoring the research project. We are therefore asking all authors to include page charges (US\$60 per printed page) in their sponsored research project budget. One printed page in the *Bulletin* is equivalent to approximately 3 double-spaced manuscript pages. The *Bulletin* will waive these charges for authors in developing countries who do not have support that will cover page charges, but will require a formal letter requesting a waiver. Articles acknowledging major financial support, or from authors in industrialized countries, will not be eligible for waivers. This policy does not apply to solicited articles. Authors contributing to special issues and supplements, which are already sponsored, are not responsible for page charges.

Manuscript copies. The contributor should keep a duplicate copy of the manuscript. Manuscripts will not be returned unless specifically requested.

Contributions should be addressed to:

Food and Nutrition Bulletin
Susan Karcz, Managing Editor
150 Harrison Ave.
Boston, MA 02111 USA
FNB@inffoundation.org

Subscribe to the *Food and Nutrition Bulletin*

Annual Subscriptions

The annual subscription cost of the *Bulletin* is US\$56.00, which includes both the quarterly issues and supplements. To subscribe, write or email:

International Nutrition Foundation
150 Harrison Ave.
Boston, MA, 02111, USA
Tel: 617-636-3771
Fax: 617-636-3727
E-mail: inffoundation.org

Subsidized Subscriptions Available

The International Nutrition Foundation (INF) is raising funds to increase the subsidized distribution of the *Food and Nutrition Bulletin* to nutrition scientists and institutions in developing countries. This effort has been supported by the United Nations University (UNU), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), the United States Agency for International Development (USAID), The Micronutrient Initiative (MI), and the International Life Sciences Institute (ILSI). Contributions have also been received from Akzo Nobel Chemicals, DSM Nutritional Products, Kraft Foods, Procter & Gamble Co., and Unilever.

If you (or your organization) are working in the field of nutrition, and are from a developing country, you may be eligible for a donated or subsidized subscription. The extent to which requests for free subscriptions can be met depends on available funds. The *Bulletin's* goal of promoting a wide geographic and institutional distribution will be taken into consideration. Individuals and institutions working in developing countries and countries in transition may apply biannually for a subsidized subscription.

Preference for subsidized subscriptions will be given to libraries. If you are affiliated with a university in a developing country, we encourage you to make this information available to the library of your institution.

Normally, individuals holding international posts working with international and bilateral agencies, international nongovernmental organizations (NGOs), and private corporations are not eligible for free subscriptions.

Subscription exchanges with journals of similar purposes and interests are welcome.

To apply for a subsidized subscription, write or email:

International Nutrition Foundation
150 Harrison Ave.
Boston, MA, 02111, USA
Tel: 617-636-3771
Fax: 617-636-3727
E-mail: inffoundation.org

Food and Nutrition Bulletin Subscription Form

Please enter my subscription to the *Food and Nutrition Bulletin* (4 issues).

Regular rates: 1 year, US\$56 2 years, US\$106 3 years, US\$150
All rates include delivery by surface mail.

Total payment enclosed: _____

Individuals are requested to enclose payment with their orders. Prices subject to change without notice. Payment must be made in US dollars only. Checks should be made payable to: International Nutrition Foundation, Inc. Subscriptions will begin with the issue following placement of the order.

Name: _____

Address: _____

Send to: Subscriptions
International Nutrition Foundation, Inc.
150 Harrison Ave.
Boston, MA 02111 USA

