

# Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children<sup>1–3</sup>

Michael B Zimmermann, Nouredine Chaouki, and Richard F Hurrell

## ABSTRACT

**Background:** In many developing countries, cereal and legume-based diets contain low amounts of bioavailable iron, which may increase the risk of iron deficiency.

**Objective:** The objective was to measure change in iron status in Moroccan children who consumed their habitual diet containing low amounts of bioavailable iron.

**Design:** The design was a prospective, longitudinal, free-living cohort study in iron-replete, nonanemic 6–10-y-old children ( $n = 126$ ). Hemoglobin, serum ferritin, and transferrin receptor were measured at baseline. The children then consumed their habitual cereal and legume-based diet for 15 mo, when their iron status was retested. We used weighed food records and direct food analysis to calculate dietary iron intake and iron bioavailability. On the basis of the change in hemoglobin and body iron stores calculated from the serum transferrin receptor-to-ferritin ratio, iron balance and iron absorption were estimated over the 15-mo period.

**Results:** Mean daily iron intake was 10.8 mg/d, 97% of which was nonheme iron. Estimated nonheme-iron bioavailability from algorithms was 1.0–4.3% adjusted for low body iron stores. Over 15 mo, the mean change in total body iron was  $-142$  mg, and mean iron absorption was estimated to be 0.22 mg/d, or 2% of dietary iron. Mean hemoglobin concentration decreased 12 g/L. At 15 mo, 75% of the cohort had deficits in tissue iron, and one-third had mild iron deficiency anemia.

**Conclusion:** Low iron bioavailability from legume and cereal-based diets is a cause of iron deficiency anemia in children in rural Africa. *Am J Clin Nutr* 2005;81:115–21.

**KEY WORDS** Iron deficiency, bioavailability, diet, anemia, children, Morocco

## INTRODUCTION

Iron deficiency anemia (IDA) is common among children in developing countries, where the prevalence is often 50% or more (1). Iron balance in childhood is maintained by adjusting the rate of iron absorption to meet the increased needs for growth and expansion of the red blood cell mass and to cover basal losses from the skin and genitourinary and gastrointestinal tracts. During the first decade of life, daily needs for absorbed iron in children increase from 0.5 to 0.8 mg (2), a relatively high requirement given their smaller body size and food intake. In many developing countries, monotonous cereal and legume-based diets contain low amounts of bioavailable iron. These diets often contain little meat, supply mainly nonheme iron, and are high in inhibitors of

nonheme-iron absorption (eg, phytic acid) and low in enhancers of absorption (eg, animal tissue and ascorbic acid) (3).

Although low iron bioavailability is thought to play a central role in the etiology of IDA in developing countries (1, 3), little direct scientific evidence supports this claim. Epidemiologic associations between serum ferritin (SF), anemia, or both and dietary components, such as animal tissue and ascorbic acid, suggest that iron bioavailability could influence iron stores (4, 5). However, correlations in most cross-sectional studies were modest, and several studies found no correlation (6, 7). Although iron bioavailability strongly influences nonheme-iron absorption from single meals (3), longitudinal studies lasting weeks or months indicate little or no response of body iron stores (estimated from SF) to changes in dietary iron bioavailability, including changes in intakes of ascorbic acid (8, 9) and meat (10). This disparity may be at least partly explained by long-term adaptation in iron absorption to maintain iron stores (11). Children in developing countries with low iron stores may be able to up-regulate iron absorption from cereal-based diets to preserve hemoglobin mass. It was argued that vitamin A deficiency or blood loss from parasitic infections, rather than iron bioavailability, are important causes of IDA (7, 12, 13).

In rural northern Morocco, the prevalence of IDA in school-age children is  $\approx 35\%$  (14). We recently had the opportunity to follow up a cohort of rural Moroccan school children who had been made iron replete by their participation in a successful efficacy trial of iron fortification (14). When that trial ended, the children resumed their customary cereal and legume-based diet at home containing no fortification iron. We measured their dietary intakes by using weighed food records and tested their iron status 15 mo later, at the beginning of a second iron fortification trial. Our aim was to determine the effects of a diet of low iron bioavailability on iron status in the cohort.

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<sup>3</sup> Reprints not available. Address correspondence to MB Zimmermann, Human Nutrition Laboratory, Swiss Federal Institute of Technology Zürich, Seestrasse 72/Postfach 474, CH-8803 Rüschlikon, Switzerland. E-mail: michael.zimmermann@ilw.agrl.ethz.ch.

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## SUBJECTS AND METHODS

### Study site

The study was done in a cluster of rural villages in the Brikha Rural Commune, in the Rif Mountains of northern Morocco. The villages are 500–700 m above sea level and have a temperate climate, with an 8-mo dry season (22–34 °C; mean rainfall: 23 cm/mo), and 4-mo damp season (10–22 °C; mean rainfall: 77 cm/mo). The villages comprise ≈450 households, with a total population of ≈3600 individuals of mixed Berber and Arab descent. The villages are isolated from commercial routes, being ≈5–10 km from the nearest through paved road. Approximately one-half of the village households do not have electricity or running water. Agriculture employs >95% of the working population, and most food consumed is produced locally on small farms (15). The main foods grown are wheat, barley, dry legumes (fava beans, chickpeas, lentils), and olives. Cereal plantings occupy 45–50% of the area suitable for agriculture, dry legumes 15–20%, with the remainder being mainly olive trees (15). There is a small amount of livestock, mainly goats destined for milk and meat production.

### Measurement of dietary iron intake and estimation of iron bioavailability

To determine food intake in the villages, 3-d weighed food records were done in 50 households randomly selected from local census rolls. The records were done by 3 trained university graduates born in the villages and fluent in the local Arab dialect. They knew the families they were surveying personally and were familiar with local food customs. Households were asked to maintain their usual food habits and their traditional ways of cooking and serving foods. To account for potential seasonal variations in the diet, 24 households were studied in the winter and 26 during the summer. The records were done under the direct supervision of an experienced member of the research team on the first day of the 3-d survey in each family.

Over 3 consecutive days, the surveyors weighed the edible portions of all food and beverages consumed by the families with use of a Kern 440-53 scale (D-72458; Kern & Sohn GmbH, Albstadt, Germany) accurate to ±1 g and calibrated with use of fixed weights each week during the study. All uneaten leftovers (mainly skin and bones from meat and stones from fruit) were weighed separately and subtracted. The age and sex of the individuals participating in each meal were recorded. Local food habits on Friday differed from the remaining days of the week. On Friday either a large couscous or a tagine are consumed, and both dishes are rich in meat and vegetables. Therefore, records were kept in half of the families on 2 weekdays and a Friday and in the other half on 3 days not including a Friday. At the end of each day, members of the household were asked if a meal or snack was consumed outside the home (this was rare). If so, its content was estimated and recorded. The completeness and coding of the records were checked by the supervisor at the end of each day.

The iron and phytic acid content of 24 local foods that formed an important part of the diet were analyzed in Zürich. These foods included legumes (lentils, chickpeas, fava beans, and white beans), olives, vegetables, cereals (whole and refined wheat flour, semolina, potato, rice). Freeze-dried food samples (500 mg) were hydrolyzed by microwave digestion (MLS 1200; Microwave Laboratory Systems GmbH, Leutkirch im Allgäu,

Germany), and the iron concentration was measured by atomic absorption spectrometry with use of a graphite furnace (SpectrAA-300/400 with GTA-96 Graphite Tube Atomizer; Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia). Phytic acid in foods was measured by using the modified Makower method (16). Triplicate portions of all food samples were analyzed and values were averaged. Analyzed values for iron (>95% of the total iron in the diet came from the 24 foods that were analyzed) and phytic acid were used with data on other nutrients from the Moroccan Food Composition Table (17) and, when necessary, the Food and Agriculture Table for Africa (18).

In this region, meals consist of 1 or 2 communal dishes placed in the center of the table from which all family members eat with their hands. We therefore estimated individual food consumption by using the unit of consumption (UC) formula used by the Department of Agriculture of Morocco (15). That is for each male older than 14 y, UC = 1.0; for each female older than 10 y, UC = 0.8; and for each male aged 14 y or younger and each female aged 10 y or younger, UC = 0.3 + [0.05 × age (in y)]. To determine individual nutrient intakes for a meal, the overall sum of a nutrient for a family was divided by the total amount of UCs participating in that meal. Vitamin A intakes were calculated as retinol activity equivalents (RAEs), using a conversion factor of 12 μg β-carotene to 1 μg retinol (19).

The algorithms of Tseng et al (20) and Reddy et al (21) were used to estimate iron bioavailability. It was assumed that only meat, fish, and poultry (MFP) contained heme iron. In meat, poultry, and fish, 50%, 40%, and 20% of iron was estimated to be heme iron, respectively, with the remainder being nonheme iron. To estimate nonheme-iron absorption for a range of body iron, results of the algorithms were adjusted for high, medium, and low body iron stores (22). It was assumed that the absorption of heme iron was stable in the presence of either enhancers or inhibitors at a rate of 23% for high iron stores, 28% for medium stores, and 35% for low stores (23).

### Cohort study

The baseline measurements for this study were done at the completion of a 1-y iron fortification trial in primary school children in the villages (14). In that trial, the addition of encapsulated ferrous sulfate to salt was highly efficacious in reducing the prevalence of IDA. The cohort for the present study was enrolled from that study population by including all 6–10-y-old children who 1) were nonanemic (hemoglobin ≥ 115 g/L), 2) were iron sufficient [SF ≥ 15 μg/L and transferrin receptor (TfR) ≤ 8.5 mg/L], 3) had a serum C-reactive protein (CRP) concentration <10 mg/L (to reduce the confounding effect of inflammation on SF), and 4) were nonmenstruating. One hundred thirty-four children met these inclusion criteria. When distribution of the iron-fortified salt stopped at completion of the efficacy trial in December 2001, the children resumed their habitual diets at home containing no fortification iron.

In April 2003, in preparation for a second set of iron fortification trials in the area, all children in the same 3 primary schools underwent another screening, with measurements of height, weight, hemoglobin, SF, TfR, serum retinol (SR), and CRP. Of the 134 children who were originally enrolled, 4 had moved away, 2 declined venipuncture, 1 had an elevated CRP, and 1 had begun menstruating. These 8 children were excluded, leaving a cohort of 126 children with complete measurements at baseline and 15 mo later. Informed written consent (or, if parents were

illiterate, oral consent) was obtained from the parents and oral assent from the children. The Swiss Federal Institute of Technology Zürich and the Ministry of Health in Rabat gave ethical approval for the studies. After the 15-mo measurements were collected, the cohort in this study joined the remainder of the children in the schools in a new iron fortification trial, which started in May 2003.

Blood from venipuncture was collected into tubes containing EDTA and transported on ice to the local laboratory. Hemoglobin was measured in whole blood (refrigerated and measured on the day of collection) with use of an AcT8 Counter (Beckman Coulter, Krefeld, Germany), using controls provided by the manufacturer. Anemia was defined as a hemoglobin concentration <115 g/L in children aged 6–11 y (1). Serum samples were divided into aliquots and frozen at –20 °C until analysis. SF and TfR were measured by using enzyme-linked immunosorbent assays (RAMCO, Houston, TX), with controls provided by the manufacturer. For SF, at concentrations of 17 and 94 µg/L, interassay CV was 24.4% and 11.2%, and intraassay CV was 9.2% and 9.1%. For TfR, at concentrations of 5.9 and 15.9 mg/L, interassay CV was 6.4% and 10.0%, and intraassay CV was 6.6% and 10.1%. Iron deficiency was defined as either SF <15 µg/L or TfR >8.5 mg/L. CRP was measured by using nephelometry (TURBOX; Orion Diagnostica, Espoo, Finland); values >10 mg/L were considered elevated. SR was measured by HPLC, and vitamin A deficiency was defined as a SR <0.70 µmol/L.

### Data analysis

Within-subject and between-subject coefficients of variation in daily iron intake were calculated and used to determine the precision of our estimate of the mean daily iron intake for the cohort, using the method of Beaton et al (24), as shown in Equation 1:

$$D_t = Z_\alpha \sqrt{CV_b^2/g + CV_w^2/gn} \quad (1)$$

where  $D_t$  is the greatest deviation from the mean as a percentage of long-term true intake (half of the 95% CI of the mean),  $Z_\alpha$  is the normal deviation for the percentage of times the measured value should be within a specified limit (1.96 for 95% confidence),  $CV_b$  is the between-subject CV,  $CV_w$  is the within-subject CV,  $g$  is the number of subjects, and  $n$  is the number of days measured.

The 4 principal components of the iron requirement in young children are basal iron losses, increase in storage iron, increase in nonstorage iron in tissues, and increase in hemoglobin iron (2). Each of these components was calculated for the individual children in the cohort.

### Basal iron losses

Daily basal losses of iron were calculated as follows (25, 26):

$$\text{Basal iron losses} = 0.538 \times \text{body surface area (BSA; in m}^2\text{)} \quad (2)$$

where

$$\text{BSA(m}^2\text{)} = \text{weight (kg)}^{0.5378} \times \text{height (cm)}^{0.3964} \times 0.024265 \quad (3)$$

For the calculation of BSA, the mean of the weights and heights from the baseline and 15-mo measurements were used.

### Body iron stores

Body iron stores were estimated from the ratio of serum TfR to SF as follows (27):

$$\text{Body iron (mg/kg)} = - [\log(\text{TfR/SF ratio}) - 2.8229]/0.1207 \quad (4)$$

Positive values indicate the amount of iron in stores, and negative values indicate the deficit in tissue iron.

### Nonstorage iron in tissues

It was assumed nonstorage iron in tissues, mainly in enzymes essential for cell function, would be conserved despite overall negative iron balance. Nonstorage iron was calculated as follows (28):

$$\text{Nonstorage iron} = 0.7\text{mg}[\text{body weight (kg) at 15 mo} - \text{body weight (kg) at baseline}] \quad (5)$$

### Iron in hemoglobin

Blood volume (BV) was calculated from weight and height at baseline and at 15 mo as follows (29):

$$\text{For boys: } \log \text{BV (mL)} = (0.6459) \log \text{weight (kg)} + (0.002743) \text{height (cm)} + 2.0324 \quad (6)$$

$$\text{For girls: } \log \text{BV (mL)} = (0.6412) \log \text{weight (kg)} + (0.001270) \text{height (cm)} + 2.2169 \quad (7)$$

The change in hemoglobin (Hb) mass (in g) was calculated as follows:

$$\Delta \text{Hb mass} = [\text{BV (L)} \times \text{Hb concentration (g/L) at 15 mo}] - [\text{BV (L)} \times \text{Hb concentration (g/L) at baseline}] \quad (8)$$

The change in iron in Hb was then calculated as follows (2):

$$\Delta \text{Hb iron (mg)} = \Delta \text{Hb mass (g)} \times 3.39 \text{ mg Fe/g Hb} \quad (9)$$

The local health records of the past 2 y were reviewed with the chief medical officer. There were no cases of malaria, and treatment of infections with *Necator americanus*, *Trichocephalus trichiura*, or *Schistosoma mansoni* and *Schistosoma hematobium* were rare and confined to individuals with a history of recent travel to endemic areas. Treatment of infections with *Ancylostoma duodenale*, often found in the Mediterranean basin, was uncommon. Because of the dry, temperate climate and clean public water supply, parasites that cause blood loss are rare in children in northern Morocco. We therefore assumed that there were negligible iron losses in the cohort other than obligatory basal losses.

The final balance equation used to estimate the amount of dietary iron absorbed over the 15-mo study was as follows:

$$\begin{aligned} \text{Dietary iron absorbed} = & \text{basal iron losses} \\ & + \Delta \text{nonstorage iron in tissues} + \Delta \text{Hb iron} \\ & + \Delta \text{body iron stores} \quad (10) \end{aligned}$$

**TABLE 1**

Dietary intakes of iron and enhancers or inhibitors of iron absorption in 6–10-y-old children consuming their customary diet in rural northern Morocco<sup>1</sup>

	Intake per 1000 kcal	Intake per day
Total dietary iron (mg)	5.7 ± 1.1	10.8 ± 2.3
Nonheme iron (mg)	5.2 ± 1.1	10.5 ± 2.3
Heme iron (mg)	0.14 ± 0.03	0.29 ± 0.07
Phytic acid (mg)	846 ± 89	1709 ± 176
Ascorbic acid (mg)	19.6 ± 11.2	39.6 ± 22.8
Meat, fish, and poultry (g)	27.7 ± 16.2	55.9 ± 33.1

<sup>1</sup> All values are  $\bar{x} \pm SD$ ;  $n = 63$ .

### Statistical analysis

Data processing and statistics were done with use of PRISM3 (GraphPad, San Diego, CA) and EXCEL 97 (Microsoft, Seattle, WA). Variables not normally distributed were logarithmically transformed before analysis. Data at baseline and at 15 mo were compared by using paired *t* tests with a Bonferroni correction for multiple comparisons. Stepwise linear regression models were calculated with the change in body iron (mg/kg body weight) from 0 to 15 mo as the dependent variable, and change in height, weight, and BSA from 0 to 15 mo as independent variables. Significance was set at  $P < 0.05$ .

## RESULTS

### Weighed food records

Fifty families and a total of 322 subjects (median age: 19 y; range: 2–74 y) participated in the 3-d weighed food records. Sixty-three subjects were 6–10-y-old children. The dietary staple was wheat flour used to make bread that was consumed at every meal. Approximately 45% was whole-wheat flour and 55% was refined wheat flour (“farine de luxe”). Overall, cereals contributed 58% of the total energy in the diet. Eighteen percent of total energy came from oil and fats; >90% of this was from olives, olive oil, or both consumed at nearly every meal. Legumes contributed 4% of total energy and included mainly fava beans along with chickpeas and lentils. MFP contributed 5% of dietary energy; the main source was fish (68% of MFP, mainly sardines).

Vegetables consisted of mainly potatoes, tomatoes, and onions and supplied <3% of total energy.

Daily mean ( $\pm$  SD) energy and protein intakes in 6–10-y-old children were 2020  $\pm$  273 kcal, and 27  $\pm$  6 g, respectively. Mean ( $\pm$  SD) vitamin A intake was 247  $\pm$  69 RAE/d, with 69% as retinol and 31% as carotenenes from plant foods. By direct analysis, the iron and phytic acid contents (mg/100 g dry weight) of the principal staples were the following: whole wheat flour, 1.91  $\pm$  0.09 mg iron and 358  $\pm$  35 mg phytic acid; refined wheat flour, 1.58  $\pm$  0.03 mg iron and 83  $\pm$  5 mg phytic acid; lentils, 6.87  $\pm$  0.90 mg iron and 589  $\pm$  28 mg phytic acid; fava beans, 4.15  $\pm$  0.56 mg iron and 595  $\pm$  25 mg phytic acid; and chickpeas, 6.34  $\pm$  0.43 mg iron and 823  $\pm$  21 mg phytic acid.

The sources of iron in the diet were as follows: cereals, 57%; legumes, 13%; vegetables, 11%; MFP, 9%; eggs, 4%; fruit, 3%; and other, 3%. The daily intakes of iron, phytic acid, ascorbic acid, and MFP in 6–10-y-old children are shown in **Table 1**. Mean ( $\pm$  SD) iron intake was 10.8  $\pm$  2.3 mg/d, 97% of which was nonheme iron. Cereals (mainly wheat flour in bread) contributed >90% of dietary phytic acid. Vegetables contributed 81% of dietary ascorbic acid. Comparing winter with summer, there were no significant differences in within-subject CV or between-subject CV in daily iron intakes and no significant differences in mean daily intakes of iron, phytic acid, or ascorbic acid (data not shown). Overall, the within-subject CV in daily iron intake was 17.1%, and the between-subject CV in daily iron intake was 21.3%. Therefore, the precision of the estimated mean daily iron intake in the cohort from the 3-d weighed food records was adequate; the 95% CI of the group mean was  $\pm$  5.8%. Because the diet was high in phytic acid and the molar ratio of ascorbic acid to iron was low ( $\approx$  1:1), estimated nonheme-iron bioavailability was only 0.3–1.3% and 1.0–4.3% for high and low body iron stores, respectively (Table 1). Calculated total dietary iron absorbed daily in 6–10-y-old children assuming high, medium, and low body iron stores is shown in **Table 2**.

### Cohort study

The changes in the cohort over the 15-mo study are shown in **Table 3**. Mean weight and height increased by 2.0 kg and 5.0 cm, respectively ( $P < 0.05$ ). Although mean hemoglobin mass decreased only 2.5%, blood volume expanded 7.5% ( $P < 0.05$ ), so that mean hemoglobin concentration fell 12 g/L ( $P < 0.01$ ). This

**TABLE 2**

Estimated iron absorption in 6–10-y-old children consuming their customary diet in rural northern Morocco, adjusted for high, medium, and low iron stores<sup>1</sup>

	Iron stores <sup>2</sup>		
	High	Medium	Low
Percentage nonheme iron absorption (%)			
Algorithm of Reddy et al (21)	0.3 $\pm$ 0.2	0.6 $\pm$ 0.4	1.0 $\pm$ 0.8 <sup>3</sup>
Algorithm of Tseng et al (20)	1.3 $\pm$ 0.3	2.9 $\pm$ 0.6 <sup>4</sup>	4.3 $\pm$ 0.9 <sup>5,6</sup>
Total absorbed dietary iron (mg/d)			
Algorithm of Reddy et al (21)	0.10 $\pm$ 0.01	0.14 $\pm$ 0.02 <sup>3</sup>	0.21 $\pm$ 0.03 <sup>5,6</sup>
Algorithm of Tseng et al (20)	0.20 $\pm$ 0.03	0.38 $\pm$ 0.07 <sup>4</sup>	0.55 $\pm$ 0.11 <sup>5,7</sup>

<sup>1</sup> All values are  $\bar{x} \pm SD$ ;  $n = 63$ .

<sup>2</sup> From Cook et al (22): high stores, serum ferritin concentrations of 65  $\mu$ g/L; medium stores, serum ferritin concentrations of 30  $\mu$ g/L; low stores, serum ferritin concentrations of 20  $\mu$ g/L.

<sup>3–5</sup> Significantly different from high stores (paired *t* tests with Bonferroni correction): <sup>3</sup> $P < 0.05$ , <sup>4</sup> $P < 0.01$ , <sup>5</sup> $P < 0.001$ .

<sup>6,7</sup> Significantly different from medium stores (paired *t* tests with Bonferroni correction): <sup>6</sup> $P < 0.01$ , <sup>7</sup> $P < 0.05$ .

**TABLE 3**

Anthropometric, biochemical, and hematologic variables in a cohort of 6–10-y-old Moroccan children who were nonanemic and iron-sufficient at baseline and consumed their habitual diet (low in bioavailable iron) for 15 mo<sup>1</sup>

	Baseline	15 mo
Age (y)	8.2 ± 1.1	9.4 ± 1.1
Height (cm)	129 ± 8	134 ± 8 <sup>2</sup>
Weight (kg)	26.3 ± 3.7	28.3 ± 3.9 <sup>2</sup>
Blood volume (L)	1.99 ± 0.24	2.14 ± 0.26 <sup>2</sup>
Hemoglobin (g/L)	128 ± 9	116 ± 9 <sup>3</sup>
Hemoglobin mass (g)	255 ± 38	248 ± 38
Hemoglobin iron (mg)	863 ± 129	841 ± 128
Serum ferritin (μg/L)	34 ± 14	13 ± 12 <sup>3</sup>
Transferrin receptor (mg/L)	6.9 ± 1.1	9.7 ± 4.5 <sup>3</sup>
Body iron stores (mg/kg)	4.1 ± 1.5	−1.1 ± 3.5 <sup>4</sup>
Total body iron stores (mg)	106 ± 40	−15 ± 76 <sup>4</sup>
No. of children with anemia (%)	0	54 [43]
No. of children with iron deficiency anemia (%)	0	42 [33]
No. of children with tissue iron deficit (%)	0	95 [75]
Serum retinol (μmol/L)	—	0.92 ± 0.15
No. of children with vitamin A deficiency (%)	—	15 [12]

<sup>1</sup> *n* = 67 girls and 59 boys. Percentages in brackets.

<sup>2–4</sup> Significantly different from baseline (paired *t* tests with Bonferroni correction): <sup>2</sup>*P* < 0.05, <sup>3</sup>*P* < 0.01, <sup>4</sup>*P* < 0.001.

increased the prevalence of anemia from 0% to 43% at 15 mo. The iron status indicators worsened: there was a significant decrease in mean SF (*P* < 0.01) and an increase in mean TfR (*P* < 0.01). Mean body iron stores in the children fell by −5.2 mg/kg (*P* < 0.001). At 15 mo, three-quarters of the cohort had deficits in tissue iron, and one-third had IDA. In the regression models, changes in height, weight, and BSA were not significantly correlated with change in body iron (in mg/kg) from 0 to 15 mo.

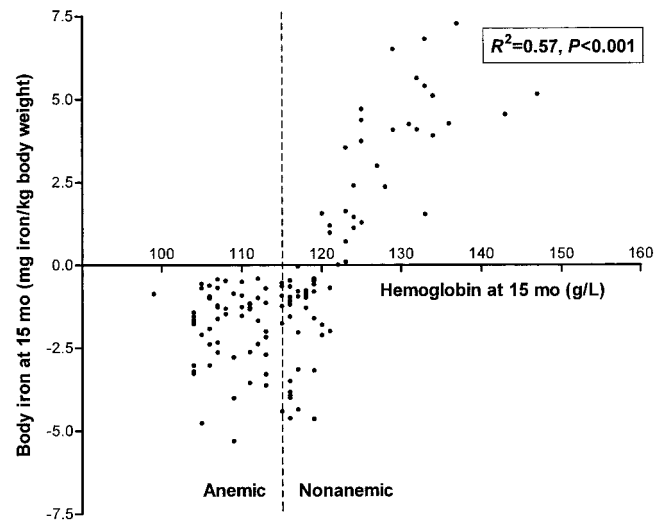
The components of iron balance in the cohort over the 15 mo study are shown in **Table 4**. The mean overall decrease in total body iron was −142 mg, because of a modest decrease in hemoglobin iron, a sharp decrease in iron stores, and a slight increase in nonstorage tissue iron. Total mean dietary iron absorption over 15 mo was 99 mg, or 0.22 mg per day. Because mean dietary intake of iron was 10.8 mg/d (Table 1), only 2% of the iron in the local diet was absorbed during the 15-mo period. Cook et al (27)

**TABLE 4**

Calculated iron balance in a cohort of 6–10-y-old Moroccan children consuming their habitual diet (low in bioavailable iron) for 15 mo<sup>1</sup>

	Total over 15 mo	Daily
Basal iron losses (mg)	241 ± 23	0.54 ± 0.05
Change in body iron (mg)		
Change in hemoglobin iron	−22.1 ± 27.5	−0.05 ± 0.06
Change in total body iron stores	−120.9 ± 85.6	−0.27 ± 0.19
Change in nonstorage iron in tissues	1.44 ± 0.52	0.003 ± 0.001
Total dietary iron intake	4.9 ± 1.0 g	10.8 ± 2.3 mg
Dietary iron absorbed (mg)	99.3 ± 92.1	0.22 ± 0.20
Dietary iron absorbed (%)	2.0 ± 1.8	2.0 ± 1.8

<sup>1</sup> All values are  $\bar{x} \pm$  SD; *n* = 126.



**FIGURE 1.** The relation between hemoglobin, anemia, and body iron stores at 15 mo in the cohort of school-age children (*n* = 126) consuming their customary diet in rural northern Morocco. Body iron stores were estimated by the method of Cook et al (27). Positive values indicate the amount of iron in stores, and negative values indicate the deficit in tissue iron. *P* values were determined by Pearson's correlation.

reported IDA developed in adults at a deficit in iron stores of −4 mg/kg. Our data indicate children develop mild anemia even with smaller deficits of tissue iron stores (**Figure 1**). Among the 54 children with anemia in the cohort at 15 mo ( $\bar{x} \pm$  SD hemoglobin = 108 ± 4 g/L), although all had deficits in iron stores, the mean iron deficit was only −1.1 mg/kg.

## DISCUSSION

This is the first prospective, longitudinal cohort study in a developing country to assess the effect of habitual diets low in bioavailable iron on iron status. Strengths of the study included the following: 1) a moderately large sample size limited to a discrete population group vulnerable to IDA, 2) a cohort free of parasitic infections that cause blood iron losses and confound measurement of SF, 3) detailed characterization of the local diet with supervised weighed food records in 2 seasons in randomly selected families and direct analysis of iron and phytic acid content of principal foods, and 4) reliable quantitative laboratory measurement of iron status, including SF, TfR, and body iron stores.

Our findings demonstrate that low iron bioavailability from a legume and cereal-based diet can be a cause of iron deficiency in children in rural Africa. The children in the cohort had sufficient dietary energy and protein, and their diet contained 5.4 mg iron/1000 kcal. This is near the usual iron content of typical Western diets, ≈6 mg/1000 kcal (2), and is consistent with data from food balance sheets for Africa from the Food and Agriculture Organization (30) reporting per capita iron intake of 14–21 mg/d. Because the absorbed iron requirement for this age group is 0.7–0.8 mg/d (2), the children's iron intake was many times higher than their iron requirement. They would have needed to absorb ≈8% of their dietary iron to meet their requirements, but they only absorbed ≈2%, or 0.23 mg/d. Iron balance was negative over the 15 mo, and most of the children exhausted their iron stores and developed iron deficiency.


There are several potential limitations to the findings in this study. First, the accuracy and precision of food records to define nutrient intake could be limited by sampling bias, variation, and measurement error. The families participating in the food survey could have modified their usual food habits, so that the intake data might not have accurately represented the habitual diet. However, well-trained, local surveyors emphasized the need to maintain usual diets and did the weighing and recording in all households. The households were randomly selected and none declined participation. Three-day weighed records can be an accurate method for assessing dietary intake of specific nutrients (31), and both the within- and between-subject variations in daily iron intake were lower than those generally reported from Western countries, as a result of the day-to-day uniformity of the local diet (31). Second, our iron balance calculations were based on several assumptions. We calculated basal losses with use of a formula derived in adults and adjusted for BSA (2). This approach might underestimate basal losses in younger children, because it gives lower values than gastrointestinal losses of iron reported in infant studies (32). There could be additional losses of iron in the cohort from occult bleeding or infection or both that went undetected. If iron losses were underestimated, our calculations would have underestimated the amount of dietary iron absorbed. Although we assumed that nonstorage tissue iron would increase, IDA can reduce the activity of iron-dependent enzymes (33), but this component contributes only minimally to iron needs. The cohort included only children with a negative CRP to minimize the confounding effect of inflammation on SF, but the timing of the rise and fall of CRP during the acute-phase response differs from that of SF. It is possible that SF was elevated by the acute-phase response in some children despite a negative CRP. Finally, the algorithm of Cook et al (27) for calculating body iron stores is derived from an adult phlebotomy study and is not validated in children. However, in a recent iron fortification trial in children it performed well (34).

Dietary iron in northern Morocco is poorly absorbed because concentrations of dietary MFP and ascorbic acid are low, and the phytic acid content is high. Legumes and cereals are the dietary staples, providing two-thirds of dietary energy, and both are rich in phytic acid. Mean daily intake of phytic acid was 1.7 g, compared with the usual phytic acid content of  $\approx 0.2$ – $0.8$  g/d in Western diets (35). Phytic acid is a strong inhibitor of iron absorption. Hallberg et al (36) found that adding 20 mg phytic acid/100 g to bread rolls decreased iron absorption by 40%. In northern Morocco, bread made from the local wheat flour contains  $\approx 350$  mg phytic acid/100 g dry weight. The 2% absorption of dietary iron in this study is consistent with results from isotope-labeled single meal studies in which iron absorption (both of native iron and fortification iron) was as low as 1–3% from meals based on whole-grain cereals and legumes, even in iron-deficient subjects (37–39). It is lower than the usual estimate of  $\sim 5\%$  iron bioavailability from plant-based diets in developing countries.

Ascorbic acid is an enhancer of iron absorption in the presence of phytic acid (37). The magnitude of the effect depends on the amount of ascorbic acid and the food matrix. An ascorbic acid-to-iron molar ratio of  $\geq 2$ :1 will usefully increase the absorption of soluble iron from low phytate foods, but a ratio of  $\geq 4$ :1 is needed to increase iron absorption from diets high in phytic acid (40). In the present study, the molar ratio of ascorbic acid to iron was  $\approx 1$ :1. Actual intakes of ascorbic acid were likely lower than

estimated; ascorbic acid is susceptible to losses during food storage and food preparation, and most Moroccan dishes containing vegetables (the source of  $>80\%$  of dietary ascorbic acid) are simmered for long periods. Muscle tissue is also a strong enhancer of nonheme-iron absorption (3). Although mean daily consumption of MFP in the children was 56 g, more than two-thirds was in the form of sardines, which are estimated to have a heme iron content of only 20%. Compared with industrialized countries, where  $\approx 10$ – $15\%$  of iron intake is heme iron (41), in the present study  $<3\%$  of dietary iron was heme iron. Nevertheless, because of its high absorption, we estimated heme iron contributed up to 30% of the total amount of iron absorbed.

We used 2 algorithms to estimate dietary iron absorption in the present study (20, 21). Of the 2 models, based on our calculation of absorbed iron, the model of Reddy et al (21) better predicted iron absorption from the local diet. When adjusted for low iron stores, it predicted an iron absorption of 0.21 mg/d, in close agreement with the calculated mean absorption of 0.22 mg/d in the cohort. Reddy et al proposed their model could predict nonheme-iron absorption from meals typical of Western diets. Although it was criticized as unsuitable for diets in developing countries (42), it performed well when applied to the northern Moroccan diet, even though the local phytic acid content was much higher than in the Western meals from which the algorithm was derived (21). A variable not considered by these absorption models is vitamin A status. Vitamin A deficiency can impair iron metabolism and bioavailability (43). The children in the cohort were marginally vitamin A deficient, and 12% of children had serum retinol concentrations  $< 0.7$   $\mu\text{mol/L}$ . Although the severity of vitamin A deficiency was only mild, it is possible that poor vitamin A status also contributed to low iron bioavailability in the cohort.

Our data highlight the challenges faced by iron fortification programs in developing countries where the diet is low in iron bioavailability. To meet a local Moroccan child's iron requirements, a food fortification program would need to supply  $\approx 0.5$  mg additional absorbed iron daily. Without a change in dietary quality, and assuming fortification iron enters the common pool and is absorbed at a level of 2%, 23 mg additional iron per day would be needed. The current flour fortification program in Morocco calls for 45 mg iron as electrolytic iron/kg flour. Assuming a relative bioavailability of 50% for electrolytic iron compared with iron in the common pool (44), a child in northern Morocco would need to consume nearly 1 kg flour/d to meet his or her needs. These data emphasize that the mere addition of iron to staple foods in developing countries, without providing an enhancer of iron absorption, is unlikely to have significant effect on iron status. The findings underscore the need for new approaches to improve iron bioavailability in food fortification. 

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Each of the authors contributed to the study design. MBZ and NC performed the fieldwork and the data collection. MBZ and RFH supervised the laboratory analysis and completed the data analysis. MBZ conducted the statistical analysis. The first draft of the manuscript was written by MBZ. All authors edited the manuscript. None of the authors had a financial or personal conflict of interest in regard to this study.

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