

Contents

Community nutrition

- The case for promoting multiple vitamin and mineral supplements for women of reproductive age in developing countries —S. L. Huffman, J. Baker, J. Shumann, and E. R. Zehner 379
- Feeding practices and malnutrition in children in rural Bangladesh —Helga Piechulek, Jorge Mendoza Aldana, and Md. Nazmul Hasan 395
- Is one nutritional benchmark appropriate? Dilemma in nutritional assessment and growth monitoring of children under five years of age in Fiji —Shoko Saito and Geoffrey C. Marks 401

Anaemia and iron deficiency

- Clinical screening may be a cost-effective way to screen for severe anaemia —E. Dusch, R. Galloway, E. Achadi, I. Jus'at, C. Sibale, C. Franco, S. Cousens, and L. Morison 409
- Assessment of the prevalence and potential determinants of nutritional anaemia in Upper Egypt —N. El-Sayed, A. Gad, L. Nofal, H. A. Zeid, H. El-Morshedy, and S. El-Waseef 417
- Development of iron-deficiency anaemia at six months of age in Jordanian infants exclusively breastfed for four to six months —Ahmad M. Faqih and Hussain S. Qazaq 422
- Adolescent schoolgirls: Daily or weekly iron supplementation? —Renuka Jayatissa and Chandrani Piyasena 429
- Increase in compliance with weekly iron supplementation of adolescent girls by an accompanying communication programme in secondary schools in Dar-es-Salaam, Tanzania —G. S. Muro, U. Gross, R. Gross, and L. Wahyuniar 435

Nutrition and the environment

- Farm commercialization and nutritional status of children: The case of the vegetables, fruits, and cash crops programme in western Nepal —Khem R. Sharma 445
- Nutrition, food, and water security —Margaret R. Biswas 454

Food science

- Testing methods for aflatoxins in foods —Kaushal K. Sinha 458

Nutrition survey summary

- Nutritional status, food-consumption frequency, and nutrient intake of pre-school children in northern Ghana —Etor E. K. Takyi 465

- Books received** 467

- News and notes** 468

The Food and Nutrition Bulletin encourages letters to the editor regarding issues dealt with in its contents.

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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

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The case for promoting multiple vitamin and mineral supplements for women of reproductive age in developing countries

Sandra L. Huffman, Jean Baker, Jill Shumann, and Elizabeth R. Zehner

Abstract

Women in developing countries often consume inadequate amounts of micronutrients because of their limited intake of animal products, fruits, vegetables, and fortified foods. Intakes of micronutrients less than the recommended values increase a woman's risk of having micronutrient deficiencies. The adverse effects of deficiencies in vitamin A, iron, and folic acid, including night-blindness in pregnant and lactating women and iron-deficiency anaemia, are well known. Low intakes of these and other nutrients, including zinc, calcium, riboflavin, vitamin B₆, and vitamin B₁₂, also have consequences for women's health, pregnancy outcome, and the health and nutritional status of breastfed children. Multiple deficiencies coexist, so the benefit of multiple micronutrient supplements is becoming increasingly apparent. Supplementation of women with multiple vitamins and minerals should be one component of a strategy to improve micronutrient status among women in developing countries. However, there are several issues for programme managers to consider before implementing programmes. Which reference standards will be used to determine nutrient levels to include in the supplements? Which nutrients will be included and in what quantities? Which factors need to be considered in purchasing supplements? These issues are discussed, and guidance is provided on the selection

of appropriate supplements for pregnant women and women of reproductive age in developing countries.

Introduction

Chronic energy deficiency and stunting among women in developing countries are the result of malnutrition during foetal growth, infancy, and childhood, with low energy intakes continuing into adulthood for many women. Micronutrient malnutrition can stem from deprivation in childhood but is primarily related to currently inadequate intakes. Although stunting caused by early malnutrition cannot be reversed in adulthood, micronutrient malnutrition can be remedied, with substantial benefits for women's and children's health.

Dietary patterns result in low intakes of several nutrients simultaneously. In addition to improving women's diets, multiple vitamin and mineral supplements should be part of a strategy to improve micronutrient status among women in developing countries. This paper provides information to facilitate the incorporation of multiple micronutrient supplements into programmes to improve the health and nutritional status of women.

Why is micronutrient malnutrition a concern among women in developing countries?

Poor dietary quality, rather than quantity, is the major determinant of inadequate micronutrient status among women in developing countries [1, 2]. Low-income populations obtain most of their calories from low-cost staple foods and less from more expensive foods, such as animal products, fruits, and vegetables, which are rich in micronutrients. Women in developing countries generally have lower intakes of animal products than women in European and other developed countries. For example, in the United States 60% of energy intake is from animal products [3]. Among pregnant women from a community study in Mexico, only 7%

Sandra Huffman is the director of the Ready to Learn Center, Academy for Educational Development, in Washington, DC. Jean Baker is the director of the LINKAGES Project, Academy for Educational Development. Jill Shumann is affiliated with Population Services International in Washington, DC. Elizabeth Zehner is a consultant with the LINKAGES Project.

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of total calories were from animal products. Tortillas provided 64% of calories, illustrating the limited diversity of the diet [3]. In a corollary study in Egypt, pregnant women obtained only 17% of their calories from animal products and 35% of their calories from the traditional bread [4]. Fruits and vegetables are often only seasonally available or of limited variety. For example, a national survey in Honduras found that among households in the poorest region, the average number of servings of vegetables per day was only 1.2 [5].

Promoting improvements in dietary intake often is based on the inclusion of small amounts of animal foods

and increasing fruits and vegetables through diets such as the one shown in table 1. This illustrates a "relatively good" Asian rice- and wheat-based diet for a woman consuming limited calories. Staple foods, including rice, wheat, millet, potatoes, and corn, provide needed energy and protein, but few micronutrients, except when fortified.

In the diet shown in table 1, 41% of the calories come from staple foods and only 9% from animal products. However, even this diet might be difficult to promote throughout the year, because of the seasonal availability of fruits (mangoes) and the relatively high cost of

TABLE 1. Vitamin A, zinc, calcium, folate, and energy in a "relatively good" Asian diet meeting RDAs for vitamin A

Food	Amount	Vitamin A (IU) ^a	Zinc (mg)	Calcium (mg)	Folate (µg)	Energy (kcal)
Rice (cooked)	3 cups (525 g)	0	1.63	99	21	598
Lentils (cooked)	½ cup (150 g)	8	1.26	19	179	115
Potato	1 potato (122 g)	0	0.36	6	11	113
Whole-wheat bread or chapatti	2 slices bread (92 g) or 1 chapatti (92 g)	0	1.38	30	43	256
Oil	2 tablespoons (28 g)	0	0.01	0	0	200
Fish (bass)	1 piece (100 g)	115	0.83	103	17	146
Mango	1 small mango (100 g)	3,894	0.04	10	14	65
Banana	1 small banana (101 g)	81	0.16	6	19	93
Kale (greens)	100 g	7,400	0.24	72	13	28
Sugar	2 tablespoons (24 g)	0	0	0	0	96
Total		11,498	5.91	345	317	1,710
Foods rich in folate						
Orange	1 large orange (184 g)	377	0.13	74	56	86
Garbanzo beans	½ cup (120 g)	29	1.27	18	80	143
Spinach (cooked)	½ cup (90 g)	7,371	0.68	122	131	21
Foods rich in zinc						
Beef	100 g	0	6.8	8	8	211
Chicken liver	1 liver (6 g)	983	0.26	0.84	46	39
Foods rich in calcium						
Cow's milk (whole)	1 cup (244 g)	307	0.9	291	12	150
Chinese cabbage	1 cup (170 g)	4,365	0.29	158	69	20
RDA for women^b						
25–50 yr old		8,000	12	1,000	400	
Pregnant		8,000	15	1,000	600	
Lactating		13,500	15	1,000	400	

RDA, Recommended daily allowance; IU, international unit; RE, retinol equivalent; AI, average intake level.

a. Conversion from RE to IU is based on 1 RE = 3.33 IU retinol; 1 RE = 10 IU provitamin A carotenoid. This level for RDA assumes food contains primarily β-carotene.

b. The most recently published RDAs are given: 1989 for zinc and vitamin A, and 1999 for calcium and folate. The 1999 RDAs set AIs rather than RDAs for calcium.

some foods (fish, lentils), which would keep them out of reach of the very poor. Although this diet exceeds vitamin A requirements (providing more than 11,000 IU of vitamin A), additional foods high in zinc, calcium, and folate would still be needed to meet the recommended dietary allowances (RDAs). Animal flesh products, such as red meat or liver, are needed to provide sufficient zinc and iron. Additional calcium-rich sources (such as Chinese cabbage, broccoli, nuts, or dairy products) are needed for this diet to meet calcium requirements. Additional legumes and dark-green leafy vegetables (such as spinach or mustard greens) are needed to provide sufficient folate.

Improving micronutrient intake through dietary approaches by diversifying diets and increasing intakes of foods high in micronutrients is an important strategy to improve women's health. However, because of economic constraints, seasonal harvests, limited production, poor infrastructure to distribute foods produced in other areas, and food-consumption patterns, other means of improving micronutrient intake are also needed. Even in the United Kingdom, a recent randomized controlled trial illustrated that fortification and supplement use have more beneficial effects on micronutrient status than do improvements in diet [6].

Fortification of staple foods is being used increasingly to improve micronutrient intake, but many nutrients missing in diets are not now included in fortificant pre-mixes for technical and other reasons. As countries expand fortification efforts, there are many logistical and quality-control measures needed for fortificants to be added and sustained at appropriate levels. It will also be necessary to ensure that the target population consumes sufficient quantities of the fortified products. However, even in developed countries, where fortification of foods is a long-standing practice, because of the low intakes of certain foods and the limited number of foods that are fortified, significant proportions of women have less than adequate intakes of micronutrients.

What is the evidence for poor micronutrient intakes among women?

The adequacy of diets for women is determined by comparing their average nutrient intakes to recommended levels of intake, such as the RDA. The RDAs for several nutrients increase substantially during pregnancy and lactation. The current diets of many women in developing countries are unable to meet even the lower RDAs for women of reproductive age. It becomes increasingly difficult to ensure adequate diets for pregnant and lactating women, with their higher requirements.

National surveys of dietary intakes are routinely conducted in the United States and Europe, but they are

less common in developing countries because of their high cost and technical difficulty. Localized studies have been conducted that indicate low intakes of micronutrients are common in many poor areas. However, several studies have shown low intakes of several nutrients among adolescent girls and adult women, including iron, vitamin A, zinc, folate, vitamin B₆, vitamin B₁₂, riboflavin, and calcium. For example, in Mexico and Argentina, adolescent girls and adult women consumed less than two-thirds of the RDA of vitamin A [7, 8]. Studies in Brazil, Guatemala, Mexico, India, Nepal, Nigeria, Malawi, Egypt, and Kenya reported mean intakes of less than two-thirds of the RDA for zinc [7, 9–11]. The World Health Organization (WHO) [12] reported that several national dietary surveys showed that the intakes of zinc and copper were unlikely to meet the requirements for most age groups in Africa, the Eastern Mediterranean, South-East Asia, and the Western Pacific. In South Africa, Indian women and rural black women consumed less than two-thirds of the RDA of folate [13]. The average dietary intake of vitamin B₆ was less than two-thirds of the RDA for pregnant and lactating women in Egypt and Kenya [14, 15]. In Mexico and Kenya, women consumed less than two-thirds of the RDA of vitamin B₁₂ [15]. In Mexico, Egypt, and Kenya, women consumed less than two-thirds of the RDA of riboflavin [7, 15]. Calcium intakes of less than two-thirds of the RDA were reported in Colombia, Thailand, Jamaica [16], and India [17].

What is the prevalence of micronutrient deficiencies in women?

When women become deficient in micronutrients, they have low levels of the micronutrients in the serum, red blood cells, or tissues. As the deficiency worsens, clinical signs can be observed. Few assessments of micronutrient deficiencies through national surveys have been conducted, other than for iron, iodine, and vitamin A. However, data from smaller-scale studies indicate that micronutrient deficiencies of zinc, folate, vitamin B₆, vitamin B₁₂, and riboflavin, among others, are evident in many subpopulations [18–27].

At least 50 million pregnant women and 320 million non-pregnant women in developing countries are anaemic, primarily due to iron deficiency [28]. Iodine deficiency and goitre are still found in women in many parts of India and Africa and in isolated parts of Latin America and Eastern Europe. In Nepal 20% of pregnant women and 27% of postpartum women [21] were vitamin A deficient (serum retinol < 20 µg/dl). In Indonesia 18% of postpartum women were deficient (< 70 µmol/L). In a national survey in Costa Rica, 25% of urban and 31% of rural women had low serum retinol levels [29]. Night-blindness was observed in Nepal in 18% of pregnant and 8% of lactating women [30],

and in Bangladesh in 1.3% to 2.4% of women of reproductive age [31].

In Indonesia a study of postpartum women found that 24% were zinc deficient [32], and a study of pregnant women in Peru found that 60% were zinc deficient (plasma zinc < 10.71 $\mu\text{mol/L}$) [33, 34]. In Egypt 33% were classified as zinc deficient (< 8.5 $\mu\text{mol/L}$) [35]. Folate deficiency is a problem in some parts of India, West Africa, and Burma [36]. In a Kenyan study, 6% to 8% of anaemia in pregnancy was related to folate deficiency among the 48% of pregnant women found to be anaemic [37]. Suboptimal vitamin B₆ status has been observed in Egypt among more than one-third of breastfeeding women, based on low breastmilk concentrations [35]. Low serum vitamin B₁₂ has been observed among pregnant and lactating women in Mexico, and low breastmilk vitamin B₁₂ was reported in Kenya [7]. In India vitamin B₁₂ deficiency is widespread because of strict limitation of animal products. Riboflavin deficiency is considered endemic in the Gambia and is common in other parts of Africa, the former Soviet Union, Indonesia, and China [38]. Studies conducted by the National Institute of Nutrition in India found that more than two-thirds of women had riboflavin deficiency [17].

What are the impacts of micronutrient deficiencies in women?

As discussed above, poor diets among many women in developing countries result in insufficient intake of several nutrients. In conjunction with infections and infestations that increase demand for nutrients, deficiencies occur that impair women's health, the outcome of their pregnancies, and the growth, development, and health of their breastfed infants.

Impact on women's health

The impact of iron and iodine deficiencies on women's health is well known. Iron deficiency affects the function of several organs. In women it impairs work capacity [18], and in adolescents it impairs learning [19]. Iodine deficiency causes goitre in women and impairs intellectual functioning [20].

Recent placebo-controlled trials illustrate the benefits of improvements in vitamin A status on women's health. Enhanced vitamin A intake reduced the severity of morbidity in women in Nepal [21, 22], reduced anaemia in pregnant and postpartum women in Nepal [23] and adolescents in Indonesia [24], and reduced rates of malaria (*Plasmodium vivax*) in women in Nepal [23].

Both increased folic acid and increased vitamin B₆ intakes have been shown in large-scale epidemiologic studies to be associated with reduced risk of fatal coronary heart disease and nonfatal myocardial infarctions

and with reduced risk of arteriosclerosis among women [25, 26]. As the morbidity and mortality from coronary disease increase among women in developing countries, including many parts of Latin America [27], these benefits will become increasingly vital. Osteoporosis is an increasing problem as the average age of women in developing countries increases. Calcium and vitamin D intake over a woman's life-span are especially important.

Other benefits to improving micronutrient status for women's health are indirect. Reduced anaemia leads to enhanced productivity, which may result in higher income and overall improvements in diet, health care, and hygiene. Improved vitamin B₆ and iron levels among mothers can increase attentiveness to child rearing, with possible benefits for children's health and development. For example, in Egypt mothers who were deficient in vitamin B₆ were less responsive to their infants' vocalizations, showed less effective intervention to infant distress, and were more likely to use older siblings as caregivers than were mothers with better vitamin B₆ status [14].

Impact on pregnancy outcome

Ramakrishnan et al. [39] summarized the relationship between micronutrient status and pregnancy outcome in a recent review. They suggested that there is strong evidence from randomized controlled trials that zinc and magnesium supplementation improves birthweight and reduces prematurity, and that supplementation with calcium improves birthweight and reduces prematurity and pre-eclampsia, especially in high-risk groups. On the basis of epidemiologic evidence, they suggested that several other nutrients affect pregnancy outcome (including low birthweight, pre-term births, premature rupture of membranes, foetal death, and pre-eclampsia). These nutrients include iron, iodine, vitamin A, folic acid, vitamin B₆, vitamin B₁₂, and vitamin D.

A review of nutritional interventions in randomized controlled trials and their effect on preventing intrauterine growth retardation was recently conducted by WHO [40]. There were only two trials of iron, four of zinc, two of magnesium, five of calcium, five of folate, and one of vitamin D that the authors considered adequately designed to assess impacts. Of these 19 trials, only 6 were conducted in developing countries, where initial micronutrient status is likely to be worse than in developed countries. The authors observed that folate supplementation appears to reduce the incidence of term low birthweight and that zinc and magnesium supplementation may have beneficial effects and should be studied further.

Randomized controlled trials have examined the effect of daily doses of calcium on the outcome of pregnancy [41]. Recent analyses of the Cochrane database of randomized controlled trials found that supplemental

calcium caused greater improvement in the health of pregnant women with low baseline calcium intake (< 900 mg/day) than of women with high calcium intake [J. Villar and M. M. Belizán, personal communication, 1998]. Among women with low baseline calcium intake, the risk of high blood pressure was reduced by half (relative risk, 0.49; 95% confidence interval, 0.38 to 0.62). The risk of pre-eclampsia was also reduced greatly (relative risk, 0.32; 95% confidence interval, 0.21 to 0.49). Among women with a high risk of hypertension, there was also a major reduction in pre-term delivery (relative risk, 0.42; 95% confidence interval, 0.23 to 0.78).

Research in Nepal found that mortality decreased by about half in women who received vitamin A for at least three months before and during pregnancy [21, 42]. The prevalence of iron-deficiency anaemia in pregnancy (haemoglobin < 11 g/dl) was reduced from 76% in controls to 69% among those receiving vitamin A [23].

Improving folate status before pregnancy is associated with reductions in neural tube defects (such as spina bifida) [43]. It is estimated that more than 200,000 such defects worldwide could be prevented by improving folate status before pregnancy [44]. In North China it is estimated that 10% of infant mortality is due to neural tube defects. High rates of neural tube defects have also been reported in South Africa: 6/1,000 in the Transkei and 3.6/1,000 in the Northern Province [13]. Randomized controlled trials in the United Kingdom, Hungary, Israel, Australia, Canada, Russia, and France reduced neural tube defects with a protective effect of 72% [45, 46]. A few other studies have shown benefits of multiple supplements in preventing cleft palate and other types of birth defects [47–49]. Improving micronutrient status, especially folate status, before pregnancy would therefore help decrease infant mortality by reducing these defects.

Multiple supplements may also be beneficial for HIV-positive women. When supplements were provided to HIV-positive pregnant women in Tanzania in a randomized controlled trial, the rates of low birthweight decreased by 44%, pre-term births (< 34 weeks of gestation) by 39%, and small size for gestational age by 43% [50]. The role of supplements in the transmission of HIV to the infant is now being assessed. However, significant improvements in maternal immune status were associated with the use of supplement.

Impact on health and nutritional status of breastfed infants

The micronutrient status of breastfeeding women affects the quality of their milk. Allen [51] suggested the categorization of nutrients for lactating women based on their relationship with breastmilk quality. Deficiencies of priority I nutrients (thiamine, riboflavin, vitamin A, vitamin B₆, vitamin B₁₂, iodine, and selenium) in lactating women result in lower concentrations in the

breastmilk and have negative effects on infants. Increasing maternal intake of priority I nutrients results in increased concentrations in breastmilk and improved infant status, since foetal storage of these nutrients is low and breastmilk is the major source for infants.

The concentrations of priority II nutrients (folic acid, vitamin D, calcium, iron, copper, and zinc) in breastmilk are relatively protected during maternal deficiency, and breastmilk concentrations are relatively unaffected by maternal supplementation. However, the mother is especially vulnerable to further depletion during lactation, and postnatal supplementation is more likely to benefit the mother than her infant.

Improving maternal iron intake during pregnancy can improve the iron status of newborns. This was shown in a placebo-controlled trial in Peru, in which iron transfer to infants was significantly increased by the supplementation of pregnant women with 60 mg of iron [52].

In a randomized controlled trial of lactating women of low socio-economic status in the United States, multivitamin supplementation increased breastmilk concentrations of vitamin B₆, vitamin B₁₂, and folate [53]. In a pre- and post-trial of supplements containing thiamine, riboflavin, niacin, and vitamin C among lactating women from the Gambia, the breastmilk contents of these vitamins were improved [54]. Improving vitamin A status during pregnancy or lactation has been shown to increase vitamin A in breastmilk in Bangladesh [55], Indonesia [56, 57], and Guatemala [58].

What is the role of multiple micronutrient supplements?

The above discussion has shown the prevalence of low intakes of many micronutrients in developing countries, associated deficiencies, and their consequences. Most supplement programmes to date have provided iron and folic acid to pregnant women; some have also distributed vitamin A or iron and folic acid to postpartum women.

However, since women have multiple deficiencies, the use of multiple supplements should be considered [59]. The US National Academy of Sciences [60] suggested that a strategy to promote increased consumption of multiple micronutrients simultaneously would be more effective than the promotion of a select few. Combining multiple micronutrients in a single delivery mechanism has been suggested as a cost-effective way to achieve multiple benefits [61] [R. Yip, personal communication, 1997].

Supplementing women with a single nutrient is an effective means to improve micronutrient status and is a long-standing approach used in clinical practice when deficiencies are found. However, some have questioned the effectiveness of nutrients combined within

a supplement because of possible interactions of the nutrients or interference in their absorption [62, 63]. Randomized controlled trials found that combined supplements were effective in improving micronutrient status for vitamin A and iron in Indonesia and Tanzania [24, 50, 64–66] and for iron and zinc in Peru, Mexico, and the Gambia [33, 34, 67–69].

What are current policies and programmes for supplements?

UNICEF collected information on government policies related to iron and folic acid supplementation and iron fortification of staple products. Questionnaires were sent to 163 countries where UNICEF has programmes, and 57 responded. Policies for the universal distribution of iron or iron and folic acid during pregnancy were evident in 49 of 57 countries (86%) [70]. Most countries distribute iron as ferrous sulphate, but Thailand and Cuba provide ferrous fumarate. In parts of India, iron, folic acid, and vitamin B₁₂ are distributed to pregnant women.

However, even though programmes exist throughout the world, the effectiveness of iron and folic acid supplementation programmes for women during pregnancy has been questioned [71]. Evaluations of programmes have shown thus far that this approach has limited benefits. Aside from limited coverage and poor compliance, the focus on pregnant women does not allow enough time to reduce iron deficiency. In addition, since developing-country diets are limited in many other essential vitamins and minerals needed for the absorption, transport, metabolism, and use of iron and folic acid, the effectiveness of efforts including only these nutrients is limited, even if they are successful in increasing supplement use among the target population. Use of iron and folate for postpartum women was reported by UNICEF for only four countries: Bangladesh, Pakistan, Oman, and Bhutan.

According to UNICEF field office reports [72], of 78 countries with vitamin A deficiency, 46 have policies to supplement postpartum mothers with high doses of vitamin A. However, 10% or more of mothers received high-dose capsules after delivery in only one-fifth of all the countries with the deficiency and only one-third of those with supplementation policies. A recent national survey in Honduras, whose public health system reaches rural areas effectively, reported that only 13% of women living in households with children 12–71 months of age (9% of whom were pregnant at the time of the survey) had received a high-dose vitamin A capsule during their last postpartum period [73]. Even where higher coverage of postpartum women has been achieved, it is difficult to enhance vitamin A stores with this one-time approach [56].

Only a few countries provide multiple micronutrient

supplements through the health system. In Cuba a multiple supplement containing 35 mg of iron is provided to pregnant women. At prenatal care visits, pregnant women in Honduras receive a multiple vitamin and mineral supplement and an iron tablet containing 60 mg of iron (ferrous sulphate) [L. Caulfield, personal communication, 1997]. The contents of the prenatal supplement have changed over the last several years and appear to be selected on the basis of bids received during the Ministry of Health's procurement process. The only country to distribute multiple vitamins on a population basis is Cuba, in response to the neuropathy epidemic related to deficiencies of thiamine and other micronutrients. National distribution of supplements to Cubans over one year of age began in 1993 [74].

The United States Government recommends that all women of reproductive age consume daily supplements containing 400 µg of folic acid [75]. The Government also recommends that pregnant women consume 30 mg/day of supplementary iron, starting with the first prenatal visit, to reduce iron deficiency during pregnancy [18].

Although there is no recognized policy in the United States concerning multiple supplement use, 98% of obstetricians and gynecologists recommend supplements to their patients during pregnancy, and 92% specifically recommend prenatal supplements [76]. The National Maternal and Infant Health Survey in 1986 found that 81% of women reported consuming supplements during their last pregnancy [77].

The National Health Interview Surveys collect information on adults in the United States regarding supplement use in the past year. In the 1987 survey, 51% of all adults reported that they had consumed some type of vitamin or mineral supplement during the past year, 39% that they had taken a supplement for more than two days during the past month, and 23% that they had taken a daily supplement during the past year [78]. Among those 25 to 34 years old, 15% of white men and 23% of white women took a daily supplement. The 1992 survey found that 27% of women took a daily supplement and 20% took daily multiple supplements [79].

A 1983 survey in Australia found that 37% of women were regular supplement users (taking a multiple vitamin or mineral supplement at least once a week) and another 13% were irregular users [80]. In Finland 14% of women used supplements in 1985, and in the United Kingdom market research found that 31% of adults consumed supplements in 1984 [80].

What issues should be considered in selecting micronutrient supplements?

The selection of an appropriate supplement for women of reproductive age requires decisions about the following:

- » Which reference standards will be used to determine nutrient levels to include in the supplements?
- » Which nutrients and what quantities will be included?
- » Which factors need to be considered in purchasing supplements?
 - availability
 - safety
 - costs
 - quality
 - acceptability and compliance

Which reference standards will be used?

Reference standards commonly used to assess the adequacy of micronutrient intakes have been set by the US Institute of Medicine (National Academy of Sciences/National Research Council), the UK Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy (UK Department of Health), and WHO. Several other European countries, the European Community, Canada, and some developing countries such as China and India also have established reference standards, but these are not often used for international comparisons of population nutrient intakes.

These reference standards are based on:

- » physiological requirements for healthy individuals (by age, sex, and reproductive status) based on:
 - intakes associated with absence of deficiency diseases or to cure deficiency diseases;
 - intakes needed to maintain nutrient balance;
 - intakes needed to maintain circulating levels or enzyme saturation or tissue concentration of the nutrient (enabling storage of the nutrient);
- » bioavailability of nutrients (estimated as the proportion of nutrients consumed that are absorbed);
- » the nature of the diet;
- » toxicity levels for the nutrient.

Because of emerging information on the health benefits of nutrients consumed at levels higher than physiologic requirements, some revisions of the reference standards now also take into account such benefits (e.g., the role of folic acid in preventing neural tube defects and coronary artery disease).

The US RDAs are recommendations designed for healthy populations in the United States and thus may underestimate the requirements of populations in developing countries [81]. The US RDAs are age and sex specific, and separate values are given for pregnant and lactating women. The most recent reference values, published in 1998, are referred to as the Dietary Reference Intakes (DRIs) and include RDAs and adequate intakes (AIs) [82, 83]. AIs are based on average intakes that appear to sustain a defined nutritional state [82] and are used when scientific evidence is too limited to develop RDAs.

In 1991 the UK Panel on Dietary Reference Values revised its previous Recommended Daily Amounts (also

known as RDAs). The United Kingdom now uses the term Reference Nutrient Intake (RNI) to state the requirements for a particular nutrient [84]. WHO and the Food and Agriculture Organization (FAO) [12] have established lower and upper limits of safe ranges of population mean intakes to meet individual basal or normative requirements for trace minerals such as zinc, copper, selenium, iodine, and magnesium. WHO/FAO are now revising the standards for other micronutrients [85–87].

The US Food and Drug Administration (FDA) of the Department of Health and Human Services sets labelling requirements for processed food or dietary supplements. Unlike the RDAs, which are age- and sex-specific, a single set of reference values, the Reference Daily Intake (RDI), is used for labelling purposes [88]. The intakes are based on the 1968 RDAs, because when labelling requirements were first established these were the most recent values available. In general, RDIs are set at the highest level for any subgroup within the 1968 RDAs. Multiple vitamin and mineral supplements are labelled based on the percentage of the RDIs (referred to as Daily Reference Values or Daily Values).

Which nutrients and quantities will be included?

Because there is no international consensus on the levels of nutrients that should be included in supplements, we have suggested a supplement composition for women of reproductive age that is shown in table 2. This formulation in part conforms with US RDI levels, since US standards are often used by supplement manufacturers to determine their formulations. Thus, these levels are more likely to be available in already manufactured supplements sold in the United States and other parts of the Western Hemisphere.

Some levels have been modified to take into account recent revisions in the US RDAs that are not accounted for in the US RDIs. Because of the widespread problem of iron deficiency in developing countries, 27 mg of iron rather than the RDI of 18 mg is suggested. This level is lower than the recommended level of 60 mg for prophylactic iron supplementation of women of reproductive age in areas of high prevalence of iron deficiency [89]. However, prophylactic iron is recommended daily for a period of one to three months, and the proposed supplement should be taken daily for longer periods of time. The 27-mg level reduces the risk of accidental iron poisoning among young children and complies with US regulations. In addition, if women consume supplements at least twice a week, they will be consuming an amount similar to that in many of the iron supplements used in weekly supplementation trials, which have resulted in improvements in iron status among adolescents and women of reproductive age [24].

Folic acid is included at the current RDA of 400 µg

TABLE 2. Proposed ranges of nutrients in a multiple micronutrient supplement

Nutrient	US RDA ^a for women of reproductive age	US RDI	Women of reproductive age	
			Proposed supplement	Proposed range of supplements
Vitamin A ($\mu\text{g RE}$) ^b	800	875	875	500–1,300
Vitamin A (IU) ^b	2,664 RE 8,000 IU β -carotene	2,914 RE 8,750 IU β -carotene	5,000	2,500–8,000
Vitamin D (IU)	200 (AI)	400	400	100–400
Vitamin E (IU)	8	30	30	8–30
Vitamin B ₁ (thiamine)(mg)	1.1	1.5	1.5	0.8–1.6
Vitamin B ₂ (riboflavin)(mg)	1.1	1.7	1.7	1.1–1.8
Niacin (mg)	14	20	20	11.5–20.0
Folate (μg)	400	400	400	400–1,000
Vitamin B ₆ (mg)	1.3	2	2	1.6–2.1
Vitamin B ₁₂ (μg)	2.4	6	6	2.0–2.6
Vitamin C (mg)	60	60	60	60–100
Zinc (mg)	12	15	15	7–25
Iron (mg)	15	18	27	15–29
Calcium (mg)	1,000 (AI)	1,000		100–1,200
Phosphorus (mg)	700	1,000		0–1,200
Magnesium (mg)	Age 19-30: 310 Age 31-50: 320	400	100	100–400
Vitamin K (μg)	65		65	0–65
Iodine (μg)	150	150		0–200
Selenium (μg)	55			30–75
Copper (mg)	1.5–3.0 (ESADI)	2.0	2.0	1.5–3.0
Manganese (mg)	2.0–5.0 (ESADI)			2.0–5.0
Fluoride (mg)	3.1 (AI)			0
Chromium (μg)	50–200 (ESADI)			0–200
Molybdenum (μg)	30–100 (ESADI)			0–250
Biotin (μg)	30 (AI)	300		30–200
Pantothenic acid (mg)	5 (AI)	10		0–10
Choline (mg)	425 (AI)			

RDA, Recommended daily allowance; RDI, reference daily intake; RE, retinol equivalent; IU, international unit; AI, average intake level; ESADI, estimated safe and adequate dietary intake.

a. The most recently published RDAs are given: 1989 for zinc and vitamin A, and 1999 for calcium and folate. The 1999 RDAs set AIs rather than RDAs for calcium.

b. 1 $\mu\text{g RE}$ = 3.33 IU retinol or 10 IU β -carotene.

rather than the lower RDI level. Copper is included because both iron and zinc can inhibit copper absorption, and copper is recommended for inclusion in supplements containing iron and zinc [90]. Calcium levels have not been recommended because of the large amounts needed to meet the RDIs and the difficulty in producing a single, small tablet that contains sufficient calcium. Some calcium would be useful, however, depending on the ability of manufacturers to include bioavailable calcium.

In addition to this proposed formulation, ranges in appropriate nutrient levels are given in table 2. The ranges can be used to choose appropriate formulations that may not meet this exact formulation but that would also be appropriate for use by women in developing countries. These ranges are generally based on the lowest

RDA, RNI, or WHO-recommended level for women of reproductive age, and the highest level of the RDI, RDA, RNI, or WHO levels for pregnant or lactating women when these are higher than the RDIs.

Providing supplements containing nutrients below the lower range would mean that the diet would have to include a larger proportion of the daily requirements to ensure adequate intake by all women. Supplements containing levels of nutrients above the upper range would not necessarily be unsafe, but they could result in inappropriate mixes of nutrients that could impair their effectiveness. In addition, higher levels could also increase costs unnecessarily.

Because pregnant women need greater amounts of iron and other nutrients, a special formulation may be useful. We have proposed a formulation for a sup-

plement for pregnant women in table 3, based in part on the US RDIs for pregnancy and lactation used in labelling prenatal supplements, with some changes to address recently revised recommendations.

In contrast to the decisions about selection of ranges in table 1 using lowest and highest values for RDAs, RNIs, and WHO values, we have selected ranges between one and two times the US RDAs. As shown in tables 2 and 3, the selection of levels for supplements can be quite arbitrary but should balance scientific knowledge with practicality. However, a study conducted in the United States found that 50% of prenatal supplements sold there were formulated inappropriately (using the criterion of nutrient levels greater than twice the RDA) [91].

A calcium–magnesium supplement could also be considered for pregnant women because of the beneficial effects of calcium in preventing pregnancy-induced hypertension and pre-eclampsia, and the beneficial

effects of magnesium on improving birthweight and reducing prematurity. However, because of logistical problems, the distribution of additional supplements along with prenatal multiple vitamin and mineral tablets has not yet been attempted on a large-scale basis.

Although it would be optimal to have at least two formulations for programmes (one for women of reproductive age and one for pregnant women), many programmes will not be able to manage this because of the associated costs (for supply, distribution, management, training, and education). The supplement for women of reproductive age could be used effectively during pregnancy as long as additional iron supplements containing 30 to 60 mg were provided in areas with a high prevalence of iron deficiency. The addition of the other nutrients to ensure that nearly all women consume 100% of the RDA for pregnancy may not warrant the additional costs of procuring two supplements.

TABLE 3. Proposed ranges of nutrients in a multiple micronutrient supplement for pregnant women in developing countries

Nutrient	US RDA for pregnant women ^a	US RDI for pregnant or lactating women	Proposed supplement for pregnant women	Range from table 2	1 to 2 times US RDA for pregnant women
Vitamin A ($\mu\text{g RE}$) ^b	800		800	500–1,300	800–1,600
Vitamin A (IU) ^b	2,664 RE 8,000 IU β -carotene	8,000	2,664 RE 8,000 IU β -carotene	2,500–8,000	2,664–5,328 RE 8,000–16,000 IU β -carotene
Vitamin D (IU)	200 (AI)	400	400	100–400	400–800
Vitamin E (IU)	10	30	30	8–30	10–20
Vitamin B ₁ (thiamine)(mg)	1.4	1.7	1.7	0.8–1.6	1.4–2.8
Vitamin B ₂ (riboflavin)(mg)	1.4	2.0	2.0	1.1–1.8	1.4–2.8
Niacin (mg)	18	25	25	11.5–20	18–36
Folate (μg)	600	800	800	400–1,000	600–1,200
Vitamin B ₆ (mg)	1.9	2.5	2.5	1.6–2.1	1.9–3.8
Vitamin B ₁₂ (μg)	2.6	8	8	2.0–2.6	2.6–5.2
Vitamin C (mg)	70	60	60	60–100	70–140
Zinc (mg)	15	15	15	7–25	15–30
Iron (mg)	30	18	60	15–29	30–60
Calcium (mg)	1,000 (AI)	1,300		100–1,200	1,000–2,000
Phosphorus (mg)	700	1,300		0–1,200	700–1,400
Magnesium (mg)	350	450	100	100–400	350–700
Vitamin K (μg)	65		65	0–65	65–130
Iodine (μg)	175	150		0–200	175–350
Selenium (μg)	65			30–75	65–130
Copper (mg)	1.5–3.0(ESADI)	2.0	2.0	1.5–3.0	1.5–6.0
Manganese (mg)	2.0–5.0(ESADI)			2.0–5.0	2.0–10.0
Fluoride (mg)	3.1 (AI)			0	3.1–6.2
Chromium (μg)	50–200(ESADI)			0–200	50–400
Molybdenum (μg)	75–250(ESADI)			0–250	75–500
Biotin (μg)	30 (AI)	300		30–200	30–60
Pantothenic acid (mg)	6 (AI)	10		0–10	6–12
Choline (mg)	450 (AI)				450–900

RDA, Recommended daily allowance; RDI, reference daily intake; RE, retinol equivalent; IU, international unit; AI, average intake level; ESADI, estimated safe and adequate dietary intake.

a. The most recently published RDAs are given: 1989 for zinc and vitamin A, and 1999 for calcium and folate. The 1999 RDAs set AIs rather than RDAs for calcium.

b. 1 $\mu\text{g RE}$ = 3.33 IU retinol or 10 IU β -carotene.

Which factors need to be considered in purchasing supplements?

Programmes generally will procure supplements from among those already available on the market. However, some of the available supplements contain inappropriate amounts of nutrients for women of reproductive age, and others may be too expensive for a national programme. To decide on what supplements should be procured, several issues need to be considered.

What supplements are currently available?

Multiple micronutrient supplements are not currently available from UNICEF, but the International Dispensary Association (IDA) and other nonprofit agencies procure them for sale to nonprofit organizations and developing-country governments. However, the micronutrient supplements available for purchase through these agencies are inappropriate because they do not contain sufficient iron, zinc, copper, vitamin A, folic acid, vitamin B₆, and vitamin B₁₂.

Supplements can also be purchased wholesale from manufacturers or distributors, or directly from pharmacies or other stores (depending on local regulations), but the cost and composition of the supplements vary greatly. Many supplements for women of reproductive age contain 18 mg of iron, but those labelled "with iron," such as Bayer One-a-Day with Iron, often include 27 mg of iron. Most prenatal supplements have 60 mg of iron as ferrous fumarate (the RDA for pregnancy is 30 mg) and 15–25 mg of zinc (the RDA for pregnancy is 15 mg). Many of the supplements available on the market in developing countries contain levels of nutrients that vary widely and that have no clear relationship with RDAs for women. The levels of nutrients in multiple supplements available in each country need to be assessed to determine whether locally available products are suitable.

Numerous preparations of minerals are available (especially of iron and calcium), and they often vary in their absorbability, use, and colour. Vitamin A can be included as either preformed vitamin A or β -carotene. Preservatives and stabilizers are often added to supplements to prevent interactions between nutrients. In some cases, minerals are encapsulated (in polysorbate or polymaltose) or iron is chelated (attached) to an amino acid, which keeps it from reacting with other elements and aids absorption.

What safety issues need to be addressed?

Some have questioned whether multiple micronutrient supplements are safe. Their use is widespread in the United States and other developed countries, and no negative consequences of using supplements with levels of nutrients normally provided in over-the-counter supplements have been reported [92–94].

However, ensuring the safety of supplements depends

on ensuring non-toxic levels of nutrients and appropriate packaging. Upper levels of safe intakes of nutrients have been published by the Institute of Medicine [82, 83], WHO [12], and the Council on Responsible Nutrition [93]. There are generally wide margins between the levels proposed in the formulations in tables 2 and 3 and the upper levels of safe intake.

Three different levels are used to assess excess intakes. The No Observed Adverse Effect Level (NOAEL) identifies intake levels not associated with adverse effects. The Lowest Observed Adverse Effect Level (LOAEL) is the intake level that has been associated with adverse effects. Tolerable Upper Limits (UL) are values reported recently by the Institute of Medicine [82], calculated from the LOAELs with the use of an uncertainty factor that takes into account the reliability of the LOAEL figures.

However, several of the supplements sold in developing countries include levels of vitamin A, fluoride, thiamine, and niacin higher than the suggested safe levels. Labels should be examined for the amounts that are included, and supplements with amounts outside the safe ranges should not be used on a daily basis. Excessive levels of vitamin A as preformed vitamin A (retinol) among pregnant women in the first trimester have been associated with birth defects. Thus, a daily limit of 10,000 IU of retinol has been suggested. However, if the vitamin A content includes both retinol and β -carotene, as is common, only the amount of retinol is of concern.

In addition to preventing toxicity from nutrients within a single tablet, it is also necessary to ensure that the combined consumption of nutrients in the diet and in supplements does not exceed toxic levels. In populations where iodine deficiency was previously prevalent, thyrotoxicosis can occur with increased iodine consumption, because the thyroid uses iodine more effectively. In such populations, toxic levels may be consumed, especially if there is inadequate quality control of the process of fortification of salt with iodine.

If supplements containing fluoride are consumed in addition to highly fluoridated water, fluoride toxicity can result. The Therapeutic Products Programme of Health Canada states that fluoride should not be given to children under three years of age, unless on the advice of a doctor or dentist, and should not be consumed in areas with fluoridated water [95].

Many anaemia-control programmes promote the use of 60 to 120 mg of iron and 250 to 400 μ g of folic acid [89] for pregnant women. The safety of the intake of iron and folic acid in addition to a multiple supplement should be considered. Iron levels exceeding 200 mg per day are not associated with health problems, but side effects are likely [28]. Women who have previously delivered infants with neural tube defects are prescribed 4,000 μ g of folic acid daily, with no negative effects reported. Thus, the amount obtained from

a daily iron and folic acid tablet in combination with a multiple supplement containing 60 mg of iron or 400 µg of folic acid does not pose a health risk.

Overdosing of nutrients from supplements can occur among children, because they require much lower levels of most nutrients, and consumption at higher levels can be toxic. In the United States a recent FDA ruling stated that any pills containing 30 mg or more of elemental iron must be packaged in individual doses such as blister packs to reduce the number of deaths and hospitalizations of children from iron poisoning [96]. Even if pills contain less than that amount, they need to be sold in childproof (difficult-to-open) bottles, because consumption of several tablets containing only 10 mg each (as found in children's vitamin and mineral supplements) can cause poisoning, and 900 mg can be lethal.

What are the relative costs of the supplements?

The raw materials of most nutrients represent only a small proportion of supplement costs. The raw materials for a supplement containing all the nutrients suggested in tables 2 and 3 cost less than about 1/10th of a US cent. The total retail cost of producing and packaging the supplement is roughly 10 times the cost of raw materials. When distribution, management, and IEC (information, education, and communication) are added in, the cost of the individual nutrients in the supplement represents only a small portion of the total cost.

Some forms of iron (ferrous fumarate) are more expensive than others (ferrous sulphate), but ferrous fumarate reacts less with other nutrients in multiple nutrient supplements. The type of vitamin A used also differentially affects other nutrients within a tablet. Information on the forms of nutrients included in supplements should be requested from manufacturers.

In our survey of supplements sold in the Washington, DC, area, the retail cost of a single multiple vitamin and mineral supplement containing most of the nutrients recommended by the RDAs ranged from US\$0.05 to \$0.13 for women of reproductive age and \$0.10 to \$0.13 for prenatal supplements. Retail costs include profit margins for distributors and retailers; wholesale costs are less. Our research suggests that it would be possible to purchase multiple supplements similar to the one recommended in table 2 for about \$0.01 each if 5 to 10 million tablets were procured.

The costs to women of reproductive age are considerably reduced if supplements are taken weekly rather than daily. Weekly or biweekly doses of iron, iodine, vitamin A, vitamin D, and riboflavin [61, 94] have been shown to be effective; however, few data exist on whether other nutrients would also work well on a weekly or biweekly basis. Theoretically, daily supplementation should not be necessary, since requirements are based on average daily intakes.

What quality issues need to be considered?

The quality of the supplement depends on many factors, including the manufacturing practices used, nutrient interactions, storage and packaging, dissolution time, and adulteration.

Good manufacturing practices. In many countries, including Canada, dietary supplements are regulated as drugs and thus have more stringent requirements (such as registry and approval of the tablets and their ingredients, sterile manufacturing environments, and expiration labels). In the United States dietary supplements are regulated as foods rather than as drugs. Manufacturers and distributors do not have to register their products or ingredients with the FDA or get approval before they produce or sell dietary supplements. The FDA does not routinely test supplements; manufacturers are responsible for ensuring that the ingredients of the supplement are safe and not contaminated, and that the amounts stated on the label match the amounts in the supplements.

WHO guidelines on good manufacturing practices are described in *Good Manufacturing Practices for Pharmaceutical Products* [97] as well as by national regulatory authorities or independent agencies such as the US Pharmacopeia, the British Pharmacopoeia, the International Pharmacopoeia, the European Pharmacopoeia, the Federal Chemical Codex, and the American Chemical Society standards. The Council on Responsible Nutrition has drafted a suggested list of good manufacturing practices for dietary supplements modeled on the good manufacturing practices for food [98].

Companies that follow the standards established by an independent quality-control organization, such as the US Pharmacopeia, can say that their product meets the standards of that organization. This tells consumers that the manufacturer claims to follow certain procedures to ensure the quality of the product. However, since the FDA does not routinely monitor products (due to lack of funds), there is no assurance that the practices have been followed. In many cases, even with well-known private label brands, whether the manufacturer follows a specified quality-control standard is not shown on the label.

An essential means of evaluating the quality of micro-nutrient supplements is to request information from manufacturers documenting their adherence to good manufacturing practices guidelines. Good manufacturing practices ensure that products are produced consistently and meet quality standards. According to WHO [97], good manufacturing practices specify that:

- » all vendors and components are validated;
- » all manufacturing processes are clearly defined and systematically reviewed;
- » critical steps of manufacturing processes and significant changes to the process are validated;
- » all necessary facilities are provided and appropriately maintained;
- » all production steps are adequately documented;

- » all workers are trained;
- » all batches are fully documented and samples retained;
- » retained samples are systematically evaluated;
- » complaints are recorded and investigated.

When procuring supplements, buyers should demand certificates of analysis, preferably issued by an independent laboratory following US Pharmacopeia or other testing protocols.

Nutrient interactions. Some concern has been raised about the negative effect of interactions between different nutrients in a multiple vitamin and mineral supplement. Although it is true that some nutrients compete to be absorbed, if sufficient quantities are given the quantity of nutrients absorbed is generally not compromised substantially. There are often benefits to combining some nutrients because of the ability of one nutrient to enhance the transport, absorption, or use of another (e.g., vitamin A is needed for iron transport, and vitamin C enhances iron absorption).

The largest concern about interactions is with minerals (iron, zinc, calcium, and copper). Several studies have reported that when supplements include both iron and zinc there may be problems with their absorption. However, results are conflicting and depend on the quantities included [99]. When pregnant women in Peru were given iron (60 mg) and folic acid (250 mg) plus zinc (15 mg), the combination was as effective as iron and folic acid alone in improving haemoglobin levels [100] and led to higher zinc levels in the pregnant woman and in the neonate, even though absorption of iron was reduced with the addition of zinc [34]. Because iron can accelerate the degradation of vitamins (especially vitamins A and C), some forms of iron are better than others in a supplement. Forms of iron that are less reactive are preferable; however, this needs to be balanced with their bioavailability. Vitamin A is often encapsulated to prevent it from interacting with other vitamins or minerals.

Storage and packaging. The shelf life of the product is affected by the packaging and the types of nutrients used (e.g., whether they are encapsulated). Conditions that affect the potency of supplements include temperature, humidity, and light. Dark bottles are often required to prevent light from oxidizing the iron or vitamin A, unless special forms of these nutrients have been used. Blister packaging offers the advantages of having each tablet sealed in an airtight space to prevent deterioration from exposure to air and humidity. The expiration date should be included on the label so that supplements used are within the effective range.

Dissolution standards. If tablets do not dissolve within the expected time period, their nutrients cannot be absorbed by the small intestine, where most absorption takes place. Dissolution standards are given in the various Pharmacopoeias and other standards.

Adulteration. Contamination of the supplement with undesirable microorganisms could be a concern if manufacturing practices are poor. No elements should be included in the product other than those listed on the label. Several recalls have been made by the FDA when supplements were found to be contaminated with lead or to contain dyes not approved by the FDA. Poor manufacturing practices can also lead to the introduction of toxic levels of nutrients by mistake. Ensuring quality control can be especially important for vitamin A, vitamin D, thiamine, niacin, and vitamin B₆, and for selenium, fluoride, iodine, and copper.

What factors influence compliance?

Compliance with iron supplementation has been studied, but little is known about compliance with multiple supplementation among populations in developing countries. With iron, the side effects, colour, stability of the supplement, information provided to the consumer, and recommended frequency of use all are known to affect compliance [101]. Tablet size is another factor likely to affect compliance.

Although calcium is an important nutrient, especially for pregnant women and adolescents, the daily amount suggested in the RDAs requires a large tablet (more than a gram). Most multiple micronutrient supplements are smaller, weighing half that amount, and most prenatal supplements contain only about 200 mg of calcium. More calcium can be packed into a tablet similar in size to a prenatal tablet, but packing of calcium reduces its bioavailability. The amount of iron in the tablet also influences compliance. Higher iron levels are associated with more symptoms, including gastrointestinal problems and nausea [102, 103].

Social marketing efforts can increase compliance with supplementation, and the messages used will need to be context specific. Special packaging and messages may be used to focus on selected target populations; this will affect compliance as well.

Conclusions

The use of multiple vitamin and mineral supplements by women in developing countries is an important strategy to improve micronutrient status and benefit women's health, pregnancy outcome, and child health.

For all women of reproductive age who have micronutrient deficiencies, increasing micronutrient intake (iron, vitamin A, folic acid, and riboflavin) will reduce anaemia, with benefits to their health and work output. Increasing intake of these and other nutrients benefits the overall health status of women, improves immunity, and reduces the severity of such infections as malaria and such chronic diseases as coronary heart disease. Improving folic acid intake before pregnancy

will reduce birth defects, such as neural tube defects, which in many countries contribute significantly to infant mortality. Improving iron status before pregnancy helps prevent the devastating effects of severe anaemia in pregnancy.

During pregnancy, both the mother and the foetus are at risk for micronutrient deficiencies, so this period deserves special attention. Iron deficiency, especially severe anaemia, must be treated during pregnancy, and it is clear that increasing the intake of other nutrients during pregnancy provides additional health benefits. Increasing vitamin A intake has been found to reduce maternal mortality, so it should be an essential component of the Safe Motherhood Initiative in vitamin A-deficient areas.

Supplementation of postpartum women will benefit their own health and help ensure the presence of many nutrients in their breastmilk. But waiting until pregnancy and the postpartum period is often too late to benefit the child's micronutrient status. For example, although vitamin A status improved in both women and infants after supplementation in pregnancy, lactation, or both, significant percentages of women and breastfed infants were still deficient after supplementation for several months postpartum (in Bangladesh and Indonesia) or when supplemented before and throughout pregnancy and the postpartum period (in Nepal).

Therefore, programmes should consider the following options, in order of priority, to use multiple vitamin mineral supplements to improve micronutrient status among women who are at high risk for deficiency in one or more micronutrients:

» Ensure that pregnant women receive daily micro-

nutrient supplements for at least 180 days of pregnancy (during the second and third trimesters);

- » Ensure that breastfeeding women receive daily micronutrient supplements for the duration of breastfeeding;
- » Ensure that adolescents and women of reproductive age who register to marry or who are beginning sexual activity receive daily micronutrient supplements;
- » Ensure that adolescents and women of reproductive age receive daily micronutrient supplements, especially during seasons when micronutrient intake in diets is lowest.

Because health systems are often unable to reach pregnant women early in gestation or women who are not pregnant, other distribution mechanisms will be needed. The effectiveness and cost of the following approaches need to be determined, especially in comparison with current programmes promoting the use of iron and folic acid supplements during pregnancy and the provision of high doses of supplementary vitamin A to postpartum women:

- » social marketing of multiple micronutrient supplements for sale through the private sector;
- » distribution of supplements at work sites and schools;
- » distribution of supplements at marriage registration or religious counselling sessions for engaged couples;
- » sales of supplements by community-based distribution workers to women of reproductive age and to pregnant and lactating women;
- » distribution of supplements to women attending family-planning clinics, growth and development clinics for well children, integrated management of childhood illness programmes, and HIV screening programmes.

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Feeding practices and malnutrition in children in rural Bangladesh

Helga Piechulek, Jorge Mendoza Aldana, and Md. Nazmul Hasan

Editorial introduction

This article is a reminder once again that there are time-important components to infant feeding. Recently the first of these, exclusive breastfeeding for four to six months, has received the greatest emphasis, but as this article reminds us, timely and appropriate complementary feeding, once breastmilk is no longer sufficient as the sole source of food, is equally important. Inappropriate timing and type of weaning diet are responsible for a high prevalence of stunting and wasting. The authors' findings indicate that effective programmes for promoting adequate infant feeding are urgently needed in Bangladesh.

Abstract

Dietary data were collected on 496 households, and anthropometric measurements were carried out on 248 children under five years of age in rural Bangladesh. The effect of socio-economic determinants on infant feeding and malnutrition was analyzed using univariate and logistic regression models. Of the children under five, 52% were stunted and 57% were of low weight-for-age. Malnutrition was significantly influenced ($p < .05$) by income, size of cropland, the education of both parents, and some infant-feeding practices, but the mother's education emerged as the primary predictor of the nutritional status of children. Breastfeeding was the norm, and 95% of the mothers perceived breastmilk to be superior to milk substitutes, but rejection of colostrum, preference for prelacteal feeds, early supplementation, and inadequate timing and type of weaning diet were widespread. Often, there was a discrepancy between the expressed infant-feeding preferences and actual practices.

Helga Piechulek is the German Technical Corporation (GTZ) team leader of the Integrated Community Family Health Development Program (ICFHDP) in Bogra, Bangladesh. Jorge Mendoza Aldana is a general practitioner and GTZ consultant in Dhaka, Bangladesh. Md. Nazmul Hasan is a professor of nutrition in the Institute of Nutrition and Food Science at the University of Dhaka.

Introduction

Malnutrition is complex in its aetiology and cumulative in its manifestations. It not only impairs physical and intellectual performance, but also causes considerable ill health and contributes significantly to child morbidity and mortality. Bangladesh ranks prominently among the countries with the highest levels of malnutrition of pre-school children (67%) [1]. Child mortality is high, with 116 children per 1,000 births dying before they reach their fifth birthday [2]. The World Health Organization (WHO) has estimated that malnutrition and its associated diseases account for over 50% of these deaths [3].

The nutritional status of infants and children under five years of age is of particular concern, since the early months of life are crucial for future growth and development. It is estimated that 50% of all children born alive in Bangladesh have low birthweight (< 2,500 g) [4]. In the following months of life, the nutritional status of these infants deteriorates further because of suboptimal feeding practices and a relative decline in energy provision [5].

Food habits and, in a more specific context, infant-feeding practices are continuously affected by factors such as the availability of food, economic well-being, and changes in social values precipitated by external influences and education. This is particularly the case in countries where poverty is not the problem of a few, but of the majority of the population, as in Bangladesh.

Considering that the relevance and focus of planned nutrition interventions could be significantly improved by a better understanding of the underlying factors of malnutrition, the Integrated Community Family Health Development Program (ICFHDP), a project implemented by the Bangladesh Government with the technical assistance of the German Technical Corporation (GTZ), carried out this study focusing on infant-feeding practices among rural women and evaluating the reasons for inadequacies in the infant diet. In addition, data on the nutritional status of children under five were collected, and its potential determinants were identified.

Materials and methods

The study, in collaboration with the Institute of Nutrition and Food Science of Dhaka University, collected data from 496 households of the rural areas of Bogra District, about 120 km northwest of Dhaka, the national capital. Data were collected in May and June 1997, the period of the year when there is a secondary harvest.

A broad spectrum of data, including household socio-economic patterns, environmental conditions, and dietary behaviour of mothers and of children under five years of age, was gathered by personal interviews using standardized open-ended questionnaires. Anthropometric measurements were also carried out on 248 children under five. The weight and length of infants were measured according to standard procedures as recommended by WHO [6].

All field personnel undertook rigorous training in data collection, including the use of questionnaires and survey forms. Selected staff members were trained in anthropometry before the actual data collection. Standardization procedures for the collection of anthropometric measures were used, in line with WHO standards, in order to correct personal and interpersonal variation [6]. A pilot survey was conducted to standardize the technique further and minimize errors. Field managers validated each information sheet before its contents were transferred onto a computerized database file.

The anthropometric indices height-for-age, weight-for-height, and weight-for-age were compared to the reference population established by the US National Center for Health Statistics (NCHS). The subroutines from the Anthropometry Software Package ANTHRO, developed by the US Centers for Disease Control and WHO, were used to generate *Z* scores for these indices. Stunting, wasting, and low weight were defined as values less than -2 SD of the respective median of the reference population. Further statistical analysis was carried out using the statistical package STATA on personal computers. Relationships among all variables were examined by running univariate and multivariate analysis.

Results

Infant-feeding practices

Breastmilk was perceived to be superior to other milk types by almost all mothers (95%), and the median duration recorded for breastfeeding was 30 months. It was found that the number of children under five years of age in the household was negatively and strongly associated with the length of breastfeeding after adjustment for some socio-economic variables.

Data on infant-feeding practices in the first few days

after delivery are given in table 1. A clear disparity between perception and actual practice in breastfeeding among mothers was noted. Thus, although 52% of women thought it correct to start breastfeeding within half an hour of birth, only 29% practised it; 28% started breastfeeding within one day, and 24% initiated breastfeeding more than two days after delivery.

Although the importance of colostrum was recognized by 88% of mothers, only 18% of them gave it immediately after birth. It is important to note that the study found that 66% of all mothers discarded colostrum in their last delivery; most of them (80%) believed that colostrum would cause abdominal discomfort to their offspring. Discarding colostrum was considered when mothers reported that the "first" or "yellowish" milk was not given to the newborn. Only 3% of women who recognized the importance of colostrum knew about its disease-preventing qualities. Multivariate analysis showed that the mother's education was a strong positive predictor of whether she gave colostrum immediately after birth ($p < .01$), keeping variables such as gender preference, number of children under five years of age in the family, income, etc. at their means (table 2).

In Bangladesh non-lacteal products are traditional supplements to maternal milk at and shortly after birth. The product preferred by 71% of women in this study was honey, followed by sugar-water solution (14%). Hence, despite the high prevalence of breastfeeding in Bangladesh, 35% of infants received complementary food in their first month of life.

The study also found that only 16% of children were exclusively breastfed at four months of age. Children in households with more cropland were less likely to be exclusively breastfed for at least four months (odds ratio, 0.89; $p = .036$). A strong preference for cow's milk as an early complementary food was identified, and

TABLE 1. Selected data on initial infant-feeding practices

Initial feeding practice	Perception (%)	Practice (%)
Initiation of breastfeeding		
Immediately-0.5 h	52	29
0.5 h-1 d	20	28
1-2 d	6	16
Later than 2 d	22	24
Use of colostrum		
Immediately	62	18
1-3 d	18	12
Discard	12	66
Pre-lacteal feeds preferred		
Colostrum	5	2
Honey	80	71
Sugar water	10	14
Other	6	13

TABLE 2. Multivariate analysis of determinants of infant-feeding practices

Dependent variable	Use of colostrum		Duration of breastfeeding		Exclusive breastfeeding		Age at weaning	
	OR ^a	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Mother's education	1.19	.01	-0.45	.06	0.95	.05	-0.12	.08
Monthly income	0.99	.74	0.00	.92	0.99	.89	0.00	.09
Size of cropland	0.99	.42	0.00	.75	0.89	.036	-0.00	.53
Children under five years of age	1.12	.04	-2.60	.002	0.16	.08	0.19	.12
Father's education	0.98	.67	0.06	.76	0.98	.78	0.01	.81

a. Odds ratio.

an increasing number of solid foods such as rice or bananas were added as the infants grew older.

Weaning practices also highlighted marked incongruities between perception and practice among mothers. Thus, although 73% of mothers considered that the optimum age for initiation of weaning was between four and six months, only 44% of mothers initiated weaning within this age boundary. However, 21% of mothers started weaning their children before four months of age. Mothers who started weaning at more than six months had fewer years of schooling than those who started at the recommended time ($F = 3.5$, $p = .042$).

Weaning diets were poorly balanced, with 45% of mothers feeding infants rice, even though only 24% perceived this as the best alternative. Although a fair proportion of mothers (25%) perceived cow's milk as part of a good weaning diet, only 16% translated this into practice. The alternative protein sources *kichuri* (rice and lentils) and *phirni* (rice, sugar, and milk) were given by a very small minority of mothers (4% and 3%, respectively). The mother's education, household income, and the amount of household cropland were strongly associated with the use of cow's milk and *kichuri* as weaning foods.

Nutritional status of children under five years of age

Malnutrition was already present among children up to five months of age, with prevalence rates of 9%, 26%, and 14% for acute, chronic, and low weight, respectively, in this group. The nutritional status of infants deteriorated rapidly from the sixth month. Overall, 19% of children under five years of age were acutely malnourished, 52% were chronically malnourished, and 57% were of low weight. The distribution of Z scores for weight-for-age, weight-for-height, and height-for-age in relation to the reference population is illustrated in figure 1.

Except in cases of acute malnutrition, girls were more likely to be malnourished than boys, but the differences were not statistically significant. Thus, 19%, 56%, and 58% of girls and 20%, 49%, and 55% of boys were wasted, stunted, and of low weight, respectively.

Univariate analysis showed that children from households with a monthly income of less than 2,000 taka (44 taka = US\$1.00 in 1997) were twice as likely to have low weight ($p = .013$) and chronic malnutrition ($p = .009$) as those from households with monthly incomes of more than 2,000 taka. A similar relationship was found in children whose family homestead was smaller than 10 decimals (1 acre = 100 decimals). Low weight was positively associated with the number of children in the household and negatively associated with the use of colostrum ($p = .04$). Acute malnutrition was positively correlated with the frequency of infant meals ($p = .001$).

Maternal years of schooling proved to be the most powerful predictor of chronic malnutrition ($p = .01$) after adjustment for several economic indicators and confounders in a multivariate analysis. Thus, a mother with more schooling was less likely to have malnourished children when covariates such as age, income, years of schooling of the head of the family, gender, size of cropland, etc., were kept at their means. Figure 2 shows the predicted frequencies of chronic malnutrition according to the education of the mother and of the head of the family. The mother's education pre-

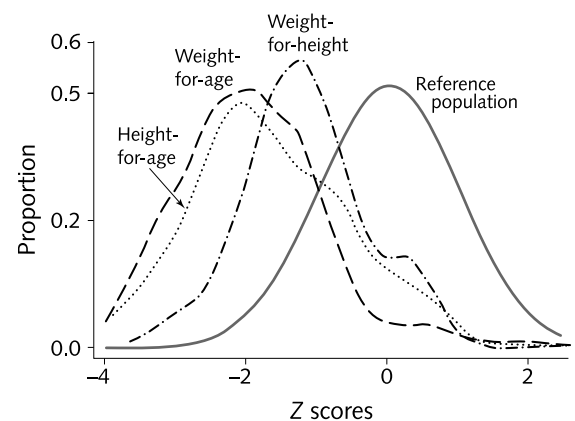


FIG. 1. Density distribution of Z scores for weight-for-age, weight-for-height, and height-for-age in relation to the reference population

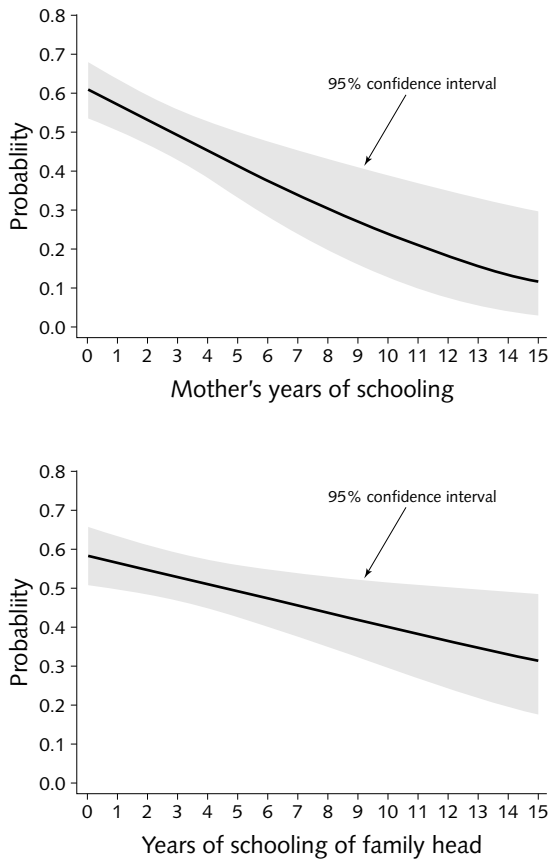


FIG. 2. Predicted values of chronic malnutrition according to years of schooling of the mother and of the head of family

dicted chronic malnutrition and also low weight better than the education of the head of the family. Although monthly per capita income and years of schooling of the head of the family had a clear negative association with malnutrition, this association did not reach statistical significance in a multivariate analysis.

Discussion

In Bangladesh the prevalent cultural norms and the lack of income-generating opportunities in rural areas confine women to the home compound, without access to education. They are, therefore, the primary caretakers of children. The results of this study have given an overall picture of poor maternal infant-feeding practices, which reinforce the negative effects of limited access to food and are influenced by socio-economic determinants, particularly years of maternal schooling.

Maternal education has proved important in a number of assessments related to child survival, feeding practices, and child malnutrition [7–12]. Our results showed that, independently of family wealth, mater-

nal education has an important effect on the use of colostrum and on advocating better feeding practices. Thus, one opportunity to improve child-feeding behaviour lies in maternal education.

Fortunately, breastfeeding is still an integral part of the culture in Bangladesh, and the results of this study confirmed the traditional preference for breastmilk despite the increased availability of manufactured milk substitutes, which are not affordable by the majority of people. This is consistent with the results of other studies [2, 4] that have reported a high prevalence of breastfeeding in rural Bangladesh. Nonetheless, a linear correlation between the duration of breastfeeding and nutritional status has been questioned by previous research [13]. There are aspects of breastfeeding that could certainly be improved, despite its high prevalence.

If breastfeeding is of paramount importance for the health of newborns and infants, the correct timing of the initiation of breastfeeding in order to gain the benefits of colostrum is essential for an improved quality of life. Ideally, the newborn should be put to the mother's breast no later than 30 minutes after delivery to make best use of the benefits of colostrum. However, it seems that most Bangladeshi newborns do not enjoy such benefits, according to our results as well as other assessments [2, 14, 15]. This disagrees with the findings of Rizvi [16], who stated that mothers discard just some drops of the "yellowish" or "watery" milk they consider as "harmful" for their offspring. Our results indicated a general avoidance of colostrum, fearing digestive distress, by 80% of mothers, with a corresponding delay in the initiation of breastfeeding.

Early supplementation of infants' diet is widespread in Bangladesh, although the Government promotes the WHO recommendation [17] that babies should be exclusively breastfed up to the age of four to six months. The early introduction of complementary food is undesirable, because it is associated with an increased risk of diarrhoea. Moreover, complementary food is of poorer quality than breastmilk and also interferes with lactation.

The WHO recommendation also implies that the adequacy of breastmilk for meeting the nutritional needs of the child is limited to the early months of life. Exclusive breastfeeding of infants more than six months of age by poorly nourished mothers is nutritionally inadequate and leads to a worsening nutritional status and growth faltering before or soon after six months [18]. In our study, the rates of malnutrition dramatically increased between 6 and 18 months of age. We also found that a significant proportion of mothers introduced solid foods as late as nine months after birth. Tradition often encourages a mother to wait until a child starts walking, asks or reaches out for food, or has teeth to chew [19, 20].

The inappropriateness of the feeding practices in infants is aggravated further by the types of food

introduced. Food items rich in carbohydrates were not only introduced earlier than protein-rich foods, vegetables, and fruits, but tended to dominate the weaning diet, mirroring the poorly balanced diet of the adult, as found in a study in rural Bangladesh [21]. Cow's milk, if given, is frequently diluted to a large extent [22].

Hence, in this context of defective feeding practices, exacerbated by negative economic and cultural settings, malnutrition is an unavoidable outcome. The extent of malnutrition, particularly of acute malnutrition, noted in this survey exceeds the national levels set by the Bangladesh Demographic and Health Survey (BDHS) 96/97 [2] and is extremely high by international standards. Significantly lower incidences of underweight children have been reported in sub-Saharan Africa: 31% compared with 56% found in this study [23].

The relationship between the socio-economic status of the family and malnutrition has also been reported in other developing countries [24, 25]. The socio-economic status of a household is important because it determines, in most cases, the availability and quality of food eaten by the family. Economic status is also closely linked to household environmental conditions, e.g., the availability of safe water, sanitation, and electricity, which in turn have been associated with the prevalence of various types of childhood disease [26]. The monthly income and the amount of land available to a family were among the most significant predictors of nutritional status in Nepal [27].

In our study, the monthly family income was positively associated with nutritional status, and the importance of the socio-economic status of a population in determining malnutrition was evident. Amtoli, the village with the highest per capita income, the highest rate of landholding, and the highest literacy rate, had the lowest prevalence of child malnutrition. Poverty, indeed, limits the ability to nourish children better on a daily basis.

The findings of this study have highlighted aspects to be considered for any nutrition programme to be

implemented in Bangladesh. Increasing the educational status of women quantitatively and qualitatively should be a cardinal point. This refers not only to the access of women to formal or informal education, but also to the delivery of suitable and appropriate nutrition messages to them. After four to six months of exclusive breastfeeding, appropriate complementary feeding is extremely important.

Emphasis placed on the mother's awareness and concern about malnutrition may lead them erroneously to introduce complementary food very early as a safeguard. Conversely, messages strongly emphasizing breastfeeding may lead mothers to believe that as long as there is some breastmilk, the child will not need any other food. Qualitative research is needed to answer these questions. In addition, a thorough and continuous analysis should be conducted of how the WHO recommendations are currently being implemented. We found that a significant proportion of mothers did not even know that their children were malnourished (fig. 3). Proper education of mothers, in addition to improving their child-feeding practices, will enable them to recognize signs of malnutrition in their children.

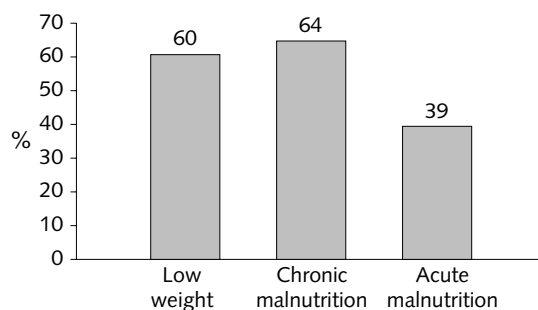


FIG. 3. Percentage of mothers who perceived their malnourished children as not malnourished according to the type of malnutrition

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Is one nutritional benchmark appropriate? Dilemma in nutritional assessment and growth monitoring of children under five years of age in Fiji

Shoko Saito and Geoffrey C. Marks

Abstract

The same criteria for assessing nutritional status of children, including internationally recommended anthropometric reference data and standard cut-off values, are commonly applied across different ethnic groups and to meet several programme objectives. The situation in Fiji is used to illustrate some issues that arise from this. Fiji has two major ethnic groups with very different physiques, Fijians and Indians. When using the same weight-for-age criteria to assess children of both ethnic groups, the results always show a much higher prevalence of underweight among Indian than among Fijian children. Yet, it is Fijian children who have a higher infant mortality rate due to infections and diarrhoeal diseases and have a higher hospital admission rate due to malnutrition. Work pressures in clinics militate against using different criteria for growth monitoring and meeting clinic reporting requirements. The implications are discussed. The "best" criteria here will differ across programme objectives but must also take account of the work setting.

Introduction

Anthropometric indicators are used throughout the world as the basis for assessing growth and nutritional status in children. Calculation of the most common indicators (weight-for-age, height-for-age, and weight-for-height) involves comparison with a reference distribution and describing how far the individual deviates from the reference median. Deviation beyond a particular cut-off value is often used in practice to screen children for an intervention or to describe the proportion of children in a population considered to be mal-

nourished. Although applying a standard approach across population groups simplifies the procedures and makes comparisons of different population groups possible, it can also present problems in practice, depending on the situation. In this paper we discuss such a case, taking Fiji as an example.

Fiji is located in the western Pacific. The population is approximately 750,000, consisting of 49% ethnic Fijians (hereafter referred to as Fijians), 46% Fiji-Indians (hereafter referred to as Indians), and 5% "others," who are made up of small proportions of part-Europeans, Chinese, and other Pacific islanders [1]. Fijians are of Melanesian origin with Polynesian influence. The large proportion of Indians today is derived from indentured labourers brought from India to work in sugar cane plantations under the British colonial administration around the turn of the century. The two major ethnic groups, Fijian and Indian, maintain their cultural identity, including dietary patterns, while living side by side. In general, Fijians are taller and more heavily built than Indians.

Fiji is better-off than other Pacific Island countries in terms of economy, development, and health status. The infant mortality rate has declined from 42 per 1,000 live births in 1974 to 17 in 1990 [2]. However, there are marked differences among ethnic groups in age-specific mortality rates (table 1). The annual neonatal mortality rate among Indians is usually high and is attributed to a high incidence of low birthweight among this group, whereas Fijian children have a higher post-neonatal mortality rate, mainly due to infections, resulting in a higher overall infant mortality rate. Protein-energy malnutrition remains one of the causes of paediatric morbidity in hospitals, and over 90% of the patients are Fijian children [3].

Growth monitoring in Fiji, in which the children's nutritional status is assessed by weight-for-age, has been evolving since the 1960s. With an excellent coverage of public health services throughout the country, growth monitoring today is a universal practice carried out concomitantly with immunization. Immunization is offered according to government guidelines, and the

The authors are affiliated with the Nutrition Programme in the Australian Centre for International and Tropical Health and Nutrition at the University of Queensland, Australia.

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TABLE 1. Mortality per 1,000 live births according to age and ethnic group, 1986–1990^a

Year	Neonatal mortality			Post-neonatal mortality			Infant mortality		
	Fijians	Indians	Others	Fijians	Indians	Others	Fijians	Indians	Others
1986	7.7	13.7	11.9	11.4	7.3	3.0	19.1	21.0	14.9
1987	7.2	10.9	10.9	9.0	3.9	4.0	16.2	14.8	14.9
1988	7.9	13.0	3.5	13.9	4.5	9.2	21.8	17.5	12.7
1989	8.3	12.2	8.7	9.0	5.4	3.3	17.3	17.6	12.0
1990	9.7	9.5	6.2	10.8	2.6	6.2	20.5	12.1	12.4

Source: ref. 2.

a. At the time of writing this article, no infant mortality rates according to ethnic group have been available since 1990.

coverage for growth monitoring is high, as indicated by 91% coverage for measles immunization, which is scheduled during the ninth month of the first year of life [4]. As in many countries, once a child's weight is plotted on a growth chart, the nutritional status is assessed, the mother and child are counselled or treated accordingly, and the data are recorded into various books, forming the basis of national statistics. Fiji uses the Centers for Disease Control/World Health Organization (CDC/WHO) weight-for-age reference distributions for the growth charts. The cut-off point for defining underweight was 80% of the CDC/WHO median until 1993, when the cut-off point was raised to 90% of the median. The same set of cut-off points has always been used for all ethnic groups.

In the field, health workers often express their concern that many apparently healthy Indian children are classified as underweight and thus at risk, whereas Fijian children generally have more health problems. Past surveys have consistently indicated a much higher proportion of underweight children among Indian children, for example, 24% compared with 8% of Fijian children in a national survey in 1981, using 80% of the Harvard standards as the cut-off point for underweight [5]. Yet, as mentioned earlier, it is Fijian children who manifest more health problems, resulting in a higher infant mortality. Using the same anthropometric criteria for the two groups presents an operational dilemma.

This paper illustrates the situation by using different cut-offs to compare mean weight-for-age, general growth patterns, and proportions of children at risk among Fijian and Indian children according to the results of the 1993 National Nutrition Survey in Fiji [6]. The appropriateness of using the same criteria for assessing the nutritional status of the two ethnic groups is discussed in terms of the patterns of stunting of the two groups and in relation to the way that anthropometry is used in Fiji. Thus, only weight-for-age is presented in this paper, although, since it is a composite indicator, it has limitations and may not be as sensitive as weight-for-height or height-for-age in predicting survival [7, 8].

Materials and methods

Study population

The study population, Fijian and Indian children under five years of age, is a subsample of the 1993 National Nutrition Survey in Fiji, which was a nationally representative survey of all age and ethnic groups. A two-stage cluster sampling scheme was used, with 60 enumeration areas and a total sample size of 4,620 required to give a precision of $\pm 5\%$ with a 95% confidence interval for the estimated prevalence of underweight among children under five years of age, with a 10% contingency for non-response. In the first stage, the sample enumeration areas were systematically selected from 1,405 enumeration areas in Fiji, with the probability of selection proportional to the population size (using 1986 census data). The second stage used households as sampling units. The listing of all households was carried out one month before the survey by public health nurses responsible for the enumeration area. On the basis of the desired total sample size and an estimated average household size from the latest census in 1986, 15 households were systematically selected from each enumeration area, with the first one being selected by a random number. All members of selected households were included in the survey. Because the household size was larger than expected, a total of 4,830 people were approached and 4,606 participated. The non-response rate was 4.6%, with 224 non-responders, who included 328 Fijian, 254 Indian, and 37 "other" children under five years of age. Ethnicity was based on the parents' report. The survey was conducted between May and November 1993. Although small, the sample was representative of the country in ethnic, geographic, urban versus rural, age, and sex distribution in comparison to the 1986 census data and 1993 population projection by the Bureau of Statistics in Fiji. For the purpose of this paper, data on "other" children are excluded because of the very small sample size.

Anthropometric data

For this analysis, birthweights, current weights, and age are used. In Fiji almost all deliveries take place at health facilities, where a nurse or midwife measures and records the birthweight on the child's Maternal and Child Health card and in the clinic record books. The birthweights of children under two years of age in the sample were obtained from clinic records or the Maternal and Child Health card. The record of birthweight was frequently missing for older children, particularly Fijians. The birthweight was available for 94% of the children under two years of age: 120 Fijian children (88%) and 99 Indian children (100%).

To obtain current weights, children under the age of five were weighed wearing light clothes on a correctly calibrated digital scale (Tanita Model 1567). Children who were unable to stand by themselves were weighed while being held in their mothers' arms, and then the mother's weight was subtracted to give the child's weight. The procedure was performed twice by two trained enumerators. When their records showed a difference of less than 0.2 kg, the average of the two values was recorded as the child's weight.

Age in months was calculated from the date of birth to the date of the survey. All but one mother were able to report the date of birth of their children. Reported dates were supported by written documents for 83% of Fijian and 95% of Indian children (Maternal and Child Health card, birth certificate, or baptism record). There was 99% agreement between the reported date of birth and the date in the documents among those with supporting documents. One child whose date of birth was unknown was excluded from the analysis. Thus, the final sample for analysis consisted of 327 Fijian children and 254 Indian children under five years of age.

Anthropometric indicators were calculated by the ANTHRO Programme, which is based on CDC/WHO reference values. Other statistical calculations were carried out with SPSS.

The anthropometric indicators are expressed as percentages of the CDC/WHO reference median, since this value has been used in Fiji for growth monitoring and in past studies. The equivalent Z scores are given in parentheses.

Results

Table 2 reports birthweights of children under two years of age, and age- and sex-specific weights and weights-for-age of children under five years of age from the National Nutrition Survey. There are no obvious trends in the distribution of children by age and sex, supporting the belief that the sample was representative.

Birthweights

The mean birthweights were $3,440 \pm 580$ g for Fijian boys, $3,400 \pm 559$ g for Fijian girls, $2,930 \pm 458$ g for Indian boys, and $2,770 \pm 530$ g for Indian girls. These values are 105.3% of the CDC/WHO reference medians (0.28 Z) for Fijian boys, 105.3% (0.58 Z) for Fijian girls, 89.7% (-0.84 Z) for Indian boys, and 85.9% (-0.89 Z) for Indian girls. The difference between ethnic groups was statistically significant ($p < .001$), while there was no difference between the sexes.

Weight-for-age distribution and nutritional status

Figure 1 shows the distribution of weight-for-age for Fijian and Indian children under five years of age based on data from the National Nutrition Survey, excluding birthweights. The mean weight-for-age of Fijian boys under five was 99.1% of the CDC/WHO median (-0.14 Z). For Fijian girls it was 99.0% (-0.17 Z). The corresponding figures were 88.1% (-1.10 Z) for Indian boys and 90% (-0.95 Z) for Indian girls. Although there was no statistically significant difference by sex within each ethnic group, there was a statistically significant difference in the mean weight-for-age between Fijians and Indians (sexes combined; $p < .001$).

This difference is also reflected in the proportion below a particular cut-off point. Figure 2 shows the proportions of children at risk, defined as below 80% and 90% of the CDC/WHO median values. There was a significant difference between Fijians and Indians in the proportion of children identified as being at risk ($p < .001$), whichever cut-off point is applied, with a much greater proportion of children at risk among Indians.

Growth patterns

Figure 3 shows the cross-sectional growth pattern of Fijian and Indian children under five years of age by

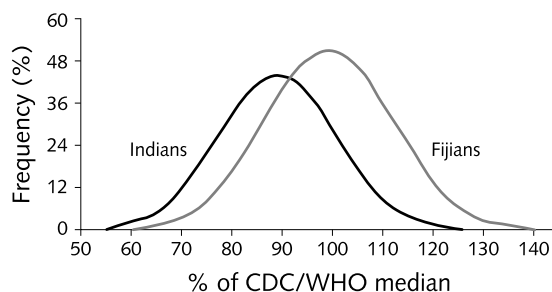


FIG. 1. Frequency distribution of weight-for-age (as percentage of CDC/WHO median) of Fijian and Indian children under five years of age. Source: ref. 6

TABLE 2. Weight and weight-for-age according to ethnic group, sex, and age

Sex and age (mo) ^a	n	Weight (kg)		Weight-for-age (% of CDC/WHO median)			
		Mean	95% CI	Mean	95% CI	Z score	95% CI
Fijian							
Male							
At birth	73	3.4	3.3 to 3.6	105.3	101.1 to 109.0	0.28	-0.02 to 0.57
0-11	43	7.3	6.8 to 7.9	103.2	98.7 to 107.7	0.18	-0.15 to 0.52
12-23	40	10.6	10.3 to 11.4	97.4	92.7 to 102.1	-0.27	-0.72 to 0.17
24-35	33	13.4	12.9 to 13.9	99.6	96.3 to 102.8	-0.12	-0.42 to 0.18
36-47	23	14.7	13.6 to 15.7	93.8	87.4 to 100.2	-0.58	-1.14 to 0.10
48-59	31	17.4	16.6 to 18.2	99.0	94.7 to 103.2	-0.13	-0.49 to 0.23
Female							
At birth	45	3.4	3.2 to 3.6	105.3	100.0 to 107.0	0.58	0.16 to 0.99
0-11	25	7.3	6.2 to 8.0	104.1	98.1 to 110.1	0.26	-0.20 to 0.72
12-23	28	10.4	10.0 to 10.8	97.7	93.7 to 101.8	-0.21	-0.59 to 0.16
24-35	36	12.2	11.6 to 12.7	95.4	91.1 to 99.7	-0.50	-0.90 to 0.10
36-47	36	15.0	14.6 to 15.5	100.1	97.2 to 103.0	-0.08	-0.32 to 0.16
48-59	32	16.5	15.7 to 17.2	98.8	94.2 to 103.4	-0.22	-0.69 to 0.15
Indian							
Male							
At birth	48	2.9	2.8 to 3.0	89.7	85.8 to 93.6	-0.84	-1.15 to -0.54
0-11	30	6.7	6.0 to 7.3	93.0	88.1 to 98.0	-0.58	-1.00 to -0.20
12-23	18	9.7	8.9 to 10.4	84.3	83.2 to 94.0	-1.54	-2.10 to -0.97
24-35	31	11.6	11.2 to 12.0	86.2	83.0 to 89.4	-1.35	-1.66 to -1.05
36-47	25	14.0	13.1 to 14.8	90.0	85.2 to 94.7	-0.91	-1.33 to -0.49
48-59	28	15.2	14.6 to 15.7	85.6	82.5 to 88.7	-1.27	-1.53 to -1.00
Female							
At birth	48	2.8	2.6 to 2.9	85.9	81.2 to 90.7	-0.89	-1.72 to -0.57
0-11	24	6.4	5.5 to 7.2	90.0	83.1 to 96.9	-0.80	-1.32 to -0.28
12-23	24	9.5	8.8 to 10.1	88.6	83.2 to 94.0	-1.05	-3.12 to -0.55
24-35	33	11.9	11.4 to 12.4	92.5	89.4 to 95.5	-0.76	-1.04 to -0.48
36-47	19	13.0	12.2 to 13.7	86.0	81.8 to 90.1	-1.33	-1.73 to -0.94
48-59	22	15.1	14.3 to 16.0	90.3	85.1 to 95.5	-0.94	-1.04 to -0.50

a. Birthweights were calculated for children under two years of age for whom records were available.

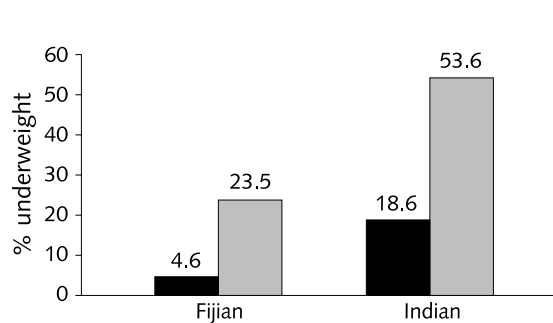


FIG. 2. Percentage of underweight Fijian and Indian children under five years of age, based on cut-off points of 80% (black bars) and 90% (gray bars) of CDC/WHO median

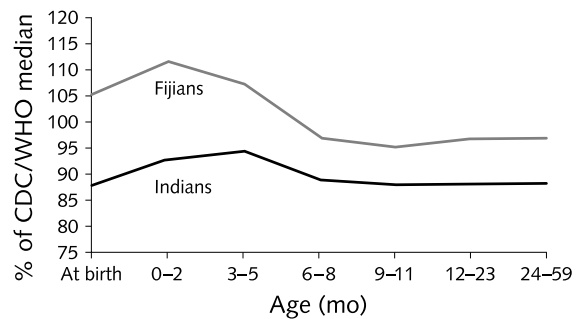


FIG. 3. Weight-for-age (as percentage of CDC/WHO median) of Fijian and Indian children from birth to five years of age. Source: ref. 6

plotting mean weight-for-age by three-month intervals from birth against the CDC/WHO median of 100%. Because the number in each group was small and the mean weight-for-age by sex within each ethnic group did not show a significant difference, the data from boys and girls were combined. The ethnic groups also have different feeding patterns, with Fijian children having a much higher rate of breastfeeding between 6 and 12 months of age than Indian children (50% vs. 19%).

As well as differing significantly in mean birthweight, Fijians and Indians differed significantly in the proportion of full-term infants with low birthweight (< 2,500 g): 4.2% among Fijians and 21.2% among Indians ($p < .001$).

Fijian children generally grew rapidly, reaching a mean weight-for-age of 111.7% (0.78 Z) of the median in the first three months. However, the weight-for-age declined with increasing age, and between six and eight months it dropped below the CDC/WHO average to 97.3% (-0.25 Z), with only minor changes thereafter. The decline of weight-for-age between the first three months and the ninth to the eleventh month was 13.4% (1.18 Z). Indian children also showed an increase of mean weight-for-age in the first six months of life, but to a lesser extent, with a subsequent decline. However, the decline from the highest in the group 3 to 5 months of age, at 94.9% of the median (-0.37 Z), to the lowest in the group 12 to 23 months of age, at 88.7% (-1.04 Z), was much smaller (8.1% or 0.79 Z) than that of their Fijian counterparts. Thereafter, they maintained a mean weight-for-age similar to that at birth until they reached five years of age.

Discussion

Indian children are at much greater risk of low birthweight than Fijian children (21.2% versus 4.2%). Indian children are significantly lighter and have a lower mean weight-for-age at all ages up to five years. Whichever cut-off point is used, the prevalence of underweight among Indian children is two to four times higher than that among Fijian children. The conventional interpretation of these findings would be that Indian children are at greater risk than Fijian children for mortality and morbidity [9, 10]. Is this so?

Individual growth charts show that many Indian children are born small but they grow in a pattern similar to that expected according to the CDC/WHO median growth curve. In contrast, Fijian children are born large, and their growth deviates downward from the expected growth, although their weight-for-age remains well within the "normal" range. Whitehead [11] and the WHO working group on infant growth [12] suggest that this may be a normal growth pattern when children are breastfed. However, a 1990 study of clinically malnourished children from the Colonial War

Memorial Hospital in Suva, the capital of Fiji [13], found that many Fijian children had a similar growth pattern before their admission and were still above the third percentile line of the growth chart at the time of admission.

There are also important differences in mortality rates between these two ethnic groups. In contrast to the usual interpretation of indicators of nutritional status, it is the Fijian children whose post-neonatal mortality rates have remained highest over the years (table 1). As the neonatal mortality rates have declined among Indians, possibly due to better management of low-birthweight babies, the gap between these two ethnic groups in post-neonatal and infant mortality rates has widened. In 1990, the last year for which data were available at the time of writing, the post-neonatal mortality rate of Fijian children was four times higher than that of Indian children [4].

This paradox highlights the dilemma that growth monitoring and nutritional assessment face in Fiji. The current cut-off to the growth monitoring of Fijian children is not serving the purpose of early detection of those who are at risk for morbidity and mortality associated with growth faltering. Adjusting the cut-off to be appropriate for Fijian children, however, would result in failure to detect and act on the stunting of the Indian children. The operational issues and implications are considered below.

Dual objectives of growth monitoring in practice

Morley [14] defines the objective of growth monitoring as "to prevent growth retardation through timely and early detection of faltering growth." Thus, the primary purpose of growth monitoring is the longitudinal assessment of nutritional adequacy in an individual child. This will be achieved by assessing growth, as reflected by the rate of change between two weight observations. Thus, growth faltering becomes an important sign to watch. However, growth-monitoring programmes are often organized to serve two important purposes of a different nature at once: to assess individual children's growth as described and to collect cross-sectional data on nutritional status for health-planning purposes at the national level. The latter requires the assessment of the nutritional status of children based on predetermined cut-off points and recording the results on various report forms. In Fiji nurses make four entries per child in different books and cards and are required to collect and send monthly figures of nutritional status to their headquarters. This creates an environment in which cross-sectional nutritional assessments are given paramount importance, and therefore may lead to a misconception of the purpose of growth monitoring. This is not a problem specific to Fiji [15-17]. In this connection, anthropometric cut-off points are applied to growth monitoring. Thus it becomes the predictive

power of the cut-off points rather than the growth pattern of the child that determines the efficiency of the programme for early detection of growth faltering and prevention of clinical malnutrition.

Universal or population group-based cut-off points?

Cut-off points are generally set to distinguish “abnormal” from “normal” in a population. Issues related to cut-off points are: On what basis are they selected? Should they be fixed or not? Rose and Barker [18] summarize that “abnormal,” or “a case,” can be determined using statistical, clinical, prognostic, or operational approaches. Each has a different interpretation, and the approach used should depend on the objectives of the programme or activity where it is applied [18, 19]. For example, a statistical approach often sets cut-offs for normality as being within ± 2 standard deviations of the mean of the reference distribution. This can provide a good basis for normative assessment and comparison of groups for equity considerations. However, “What is common is not necessarily good” [18], and statistical abnormality does not necessarily relate to morbidity, mortality, or other functional outcomes. With a clinical approach to setting cut-offs, “symptomless” does not mean that an individual is free of a latent condition, and so this is not a particularly strong basis. Ideally, for the purposes of early prevention of morbidity and mortality in clinics in Fiji, cut-offs for nutritional assessment would be based on some understanding of the prognosis at different levels of the indicator—either in terms of risk of morbidity or mortality (so those at highest risk could be selected) or in terms of likely benefit from treatment. Unfortunately, setting cut-offs on this basis generally requires a costly prospective study [20], and for anthropometry the results seem to have limited generalizability [21]. In an operational approach, the aim is to decide the cut-off where “Action is better than inaction” [18], taking account of the resources available and the extent of the problem.

An operational approach also requires recognizing the trade-offs. When a cut-off point distant from the reference median is used, it reduces false-positive diagnoses (those that are not really at risk or likely to benefit from treatment) and has a high specificity. The total number of children identified as being at risk is reduced, but children who are in fact at risk will be missed. On the other hand, a cut-off point closer to the reference median will reduce false negatives (those who are at risk but are not identified as such), but this also means likely inclusion of children who are not really at risk. In this regard, the availability of resources has been advocated as a basis for setting the cut-off, setting a level that identifies the most needy children [22, 23].

Another critical issue is whether there should be one set of cut-off points across population groups. Some authors argue for fixed cut-off points on the basis that

if a cut-off point exists that indicates an associated functional risk, then a child identified as being at risk according to the cut-off point requires an intervention [24, 25]. The use of universal cut-off points has the obvious advantage of allowing comparison of results from different populations for priority setting. However, as the case of Fiji shows, the use of universal cut-offs may also create a problematic situation. The widely used cut-off point of 80% of the median identifies more Indian children at risk and possibly misses many Fijian children at risk. A cut-off of 90% will identify more Fijian children as being at risk than the 80% cut-off point, thereby increasing their chance of receiving a timely intervention at a much earlier stage. In fact, the 90% cut-off point is based on Gomez’s classification [26], which is criticized for its over sensitivity owing to the fact that it was derived from hospitalized children [24]. However, given the higher mortality rate and the growth pattern among Fijian children, the use of a higher cut-off point seems reasonable. On the other hand, this introduces another problem. Half of the Indian children are identified as being at risk. Since it is the health-care provider’s responsibility to act if the child is found to be at risk, this will greatly stretch resources. Another concern is that it might cause unnecessary anxiety for the mothers of at-risk children, which is often overlooked as an important issue.

In the past, the use of universal reference data has been debated at great length. Jelliffe and Jelliffe once questioned the appropriateness of the use of the same reference data for Fijian and Indian children [19]. WHO currently recommends the universal use of CDC/WHO reference data, while recognizing their limitations [27]. This is based on accumulated data indicating that the effect of ethnic differences on growth is smaller than that of socio-economic environment [27, 28].

Although this has settled the debate in a wider context, Fiji still faces a dilemma from an operational point of view, because the two major ethnic groups present an unconventional correlation between anthropometric and health indices. It appears from the results that the two ethnic groups have different patterns of stunting. The Indian children on average have lower birthweights than the Fijians and systematically track at a lower level, below the reference median, but following the same pattern as the CDC/WHO reference distributions. Although it cannot be tested here, these observations are consistent with the notion that size at critical ages (e.g., birth) determines the possible trajectory for subsequent growth [29–33]. Further, it appears that the risk of morbidity and mortality is more closely related to deviation from this group-specific pattern of growth than it is to deviation from the CDC/WHO reference patterns.

Thus the issue needs to be viewed in terms of the main objectives of the assessment. Habicht and Pelletier point out that the “best indicator” depends on the context:

how it is to be used [34]. In the case of Fiji, we have the same anthropometric data being used to achieve two quite different purposes. The clinics are screening children primarily to identify those at risk, those who will benefit from additional treatment, or both. Based on the foregoing, there is a strong case for clinics to use different cut-off points for Fijians and Indians. In contrast, the data reported to and collated by the Ministry of Health at the national level are used more to monitor changes in population groups over time and to compare these groups. Although the latter has an element of trying to reflect the levels of "malnutrition" in these populations, in practice the primary considerations are generally resource allocation and thus equity. This would be difficult to achieve if different cut-off points were used for the two ethnic groups, because there would be no common basis for comparison.

Ideally, the "best" cut-off points for use in the clinics would be determined by investigating the relationship between nutritional status and morbidity and mortality for both ethnic groups. Considering the observed levels of risk for the two population groups, the outcome is likely to be close to 80% for Indians and 90% for Fijians. These cut-offs identify a similar proportion of children as underweight in both ethnic groups and are consistent with the difference in mean weight-for-age in children under five years of age, which is approximately 10% of the CDC/WHO median. Until it is possible to conduct such a study, these separate cut-off points could be used in the clinics. For comparability of data at the national level, a practical solution would be to aggregate weight-for-age data in the categories < 80%, 80% to 90%, and > 90%. This uses the same cut-offs as for clinics but also provides a basis for comparison across groups and across time.

Conclusions

Debate about the use of anthropometric indicators for

assessing children's nutritional status in different countries or ethnic groups has generally revolved around the appropriateness of growth reference data. In recent years, there has been widespread acceptance of the advantages and appropriateness of adopting a single set of reference data and cut-off values across countries. A result of this has been the tendency to apply the same nutritional benchmarks across ethnic groups and for every purpose. Fiji is an example of a situation where two major ethnic groups exist with significant differences in cross-sectional growth patterns, and where the level of risk for morbidity and mortality at a given level of weight-for-age appears to differ across the ethnic groups. The situation in Fiji also illustrates the outcome of work pressures in clinics that often militate against applying assessment based on the principles of growth monitoring for discussions with mothers and screening for treatment, while using different criteria to meet the reporting requirements to headquarters—a common situation. Recent WHO recommendations stress the importance of considering the purpose of carrying out nutritional assessment when choosing nutritional indicators, including consideration of reference values and cut-off values [27]. The example of Fiji shows that this may include recognition of ethnic group differences for some assessment objectives. The example also illustrates the need to consider the practical aspects of the work setting and target group when defining nutritional assessment criteria.

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Clinical screening may be a cost-effective way to screen for severe anaemia

Erin Dusch, Rae Galloway, Endang Achadi, Idrus Jus'at, Chakunja Sibale, Ciro Franco, Simon Cousens, and Linda Morison

Abstract

Clinical screening for pallor is one of the most common methods of screening for anaemia. Health workers examine the face, inner lower eyelids or conjunctiva, palms, nail beds, and other body parts for paleness or pallor that may be a sign of anaemia. MotherCare found 17 studies that evaluated the sensitivity and specificity of using pallor to identify individuals with anaemia. The focus was to review the sensitivity of pallor screening to detect individuals with severe anaemia and to make recommendations to improve the sensitivity of screening for pallor. The studies confirmed that sensitivities increased as the haemoglobin level decreased.

Introduction

Iron deficiency and iron-deficiency anaemia, as measured by low haemoglobin, are the most prevalent nutritional deficiencies in the world. Anaemia is the most serious manifestation of iron deficiency, and it is estimated that for every person with anaemia, there is at least one other with iron deficiency [1]. Women of reproductive age and small children are at greatest risk, with an estimated 50% to 60% of pregnant women and 40% of small children suffering from anaemia [2–4]. Anaemia during pregnancy is a major health concern because it has been associated with increased risks of maternal morbidity [5], maternal mortality [6–9], and

poor birth outcomes, including stillbirth, prematurity, low birthweight, and perinatal and neonatal mortality [1–2, 6, 10–12]. It is estimated that favourable pregnancy outcomes are compromised by 30% to 45% when women have anaemia [3, 13, 14].

Because the consequences of anaemia increase as haemoglobin falls, the prevalence of severe anaemia (haemoglobin < 7 g/dl) should be of concern and warrants special actions. Although severe anaemia makes up a relatively small proportion of overall anaemia in most countries, a public health problem exists when even 2% of pregnant women have severe anaemia because of the poor birth and delivery outcomes associated with very low haemoglobin [15].

Where the cost of screening is affordable, it is possible to screen for iron deficiency several times during pregnancy and instruct women to take iron pills as needed. In these situations, other causes of anaemia (e.g., vitamin B₁₂ and folic acid deficiencies) can be assessed and treated appropriately. Given the life-threatening consequences of severe anaemia, it is also desirable to identify women with severe anaemia so they can be given more iron and treated for other causes of anaemia, such as parasitic infections. Women with severe anaemia should receive follow-up and be referred to a higher-level health facility if their haemoglobin values do not reach a safe range before labour and delivery. A dilemma exists in developing countries, because to identify severe anaemia, blood samples would need to be collected for every woman attending prenatal care sessions. In these countries, where screening supplies are costly and not consistently available and where taking a fingerprick sample of blood may be unacceptable, other methods are needed to identify women with severe anaemia.

One low-cost method for identifying cases of anaemia is by clinical examination for pallor, although this method is generally recognized as having low sensitivity* for all anaemia (haemoglobin < 11 g/dl). Although the

Erin Dusch was formerly affiliated with the MotherCare Project/John Snow, in Arlington, Virginia, USA, and is currently a program manager for Asia with Helen Keller International in New York. Rae Galloway is with the BASICS Project/John Snow. Endang Achadi and Idrus Jus'at are with the MotherCare Project in Indonesia. At the time of the research, Chakunja Sibale and Ciro Franco were with Project HOPE/Malawi. Simon Cousens and Linda Morison are with the London School of Hygiene and Tropical Medicine.

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

* Sensitivity is the ability to correctly identify those who have the disease and is calculated by dividing the number of true

TABLE 1. Studies of pallor in moderate to severe anaemia (haemoglobin < 8 g/dl)

Study	General description: <i>n</i> , country, examiners	Gold standard	Hb cut-off and sensitivity and specificity of examination	Examination sites
Yip [1] Iron deficiency: contemporary scientific issues and international programmatic approaches (1994)	<i>n</i> = 743 Ethiopian refugee women in Somalia No details on training or examiners	HemoCue	Hb < 7 g/dl Sensitivity 53.4% Specificity 90.6%	Conjunctiva, tongue, nail beds, palms
Gujral et al. [29] Agreement between hemoglobin estimation and anemia recognition card in assessment of anemia in pregnant women (1989)	<i>n</i> = 211 pregnant women in India Trained medical interns examined women	Cyanmethaemoglobin method	Hb < 8 g/dl Sensitivity 67% Specificity 41%	Lips, tongue, nail beds using anaemia recognition card
Meda et al. [34] Anaemia among women of reproductive age in Burkina Faso (1996)	<i>n</i> = 251 women of reproductive age (non-pregnant, pregnant, and lactating) in Burkina Faso Trained fieldworkers examined women	HemoCue	Sensitivity Hb < 7 g/dl: 100% Hb 7 to < 10 g/dl: 43% Hb < 11 g/dl: 16%	Conjunctiva
Ghosh and Mohan [30] Screening for anemia (1978)	<i>n</i> = 886 (528 children, 207 adults, 151 pregnant women) in India 4 fieldworkers with some education and training in basic health care examined subjects	Cyanmethaemoglobin method	Sensitivity Hb < 6 g/dl: 100% Hb 6-9 g/dl: 66% Hb 9-11 g/dl: 50% Specificity Children with Hb < 11 g/dl: 80%	Tongue, lips, and nails using anaemia recognition card
Luby et al. [24] Using clinical signs to diagnose anaemia in African children (1995)	<i>n</i> = 1,104 children under 5 yr in rural Malawi 3 non-physician health-care workers with training in two hospital-based outpatient clinics examined children	HemoCue	Sensitivity Hb < 5 g/dl: 93% Hb 5-8 g/dl: 66% Specificity Hb < 5 g/dl: 57% Hb 5-8 g/dl: 68%	Conjunctiva, tongue, palm, nail beds

Hb, Haemoglobin.

positives (TP) by the number of true positives plus the number of false negatives (FN): $TP/(TP + FN)$. Specificity is the ability to identify those who do not have the disease and is calculated by dividing the number of true negatives (TN) by the number

of true negatives plus the number of false positives (FP): $TN/(TN + FP)$. Sensitivity and specificity are determined by comparing the results of the screening test with a "gold standard" or definitive diagnostic procedure (e.g., a laboratory test) [16].

World Health Organization (WHO) [17, 18] recommends that all pregnant women be clinically screened (using pallor) for anaemia as part of their antenatal check-up, there have been no recommendations for using pallor screening to identify women with severe anaemia. The purpose of this paper is to review the literature and results from recent field trials to determine if this method is useful in identifying severe anaemia and to make recommendations on the use of pallor screening to detect severe anaemia.

Methods

A Medline search of the literature from 1978 to 1997 was conducted to identify studies for review. Key words used to conduct the search included "anaemia and pallor," "anaemia and clinical signs," "testing, sensitivity, and anaemia," and "anaemia and physical examination." Because it was of interest to show how clinical signs can be used to identify anaemia, studies were used in which the sensitivities of the examinations were reported or could be calculated. In addition to the literature review, iron specialists and journal article authors were contacted. Although we surveyed the literature for all studies evaluating the usefulness of pallor screening, we will focus only on studies in which the sensitivity of detecting moderate to severe anaemia (haemoglobin < 8 g/dl) was examined. However, we used all studies to determine ways to improve pallor screening. These are included in the Discussion section and in tables 2 and 3.

In two anaemia prevalence surveys supported by MotherCare (a programme of John Snow, Inc., funded by the US Agency for International Development), involving over 600 pregnant women, pallor screening was conducted before haemoglobin values were taken using the HemoCue.

Results

Seventeen studies were identified in which pallor was used as a screening tool to identify anaemia [1, 19–34]. Four of these studies were conducted with Western populations [19–22] and 13 studies with developing country populations [1, 23–34]. Five studies included mixed populations of men, women, pregnant women, adolescents, and children [27, 28, 30, 31, 33]. Three studies were conducted on women only [1, 29, 34]. Two studies included only men [20, 23], and two studies were conducted on children under five years of age [24, 25]. Four studies were conducted on adult men and women [19, 21, 22, 32], and one study gave little description of the study population [26]. Four of the five published studies that looked at severe to moderate anaemia included women [1, 29, 30, 34]. The fifth study

involved children only [24]. Physicians and other health workers of various skill levels and experience conducted the screenings. The studies varied in the amount of detail they provided on training, examination procedures, and environmental conditions during the examinations. Most of these studies examined the paleness of several different areas, such as the face, nail beds, tongue, palms, and conjunctivae and compared them against a gold standard for haemoglobin, including copper sulphate, the cyanmethaemoglobin method, the Coulter counter, the haemoglobinometer, and the HemoCue instrument, which uses the cyanmethaemoglobin reagents (for a description of these methods, see ref. [35]).

The majority of studies define anaemia according to haemoglobin levels, although two studies used haematocrit values [19, 23]. The research showed that in conducting clinical screenings of moderate to severe anaemia (haemoglobin < 8 g/dl), using pallor of a variety of body sites, the sensitivity is relatively high: from 53% to 100%. A more detailed review of the literature is shown in tables 1 to 3.

Review of the literature

Women

Yip [1] and other investigators assessed pallor of the conjunctiva, tongue, nail beds, and palms in 743 Ethiopian refugee women in Somalia. For the clinical examination, women were classified as "definitely anaemic," "probably anaemic," or "normal." Fifty-three percent of women with severe anaemia (haemoglobin < 7 g/dl) were identified; the specificity was 90.6%.

Gujral et al. [29] looked at the sensitivities and specificities of clinical examinations of 211 pregnant women from rural and tribal villages in Gujarat, India, using an anaemia recognition card developed by the Voluntary Health Association of India to help community health workers identify anaemia by signs of pallor in the lips, tongue, and nails. Sixty-seven percent of women with anaemia (haemoglobin < 8 g/dl) were identified; the specificity was 41%.

Meda et al. [34] studied anaemia among women of childbearing age in Bobo-Dioulasso, Burkina Faso. Fieldworkers were trained to examine the conjunctiva for signs of anaemia in 251 women. Haemoglobin concentration was estimated by HemoCue and then validated by a Coulter counter. Haemoglobin cut-offs followed WHO guidelines. The overall sensitivity of determining anaemia (haemoglobin < 11 g/dl) from pallor was only 16%. The sensitivity increased to 43% in the 20 women with haemoglobin < 10 g/dl. However, the sensitivity was 100% in the three women with severe anaemia (haemoglobin < 7 g/dl).

In MotherCare-supported research in Malawi, 197

TABLE 2. Studies of pallor in developing countries

Study	General description: <i>n</i> , country, examiners	Gold standard	Hb cut-off and sensitivity and specificity of examination	Examination sites
Ekunwe [33] Predictive value of conjunctival pallor in the diagnosis of anaemia (1997)	<i>n</i> = 2,697 men, women, children in Lagos, Nigeria (1,449 males, 1,248 females) Researcher conducted examinations	Oxyhaemoglobin	Hb < 10 g/dl Sensitivity 25% Specificity 89%	Conjunctiva
Thaver and Baig [25] Anemia in children: Part I. Can simple observations by primary care provider help in diagnosis? (1994)	<i>n</i> = 947 children 6–60 mo old in squatter settlement in Karachi, Pakistan Physicians with training to detect pallor examined children	HemoCue	Hb < 11 g% Sensitivity Specificity Conjunctiva 74.1% 64.5% Tongue 20.2% 94.1% Nails 14.9% 96.1% Palms 32.0% 89.7% Combination of conjunctiva and: Palms 78.6% 62.1% Nails 75.6% 63.5% Tongue 75.2% 63.1%	Conjunctiva, tongue, nail beds, palms
Khan et al. [28] Relationship of intestinal parasitism, malaria, and undernutrition to prevalence of anemia in an urban community (1990)	<i>n</i> = 113 men, women, and children in urban India No details on training or examiners	Not given	WHO cut-off levels for Hb Sensitivity 31.9%	Conjunctiva, skin, nail beds
Sanchez-Carrillo [26] Bias due to conjunctiva hue and the clinical assessment of anemia (1989)	<i>n</i> = 219 subjects in clinic in Mexico 657 independent observations: 179 described as “pink” and 478 as “reddish” 3 non-clinicians examined patients	Not given	Hb < 9.9 g/dl 52% of subjects with pinkish hue and 38.9% with reddish hue were correctly classified as anaemic or non-anaemic	Conjunctiva
Sanchez-Carrillo et al. [27] Test of a non-invasive instrument for measuring hemoglobin concentration (1989)	<i>n</i> = 200 (118 females, 82 males, all > 12 yr old) in Mexico 2 medical professionals and 1 non-medical professional examined patients	Not given	Hb < 13 g/dl Sensitivity 63% Specificity 72% Hb < 11 g/dl 97% identified correctly	Conjunctiva using colour shades

women were examined for conjunctival pallor. The sensitivity for identifying women with severe anaemia (haemoglobin < 7 g/dl) was 83.3% (*n* = 5), and the specificity was 80%. The sensitivity for moderate anaemia (haemoglobin 7–10.9 g/dl) was 25%. In Indonesia, 409 pregnant women were examined; the sensitivity of conjunc-

tival pallor for severe anaemia was 100% (*n* = 5), and the specificity was 80%.

Pregnant women, adults, and children

Ghosh and Mohan [30] used the anaemia recognition

TABLE 2. Studies of pallor in developing countries (*continued*)

Study	General description: <i>n</i> , country, examiners	Gold standard	Hb cut-off and sensitivity and specificity of examination	Examination sites
Wurapa et al. [31] Evaluation of conjunctival pallor in the diagnosis of anemia (1986)	<i>n</i> = 627 were examined for pallor and had haemoglobin estimates (269 males, 358 females, including children); 951 were examined for pallor (406 males, 545 females, including children) in Zambia 2 physicians examined different groups of subjects	Coulter counter	Hb \geq 10 g/dl; <i>n</i> = 627 Sensitivity Physician A 32.7% Physician B 4.0% Both 18.6% Specificity Physician A 90.4% Physician B 100% Both 95.8%	Conjunctiva
Glass et al. [23] The value of simple conjunctival examination in field screening for anemia (1980)	<i>n</i> = 64 male agricultural workers on a Guatemalan coffee plantation 3 physicians and 2 medical students examined men	Cyanmethaemoglobin method	Hct < 36% or Hb < 11.9 g/dl Sensitivity and specificity > 70%	Conjunctiva
Jacobs et al. [32] Observer bias and error in the integumentary clinical diagnosis of chronic anemia (1979)	<i>n</i> = 74 white patients (44 women, 30 men; mean age, 65.6 yr) in hospital in South Africa Researchers examined patients	Cyanmethaemoglobin method	Men: Hb < 14.3 g/dl Women: Hb < 12.3 g/dl Sensitivity and specificity not given	Conjunctiva, oral mucosa

Hb, haemoglobin; Hct, haematocrit.

card to detect anaemia in 886 children, adults, and pregnant women in India. The fieldworkers were able to correctly identify 100% of patients with severe anaemia (haemoglobin < 6 g/dl), 66% of those with moderate anaemia (haemoglobin 6–9 g/dl), and 50% of those with mild anaemia (haemoglobin 9–11 g/dl). In addition, 70% to 80% of all subjects with haemoglobin above 11 g/dl were correctly identified as non-anaemic.

Children under five years of age

Luby et al. [24] evaluated the ability of health workers in rural Malawi to identify anaemia in 1,104 children under five years of age by examining pallor of the conjunctiva, tongue, palms, and nail beds. Health workers used their own judgement to assess severity of pallor and graded pallor as “definite,” “probable,” or “absent.” The sensitivity and specificity of pallor were highest (93% and 57%, respectively) for severe anaemia (haemoglobin < 5 g/dl in children). The sensitivity and

specificity of pallor screening were 66% and 68%, respectively, for moderate anaemia (haemoglobin 5–8 g/dl in children).

Discussion

Although pallor has been extensively used in the antenatal care setting to identify cases of anaemia, it is widely known not to be useful for identifying the entire spectrum of anaemia from mild to severe (i.e., haemoglobin < 11 g/dl for pregnant women). In fact, some researchers feel that screening is not useful because of individual variability [personal communications from Dr. James Cook of the University of Kansas, USA, 1995, and Dr. Alan Fleming of the University Teaching Hospital in Lusaka, Zambia, 1996]. The authors of this review agree that there is wide variation in results (16%–78% sensitivity) in identifying those with anaemia but disagree that this variation is due only to subject differences.

TABLE 3. Studies of pallor in Western countries

Study	General description: <i>n</i> , country, examiners	Gold standard	Hb cut-off and sensitivity and specificity of examination	Examination sites
Sheth et al. [22] The relation of conjunctival pallor to the presence of anemia (1997)	<i>n</i> = 302 (171 males and 131 females) in racially diverse hospital setting in Toronto, Canada 2 medical students and 1 physician examined patients	Not given	Hb < 9 g/dl Sensitivity 54.5% Specificity 74.9% Values converted from likelihood ratios	Conjunctiva
Nardone et al. [19] Usefulness of physical examination in detecting the presence or absence of anemia (1990)	<i>n</i> = 103 white patients (98 men and 5 women; mean age, 60 yr) in a veterans' hospital in Oregon, USA 3 physicians examined patients	Not given	Hct 40%, Hb < 13 g/dl Sensitivity: palms 53%; pallor at any site 65% Specificity: palmar creases 100%; pallor at any site 95% Hct 35%, Hb < 12 g/dl Sensitivity: palms 64%; pallor at any site 77% Specificity: palmar creases 99%; pallor at all 3 94% Hct 30%, Hb < 10 g/dl Sensitivity: palms 67%; pallor at any site 80% Specificity: palmar creases 98%; pallor at all 3 87%	Conjunctiva, palms, palmar creases, face, nail beds
Strobach et al. [20] The value of the physical examination in the diagnosis of anemia (1988)	<i>n</i> = 50 white men in a veterans' hospital in Missouri, USA Physicians examined patients	Not given	Hb < 10 g/dl Conjunctival pallor 81% Absence of nail bed blanching 81% Nail bed pallor 56% Palmar crease 50%	Conjunctiva using colour tint selector, nail beds, palmar creases
Gjorup et al. [21] A critical evaluation of the clinical diagnosis of anemia (1986)	<i>n</i> = 180 (108 women and 72 men; median age, 69 yr) in a hospital in Denmark 3 physicians examined patients	Hemalog 8	Men: Hb 8.0–11.0 g/dl Women: Hb 7.0–10.0 g/dl Sensitivity range 27%–44% Specificity range 88%–95%	Skin, nail beds, conjunctiva

The accuracy of the measurement depends also on the body part being examined and the skill of the physician or health worker.

On the basis of the studies reviewed here, there may be several ways to improve the sensitivity of clinical signs to identify anaemia. It appears that the accuracy of pallor screening is not dependent on years of clinical experience [19, 29, 31]. Instead, the sensitivity of pallor screening can improve when health workers are trained specifically to identify anaemia using clinical signs and to use the best body sites and environmental settings while doing the examination [25, 30, 36].

Two studies [24, 25] found that health workers at all levels (e.g., in the community and at facilities) with training were successful in identifying signs of anaemia, especially moderate to severe levels. Immediate feedback, in which workers detect pallor and compare their results with an accurate test for haemoglobin, appears to be an important component of training [23–27]. In Kenya using clinical signs to detect anaemia was more accurate when health workers knew their own haemoglobin values and were able to use their own palms to compare with the palms of children [Dr. Jane Zucker, Centers for Disease Control and Prevention,

Georgia, USA, personal communication, 1996].

The use of different sites to improve the sensitivity of pallor screening is not conclusive. Generally, it appears that the use of a combination of sites (conjunctiva, nail beds, and palmar creases) can improve sensitivity. When only a single site is used, the conjunctiva is best in adults because it is not affected by race and requires less training to obtain accurate results. For children, nail beds and palmar pallor seem the most useful, possibly because the surface area of the conjunctiva is too small in children.

Environmental conditions may affect the accuracy of pallor screening. For example, examining subjects in a well-lit room or in daylight is preferred. In areas where women cook indoors, smoke may cause an unusually high incidence of conjunctivitis, causing hyperaemia or an unusual amount of blood flow to this part of the body. The use of tobacco or betel leaf can discolour the tongue, mouth, and hands, making clinical examination difficult [30].

Several studies [20, 27, 29, 30] used comparison aids to increase the sensitivity of pallor screening. Only one study [20] showed that a comparison guide, in this case a colour tint selector, produced high sensitivities in the detection of moderate anaemia. Even if comparison aids increase sensitivity substantially, they may be expensive and difficult to maintain.

Pallor screening is usually conducted to target limited supplies of iron and folate supplements. This is not an acceptable public health strategy. Instead, health workers need to be aware that pallor screening is not sensitive enough to detect the majority of pregnant women with anaemia and that enough iron and folate pills should be procured so that all women receive the recommended number of pills during pregnancy. Where the prevalence of anaemia is less than 40%, women should take 60 mg of elemental iron and 400 µg of folic acid daily for six months during pregnancy. Where the prevalence of anaemia is over 40%, women should take 60 mg of elemental iron and 400 µg of folic acid daily for six months during pregnancy and for three months postpartum [15].

It is clear from the small number of studies reviewed here and the MotherCare field studies that pallor screening can be used as a tool for identifying women with severe anaemia. In three of the four studies that looked at cases of severe anaemia (haemoglobin < 7 g/dl), it was possible to correctly identify 93% [24] and 100% [30, 34] of individuals with severe anaemia. In the fourth

study, researchers were only able to detect 53% of women with severe anaemia [1]. Gujral et al. [29] were able to identify 67% of pregnant women at a haemoglobin cut-off of < 8 g/dl.

Women who are identified with severe anaemia through pallor screening ought to be targeted for special follow-up. Pregnant women with pallor who are at less than 36 weeks of gestation should be given a higher dosage of iron and folate tablets and, like their less anaemic counterparts, counselled on the importance of taking them and asked to return within 30 days to be reexamined and questioned about pill-taking. Since women with anaemia have fatigue and often feel noticeably better after taking iron pills for a couple of weeks, they should be asked about their energy levels, which can also be a proxy for compliance. Although some women with less severe anaemia will be classified as being severely anaemic (false positives) according to pallor screening, follow-up efforts with women will also be beneficial, since most of these women will be mildly anaemic or iron deficient [1]. If a woman does not respond to iron and folate therapy (e.g., if she still exhibits pallor or complains about being tired), either compliance is low or there are other causes of anaemia that should be treated. These women should be referred for a haemoglobin test. Meda et al. [34] found that verification of low haemoglobin can be used to further convince women to take their iron pills. Women with pallor who are at more than 36 weeks of gestation should be referred immediately for a haemoglobin test, and the appropriate action should be taken, depending on their haemoglobin status.

In conclusion, from this review and from MotherCare field trials, the recommended number of iron pills should be given routinely to all pregnant women, and pallor screening should be used to identify women with severe anaemia. Operations research to investigate the usefulness of other ways to identify severe anaemia (e.g., asking about breathlessness and observing breathlessness at rest) should be conducted. Health workers should also be trained to use pallor screening correctly to identify women with severe anaemia (haemoglobin < 7 g/dl) and provide appropriate follow-up based on their stage of gestation. Ministries of Health need to make a new commitment to solving the problem of anaemia by ensuring supplies of iron and folate, by developing effective messages to ensure compliance, and by training health workers to improve their counselling skills.

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Assessment of the prevalence and potential determinants of nutritional anaemia in Upper Egypt

Nawal El-Sayed, Ashry Gad, Laila Nofal, Hamdy Abou Zeid, Hala El-Morshedy, and Safaa El-Waseef

Abstract

Iron deficiency is the most common type of malnutrition worldwide. This community-based study was conducted to assess the prevalence of anaemia in Minia, Assiut, and Sohag Governorates of Upper Egypt and to analyse the nature of the problem to help prioritize the control intervention strategy. The study was conducted on children aged 6 to 71 months. The two-stage cluster sampling method was used, and 2,700 pre-schoolers (900 in each Governorate) were enrolled. Data were collected on sociodemographic characteristics, morbidity profile, dietary characteristics (breastfeeding and qualitative and quantitative food-consumption patterns), haemoglobin concentration by the cyanmethaemoglobin method, and urine and stool analysis (for every fifth child). The analysis, presentation, and interpretation of data followed the guidelines and indicators recommended by the World Health Organization/UNICEF. Anaemia was very highly prevalent among all pre-schoolers (69%). Minia Governorate showed the highest trend in prevalence of anaemia, followed by Sohag and then Assiut. The prevalence of anaemia was slightly higher in most rural sites and was higher among girls (70%) than boys (68%). The highest prevalence was seen in the second year of life. Severe anaemia was observed among 5.5% of pre-school children. Breast-feeding pattern, economic status, parasitic load, and the anaemia state of mothers were all significantly associated with the risk of anaemia. A set of strategic recommendations and implications for action was proposed.

Nawal El-Sayed is a professor of nutrition, Ashry Gad is an associate professor of epidemiology, Laila Nofal is a professor of biostatistics, Hamdy Abou Zeid is an associate professor of family health, and Hala El-Morshedy is an associate professor of tropical health at the High Institute of Public Health in Alexandria, Egypt. Safaa El-Waseef is a lecturer in nutrition at the Home Economics Department, Faculty of Specific Education, at the Suez Canal University.

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

Introduction

Anaemia is the most common type of malnutrition worldwide. Nutritional anaemia refers to a condition in which the haemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients (usually iron, less frequently folate or vitamin B₁₂), regardless of the cause of such deficiency.

It is generally held that at least half of anaemia worldwide is due to nutritional iron deficiency [1]. Nearly two billion people are estimated to be anaemic, and even more are iron deficient. Between 40% and 50% of children under five years of age in developing countries are iron deficient [2].

The adverse functional effects of anaemia in children include impaired cognitive function [3], reduced physical work capacity and productivity, lowered cellular immunity, increased morbidity, and child growth deficits [4].

Major nutritional studies done in Egypt over the last 30 years revealed that anaemia is a moderate to severe public health problem among pre-school children. However, most of these studies were conducted in lower Egypt, and data regarding Upper Egypt are scarce. The aim of this work was to assess the prevalence of anaemia among pre-school children in Upper Egypt and to study the potential risk factors that could help in prioritizing the control intervention.

Subjects and methods

Study type and site

This community-based study was carried out in three of the six Governorates of Upper Egypt. The chosen Governorates were Minia (3.3 million), Assiut (2.9 million), and Sohag (3.1 million). They are representative of Upper Egypt, being at the middle and adjacent to each other on the Nile River [5–7].

Target population

The population targeted was pre-school children aged 6 to 71 months.

Sampling

A two-stage cluster sampling technique was used to select the study sample from each Governorate. In the first stage, 30 clusters were selected based on probability proportionate to the size of the target population in the different districts. All districts of each Governorate were represented in the study. In the second stage, from a random starting point in each household cluster, the investigators went door to door until 30 children (one per family), corresponding to the predetermined age, were selected. This sample size (900 child from each Governorate) ensured with a probability of 95% that the estimated prevalence would be within 5% of the true prevalence, irrespective of the prevalence value and assuming a design effect of 2.

Data collection

Field activities were performed during late 1997. Data were collected from all participants by the following methods.

Questionnaire interview with the child's mother

The questionnaire design followed the guidelines recommended by the World Health Organization (WHO)/UNICEF on assessment of anaemia [1]. Mothers were interviewed about the child's characteristics, medical history to exclude other causes of anaemia, socio-economic status [8], morbidity pattern, and dietary characteristics (breastfeeding, complementary feeding, and daily diet of the child, by using food-frequency and 24-hour dietary recall methods) [9].

Laboratory investigation

Venous or capillary blood samples were drawn from all children and their mothers for measurements of haemoglobin concentration. Anaemia was diagnosed by measuring the concentration of haemoglobin in circulating red blood cells by the cyanmethaemoglobin technique [10].

Urine and stool samples were collected from every fifth child to assess the state of parasitic infection. Urine was examined by the sedimentation centrifugation technique [11]. Stools were examined by the Merthiolate iodine formaldehyde concentration technique [12].

Data analysis

Dietary data were analysed with a Lotus 123 computer package. The nutritive value of the consumed diet was computed from Egyptian food-composition

tables [13]. The nutritional adequacy of the diet was calculated by reference to the recommended daily allowances [14].

The data were analysed by Epi-Info 6.D and SPSS/PC+. Children with haemoglobin levels below 11 g/dl were considered anaemic. WHO criteria for grading the severity of anaemia were followed [1].

Simple logistic regression analysis was performed to identify the independent variables (demographic, socio-economic, and child characteristics) significantly related to the outcome variable (anaemia). All variables found to be significant were included in a multivariate analysis model, using the SPSS forward stepwise logistic regression procedure.

Maximum likelihood estimates of combined odds ratios and their attendant 95% confidence intervals, adjusted for confounders, were obtained by logistic regression.

Results

The total number of enrolled pre-schoolers was 2,700. The questionnaire was completed by 2,670 of the mothers (98.5%), and 2,577 agreed to a haemoglobin estimation (95.4%).

As shown in table 1, 58.4% of the subjects were male and 41.6% were female. Their mean age was 30.4 ± 17.9 months. Eighty-eight percent belonged to a low or very low socio-economic class.

About one-third (35.9%) of the children were still being breastfed at the time of the study. Among those who were not being breastfed at the time of the study, prolonged breastfeeding was the common rule (65.3% for more than one year and 20.4% for more than two years). During the preceding two weeks, two-fifths of the children had diarrhoea (40.4%) or fever (42.4%). *Entamoeba histolytica* was the most common protozoal infestation (20.1%), followed by *Giardia lamblia* (12.4%). Other parasitic infestations were rare.

The mean daily energy intake was only 82% of the recommended value for children under four years of age and 64% of the recommended value for children four years old or more (table 2). The total daily protein intake exceeded the recommendations (194% and 133% in younger and older children, respectively). However, only 31% of the total protein was animal protein. The total iron intake was inadequate for both age groups (67% and 79% of the recommended values, respectively), and haem iron constituted only 18% of total iron intake. Vitamin A intake was deficient in both age groups (59% and 62% of the recommended values, respectively). Vitamin C intake was high and exceeded the recommendation (153% and 184% of the recommended values, respectively).

The prevalence of anaemia was 69%: 68% for boys and 70% for girls (table 3). The highest prevalence was in the second year of life (72%) and the lowest was in

TABLE 1. Sociodemographic and morbidity characteristics of the study sample of pre-school children in Upper Egypt, 1997

Characteristic	No.	%
Residence		
Al-Minia	892	33
Assiut	883	33
Sohag	895	34
Sex		
Male	1,560	58
Female	1,110	42
Age (mo)		
< 12	469	18
12–23	716	27
24–35	561	21
36–47	415	16
48–59	285	11
60+	224	8
Mean age \pm SD: 30.4 \pm 17.9 mo		
Socio-economic level		
High	32	1
Middle	312	12
Low	926	35
Very low	400	53
Age of stopping breastfeeding (mo)		
Never breastfed	55	3
< 6	105	6
6–11	81	5
12–23	1,104	65
24+	345	20
Morbidity		
Diarrhoea (last 2 wk)	1,080	40
Vomiting (last 2 wk)	293	11
Persistent cough (last 2 wk)	430	16
Fever (last 2 wk)	1,132	42
Measles (last 6 wk)	49	2
Protozoal infection		
<i>Giardia lamblia</i>	62	12
<i>Entamoeba histolytica</i>	100	20
Helminthic infection		
<i>Hymenolepis nana</i>	24	5
<i>Enterobius vermicularis</i>	17	3

the fifth year (66%). Severe anaemia (haemoglobin < 7 g/dl) was seen in 5.5% of the children.

In Minia anaemia was significantly more prevalent in rural (81.1%) than urban (70.4%) areas. However, there was no significant difference in the prevalence of anaemia between urban and rural areas in the other two Governorates.

The results of stepwise multiple logistic regression (table 4) showed that children in Assiut and Sohag were at less risk for developing anaemia (regression coefficient, 0.38 and 0.56, respectively) relative to children in Minia. Young children who were never breastfed or had prolonged breastfeeding had a high risk of anaemia (regression coefficient, 4.95). Children of high eco-

nomic class were at less risk of anaemia (regression coefficient, 0.95). Children of anaemic mothers had about double the risk of anaemia (regression coefficient, 1.85; 95% confidence interval, 1.15–2.99).

Discussion

Anaemia has major social, educational, and economic implications for the affected populations. The prevention and control of anaemia are of crucial significance. In our study, more than two-thirds (69%) of pre-school children in Upper Egypt had anaemia, constituting a severe public health problem. This high prevalence rate may be due to low dietary iron intake (67% of the recommended value for children under four and 79% for children four or more years old) and the small contribution of haem iron (18%) to the total iron intake. The low vitamin A intake (59% and 62% of recommended values for younger and older children, respectively) may be another contributing factor to this high prevalence of anaemia. Vitamin A may be needed in haematopoiesis [4]. Infectious and parasitic diseases among these children contribute directly or indirectly to their vulnerability to nutrient deficiencies by causing loss of nutrients, decreasing appetite, and decreasing the efficiency of absorption, conservation, and utilization of nutrients [14]. In addition to the high requirements for iron in early childhood [10], infants and children require iron for their expanding red cell mass and growing body tissues.

Anaemia was more prevalent in the second year of life (72.1%). This may be due to prolonged breastfeeding and delayed or insufficient introduction of complementary foods rich in iron. A peak of anaemia in the second year of life has also been reported by other investigators in Egypt [15, 16] and other countries [17]. Anaemia was slightly more prevalent among girls (70%) than boys (68%). The culture in Upper Egypt may give more attention to boys than girls in all aspects of care, including nutritional care.

Children in Assiut and Sohag were at less risk of anaemia than those in Minia, possibly because of the high fertility rate, greater menstrual blood loss, and high prevalence of anaemia among mothers in Minia. Low economic status was significantly associated with a higher risk of anaemia (table 4). Many studies have noted a positive relationship between income and both qualitative and quantitative adequacy of the diet [18]. Investigators in Montreal, Canada, concluded that infants from low socio-economic backgrounds were at high risk of anaemia [19]. Sargent et al. in Massachusetts, USA, examined the association between community rates of iron-deficiency anaemia in children and sociodemographic characteristics and found that community iron deficiency was associated with low socio-economic status [20].

TABLE 2. Daily intake (mean \pm SD) and percent adequacy of selected nutrients of pre-school children in Upper Egypt, 1997

Nutrient	< 4 yr old		\geq 4 yr old	
	Intake	% adequacy	Intake	% adequacy
Energy (kcal)	1,039 \pm 460	82	1,148 \pm 436	64
Total protein (g)	30.8 \pm 15.08	194	32.3 \pm 16.0	133
Animal protein (g) ^a	9.4 \pm 8.6	–	9.1 \pm 10.1	–
Vegetable protein (g)	21.5 \pm 12.3	–	23.2 \pm 10.7	–
Iron (mg)	6.7 \pm 4.3	67	7.9 \pm 5.3	79
Haem iron (mg) ^b	1.2 \pm 1.8	–	0.9 \pm 1.2	–
Non-haem iron (mg)	5.6 \pm 3.8	–	7.0 \pm 5.0	–
Vitamin C (mg)	60.8 \pm 133	153	83 \pm 143	184
Vitamin A (RE)	236 \pm 268	59	305 \pm 333	62

a. Animal protein was 31% of total protein.

b. Haem iron was 18% of total iron.

TABLE 3. Prevalence of anaemia among pre-school children in Upper Egypt according to age and sex, 1997

Age (mo)	Boys		Girls		Total	
	No.	%	No.	%	No.	%
< 12	243	68	201	71	444	69
12-23	389	73	291	70	680	72
24-35	344	66	204	65	548	66
36-47	231	65	177	70	408	67
48-59	167	62	111	70	278	66
60+	137	66	82	70	219	67
Total	1,511	68	1,066	70	2,577	69
χ^2	9.03		2.02		7.72	
Mean haemoglobin (g/dl) \pm SD	10.15 \pm 1.79		10.09 \pm 1.9			

TABLE 4. Stepwise multiple logistic regression analysis of anaemia among pre-school children in Upper Egypt, 1997

Independent variable	RC	95% CI
Residence		
Assiut	0.38	0.27–0.53
Sohag	0.56	0.39–0.79
Breastfeeding		
Never breastfed	4.95	1.24–19.85
6+ mo	4.85	1.42–16.58
Economic level	0.95	0.90–0.99
Mother's anaemia	1.85	1.15–2.99

RC, Regression coefficient; CI, confidence interval

Children born to anaemic mothers were more prone to develop anaemia (regression coefficient, 1.85). Previous studies in Egypt reported the same finding [21]. In prospective studies in Spain, Colomer et al. found the same results among newborn infants (regression coefficient, 6.57) [22].

Breastfeeding delays the onset of anaemia, but prolonged breastfeeding without timely complementary feeding can lead to anaemia [10]. Our results are consistent with this statement. Term infants who are breastfed exclusively for the first six months may not be at risk for iron depletion or for development of anaemia [23].

Conclusions and recommendations

Anaemia is a severe public health problem among pre-school children in Upper Egypt. Many risk factors contribute to this situation, including low socio-economic class, prolonged breastfeeding without efficient complementary feeding, anaemic mothers, and high morbidity from infectious and parasitic diseases. This situation necessitates an urgent intervention programme of iron supplementation targeted to pre-schoolers (who may receive the supplement at the same time as immunizations) and women of childbearing age. Suitable food-based approaches, including food fortification and diversification, together with other public health measures directed towards parasitic control and maternal care, also must be developed.

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Development of iron-deficiency anaemia at six months of age in Jordanian infants exclusively breastfed for four to six months

Ahmad M. Faqih and Hussain S. Qazaq

Editorial comment

This paper has two particularly interesting findings: (1) The rate of iron-deficiency anaemia at four months of age was rather high, which challenges the current thinking that there are adequate iron stores to protect against iron deficiency up to six months of age. (2) Longer exclusive breastfeeding had a protective effect against iron-deficiency anaemia. It is somewhat surprising to see the rather large difference in iron status between those with four months of exclusive breastfeeding and those with five months. It should be noted that this is an observational study, not a controlled study. It is possible that infants with a shorter period of exclusive breastfeeding differ from those with a longer period of exclusive breastfeeding in other factors. For example, the iron status of the mother during pregnancy will affect iron stores at birth. Ideally, some important factors such as birthweight can be used as covariates to control for the potential difference among the three groups compared, since low-birthweight infants have lower iron stores. Other factors might be infection in the infant and the effects of deficiencies in other haematopoietic nutrients. This is an interesting and provocative study, which may help to promote longer periods of exclusive breastfeeding. Given the nature of the study, with its relatively small sample size, and the potential significance of the study, larger studies repeated elsewhere are needed.

Abstract

The iron status at six months of age for a total of 66 three-month-old, full-term, normal-birthweight Jordanian

infants who were exclusively breastfed for four, five, or six months postpartum is reported here. Three groups of infants were identified who were exclusively breastfed for four months (n = 14), five months (n = 15), and six months (n = 37). Iron status was evaluated by haemoglobin, plasma ferritin, and mean corpuscular volume. The prevalences of nutritional anaemia (haemoglobin < 10.5 g/dl), iron-deficiency anaemia (haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml), and depletion of iron stores (plasma ferritin < 12 ng/ml) were significantly higher (p < .05) in infants exclusively breastfed for four months than in the other two groups. Significant differences were not observed between the latter two groups in the prevalence of any of these indices. Under the conditions of this study, which was carried out in a community of low to medium socio-economic status where iron-fortified weaning foods were not used, the data strongly suggest that exclusive breastfeeding for five or six months is protective against the development of nutritional anaemia or iron-deficiency anaemia at six months of age. The weaning foods used in this community have low iron content and bioavailability.

Introduction

Iron-deficiency anaemia is the most common nutritional disorder in the world [1, 2]. From a public health point of view, the importance of iron-deficiency anaemia prevention arises from the seriousness of its consequences for the health of infants, children, and women of reproductive age, requiring highly coordinated efforts to prevent its development during such sensitive periods of life [3, 4].

At birth the average amount of total body iron in full-term infants is about 75 mg per kilogram of body weight [5, 6]. These iron stores are normally adequate to meet the infant's needs for about the first four months of life [7], or for a rather longer period of about six months in breastfed infants, as stated by Fairweather-Tait in 1992 [8].

Whereas iron-deficiency anaemia is specific to iron

Ahmad M. Faqih is a professor of nutrition in the Department of Nutrition and Food Technology in the University of Jordan in Amman. Hussain Qazaq is affiliated with the Nutrition, Maternal and Child Health Directorate in the Ministry of Health in Amman.

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deficiency, nutritional anaemia can be precipitated by the deficiency not only of iron but also of any of several other nutrients, including vitamin B₆, vitamin C, vitamin E, folic acid, copper, and protein [9, 10], singly or in combination. In developing countries, however, it is generally accepted that the major cause of anaemia is iron deficiency [1].

Pisacane et al. observed that the prevalence of anaemia (haemoglobin < 11 g/dl) was significantly lower among 12-month-old Italian infants who were exclusively breastfed for 6.5 months than among those exclusively breastfed for 5.5 months [11]. In contrast, Dewey et al. found that exclusive breastfeeding did not prevent the development of anaemia at four or six months of life: 25% of the four-month-old exclusively breastfed infants (*n* = 22) developed anaemia (haemoglobin < 10.3 g/dl), a proportion which was not significantly different from a prevalence of 32% observed in exclusively breastfed infants at six months [12].

In this paper, we report the prevalence of nutritional anaemia and iron-deficiency anaemia for infants at six months of age as affected by the duration of exclusive breastfeeding for the first four, five, or six months of life.

Subjects and methods

Subjects

One hundred seventeen full-term, appropriate-for-gestational-age infants (63 boys and 54 girls) who were born to mothers willing to continue exclusive breastfeeding for at least the first four months after birth participated in the study between August 1994 and October 1995. The participants represented a random sample of mothers who attended the training at the Maternal and Child Health Centre at Wadi Srour in Amman. Mothers were informed about the study protocol, including the need for drawing four blood samples from the infants at 3, 6, 9, and 12 months of age. The mother's consent was required for participation. The selected group of infants formed the basis for a nine-month longitudinal study (3–12 months of age) that was conducted to test the effectiveness of applying certain intervention strategies at six months of age for the prevention of iron-deficiency anaemia [13, 14]. Only 66 infants (32 boys and 34 girls) completed the study. Forty-nine dropped out for one reason or another, and two three-month-old infants (one boy and one girl) were excluded because their haemoglobin levels were below 9.5 g/dl at three months of age.

Diet

All infants were exclusively breastfed for the first four, five, or six months, beyond which solid foods were

introduced according to the desire of their mothers. When the infants were six months old, the mothers were asked if iron-fortified cereals or formulas were given. Solid food was introduced at four, five, or six months of age for 21%, 23%, and 56% of the study infants, respectively.

Anthropometry

The infants' body weight, length, and head circumference were measured monthly. Body weight was measured to the nearest 0.01 kg with a paediatric scale (Seca, Germany). Recumbent length was measured to the nearest millimetre with a wooden measuring board (Shorr, USA). Head circumference was measured to the nearest millimetre with a narrow, flexible, and non-stretchable tape made of fibreglass. Measurements were compared with the respective median National Center for Health Statistics (NCHS) references [15]. Growth was assessed by weight-for-age, length-for-age, and weight-for-length, in addition to growth velocity expressed as gain in body weight per day.

Iron status

Under medical supervision, a 2-ml blood sample was drawn by qualified personnel from a superficial vein of the forearm of the infant, using sterile butterfly-gauge 23 syringes (Betcon Dickinson, Ontario, Canada). Blood collected in sterile vacuum tubes with EDTA was analysed for complete blood count using a Coulter counter (Seorno, Japan). A portion of blood was used for obtaining plasma which was kept frozen at -18°C until further analysis for the determination of plasma ferritin by radioimmunoassay with a commercial kit (Incstar, USA).

Infants at three or six months of age were considered to have anaemia if their haemoglobin fell below 9.5 or 10.5 g/dl, respectively [5]. The combined cut-off points that indicate iron-deficiency anaemia were based on the infant's age. At three months the cut-off points were haemoglobin < 9.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml. At six months they were haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml.

Statistical analysis

A two-tailed Student's *t* test, analysis of variance (ANOVA), and a two-sample binomial test were used to detect significant differences between the study groups.

Results

The haematological findings at six months of age are

presented in table 1. All red blood cell indices tended to be higher for female infants than for male infants, with statistically significant differences only for haemoglobin (11.5 ± 0.8 vs. 10.9 ± 1.0 g/dl) and mean corpuscular haemoglobin concentration (33.6 ± 1.0 vs. 32.8 ± 1.4 ; $p < .01$).

Table 2 shows that at six months of age, irrespective of sex, markedly more infants exclusively breastfed for four months were anaemic (haemoglobin < 10.5 g/dl) than those exclusively breastfed for five or six months (57%, 20%, and 11% of infants, respectively). Although differences between the latter two groups were not statistically significant, there is a clear tendency in favour of exclusive breastfeeding for six months rather than five months. However, even this tendency disappears

TABLE 1. Haematological values (mean \pm SD) at six months of age for infants exclusively breastfed for four to six months, according to sex

Value ^a	Boys ($n = 32$)	Girls ($n = 34$)
Hb (g/dl)	10.9 ± 1.02	11.5 ± 0.79^b
Hct (%)	33.1 ± 2.77	34.0 ± 2.56
MCV (fl)	74.9 ± 7.15	76.1 ± 5.01
MCH (pg/dl)	24.6 ± 2.71	25.5 ± 1.68
MCHC (%)	32.8 ± 1.38	33.6 ± 1.03^c
PF (ng/ml)	42.3 ± 40.69	53.2 ± 59.36
RBC (10^6 /ml)	4.4 ± 0.44	4.5 ± 0.37

a. Hb, Haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PF, plasma ferritin; RBC, red blood cell count.

b. $t = 2.6$, $p < .01$.

c. $t = 2.5$, $p < .01$.

TABLE 2. Percentage of exclusively breastfed infants who developed nutritional anaemia, iron-deficiency anaemia, and depletion of iron stores at six months of age, according to the duration of exclusive breastfeeding

Condition ^b	Duration of exclusive breastfeeding (mo) ^a					
	4 ($n = 14$)		5 ($n = 15$)		6 ($n = 37$)	
	No.	%	No.	%	No.	%
Nutritional anaemia	8	57 ^c	3	20 ^d	4	11 ^d
Iron-deficiency anaemia	4	29 ^c	0	0 ^d	0	0 ^d
Depletion of iron stores	6	43 ^c	0	0 ^d	3	8 ^d

a. Rows with different superscript letters differ significantly at $p < .005$.

b. Nutritional anaemia is defined by haemoglobin < 10.5 g/dl; iron-deficiency anaemia by haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml; and depletion of iron stores by plasma ferritin < 12 ng/ml.

in the case of iron-deficiency anaemia, since none of the infants exclusively breastfed for five or six months developed iron-deficiency anaemia.

Table 2 also shows the prevalence of depleted iron stores (plasma ferritin < 12 ng/ml) according to the duration of exclusive breastfeeding. None of the infants exclusively breastfed up to five months and only 8% of those exclusively breastfed up to six months had depleted iron stores. However, a markedly higher percentage (43%) of infants exclusively breastfed for four months had depleted iron stores ($p < .05$).

Table 2 also shows that at six months of age, the prevalence of nutritional anaemia was markedly higher than that of iron-deficiency anaemia among infants who were exclusively breastfed for four months (57% vs. 29%; $p < .05$). Similarly, the prevalence of nutritional anaemia after five months (20%) or six months (11%) of exclusive breastfeeding tended to be higher than that of iron-deficiency anaemia, which disappeared completely (0%) after five or six months of exclusive breastfeeding.

Table 3 shows the mean values of haemoglobin, plasma ferritin, and mean corpuscular volume at six months of age for infants exclusively breastfed for four to six months according to the duration of exclusive breastfeeding and iron status.

Growth performance, based on the limited sample size, was not adversely affected by nutritional anaemia or iron-deficiency anaemia. Indeed, none of the study infants were underweight, stunted, or wasted (data not shown).

Better iron status with regard to nutritional anaemia and iron-deficiency anaemia in female infants has also been observed in an ongoing study by Faqih and co-workers of 9- to 36-month-old Jordanian infants whose mothers attend the same Maternal and Child Health Centre at Wadi Srouf. Of the 51 girls in the study, 9.8% had nutritional anaemia and 7.8% had iron-deficiency anaemia, as compared with 21.7% and 17.4% of the 46 boys (unpublished data). None of the infants have participated in any other studies.

Discussion

The results presented in table 2 strongly suggest that in Jordan exclusive breastfeeding for five to six months has a better protective effect against the development of nutritional anaemia and iron-deficiency anaemia than a shorter period of four months of exclusive breastfeeding. About 29% of Jordanian infants who were exclusively breastfed for four months developed iron deficiency at six months of age, as compared with none of the infants who were exclusively breastfed for five or six months. The high bioavailability of iron in breastmilk, ranging from 50% to 70% [16] may partly explain these results. In addition, the earlier introduction of

TABLE 3. Haemoglobin, plasma ferritin, and mean corpuscular volume in infants exclusively breastfed for four to six months, according to the duration of exclusive breastfeeding and iron status^a

Duration (mo)	Iron status	No.	Hb(g/dl)		PF(ng/ml)		MCV(fl)	
			Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
4 (n = 14)	NA	8 (4 with IDA)	9.7 \pm 0.9	8.4–10.4	35.8 \pm 47.0	5.0–161.3	71.0 \pm 8.3	59.5–80.5
	ID	2	12.1 \pm 1.15	10.9–13.2	8.0 \pm 2.15	5.8–10.1	76.7 \pm 3.55	73.2–80.3
	No NA or ID	4	11.6 \pm 0.4	11.0–11.8	63.0 \pm 34.8	30.5–111.3	80.7 \pm 4.7	75.1–86.5
5 (n = 15)	NA	3 (0 with IDA)	10.0 \pm 0.6	9.3–10.4	72.0 \pm 71.5	17.5–153	70.5 \pm 5.2	64.7–74.7
	ID	0						
	No NA or ID	12	11.6 \pm 0.7	10.5–13.2	64.6 \pm 73.8	12.1–277.0	79.5 \pm 4.4	73.4–86.9
6 (n = 37)	NA	4 (0 with IDA)	10.2 \pm 0.1	10.1–10.4	61.2 \pm 61.3	26.4–152.9	74.6 \pm 3.0	72.0–77.6
	ID	3	11.5 \pm 0.8	10.7–12.2	8.2 \pm 2.3	5.7–10.1	71.3 \pm 3.6	67.4–74.5
	No NA or ID	30	11.5 \pm 0.6	10.5–13.3	54.6 \pm 48.9	13.7–187.2	77.4 \pm 5.2	67.6–88.3

a. Hb, Haemoglobin; PF, plasma ferritin; MCV, mean corpuscular volume; NA, nutritional anaemia (Hb < 10.5 g/dl); IDA, iron-deficiency anaemia (Hb < 10.5 g/dl, MCV < 70 fl, PF < 12 ng/ml); ID, iron deficiency (depletion of iron stores [PF < 12 ng/ml] but Hb > 10.5 g/dl).

solid foods associated with the shorter period of exclusive breastfeeding (four vs. five or six months) necessitates the ingestion of certain food factors that inhibit iron absorption, such as phytates in cereals and tannins in tea [2]. Bread and tea are consumed as weaning foods by Jordanian infants at quite an early age [17, 18]. In addition, according to their mothers, iron-fortified baby foods were not given to the study infants. Thus, in Jordan, where iron-fortified formula or cereals are not generally given to weanlings, especially in families of low to medium economic status, breastfeeding is the main preventive measure against iron-deficiency anaemia.

Upon further analysis, Dewey et al. [12] observed that none of the infants with birthweights over 3,000 g had depleted iron stores (plasma ferritin < 12 μ g/L) at six months of age, whether they were exclusively breastfed for six months or whether they were exclusively breastfed for four months, then given continued breastfeeding in addition to iron-fortified foods. Only 5.3% (1/19) of the exclusively breastfed infants had anaemia (haemoglobin < 10.3 g/dl), as compared with 18% (4/22) of those who were exclusively breastfed for four months, then given breastmilk plus iron-fortified foods. This suggests that exclusive breastfeeding for six months was protective against the development of anaemia in infants with birthweights over 3,000 g, irrespective of the fact that iron-fortified food was given to the other

group between four and six months of age. The results are consistent with our study, although we did not include low-birthweight infants.

Excluding the intake of iron-fortified weaning foods, which were not used in this study, typical weaning foods consumed by the Jordanian infants up to six months of age were conducive to iron deficiency at that age. This is further supported by the results of national [18] and regional [19] studies. Meat, poultry, or fish are given seldom, if at all, to Jordanian infants at six months of age, especially by poor families. For example, during the period from 8 to 24 months of age, about 54% of the infants never received any meat, and about 80% never received any infant formula [19].

Meat provides haem iron, which has a high bioavailability besides its enhancing effect on dietary non-haem iron [20]. In the UNICEF national study in Jordan, rice pudding was introduced in the second month; cooked rice, milk, yoghurt, and *labneh* (concentrated yoghurt with about 70% water) were introduced in the fourth month; and pasta and cereal grains were introduced in the sixth month at a rate of one to two servings per week. Lentils, broad beans, and chickpeas were introduced mainly in the fifth month.

More strikingly, Khatib and Hijazi [19] observed that the proportion of infants who had received the following foods during the fifth and sixth months (and during

the third and fourth months as given in parentheses) were: meats, 8.5% (1.6%); rice, 69% (56.2%); bread and biscuits 78.1% (52.4%); legumes, 43.5% (11.3%); eggs, 51.6% (33.3%); cow's milk and yoghurt, 65.0% (45.8%); vegetables, 47.6% (14%); tea, 2.0% (15.5%); juices, 62.4% (42.6%); and family dishes, 17.5% (6.2%).

In Jordan bread, biscuits, or crackers are soaked in tea and are eaten together as a weaning food [17]. The strong inhibitory effect of tea on iron absorption is well documented [9, 20–22]. This effect is expected to increase when tea is consumed with bread, which has a high phytate content. Currently, locally available wheat flour is not fortified with iron. Two types of wheat flour are produced: the “unified” type, with a low extraction rate of about 72%, and the “brown” type, with a higher extraction rate of about 80%. The brown flour is subsidized by the government and is sold at a lower price and consumed at a higher rate than the “unified” flour. This means a higher phytate intake, with more hindrance to iron absorption.

The high phytate content of lentils and the high polyphenol content of spinach, lentils, and eggplants is associated with poor absorption of non-haem iron [23]. Data on weaning foods in Jordan, in addition to our own experience, indicate that solid weaning foods introduced to infants between four and six months of age are also based on cow's milk: pudding, yoghurt, and *labneh*. Cow's milk is a poor source of iron. Eggs are also given as weaning foods in Jordan. They not only have little bioavailable iron but also reduce the bioavailability of food non-haem iron [20]. However, fruit juices and certain other vegetables, especially tomatoes, are available in Jordan at affordable prices. They enhance non-haem iron absorption due to their vitamin C content and thus ameliorate the inhibitory effect of the above-mentioned weaning foods. Otherwise, the prevalence of iron deficiency among the study infants might have been higher.

In infancy the main determinants of iron requirements are iron stores at birth, growth requirements, and the need to replace iron losses [7]. Dewey et al. [12] concluded that birthweight was a critical risk factor in the development of anaemia (low haemoglobin) and in the depletion of iron stores (low plasma ferritin). In our study, iron stores at birth were assumed to be the same among the study groups, since all infants were full term and of normal birthweight (> 2,500 g). Also, it was assumed that the iron status of their mothers during pregnancy was not low enough to have influenced the infants' iron stores at birth. This is presumably due to the fact that all pregnant women attending Jordanian Maternal and Child Health Centres, in particular the Wadi Srou Centre, routinely receive free iron sulphate and folic acid tablets and dietary counselling, starting during the first trimester. Furthermore, mothers who are diagnosed as anaemic during pregnancy are treated with iron and folic acid. The rela-

tion between maternal and infant iron status seems to be controversial when, at least, mothers are not severely anaemic during pregnancy. For example, a cross-sectional study of 47 paired samples at term was carried out in urban mothers of low social class in northern Jordan [24]. No correlation between maternal and cord serum ferritin was observed, even when two cut-off points for low maternal ferritin at the 50th and the 25th percentiles were examined. This is in agreement with the observations of Bhargara et al. [25] in low- to medium-income urban Indian mothers and with certain other studies [7, 26], but it is in conflict with others [27, 28]. Stekel [7] concluded that “It is only under unusual circumstances of severe iron deficiency in the mother that iron endowment of the newborn may be affected.” A correlation between the iron status of mothers and their infants during the first year of life was found in certain prospective studies [29–33], but not in others [34, 35].

The prevalence of anaemia among the mothers attending the Maternal and Child Health Centres in 1990 and 1991 was about 25% [36], a figure not much different from the national average of 29% for Jordanian women of child-bearing age in 1994 [37]. These figures suggest that Jordanian women have better iron status than women worldwide (an average of 37% are anaemic), African women (44% anaemic), or Asian women (45% anaemic) [38]. However, neither anthropometric measurements nor further in-depth analysis of the data from the Jordanian national study was carried out.

At birth the infant of normal weight has iron stores, in the form of 75 mg of ferritin per kilogram of body weight, that supply most of the requirements for iron during the first four to six months of life. Daily iron requirements to satisfy infant growth and basal losses are estimated at 0.5 mg of iron absorbed up to 6 months of age and at 0.9 mg of iron during the subsequent 6 to 12 months [7]. In this study, the growth velocity of infants three to six months of age was within the normal range of 15 to 20 g/day and did not differ significantly among the study groups. Therefore growth velocity should not be considered as a variable that might have confounded the results. Iron losses are mainly in the stools and due to occult blood provoked by cow's milk [39], enteric infection, and parasitic infestation (mainly hookworm and schistosoma, and to lesser degree giardia [40]). In Jordan the main parasites related to iron status are *Entamoeba coli*, giardia, and pinworms, but not hookworms, schistosoma, or malaria. In a 1994 UNICEF national study of 8- to 11-year-old schoolchildren, the prevalences of giardia, ascariis, *Hymenolepis nana*, and enterobius were 8.4%, 1.5%, 1.3%, and 1.9%, respectively [41]. *Entamoeba coli* was the most common, with a prevalence of 14.3%. Equally important, anaemia was not considered to be related to the presence of intestinal parasites. Likewise, parasites were not considered an important aetiological factor in the high

prevalence (65.5%) of anaemia (haemoglobin < 11 g/dl) that was observed in 6- to 12-month-old infants among the underprivileged Palestinians living in Jordan and served by the United Nations Relief and Works Agency [42]. We did not study intestinal parasites, but the results obtained suggest that exclusive breastfeeding until six months of age was, if anything, important against the negative effects that might have influenced the infant's iron status.

The fact that the prevalence of nutritional anaemia was always higher than that of iron-deficiency anaemia after four, five, or six months of exclusive breastfeeding strongly suggests that nutritional anaemia is caused by a deficiency of haematopoietic nutrients other than iron, such as folic acid, vitamin A, pyridoxine, vitamin B₁₂, copper, or protein [9, 10]. Further work is needed to determine which deficiencies are relevant under the local conditions of this study, which was limited to the region served by the Maternal and Child Health Centre of Wadi Srou in Amman.

Conclusions

The data presented in this study strongly suggest that exclusive breastfeeding for five or six months rather

than for four months is protective against the development of nutritional anaemia or iron-deficiency anaemia up to at least six months of age.

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Adolescent schoolgirls: Daily or weekly iron supplementation?

Renuka Jayatissa and Chandrani Piyasena

Editorial introduction

This paper was accepted after revision to help ensure that all adequately designed studies of weekly and daily iron supplementation of vulnerable groups are available for further peer review after publication. Although this study was too short to be an adequate test of preventive supplementation, there was no significant difference in haemoglobin response to weekly and daily iron. A greater response of ferritin to daily than to weekly iron in the short period of eight weeks is to be expected. However, for a long-term preventive programme, the slower ferritin response is not disadvantageous, since iron stores will slowly rise to desirable levels with several months of weekly supplementation [1]. With daily supplementation, there is a tendency to initially undesirable high serum ferritin levels that only gradually become normal.

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Abstract

In Sri Lanka 36% of all adolescents have inadequate iron intakes. Daily and weekly iron supplementation of 659 adolescent schoolgirls, divided into three groups, was studied in an eight-week double-blind trial. One group received 60 mg of iron, 250 µg of folic acid, and 100 mg

of vitamin C daily. The second group was given the same doses on a weekly basis. The third group was given a placebo. All of the participants were dewormed at the beginning of the study. Anaemia was more common among older adolescents. Haemoglobin levels increased significantly at the end of the study. The prevalence of anaemia was reduced from 25% to 9.5% by weekly supplementation and from 18.5% to 8.6% by daily supplementation. The difference in haemoglobin levels between the two groups receiving supplementation was not significant. The daily administration of iron produced a greater increase in serum ferritin than weekly administration. The unit cost of weekly supplementation was 3.24 SLR (Sri Lankan rupees), equal to \$US0.05. On the basis of these results, long-term weekly doses of iron are suitable for the prevention of iron-deficiency anaemia in adolescents. Use of the school as the administration channel ensures compliance.

Introduction

Iron deficiency is estimated to cause anaemia in two billion people worldwide, and another billion are iron deficient. Inadequate iron is the most prevalent nutritional deficiency. An iron-supplementation programme has the potential to prevent iron deficiency in substantial segments of the population, as demonstrated in other countries [1].

Adolescence has been defined by the World Health Organization (WHO) as the period between the ages of 10 and 19 years [2]. It is also a period of increased nutritional requirements. In addition to the increased iron needs of the expanding red cell mass and myoglobin in newly gained muscle tissue, adolescent girls require up to 15% more iron to compensate for menstrual blood losses [3]. The haemoglobin cut-offs used to define anaemia in adolescents over 12 years and under 12 years of age living at sea level are 12 and 11.5 g/dl, respectively [4]. The prevalence of anaemia was 36% among adolescents in Sri Lanka [5].

Preventing and correcting iron-deficiency anaemia among adolescents is urgent because of its negative con-

Renuka Jayatissa is affiliated with the Public Health Nutrition Unit in the London School of Hygiene and Tropical Medicine in London. Chandrani Piyasena is affiliated with the Nutrition Department in the Medical Research Institute of the Ministry of Health in Colombo, Sri Lanka.

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sequences, which include decreased immunity, increased morbidity, and impaired cognitive performance [6]. Adolescent girls are an important target group because they are future mothers, and they can often be reached relatively easily through schools. Although in principle prevention and treatment of nutritional iron deficiency is simple, i.e., increasing available iron through diet and preventing abnormal iron losses, achieving these goals is not easy. Regular provision of supplementary iron and folate tablets to adolescent girls from the onset of menses has proved to be effective [7]. However, the health infrastructure required for delivery of these supplements is often inadequate for the implementation of this approach.

The daily administration of 60 mg of iron results in a rapid decline in percent of iron absorbed. It also loads the intestinal epithelium with iron and often causes adverse gastrointestinal symptoms. Since the renewal time of the intestinal mucosa is five to six days, a similar weekly dose of iron should be efficacious, given adequate time and compliance, and side effects would be minimized. Large-scale programmes to combat iron deficiency among adolescents have seldom been attempted because of the costs of daily supplementation and the extra managerial burden for the health sector [8]. To improve the iron status of adolescents, alternative approaches that are less costly and less burdensome to the health sector are needed.

Supplementation using intermittent dosing schedules may offer such an alternative for large-scale programmes targeted to children. Studies in pre-school children [9], adolescents [10], and pregnant women [11] showed that intermittent iron supplementation was as effective in improving iron status as daily supplementation. Supplementation on a weekly instead of a daily basis decreases programme cost and might increase compliance. For these reasons, we tested the hypothesis that weekly iron supplementation would be as effective as daily iron supplementation under real-life community conditions in Sri Lanka when schools were used as the distribution channel.

Materials and methods

The study was designed as a double-blinded, placebo-controlled clinical trial. It was conducted in a girls' schools with classes up to year 10 in the district of Colombo, Sri Lanka. The girls were from 10 to 17 years of age and were distributed among five classes (years 6–10). Some schools with classes up to year 13 were closed during the study period. The sample size was calculated on the basis of the formula given by Pocock [12]. To achieve significant results at the 5% level with a 5% drop-out rate at a power of 90%, 231 girls were assigned to each group. Nine schools were selected randomly.

In each school, one grade from years 6 to 10 was randomly selected for the study. Three parallel classes from each school were studied. When three parallel classes of the same grade were not available in the same school, three consecutive classes were selected (e.g., years 6, 7, and 8). All selected classes in nine schools were invited to participate in the study. Subjects were excluded if they had chronic infectious diseases or cardiopathies, if they had taken supplements or medications containing iron during the previous month, or if they had a haemoglobin level less than 10 g/dl with a blood picture showing any other kind of anaemia. One class in each school in each treatment group (nine classes for each treatment), with a total population of 690 girls 10 to 17 years of age, was enrolled for the study.

The Institute's ethical review committee approved the study. The girls excluded because of blood findings indicative of an anaemia not due to iron deficiency were referred for further investigation. After permission had been obtained from the education authorities, the schools were visited and the principals and teachers of the selected classes were briefed about the study. The girls selected to participate received letters explaining the purpose of the study to obtain the consent of their parents. After written consent had been obtained, the fieldwork was carried out from September to December 1997.

Iron and folate tablets distributed by UNICEF were used in the study. Each tablet contained 60 mg of elemental iron and 250 µg of folic acid. Supplements and placebos had the same colour and shape and were not distinguishable by sight. Therefore, the girls, teachers, and interviewers were not aware of differences among the treatment regimes.

All the girls who participated in the study were given the specified supplementation regime for each school as follows. Those in class 1 (243 girls) were given a daily dose of ferrous sulphate (60 mg elemental iron) and 250 µg of folic acid in a combined tablet (iron/folate) and 100 mg of vitamin C five days per week, Monday through Friday. Those in class 2 (230 girls) were given the same dose of iron/folate and vitamin C, but only once a week on Monday, and they were given a placebo replacement for the iron/folate and vitamin C during the other four days. Those in class 3 (217 girls) were given the placebo replacement for iron/folate and vitamin C five days per week, Monday through Friday.

Fingerprick samples of blood were collected to measure the haemoglobin level by the HemoCue method. The instrument was standardized before each session. The readings were recorded immediately on the questionnaire. The samples were taken with disposable needles in the morning before the interval snack, when the participants were in a seated position. About 5% of the samples were duplicated, and the variability was 0.89 g/L.

In one school chosen at random, 5 ml of antecur-

bit venous blood was collected from each participant with minimum stasis to measure serum ferritin and haemoglobin levels. Disposable syringes and needles were used for venipuncture. The blood was collected in the specified bottle and transported to the laboratory within two to three hours. The serum was separated on the same day and refrigerated at 4° to 8°C. After the final blood collection, serum ferritin was determined in the samples by enzyme immunoassay using a commercial kit. Duplicate analyses were performed for 5% of the samples, and the variability was 0.54 µg/L. Control sera with a mean ferritin value of 31.2 ± 6.2 µg/L were provided by the manufacturer.

On the same day that the blood samples were drawn, height and weight were measured using standard anthropometric techniques. The electronic weighing scales (with precision up to 100 g and with a digital display) and anthropometric rods (with precision up to 10 mm) were used for all measurements. All the equipment was standardized each morning before the measuring session. The girls were measured in the morning without shoes. To detect inter- and intra-observer variability, duplicate measurements were obtained by the same measurer and recorder in 5% of the sample and by a different measurer and recorder in 10% of the sample; the variability was 0.3 to 0.5 cm for height and 0.1 kg for weight.

To eliminate a major source of variation among subjects, anthelmintic therapy, consisting of a single 500-mg dose of mebendazole, was given to all the girls on the first day, without prior screening.

The girls who participated in the study were given letters to be submitted to their family doctor or to any other doctor if they happened to seek treatment during the study. They were requested to avoid any additional iron or vitamin preparations.

A two-week supply of supplement and placebo tablets was issued to each class teacher. The tablets for each day were packed in a sealed polythene bag labelled with the date and the class. Weekends and public holidays were excluded. If a girl was absent, the teacher was asked to keep the packet and turn it over to the officer who visited the school next. The teachers administered the tablets at the time of the marking of attendance registers around 9 a.m. under direct supervision, to make sure the girl swallowed the tablets.

The teachers maintained the daily record sheets of the students and recorded whether the students were present or absent, the tablets administered, and any complaints. Written guidelines were given to each teacher. The schools were visited once every two weeks. At this time the tablets for the next two weeks were left and the balance from the previous two weeks was collected.

On the day after the last dose was administered, i.e., after eight weeks, another blood sample was collected. The participants who had had signs of vitamin deficiencies

on the previous examination were treated, and other medical conditions such as dental caries were referred for treatment. All the girls were given oral health education information on iron-deficiency anaemia, its prevention, and its dietary management. In addition, written information was distributed in the form of pamphlets.

Results

A total of 690 girls were enrolled in the study. The drop-out rate was 4.5%. The reasons for dropping out were side effects (16 girls, 52%), leaving school (10 girls, 32%), or doctor's advice not to take the tablet with other treatment (5 girls, 16%). Side effects were reported by 16 girls: 11 in the daily supplementation group and 5 in the weekly group. The side effects reported included constipation, sleepiness, abdominal pain, rash, and nausea. Usually the side effects became milder after a couple of days and eventually disappeared. In some cases, however, they caused the girls to discontinue treatment. The girls who dropped out did not differ significantly from those who remained in the study in weight, height, age, initial haemoglobin concentration, and other factors. The compliance was 100% on the days when the girls were in school, unless the girls were drop-outs.

Of the 659 girls who completed the study, 139 (21%) were anaemic. The numbers of girls in the weekly, daily, and placebo treatment groups were 220 (33%), 222 (34%), and 217 (33%), respectively.

Though the groups were randomly allocated by classes, not by individuals, the similarity of the subjects' characteristics among the groups was assessed by comparing the variables age, level of mother's education (completed in school), number of siblings, birth order, nutritional status (determined by body mass index), and age of menarche (table 1). The girls in the group that received daily supplementation were significantly younger than those in the other two groups.

The factors affecting the initial haemoglobin values were compared at the beginning of the study to detect any confounding effect of these factors on the supplementation (table 2). The girls were classified as anaemic or non-anaemic according to the age-specific cut-offs for initial haemoglobin values. The anaemic and non-anaemic groups did not differ in age, mother's years of education, number of siblings, birth order, nutritional status, and age of menarche.

Initial haemoglobin ($F = 0.46, p = .6$), final haemoglobin ($F = 1.6, p = .2$), and the change in haemoglobin ($F = 1.5, p = .2$) did not differ among the three treatment groups (table 3). Daily and weekly supplementation had a greater effect on haemoglobin levels than placebo.

The overall prevalence of anaemia at the start of the study was 21.1%, and there were no significant differences among the treatment groups in the initial hae-

moglobin levels (table 4). The prevalence of anaemia was reduced from 25% to 9.5% by weekly supplementation and from 18.5% to 8.6% by daily supplementation. In the placebo group the prevalence decreased from 19.8% to 13.4%.

The three treatment groups did not differ in initial

ferritin levels ($F = 0.51, p = .6$), but there were significant differences among the groups in final ferritin levels ($F = 3.67, p = .03$) and the change of ferritin levels ($F = 4.11, p = .02$) after supplementation (table 5). Ferritin increased most in the daily treatment group, much less in the weekly treatment group, and not at all in the placebo group.

TABLE 1. Characteristics of subjects according to treatment group^a

Characteristic	Weekly treatment (<i>n</i> = 220)	Daily treatment (<i>n</i> = 222)	Placebo (<i>n</i> = 217)
Age (yr) ^b	13.6 ± 1.3	13.1 ± 1.4	13.6 ± 1.1
Mother's education (yr) ^c	10.0 (8.0–11.0)	11.0 (9.0–11.0)	10.0 (8.5–11.0)
Body mass index (kg/m ²) ^d	17.4 ± 3.0	17.2 ± 2.9	17.3 ± 2.9
No. of siblings ^e	2.0 (1.0–2.0)	2.0 (1.0–3.0)	2.0 (1.0–3.0)
Birth order ^f	2.0 (1.0–2.0)	2.0 (1.0–2.0)	2.0 (1.0–3.0)
Age of menarche (yr) ^g	12.4 ± 1.1	12.2 ± 1.1	12.1 ± 1.4

a. Plus-minus values are means ± SD. Other values are medians (25th and 75th percentiles).

b. $F = 14.2, df = 2, 656, p = .000001$.

c. Kruskal-Wallis = 1.3, $df = 2, p = .5$.

d. $F = 0.4, df = 2, 656, p = .3$.

e. Kruskal-Wallis = 0.4, $df = 2, p = .8$.

f. Kruskal-Wallis = 1.6, $df = 2, p = .4$.

g. $F = 1.9, df = 2, 440, p = .2$.

TABLE 2. Characteristics of subjects according to initial anaemia status^a

Characteristic	Anaemic ^b	Non-anaemic
Age (yr) ^c	13.6 ± 1.2	13.4 ± 1.7
Mother's education (yr) ^d	11.0 (8.0–11.0)	11.0 (8.0–11.0)
Body mass index (kg/m ²) ^e	17.2 ± 2.7	17.3 ± 3.0
No. of siblings ^f	2 (1.0–3.0)	2 (1.0–2.5)
Birth order ^g	2 (1.0–2.0)	2 (1.0–2.0)
Age of menarche (yr) ^h	12.3 ± 1.1	12.2 ± 1.2

a. Plus-minus values are means ± SD. Other values are medians (25th and 75th percentiles).

b. Under 12 years of age, < 11.5 g/dl haemoglobin; 12 years of age or more, < 12.0 g/dl haemoglobin.

c. $F = 2.8, p = .09$.

d. Kruskal-Wallis = 0.02, $df = 1, p = .9$.

e. $F = 0.07, p = .8$.

f. Kruskal-Wallis = 3.2, $df = 1, p = .07$.

g. Kruskal-Wallis = 0.3, $df = 1, p = .6$.

h. $F = 0.2, p = .6$.

Discussion

All over the world, more than 1.6 billion people are affected with anaemia. More than half of these cases are preventable and treatable iron-deficiency anaemia [1]. Iron-deficiency anaemia in adolescent girls is associated with poor pregnancy outcomes and contributes to intrauterine growth retardation, low birthweight, increased maternal and perinatal mortality, and low iron stores of the infant at birth [13].

The average age at first marriage in Sri Lanka is 24.4 years [2]. One-third of the adolescents in Sri Lanka suffer from anaemia [5]. When there is a high prevalence of anaemia in the population, supplementation will be reduced to treat the most severely affected individuals. Therefore, the control of anaemia in adolescence is the need of the day [14].

Because the teachers gave the tablets to the girls, compliance was high. Side effects do not appear to have played a major role in lack of compliance in taking iron tablets. It should also be noted that drop-outs due to side effects were higher among the daily group than the weekly group. This finding is comparable to that from a study in Indonesia [10].

At the start of the study, the overall prevalence of anaemia was 21.1%. Supplementation with iron, folic acid, and vitamin C for eight weeks caused a rise in haemoglobin concentration and a fall in the prevalence of anaemia in both the weekly and the daily supplementation groups. When the groups receiving daily and weekly supplementation were compared, no difference in treatment effect on haemoglobin concentrations was

TABLE 3. Mean ± SD haemoglobin concentration (g/dl) before and after eight weeks of treatment

Treatment group	Before treatment ^a	After treatment ^b	Change ^c
Weekly (<i>n</i> = 220) ^d	12.8 ± 1.3	13.1 ± 1.0	0.4 ± 1.2
Daily (<i>n</i> = 222) ^e	12.9 ± 1.1	13.2 ± 0.9	0.4 ± 1.1
Placebo (<i>n</i> = 217) ^f	12.9 ± 1.1	13.1 ± 1.1	0.2 ± 1.2

a. $F = 0.46, p = .6$.

b. $F = 1.6, p = .2$.

c. $F = 1.5, p = .2$.

d. $t = 4.9, df = 219, p = .00000$.

e. $t = 5.2, df = 221, p = .00000$.

f. $t = 2.8, df = 216, p = .005$.

TABLE 4. Prevalence of anaemia^a [no. (%)] before and after eight weeks of treatment

Treatment group	Before treatment ^b		After treatment ^c	
	Anaemic	Non-anaemic	Anaemic	Non-anaemic
Weekly (<i>n</i> = 220)	55 (25.0)	165 (75.0)	21 (9.5)	199 (90.5)
Daily (<i>n</i> = 222)	41 (18.5)	181 (81.5)	19 (8.6)	203 (91.4)
Placebo (<i>n</i> = 217)	43 (19.8)	174 (80.2)	29 (13.4)	188 (86.6)
Total	139 (21.1)	520 (78.9)	69 (10.5)	590 (89.5)

a. Under 12 years of age, < 11.5 g/dl haemoglobin; 12 years of age or more, < 12.0 g/dl haemoglobin.

b. $\chi^2 = 3.15$, *df* = 2, *p* = .21.

c. $\chi^2 = 3.0$, *df* = 2, *p* = .2

found. In a subsample, daily administration of iron produced a significantly greater increase in serum ferritin than weekly supplementation. These findings compare well with those of similar studies in other countries [9, 15, 16].

Besides the highly significant increase in haemoglobin in the iron-supplemented groups, there was a small but significant increase in haemoglobin in the placebo group. However, there was no increase in ferritin level in the placebo group; the mean ferritin concentration actually decreased by an average of 2.8 µg/L. The rise in haemoglobin in the placebo group may have been the result of the deworming. A positive effect of deworming on haemoglobin status was reported previously in a study of pregnant women on a Sri Lankan plantation [17].

A significantly higher proportion of older adolescents were anaemic. Although the prevalence of anaemia was markedly reduced by supplementation for eight weeks, 10.5% of all the girls remained anaemic. Whether a more prolonged supplementation would further reduce the prevalence of anaemia needs to be investigated. It is possible that adding other nutrients, such as vitamin A [10], would improve the results.

The unit cost of weekly supplementation, including anthelmintic treatment, was 3.24 SLR (Sri Lankan rupees), equal to \$US.05. This was one-quarter the cost of daily iron supplementation.

The following conclusions can be drawn. In this population of mildly anaemic girls (all of whom were treated for possible hookworm infection), there was only a small response to iron supplementation. The effects of weekly and daily supplementation were not distinguishable. Weekly supplementation is an economically advantageous and simple intervention to improve the haemoglobin status of adolescent girls. Weekly supplementation generated fewer complaints of side effects, and compliance was high. The greater rise in serum ferritin with daily administration of iron is of no practical

TABLE 5. Mean ± SD serum ferritin level (µg/L) in a subsample of subjects before and after eight weeks of treatment^a

Treatment group	Before treatment ^b	After treatment ^c	Change ^d
Weekly (<i>n</i> = 9) ^e	66.6 ± 32.9	82.7 ± 34.8	16.1 ± 21.3
Daily (<i>n</i> = 22) ^f	54.1 ± 26.7	92.3 ± 39.9	38.2 ± 41.1
Placebo (<i>n</i> = 11) ^g	59.1 ± 34.9	56.3 ± 30.4	-2.8 ± 31.2

a. Log transformation was done before the statistical analysis.

b. *F* = 0.51, *p* = .6.

c. *F* = 3.67, *p* = .03.

d. *F* = 4.11, *p* = .02.

e. *t* = 2.27, *df* = 8, *p* = .05.

f. *t* = 4.36, *df* = 21, *p* = .0003.

g. *t* = 0.29, *df* = 10, *p* = .77.

significance as long as the preventive supplementation programme is continued. Experience from other studies indicates that even iron stores, as indicated by serum ferritin, reached similar levels with daily and weekly iron supplementation [18]. Moreover, the initially high ferritin levels with daily iron administration may be disadvantageous [19]. Administration of iron supplementation by teachers without close medical supervision is a viable possibility. A weekly iron-supplementation programme aimed at controlling anaemia among adolescents should be encouraged. It is recommended that preventive iron supplementation be provided through schools on a long-term basis.

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Increase in compliance with weekly iron supplementation of adolescent girls by an accompanying communication programme in secondary schools in Dar-es-Salaam, Tanzania

Grace S. Muro, Ursula Gross, Rainer Gross, and Lely Wahyuniar

Abstract

Insufficient compliance has been identified as a major contributing factor to the low effectiveness of iron-supplementation programmes. An experimental community trial was conducted to observe the effect of a communication programme on compliance with weekly iron supplementation in urban Tanzanian adolescent schoolgirls. A sample of 237 girls aged 14 to 17 years was randomly recruited from five schools in Dar-es-Salaam, and randomly assigned to three groups. Group A (schools 1 and 2) received one tablet weekly containing 65 mg of elemental iron with 0.25 mg of folic acid for eight consecutive weeks and participated in weekly communication sessions. Group B (school 3) received the same supplementation without communication sessions. Group C (schools 4 and 5) served as the control group, without supplementation or communication. Reported and observed compliance was checked by pill counting and stool analysis. Haemoglobin levels were determined by the cyanmethaemoglobin method before and after intervention. A knowledge test on iron-deficiency anaemia (causes, effects, and treatment) was carried out before and after intervention. Venn diagrams were drawn to identify the most influential persons. Three focus group discussions with the girls and the teachers were conducted at different stages during the study. In the group receiving supplementation and communication, the prevalence of anaemia decreased significantly ($p < .001$) from 49% to 5% in school 1 and from 54% to 23% in school 2. The prevalence of anaemia did not change significantly in schools 3 and 4 but increased significantly in school 5. The reported and observed compliance was

90% and 94%, respectively, in school 1, 89% and 75% in school 2, and 48% and 50% in school 3. The participants' knowledge of iron-deficiency anaemia increased significantly in all schools after the intervention ($p < .001$) but was highest in schools 1 and 2, which received supplementation and communication. It is concluded that comprehensive communication strategies can influence the ingestion of iron supplements and therefore help to reduce anaemia in adolescents.

Introduction

Iron-deficiency anaemia is prevalent throughout the world, particularly in developing countries [1, 2]. It has been calculated that nearly 1% of the gross domestic product of the developing countries is lost because of reduced productivity and cognitive performance caused by iron deficiency [2]. In Tanzania the prevalence of anaemia in pregnant women and children under five years of age is as high as 80% and 45%, respectively [3]. The prevalence of anaemia in women increases during adolescence because of the growth spurt and menstrual losses [4, 5], coupled with inadequate diet [6] and often aggravated by infectious diseases such as malaria, schistosomiasis, and hookworm infection [7].

The cornerstone intervention in health service for iron deficiency has been supplementation of pregnant women and children with iron tablets, but the effectiveness of this approach so far has not been encouraging [8, 9]. One reason for the poor results is that during the relatively short period of fast growth, particularly during pregnancy and infancy, iron requirements are very high. Thus it is not always possible to absorb an adequate amount of supplementary iron, especially for those women who are iron deficient before pregnancy. Trying to catch up on iron intake during pregnancy is too little, too late [10].

One strategy is to secure an adequate iron status before pregnancy. Adolescent girls are an important target group, since their high iron requirements lead to depleted iron stores even before pregnancy [5, 9].

Grace Muro is a staff member of the Tanzania Food and Nutrition Centre in Dar-es-Salaam. Ursula Gross and Rainer Gross are currently visiting professors in the Department of Nutrition at the Faculty of Public Health, University of São Paulo, Brazil. Lely Wahyuniar is a staff member of the SEAMEO-TROPED Regional Center for Community Nutrition at the University of Indonesia in Jakarta.

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Targeting adolescent girls not only would have an immediate curative effect but also might have a longer-term preventive effect on iron deficiency during pregnancy [11]. In many societies, girls can be reached relatively easily through schools, and an improved and secured iron supply can be achieved by periodic supplementation under supervised conditions [7, 10]. Muhilal et al. [12] reported that 12 weeks of supervised supplementation with iron tablets reduced the prevalence of anaemia by an average of 24% in schoolchildren. Brabin and Brabin [7] recommended that health services switch from curative to preventive iron supplementation and target adolescent girls rather than children or post-adolescent and pregnant women. In this way a greater impact on reproductive health and reproductive success could be achieved than by supplementation during pregnancy alone. Supervised supplementation at schools and work sites ensures that the iron tablets are properly used, and potential gains in work productivity from correction of iron-deficiency anaemia can justify the investment for such an effort [9].

More recently, weekly iron supplementation has been recommended for prevention. In particular, satisfactory efficacy was shown in adolescent girls in Indonesia [13]. Weekly administration has the advantage of fewer side effects [14] and lower costs [15], and this approach therefore seems to be more efficient than daily supplementation. Nevertheless, independently of the method of administration of iron supplements, adequate information and motivation has to be an essential part of supplementation programmes [9].

In a plantation in Sri Lanka, Atukorala et al. [16] found that compliance could be increased if both the beneficial effect of supplements and the possible adverse side effects were clearly explained. Studies of nutrition education for school-aged children in the United States [17] found that intervention was effective if it was relevant and behaviourally focused and if sufficient time was devoted to the education. Contento et al. [18] reported that interventions that focused on specific behaviours resulted in more behavioural changes than general nutrition education programmes. Lytle [17] showed that nutrition education interventions were more likely to be effective when they employed educational strategies that were directly relevant to a behavioural focus and were derived from appropriate theory and research.

In summary, focused communication programmes could increase compliance with oral iron supplementation and, therefore, the effectiveness and efficiency of such programmes for adolescent schoolgirls. Nevertheless, despite this commonly accepted concept, there is little scientific evidence available on motivational programmes for iron supplementation in adolescent schoolgirls in developing countries. This study was conducted to evaluate weekly iron supplementation strategies in Tanzanian adolescent schoolgirls, with and without an

accompanying communication programme, for compliance and effectiveness.

Subjects and methods

Sample selection

The study was carried out between August and November 1996 in Dar-es-Salaam, Tanzania. All five public secondary schools with adolescent schoolgirls within the city centre participated in the study. Prospective participants were randomly selected from the different classes and age groups of the identified schools. Girls were eligible if they were between 14 and 17 years of age and had already had their menarche. Written informed consent from the girls and their parents was required. Both anaemic and non-anaemic girls were included. Malaria was not a factor of exclusion, because this disease is endemic in the region. However, girls suffering from infections with fever at the time of the interview were excluded. According to sample size calculation, 240 girls should have participated in the study [19]. However, in the end, a total of 237 subjects from a potential roster of 3,517 participated in the study, mainly because of consent problems (missing parental approval).

The study population consisted of three groups. Group A received supplementary iron with a communication programme, group C received supplementary iron without a communication programme, and group B received no intervention. Each group should have contained a similar number of girls. However, this goal was not reached, because after the schools had been randomly assigned to the three groups, the parents of the girls in school 4, who were assigned to receive iron supplementation without communication, did not approve of their daughters receiving iron tablets. They only allowed measurement of haemoglobin concentrations and anthropometric data. Hence, the girls from this school were added to the non-intervention group. Table 1 shows the final number of girls in each group. Seventy-eight girls were enrolled in group A (schools 1 and 2: iron supplements along with communication sessions), 39 girls in group B (school 3: iron supplementation without communication sessions), and 120 girls in group C (schools 4 and 5: neither iron supplementation nor communication sessions). Furthermore, the intended equal representation of each grade (grades 1–3) was not reached because of restrictions on participation. In school 2, none of the students in the second grade received parental permission to participate in the study, and the majority of the third-grade students in schools 3 and 4 were more than 17 years old. There was no immediate remedy to the inconsistency, since the school year was coming to an end. An operational framework of the study is depicted in figure 1, showing the interventions performed in the three groups.

TABLE 1. Number of participants according to treatment, school, and grade

Treatment	School	Grade	No. of participants		
			Grade	School	Treatment
A: Intervention (iron + communication)	1	1	17	39	78
		2	12		
		3	10		
	2	1	20	39	
		2	–		
		3	19		
B: Intervention (iron only)	3	1	23	39	39
		2	13		
		3	3		
C: Control (no intervention)	4	1	16	40	120
		2	17		
		3	7		
	5	1	27	80	
		2	27		
		3	26		

Haemoglobin measurements

The haemoglobin concentrations of all 237 subjects were measured twice, at baseline and after the eighth week of intervention. Blood was obtained by skin puncture of a left-hand finger using an automatic skin puncture device (Autoclix-Lancet, Boehringer, Mannheim, Germany.) Haemoglobin level was determined by the cyanmethaemoglobin method [20] with the Compur Minilab (Bayer Diagnostic, Munich, Germany).

Distribution of iron supplements

Once weekly, each girl in groups A and B received an iron pill containing 65 mg of elemental iron in the form of iron sulphate and 0.25 mg of folic acid. For girls in group A (schools 1 and 2), the trained class teachers distributed the pills during the additional communication sessions. Group B subjects also received the pills from the classroom teachers. All girls were instructed to take the pills after school, so the teachers were not able to observe the girls taking the pills. A Supplementation Record Book was provided to each class leader for the weekly collection of information about ingestion of the tablets, eventual side effects, illnesses, and absenteeism throughout the whole supplementation period.

Group C did not receive iron pills or participate in the communication sessions during the eight-week period. At the end of the study, all girls who were still anaemic, including those in the control group, received iron pills with detailed explanations as to why and how to take them.

Focus group discussions

In schools 1 and 2 (group A), focus group discussions were held with the girls on two occasions, and a third focus group discussion took place with the two teachers and the two headmasters of these schools.

Before the intervention began, focus groups were organized in schools 1 and 2. Each school had four groups, two for girls 14 and 15 years old and two for girls 16 and 17 years old, for a total of eight groups. Each group had 9 or 10 randomly chosen members.

The first round of focus group discussions aimed to identify gaps in the girls' knowledge and vocabulary related to iron-deficiency anaemia. This information was used for the development of materials, teacher training, and the formulation of a pre- and post-knowledge test. During the first round of focus group discussions, Venn diagrams [21] were built up. A Venn diagram (John Venn, English logician, 1834–1923) uses circles and rectangles to present various types of mathematical sets and to show the relationships among them. In a Venn diagram, separate sets may be represented by two or more separate circles and overlapping sets by two or more overlapping circles [22]. Venn diagrams are currently used in qualitative research (e.g., focus group discussions) to identify social relationships. Different persons and institutions are represented as circles or rectangles, and the importance of a person or institution to the target is represented by its distance from the target (in this case, adolescent girls) [21]. The shorter the distance, the greater the importance. In this study, a pre-identified list of potentially influential persons

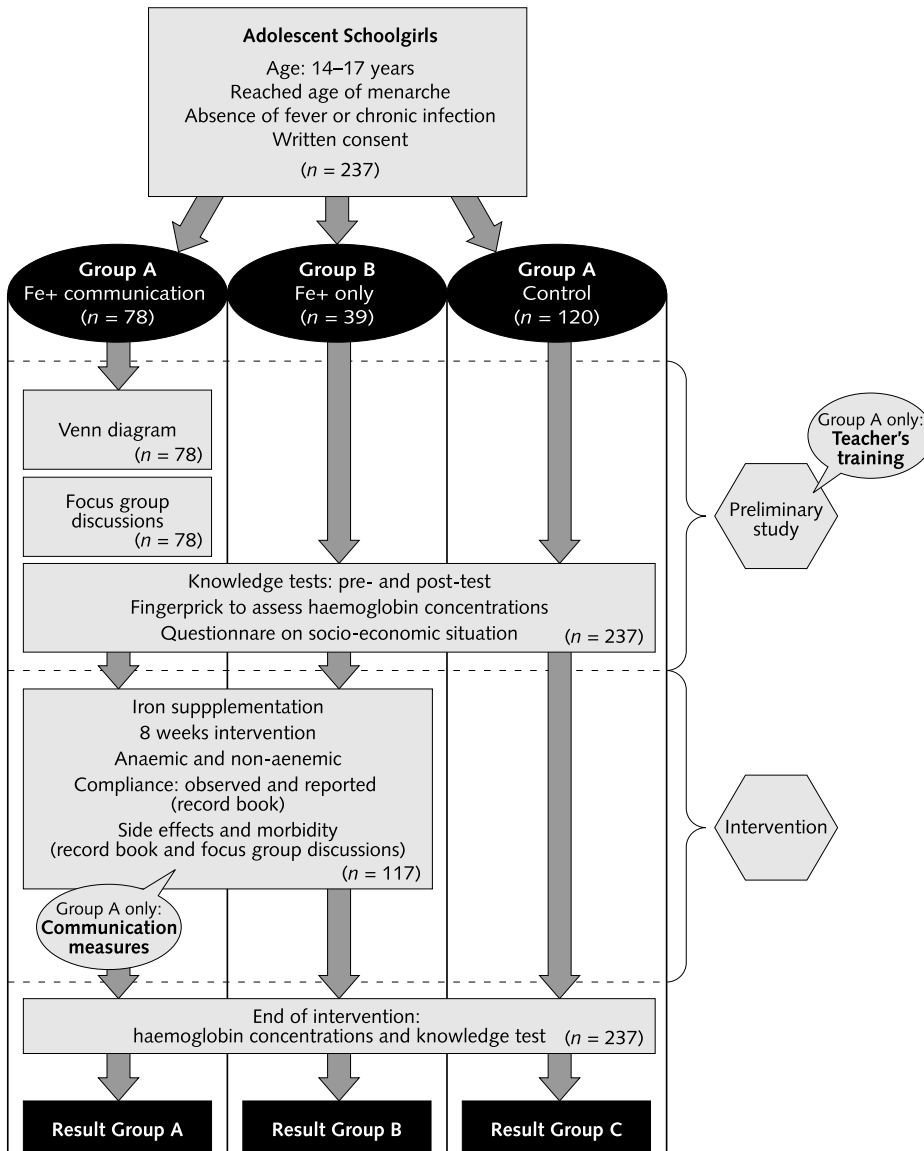


FIG. 1. Operational framework

was provided to the girls. Since this list was not exhaustive, the girls added persons from their family or the social environment whom they trusted and from whom they would seek advice. The purpose of the Venn diagram was to identify the most influential persons whom the girls trusted and listened to. The diagram was drawn on paper by the girls of the focus group. The distance between the indicated persons and the students represented the intensity of their relationship. The distance was measured with a tape and recorded for further statistical analysis. An example of a Venn diagram is shown in figure 2.

A second round of focus group discussions (six groups) was held with the girls in both schools after

four weeks of intervention. The first objective was to learn the reasons for the participants' intention to continue the iron supplementation. The second objective was to learn about potential barriers to continuation and ways to overcome them. Schoolgirls who had experienced side effects were purposely included in the discussion groups. The rest of the participants were randomly selected and equally distributed among the three groups in the respective schools. The third objective was to discuss eventual reactions from the girls' parents.

After the end of the intervention, another focus group discussion was held with the two school heads and the four implementing teachers in the two schools

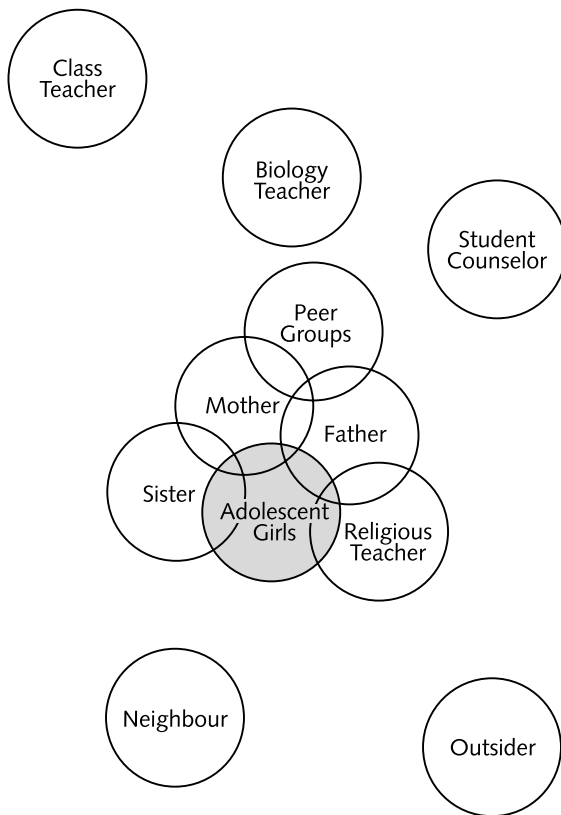


FIG. 2. Venn diagram showing most influential persons to 14- and 15-year-old adolescent girls

of group A. The discussions were focused on the implementation of and possible improvements to the pilot supplementation programme.

Communication sessions

During the intervention period, weekly communication sessions were conducted with the girls of group A, before distribution of the tablets. The sessions lasted approximately 45 to 60 minutes and were implemented by the trained teachers. The training of the four teachers (one designated teacher and her substitute from each school of group A) aimed to increase their knowledge of iron-deficiency anaemia and its control. The teachers were provided with two posters, one booklet, and one brochure on anaemia. During the communication session, all girls in group A received the same educational materials, with the exception of the posters. Motivation and commitment-building strategies were applied by the teachers to increase the girls' knowledge and to initiate a change in their attitudes. Technical knowledge was also imparted through a short play and a poem on anaemia that were each performed once at each school of group A.

Compliance

"Compliance" was defined as the proportion of distributed pills that were consumed by the subjects. Thus, during an eight-week period, the consumption of eight pills would represent 100% compliance.

"Reported compliance" was the percentage of subjects who reported that they received and ingested the iron supplement. This information was documented in the Supplementation Record Book kept by the class leader.

"Observed compliance" was the proportion of subjects who had a positive stool test related to iron ingestion. Stool samples were obtained during the third and fifth weeks of intervention on the day after the pills were distributed. Half of the girls in the supplementation groups were randomly chosen to provide a sample during the third week, and the other half provided a sample during the fifth week. The girls were provided with small plastic containers for the samples. The test for ingested iron was performed by the method of Afifi et al. [23].

Side effects

Information on possible side effects was collected from the Supplementation Record Book throughout the whole supplementation period. The aim was to record all negative side effects as well as feelings of well-being perceived by the participants. The rate of reported side effects in group A was higher, possibly because this group had received information about possible side effects. Furthermore, the list of side effects was used during the second focus group discussion to find out if the reported side effects could have been related to lower compliance or to higher drop-out rates.

Personal questionnaire

Each girl completed an additional questionnaire that asked for information on menstrual periods, socio-economic status, environmental sanitation, and personal hygiene. The classification of the socio-economic status of a family was based on the income of the father. However, because of difficulties in measuring income, the father's occupation was used as a proxy for income. The cluster of high-income fathers consisted of professionals such as administrators, accountants, engineers, and high-ranking officers. The middle-income fathers included teachers, technicians, clerical officers, etc. Low-income fathers were employed as cleaners, messengers, and drivers.

Pre- and post-knowledge test

A 25-question pre-knowledge test was conducted during the first week in all groups to measure the students'

knowledge of the causes, effects, and treatment of anaemia. The percentage of correct answers was used for statistical evaluation. The test was repeated after the intervention to measure increases in knowledge.

Statistical analysis

Data analysis was carried out using SPSS/PC+ version 4.0 (SPSS, Chicago, Ill, USA). For normally distributed data, the differences between and within treatment groups were compared with unpaired and paired *t* tests, respectively. Non-parametric tests were used when values were not normally distributed, as for haemoglobin levels after intervention and differences between initial and final haemoglobin. Differences between groups were tested by the Mann-Whitney *U* test or the Kruskal-Wallis *H* test and differences within groups by Wilcoxon's matched-pairs signed-rank test. The chi-squared test was used to test for associations between variables. Differences were considered statistically significant when $p < .05$.

Ethical considerations

The ethical guidelines of the Council for International Organizations of Medical Sciences [24] were followed. Informed consent was collected from all persons involved, including school heads, teachers, schoolgirls, and parents. The Ethical Review Committee of the Regional SEAMEO-TROPED Center for Community Nutrition at the University of Indonesia in Jakarta and the Tanzania Food and Nutrition Research and Ethics Committee approved the research proposal.

Results

Socio-economic status

Family income was classified according to the father's occupation. Of the 237 girls studied, 44% came from high-income, 32% from middle-income, and 25% from low-income families. There were no significant differences among the schools in the distribution of income levels.

Reported compliance

The reported compliance in school 1 was high (fig. 3a). Among 39 participating girls in school 1, 34 (87%) received the first pill; 5 girls did not want to take the supplement. The lowest compliance (69%) was observed at the administration of the second tablet. The main reason for the drop in compliance was that the parents increasingly disapproved of their daughters' participation in the study after a letter in a local newspaper had claimed that the tablets were contraceptives. Compli-

ance increased to 90% with the third supplement after the parents received additional information sessions regarding the content of the iron tablets and the benefit of their impact. Furthermore, the researcher reassured the implementing teachers, as well as the schoolgirls, that the supplements were not contraceptives. The reported compliance was 100% for the fourth and fifth pills but decreased for the sixth, seventh, and eighth pills (95%, 90%, and 85%, respectively). This decrease was due to the absence of the girls as they prepared for their final examinations. There were no more formal classes, which made it difficult to assemble the girls for the communication sessions and the distribution of pills.

The reported compliance in school 2 was satisfactory, particularly with the first, second, third, sixth, and seventh supplements (fig. 3b). In this school no initial refusal due to the letter in the newspaper could be observed. The decreased compliance with the fourth (74%) and fifth (59%) tablets was due to the late arrival of the teacher responsible for the communication and distribution session, so that some of the girls left school without receiving the supplement. The decreased compliance with the eighth pill (90%) was due to absenteeism of the girls for the same reasons as in school 1.

The reported compliance in school 3 (iron only) was very low compared with that in the other two schools (iron plus communication)(fig. 3c). The percentage of participants who reported having ingested the tablets ranged from 18% to 90% per pill distribution day. Only 56% took the first supplement. The rest of the girls refused to take the tablets from the teacher, although they had signed consent letters. Also in this school, because of the negative newspaper publicity, the girls and their parents feared that the pills were contraceptives. The coverage was even lower for the second pill (28%). After the girls were reassured by the researcher and the teacher that the pills were not contraceptives but contained iron to reduce anaemia, the willingness to take the third iron pill increased to 67%. The administration of the fourth pill had a reported compliance rate of 90%. Since the schools were located within walking distance of each other, a spillover of information from school 2 could not be avoided. The girls in school 3 anticipated the day that the communication activity (role playing) that had taken place in school 2 would take place in their school. Since this did not happen, the reported compliance decreased to 51% for the fifth and sixth tablets, 28% for the seventh, and 18% for the eighth.

In school 3 the teacher and her deputy had not been previously trained for further motivation sessions. Therefore they had no additional perceptions to transmit to the girls about the importance of reducing anaemia through iron supplementation. Lack of commitment of the implementing teacher and her deputy may explain the increasing absenteeism of the girls in school 3. Reported compliance decreased from 51% in the

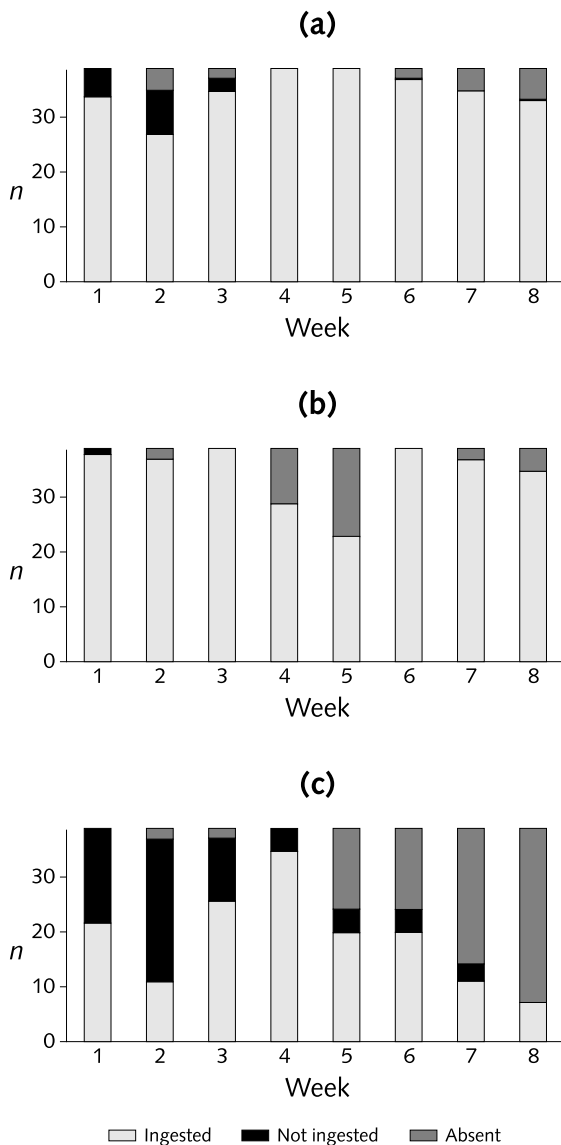


FIG. 3. Reported compliance in school 1 (a), school 2 (b), and school 3 (c)

fourth week to only 18% in the eighth week. An additional reason for absenteeism was preparation for final examinations. However, despite the examinations, the reported compliance in group A (iron plus communication) during the eighth week remained 85% and 90% in schools 1 and 2, respectively.

Observed compliance

The observed compliance during the third week, as indicated by positive iron stool tests, was 100% in school 1, 93% in school 2, and 60% in school 3. In the fifth week, the observed compliance declined to 87% in

school 1, 56% in school 2, and only 40% in school 3 (iron without communication). The reason given by the girls whose samples tested negative was that they forgot to take the tablets.

Negative and positive effects perceived by participants

Five girls in school 1 (13%) and three in school 2 (8%) reported increased appetite. Four girls, one each from schools 1 and 2 and two from school 3 (iron only), reported stomach irritation. Several students in all three schools reported an increase in activity and less sleepiness, and none complained about negative side effects such as nausea and vomiting. School 3 had the highest percentage of girls who did not report any effects (69%). However, in schools 1 and 2, in which communication sessions were conducted, 52% and 46%, respectively, reported no side effects.

Haematological response

There were no significant differences among the groups in mean baseline haemoglobin. After eight weeks of intervention, the haemoglobin level increased significantly only in the iron plus communication groups ($p < .001$, Wilcoxon's paired t test), whereas it decreased significantly in the control group. The difference in haemoglobin between groups A (iron plus communication) and C (control) was significant ($p = .04$, Mann-Whitney U test).

Table 2 shows the impact of the intervention on anaemia. At the beginning of the study, the overall prevalence of anaemia was 48%, ranging from 30% in school 5 to 70% in school 4. After intervention the prevalence of anaemia decreased significantly to 5% in school 1 and 23% in school 2 ($p < .001$, χ^2 test).

Pre- and post-knowledge test

To measure increases in knowledge during the intervention, the same test of knowledge was administered in all five schools (table 3). After eight weeks, the mean knowledge scores in all schools increased significantly as compared with the pre-test scores ($p < .001$, Wilcoxon test). The greatest increases in knowledge occurred in schools 1 and 2, with median increases of 25% and 10%, respectively). The amount of increase in knowledge differed significantly among schools ($p < .001$, Kruskal-Wallis test).

Venn diagram

Six focus groups (10 participants per group) from schools 1 and 2 identified the most influential persons whom the girls trusted and whom they would follow or listen to. The Venn diagrams consistently recorded

TABLE 2. Number and percentage of schoolgirls with anaemia before and after intervention according to school

School	Total no.	Anaemic before intervention		Anaemic after intervention	
		No.	%	No.	%
1: Iron + communication	39	19	49	2	5 ^a
2: Iron+ communication	39	21	54	9	23 ^a
3: Iron	39	21	54	21	54
4: Control	40	28	70	28	70
5: Control	80	24	30	27	34
Total	237	113	48	87	37

a. $p < .001$, χ^2 test.

significantly larger distances for people in the social environment than for people in the family environment ($p = .001$, unpaired t test). The parents (father, mother, or both) emerged as the most powerful persons.

Discussion

The aim of this study was not to show the efficacy or physiological effectiveness of iron supplementation, which would require strict supervision of the intake of the iron pills. The objective was rather to study the effectiveness, which includes compliance with the iron supplement intake. In particular, the effect of communication intervention was assessed, using the involvement of schoolteachers in the distribution of tablets and the information and motivation sessions.

Two major factors influence compliance with supplementation programmes: the availability of the tablets and the willingness of the participants to ingest them. Comprehensive communication should address both factors simultaneously.

Effective comprehensiveness of the communication programme was indicated by the fact that in the two schools with communication interventions (group A), the prevalence of anaemia was significantly reduced in a relatively short time. In school 3, in which no training of the implementing teachers took place, reported and observed compliance were much lower, and therefore iron supplementation had no observed effect. The distribution of iron supplements alone did not guarantee the reduction of anaemia in the girls.

Compliance with the distribution of tablets

Several measures were undertaken to guarantee the participation of the school staff. The headmasters were important for the success of the supplementation measures. They were essential for the administrative support of the distribution of supplements. Their status as the highest authority of the schools facilitated the

promotion of the supplementation programmes with the teachers, parents, and schoolgirls.

In discussions it became obvious that the involvement of natural science teachers was particularly helpful because of their basic knowledge of physiology, nutrition, and health. Therefore, these teachers should be trained to implement a supplementation programme. The training and motivation of teachers is important in bringing about a multiplier effect of an iron supplementation programme among adolescent schoolgirls. A well-planned communication strategy applied to secondary school students is a precondition for high compliance, which leads to high effectiveness of iron supplementation.

The intake of iron supplement in the school creates severe ethical and practical problems, particularly if the supplement has to be taken in the presence of a teacher. In the long run, voluntary intake after motivational strategies seems to be more effective than supervised intake. In this intervention, the girls perceived unsupervised ingestion of the iron supplements as "becoming an adult." They described tablet ingestion as "cool," and a certain peer pressure was created.

Compliance of schoolgirls

According to the Venn diagrams, parents had the most

TABLE 3. Summary of pre- and post-knowledge test performance for all schools

School	<i>n</i>	Median % correct answers		
		Before	After	Difference ^a
1	39	41	58	25
2	39	45	58	10
3	39	53	63	6
4	40	29	38	7
5	80	49	52	3

a. All before–after differences are significant ($p < .001$, Wilcoxon test).

influence on the girls. This fact was not considered at the beginning of the study. The denial of this fact created severe problems, since the parents were suspicious that the girls would receive not iron supplements but contraceptives. Only after intensive additional information and communication were provided to the parents did they give permission for their daughters to participate in the study. Parents must be included as communication channels and act as motivators to bring about and maintain a high compliance rate. For the identification of interpersonal channels, the Venn diagram proved to be an appropriate instrument.

Side effects

Negative side effects of taking iron are the most frequently suggested explanation for non-compliance with iron regimes [25]. In a study by Bonnar et al. [26], one-third of the women did not take their iron prophylaxis because of the fear of gastrointestinal side effects, although only 3% of these women actually reported to have experienced such upset.

The results of our study showed that awareness of possible side effects did not frighten the adolescents, as mentioned in the focus group discussions. All participants considered the advantages of the iron supplement to be greater than possible side effects. The one girl who had diarrhoea and the three who had stomach irritation said they would continue taking the iron pills despite the effects. They argued: "We know about the need to elevate our haemoglobin levels because of menstrual losses, exposure to mosquitoes, and the dirty environment, so we want to have enough iron stores in case we get infected. At home, we rarely eat foods like meat, fish, or liver, and we want to be on the safe side because blood transfusions in severe anaemic cases might contaminate one with diseases such as AIDS." Furthermore, the girls mentioned that they were not worried about the side effects because they had been informed about the symptoms. Moreover, if side effects occurred, they did not last long, according to the students.

These findings lead to the suggestion that the positive attitude towards the supplementation was fostered by the information sessions, since the teachers considerably increased the girls' knowledge of iron-deficiency anaemia, as shown by the differences between the pre- and post-knowledge test scores. These teachers were motivated to transfer their newly gained knowledge through different means and ways of communication to the adolescents. Charoenlarp et al. [27] reported that high drop-out rates could be attributed to lack of motivation of health personnel responsible for distributing medication to the participants in an iron-supplementation study. Insufficient correct knowledge of iron-deficiency anaemia by health personnel might be another factor contributing to low compliance.

Among different classes and age groups, the interest and motivation increased in the group receiving iron plus communication (as expressed by the compliance data), despite the fact that they had to gather voluntarily in their free time for the communication sessions, which were sometimes changed without clear announcements by the teachers. The girls had to come to school for the communication sessions, even when they had no formal classes and were preparing for their final examinations.

The positive effect of being more active, which was observed by 26% of the girls in school 1 and 23% of the girls in school 2, as compared with 18% in school 3 (iron only), may be due to increased haemoglobin levels, or it may be just a psychological effect, since they had been informed about the positive effects of ingesting iron pills. Two studies of female factory workers in Indonesia found that the work output of anaemic women was lower, even if they performed only very light activities [28, 29]. They also spent fewer hours on homework and leisure than their non-anaemic colleagues [28].

The sleepiness mentioned in all three groups might be related to the stress of final examinations. The lowest percentage of girls who reported negative or positive side effects was in group B (31%), who had no communication sessions and were not informed about possible effects of the iron supplement, as compared with 48% (school 1) and 54% (school 2) of girls in group A. However, as shown above, the well-informed and motivated participants could tolerate negative side effects without reducing compliance.

Conclusions

A high prevalence of anaemia (48%) justifies the urgent need for an adequate design of an iron-supplementation programme for adolescents in Tanzania. The lack of effective supplementation programmes in pregnant women requires new ways to alleviate iron-deficiency anaemia. Preventive iron supplementation in adolescence, which increases low iron stores and maintains high iron stores, has been shown to be a feasible alternative, as long as effective communication measures accompany the distribution of tablets.

Adequate communication strategies require the development of adequate communication tools and messages, with participation of the target groups. Moreover, as the problems of compliance during the first weeks of the study showed, the selection and utilization of the most effective channels of communication are of essential importance. When schoolchildren are the target, parents—who were the most influential channel for the girls in this study—need to be well integrated from the beginning of the planning of a supplementation programme. However, under different sociocultural conditions, different channels may have more influence

on the opinions, practices, and behaviours of school-girls. Therefore, instruments such as the Venn diagram must be utilized to identify these channels before the development of communication tools and messages.

In many countries, most of the economic efforts within the education sector are devoted to infrastructure development, teacher training, and curriculum development. However, all expenditures lead to very limited results as long as the learning capacity of school-children is physiologically constrained by micronutri-

ent deficiency. The study showed that weekly iron supplementation in adolescent schoolgirls can reduce iron-deficiency anaemia and therefore help to improve their cognitive performance as long as comprehensive communication measures are included. Therefore, appropriate communication programmes should be developed to accompany weekly supplementation programmes to improve the cognitive performance of schoolchildren and reduce the number of women who enter pregnancy with depleted iron stores.

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Farm commercialization and nutritional status of children: The case of the vegetables, fruits, and cash crops programme in western Nepal

Khem R. Sharma

Abstract

There are two conflicting views regarding the effects of commercialization of subsistence agriculture on health and nutrition. While the proponents of agricultural commercialization argue that commercialization raises income, increases food availability, and improves the health and nutritional status of rural households, the critics claim that shifting resources from subsistence to commercial crops reduces food security and increases women's work burden, thereby affecting the health and nutrition of farm families, especially that of women and children. The literature also fails to provide clear evidence whether the commercialization of subsistence agriculture results in an improvement or a deterioration in health and nutrition. This paper examines the effects of farmers' participation in a USAID-sponsored vegetables, fruits, and cash crops (VFC) programme on the nutritional status of children in western Nepal. According to a simple dichotomous comparison between the participating and non-participating households, the VFC programme had a positive impact on the nutritional status of children, especially in terms of standardized weight-for-age and weight-for-height measures. However, according to multivariate regression analysis, the VFC programme did not have a notable impact on children's nutritional status. The results suggest that household demographics, the nutrition and demographics of mothers, individual child demographics, and a complex set of other unknown factors play a greater role in children's nutritional performance than household economic strategies.

Introduction

More than 80% of Nepal's 22 million people still depend on agriculture for income and employment, and

nearly half of them live in poverty. Despite substantial investments in agriculture, food production has not been able to keep pace with population growth. Consequently, the country has turned from being a net exporter of food grains up to the late 1970s to being a net importer of food in recent years. Given the lack of adequate employment in the non-agricultural sector, the growing population has exerted increasing pressure on limited land and forest resources, rendering subsistence agriculture less and less environmentally sustainable. Despite some improvements in health services in recent years, health and nutritional problems are still widespread in the country, especially among children. The Nepal Multiple Indicator Surveillance (NMIS) study in 1995 found that 63% of children aged 6 to 36 months suffered from chronic malnutrition (low height-for-age or stunting) [1]. Nearly half of the children had general malnutrition (low weight-for-age) [1, 2], and 5.5% of them had acute malnutrition (low weight-for-height or wasting) [1]. Compared with the 1975 national data, the prevalence of both chronic and general malnutrition has increased in recent years [1].

The primary goals of the country's development programmes have been increasing agricultural productivity and household income and reducing rural poverty. In order to realize these goals, both the government and the donor agencies have recognized the importance of agricultural commercialization. Among many other donors, the US Agency for International Development (USAID) has played a key role in Nepal's development activities. The USAID agricultural programmes in Nepal are geared towards increasing rural incomes through market-oriented agricultural production. One such programme is the vegetables, fruits, and cash crops (VFC) programme initiated under the Rapti Development Project in western Nepal. The VFC programme was first implemented in 1985 in three communities: Satabariya in Dang District, and Jinabang and Thabang in Rolpa District. Subsequently, the VFC activities have been launched in the other districts of Rapti Zone. In 1990 USAID commissioned a study to examine the effects of the VFC programme on income, expenditure,

The author is an assistant researcher/professor in agricultural and resource economics at the University of Hawaii in Manoa, Hawaii, USA.

resource allocation, and health and nutrition in participating farm households.

The effect of technological change or commercialization of subsistence agriculture on health and nutrition continues to be an important but debatable issue. The optimistic view is that agricultural commercialization creates employment, generates income and rapid agricultural growth, and increases food availability and affordability, thereby leading to an improvement in the health and nutritional status of the population [3, 4]. However, as noted by von Braun and Kennedy [5], it is widely argued in the literature that technological change or commercialization of subsistence agriculture reduces food security at the household or community level, resulting in a deterioration of the nutritional status of the poor. Critics also argue that agricultural commercialization may limit women's access to resources and control over household income [6] and increase their work load [7], thus affecting the well-being of themselves and their children.

A few empirical studies have suggested that shifting resources from traditional to commercial crops may adversely affect household food security, health, and nutrition. However, a review of empirical work [8, 9] has shown that many of the studies on which these generalizations are based either are conceptually flawed [10, 11] or are based on very small, potentially biased samples [12]. The impact of commercialization on food availability also depends on whether the cash crop is a food or a non-food crop. For example, market-oriented food production (e.g., vegetables, fruits, potatoes, and improved cereals) can directly improve household-level food availability. The cash income obtained from selling these products can also increase the farmer's ability to acquire additional food for the family.

Against this background, the main objective of this study was to examine the effects of the VFC programme on the nutritional status of children in western Nepal. The study also contributes to the literature by providing additional evidence that commercialization of subsistence agriculture does not result in a deterioration of health and nutritional status.

Theoretical framework

The nutritional effects of farm commercialization are mediated through a set of complex factors at the community, household, and individual levels. The nutritional impacts of commercialization depend on various exogenous factors, such as population demographics, the availability of new technologies, government policies, infrastructure development, and health environment, which can affect the farmer's decision to participate in market-oriented production. A number of indirect consequences of commercialization, such as changes in time allocation of men and women, control

over household resources and incomes, food availability, and expenditure pattern, also affect the nutritional status of the family. Figure 1 presents a simplified overview of pathways through which commercialization can influence resource allocation, income, expenditure, and ultimately the health and nutritional status of individuals in the household. A detailed description of the interdependencies of determinants and consequences of commercialization can be found in the literature [5, 13]. In order to determine whether agricultural commercialization results in an improvement or a deterioration in health and nutrition, it is important to examine the relationships among health and nutritional consequences and their determinants.

The effect of commercialization on nutrition depends on circumstances. Where subsistence food production is maintained [14] and the incremental income from cash crops is spent on nutritious foods [15], commercialization tends to result in an improvement in nutrition. However, farm commercialization can result in a deterioration of nutritional status when subsistence food supply decreases, food prices increase, and purchased foods of poor nutritional value substitute for more nutritious home-produced foods [16]. The impact of change from subsistence to commercial agriculture on nutritional status also depends on who controls the household income. If male members of the households have more control than females over income from cash cropping, then nutritional benefits may not be realized because of differences between men and women in expenditure patterns [17]. Women's income has been positively linked to food intake in Panama [18] and the Philippines [19]. In Ghana it has been associated with improved nutritional status of children [20]. However, although a review of the literature shows a lack of consistent relationship between mothers' work and children's nutrition [21], if commercialization increases women's time spent in farming, decreased time for child care can offset the beneficial effects of women's income on the children's nutrition [19].

Methods

Description of study communities

This paper is based on the data collected for a study entitled "Gender and farm commercialization of subsistence agriculture in Nepal" [22]. Three communities in Rapti Zone were selected for the study: Satabariya in Dang District, and Jinabang and Thabang in Rolpa District. These communities are quite different from each other in their geographic and ethnic characteristics. Satabariya is located in the low plains of the Deukhuri Valley, 10 km west of Lamahi Bazaar along the east-west highway. Jinabang is about 2,000 m above sea level and is about a three-day walk from Tulsipur,

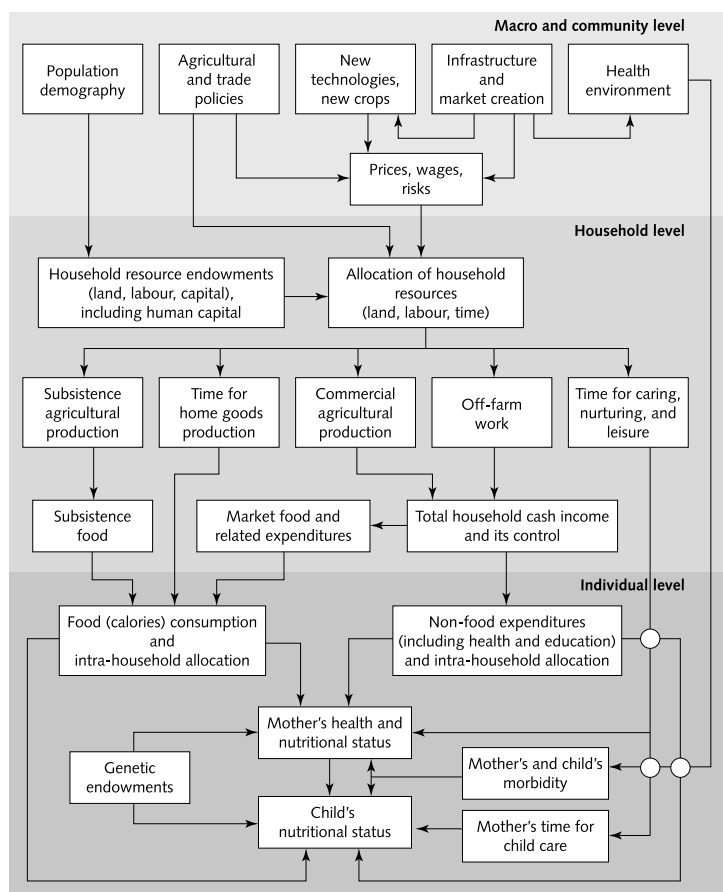


FIG. 1. Nutritional effects of farm commercialization (modified from von Braun et al. [13])

the headquarters of Rapti Zone, and a two-day walk from Liwang, the District headquarters of Rolpa. Thawang is 2,200 m above sea level and is a four-day walk from Tulsipur and a two-day walk from Liwang. The predominant ethnic groups are the Tharus in Satabariya, the Chhetris in Jinabang, and the Kham Magars in Thabang. The Tharus and the Chhetris are Indo-Aryan cultural groups, and the Kham Magars are a Tibeto-Burman cultural group. In general, women belonging to the Tibeto-Burman cultural groups enjoy considerably higher socio-economic status in the household (such as in deciding the time for marriage and the marriage partner and in selecting economic activities they wish to pursue) as compared with their counterparts in the Indo-Aryan cultural groups [23].

In these three communities, farmers are engaged in producing subsistence foods (cereals and livestock), mostly for home consumption, and vegetables, fruits, and other cash crops for home consumption and for sale in local markets. A detailed description of VFC activities in each of these communities is provided by Paolisso and Regmi [22].

Selection of households

Because of the lack of baseline data on households, it was not possible to examine the effects of the VFC programme by a longitudinal approach comparing the situations before and after the programme. Therefore, a cross-sectional comparison approach was used. In each community, 44 households were selected to participate in the VFC programme and 44 were selected as controls. The overall sample consisted of 264 households. In selecting the households, a number of socio-economic factors, such as landholding and household size, were considered to ensure that the two groups were comparable. More detailed information on the study households and communities is available in Paolisso and Regmi [22].

Data collection techniques

The sample households were surveyed four times between February 1991 and January 1992. Information was collected on household demographics (the number

of persons in the household and their sex, age, marital status, and educational level), socio-economic status (landholding, source and level of income, and expenditure patterns), and farming system (acreage, use of inputs, production, sales, and purchases for both subsistence and VFC crops). To assess the impact of the VFC programme on health and nutritional status, anthropometric measures and morbidity patterns were also recorded for women 15 to 49 years of age and children 6 to 36 months of age in the selected households four times during the study period, in April 1991 (round 1), July 1991 (round 2), October 1991 (round 3), and January 1992 (round 4). The survey also gathered data on intra-household time allocation by the random-spot observation technique. With this technique, a different set of eight randomly selected sample households in each community (four VFC and four non-VFC) was visited daily between 6:30 a.m. and 6:30 p.m., and the activities of men and women (15–49 years of age) at the time of the visit were recorded. See Paolisso and Regmi [22] for further details on data-collection techniques.

After incomplete observations had been deleted, the total number of observations included in the analysis of children's nutritional status was 511, involving 140 households, 162 mothers, and 195 children. Of these 195 children, 29% were observed in all four survey rounds, 26% in three, 23% in two, and 22% in only one round.

Analytical procedures

Given the lack of direct observations on food intake by individuals in the household, analysis is based on the assumption that agricultural commercialization influences household income, household and per capita food expenditures, and time allocated for caring and nurturing, which when combined with various community, household, and individual characteristics determine the nutritional status of the household or individual. In this study the nutritional status of children is expressed in terms of standardized Z scores for height-for-age, weight-for-age, and weight-for-height measures. These scores are constructed from anthropometric measurements of children aged 6 to 36 months with the ANTHRO 1.01 programme [24]. Height-for-age is interpreted as indicative of longer-term nutritional status, weight-for-height is the shorter-term indicator, and weight-for-age is a composite of the longer-term height-for-age and the shorter-term weight-for-height indicators.

The anthropometric measurements of children from VFC and non-VFC households were compared by a simple one-way analysis of variance (ANOVA) procedure. This straightforward dichotomous comparison (not controlled for other relevant factors) may largely reflect the effect of commercialization-led changes in income and expenditure patterns and food availabil-

ity on nutritional status. However, according to the theoretical framework outlined in figure 1, the nutritional status of children depends on a complex set of variables other than consumption patterns, such as maternal health and nutrition, time spent on child care, and the household and community health and sanitation environment affecting the morbidity pattern. These complex relationships are analysed by a multivariate regression technique in which the variations in each of the three measures of children's nutritional outcomes are explained in terms of a number of variables. These variables include income and expenditure patterns; household, mother, and child demographics; the mothers' health and time allocation; the children's morbidity patterns; and seasonal and community dummy variables.

For several reasons, some caution should be used when interpreting the multivariate regression results and drawing conclusions from the analysis. First, it should be noted that a number of explanatory variables, such as the proportion of household income derived from VFC production, the mothers' health and time allocation, and children's sickness, may be simultaneously determined with nutritional outcomes of the VFC programme. This may influence their estimated effects on children's nutrition if the possibility of endogeneity of these variables is not accounted for appropriately, such as by using the instrumental-variables estimator. However, children's nutritional equations are estimated with the ordinary least-squares technique in view of the lack of relevant instrumental variables in the data. Perhaps for the same reason and for analytical simplicity, several previous studies also used the ordinary least-squares method rather than the instrumental-variables method to assess the effect of agricultural commercialization on children's nutritional outcomes [5, 13, 25–27]. Second, because of the lack of data, some relevant explanatory variables could not be included in the analyses, such as women's control over household income, household decision-making patterns, gender-specific expenditure and resource allocation patterns, and household health and sanitation environment. Third, given differences across communities, it might have been more appropriate and interesting to estimate separate regression equations for each community for each season rather than using dummy variables, but this was not feasible because of an inadequate sample of children.

Results and discussion

ANOVA results

Table 1 shows the straightforward comparisons of children's height-for-age, weight-for-age, and weight-for-height Z scores for households that were actively involved

TABLE 1. Anthropometric measurements of children aged 6 to 36 months according to sex and community

Community	<i>n</i> ^a		HAZ		WAZ		WHZ	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Satabariya								
VFC	43	59	-1.52	-1.11	-1.15	-1.32	-0.20	-0.70
Non-VFC	47	51	-1.21	-1.26	-1.89	-1.40	-1.35	-0.61
<i>F</i> -value ^b			1.14	0.32	11.27***	0.13	22.15***	0.13
Jinabang								
VFC	36	53	-2.00	-1.83	-1.75	-1.74	-0.56	-0.73
Non-VFC	40	62	-2.16	-1.82	-2.28	-1.69	-1.14	-0.61
<i>F</i> -value ^b			0.68	0.002	7.44***	0.16	4.40**	0.26
Thabang								
VFC	33	32	-1.87	-1.69	-1.33	-1.34	-0.25	-0.23
Non-VFC	34	21	-1.82	-1.96	-1.53	-1.95	-0.47	-0.83
<i>F</i> -value ^b			0.03	0.98	1.14	7.72***	0.71	2.60
All communities								
VFC	112	144	-1.78	-1.50	-1.39	-1.48	-0.33	-0.61
Non-VFC	121	134	-1.70	-1.63	-1.91	-1.61	-1.04	-0.65
<i>F</i> -value ^b			0.28	0.83	17.83***	1.12	20.96***	0.06

HAZ, Height-for-age Z score; WAZ, weight-for-age Z score; WHZ, weight-for-height Z score.

a. Number of observations from all survey rounds.

b. *F*-values test the null hypothesis that the children's nutritional status is independent of the farmers' involvement in the VFC programme.

** $p \leq .05$.

*** $p \leq .01$.

in the VFC programme versus those not involved in the programme. The height-for-age values of children aged 6 to 36 months were quite similar in the two groups of households. However, the results tend to show a positive relationship between participation in the VFC programme and children's nutritional status as indicated by weight-for-age and weight-for-height, with some notable community-specific gender effects. For example, in the less gender-egalitarian Satabariya and Jinabang communities, commercialization resulted in an improvement in the nutritional status of boys, whereas in the more gender-egalitarian Thabang community, commercialization improved the nutritional status of girls. These simple results suggest that farm commercialization tended to reduce acute (low weight-for-height) and general (low weight-for-age) malnutrition in children and that it had no effect on chronic (low height-for-age) malnutrition.

Multivariate regression results

The results of multivariate regression analyses of children's nutritional indicators are presented in table 2. Also presented are the mean values of dependent and independent variables involved in the analyses. In terms of the coefficient of variation (R^2), the estimated models show relatively higher levels of explanatory power compared with previous nutritional studies of children in

the context of agricultural commercialization [5, 13, 25–27]. The regression results provide valuable insights into the role of various factors in determining children's nutritional status.

Except for a moderate unexpected negative association between per capita expenditure and the weight-for-height measure, none of the income and expenditure variables expected to be influenced by commercialization had a significant effect on children's nutritional status. Although VFC households had higher levels of per capita income and expenditure than non-VFC households, the lack of positive relationship between expenditure and nutritional status suggests that higher incomes do not necessarily result in an improvement in nutritional status. Two plausible explanations, as suggested in other nutritional studies, are that households may allocate incremental incomes from cash cropping to more expensive but not necessarily more nutritious foods, and that they allocate incomes to non-food items [26, 28]. The proportion of total expenditure spent on food had a positive but non-significant effect on child nutrition. Similarly, although the coefficient is positive, the proportion of income derived from VFC production had no significant effect on children's nutritional measures. In this study, few households were dedicated solely to cash cropping, and most of them (including those actively involved in the VFC programme) still continued subsistence food production.

TABLE 2. Determinants of nutritional status of children aged 6 to 36 months

Determinant	Mean \pm SD of independent variable	Dependent variable		
		HAZ	WAZ	WHZ
Mean \pm SD of dependent variable		-1.64 \pm 0.05	-1.60 \pm 0.04	-0.66 \pm 0.06
Independent variables				
Total per capita expenditure (NRs/yr) ^a	4.77 \pm 0.15	0.035	-0.041	-0.085*
Total per capita expenditure squared	34.99 \pm 3.06	-0.002	0.001	0.003
Food share in total expenditure	0.73 \pm 0.01	0.505	0.421	0.181
VFC share in total household income	0.24 \pm 0.01	0.025	0.503	0.582
Household size	9.34 \pm 0.24	0.002	0.028***	0.034***
Dependency ratio ^b	1.13 \pm 0.02	-0.195*	-0.084	0.035
Educational level of household head (1 = literate, 0 = illiterate)	0.69 \pm 0.02	-0.108	0.111	0.206*
Mother's time in agriculture (h/day)	3.56 \pm 0.09	0.028	-0.075***	-0.124***
Mother's educational level (1 = literate, 0 = illiterate)	0.09 \pm 0.01	-0.055	0.513***	0.686***
Mother's age (yr)	27.02 \pm 0.30	0.023*	-0.006	-0.033**
Mother's height (cm)	152.22 \pm 0.27	0.008	0.009	0.006
Mother's body mass index ^c	20.15 \pm 0.09	0.050*	0.076***	0.054*
Child's age (mo)	19.48 \pm 0.40	-0.082***	-0.068***	-0.096***
Child's age squared	461.70 \pm 16.64	0.002***	0.001**	0.002**
Sex (1 = male, 0 = female)	0.46 \pm 0.02	-0.209**	-0.081	0.015
Birth order (1 = first, 2 = 2nd, etc.)	2.67 \pm 0.07	-0.007	-0.044	-0.023
Sickness (1 = yes, 0 = no)	0.38 \pm 0.02	-0.127	-0.135	-0.070
Satabariya (1 = Satabariya, 0 otherwise)	0.39 \pm 0.02	0.788***	-0.441**	-1.237***
Jinabang (1 = Jinabang, 0 otherwise)	0.37 \pm 0.02	0.088	-0.084	-0.244
Round 2 (1 = round 2, 0 otherwise)	0.23 \pm 0.02	0.129	0.072	-0.018
Round 3 (1 = round 3, 0 otherwise)	0.28 \pm 0.02	0.070	-0.001	-0.056
Round 4 (1 = round 4, 0 otherwise)	0.24 \pm 0.02	0.645***	0.476***	0.086
Constant		-4.430***	-3.551***	-0.063
R ²		0.173	0.253	0.218
Adjusted R ²		0.136	0.219	0.183
F-value		4.646***	7.493***	6.181***

HAZ, height-for-age Z score; WAZ, weight-for-age Z score; WHZ, weight-for-height Z score.

a. Expenditure is used as a proxy for income.

b. The number of people aged ≤ 14 or ≥ 60 years divided by the number of people aged 15–59 years.

c. The weight in kilograms divided by the height in metres squared.

* $p \leq 0.10$.

** $p \leq 0.05$.

*** $p \leq 0.01$.

For example, among the VFC households cash cropping accounted for about 25% of cultivated land and household labour allocated to agriculture, implying that at least 75% of household resources were devoted to subsistence agriculture. This result is consistent with other findings showing that commercialization does not lead to a deterioration of nutritional status if subsistence production is maintained [29, 30]. The weak association between income and expenditure variables and anthropometric measures indicates that the child's nutritional status is influenced more heavily by char-

acteristics of the household, the mother, and the child than by income and expenditure measures *per se*.

Household size has a positive impact on children's nutritional indicators, especially weight-for-age and weight-for-height measures. The positive association between household size and children's nutrition can be attributed to the larger amount of time available for child care in large, extended families than in small, nuclear families. Positive economies of scale in household nutrition were also reported by von Braun et al. [13]. Among other household characteristics, house-

hold composition (the ratio of persons aged 14 or below and 60 and above to those aged between 15 and 59) had a moderate negative effect on children's height-for-age (a longer-term indicator), whereas the educational level of the head of the household had a moderate positive effect on weight-for-height (a shorter-term indicator).

Mothers' time spent in agricultural activities, literacy level, health and nutritional status, and age had significant impacts on children's anthropometric status. The amount of time spent by mothers in agricultural activities was highly negatively associated with children's weight-for-age and weight-for-height. Spending more time in agricultural activities will leave less time for household activities, including child care. Kumar [25] found a positive relationship between women's time spent in household activities and pre-schoolers' weight-for-age and weight-for-height. Although women in VFC households spent significantly more time on VFC activities than those in non-VFC households, the two groups of households were very similar in the total time spent by women in VFC and subsistence agricultural activities. Furthermore, the VFC activities accounted for only about 10% of women's time in agriculture. Thus the negative association between mothers' time spent in agricultural activities and children's nutrition cannot be attributed to farm commercialization alone.

Mothers' education had a strong positive impact on children's weight-for-age and weight-for-height. The effect can be explained by the superior abilities of literate mothers to provide their children with better feeding, medical care, and sanitation. Similarly, mothers' nutritional status, as measured by body mass index, also had a positive impact on children's nutritional status, especially the weight-for-age measure. Babies born to healthier mothers were likely to be longer and weigh more at birth and grow faster. Although the mother's height is treated in the literature as a positive indicator of the household's long-term socio-economic and nutritional status [31], its effect on children's nutrition was positive but non-significant in this study. The effect of mothers' age on children's nutrition was mixed, with the long-term height-for-age measure tending to improve and the short-term weight-for-height indicator tending to deteriorate with mothers' age.

Among the children's characteristics, age was inversely associated with all three nutritional measures. However, as indicated by the significantly positive coefficient for the age-squared term, the relationship between age and anthropometric measures was not linear but U-shaped. Despite a preference for sons over daughters in Nepali society (sons are considered economic insurance against the insecurities of old age, whereas daughters' economic value is restricted to their childhood years), the results do not support a commonly accepted view that sons have a better nutritional status than

daughters. In fact, girls appeared to be slightly better-off than boys in terms of height-for-age. A plausible explanation is that girls receive the same care and nutrition as boys during the early years and the discrimination occurs only during middle and late childhood and adolescence. Since this study was limited to children aged 6 to 36 months, the lack of evidence of gender discrimination in child nutrition is not surprising. Martorell et al. [32] also found a lack of evidence for gender discrimination according to anthropometric measurements of children 3 to 10 years of age in Nepal. Both birth order and morbidity have a negative but non-significant effect on children's nutrition.

Children's nutritional measures were also found to be related to community and seasonal variables. For example, compared with children from Thabang, children from Satabariya were better-off in terms of the height-for-age measure but worse-off in terms of the weight-for-age and weight-for-height measures. These differences could be attributable to genetic differences between the two ethnic groups. The Tharu children in Satabariya were taller and leaner, whereas the Kham Magar children in Thabang were shorter and stouter. Similarly, children were nutritionally better-off during January (round 4) than at other times of the year. This can be attributed to increased food availability during this season.

Conclusions

This study examined the impact of a farm commercialization programme, the vegetables, fruits, and cash crops (VFC) programme, on the nutritional status of children aged 6 to 36 months in rural households in western Nepal. Although a simple comparison of VFC versus non-VFC households (not controlled for other factors influencing children's nutrition) showed a positive relationship between VFC involvement and some measures of children's nutritional status, multivariate regression analysis found no notable impact of the VFC programme on children's nutrition. To summarize, the results were consistent with those of similar previous studies finding that children's nutritional status is more strongly influenced by household, mother, and child characteristics than by household economic strategies. These factors should be carefully considered in the design of any programme to improve children's health and nutrition.

Although commercialization may provide much-needed cash income, maintenance and improvement of subsistence food production are indispensable for a country like Nepal. Despite the implementation of the VFC programme, the study households still allocated most of their resources to subsistence agriculture for several reasons, including farmers' limited ability to cope with increased risks associated with commercial pro-

duction, and high transaction and marketing costs due to limited markets and poor infrastructure. Given these factors and the increasing food demand for a growing population, there is an enormous need to increase subsistence production by promoting appropriate technologies, rather than giving too much emphasis to commercialization *per se*.

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Nutrition, food, and water security

Margaret R. Biswas

Abstract

Although nutritionists have long been aware of the importance of clean drinking water and sanitation, water is becoming part of the international political agenda only after a slow realization of its scarcity. This is mainly because water has been taken for granted in industrialized countries except during periods of drought. In many areas of developing countries, water shortages already exist. Even with improved management, new sources of water will have to be developed at higher costs per project. Provision of clean water and sanitation has been rendered difficult by rapid urbanization since the middle of the twentieth century. Although cities have managed to provide a water supply, they have not been able to provide sewage and wastewater treatment. Meanwhile, irrigated agriculture uses nearly 70% of world water. In the future, food security will become even more dependent on irrigation. Poor management, due mostly to low salaries and political interference, is one of the main reasons for inefficient water systems. Underpricing of water in towns and on farms discourages conservation. Furthermore, people who do not have access to tap water in developing countries pay 10 times more than those who have taps.

Introduction

Despite the fact that water is essential to food and health, it has never been perceived as being of great international significance, while food and health have often dominated the international political agenda since World War II. Water-related diseases are the cause of 80% of infectious illness in developing countries, affecting about two billion people annually [1]. The deaths of over two million children from such diseases each year could be averted with adequate water supply and sanitation. If this incidence of illness were attributed to any other

cause, there would be a universal clamour for action, as with cancer and AIDS. The provision of safe drinking water and the proper disposal of human excreta have greater impact on public health and national development than any other kind of intervention.

Although nutritionists have long been aware of the importance of clean water for drinking and sanitation, water is becoming part of the international political agenda only after a slow realization of its scarcity. This is mainly because water has been taken for granted in industrialized countries except during periods of drought. Although prolonged drought is often a matter of life and death in the third world, it is a temporary inconvenience in the developed countries.

Water is a particularly critical issue for most developing countries, which are located in tropical and subtropical climates. Water supply for megacities in the third world is already a serious problem.

Water was not a major issue at the United Nations Conference on Environment and Development held in Rio de Janeiro in June 1997, though water scarcity was already a reality in a number of countries. Efforts by the World Water Conference in Buenos Aires in 1977 to avert a water crisis went unheeded. The conference did, however, result in the International Drinking Water Supply and Sanitation Decade from 1981 to 1991, which placed these issues higher on the agendas of many developing countries and development agencies than they might otherwise have been. During the decade 1981–1991, about 1,600 million people were provided with safe water and about 750 million with excreta disposal facilities [2]. In spite of this, the World Health Organization (WHO) estimates that approximately one billion people lack safe water and more than two billion do not have adequate excreta disposal facilities.

Water crisis

Water requirements are rising worldwide, first because of increased population and second because of the increased demands for water as more people attain higher

The author is an international consultant in food and nutrition in Oxford, UK.

standards of living. The National Water Study for England and Wales indicated that water requirements will increase by 25% by the year 2020, despite an almost stationary population, primarily because of greater use of washing machines and dishwashers [3].

Water is considered scarce when supplies are less than 1,000 cubic metres per year per capita. Water stress occurs when supplies fall below 1,700 cubic metres but above 1,000 cubic metres per person per year. The Food and Agriculture Organization (FAO)[4] indicated that the countries listed in table 1 will be water scarce. Countries that can slow population growth will have less water scarcity than is now estimated.

The World Bank estimates that by 2025, 52 countries will have water scarcity or water stress [1]. India will have chronic water shortage everywhere, while China will have a state of water stress. However, scarcity already exists in many areas, such as the North China plain. Some of these water shortages can be alleviated with better management.

Even with improved management, new sources of water will have to be developed. Since most of the cheap and easily available sources of water have been exploited, the average costs of most new projects will be two or three times higher than the present costs of projects. Costs will increase dramatically, especially if drainage and sanitation are included. Beijing is already drawing its water from a source over 1,000 km away, and Mexico City may have to pump water over a height of 2,000 metres. With the growing demand for water, countries that share rivers are increasingly in conflict.

Urban water supply

Provision of clean water and sanitation to the world's increasing population has been rendered difficult by rapid urbanization since the middle of the twentieth

century. The Global Report on Human Settlements [5] estimated that nearly 45% of the total population of the world lived in urban areas in 1995, as compared with 30% in 1950. The megacities (those with over 10 million residents) in developing countries have recently received most of the financial and other national and international resources, although only 3% of the global population live in them.

Table 2 shows the high projected growth rates of the 10 largest megacities from 1995 to 2015. In 1995 three of these cities were in developed countries. In 2015 only Tokyo will remain among the 10 largest. The growth of Tokyo and New York occurred over a century, but most urban growth in developing countries has occurred since 1950. Although these cities have managed to provide a water supply, they have not been able to provide sewage and wastewater treatment. Only 2% to 4% of sewage in major cities in Latin America receives adequate treatment [6]. In the developing world as a whole, more than 95% of urban sewage is discharged untreated into water [7].

The major problem in the developing countries is that new sources of water that can be easily exploited are simply not available. Often additional supplies can only be obtained by diverting water from other uses, such as agriculture. Further development of new sources is technically complex and more expensive than existing projects. Construction, operation, and maintenance of water supply systems and waste treatment systems are constrained by lack of funds. Inadequate pricing of water and inefficient billing further complicate the financial situation.

Health issues

In most developing countries, urban dwellers do not trust the quality of the water provided by water systems.

TABLE 1. Water-scarce countries (less than 1,000 cubic metres available per capita per year)

Since 1955	Since 1990	By 2000	By 2025
Bahrain	Algeria	Botswana	Comoros
Barbados	Burundi	Egypt	Ethiopia
Djibouti	Cape Verde	Hungary	Haiti
Jordan	Israel	Libya	Iran
Kuwait	Kenya	Mauritania	South Africa
Malta	Malawi	Morocco	Peru
Singapore	Qatar	Oman	Tanzania
	Rwanda	Sudan	Zimbabwe
	Saudi Arabia	Syria	Cyprus
	Somalia		
	Tunisia		
	United Arab Emirates		
	Yemen		

Source: ref. 4.

TABLE 2. Population of largest urban agglomerations (in millions)

1995			2015		
Rank	City	Population	Rank	City	Population
1	Tokyo, Japan	26.8	1	Tokyo, Japan	28.7
2	São Paulo, Brazil	16.4	2	Bombay, India	27.4
3	New York, USA	16.3	3	Lagos, Nigeria	24.4
4	Mexico City, Mexico	15.6	4	Shanghai, China	23.4
5	Bombay, India	15.1	5	Jakarta, Indonesia	21.2
6	Shanghai, China	15.1	6	São Paulo, Brazil	20.8
7	Los Angeles, USA	12.4	7	Karachi, Pakistan	20.6
8	Beijing, China	12.4	8	Beijing, China	19.4
9	Calcutta, India	11.7	9	Dhaka, Bangladesh	19.0
10	Seoul, South Korea	11.6	10	Mexico City, Mexico	18.8

Source: ref. 5.

Therefore, there is a large increase in the resort to boiled water, water filters, and bottled water. Bottled water, however, may have equal health risks. Either standards for bottling do not exist, or they may not be enforced.

Domestic use of water results in wastewater, which can be treated and re-used for purposes such as agriculture, releasing higher-quality water for selected uses. Even if wastewater is not used, it should be treated for health reasons. Without increased treatment of sewage, diseases will continue to spread among the poor.

Gender issues

Water scarcity directly affects women and children, since they are frequently the ones who must carry water for long distances. Increased time for collection of water allows less time for child care, household chores, and food production. An inadequate amount of water for drinking, washing clothes, bathing, and sanitation contributes to malnutrition, as does a lack of adequate water for agricultural production.

Water for agriculture

Water for food production is as significant to nutritionists as drinking water. The most intensive use of water occurs in food production, especially irrigated agriculture. The high-yielding varieties rely heavily on irrigation to provide the necessary water for their growth. Irrigated agriculture uses nearly 70% of world water—over 90% in agricultural economies in the arid and semi-arid tropics, but less than 40% in the temperate regions [8]. At present 40% of world food production is derived from irrigated agriculture on 17% of cultivated land. In the future, food security will become even more dependent on irrigation. Some experts argue that 80% of additional food production will need to be contributed by irrigated agriculture. Serageldin [1] estimated

that one-half to two-thirds of the increase in food production in the future will come from irrigated land.

Water management

Poor management, due mostly to low salaries and political interference, is one of the main reasons for inefficient water systems. Managing water utilities requires professional competence free of political interference if they are to function efficiently. Government failure to manage water effectively is the main reason for its misallocation and waste. There are different agencies for various uses, such as irrigation or transportation, and other departments concerned with health and the environment. They do not coordinate their work, and hence decision making is fragmented.

Water pricing

Water is seldom regarded as an economic commodity by governments or users. It is usually underpriced whether in town or on farms. If farmers pay little or nothing for water, they have little reason to conserve. Similarly, prices in towns provide no reason for conservation. Calcutta has no water tariffs. A review of municipal water supply projects financed by the World Bank found that the price charged was only 35% of the cost of supplying the water [1]. Irrigation charges are usually much less. It is also difficult to measure the volume of water that each farmer receives.

In most cities in developing countries, people who do not have access to tap water often pay 10 times more for it than people who have taps. Not only do the urban poor pay higher prices for water, they spend a high proportion of their income on water [9]. In Port-au-Prince, Haiti, the poor spend 20% of their income on water. In Onitsha, Nigeria, the poor spend about 18% of their income on water, and upper-income households spend

2% to 3%. In Jakarta, Indonesia, only 14% of households receive water from the municipal system, and 32% purchase water from street vendors. Households purchasing water from vendors may pay 25 to 50 times as much as households connected to the municipal system. This trend is evident in many cities, including Karachi, Pakistan; Jakarta, Indonesia; Port-au-Prince, Haiti; Nouakchott, Mauritania; Dhaka, Bangladesh; Tegucigalpa, Honduras; and Onitsha, Nigeria [9]. In Mexico City the poor who buy their water from vendors pay five times as much for water as the rich [6].

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Conclusions

Nutritionists should be concerned with water for food production as well as with water for drinking and sanitation. As Asit Biswas states, “Potential solutions like water pricing and better water conservation and irrigation management practices have to be taken seriously. The days of providing lip-service to these critical issues are now truly over” [10].

Testing methods for aflatoxins in foods

Kaushal K. Sinha

Abstract

*Aflatoxins, a group of mycotoxins produced mainly by *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare, have been recognized as one of the major contaminants of food throughout the world. Because of their acute hepatocarcinogenic nature, these groups of fungal food contaminants have received considerable attention in recent years. Several analytical and immunological methods are available for testing and estimating aflatoxins in different food commodities. However, most of these analytical methods are actually minor modifications of the basic methods officially adopted for specific commodities. They differ only in the solvents used to extract the toxins and in the techniques used for estimating the intensity of fluorescence of the analysed toxins. All analytical methods for aflatoxins involve basically the same steps: sampling and sample extraction, clean-up, work-up, detection, and confirmation, as well as estimation of the toxin. Various types of high-performance chromatographic approaches are most commonly used. A rapid qualitative assessment has also been reported with the help of minicolumn methods. By using monoclonal and polyclonal antibodies, several sensitive and specific enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA) have been developed for aflatoxin analysis. ELISA techniques are more rapid, more sensitive, and simpler to use than the conventional analytical methods. Several commercial ELISA-based aflatoxin kits using monoclonal antibodies are also available for aflatoxin analysis.*

Introduction

Aflatoxins are extremely biologically active secondary

metabolites that are produced mainly by the toxigenic strains of two important fungi, *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare. These groups of compounds are receiving worldwide attention from researchers, the food industry, and the general public, mainly for two reasons. First, aflatoxins (particularly aflatoxin B₁) not only are toxic to animals and humans but also are the most carcinogenic natural compounds known. Second, there is a high incidence of aflatoxins in food and feed throughout the world [1–3].

In view of their common occurrence and toxicity, several analytical and immunological methods were devised after aflatoxins were discovered to be the causative agent of Turkey-X disease in England in 1960. However, most of these analytical methods are actually minor modifications of the basic methods officially adopted for specific commodities. They differ only in the solvents used to extract the toxins and in the techniques used to estimate the intensity of the fluorescences of the analysed toxins.

All analytical methods for aflatoxins involve basically the same steps: sampling and sample extraction, clean-up, work-up, detection, and confirmation, as well as estimation of the toxin. The use of thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas–liquid chromatography (GLC), or fluorotoxinmeter (FTM) is common for the detection and quantification of aflatoxins.

Rapidity in analysis is another factor that has drawn worldwide attention recently. When a large number of samples have to be analysed within a short period, minicolumn methods, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA) techniques can be used. However, any method recommended for aflatoxin analysis should be economical and convenient to the handlers, taking into account their available laboratory facilities, as well as providing greater accuracy in the results. Detailed methods for aflatoxin analysis are discussed below.

The author is affiliated with the Department of Botany at T.M. Bhagalpur University in Bhagalpur, India.

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

Sampling and sample preparation

Sampling and sample preparation are the most important steps before the sample is subjected to chemical analysis for the presence of aflatoxins. It is obvious that aflatoxins are present in only a few kernels and grains and have highly skewed distribution in food and feed commodities. This results in extreme variations in analytical results if the sample taken for analysis is not representative of the bulk [4–7]. Coker et al. [8] have shown the variations in the levels of aflatoxins analysed in 20 subsamples taken from a 54-kg lot of groundnut kernels. They have also suggested different sampling plans on the basis of the nature and size of kernels or grains and other food items.

Sampling accuracy can be increased by taking a large number of representative samples and dividing them into three equal parts. Differences in weight have also been considered, depending on the regulations of a particular country. The United Kingdom has recommended a weight of 10.5 kg per sample, whereas the US Department of Agriculture requires 66 kg per sample, a much larger amount. However, an average weight of 5 to 10 kg per sample has been adopted by most countries.

Proper grinding and subdivision of the sample are also important before determination of aflatoxin. Rotary sample divisors, spinning riffles, and cascade samplers can be used to prepare representative subsamples [7, 9]. The size of the subsamples may vary from 20 to 100 g. Most of the methods, however, require a 50-g sample for aflatoxin assay, which appears to be the best in terms of economy in using solvents.

Aflatoxin analysis

Three types of analytical methods have been suggested for aflatoxins: biological, chemical, and immunochemical. Biological methods are qualitative, nonspecific, and time consuming, since they are based on reactions occurring from the exposure of biological test organisms, such as chick embryos, day-old ducklings, brine shrimp, *Salmonella* and *Bacillus* spp., and ochra seedlings, to aflatoxins. A duckling test is useful for a one-time dose response and as a confirmatory test for aflatoxin B₁ exposure. However, biological assays are only useful for indicating the presence of a toxin in the system.

Isolation, identification, physiology, chemical nature, and immunochemical responses are essential for routine analysis of aflatoxins. These are invariably quicker, cheaper, more specific, more sensitive, and more reproducible than biological analyses. Although several analytical methods have been developed, most of them differ in the solvents used and the measurement of fluorescence. In most cases organic solvents are mixed with a given ratio of a more polar solvent, such as water, so

that the aqueous solvents penetrate the hydrophilic tissues and effectively extract aflatoxins. Methanol/water (55:45, 80:20), acetone/water (85:15), acetonitrile/water (90:10), and chloroform/water (250:25) are some of the common solvents used to extract aflatoxins from agricultural commodities. An assay method for aflatoxins in milk has been developed separately by Lafont and Siriwardana [10].

Clean-up

Purification and clean-up of the aflatoxins from the interfering substances in the extract are essential before determination of aflatoxin. Liquid–liquid partitioning by suitable solvents in a separating funnel, as well as removal of plant pigments from the extracts, is performed for this purpose. Hexane or petroleum ether is used to separate fats and oils from the extract, and lead acetate solution or cupric carbonate is used to remove pigments and other compounds [11].

Column chromatography can also be used for this purpose. A glass column is packed with one or more adsorbent materials, and the crude extract is added to the top of the column. The column is then eluted with a series of solvents or solvent mixtures that are designed first to wash off the interfering substances and then to elute aflatoxins separately. The other interfering compounds remain strongly bound on the column. The purified extract is subsequently subjected to work-up for qualitative and quantitative analyses through chromatography and immunochemical assays.

Work-up

After the clean-up step, the extract must be worked up to make it suitable for the estimations. The purified pooled extract is subsequently transferred into 10 to 50 ml of chloroform. This chloroform extract is then passed through the bed of anhydrous sodium sulphate to remove any moisture present in the extract. The dried chloroform extract is then evaporated to near dryness in a rotary evaporator at 50°C. Alternatively, evaporation can be carried out with the use of a steam bath, preferably under a stream of nitrogen. Finally, the concentrated chloroform extract is used for estimations.

Detection and confirmation

Since aflatoxins are fluorescent under long-wave ultraviolet (UV) light, they can be detected at very low levels. Detection or qualitative estimation of aflatoxins is usually done on TLC plates or on a minicolumn, which requires only the qualitative standards. TLC has been the most widely used method for both qualitative and quantita-

tive estimations of aflatoxins since the initial discovery of aflatoxins in 1960 [12]. Glass plates coated with a uniform layer of silica gel (Kieselgel G) were used for TLC, and chloroform/methanol and chloroform/acetone were the solvents [13]. Stubblefield et al. [14] used water/acetone/chloroform (1.5:12:88, v/v) as the solvent system for better resolution of different components of aflatoxins. Reddy et al. [15], however, obtained the best resolution in a toluene/isoamyl alcohol/methanol (90:32:2, v/v) solvent system. The developed TLC plates are then examined under long-wave UV light (365 nm), and the fluorescent spots are compared with the spots of standard aflatoxins for their colours and relative flow values.

Confirmatory tests are needed after qualitative detection of aflatoxins on TLC plates, because many compounds can behave like aflatoxins. To eliminate such false results, the identity of aflatoxin in positive samples needs to be confirmed. Most of the confirmatory tests involve the formation of a derivative that has different properties (colour of fluorescence, polarity, etc.) from that of the original toxin. When detection is done by TLC, the derivatives can be formed on the same plate, either by spotting a reagent on the toxin extract before development or by spraying a reagent after development. Przybylsky [16] suggested the use of trifluoroacetic acid in the former approach, which forms the hemiacetal derivatives (B_{2a}) of aflatoxin B_1 after reaction and can be detected at a lower relative flow value than that of standard aflatoxin B_1 . For additional confirmation, 50% sulphuric acid is sprayed on the developed plates and reacts with the blue and green fluorescent aflatoxins to give yellow fluorescent derivatives. Other confirmatory tests for aflatoxins have been suggested [17, 18].

Quantitative estimations

Quantitative estimations of aflatoxins can be done by any of the following methods.

Thin-layer chromatography and fluorodensitometric methods

As discussed earlier, TLC has been the most widely used method for quantitative estimations of aflatoxins. Developed TLC plates are examined under UV light, and aflatoxin concentrations are estimated by visual comparison of the fluorescent intensity of the spots in the sample extracts with those of the appropriate aflatoxin standards chromatographed on the same plate. Coomes et al. [12] described this visual method of estimation as sensitive, and concentrations of aflatoxin as low as 3 to 4 μg per kilogram can be detected. However, visual estimations present problems of accuracy and precision [11]. The coefficient of variation with this method may range from 20% to 30%.

Direct measurements of aflatoxins on TLC plates by fluorodensitometer are more accurate and precise than visual estimates [19]. The detection limit is 1 $\mu\text{g}/\text{kg}$. Although fluorodensitometers are commercially available, their high cost precludes their use in many laboratories, and investigators continue to compare the fluorescent spots visually [20].

TLC methods for aflatoxin estimation have been subjected to an extensive evaluation by the Smalley Check Sample Program [21] and the International Mycotoxin Check Sample Program [22]. These studies demonstrated the lack of precision associated with commonly used TLC methods; the coefficient of variation ranged from 30% to 122%. The use of TLC methods for aflatoxin estimations has also been reviewed [23, 24].

High-performance thin-layer chromatography

HPTLC is a comparatively new method that has been reviewed comprehensively by Coker [9]. It uses an automated sample applicator, a scanner, and a computing integrator, all of which lead to improved precision in the quantification of aflatoxin. If an automated densitometer/scanner is used for this purpose, the position of spots will be accurate. This can be done by using an automated spotting technique. The other advantage of this method is that only 1 μl is sufficient for spotting, as against 10 to 20 μl required for the commercial TLC method. Concentrations of aflatoxins as low as 5 pg can be detected through HPTLC [20].

High-performance liquid chromatography

The HPLC system of aflatoxin estimation has high precision, high sensitivity, and high automation. This method retains two phase systems: normal phase (liquid/solid, polar stationary phase) and reverse phase (liquid/liquid, polar mobile phase) in conjunction with UV absorption and fluorescence detection. Reverse-phase HPLC is widely used for aflatoxin analysis [20].

HPLC quantitation of aflatoxins was initially used by DeVries and Chang [25] and Tarter et al. [26]. They used trifluoroacetic acid derivatization in reverse-phase HPLC and detected aflatoxins B_1 , B_2 , G_1 , and G_2 even down to 5 pg . Subsequently post-column derivatization methods were also developed using fluorescence detection of an iodine derivative of aflatoxin B_1 [27, 28]. Coker and Jones [29] later published a comprehensive review of HPLC-based methods recommended for estimation aflatoxin. HPLC methods, however, involve complex steps for extraction and clean-up, besides being time consuming and requiring experience.

Minicolumn methods

When a large number of samples require rapid screening,

minicolumn methods are used more often in quality-control laboratories. They are simple, rapid, and less expensive than TLC and other methods of aflatoxin analysis. Since the first use of the minicolumn method as a rapid screening procedure for detection of aflatoxin in groundnuts [30], several improved procedures have been recommended [18, 31–33]. The minicolumn method of Romer [32] has been adopted by the Association of Official Analytical Chemists for aflatoxin analysis in groundnuts, groundnut products, and various other commodities. In this method, aflatoxins are extracted from the commodities by acetone/water (85:15, v/v), and the interfering compounds are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are subsequently extracted from the aqueous phase with chloroform, and the chloroform extract is then applied to the top of a minicolumn containing successive layers of neutral alumina (top), silica gel, and florosil (bottom), with a calcium sulphate drier at both ends. The column is developed with chloroform/acetone (9:1), and the aflatoxins are trapped as a tight band at the top of the florosil layer, which can be detected under UV light as a blue fluorescence. The fluorescence can be measured directly by inserting the developed minicolumn into a fluorotoxinimeter and can ultimately be calculated to give the total amount of aflatoxins present in the sample. Reference minicolumns are commercially available [20]. A minicolumn confirmation method for aflatoxin has also been reported [34].

One of the advantages of the minicolumn method is that the remaining chloroform extract is sufficiently clean to be used for a TLC presumptive test. However, this method is less accurate than other analytical methods [35].

Immunochemical methods

In addition to the above analytical methods, efforts have been made to develop some simpler and more specific methods for aflatoxin determination. The use of an immune response, with quantification of the reaction by competitive binding of either radiolabelled aflatoxin or enzyme-linked aflatoxin, has also been explored [36–39]. There has been increased progress in the development of various ELISA methods for the determination of aflatoxins, using monoclonal and polyclonal antibodies [40]. The use of affinity chromatography for detecting and determining aflatoxins is also known [41].

It is important to consider the cross-reactivity of the antibody during screening of samples for the presence of aflatoxins. The accuracy of the immunoassay of aflatoxins in naturally contaminated samples is affected by the specificity of the antibody used and by the presence of specific toxin in the sample [20]. Zhang and Chu [42] and Hefle and Chu [43] developed polyclonal and monoclonal antibodies, respectively, that show good cross-reactivity with aflatoxin B₁ and G₁. A membrane-

based enzyme immunoassay test for aflatoxin B₁ has been developed by Singh and Jang [44].

Radioimmunoassay

In this method, there is a simultaneous incubation of the sample extract or known standard with a specific antibody and a constant amount of labelled toxin. Free toxin and bound toxin are then separated by an appropriate technique, and the radioactivity in these fractions is determined [45]. The toxin concentration in the unknown sample is determined by comparing results with the standard curve established by plotting the ratio of radioactivity in the bound fraction and free fraction versus log₁₀ concentration of the non-labelled toxin. RIA can detect 0.25 to 0.50 µg of purified aflatoxin per assay in the standard preparation. The lowest detection limit for aflatoxin in the sample varies from 2 to 5 µg/kg.

RIA is, however, not widely used because it requires labelling of the toxins with tritium, which is difficult and expensive; disposal of radioactive waste is a problem; it requires a scintillation counter, which is also expensive; and only a small number of samples can be processed [46].

Enzyme-linked immunosorbent assays

Two types of ELISA can be used for the analysis of aflatoxins: direct and indirect. Both methods involve the separation of free (unreacted) aflatoxin in a liquid phase from the bound toxin in a solid phase. Direct ELISA involves the use of an aflatoxin–enzyme conjugate, whereas indirect ELISA involves the use of a protein–aflatoxin conjugate and a secondary antibody, such as goat anti-rabbit IgG, to which an enzyme has been conjugated. The most commonly used enzyme for conjugation is horseradish peroxidase, although alkaline phosphatase can be used [45, 47].

Direct ELISA

In direct ELISA, a specific antibody is initially coated to a solid phase such as a microtitre plate [48, 49]. The sample extract or standard aflatoxin is then incubated simultaneously with the enzyme conjugate or separately incubated in two steps. The amount of enzyme bound to the plate is determined after appropriate washings by incubation with a specific substrate solution. The resulting colour is subsequently measured by visual comparison with the standard toxin or by a spectrophotometer. Since this method is based on the competition for antibody-binding sites, the free toxin concentration is inversely related to the antibody-bound enzyme conjugate.

Mehan [20] compared the efficacy of several direct ELISA methods recommended for the analysis of aflatoxin B₁ in groundnuts and groundnut products. A simple

ELISA protocol was developed by Chu et al. [49], which takes about an hour for the analysis of aflatoxin B₁ in several food commodities. Although clean-up treatment is not necessary [50], the sensitivity of ELISA can be improved when it is included (for example, extraction with hexane)[51]. The results of a collaborative study reflected positive responses of the direct ELISA method [52]. Azimahtol and Tey [53] developed a direct competitive ELISA based on detecting high-affinity, specific polyclonal antibodies for aflatoxin B₁ in cereals, peanuts, and peanut butter.

Indirect ELISA

In indirect ELISA, aflatoxin–protein conjugate (KLH–aflatoxin B₁) is coated onto the microtitre plate, and the sample or standard aflatoxin is added to the wells, followed by an aliquot of an anti-aflatoxin antibody. The amount of antibody bound to the plate is detected by the addition of goat anti-rabbit IgG conjugated to alkaline phosphatase, followed by reaction with *p*-nitrophenyl phosphate to give a coloured product. The toxin is determined by comparing the colour of this reaction product with that of the standard curve prepared from known toxin concentrations.

Ramakrishna and Mehan [54] reported both direct and indirect ELISA methods for analysis of aflatoxin B₁ in groundnuts. Both methods detected concentrations of aflatoxin B₁ as low as 20 pg per well. However, indirect ELISA takes longer than direct ELISA—about 5.5 hours. The other advantages of direct over indirect ELISA are that it uses a single conjugated protein, requires one less incubation and washing step, and is less variable.

Several commercially available ELISA kits for the analysis of aflatoxins in food have been developed in the United Kingdom, Japan, France, the United States, Australia, and other countries. Mehan [20] summarized the feasibility of using these kits for the analysis of aflatoxin in groundnuts. Some of the ELISA methods have been designed as rapid screening methods, which are suitable when aflatoxin levels are below 20 µg/kg (e.g., the Agriscreen test for aflatoxin B₁, kit developed by Neogen in the United States).

Although commercial ELISA kits are suitable for aflatoxin monitoring and programmes and for testing agricultural exports or imports, their high cost may restrict their use in most analytical laboratories in developing countries.

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Nutritional status, food-consumption frequency, and nutrient intake of pre-school children in northern Ghana

Etor E. K. Takyi

Introduction

Northern Ghana, a sub-sahelian savannah region, is characterized by food shortage as a result of adverse climatic and ecological conditions. A 1987–1988 agro-ecological nutrition survey, based on a Z score cut-off point of -2 SD, indicated that malnutrition was particularly high in the savannah agroecological zones of northern Ghana [1]. The rates were 36% for chronic malnutrition (stunting) and 9.5% for acute malnutrition (wasting). This project is part of a larger project on the bioavailability of carotenoids in leafy vegetables. It involved 519 children 2.5 to 6 years of age whose parents or caregivers had consented to their participation. The objective of the study was to assess the nutritional status and nutrient intakes of pre-school children in Saboba, a rural community in northern Ghana, to determine their adequacy.

Methods

Nutritional status was assessed by anthropometric measurements of age, weight, and height and by measurements of haemoglobin and vitamin A status. Haemoglobin was measured by a haemoglobin photometer (HemoCue AB, Angelholm, Sweden), and vitamin A was determined by high-performance liquid chromatography (HPLC).

Nutrient intake was assessed by a food-frequency questionnaire and a 24-hour dietary recall on two non-consecutive days, one of which was on a weekend. Socio-cultural studies were carried out to identify any adverse

practices that could contribute to vitamin A deficiency in the areas. Stool examinations were carried out by direct smear and the Kato technique [2] to identify worm infestation.

Results

Anthropometric findings

According to anthropometric measurements, 66.7% (346) of the children were of normal nutritional status, 27% (140) were stunted, 4.4% (23) were wasted, and 1.9% (10) were wasted and stunted (table 1). Stunting was the most predominant form of growth faltering in all age groups. The percentage of stunting increased with age up to 60 months and then decreased to the level for children 48 to 60 months of age.

Dietary findings

The food-frequency questionnaire showed that whole maize was the most important cereal, with brown rice and sorghum (guinea corn) being equally important (table 2). The most commonly consumed green leaf was kapok (*Ceiba* sp.), followed by cowpea (*Vigna* sp.) and cassava (*Manihot* sp.) leaves. The most commonly eaten meats were beef, goat, and poultry. Mangoes, oranges, and pawpaws in season were the most important fruits.

Biochemical findings

The mean haemoglobin level was 10.3 g/dl. On the basis of a cut-off point of 12 g/dl, 92% of the children were anaemic. The mean level of vitamin A was 16.8 $\mu\text{g}/\text{dl}$; 16.3% had deficient levels of vitamin A ($< 0.35 \mu\text{mol}/\text{L}$). Only 28.8% of the children had adequate levels of vitamin A (0.70–1.75 $\mu\text{mol}/\text{L}$), with a staggering 54.9% having low levels (0.35–0.70 $\mu\text{mol}/\text{L}$).

The author is a senior research fellow and head of the Nutrition Unit in Noguchi Memorial Institute for Medical Research in the University of Ghana, Legon.

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

TABLE 1. Nutritional status, haemoglobin levels, and vitamin A levels of pre-school children in northern Ghana

Age (mo)	Nutritional status (%)				Haemo globin (g/dl)	Vitamin A (µg/dl)
	Normal	Stunted	Wasted	Wasted and stunted		
24–36 (n = 85)	69.4	15.3 ^a	12.9 ^a	2.4 ^a	9.8 ± 1.5	16.8 ± 6.9
37–48 (n = 118)	66.1 ^a	27.1 ^{b,c}	5.1 ^{b,c}	1.7 ^a	10.2 ± 1.4	17.0 ± 7.3
49–60 (n = 157)	65.0 ^a	33.1 ^{c,e}	1.3 ^{c,e}	0.6 ^a	10.3 ± 1.3	17.0 ± 6.8
> 60 (n = 159)	67.3 ^a	27.0 ^{d,e}	2.5 ^{d,e}	3.1 ^a	10.6 ± 1.1	16.4 ± 7.4
Total (n = 519)	66.7	27.0	4.4	1.9	10.3 ± 1.3	16.8 ± 7.1

Values in the same column followed by the same superscript numbers are not significantly different ($p > .05$).

TABLE 2. Nutrient intake (a) and food-consumption frequency (b) of pre-school children in northern Ghana

(a)			(b)	
Nutrient	RDA	% of children meeting RDA	Food	% of children consuming at least once per day
Energy	1,550 kcal	26.5	Cereals	
Protein	17.5 g	92.6	Tz (1:1 mixture of dry cassava flour and corn flour)	84.3 ^a
Iron	7 mg	97.4	Maize (whole)	83.0 ^a
Calcium	420 mg	21.7	Rice (brown)	39.5 ^b
Vitamin C	40 mg	9.5	Sorghum	38.6 ^b
Vitamin A	400 RE	10.3	Millet	31.0 ^c
			Groundnuts	
			Raw, fresh	59.2 ^a
			Roasted	50.7 ^b
			Dry, raw	58.5 ^a
			Leafy vegetables	
			Kapok	58.1 ^a
			Cowpea	26.0 ^b
			Cassava	22.8 ^b
			Meat	
			Beef	56.5 ^a
			Mutton	36.2 ^b
			Poultry	40.3 ^{b,c}
			Goat	50.2 ^{b,d}
			Fruits	
			Mango	61.2 ^a
			Orange	60.4 ^a
			Pawpaw	29.7 ^b

Values followed by the same superscript numbers are not significantly different ($p > .05$).

Discussion

Nutrient intake measurements showed that the total caloric intake was low. For more than 90% of the children, the intakes of protein and iron met the recommended daily allowances (RDAs). The intakes of calcium, vitamin A, vitamin C, and provitamin A were unacceptably low in comparison with the RDAs. Iron intake was adequate, but there was a high incidence of anaemia (92%) due to the high incidence of malaria (32% of all clinical cases) and hookworm infestation (20%), the low intake of vitamin C, and the apparently low bioavailability of iron from cereals. Furthermore, much of the protein (50%) was from plant sources. This could affect protein quality, since plant proteins are deficient in one or more essential amino acids. Socio-cultural studies did not find any factors in the communities that could significantly contribute to vitamin A deficiency. It is concluded that the poor nutritional status of the children was due to low intakes of essential nutrients, compounded by various infections.

Acknowledgements

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Books received

Antioxidant food supplements in human health. Edited by Lester Packer, Midori Hiramatsu, and Toshikazu Yoshikawa. Academic Press, San Diego, Calif., USA, 1999. (ISBN 0-12-543590 8) 511 pages, hardcover. US\$99.95.

There is considerable recent basic research on nutrient antioxidants (antioxidants that are either vitamins or commonly found in the diet) and natural source antioxidants (extracts from various sources that are rich in a wide range of antioxidants). This evidence is reviewed by leading authorities. The first chapter reviews the evidence in detail and concludes that a causal relationship between vitamin E and lung cancer has not been demonstrated. In the second chapter, smoking is shown to increase oxidative stress, but whether antioxidant vitamins help to prevent this is not clear. Chapters 3 and 4 deal with the antioxidant properties of vitamins C and E. Those of vitamin E and selenium interactions are covered in chapters 5–7, α -lipoic acids in chapters 8 and 9, coenzyme Q₁₀ in chapters 11 and 12, carotenoids in chapters 13 to 15, and flavonoids in chapters 16 to 18. Succeeding chapters deal with antioxidants from natural sources, including pycnogenols

and procyanadine in ancient pine bark, ginkgo extract, wine, herbs, tea, oyster extracts, and papaya extract. This book provides comprehensive coverage of a research area increasingly recognized to be of major health significance.

Food lipids and health. Edited by Richard E. McDonald and David B. Min. Marcel Dekker, New York, 1996. (ISBN 0-8247-9712-4) 473 pages, hardcover. US\$150.00.

Dietary lipids are recognized to be among the important determinants of human health. There is need for an authoritative summary of current data, their interpretation, and recommendations based on them. The chapters on food lipids and atherosclerosis, cancer, the immune response, and bone health are concise and offer good coverage. The rest of the book is devoted to regulatory, food technology, and marketing issues. It will meet the needs of food scientists, but public health workers need a book that goes into the epidemiological aspects of the relationship between food lipids and health as well.

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The International Union of Food Science and Technology (IUFOST) has approved the following code of professional behaviour:

In the practice of their professions, food scientists and technologists shall

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2. Honour contractual obligations to their employers whether written or inferred from the laws or customs of their own countries;
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4. Uphold the traditional values of science and the standing and dignity of the profession of food science and technology;
5. Assist in the maintenance of professional standards and the extension of their usefulness;
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7. Avoid unethical exploitation of their professional status by endorsement of misleading or fraudulent advertising or product certification;
8. Protect the public interest in the wholesomeness and safety of any food or drink with which they are concerned;
9. Declare any conflict of interest while acting as consultants;
10. Maintain professional competence by continuing awareness of relevant new information and developments;
11. Respect the character and integrity of other members of the profession giving due recognition to the work of others without distortion or discrimination;
12. Attend to the proper guidance and training of subordinates;
13. Refuse any favours or gifts which might prejudice professional judgement nor offer such;
14. Pursue by every ethical and practical means the improvement of public knowledge of basic facts of food safety and sound nutrition.

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The editors of the *Food and Nutrition Bulletin* welcome contributions of relevance to its concerns (see the statement of editorial policy on the inside of the front cover). Submission of an article does not guarantee publication—which depends on the judgement 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 may be in English, French, or Spanish. If French or Spanish is used, the author should submit an abstract in English if possible.

Format. Manuscripts should be typed or printed on a word processor, **double-spaced**, and with ample margins. Only an original typed copy or a photocopy of equivalent quality should be submitted; photocopies on thin or shiny paper are not acceptable.

When the manuscript has been prepared on a word processor, a diskette, either 3½- or 5¼-inch, should be included with the manuscript, with an indication of the disk format and the word-processing program used.

Length. Ordinarily contributions should not exceed 4,000 words.

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Note à l'intention des auteurs

La rédaction du *Food and Nutrition Bulletin* recherche des articles traitant de sujets correspondant à ses thèmes (voir au verso de la couverture la politique éditoriale de cette revue). La remise d'un manuscrit ne signifie pas sa publication, qui dépend de l'opinion de la rédaction et des réviseurs sur son intérêt et sa qualité. Tous les manuscrits susceptibles d'être acceptés sont révisés par des pairs. Les auteurs sont invités à se pencher sur les récents numéros du *Bulletin* pour prendre connaissance de son contenu et de son style.

Langues. Les manuscrits peuvent être rédigés en anglais, en français ou en espagnol, et dans ces deux derniers cas, l'auteur ajoutera, si possible, un résumé en anglais.

Format. Les manuscrits doivent être dactylographiés ou imprimés sur une machine de traitement de texte, en **double interligne**, avec une marge suffisante. Ne doit être présenté qu'un exemplaire original dactylographié ou une photocopie de qualité équivalente.

Lorsque le manuscrit a été préparé sur une machine de traitement de texte, une disquette de 3,50 ou de 5,25 pouces devrait dans toute la mesure possible y être jointe en précisant son format et le programme utilisé.

Longueur. Les manuscrits ne doivent pas, normalement, dépasser 4000 mots.

Résumé: Un résumé de 150 mots maximum doit accompagner le manuscrit. Il devra donner les buts de l'étude ou des recherches, les procédures de base (sujets de l'étude ou animaux expérimentaux et méthodes d'observation et d'analyse), les principaux résultats (fournir des données spécifiques et indiquer dans la mesure du possible leur importance statistique) ainsi que les principales conclusions. Veuillez mettre en relief les aspects nouveaux et importants de l'étude ou des observations. Prière de *ne pas* inclure des informations qui ne figurent pas dans le corps de l'article. Dans le résumé, ne citez aucun ouvrage de référence et n'utilisez ni abréviations ni sigles.

Tableaux et figures. Ils doivent être reportés sur des feuillets séparés. Les tableaux doivent être dactylographiés ou imprimés en double interligne. Veuillez soumettre uniquement des figures originales, des dessins à l'encre de Chine ou des photographies tirées sur papier glacé. Les labels qui apparaissent sur les figures doivent être dactylographiés ou gravés ou imprimés de manière professionnelle et non pas écrits à la main.

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Abréviations. Prière d'expliquer les abréviations utilisées à moins qu'elles ne soient évidentes.

Références. Les références doivent apparaître à la fin de l'article, en double interligne également. Les documents non publiés ne doivent pas figurer dans les références pas davantage que les documents présentés à des fins de publication mais qui n'ont pas encore été acceptés.

Veuillez numéroter les références dans l'ordre où elles sont mentionnées dans le texte. Identifiez au moyen d'un chiffre arabe placé entre crochets les références dans le texte, les tableaux et les légendes des figures. Les références citées uniquement dans les tableaux ou les légendes des figures doivent être numérotées en fonction de la première fois où il est fait mention du tableau ou de la figure appropriée dans le texte. **Assurez-vous que les références sont complètes.**

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—*article de journal type* (énumérer tous les auteurs):

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

—*auteur d'une société:*

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. Livre ou autre monographie

—*auteur(s) à titre personnel:*

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

—*auteur d'une société:*

4. American Medical Association, Department of Drugs. AMA drug evaluations. 3e éd. Littleton, Mass. (E.-U.): Publishing Sciences Group, 1977.

—*éditeur, compilateur, président en tant qu'auteur:*

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

—*chapitre d'un ouvrage:*

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Los editores del *Food and Nutrition Bulletin* agradecen el envío de contribuciones pertinentes al tema de la revista (vea la política editorial de esta revista en el interior de la tapa anterior). La presentación de un artículo no es garantía de su publicación, la cual dependerá del criterio de los editores y evaluadores en lo que respecta a su pertinencia y calidad. Los manuscritos con posibilidades de ser aceptados serán sometidos a evaluación por pares. Se ruega a quienes deseen colaborar que consulten números recientes de *Food and Nutrition Bulletin* para cerciorarse de su contenido y estilo.

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Cuando el manuscrito ha sido preparado usando tratamiento de textos, deberá enviarse junto al manuscrito un disco floppy, bien en 3½ pulgadas, bien en 5¼ pulgadas, indicando el formato del disco y el programa de tratamiento de textos que ha utilizado.

Longitud. Las contribuciones ordinarias no deberán exceder las 4.000 palabras.

Resúmenes. Se adjuntará al manuscrito un resumen que no exceda de 150 palabras, y que indicará el objetivo del estudio o investigación, métodos básicos (individuos, animales seleccionados en experimentos y métodos de observación y análisis), descubrimientos principales (si fuera posible aportando datos específicos y su significado estadístico), y las conclusiones principales. Se enfatizarán los aspectos nuevos e importantes del estudio u observaciones. Rogamos *no* incluya informaciones que no se hayan dado en el cuerpo del artículo. En el resumen no se citarán referencias ni se usarán abreviaturas ni siglas.

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