Original Research

Randomized Placebo-Controlled Trial of Guava Juice as a Source of Ascorbic Acid to Reduce Iron Deficiency in Tarahumara Indigenous Schoolchildren of Northern Mexico

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Objective: Assess the efficacy of a 10-week consumption of guava juice on the iron status of children with mild iron deficiency anemia.

Methods: Ninety-five boarding school children aged 6–9 years identified as anemic were randomly allocated to receive 300 mL of natural guava juice containing ~200 mg of ascorbic acid (AA) or placebo (guava-flavored juice free of AA) with the main meal (5 d/wk). Information about dietary intake was collected at weeks 3, 5, and 7 at school and household levels. Changes in hemoglobin (Hb) and plasma ferritin (PF) among the subsample iron deficient at baseline ($n = 33$) were the main outcomes.

Results: Iron and phytic acid intakes at school and at home did not differ between groups. Baseline Hb and PF were 11.9 ± 0.5 g/dL and 8.2 ± 3.6 ng/mL for the guava, and 11.4 ± 1.1 g/dL and 7.4 ± 4.6 ng/mL for the placebo group (Hb: $p = 0.08$; PF: $p = 0.31$); at week 10 of the study, corresponding values were 13.1 ± 0.9 g/dL and 17.9 ± 10.3 ng/mL ($n = 16$), and 12.3 ± 1.3 g/dL and 15.4 ± 5.8 ng/mL ($n = 12$) (Hb: $p = 0.05$; PF: $p = 0.21$). With analysis of variance (ANOVA) for repeated measures, the guava group had 0.64 g/dL higher Hb (CI_{95} , 0.18–1.11; $p = 0.01$) and 2.47 ng/mL higher PF (CI_{95} , –1.04 to 5.98; $p = 0.12$) compared with the placebo group.

Conclusion: Guava juice providing 200 mg AA at one meal on each school day had a marginal effect on Hb and PF concentrations in children consuming high-phytate diets fortified with iron.

INTRODUCTION

Dietary iron absorption is determined by its heme iron (HI) and nonheme iron (NHI) content, by the type and quantity of dietary factors that influence iron absorption, and by the individual's iron status [1]. Whereas HI is highly bioavailable independent of dietary composition [2], NHI absorption can be as low as 2%, depending on the enhancing or inhibiting effects of food components present in a given meal [3–5]. In rural Mexico, NHI constitutes a very large proportion of the dietary iron consumed, as animal sources of HI are scarce [6,7].

Higher bioavailability of NHI can be achieved by increasing the dietary content of iron absorption enhancers such as vitamin C or ascorbic acid (AA) [8], fish, and meat [9], or by decreasing the content of inhibitors such as calcium [10,11], phytates [12,13], and polyphenols [14,15].

AA has long been recognized as a powerful enhancer of NHI absorption [8,16], and a dose-effect relationship between AA intake and iron absorption has been documented [17]. The principal mechanisms appear to be reduction of ferric to ferrous iron, which seems to be a requirement for the prevention of formation of insoluble and unabsorbable iron compounds, and

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Abbreviations: AA = ascorbic acid, ANOVA = analysis of variance, CDI = Commission for the Development of Indigenous Peoples, CRP = C-reactive protein, CV = coefficient of variation, Hb = hemoglobin, HI = heme iron, ID = iron deficiency, IDA = iron deficiency anemia, NHI = nonheme iron, PF = plasma ferritin, RDA = recommended dietary allowance, UL = upper limit.

uptake of iron into the mucosal cells [18]. In addition, AA counteracts the effects of inhibitors such as phenolic compounds [15], calcium [19], and phytates [13,15].

Several studies have shown the pronounced enhancing effects of added AA on the absorption of dietary NHI when foods or drinks rich in or supplemented with AA were tested [20–23].

AA, natural or synthetic, is a remarkably safe substance [24], even in premature infants [25], as excess intake is excreted. Adverse effects, mostly minor gastrointestinal upset, have been reported, but only with very large doses ($>3\text{--}4\text{ g/d}$) [26–28]. Renal calcium oxalate stone formation [29,30] and the development of iron overload [31,32] have been dismissed as important concerns related to AA consumption.

Major dietary sources of AA include guava, papaya, mango, citrus fruits, and various berries [33]. Small amounts of AA are present in vegetables such as broccoli, bell peppers, potato, cabbage, tomato, spinach, and cauliflower, but the cooking process destroys it partially or completely, depending on the time and method of food preparation [34]. Currently, the daily recommended dietary allowance (RDA) (and upper limit [UL]) for children aged 4–8 years and 9–13 years is 25 mg (UL 650) and 45 mg (UL 1200), respectively [35].

Guava (*Psidium guajava*), a fruit with an exceptionally high AA content, grows in tropical/subtropical regions and thrives especially well in Mexico; it is likely native to this region. The AA content depends on the variety (50–400 mg/100 g) and declines by $\sim 50\text{ mg}$ as it hits its peak ripeness; it also decreases with canning and storage [36,37]. Mexican species have 180–240 mg/100 g of AA [38]. Guavas also contain some carotenes (200–400 IU/100 g) and iron (0.30–0.70 mg/100 g) [36].

Iron deficiency (ID) has long been recognized as the most common nutritional disorder worldwide and is a major cause of nutritional anemia in children [39]. Anemia is a problem in Tarahumara indigenous schoolchildren of northern Mexico with a prevalence of $\sim 25\%$ (10% with iron deficiency anemia [IDA]) [40]. Of the strategies devised to tackle ID and IDA [41], food-based interventions have been relatively neglected, in part because of the urgent need to rapidly improve iron and hemoglobin (Hb) status through fortification and supplementation programs, but also because no practical approaches have so far proved successful.

Increased intake of AA is commonly included in nutritional advice to treat and prevent ID/IDA [41–43], but only one long-term community level efficacy trial has examined its impact. This study assessed the effect of administration of 25 mg AA twice daily in the form of limeade with meals for 8 months to nonanemic iron-deficient adult women, and found no improvement in various indicators of iron status [44]. The authors suggested that in diets with high phytic acid content and iron-fortified foods, the ratio of AA to iron would need to be at least 12:1 (by weight) for the AA to have an impact on iron status.

The present randomized controlled trial aimed to assess the efficacy of drinking natural guava juice containing $\sim 200\text{ mg}$ of AA with the main meal from Monday through Friday during a 10-week period on the iron status of indigenous schoolchildren with mild IDA who were consuming a school diet with iron-fortified foods and high in phytic acid content.

MATERIALS AND METHODS

In addition to the children's verbal assent, written informed consent was obtained from parents/tutors. The proposal was approved by the Ethics Committee of Research at the Mexican Institute of Social Security (2005/1/I/029) and by the Mexican Commission for the Development of Indigenous Peoples (CDI) in Chihuahua, the institution under whose aegis the study was conducted. The CDI promotes the educational and nutritional development of indigenous children living in highly marginalized areas with extreme poverty through boarding schools that accommodate 100–150 children, providing free primary education, housing, and food [45].

Study Design

A school-based efficacy trial with random assignment to receive guava or placebo was conducted to examine the effect of drinking 300 mL of natural guava juice containing $\sim 200\text{ mg}$ of AA with the main meal (midday) for 10 weeks on the Hb and plasma ferritin (PF) concentrations of indigenous Tarahumara boarding school children with mild IDA.

A total of 38 indigenous boarding schools are located in Guachochi, the most predominantly indigenous municipality of northern Mexico [46]. Four were chosen in consultation with CDI officials to represent the range of socioeconomic and geographic (particularly taking into account altitude differences) variability present in the district, and to provide enough children to fulfill sample size needs. Children attending CDI schools usually come to school on Mondays and return home on Fridays.

From the 519 children registered in the 4 selected schools, 295 aged 6–9 years were screened for mild anemia (capillary Hb, $9\text{--}11.5\text{ g/dL}$ at sea level). A total of 95 anemic children were identified by using altitude-adjusted cutoffs [47], rendering a prevalence of anemia that ranged between 27% and 42% among the sampled schools.

The anemic children were randomly allocated into intervention ($n = 50$) or placebo groups ($n = 45$). The efficacy trial focused only on those children with ID at baseline, defined as $\text{PF} < 12\text{ ng/mL}$ corresponding to the fifth percentile of a reference distribution for children aged 6–15 years [48] with normal C-reactive protein ($\text{CRP} < 10\text{ mg/dL}$) and no history of respiratory or gastrointestinal infection within the 2 weeks preceding blood extraction (Fig. 1). Thirty-three children met these criteria: 17 in the guava group and 16 in

Fig. 1. Selection of children from the 4 boarding schools included in the trial, Sierra Tarahumara, 2006.

the placebo group. Children with moderate or severe anemia (Hb < 9 g/dL), those who had received iron supplementation within 3 months before the study, and any who suffered from hematologic or chronic disease were excluded. All children infected with intestinal parasites were given individualized and supervised antiparasitic treatment at the beginning of the study to avoid the chance that parasitic infection might modify the results.

Sample Size

The sample size estimation was based on the assumed biologically significant increase of 5 ng/mL in PF concentration [44] in the guava group after 10 weeks, setting an alpha value of 0.05 and a beta value of 0.20. Data on PF to estimate background variability were obtained from a previous study of boarding schoolchildren in the same region [40]. The calculation resulted in a required sample size of 16 children per group. However, because of our inability to screen for PF in a timely fashion in this isolated rural area, mostly because of logistic and financial constraints, we had to enter children into the study on the basis of Hb levels, as mentioned previously. We then chose a sample size for the total number of schools

and the total number of children entering the study based on an estimation of likely IDA levels in this population.

Interventions

Different types of beverages are drunk with meals at the boarding schools; for breakfast and dinner, the most common are *atoles* (thick drinks made of cereals), and for lunch, artificial fruit drinks of various flavors are most often provided. Thus, we decided to offer the supplement with the main meal at midday during schooldays (Monday to Friday).

Guava Juice. The AA content of fresh guavas from a batch similar to those used in the trial was determined at 3 maturity levels (pre-ripe, ripe, and post-ripe) at the Department of Food Science and Technology of the National Institute of Nutrition in Mexico City, using atomic absorption spectrometry according to the analytical technique recommended by the Association of Official Analytical Chemists International. The guava juice was prepared by mixing guava, water, and a small amount of sugar in an electric blender, such that a 300 mL glass provided an overall estimated 200 mg of AA.

Artificial Guava Juice (Placebo). Children in the placebo group were given 300 mL of artificially flavored guava juice,

which contained no ascorbic, citric, malic, or other acids that promote iron absorption [49].

Data Collection

Baseline Data. An individual questionnaire was administered at the beginning of the trial to register child characteristics such as age, school grade, weight, and height. Children were screened for fecal parasites using direct microscopy, and diagnoses were made with at least 1 positive result from 2 serial samples as reported by 2 laboratory technicians; those infected were given individualized and supervised treatment with oral mebendazole for helminths and metronidazole for protozoa before the trial.

Dietary Assessment. The mean daily dietary intake of energy, protein, iron (HI, NHI, fortified), phytic acid, and calcium was calculated using weighed food records [50].

At the boarding school, children receive 3 meals on school days: breakfast (07:00 hours), the midday meal (12:30 hours) and dinner (18:00 hours). The type and quantity of ingredients used by the indigenous female cooks to prepare food recipes and drinks and the mode of preparation (i.e., cooking time, dilution with water, salt added, etc.) were recorded through direct observation. For instance, “sopa de fideo” (noodle soup) was a blend containing a certain quantity (depending on the number of children expected to eat at each meal time) of wheat flour noodles, onions, tomatoes, sunflower oil, bouillon cubes, and salt. After the quantity in grams of each food item used to prepare the mix was recorded, the weight of all foods was summed up, and the proportion of each ingredient was calculated from the total weight. Then the standard food ration served to the children was weighed, and the proportion in grams of each ingredient in the mix estimated. The actual amount of food eaten by each child was adjusted for additional servings and leftovers of foods/drinks using Salter weighing scales with 10 g precision for solids and measuring cups with milliliter scale divisions for liquids. All 3 meal times were added together, and the daily energy and nutrient content was calculated using the food composition database constructed for all foods used in the schools and households. Assessment of the diet served to children at school is provided in another publication [51].

Complete data were available for a subsample of 31 children of the 95 anemic children (guava 18; placebo 13) for 9 days from 3 nonconsecutive weeks (Tuesday, Wednesday, and Thursday of weeks 3, 5, and 7). Mondays were excluded, as many children arrived to the boarding school by noon, and Fridays, because most left for home after the midday meal.

The dietary intake at the household was assessed using 48-hour recall questionnaires administered to a subsample of 22 mothers of participating children (guava 13; placebo 9). They were interviewed Monday mornings about what children ate or drank over the previous weekend, including foods utilized, preparation methods, and meal times. Mothers were asked

about the quantities of food prepared and offered to and consumed by the child using both traditional and volumetric measures, which were later converted into net weight or volume for data analyses. The questions were translated into *Rarámuri*, the indigenous language, for those who did not speak Spanish. Direct observations at indigenous households were carried out to record traditional food preparations, including the staples, maize tortillas, and beans. Daily nutrient intake calculations were similar to those at school, but were based on 6 days from 3 nonconsecutive weekends (Saturday and Sunday of weeks 3, 5, and 7).

Dietary data were collected by nutritionists trained by the principal investigator. They lived at the boarding schools throughout the study period and were responsible to prepare and serve the guava and placebo drinks to the children, and to collect all dietary data at school and household levels.

The food composition was obtained from 3 main sources: (1) the Latin American Food Composition Table [38]; (2) the U.S. Department of Agriculture Nutrient Database for Standard Reference [33]; and (3) the food product labels. Phytate content was mainly estimated using data published by Wyatt and Triana-Tejast from foods commonly consumed in northern Mexico [52], which conform to the staples of the Tarahumara diet in both schools and households.

HI was determined on the basis of iron originating from meat, fish, and poultry; NHI was obtained by summing the iron from all other foods; and fortified iron was calculated from the fortified industrialized products used. Dietary iron density, expressed as milligrams per 1000 kilocalories, was calculated by dividing the total milligrams of iron consumed by the child by the total calories consumed during the day and multiplying the result by 1000.

The number of days of food intake data needed to calculate intake was 7, and was based on the estimate of energy content, considering a 95% confidence level, 20% precision, and a coefficient of variation (CV) for energy of 25% [53]. In this trial, we used 9 and 6 days for the school and household diets, respectively.

Compliance, Morbidity, and Adverse Effects. A daily registry was used to document the children’s school attendance and their ingestion of guava or placebo drinks. A questionnaire was administered on Fridays to document general symptoms, including headache, abdominal pain, nausea, vomiting, malaise, anorexia, dizziness, fever (sublingual temperature $>38^{\circ}\text{C}$), and diarrhea (watery stools for >2 consecutive days), as possible side effects.

Biochemical Data. At the screening visit, the middle finger of all children at the boarding schools whose parents consented was pricked to obtain a drop of capillary blood for Hb measurement using a new portable photometer, HemoCue 201+ (HemoCue AB, Ängelholm, Sweden), which performs a self-calibration, requiring no intervention by the operator; the average of 2 consecutive Hb readings was calculated.

Anemia was diagnosed on the basis of recommended Hb cutoff points at sea level (6–11 years: <11.5 g/dL) [54] adjusted for altitude [47]. From all who were anemic, nonfasting blood was drawn from the cubital vein of the right arm directly into a 5 mL vacutainer tube containing K₂-EDTA (Becton Dickinson, Franklin Lakes, NJ). The tube was opened, and a drop of blood was collected by capillary action into the microcuvette and placed in the HemoCue 201+ photometer. Hb concentration was determined using the average of 2 consecutive readings. Blood samples then were transported on dry ice for 2–4 hours across unpaved/paved mountain roads to the participating hospital, where plasma was separated by centrifugation at 4500 rpm for 3 minutes, placed in 1.5 mL Eppendorf tubes, and frozen at –20°C before it was sent to the Nutrition Research Laboratory in Mexico City for PF determination.

PF was quantified using a 2-site solid-phase immunoradiometric assay (IRMA-Mat Ferritin, DiaSorin Inc, Stillwater, MN); the coefficient of variation (CV) was 7.2%. C-reactive protein was measured qualitatively by a latex slide test. Positive values were excluded from analyses on the assumption that ferritin levels were thereby elevated as the result of infection or inflammation [55]. Hb, PF, and CRP were measured at baseline and at the end of follow-up (week 10).

Statistical Analyses

Statistical procedures were conducted using Minitab statistical software, version 14.5 (Minitab Inc, State College, PA). Guava and placebo groups were compared at baseline with respect to age, sex, weight, height, and parasite infections using Student's *t*-test and Pearson's Chi² or Fisher's exact test for continuous and categorical data, respectively.

Student's *t*-test and analysis of variance (ANOVA) with Bonferroni tests were used to compare daily dietary energy and nutrient intakes, including iron, AA, and phytic acid, as well as the biochemical parameters Hb and PF between groups at baseline and at week 10.

ANOVA for repeated measurements analysis was used to evaluate changes in Hb and PF. Mean differences and 95% confidence intervals are presented; the significance level was set at $p < 0.05$.

Microsoft Office Excel (Microsoft Corporation, Redmond, WA) was used to calculate dietary intake, and data were analyzed using the Statistical Package for the Social Sciences (SPSS) computer software, version 14.0 (SPSS Inc, Chicago, IL).

RESULTS

Baseline Characteristics

The amount of AA found to be in the guava juice varied, depending on ripeness (pre-ripe 184.7, ripe 319.9, post-ripe 115.5 mg/100 g flesh). Overall, based on our estimation of how

ripe the guavas used tended to be, and accounting for an estimated 5% loss due to processing, we calculate that the children received an average of about 200 mg/300 mL drink offered at the midday meals.

Baseline characteristics of ID children stratified by treatment group are presented in Table 1. All variables, including mean BMI and BMI percentile, were very similar between the guava and placebo groups. The mean number of days that children received guava (45.2 days of 50 total possible days in 10 weeks; 90.5%) or placebo (46.8 days; 93.6%) during the 10-week period was also similar ($p > 0.05$).

Adverse Effects

The number of children who presented with fever (median 0, range 0–3 for both groups) or diarrhea (median 0, range 0–5 for guava; and median 0, range 0–4 for placebo) was comparable between groups. Headache, stomachache, and malaise accounted for 84% of these potential side effects, but no differences were noted between groups. Mild abdominal pain was the most common side effect that could potentially be attributed to ingestion of AA; it was reported by 3 of the 16 children in the experimental group and by 2 of the 12 children in the placebo group during the trial.

Dietary Intake

A comparison of the intake of selected nutrients between the guava and placebo groups at boarding school and household

Table 1. Comparison of Baseline Characteristics of School-children in the Guava and Placebo Groups, Sierra Tarahumara, 2006

Variable ^a	Guava (n = 17)	Placebo (n = 16)	<i>p</i> Value ^b
Weight, kg	21.3 ± 2.9	21.3 ± 4.7	0.97
Height, cm	116.8 ± 6.2	116.1 ± 11.2	0.82
Body mass index, kg/m ²	15.6 ± 1.5	15.6 ± 0.95	0.94
Body mass index, percentile ^c	45.5 ± 30.6	48.0 ± 20.9	0.78
Age, y	7.2 ± 1.1	6.8 ± 1.4	0.34
Sex, %			
Male	52.9	62.5	0.72
Female	47.1	37.5	
School year, %			
Preschool–first	47.1	56.3	0.73
Second–third	52.9	43.8	
Parasite infection, %			
Yes ^d	58.8	50.0	0.73
No	41.2	50.0	

^a Mean ± SD for continuous and percentages for categorical variables.

^b Student's *t*-test for continuous and Pearson/Fisher testing for categorical variables.

^c Computed using the Centers for Disease Control and Prevention (CDC) online BMI percentile calculator for children.

^d Presence of *Entamoeba histolytica/dispar*, *Giardia lamblia*, or *Ascaris lumbricoides*.

Table 2. Comparison of Dietary Intake of Selected Nutrients at Boarding School and Household Levels in Schoolchildren Receiving Guava (Experimental Group) or Placebo, Sierra Tarahumara, 2006*

Nutrient	Boarding School ¹			Household ²		
	Experimental ³ (n = 18)	Placebo (n = 13)	Total (n = 31)	Experimental (n = 13)	Placebo (n = 9)	Total (n = 22)
Energy, kcal	2048 ± 184	1987 ± 268	2023 ± 221	1307 ± 203	1318 ± 173	1311 ± 203
Protein, g	78 ± 9	73 ± 11	77 ± 10	47 ± 13.9	47 ± 9.0	47 ± 14.0
Iron, mg	25.2 ± 2.7	23.9 ± 4.0	24.7 ± 3.3	19.1 ± 1.7	20.4 ± 2.6	19.4 ± 2.4
Heme	0.76 ± 0.1 ^a	0.67 ± 0.1 ^b	0.72 ± 0.1	0.12 ± 0.0	0.14 ± 0.0	0.13 ± 0.0
Nonheme	8.0 ± 1.1	7.2 ± 1.3	7.7 ± 1.3	17.8 ± 1.7	19.0 ± 2.8	18.4 ± 2.1
Fortified	16.4 ± 1.6	15.9 ± 2.8	16.2 ± 2.1	1.2 ± 0.5	1.3 ± 0.5	1.2 ± 0.5
Iron density ⁴	12.3 ± 0.42	11.9 ± 0.51	12.1 ± 4.8	14.9 ± 2.7	15.3 ± 1.9	15.0 ± 2.4
Phytic acid, mg	3010 ± 610	3069 ± 651	3045 ± 631	3286 ± 846	2690 ± 1018	2920 ± 975
Calcium, mg	1184 ± 96	1165 ± 154	1177 ± 122	732 ± 214	770 ± 117	746.7 ± 181
Ascorbic acid, mg	97 ± 17	88 ± 22	93.7 ± 20.0	62 ± 31	49 ± 22	57.6 ± 28.3

* One-way ANOVA and Bonferroni tests: different superscript letters (a, b) indicate statistical significance ($p < 0.05$) between experimental and placebo groups in the boarding school and the household.

¹ Mean daily intake for 9 days (Tuesdays, Wednesdays, and Thursdays from weeks 3, 5, and 7) based on weighing.

² Mean daily intake for 6 days (Saturdays and Sundays from weeks 3, 5, and 7) based on recall.

³ Does not include the 200 mg of AA and 0.5 mg iron contained in the guava juice.

⁴ Dietary iron density in mg/1000 kcal = (daily iron intake in mg/daily energy intake in kcal) × 1000 kcal.

levels is presented in Table 2. For both groups, the boarding school diet provided a mean caloric intake that was approximately 700 kcal higher. Thus protein intake was 60% greater, and iron intake 5 mg superior to the home diet. However, iron density was 3.2 mg/1000 kcal higher at the household level (15.3 vs 12.1; $p < 0.01$), although it was similar between groups in both the school and the house. Excluding the supplement, intakes of AA were also 56% higher at school than at home in the guava group and 79% higher in the placebo group. The guava group consumed slightly more HI when at school. However, no other differences in caloric or nutrient intake were seen between the guava and placebo groups. Intakes of phytic acid were ~3 g/d irrespective of group or location.

Biochemical Results

Blood specimens were available for 17 guava and 16 placebo children at baseline, but only 16 and 12 children, respectively, were eligible to be included in the final analysis. Five children were thus excluded: 3 because of positive CRP (1 guava and 2 placebo), and 2 (placebo) because plasma was not available at the end of the trial (children were not present during the week of blood sampling).

The mean venous Hb and PF at baseline and at week 10 were not different between guava and placebo groups. Also, increments of Hb (guava 1.1, placebo 0.9 g/dL; $p = 0.26$) and PF (guava 9.6, placebo 7.9 ng/mL; $p = 0.32$) from baseline to week 10 did not reach statistical significance between groups (Table 3).

However, after individual variability was controlled by use of ANOVA for repeat measures, a significant difference in the

increment of Hb was observed. Venous Hb increased by 0.65 g/dL (95% confidence interval [CI₉₅], 0.18–1.11; $p = 0.01$) more in the guava than in the placebo group. PF also increased by 2.47 ng/mL, following the same trend, but statistical significance was not achieved (CI₉₅, –1.04 to 5.98; $p = 0.12$).

DISCUSSION

We conducted a placebo-controlled efficacy trial to determine the effects of drinking natural guava juice containing ~200 mg of AA with the main meal 5 days per week for 10

Table 3. Venous Hemoglobin and Plasma Ferritin of Schoolchildren with Mild Iron Deficiency Anemia at Baseline and Week 10 in the Guava and Placebo Groups, Sierra Tarahumara

Measurement	Mean ± SD (n)		<i>p</i> *
	Guava	Placebo	
Hemoglobin, g/dL ¹			
Baseline	11.9 ± 0.5 (17)	11.4 ± 1.1 (16)	0.085
Week 10	13.1 ± 0.9 (16)	12.3 ± 1.3 (12)	0.055
Δ week 10–baseline	1.1 ± 0.9 (16)	0.9 ± 1.0 (12)	0.265
Plasma ferritin, ng/mL ²			
Baseline	8.2 ± 3.6 (16)	7.4 ± 4.6 (12)	0.316
Week 10	17.9 ± 10.3 (16)	15.4 ± 5.8 (12)	0.214
Δ week 10–baseline	9.6 ± 11.9 (16)	7.9 ± 7.0 (12)	0.320

* Student's *t*-tests were used to detect significant differences between groups.

¹ Venous Hb was measured using the Hemocue 201+.

² No samples had elevated C-reactive protein; includes only children without history of respiratory/diarrheal infection within 2 weeks of blood testing.

weeks on the iron status of indigenous Tarahumara school-children with mild IDA. Our main finding is that, after control for individual variability was attained by using a repeated measurement analysis, Hb concentration increased by 0.64 g/dL and PF by 2.47 ng/mL more in the experimental group than in placebo counterparts, although only the former was statistically significant.

The time frame used to conduct the trial was based on constraints derived mainly from the school calendar. Although indigenous children were supposed to start school at the beginning of September and finish in the middle of December, most came in mid-September and left in early December; therefore, if the time required for initial setup and final measurements of the study is accounted for, it can be seen that 10 weeks of effective follow-up was provided. The fact that children had to leave for Christmas vacation and come back in the middle of January limited the possibility of continuing the trial. Moreover, Garcia et al. [44] had reported the largest PF increase at week 16, and because we were using a considerably higher ratio of AA to iron, we thought that a 10- to 12-week period would be sufficient to detect the effect.

Garcia et al. [44] failed to achieve a positive effect on iron status (experimental vs control: baseline Hb 13.7 vs 13.9 g/dL and PF 6.4 vs 6.2 ng/mL; after 8 months, Hb 14.0 vs 13.7 g/dL and PF 9.0 vs 8.7 ng/mL) with administration of AA in the form of limeade to ID nonanemic adult women. The differences between the studies likely explain the differing results. Although previous investigators had included only subjects with mild ID (they were not anemic) who received an AA dose of only 50 mg daily, in the present study we included only children with IDA, and we administered a dose that was 4 times higher. Therefore, our study subjects were more prone to improvement because iron absorption depends on both initial iron status and the amount of AA in the diet [17,56].

As advised by Garcia et al., our subjects consumed diets with high phytic acid content, supplemented with both AA (guava) and iron (via fortification). The authors also suggested a dietary ratio of 12:1 AA/iron. However, a considerably higher ratio of AA to iron (by weight) has been recommended to increase iron absorption from a meal rich in fortified iron and phytates [5,44,57–60]. At the boarding school level, the ratio reached in the guava group was ~24:1 for the main meal (midday), compared with 4:1 in the placebo group. At breakfast and dinner, both groups achieved ratios of ~4–5:1. At home, the daily AA/iron ratio was estimated to be ~2–3:1 in both groups. Based on the small magnitude of the difference in increased Hb and PF concentrations between groups reported in our study, it is probable that even with the 12:1 ratio proposed by Garcia et al., no effect at all would have been achieved, but more research is needed to establish the optimum ratio.

Guava was used in this study because it is one of the few foods with exceptionally high AA content that is available

locally at low cost and is tasty and feasible to prepare and consume easily with meals. Fresh raw guavas were utilized because evidence indicates that industrial processing into jam or juice [61], or giving it dried [62], decreases the AA content considerably. Guava is a local product cultivated on a small scale at the bottom of the gorges of the Sierra Tarahumara [63]. Guavas are very much appreciated by the local population for seasonal domestic consumption. Nevertheless, we recognize that the use of electrical devices such as blenders in preparation of the juice complicates such an intervention at the household level in a low-income setting; yet it seems feasible at the school level where, in addition to dry commodities, HI-rich food is offered. Even at the household level, consumption of adequate quantities of guava with the main meal is feasible if people have been informed of its potential impact. However, long-term compliance with this strategy for reducing IDA seems difficult.

We were unable to measure other more sensitive biochemical indicators of iron status such as transferrin receptors [64]. However, PF was used to assess ID because it has been considered a good indicator of ID [55,65]; in addition, we made special efforts to ensure that PF values truly reflected iron stores by excluding children with any history of infection or with a positive CRP test [66,67]. Nevertheless, despite a statistically significant improvement in Hb in the guava group (although of limited clinical importance), the increment in PF concentrations was only marginal compared with placebo. A sample size of 16 subjects per group was calculated in advance to detect differences, but at the end of the study, only 12 individuals in the placebo group were available. Therefore, we cannot rule out the possibility of a type II error. In addition, the superior improvement in Hb compared with PF in both groups may reflect the presence of other causes of nutritional anemia such as B₁₂ deficiency [40], which is currently being tackled by the school diet alone.

Despite limitations such as small sample size and lack of measurement of more sensitive iron status indicators, this study had some strengths. These include the similarities of studied groups in terms of baseline characteristics and intestinal parasite infections, which were caused mostly by helminths (principally *Ascaris lumbricoides*) or protozoa (mainly *Giardia lamblia*) that do not interfere with iron absorption and were treated before the study started. Compliance was high and similar for both groups (guava 90.5%; placebo 93.6%; $p = 0.38$); morbidity rates were also comparable. This study started right after the usual 2- to 3-month summer vacation period, during which children had been eating the household diet, which likely depleted some children's iron stores sufficiently to affect Hb concentrations. Thus, when they entered the study and started to eat a moderately bioavailable diet with higher caloric, protein, iron (including HI), and AA intake, the iron status of both groups was likely to improve.

As anticipated, nutrient intake was higher (in general by 50%–70%), including iron intake (20%–30% higher), in the

boarding school diet than in the household diet. Yet the children's dietary intake did not differ statistically between groups at the school or in the household. Even though the household diet contained on average more dietary iron than the school diet per 1000 kcal (15.3 vs 12.1 mg), the mean daily caloric intake was considerably lower at home (~1300 vs 2000 kcal), thus overall iron intake was higher at school (~25 vs 20 mg). The iron content of both household and school diets appears high compared with the 6 mg/1000 kcal documented in the Western diet [68,69], pointing toward a possible overestimation. This is especially true for the household diet, where the accuracy and precision of 48-hour recalls can be questioned, both because it is difficult to interview Tarahumara mothers, and because they may not have reported their usual diet accurately. However, our data are consistent with those of other studies that have reported similar iron intakes as in the Tarahumara [5,68]. For the school diet, we used individual food weighing by trained personnel; this approach is much more accurate and reliable than is the use of recall data [70].

Nonetheless, an important limitation relates to our failure to compare dietary data only between guava (17) and placebo (16) children with ID at baseline. This is because we did not know which children were deficient at the beginning of the trial, and because we could not assess the dietary intake of all 95 anemic individuals because of the workload involved, we had to take a sample. As a result, we measured and present dietary data from ID and non-ID children, because presenting results only for those deficient at baseline dramatically shrinks the sample, producing very imprecise estimates.

As it was seen in the children from the placebo group, the school diet alone seemed sufficient to improve the Hb concentration and to replete the children's iron stores, although it did so rather slowly. From this point of view, the AA given in the guava juice had only a small additive effect on Hb and PF concentrations. Its impact was probably attenuated by the presence of HI in the same meal, as other authors have noted [71,72]. The approach we used in this study could have led to different results if the home diet had been utilized instead, but it would have been ethically questionable to exclude the children from the school diet. The feasibility of attempting to carry out such a trial at the household level in this area is questionable because of serious logistical difficulties.

CONCLUSION

Guava juice used as a source of AA demonstrated marginal beneficial effects of small clinical significance on Hb and PF concentrations of indigenous children with IDA fed diets rich in NHI and phytic acid. We suggest that further research is needed to examine the impact of foods this high in AA on diets lacking HI. On its own, however, this study is inadequate to question accepted limitations, including poor long-term

compliance except for animal sources, with food-based strategies designed to treat individuals with ID/IDA at the community level.

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