

## Effects of discontinuing coffee intake on iron status of iron-deficient Guatemalan toddlers: a randomized intervention study<sup>1-3</sup>

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**ABSTRACT** Coffee is one of the first liquids given to infants in Guatemala. To evaluate whether this practice has an adverse effect on iron status, 160 children 12–24 mo of age who had received coffee for  $\geq 2$  mo and had at least one indicator of iron deficiency were stratified by initial hemoglobin concentration (anemic, or nonanemic, ie, hemoglobin  $\geq 105$  g/L) and randomly assigned to a control (continuation of coffee; coffee) or intervention (provided with a substitute consisting of sugar and coloring; substitute) group for 5 mo. Anemic children were provided with iron supplements for 2–3 mo. Hematologic and anthropometric measurements were made before and after the intervention and dietary and morbidity data were collected every 2 wk. A total of 139 children completed the study: 45 coffee, nonanemic; 56 substitute, nonanemic; 19 coffee, anemic; and 19 substitute, anemic. Compliance with the procedures was good: median coffee intake was 891 mL/wk in the coffee group compared with 18 mL/wk in the substitute group ( $P = 0.0001$ ). There was no significant effect of discontinuing coffee consumption on changes in hemoglobin, hematocrit, ratio of zinc protoporphyrin to heme or plasma iron, zinc or copper in either nonanemic or anemic children, or plasma ferritin in children who did not take iron supplements. In children who took iron supplements, change in plasma ferritin was significantly greater in the substitute group than in the coffee group (106% compared with 1%,  $P < 0.05$ ). This implies that coffee interferes with the utilization of supplemental iron. It is likely that the amount and strength of coffee consumed by Guatemalan toddlers are too low to significantly affect the other indexes of iron status. *Am J Clin Nutr* 1997;66:168–76.

**KEY WORDS** Coffee, iron metabolism, anemia, hemoglobin, hematocrit, zinc, copper, ferritin, children, Guatemala, toddlers

### INTRODUCTION

Coffee is a beverage consumed widely throughout the world. Caffeine, a natural constituent of coffee, has been the subject of numerous studies because of its pharmacologic effects (1–3). However, caffeine is not the only metabolically active substance in coffee; there are many other compounds that also have physiologic effects (4). For example, it is well documented that the polyphenols (tannins) in coffee bind to iron in the intestinal lumen, forming an insoluble complex and thereby

inhibiting iron absorption (5, 6). Other components of coffee such as chlorogenic acid are also thought to interfere with iron absorption (7).

In addition, there may be other effects of coffee on iron metabolism aside from impaired absorption. In studies with rats, hemoglobin and hematocrit values of pups of dams who consumed coffee during pregnancy and lactation were significantly lower, whereas liver iron concentrations were significantly higher, than in pups of control rats (8). These findings suggested that coffee may interfere with the mobilization of iron from the liver to sites of hematopoiesis. Liver zinc and copper concentrations were also elevated in pups of dams who had consumed coffee.

Several observational studies in humans have shown an inverse association between coffee intake and indexes of iron status. In adult, nonpregnant women in France, serum ferritin values were negatively correlated with coffee intake (9). Women in Costa Rica who drank three or more cups of coffee per day during pregnancy and lactation were more likely to be anemic and had infants with lower hemoglobin and hematocrit values than mothers who did not drink coffee even though both groups used prenatal iron supplements and were very similar in socioeconomic status, maternal anthropometric indexes, and dietary intake (10). In Zaire, hemoglobin and hematocrit values were lower in coffee-drinking preschoolers than in those who did not drink coffee (11), although these investigators did not report whether these results remained significant after potentially confounding variables were controlled for.

In many parts of Latin America coffee is commonly consumed by children (12, 13). In Guatemala, for example, coffee is one of the first foods given to infants, often in the form of

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bread soaked in coffee (14). In a survey of 100 mothers of children 5–36 mo of age recruited through outpatient clinics at three hospitals serving low-income families in Guatemala City, we found that 72% of the children consumed coffee (15). Of those given coffee, 78% began to consume it by 10 mo of age. In a second preliminary study to examine whether coffee intake was associated with indexes of iron status in preschoolers (16), venous blood samples were collected from  $\approx 85$  children 6–35 mo of age living in low-income neighborhoods in Guatemala City. Special efforts were necessary to recruit a sufficient number of children who did not drink coffee. Because those who did not drink coffee tended to be younger than the coffee drinkers, we restricted the analysis to children  $> 8$  mo of age. In these 66 children, the percentage with hematocrit values  $< 0.38$  was 46% in the group who drank coffee compared with 12% in those who did not ( $P = 0.014$ ). The percentage of children with plasma ferritin values  $< 12 \mu\text{g/L}$  did not differ significantly between groups but there was a trend in the expected direction (52% in coffee compared with 35% in the no coffee group). The difference in hematocrit between groups remained significant after several potentially confounding variables were controlled for.

Although the findings in the above observational studies all point in the same direction, an experimental design was considered necessary to determine whether there is a causal association between coffee consumption and iron status. Ethically, however, we could not randomly assign children who did not consume coffee to be given coffee. An alternative strategy was to eliminate coffee from the diet of those who already consumed it. With this approach, the objectives of the present study were to determine the effects of discontinuation of coffee intake among Guatemalan toddlers 12–24 mo of age on 1) iron status and other hematologic indexes, including plasma zinc and copper concentrations; 2) growth; 3) morbidity; and 4) behavioral outcomes, including sleep patterns and cognitive development. This paper presents the hematologic results; the growth and morbidity outcomes are reported elsewhere (17).

## SUBJECTS AND METHODS

### Study design

Several pilot studies (results not reported) were conducted to develop a coffee substitute for Guatemalan toddlers and assess its acceptability. We then initiated a randomized intervention trial with two groups: 1) the control (coffee) group, which continued to drink coffee during the 5-mo intervention period; and 2) the intervention (substitute) group, which was provided with a substitute for coffee consisting of a premeasured portion of brown-colored sugar that was to be mixed with hot water in the home. The amount of sugar this provided ( $\approx 6 \text{ g}/100 \text{ mL}$ ) was similar to the average amount used in preparing coffee for children. We chose this substitute, rather than more nutritious beverages such as juice or milk, to test specifically the effect of coffee independent of any alterations in nutrient intake. In the acceptability trials, mothers reported that their children liked the substitute as much as or more than coffee. Mothers in the randomized trial were not told the true purpose of the study, but to be eligible to participate they had to be willing to stop giving coffee to their children if assigned to the substitute group. The study protocol was approved by the institutional review board

at the University of California, Davis, and the Human Subjects Committee of the Center for Studies of Sensory Impairment, Aging and Metabolism in Guatemala.

The selection criteria for the study were that the children 1) were 12–24 mo of age; 2) had consumed coffee for  $\geq 2$  mo, with an intake of  $> 90 \text{ mL/d}$ ; and 3) were likely to be iron deficient at baseline. We selected children who were likely to be iron deficient to maximize the chances of observing a positive effect of discontinuing coffee. Children were considered likely to be iron deficient if their hemoglobin concentration was  $\leq 115 \text{ g/L}$ , hematocrit was  $\leq 0.35$ , or the ratio of zinc protoporphyrin to heme (ZPP:H) was  $> 80 \mu\text{mol/mol heme}$ . These criteria for hemoglobin and hematocrit were adjusted upward relative to the standard cutoffs of 110 g/L and 0.33, respectively, to correct for the altitude of Guatemala City (1300–1500 m) (18).

Children were categorized into two groups: 1) those who were initially frankly anemic (defined herein as a hemoglobin concentration  $< 105 \text{ g/L}$ ), or 2) those who were either mildly anemic or not anemic (hemoglobin  $\geq 105 \text{ g/L}$ ; herein labeled as “nonanemic”). After this stratification, children were randomly assigned to the coffee or substitute group. Anemic children were provided with iron supplements (15 mg Fe/d as ferrous sulfate in Fer-N-Sol; Mead Johnson, Evansville, IN) for 2–3 mo because it was not considered ethical to leave this group untreated during the 5-mo study. Although some of the nonanemic children had a hemoglobin concentration  $< 115 \text{ g/L}$ , their status was not considered severe enough to warrant immediate iron supplementation.

Recruitment was done by door-to-door canvassing in selected low-income neighborhoods of Guatemala City during March–August, 1994. At the time of recruitment, children were screened for eligibility, a blood sample was taken, and anthropometric measures were completed. Of the 211 children screened, 160 were eligible for the study. Eligible subjects were then stratified into anemic and nonanemic groups, and random assignment was performed in blocks of 20 by using a table of random numbers. Subjects were visited in the home shortly after recruitment to obtain demographic data, evaluate dietary intake with a food-frequency questionnaire, and assess baseline sleep patterns. At this visit, subjects in the substitute group were given their first 2-wk supply of the coffee substitute and instructed in its use. Every 2 wk, all subjects were visited in the home to assess the child's intake of fluids, compliance with use of the substitute (if assigned to that group), use of iron supplements, morbidity since the previous visit, and sleep patterns. At the end of the 5-mo study period, the final blood sample was collected, anthropometric and food-frequency assessments were repeated, child behavioral development was assessed, and an exit interview was conducted with mothers to determine their reaction to the study.

### Blood sampling and analysis

Blood samples were collected by venipuncture. Hemoglobin was measured immediately by using the HemoCue instrument (HemoCue, Mission Viejo, CA). Two microhematocrit tubes were filled for subsequent determination of hematocrit. Aliquots of the remaining blood were placed into tubes with heparin and transported on ice to a central laboratory, where ZPP:H was determined by using a ProtoFluor Z hematofluorometer (Helena Labs, Beaumont, TX) (19). The remaining

blood was centrifuged at  $4192 \times g$  (5000 rpm) for 5 min at room temperature and the plasma was frozen at  $-20^\circ\text{C}$ . Subsequent analyses were performed at the University of California, Davis, and included plasma ferritin (by radioimmunoassay; Diagnostic Products Company, Los Angeles), C-reactive protein (The Binding Site, Birmingham, United Kingdom), and plasma iron, zinc, and copper [by atomic-absorption spectrophotometry according to the methods described by Clegg et al (20)]. C-reactive protein values were used as an index of an active acute phase response to infection or inflammation, processes that can increase ferritin concentrations (21, 22).

### Anthropometry

Weight was measured to the nearest 1 g by using a digital platform balance (Cardinal Detecto model 8435; Webb City, MO). Recumbent length was measured by two individuals to the nearest 1 cm by using an infant length board. Z scores for weight-for-age, length-for-age, and weight-for-length were calculated by using WHO/CDC reference data (EPI-INFO version 6.03; Centers for Disease Control and Prevention, Atlanta).

### Dietary intake

Dietary patterns were evaluated by using a food-frequency questionnaire. During the intervention, data on intake of liquids were collected at each home visit by using a 2-wk recall of frequency and approximate amount consumed for each item. At recruitment and at each home visit, detailed information was obtained on the type of coffee used in the household, the amount of water used in its preparation, and any additional items added (eg, milk, sugar, or more water) in preparing coffee for the child. This information was used to calculate the dose of actual dry coffee per kilogram body weight of the child. In this calculation, the weight of instant coffee was multiplied by a factor of two relative to ground coffee (based on the fact that caffeine concentration per gram dry weight of instant coffee is about twice that of ground coffee).

Because some brands of coffee in Guatemala contain substantial amounts of noncoffee fillers, such as roasted grains, samples of each brand were analyzed for caffeine content as a marker of the strength of the coffee. Although caffeine is not the component suspected of influencing iron status, we assumed that its concentration reflects the proportion of coffee to fillers in each brand. Caffeine content was measured by HPLC using a Beckman Gold System apparatus (Beckman, San Ramon, CA) with an ultraviolet detector. Liquid coffee, prepared as for consumption, was added in a proportion of 1:8 to the mobile-phase solvents. Using a C-18 column with a mobile-phase solvent mixture of methanol:methylene chloride (90:10, vol:vol) with a flow rate of 1 mL/min, the absorbance was read at 254 nm in relation to caffeine standards prepared in the solvents from reference standard caffeine (Sigma Diagnostics, St Louis). These data were used to calculate another index of coffee dose based on caffeine concentration.

### Morbidity

A continuous record of child morbidity during the intervention period was obtained by interviewing the mother at each home visit. At such visits, mothers were given a 2-wk grid with pictures of common conditions (diarrhea, earache, fever, vomiting, cough, runny nose, and rash) on which to mark all

symptoms of illness. This form did not require that the mothers be literate. Fieldworkers used this information when interviewing mothers to help make the morbidity record as complete as possible.

### Data analysis

Data were analyzed by using PC SAS (SAS Institute, Cary, NC). Baseline characteristics were compared between the coffee and substitute groups by using the chi-square statistic for categorical variables and Student's *t* test for continuous variables. Analysis of covariance was used to compare changes in outcome variables during the intervention, with treatment group (coffee or substitute) and initial anemia status as categorical main effects, baseline value of the outcome as a continuous covariate, and the interaction between treatment group and initial anemia. Pearson's correlation coefficients and stepwise multiple-regression analysis were used to examine the relations between maternal prenatal coffee intake and child iron status at baseline. In all analyses, logarithmic transformations were utilized for variables that were not normally distributed.

## RESULTS

### Sample and characteristics of subjects

The number of subjects recruited and the number of dropouts is shown in **Table 1**. The overall attrition rate was 13%, and was slightly higher in the control group (17%) than in the intervention group (10%). The final sample included 139 children, 101 of whom had an initial hemoglobin concentration  $\geq 105$  g/L and 38 of whom had an initial hemoglobin concentration  $< 105$  g/L. All but one of the 21 subjects who dropped out did so because they moved out of the area; the remaining child could not complete the study because of hospitalization. There were no significant differences in socioeconomic status or maternal characteristics between those who completed the study and those who did not.

Characteristics of subjects who completed the study are shown in **Table 2** by initial anemia status and treatment group. The random-assignment procedures resulted in generally similar characteristics between study groups (within nonanemic or anemic subgroups), except for one of the indexes of socioeconomic status (a score based on the type of floor), which was significantly higher in the substitute group than in the coffee group (within the nonanemic subgroup only). This variable was unrelated to any of the outcome measures. The average age of the children at the beginning of the study was  $\approx 17$  mo. The

**TABLE 1**  
Sample sizes

	Hemoglobin status	Coffee group	Substitute group	Total sample
Nonanemic (initial hemoglobin $\geq 105$ g/L)				
	Recruited	54	62	116
	Dropped out	9	6	15
	Final sample	45	56	101
Anemic (initial hemoglobin $< 105$ g/L)				
	Recruited	23	21	44
	Dropped out	4	2	6
	Final sample	19	19	38

TABLE 2  
Characteristics of subjects

	Nonanemic		Anemic	
	Coffee group (n = 45)	Substitute group (n = 56)	Coffee group (n = 19)	Substitute group (n = 19)
Maternal age (y)	26 ± 6 <sup>1</sup>	26 ± 6	26 ± 5	28 ± 7
Parity	3.3 ± 1.8	2.8 ± 1.9	3.8 ± 2.1	2.9 ± 1.9
Maternal education (y)	4.0 ± 3.4	5.1 ± 3.2	5.1 ± 3.5	5.2 ± 3.7
Maternal coffee intake during pregnancy (cups/d)	1.8 ± 1.1	2.2 ± 1.3	2.3 ± 1.4	2.6 ± 1.6
Possessions score	5.1 ± 1.6	5.1 ± 1.7	4.4 ± 1.6	4.9 ± 2.1
Floor score	1.6 ± 0.7	1.9 ± 0.7 <sup>2</sup>	1.6 ± 0.6	1.8 ± 0.7
Child age (mo)	17.1 ± 3.5	17.6 ± 3.8	16.1 ± 3.6	17.1 ± 3.2
Child sex (% male)	51	38	53	47
Birth weight (kg)	3.1 ± 0.5	3.1 ± 0.6	2.9 ± 0.5	2.7 ± 0.8
Initial Z scores				
Weight-for-age	-1.5 ± 0.8	-1.4 ± 0.9	-1.5 ± 1.1	-1.5 ± 0.8
Length-for-age	-2.0 ± 1.2	-2.2 ± 1.3	-2.4 ± 1.4	-2.0 ± 1.0
Weight-for-length	-0.3 ± 1.0	0.0 ± 0.9	0.0 ± 1.3	-0.4 ± 1.2
Still breast-fed (%)	56	45	37	47
Age began consuming coffee (mo)	8.2 ± 3.4	7.3 ± 3.9	8.3 ± 3.7	7.1 ± 3.2
Type of coffee (% consuming ground rather than instant)	62	73	63	68
Have milk with coffee (%)	7	4	5	0
Initial coffee intake				
(mL/d)	162 ± 92	193 ± 120	166 ± 95	207 ± 145
(g · kg <sup>-1</sup> · d <sup>-1</sup> )	0.40 ± 0.34	0.51 ± 0.53	0.51 ± 0.39	0.35 ± 0.27
Initial caffeine intake				
(mg/d)	84 ± 121	89 ± 166	107 ± 160	55 ± 82
(mg · kg <sup>-1</sup> · d <sup>-1</sup> )	8.8 ± 11.4	9.7 ± 17.7	11.0 ± 15.2	6.1 ± 9.1

<sup>1</sup>  $\bar{x} \pm SD$ .

<sup>2</sup> Significantly different from coffee group within the nonanemic subgroup,  $P < 0.05$ .

initial Z scores indicate a high prevalence of stunting, with an average length-for-age  $> 2$  SD below the National Center for Health Statistics median (23). About one-half of the children were still being breast-fed. The average age at which coffee was introduced was 7–8 mo. The majority of families used ground rather than instant coffee. Very few of the children received milk with their coffee. Initial coffee intake averaged 160–200 mL/d and did not differ significantly among the four subgroups. When calculated as dry coffee per kilogram body weight, the initial dose was 0.35–0.51 g · kg<sup>-1</sup> · d<sup>-1</sup>, depending on the subgroup. Caffeine concentration of the various brands ranged from 7 to 56 mg/g dry coffee. Average initial caffeine intake was 55–107 mg/d (6.1–11.0 mg · kg<sup>-1</sup> · d<sup>-1</sup>), depending on the subgroup.

#### Compliance with the study

In general, compliance with the study procedures was good. However, coffee intake was not completely discontinued by all of the children in the substitute group: 28% continued to drink coffee (usually in addition to the substitute) during at least three of the 10 2-wk intervals during the study period (henceforth referred to as “noncompliers”). Six others (8%) in this group did not accept the substitute consistently, but did not return to drinking coffee. In the coffee group, there were some children who discontinued coffee intake: 20% reportedly did not drink coffee during at least four of the 10 2-wk intervals (also referred to as noncompliers).

The intake of various liquids during the intervention, averaged over all of the 2-wk intervals during the 5-mo

study period is given in **Table 3**. In the coffee group, the mean volume of coffee consumed by nonanemic children was significantly lower ( $P < 0.01$ ) during the study period (117 mL/d) than the amount reported at baseline (162 mL/d), whereas for anemic children coffee intake did not change significantly ( $\approx 160$  mL/d). Median coffee intake was close to zero in the substitute group. Mean intake of the substitute by children in that group exceeded the amount of coffee consumed by children in the coffee group: 231 compared with 127 mL/d (combining anemic and nonanemic children), which indicated that most of the children readily accepted the substitute. Intakes of nutritive fluids such as atoles (cereal-based beverages), milk, soft drinks, Incaparina (a fortified cereal-based beverage produced by Alimentos SA, Guatamala City), and juices were quite similar between groups, but children in the substitute group consumed less water. This compensated for the large volume of substitute consumed, with the result that total liquid intake was not significantly different between groups. Data shown elsewhere (17) indicate that there were no significant differences between groups in initial food intake or in the change in frequency of consumption of each food type during the intervention.

#### Effect of the intervention on hematologic indexes

There were no significant differences between the coffee and substitute groups in initial mean values for hematocrit, ZPP:H, ferritin, hemoglobin, or plasma iron, zinc, or copper (**Table 4**) within either the nonanemic or anemic subgroups. The percent-

TABLE 3

Intake of liquids during the study.

	Nonanemic		mL/d	Anemic	
	Coffee group (n = 45)	Substitute group (n = 56)		Coffee group (n = 19)	Substitute group (n = 19)
Coffee <sup>1</sup>	117 ± 74 [111] <sup>2</sup>				
Substitute <sup>1</sup>	0	12 ± 20 [3]			
Atoles <sup>3</sup>		249 ± 196	173 ± 78 [162]		15 ± 33 [0]
Milk	300 ± 279	308 ± 315	0		177 ± 85
Soft drinks	238 ± 282	242 ± 266	307 ± 280		296 ± 387
Water <sup>1</sup>	138 ± 75	150 ± 99	271 ± 381		251 ± 254
Incaparina <sup>4</sup>	133 ± 104	97 ± 91	162 ± 104		133 ± 108
Juices	107 ± 170	114 ± 220	125 ± 146		85 ± 77
Total liquids	12 ± 43	8 ± 20	85 ± 211		117 ± 252
	1044 ± 434	1180 ± 557	11 ± 20		9 ± 16
			1143 ± 570		1083 ± 548

<sup>1</sup> Significantly different between coffee and substitute groups,  $P < 0.05$  (main effect, regardless of initial anemia status).

<sup>2</sup>  $\bar{x} \pm$  SD; median in brackets.

<sup>3</sup> Cereal-based beverages.

<sup>4</sup> A fortified, cereal-based beverage produced by Alimentos SA, Guatemala City.

ages of children with abnormal values for the first four indexes are shown in Figures 1, 2, 3, and 4. In the nonanemic subgroup, about one-half of the children had an initial hemoglobin

concentration  $< 115$  g/L, one-fourth had an initial hematocrit  $< 0.35$ , more than one-half had an initial ZPP:H  $> 80$   $\mu$ mol/mol heme, and two-thirds had an initial ferritin concentration

TABLE 4

Hematologic values before and after the study period<sup>1</sup>

Hematologic value	Nonanemic		Anemic	
	Coffee group (n = 45)	Substitute group (n = 56)	Coffee group (n = 19)	Substitute group (n = 19)
Hemoglobin (g/L)				
Initial	114 ± 5	114 ± 7		
Final	119 ± 11		93 ± 8	
Change	5 ± 11	117 ± 10	111 ± 11	94 ± 10
Hematocrit (l)		4 ± 9	18 ± 14	111 ± 10
Initial				17 ± 14
Final	0.361 ± 0.021	0.360 ± 0.021	0.327 ± 0.024	
Change	0.375 ± 0.026	0.371 ± 0.030	0.368 ± 0.032	0.328 ± 0.021
ZPP:H ( $\mu$ mol/mol heme)	0.014 ± 0.029	0.011 ± 0.033	0.041 ± 0.029	0.371 ± 0.031
Initial				0.043 ± 0.031
Final	86.4 ± 30.5	92.3 ± 35.3	151.8 ± 68.8	
Change	93.4 ± 38.7	105.8 ± 70.3	106.9 ± 49.3	191.6 ± 115.6
Ferritin ( $\mu$ g/L) <sup>2</sup>	7.0 ± 34.6	13.5 ± 60.5	-44.9 ± 62.2	114.0 ± 51.6
Initial				-77.6 ± 100.4
Final	14.5 ± 14.4 [25]	12.4 ± 10.0 [39]	6.8 ± 5.2 [11]	
Change	13.5 ± 9.7 [25]	14.2 ± 13.6 [39]	13.4 ± 14.4 [11]	4.9 ± 7.7 [12]
Plasma iron ( $\mu$ g/L) <sup>3</sup>	-0.9 ± 14.8 [25]	1.8 ± 9.5 [39]	6.5 ± 15.1 [11]	10.0 ± 7.5 [12]
Initial				5.1 ± 11.2 [12]
Final	900 ± 338 [29]	887 ± 570 [43]	513 ± 183 [16]	
Change	751 ± 279 [29]	847 ± 449 [43]	712 ± 403 [16]	571 ± 296 [17]
Plasma zinc ( $\mu$ g/L)	-149 ± 379 [29]	-40 ± 660 [43]	199 ± 424 [16]	769 ± 405 [17]
Initial				199 ± 404 [17]
Final	675 ± 129	636 ± 116	682 ± 197	
Change	670 ± 117	634 ± 109	637 ± 146	641 ± 139
Plasma copper ( $\mu$ g/L)	-6 ± 103	-2 ± 104	-45 ± 112	600 ± 95
Initial				-41 ± 81
Final	1539 ± 358	1410 ± 386	1437 ± 388	
Change	1363 ± 333	1322 ± 308	1359 ± 428	1491 ± 384
	-176 ± 353	-88 ± 407	-77 ± 367	1442 ± 343
				-49 ± 443

<sup>1</sup>  $\bar{x} \pm$  SD; n in brackets. There were no significant differences between the coffee and substitute groups within either the nonanemic or anemic groups.

<sup>2</sup> Subjects with elevated C-reactive protein concentrations at either the initial or final blood sampling were excluded: nonanemic, coffee—12 initial, 10 final; nonanemic, substitute—11 initial, 8 final; anemic, coffee—4 initial, 5 final; anemic, substitute—5 initial, 2 final.

<sup>3</sup> Subjects with hemolyzed plasma samples at either the initial or final blood sampling were excluded.

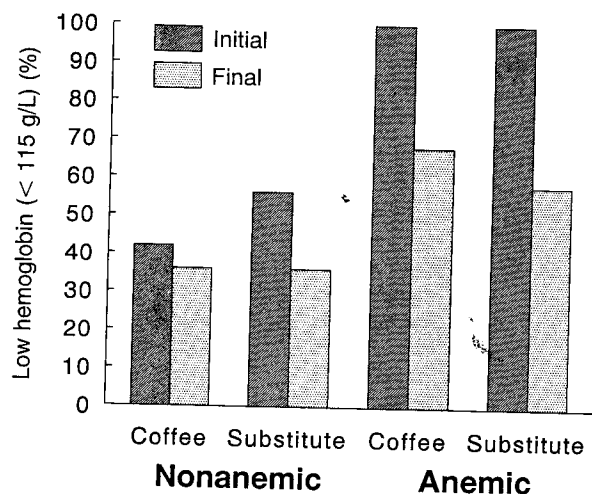


FIGURE 1. Percentage of children with low hemoglobin concentrations (< 115 g/L) among nonanemic and anemic children assigned to the coffee or substitute group.

< 12  $\mu\text{g/L}$ . As expected, these percentages were much higher in the anemic group, with the vast majority having abnormal values initially.

There was no significant effect of the intervention on these outcomes in either the nonanemic or anemic subgroups. In the nonanemic children, hemoglobin and hematocrit values increased slightly during the study and there was a decrease in the percentage with low values in both the coffee and the substitute groups. There was little change in mean ZPP:H or ferritin concentrations in either group, although the percentage with low ferritin concentrations decreased to  $\approx 50\%$ . Neither the mean change in these four indexes (Table 4), nor the change in the percentage with abnormal values (Figures 1-4) differed significantly between study groups. Of the nonanemic children whose initial hemoglobin concentration was < 115 g/L, similar percentages of those in the coffee and substitute groups had a final hemoglobin concentration  $\geq 115$  g/L (53% compared with 51%, respectively). Likewise, of those with low ferritin or abnormal ZPP:H values, there was no significant

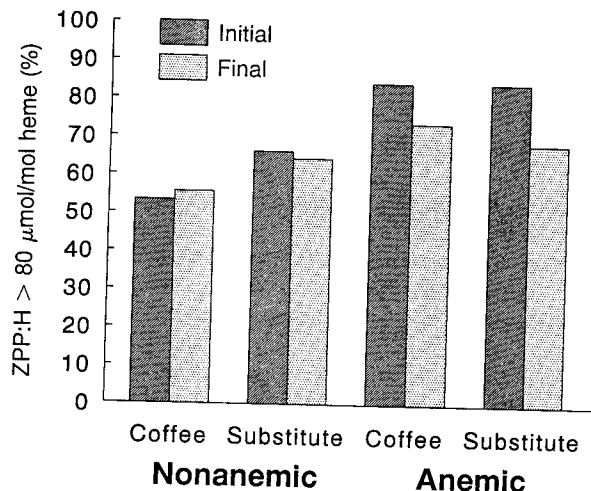


FIGURE 3. Percentage of children with abnormal ZPP:H (> 80  $\mu\text{mol/mol heme}$ ) among nonanemic and anemic children assigned to the coffee or substitute group.

difference between treatment groups in the percentages with normal values after the intervention.

In the children initially classified as anemic, hemoglobin, hematocrit, and ferritin values increased considerably and ZPP:H values declined during the study in both the coffee and the substitute groups, with corresponding decreases in the percentages with abnormal values. Neither the mean change nor the change in the percentage with abnormal values differed significantly between coffee and substitute groups. Similarly, there were no significant differences between treatment groups in the change in plasma iron, zinc, or copper concentrations, or in the change in the percentage with values below the cutoffs of 0.4, 0.8, or 0.6 mg/L, respectively.

Additional statistical analyses were performed to evaluate whether the above results would differ if we 1) excluded children who were considered noncompliers in either the substitute or coffee group (as defined above), 2) limited the comparison to children whose initial coffee or caffeine intakes were

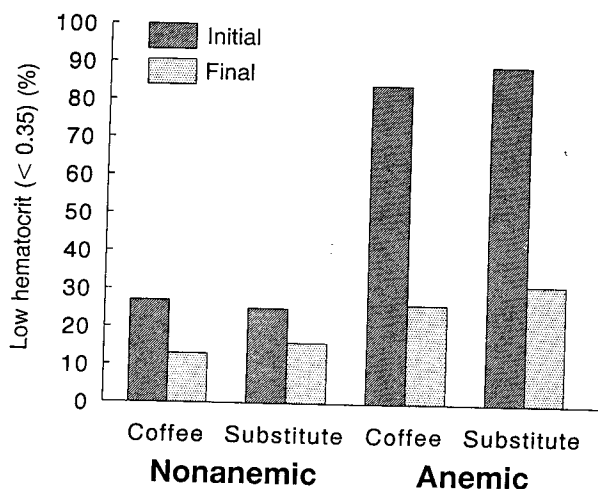


FIGURE 2. Percentage of children with low hematocrit (< 0.35) among nonanemic and anemic children assigned to the coffee or substitute group.

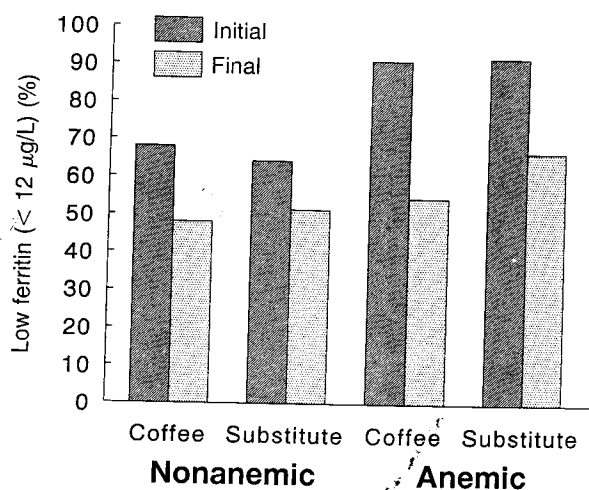


FIGURE 4. Percentage of children with low ferritin concentrations (< 12  $\mu\text{g/L}$ ) among nonanemic and anemic children assigned to the coffee or substitute group; excludes subjects with elevated C-reactive protein concentrations at either the initial or final blood sampling.

above selected thresholds, 3) tested for interaction effects with child sex or initial iron status, or 4) included a variable in the model to account for the potential effects of maternal coffee intake on children who were still breast-fed. None of these analyses resulted in significant differences between treatment groups.

The potential influence of iron supplementation was also evaluated. During the home visits, all mothers were asked whether the child received any vitamin or mineral supplements. If a child received iron supplements during two or more of the 10 2-wk intervals, they were categorized into the iron-supplemented group ( $n = 42$ ). As mentioned in SUBJECTS AND METHODS, children who were initially categorized as anemic ( $n = 38$ ) were supposed to receive iron supplements for 2–3 mo during the intervention, but only 23 of their mothers actually gave them these supplements. On the other hand, some of the children ( $n = 19$ ) in the nonanemic group were given iron supplements by their parents even though these were not provided by the study. When actual use of iron supplements was included in the statistical models, it did not alter the findings regarding the lack of effect of the intervention on hemoglobin, hematocrit, or ZPP:H values. In other words, there was no significant difference between the coffee and substitute groups in the change in hemoglobin, hematocrit, or ZPP:H values during the intervention in either the iron-supplemented children or the nonsupplemented children (Table 5).

However, there was a significant interaction effect between treatment group and iron supplementation for change in plasma ferritin concentration. In children given iron supplements ( $n = 28$  for those without elevated C-reactive protein either at the initial or final blood sampling), ferritin concentration more than doubled in those assigned to the substitute group but showed much less change in the coffee group (Table 5). Among those not given iron supplements ( $n = 59$  with normal C-reactive protein concentrations), there was no significant difference in the change in ferritin concentration between treatment groups. This interaction was significant when initial ferritin concentration and anemia status were controlled for (ie, anemic or nonanemic; Figure 5).

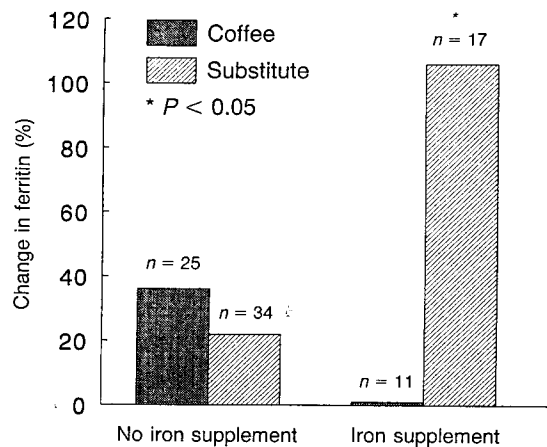


FIGURE 5. Percentage change in ferritin: interaction between coffee and iron supplementation, with initial ferritin concentration and anemia status controlled for; excludes subjects with elevated C-reactive protein concentration at either the initial or final blood sampling.

#### Association of child iron status with maternal prenatal coffee intake

Maternal coffee intake during pregnancy was associated with several indicators of iron status of the child at baseline. There was a marginally significant inverse correlation of child hemoglobin with maternal coffee intake ( $r = -0.14$ ,  $P < 0.10$ ). Maternal coffee intake was positively associated with child ZPP:H (indicating greater risk of iron deficiency;  $r = 0.19$ ,  $P < 0.05$ ) and negatively associated with child ferritin concentration (excluding those with elevated C-reactive protein;  $r = -0.23$ ,  $P < 0.05$ ). The latter two associations remained significant after completing step-wise regressions that included maternal characteristics (age, parity, education, and socioeconomic status), and child age, sex, birth weight, weight gain since birth, breast-feeding status, the age at which coffee was introduced, coffee intake at baseline, and intake of meat, eggs, beans, tortillas, bread, fruit, vegetables, juices, milk, Incapa-

TABLE 5

Hematologic values before and after the study period in children who were or were not given iron supplements<sup>1</sup>

	Iron supplemented		Not supplemented	
	Coffee group ( $n = 18$ )	Substitute group ( $n = 24$ )	Coffee group ( $n = 46$ )	Substitute group ( $n = 51$ )
Hemoglobin (g/L)				
Initial	104 ± 11	102 ± 14	110 ± 12	112 ± 9
Final	115 ± 11	115 ± 10	118 ± 12	116 ± 10
Hematocrit (l)				
Initial	0.347 ± 0.032	0.339 ± 0.027	0.353 ± 0.025	0.358 ± 0.022
Final	0.376 ± 0.031	0.375 ± 0.029	0.372 ± 0.027	0.370 ± 0.030
ZPP:H (μmol/mol heme)				
Initial	124 ± 63	150 ± 110	99 ± 49	102 ± 52
Final	98 ± 39	98 ± 51	97 ± 44	113 ± 72
Ferritin (μg/L) <sup>2</sup>				
Initial	11.9 ± 14.9 [11]	6.7 ± 8.3 [17]	12.3 ± 12.0 [25]	12.6 ± 10.2 [34]
Final	15.6 ± 13.5 [11]	14.3 ± 9.1 [17] <sup>2</sup>	12.6 ± 10.1 [25]	12.7 ± 14.0 [34]

<sup>1</sup>  $\bar{x} \pm SD$ ;  $n$  in brackets. Subjects with elevated C-reactive protein concentrations at either the initial or final blood sampling were excluded.

<sup>2</sup> Significantly different change between coffee and substitute groups within the iron-supplemented children with initial ferritin and anemia status controlled for,  $P < 0.05$  (see Figure 5).

rina, atoles, and soft drinks. In fact, in the final step-wise-regression model, only two variables were significant predictors of child ferritin concentration ( $R^2 = 0.11$ ,  $P < 0.01$ ): child sex (girls had higher concentrations) and maternal prenatal coffee intake. In the regression model for child ZPP:H ( $R^2 = 0.23$ ,  $P = 0.0001$ ), the significant predictors were age (older children were less iron deficient), breast-feeding status (children were less iron deficient if still breast-fed), intake of Incaparina and vegetables (lower intakes were associated with less iron deficiency), and maternal prenatal coffee intake.

## DISCUSSION

These results indicate that there was no significant main effect of discontinuing coffee intake on iron status of iron-deficient Guatemalan toddlers, despite relatively good compliance with use of the coffee substitute. However, in children who took iron supplements, ferritin concentration doubled in those who discontinued coffee but did not increase among those in the coffee group, suggesting that coffee may interfere with the utilization of supplemental iron.

There are several potential explanations for the negative findings in the group as a whole. First, it is possible that the amount or strength of the coffee typically consumed by children in Guatemala is not sufficient to alter hematologic indexes. When coffee is prepared for children, it is sometimes diluted with additional water or milk. However, even after taking this into account, the amount of dry coffee consumed by children in this study averaged  $\approx 0.4\text{--}0.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at baseline, which is similar to the dose per kilogram among pregnant women in Costa Rica in whom the association of coffee with hematologic status was highly significant (10). On the other hand, the noncoffee fillers added to many brands of inexpensive coffee in Guatemala reduce the dose of actual coffee consumed. We attempted to assess this by measuring the caffeine concentration of each brand. Although many of the study children drank coffee with a relatively low caffeine concentration, the average was not nearly as low as that reported for home-prepared coffee in the rural highlands of Guatemala (24). The average caffeine intake among our subjects was  $\approx 9 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  at baseline, which is 5–13 times greater (per kilogram) than the estimated intake of 10-y-old children in the United States (25). Furthermore, when we examined the effect of the intervention after excluding children whose initial coffee or caffeine intake was relatively low, we still did not find any significant effect of discontinuing coffee consumption. Therefore, the lack of a main effect of coffee on iron status is not likely to be due to an insufficient dose.

A second potential explanation is that, for ethical reasons, we could not adequately test the effect of discontinuing coffee among children who were most likely to respond, ie, those who were truly anemic at the beginning of the study, because these children were provided with iron supplements. However, it should be noted that even in the nonanemic subgroup, about two-thirds of the children had low iron reserves initially, yet there was no significant effect of the intervention on ferritin concentration. Thus, it seems unlikely that we missed detecting an effect of coffee by treating the anemic subgroup with iron.

A third alternative is that the study procedures resulted in improved iron status in both groups, reducing the chance of

detecting an effect of discontinuing coffee consumption. The home visits every 2 wk focused considerable attention on the target child, which may have led to an overall improvement in child feeding and child care practices. The data show that iron status improved over the 5-mo period; there was a decline in the percentage of children in the nonanemic group with abnormal values for hemoglobin, hematocrit, and ferritin. However, it is difficult to tell if this was due to the study procedures, seasonal changes, or the greater age of the children at the end of the study (although the last is unlikely because the magnitude of the change was much larger than expected over that age range, based on the baseline regression coefficients between hematologic indicators and age).

Comparison of these results with those of previous studies in both rats (8) and humans (10) suggests that the effect of coffee on iron status is likely to be greater during the prenatal and early postnatal period than during childhood or adulthood. Although the experimental design of the present study made it a more rigorous test of the hypothesis than previous human studies using observational designs, one cannot ignore the consistent experimental evidence from animal models. It is well established that coffee intake reduces iron absorption even in adults (5, 6). What is less clear is whether this reduction in absorption is sufficient to impair iron status at all ages, or only in the most vulnerable groups, such as pregnant women. The rat studies suggest that there may be other mechanisms, besides impaired absorption, by which coffee interferes with iron metabolism. Again, these effects may disappear or be too subtle to detect after the prenatal and early postnatal periods. In this study, the significant associations between maternal prenatal coffee intake and child iron status indicate that exposure via the mother (during pregnancy or lactation) has a greater influence than does the direct consumption of coffee by the child.

The significant interaction effect between coffee intake and iron supplementation on ferritin concentration may reflect the inhibitory effect of coffee on iron absorption. Unfortunately, we do not have information on whether the iron supplements were given to children shortly before or after consumption of coffee, or at other times of the day. Morck et al (6) showed that coffee inhibited iron absorption when consumed with the meal or 1 h later, but not when consumed 1 h before the meal. Further research is needed to evaluate whether the effectiveness of iron-supplementation programs could be compromised by coffee intake.

Measurements of plasma iron, zinc, and copper concentrations were included in this study because of previous research showing apparent hepatic sequestration of iron, copper, and zinc in rat pups nursed by coffee-consuming dams (8) and because of the fact that an anemia that mimics that of iron deficiency is produced by copper deficiency (26). Internal sequestration of copper that produces a functional copper deficiency would be a plausible mechanism for low hemoglobin concentrations. However, concentrations of all three trace elements generally did not change over time and did not differ by study group. Although there are important limitations in the nutritional interpretation of plasma concentrations of zinc and copper (27), these results do not support the hypothesis that customary coffee intake by Guatemalan toddlers has an adverse effect on zinc or copper status.

To conclude, these findings do not provide a rationale for recommending that coffee be avoided by young children, ex-

cept perhaps among those receiving iron supplements. However, this does not necessarily imply that coffee intake among toddlers is benign. We reported elsewhere (17) that children in this study who discontinued coffee consumption experienced a modest improvement in growth compared with the control group. There may be other nutritional effects that we were unable to document. Certainly, there are more nutritious beverages for toddlers that can be recommended instead of coffee, such as vitamin C-rich juices (which can enhance iron absorption) and milk, regardless of whether coffee itself has any deleterious health consequences. Further research is needed to determine whether using more nutritious substitutes is culturally acceptable and economically feasible in such populations. †

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