

# Diet and iron status of nonpregnant women in rural Central Mexico<sup>1-3</sup>

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## ABSTRACT

**Background:** Few studies have examined the relation of iron status to diet in populations from developing countries with high levels of iron deficiency and diets of poor quality.

**Objective:** The objective was to identify nutrients, dietary constituents, and foods that are associated with better iron status in a rural Mexican population.

**Design:** A prospective cohort study was conducted in rural central Mexico. The subjects were 125 nonpregnant women aged 16–44 y. During the 12 mo before blood collection, food intakes were assessed repeatedly by a combination of dietary recalls, food weighing, and food diaries [mean ( $\pm$ SD) days of food intake data:  $18.8 \pm 5.9$  d]. Hemoglobin, hematocrit, and plasma ferritin were measured at the end of the study.

**Results:** Higher plasma ferritin concentrations were associated with greater intakes of nonheme iron and ascorbic acid after control for age, BMI, breast-feeding, season, and the time since the birth of the last child. Higher ascorbic acid intakes, but not higher intakes of heme and nonheme iron, predicted a lower risk of low hemoglobin and hematocrit values after control for the background variables. Consumption of the alcoholic beverage *pulque* predicted a lower risk of low ferritin and low hemoglobin values. Seasonal variation in ferritin, hemoglobin, and hematocrit values was observed.

**Conclusion:** Better iron status was associated with greater intakes of foods containing nonheme iron and ascorbic acid. *Pulque*—a beverage containing iron, ascorbic acid, and alcohol—may influence the iron status of women in rural central Mexico. *Am J Clin Nutr* 2002;76:156–64.

**KEY WORDS** Iron status, nonpregnant women, diet, Mexico

## INTRODUCTION

An estimated 5000 million people worldwide are iron deficient, and 52% of the world's pregnant women are thought to be anemic (1). Iron absorption is strongly affected by the form of iron consumed and by other constituents in the diet (2, 3). Heme iron, which is highly bioavailable, is often consumed in small amounts by persons in developing countries, who obtain most of their iron from plant products; this nonheme iron is often poorly absorbed because the diets in developing countries are often high in constituents that impair iron absorption. Therefore, one approach to preventing iron deficiency is to improve the bioavailability of nonheme iron by either reducing

intakes of iron inhibitors (phytates, polyphenols, and calcium) or increasing intakes of ascorbic acid, meat, and other dietary constituents that improve iron absorption.

The scientific understanding of iron absorption comes largely from experimental research that has focused on single foods or simple meals that were labeled with radioactive or stable isotopes of iron (2). However, recent work based on more complicated models (including extended time periods, complex meals, and multiple enhancers or inhibitors) suggests that the single-meal approach can result in flawed estimates of the magnitude of inhibition and enhancement of iron absorption (2, 4).

Relatively few studies have examined the relation of usual diets to iron status in free-living populations, the individual diets of whom vary in content, in the times at which they are eaten, and in the techniques used to prepare them. An exception is a study conducted by Fleming et al (5), who examined the relation of diet to iron stores in elderly participants in the Framingham Heart Study. Higher iron stores were associated with lower intakes of coffee and with greater intakes of heme iron, supplemental iron, ascorbic acid, alcohol, meat, processed meat, and fruit. In developing countries, where iron deficiency is highly prevalent, the sparse literature is due in part to the large number of days of dietary data required to detect such associations.

The current study examined the relation of usual diet to the iron status of 125 nonpregnant women living in the Solís Valley of rural central Mexico. We used extensive dietary data to 1) characterize the relation of hemoglobin, hematocrit, and ferritin to prior consumption of heme and nonheme iron and to intakes of dietary constituents known to either enhance or inhibit iron absorption and 2) examine the relation of iron sta-

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tus to consumption of those foods that are major dietary sources of iron or of dietary constituents expected to influence iron absorption.

## SUBJECTS AND METHODS

### Mexico Nutrition Collaborative Research and Support Program in Human Nutrition

Data were collected as part of the Mexico Nutrition Collaborative Research and Support Program in Human Nutrition (NCRSP), a prospective cohort study of the relation of diet to human function (6). Fieldwork was conducted between 1982 and 1986 in 6 small communities located in the Solís Valley, a rural area of central Mexico. The Mexico NCRSP was a collaboration between the University of Connecticut and the Instituto Nacional de la Nutrición Salvador Zubirán in Mexico City. The research protocols were approved by committees on the use of human subjects at both institutions.

#### Solís Valley

The Solís Valley is situated in the central highlands of Mexico, a high-altitude environment located  $\approx 2400$  m (7900 ft) above sea level. The climate is marked by a rainy season that lasts from late May through mid-September and by a dry season that lasts from the time of risk of frost in October through March. At the time of the study, the important local economic activities were subsistence corn (maize) farming and low-skill wage labor. About 25% of males are engaged in seasonal migration and leave the valley during the summer months to work elsewhere in Mexico and in North America. Households in this region obtain food by a combination of subsistence agriculture (primarily through the production of corn and animal products), barter, purchase, and gathering of wild or semiwild fruit and vegetables. The most commonly gathered foods are *quelites* (wild greens), *nopales* (cactus leaves), and *tanás* (cactus fruits) (7). Most foods, other than tortillas, are prepared by stewing in cooking pots that are usually made of aluminum.

#### Subjects

The data collection phase of the Mexico NCRSP was between January 1984 and June 1986. Subjects were selected because of the presence in the household of 1) a pregnant women, 2) a preschool-aged child (aged 18 mo), or 3) a school-aged child (aged 7–8 y). Because refusals to participate were rare, the subjects enrolled in the study represented nearly all those identified by census as eligible for inclusion. The women identified as pregnant were followed prospectively until their infants reached the age of 8 mo. The mothers of preschoolers and school-aged children were followed for  $\approx 12$  mo.

The analytic sample consisted of 125 nonpregnant mothers aged 16–44 y for whom  $> 10$  d of dietary data had been collected and hemoglobin, hematocrit, and ferritin had been measured, ie, 59.5% of the 210 women who enrolled in the study. The reasons for the elimination of the other 85 women were as follows: 13 dropped out; 27 did not provide a blood sample; 40 had a missing ferritin, hematocrit, or hemoglobin value; 4 had fewer than 10 d of dietary data collected before their blood samples were taken; and 1 because of a very high white blood cell count ( $12800/\mu\text{L}$ ). Missing blood data were almost entirely due to the difficult logistics of blood collection and transport.

In a comparison of the analytic and larger samples, no statistically significant differences were seen in household energy needs ( $t = -0.58$ ,  $P = 0.5635$ ;  $n = 194$ ) or maternal education ( $t = -0.84$ ,  $P = 0.4041$ ;  $n = 174$ ). The subjects in the analytic sample tended to come from somewhat wealthier households ( $t = -3.11$ ,  $P = 0.0021$ ;  $n = 194$ ) and from communities that were less remote ( $\chi^2 = 12.51$ ,  $P = 0.0284$ ;  $n = 210$ ).

#### Blood collection and analysis

The time of blood collection was scheduled at the end of the study, which for most women was  $\approx 12$  mo after enrollment. The subjects fasted overnight and were then transported to the clinic in Solís for phlebotomy during the early morning hours. Venous blood was drawn into tubes containing EDTA as the anticoagulant. The blood samples were transported on ice to the Instituto Nacional de la Nutrición Salvador Zubirán in Mexico City within  $\approx 4$  h of being drawn. Hemoglobin, hematocrit, and the mean corpuscular volume were measured with an electronic counter (model ZF; Coulter Electronics, Hialeah, FL). Quality-control measures included assessment of duplicate samples that were checked once per week and controls that were analyzed daily between samples. Plasma ferritin was assessed in duplicate with a radioimmunoassay kit (Ferrizyme; Abbott Laboratories, Berkeley, CA). Plasma vitamin B-12 and folate values were available for a subset of 43 mothers. Details of the laboratory procedures were provided by Black et al (8).

#### Food intake

Staff nutritionists assessed dietary intake during 2 consecutive 24-h periods per month (8, 9). At the end of each 24-h period, the subjects provided a verbal list of all the foods and recipes consumed by the household; the subjects also identified each ingredient in each recipe and provided an estimate of the quantity used, using the actual ingredients and commonly used household utensils whenever possible. The nutritionist then weighed and recorded each ingredient still available in the home or provided an estimate of the amount consumed if necessary. The subjects also kept a simple food diary for recording the number of tortillas consumed throughout the day. Validation studies during the preliminary phase of the Mexico NCRSP showed strong agreement between estimates of energy intake provided by this technique and those provided by direct weighing.

Daily nutrient intakes were estimated by using the International Minilist (University of California, Berkeley), a nutrient database created specifically for use by the NCRSP projects (10). The retinol activity equivalents of plant products were calculated by dividing the published retinol equivalents for the plant product by 2, which roughly corresponds to the new suggested conversion ratio of  $12 \mu\text{g} \beta$  carotene to  $1 \mu\text{g}$  retinol (11). The dietary data were restricted to those daily intakes that occurred after the birth of the mother's last child and before the day of phlebotomy. Mothers provided  $18.8 \pm 5.9$  d of dietary data.

Ethanol intakes were also estimated from the dietary intake data. In all cases, the only source of ethanol was *pulque*, a mildly alcoholic beverage ( $\approx 4.8\%$  ethanol) that is produced from the sap (*aquamiel*) of the maguey plant (*Agave* sp.) (12). Daily alcohol intakes were calculated by using a value of 47 g ethanol/L *pulque*, the midpoint of the range (29–65 g/L) provided by Steinkraus (12).

**TABLE 1**  
General characteristics of the study subjects<sup>1</sup>

	NPNB women (n = 66)	Breast-feeding women (n = 59)
Age (y)	34.8 ± 6.4	30.3 ± 6.3 <sup>2</sup>
Weight (kg)	61.8 ± 11.2	57.2 ± 7.9 <sup>2</sup>
Height (cm)	153.1 ± 4.7	152.9 ± 5.3
BMI (kg/m <sup>2</sup> )	26.2 ± 3.9	24.5 ± 2.9 <sup>4</sup>
Ferritin (mg/L) <sup>5</sup>	16.4 ± 2.6	21.9 ± 2.4 <sup>6</sup>
Hemoglobin (g/L)	13.0 ± 1.3	13.3 ± 1.2
Hematocrit (%)	40.3 ± 4.6	41.6 ± 4.6
MCV (fL)	94.3 ± 4.8	94.1 ± 5.3

<sup>1</sup>x ± SD. NPNB, nonpregnant, non-breast-feeding; MCV, mean corpuscular volume.

<sup>2-4,6</sup>Significantly different from NPNB women: <sup>2</sup>P = 0.0001, <sup>3</sup>P = 0.0087, <sup>4</sup>P = 0.0058, <sup>6</sup>P = 0.0729.

<sup>5</sup>Geometric  $\bar{x}$  ± SD.

### Statistical analyses

All statistical analyses were conducted by using SAS software (version 8.02; SAS Institute, Cary, NC). Box plots and stem-and-leaf plots were used to examine the distributions of variables. The descriptive statistics included means, medians, and quartiles. Multiple regression models (PROC REG) were used to investigate the relation of dietary intake to plasma ferritin. The dependent variable plasma ferritin was transformed (natural log) to improve a very skewed distribution. Multiple logistic regression (PROC LOGISTIC) was used to investigate the relation of dietary intake to low ferritin, low hemoglobin, and low hematocrit.

The residual method was used to create intake variables that were uncorrelated with nonheme iron intake (13). The resulting residual variables represent intakes above or below those that are usual for a given nonheme iron intake. These models are more clearly interpretable than are standard multiple regression models (13). All models included terms for maternal age, breast-feeding status, BMI, season at blood measure, and the time since the birth of the last child (background variables). Interaction effects and curvilinear relations were investigated by using centered data, and residual plots and influence statistics were used to assess the adequacy of the multiple regression and logistic regression models (14, 15).

An all-possible-regression approach (PROC RSQUARE) was used to investigate the relation of plasma ferritin values to intakes of various foods. Mallows's  $C_p$  (16) and  $R^2$  were used to identify the most parsimonious model that also contained the background variables.

Seasonal variation in blood variables and diet was investigated by using PROC LOESS. Unlike classic regression, the LOESS technique does not require researchers to specify a function (eg, linear or quadratic) when modeling the data (17). Instead, LOESS uses weighted least squares to fit linear or quadratic functions to the centers of neighborhoods of points. LOESS curves were fitted by using the direct method and iterative reweighting to improve robustness of fit and resistance to outliers.

Because of the high-altitude environment in which the subjects lived, adjusted cutoffs for low hemoglobin (<130 g/L) and hematocrit (<38.7%) were used (18). Iron deficiency was defined as a plasma ferritin concentration <15 µg/L, and normal values for mean corpuscular volume were specified as 81.9–98.9 fL (19). A low plasma folate concentration was defined as <7 nmol/L and a low vitamin B-12 concentration as <150 pmol/L (20).

### RESULTS

Some general characteristics of the 125 subjects [59 breast-feeding women and 66 nonpregnant, non-breast-feeding (NPNB) women] are shown in Table 1. The NPNB women were somewhat older, were heavier, and had higher BMIs than did the breast-feeding women. The median time since the birth of the last child was 7.8 mo for the breast-feeding women and was 30.1 mo for the NPNB women ( $P < 0.0001$ , Wilcoxon's test). A high proportion of all women (35.2%) had plasma ferritin values that fell below 15 µg/L, which indicates iron deficiency. More than one-third of the mothers (38.4%) were anemic on the basis of an altitude-adjusted hemoglobin cutoff of 130 g/L, and 30.4% had a low hematocrit on the basis of a cutoff of <38.7%. The NPNB women tended to have lower plasma ferritin concentrations ( $P = 0.0729$ ). Hematocrit and hemoglobin values and the rates of low values were not significantly different between the breast-feeding and NPNB women.

Iron deficiency anemia was determined in 17.6% of the mothers, and another 17.6% of the mothers were iron deficient (low ferritin) but not anemic; 20.8% of the mothers were anemic but had normal ferritin concentrations. Mean corpuscular volume values were generally high, which is atypical of an iron-deficient population; no mother had a value <81.9 fL.

### Plasma folate and cobalamin

Plasma vitamin B-12 and folate values were available for 43 of the 125 women. Previous research showed high rates of vitamin B-12 deficiency in this population (8, 21). On the basis of a cutoff of <150 pmol/L, 41.9% of the subjects had low plasma vitamin B-12 values. On the basis of a higher cutoff of <260 pmol/L, 86.0% of the subjects were classified as having low vitamin B-12 status (22). The subjects with a low hematocrit had lower plasma vitamin B-12 values (geometric  $\bar{x}$ : 113.8 pmol/L;  $n = 14$ ) than did those with a normal hematocrit (geometric  $\bar{x}$ : 188.8 pmol/L;  $n = 29$ ) ( $P = 0.0251$ ), but no significant differences in plasma B-12 concentrations were seen when women with low hemoglobin values were compared with those with normal values. Plasma folate values were generally normal, and only one subject (2.3%) had a low value (<7 nmol/L). Plasma folate was unrelated to anemia, whether defined as a low hemoglobin concentration ( $t = 1.61$ ,  $P = 0.1147$ ) or a low hematocrit value ( $t = 0.86$ ,  $P = 0.3952$ ).

### Seasonality and iron-status measures

On the basis of cross-sectional data, hematocrit values were lowest among the subjects whose measurements were made during early summer or late spring (Figure 1). Ferritin values were highest in September, which corresponded to the end of the rainy season and the period just before the maize harvest. From October to January, ferritin values tended to decrease, whereas hematocrit values increased. From February to May, both hematocrit and ferritin were lower than during December and January. Hemoglobin mirrored hematocrit, and values were highest in December and January (data not shown).

### Dietary constituents and iron metabolism

The intakes of iron and other dietary constituents with the potential to influence iron absorption are shown in Table 2. Nearly all iron was consumed in the nonheme form (median: 98.9% of total iron). Additionally, phytate intakes were extremely

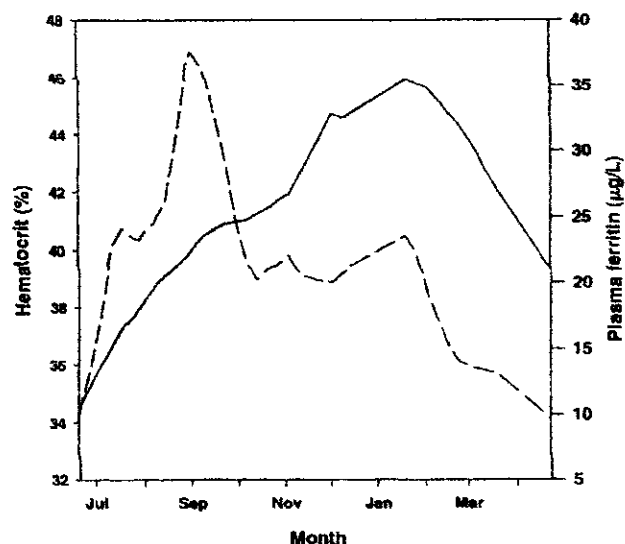


FIGURE 1. Relation of season (rainy: late May through mid-September; dry: time of risk of frost in October through March) to hematoerit (—) and plasma ferritin (---) values. The curves were fitted by locally weighted regression (PROC LOESS, version 8.02; SAS Institute, Cary, NC).

high (median: 4170 mg/d), or >10 times the value of 395 mg/d reported for young US women (23). The interquartile range for molar ratios of phytate to iron was high, 22.2–28.9; high ratios are associated with strongly negative effects of phytate on non-heme iron absorption. Nearly all of the subjects (92.0%) consumed coffee but most drank the beverage on <35% of days. Additionally, most coffee consumption was confined to the early, "light" breakfast meal (*desayuno*), at which time relatively little iron was consumed.

TABLE 2  
Intakes of energy and dietary constituents that might affect iron absorption or iron metabolism in the women from rural central Mexico<sup>1</sup>

	Value
Energy (%) <sup>2</sup>	97.5 (85.1–113.0)
Total iron (mg)	16.2 (13.1–19.6)
Heme	0.17 (0.08–0.35)
Nonheme	16.1 (13.0–19.1)
Ascorbic acid (mg)	32.0 (22.5–48.8)
Meat, fish, and poultry (g)	27.6 (15.3–43.6)
Phytate (mg)	4170 (3454–5463)
Calcium (mg)	1683 (1376–2090)
Instant coffee (g)	1.4 (0.5–2.7)
Alcohol from <i>pulque</i> (g)	9.0 (0–23.7)
Phytate:iron	26.4 (22.2–28.9)
Folic acid (µg)	321 (249–348)
Vitamin B-12 (µg)	1.08 (0.61–2.00)
Vitamin B-6 (mg)	1.50 (1.24–1.85)
Riboflavin (mg)	0.82 (0.65–1.05)
Total vitamin A (µg RAE)	155.7 (123.1–226.3)
From animals	62.9 (38.8–119.8)
From plants	79.1 (57.6–108.8)
Vitamin E (TE)	4.1 (3.3–5.4)

<sup>1</sup> Median values; interquartile range (25th and 75th percentile of intake) in parentheses. *n* = 125. RAE, retinol activity equivalents; TE, tocopherol equivalents.

<sup>2</sup> Percentage of estimated requirement.

TABLE 3  
Principal food sources of iron, ascorbic acid, phytate, and calcium<sup>1</sup>

Food source	Quantity
Iron (mg)	
Total	16.2 (13.1–19.6)
Tortillas	8.2 (6.8–11.1)
Legumes	3.2 (2.1–4.2)
Pulque	1.1 (0.0–3.0)
Meat	0.4 (0.2–0.9)
Bread and pasta	0.3 (0.2–0.8)
Other vegetables	0.2 (0.1–0.4)
All other	1.4 (1.1–2.0)
Ascorbic acid (mg)	
Total	32.0 (22.4–48.8)
Pulque	5.7 (0.0–15.1)
Tomatoes	4.1 (2.5–6.2)
Potatoes	3.0 (1.7–4.5)
Green chiles	2.1 (1.2–3.8)
Quelites	1.5 (0.1–3.6)
Nopales	1.4 (0.7–2.3)
Legumes	0.9 (0.6–1.3)
Tomatillos	0.8 (0.4–1.4)
Other vegetables	3.4 (2.2–5.5)
Fruit	0.6 (0.0–2.3)
Dairy	0.3 (0.0–0.8)
All other	1.0 (0.2–2.3)
Phytate (mg)	
Total	4170 (3454–5455)
Tortillas	3713 (3045–4972)
Legumes	371 (245–472)
All other	111 (80–162)
Calcium (mg)	
Total	1683 (1376–2084)
Tortillas	1359 (1114–1820)
Dairy	45 (3–112)
Legumes	35 (22–54)
Pulque	20 (0–60)
All other	109 (71–153)

<sup>1</sup> Median values; interquartile range (25th and 75th percentile of intake) in parentheses. *n* = 125.

Dietary constituents that might enhance iron absorption were consumed in relatively low amounts. Consumption of meat, fish, and poultry was low (median: 27.6 g/d), as indicated by heme intakes, and some individuals consumed almost no animal flesh. The median ascorbic acid intake was 42.2% of the recommended dietary allowance (RDA; 24). Vitamin A intakes, which have been reported to improve the absorption of nonheme iron, were also low (median: 17.3% of the RDA) (25, 26). Most of the vitamin A consumed came from plant sources (median: 71.1%).

Several vitamins have been reported to influence hemopoiesis, eg, ascorbic acid, folate, vitamin B-12, riboflavin, vitamin B-6, and vitamin A (3). Folate intakes expressed as a percentage of the RDA were relatively high (median: 147.1%). In comparison, median intakes of riboflavin (median: 58.5%) and vitamin B-12 (median: 48.8%) were low. Intakes of vitamin B-6 (median: 83.2%) appeared to be relatively adequate.

#### Foods and dietary constituents that can influence iron metabolism

The principal food sources of iron, ascorbic acid, phytate, and calcium in the diets are shown in Table 3. Tortillas provided >50% of the iron consumed and were also the principal source of

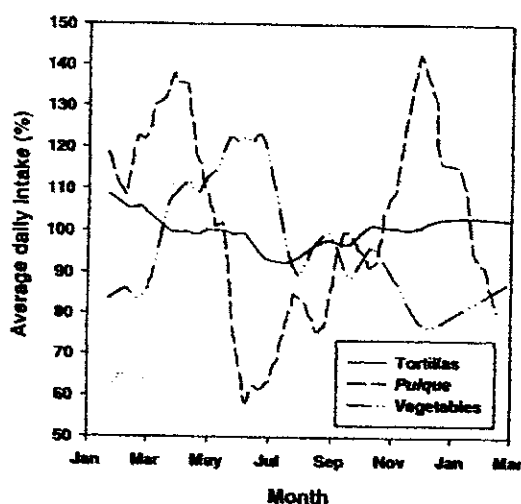


FIGURE 2. Relation of season (rainy: late May through mid-September; dry: time of risk of frost in October through March) to intakes of tortillas, pulque, and vegetables that are important sources of ascorbic acid. Individual daily intakes are expressed as the percentage deviation from the subject's average intake across all recalls. The curve was fitted by using PROC LOESS (version 8.02; SAS Institute, Cary, NC).

calcium and phytate. Legumes were the second most important source of both iron and phytate. *Pulque* was the third most important source of iron in the diet and was the most important source of ascorbic acid. This traditional beverage was the sole source of alcohol for these women. Ascorbic acid was obtained from a wide range of foods other than *pulque*, including tomatoes, green chiles, *quelites* (wild greens), *nopales* (prickly pear cactus leaves), and tomatillos (husk tomatoes). Fruit consumption provided negligible amounts of ascorbic acid for most individuals.

Eggs and dairy products were the principal sources of preformed vitamin A (data not shown). On the basis of values in the International Minilist food-composition table, the principal sources of provitamin A carotenoids were tortillas, *nopales*, and *quelites*, whereas tortillas and legumes were the principal sources of vitamin B-6, riboflavin, and folate. Although no data exist on the folate content of *pulque*, a high correlation between *pulque* consumption and plasma folate ( $r = 0.58$ ,  $P < 0.0001$ ;  $n = 43$ ) suggests that the alcoholic beverage may be a particularly important source of folate.

The seasonal variation in intakes of tortillas, *pulque*, and the ascorbic acid-containing vegetables tomatoes, tomatillos, *nopales*, and *quelites* is shown in Figure 2. Curves were fit from all days of dietary intake by using PROC LOESS. Daily intakes were expressed as a percentage of the individual mean intake. Therefore, the graph reflects individual variation in intakes across time. Intakes of tortillas and *pulque* were lowest from May through September, as were energy intakes (data not shown). Intakes of the ascorbic acid-containing vegetables were greatest from March through July. *Pulque* consumption increased soon after the start of the rainy season (in late May), whereas meat consumption was lowest in August (data not shown).

### Predictors of plasma ferritin

#### Background variables

All multiple regression models controlled for age, BMI, breast-feeding, the time since the birth of the last child, and sea-

son (with the use of linear and quadratic terms for the date of measure). In a model containing only those variables, higher plasma ferritin values (log) were associated with breast-feeding ( $P = 0.0017$ ), greater age ( $P = 0.0420$ ), and more days since the birth of the last child ( $P = 0.0465$ ). The quadratic term for season was not significant ( $P = 0.0732$ ). Together, the 6 variables explained 19% of the variance in ferritin values. No interaction was observed between breast-feeding and the time since the birth of the last child.

#### Food-based models and pulque intake

An all-possible-regressions approach (PROC RSQUARE) was used to investigate the relation of plasma ferritin to intakes of the 15 foods listed in Table 3. With the background variables in the models, 5 foods consistently dominated the models: *pulque* (+), *quelites* (+), *nopales* (+), tomatoes (+), and tomatillos (+). On the basis of Mallows's  $C_p$ , the most parsimonious model ( $R^2 = 0.30$ ) contained the background variables plus *pulque* intake ( $t = 4.20$ ,  $P < 0.0001$ ) (27). *Pulque* intake was a much stronger predictor than were all other food variables. A logistic regression model that contained the background variables and *pulque* intake indicated a 5% reduction in risk of a low ferritin concentration with each additional milligram intake of ascorbic acid from the alcoholic beverage [odds ratio (OR): 0.95; 95% CI: 0.91, 0.99;  $P = 0.0294$ ].

#### Dietary constituent models

An all-possible-regressions approach (PROC RSQUARE) was used to investigate the relation of plasma ferritin to intakes of the micronutrient and dietary constituents in Table 3. Alcohol intake was excluded because it is synonymous with *pulque* intake. After the background variables and the 2 iron variables (heme and nonheme iron) were forced into the regression model, the most parsimonious model contained ascorbic acid intake (27). This "best" multiple regression model is shown in Table 4. The heme iron and ascorbic acid intake variables were adjusted for nonheme iron intake by using the residual method and represent intakes independent of the amount of nonheme iron consumed (13). Higher ferritin concentrations were predicted by greater intakes of nonheme iron ( $P = 0.0030$ ) and ascorbic acid ( $P = 0.0395$ ). After exclusion of the seasonality variables, the relations between plasma ferritin and intakes of nonheme iron ( $P = 0.0012$ ) and ascorbic acid ( $P = 0.0304$ ) were somewhat strengthened. No interaction was seen between ascorbic acid and nonheme iron intake.

A logistic regression model that contained the same independent variables as shown in Table 4 indicated that the risk of a low plasma ferritin concentration was unrelated to the intake of nonheme iron (OR: 0.92; 95% CI: 0.83, 1.01;  $P = 0.0867$ ), heme iron (OR: 1.26; 95% CI: 0.77, 2.05;  $P = 0.3569$ ), and ascorbic acid (OR: 0.98; 95% CI: 0.94, 1.00;  $P = 0.1268$ ).

### Predictors of low hemoglobin and hematocrit

#### Background variables

All the logistic regression models contained the same background variables used in the plasma ferritin models. In the models restricted to the background variables, seasonal differences were seen in the risk of low hemoglobin ( $P = 0.0043$ ) and hematocrit ( $P < 0.0001$ ) values. Low hemoglobin and hematocrit values were unrelated to differences in age, BMI, breast-feeding, and the time since the birth of the last child (data not shown).

TABLE 4

Multiple regression model for predicting log plasma ferritin after control for nonheme iron intake<sup>1</sup>

Variable	Parameter estimate	SE	P
Intercept	0.13987	0.76049	0.8544
Age (y)	0.02235	0.01228	0.0714
Breast-feeding: 1 = yes, 0 = no	0.69569	0.22680	0.0027
BMI	0.02815	0.02316	0.2266
Time since the birth of the last child	0.02261	0.00950	0.0190
Date of blood measure <sup>2</sup>			
Linear term	-0.00122	0.00089	0.1729
Quadratic term	-0.00002	0.00001	0.0743
Nonheme iron (mg)	0.04755	0.01569	0.0030
Heme iron (mg) <sup>3</sup>	-0.05873	0.08792	0.5055
Ascorbic acid (mg) <sup>3</sup>	0.43122	0.20705	0.0395

<sup>1</sup> $n = 125$ ,  $R^2 = 0.278$ , root mean square error = 0.80358.<sup>2</sup>Measured as the number of days since 1 November 1985; variables are centered.<sup>3</sup>Skewed distribution was normalized by using log transformation. The residual intake was controlled for nonheme iron intake.

### Dietary constituent models

Several different logistic regression models were used to investigate the potential influence of vitamin A (plant and animal), riboflavin, folate, vitamin B-6, and vitamin B-12 intakes on hemoglobin and hematocrit. No models improved on the basic model used in the ferritin analyses. When the same independent variables as shown in Table 4 were used, the risk of a low hemoglobin value was reduced by 7% for each additional milligram of ascorbic acid consumed (OR: 0.93; 95% CI: 0.89, 0.97;  $P = 0.0009$ ). Intakes of nonheme (OR: 0.95; 95% CI: 0.86, 1.05;  $P = 0.2962$ ) and heme (OR: 1.21; 95% CI: 0.74, 1.98;  $P = 0.4475$ ) iron were unrelated to the risk of a low hemoglobin value. For hematocrit, a similar result was observed: each additional milligram of ascorbic acid consumed was associated with a 7% reduction in the risk of a low value (OR: 0.93; 95% CI: 0.89, 0.97;  $P = 0.0020$ ). Intakes of nonheme (OR: 0.99; 95% CI: 0.88, 1.11;  $P = 0.9047$ ) and heme (OR: 1.42; 95% CI: 0.76, 2.68;  $P = 0.2736$ ) iron were unrelated to hematocrit.

### Pulque, iron, ascorbic acid, and poor iron status

Because *pulque* is a major source of both ascorbic acid and nonheme iron, we investigated the relations of the iron-status measures to *pulque* intake and to the intake of ascorbic acid and nonheme iron from all other foods. Low hemoglobin values were concentrated among individuals with low intakes of ascorbic acid from foods other than *pulque* (Figure 3); the same finding was observed for hematocrit (data not shown) but not for ferritin. The highest intakes of ascorbic acid not from *pulque* were concentrated among those who consumed the lowest quantities of *pulque*. In other words, the total ascorbic acid intake had considerable heteroscedasticity (unequal variance) across the range of *pulque* intakes, which in regression models leads to biased SEEs (13, 28). Therefore, we restricted these analyses to logistic regression, which does not have an assumption of homoscedasticity.

The results of the logistic regression models containing the background variables and intakes of ascorbic acid from *pulque* and of intakes of ascorbic acid and nonheme iron from all other foods are shown in Table 5. Each milligram intake of ascorbic acid from *pulque* was associated with a 5% decrease in the risk of low ferritin and low hemoglobin values, and each milligram intake of ascorbic acid from other foods was associated with a 7%

decrease in the risk of low hemoglobin and low hematocrit values. Nonheme iron intake from foods other than *pulque* was unrelated to the risk of low ferritin, hemoglobin, or hematocrit values.

### DISCUSSION

Prior research showed that iron deficiency and anemia are common in Mexico's rural Solis Valley (8, 29, 30). In the current study, 35.2% of the 125 nonpregnant women had low iron stores and 38.4% had anemia (low hemoglobin). Despite the high rate of iron deficiency in this population, the median daily iron intake was not especially low (16 mg/d). However, nearly all of the iron consumed (usually >98.9%) was in the nonheme form, which is much less bioavailable than is heme iron. Additionally, the absorption of nonheme iron is strongly influenced by other constituents in the meal. Therefore, low iron stores in this population can be

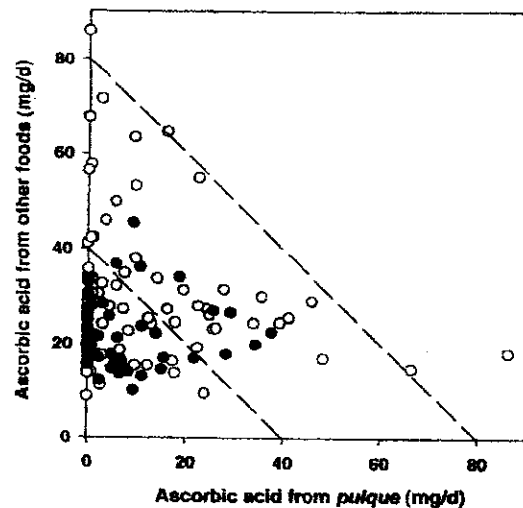


FIGURE 3. Relation of ascorbic acid from *pulque* to ascorbic acid provided by other foods in subjects with normal (○) and low (●) hemoglobin values. The highest intakes of ascorbic acid from foods other than *pulque* were concentrated among individuals with low intakes of the alcoholic beverage. The dashed lines indicate equivalent total ascorbic acid intakes. Low hemoglobin concentrations are concentrated among those with low total ascorbic acid intakes.  $n = 125$ .

TABLE 5

Logistic regression models for predicting low ferritin, hemoglobin, and hematocrit values<sup>1</sup>

Iron variable	Food source	Micronutrient (mg/d)	OR (95% CI)	P
Low ferritin	<i>Pulque</i>	Ascorbic acid	0.95 (0.91, 0.99)	0.0449
	Other foods	Ascorbic acid	0.99 (0.96, 1.02)	0.5744
	Other foods	Nonheme iron	0.99 (0.88, 1.12)	0.9232
Low hemoglobin	<i>Pulque</i>	Ascorbic acid	0.95 (0.91, 0.99)	0.0239
	Other foods	Ascorbic acid	0.93 (0.88, 0.97)	0.0024
	Other foods	Nonheme iron	1.10 (0.97, 1.25)	0.1481
Low hematocrit	<i>Pulque</i>	Ascorbic acid	0.96 (0.92, 1.00)	0.0828
	Other foods	Ascorbic acid	0.93 (0.88, 0.98)	0.0045
	Other foods	Nonheme iron	1.14 (0.98, 1.32)	0.0824

<sup>1</sup>n = 125. All models controlled for age, breast-feeding, time since the birth of the last child, BMI, and season.

conceptualized to be a problem of poor iron bioavailability rather than of low iron intake per se.

With respect to anemia, most of the women with low hemoglobin or hematocrit values had a normal plasma ferritin concentration. High rates of non-iron deficiency anemia in this population are probably due to other nutritional deficiencies, particularly a vitamin B-12 deficiency (8, 21). Folate deficiency does not appear to be a problem in this population (8, 21). Anemia in the Solís Valley has been shown to be insensitive to iron supplementation, despite improvements in iron stores (30).

In an earlier article, Black et al (8) reported seasonal variation in the iron status of adults in the Mexico NCRSP. Our analyses, which used many of the same subjects as did Black et al but had a different analytic approach, suggest the same (Figure 1). Seasonality in iron variables is consistent with the complexity of iron absorption, iron mobilization, and erythropoiesis, as shown in the clinical trials that were conducted in the Solís Valley (30, 31). However, because our blood data are cross-sectional, we cannot conclusively state that there was seasonal variation in ferritin, hemoglobin, or hematocrit. Additionally, the sharp increase in ferritin concentrations seen in August and September was physiologically unlikely, unless there was infection or inflammation during the rainy season. Nevertheless, seasonality in blood variables is consistent with temporal variation in the diet, as shown in Figure 2.

Iron deficiency and anemia occur in this population within a dietary context that is much constrained by economic and environmental conditions that limit food choice (7). Together, maize tortillas and legumes provided nearly 75% of the iron in the diet (median: 73.9%). However, both foods are also very high in phytate. Phytate intake has a negative and nonlinear effect on iron absorption, and the incremental effect of each additional milligram intake of phytate is greatest at low intakes and least when the consumption of phytate is already high (32). Additionally, tortillas contain significant quantities of calcium, a second inhibitor of iron absorption, which is added by soaking maize kernels in a calcium hydroxide solution (33). Not surprisingly, the iron in maize tortillas, when consumed as a single food, is poorly absorbed ( $\approx 1.9\%$ ) (34). Additionally, tortillas are typically eaten at every meal, which ensures the ingestion of large amounts of calcium and phytate with each meal. As a result,  $\geq 300$  mg Ca, the range associated with maximal inhibition of heme and nonheme iron absorption, is frequently consumed at meals (33). Therefore, although tortillas and legumes were the

principal sources of iron, the 2 foods were in all likelihood the principal reasons for the inhibition of iron absorption.

*Pulque*, after tortillas and legumes, was the third most important source of nonheme iron in the diet of the mothers (median: 6.3% of nonheme iron). The traditional beverage was usually consumed with meals and was also the principal source of ascorbic acid in the diet (median: 15.2% of total ascorbic acid). A half liter of *pulque*, the amount most commonly consumed at a meal, contains  $\approx 30$  mg ascorbic acid. The enhancing effects of ascorbic acid on iron absorption, in contrast with both phytate and calcium, are thought to be linear and to increase with greater phytate intakes (33). *Pulque* also contains significant amounts of ethanol, which may also enhance iron absorption; the beverage is also a good source of riboflavin and several other B vitamins and contains significant quantities of steroidal saponins, many of which are bioactive (35-37).

Consistent with the experimental literature, our analyses suggest that variability in ascorbic acid intakes influenced the iron status in this rural Mexican population. Multiple regression models showed that higher ferritin values were associated with greater intakes of nonheme iron and ascorbic acid after control for age, BMI, breast-feeding, and the time since the birth of the last child. In the multiple logistic regression models, higher ascorbic acid intakes predicted a lower risk of low hemoglobin and low hematocrit values. In other analyses, we examined the relations of ferritin, hemoglobin, and hematocrit to intakes of nonheme iron and ascorbic acid from both *pulque* and all other foods combined. Independent of *pulque* intake, the consumption of ascorbic acid from other foods was associated with a lower risk of low hemoglobin and low hematocrit values.

The potential benefits of ascorbic acid on iron absorption in this population were examined by Garcia Obregon (31). In a field trial conducted in the Solís Valley, the addition of 25 mg ascorbic acid to each of 2 meals during a 14-d period improved iron absorption from a baseline of 6.6% to 22.9%. Other research showed substantial increases in iron bioavailability when ascorbic acid was added to maize-based meals (38-40). However, a community trial conducted in the Solís Valley showed no effect of an ascorbic acid (limeade) supplement on the ferritin and hemoglobin concentrations of iron-deficient, nonanemic women beyond that provided by a placebo (a lime-flavored drink) (31). Although the supplements were provided at 2 meals per day during an 8-mo period, the small sample size and seasonal effects may have contributed to the lack of a statistically significant result.

In conclusion, iron deficiency was common among nonpregnant women residing in the Solís Valley of central Mexico at the time of this study. Although the data were collected 15 y ago, more recent research in this region has shown that the dietary and nutritional conditions remain much the same (30, 41). Our analyses showed that better iron status was associated with higher intakes of nonheme iron, ascorbic acid, and foods that contain ascorbic acid. In this population, however, those frequently consumed foods that contain significant amounts of ascorbic acid are also the main sources of other vitamins and dietary constituents that are frequently consumed in limited amounts. Therefore, the associations with ascorbic acid intake are potentially confounded by intakes of other dietary constituents, and the role of these foods in the context of the restricted range of foods in the local diet cannot be ruled out.

From a public health perspective, the most problematic food in the diet of the women residing in the Solís Valley is *pulque*. Previously, we showed that large intakes of this locally brewed alcoholic beverage are associated with poor infant outcomes (42). However, *pulque* is also an important source of ascorbic acid, nonheme iron, and folate and several other B vitamins. Therefore, *pulque* consumption may improve a mother's iron status while placing her unborn child at increased risk of the negative effects of fetal exposure to alcohol (43). To address this situation, interventions to reduce the heavy consumption of *pulque* and to increase the intakes of other foods that contain ascorbic acid are clearly indicated. Clinical trials using specific foods or food groups would provide useful information for the development of appropriate dietary interventions.

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