The Journal of Nutrition 154 (2024) 3740–3748

THE JOURNAL OF NUTRITION

journal homepage: https://jn.nutrition.org/

Community and International Nutrition

Vitamin C-Rich Guava Consumed with Mungbean Dal Reduces Anemia and Increases Hemoglobin but not Iron Stores: A Randomized Controlled Trial of Food-to-Food Fortification in Indian Children

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A B S T R A C T

Background: Adding vitamin C-rich fruit to staples containing iron could be an effective strategy to improve iron bioavailability and thereby reduce iron-deficiency anemia in children.

Objectives: We aimed to assess the effect of consuming a mungbean-based meal with or without guava fruit on body iron stores, hemoglobin concentration, and anemia of children as part of a school feeding program.

Methods: We conducted a 7-mo randomized, controlled trial with 6- to 10-y-old school children ($n = 200$; 46% anemic, 71% iron-deficient) from a rural community in Haryana, North India. Children were assigned to 2 treatment groups to daily receive either a meal of mungbean dal only (3.0 mg iron; vitamin C:iron molar ratio ~0.5:1), or mungbean dal with fresh guava (3.2 mg iron; ~170 mg vitamin C; molar ratio ~18:1). Meals were served every school day under supervision. The primary outcome was body iron stores, whereas concentrations of hemoglobin and other iron indicators were secondary outcomes.

Results: Daily consumption of mungbean dal along with guava did not result in an overall improvement of body iron stores [mean treatment effect: 0.65 mg/kg body weight; 95% confidence interval (CI): −0.34, 1.63; $P = 0.197$]. However, compared with children who consumed mungbean dal only, children in the guava group showed a larger increase in hemoglobin concentration (3.7 g/L; 95% CI: 1.6, 5.6; $P = 0.001$), and a larger drop in the prevalence of anemia (−51%; 95% CIs: −74, −10; $P = 0.022$) and iron-deficiency anemia (−56%, 95% CI: −83, 13; $P = 0.087$). These effects were more pronounced in children who were iron deficient at study start.

Conclusions: Addition of guava to a mungbean-based meal containing a moderate amount of iron increased hemoglobin and reduced anemia but did not provide enough additional absorbed iron to also increase body iron stores. Food-to-food fortification by inclusion of vitamin C-rich fruits in iron-containing school meals may help alleviate the burden of anemia in children.

Trial registration number: This trial was registered at https://clinicaltrials.gov/ct2/show/NCT01191463.

Keywords: iron, anemia, vitamin C, guava, mungbean, school feeding, children, India

Introduction

Anemia is the most prevalent public health problem in children in India, with a prevalence of 67% among preschoolers, and a slightly higher prevalence in rural areas [1]. Anemia in children is associated with impaired physical and cognitive development, increased morbidity, and higher mortality [2–7]. Periodic dietary surveys conducted in public primary school children in Haryana State indicate their diets to be monotonous. They consume primarily starchy staples, that is, rice, wheat, and

Abbreviations: AGP, α1-acid glycoprotein; BW, body weight; CI, confidence interval; CRP, C-reactive protein; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; SF, serum ferritin; sTfR, soluble transferrin receptor.

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https://doi.org/10.1016/j.tjnut.2024.10.042

Received 13 June 2024; Received in revised form 30 September 2024; Accepted 24 October 2024; Available online 30 October 2024

potato, with minimal intake of legumes and milk, negligible amounts of vegetables and fruit, and no consumption of meat, fish, and poultry [8]. Consequently, diets of North-Indian children are low in iron and vitamin C content, and high in antinutritional factors, that is, phytic acid and polyphenols, resulting in low iron bioavailability. The key etiologic factors for anemia among school-aged children in rural North India are identified as low iron intake, followed by low iron bioavailability and a high prevalence of helminth infections [9–12].

To control soil-transmitted helminth infections, the Indian government has initiated the biannual distribution of albendazole tablets among school children. However, the low intake and bioavailability of iron remains to be addressed. Vitamin C has shown to be a potent enhancer of iron absorption in short-term human studies, because it counteracts the inhibitory effect of phytic acid, polyphenols, and calcium [13–15]. It is suggested that a vitamin C:Fe molar ratio in excess of 4:1 is needed to counteract any inhibitory effects [13]. Use of natural vitamin C has been recommended over the pure chemical form, because the latter is highly unstable during food processing and storage [16]. Increasing the dietary intake of vitamin C from natural sources may therefore be a promising and sustainable dietary approach for increasing iron absorption and counteracting iron-deficiency anemia (IDA), in addition to antihelminth treatment.

Guava (*Psidium guajuva*), a local Indian all-season fruit that can be eaten whole, stands out for its high content of ascorbic acid of 200–300 mg per 100 g fresh weight, depending on the variety and season. Compared with other fruits, it is only surpassed in vitamin C content by aonla (*Emblica Officinalis*, Indian gooseberry). However, aonla is a seasonal fruit, and, due to its pungent taste, it is primarily utilized for herbal preparations and preserved products. Mungbean (*Vigna radiata* L.) is a commonly consumed basic food in the Indian diet, with its iron content ranging between 4 and 8 mg per 100 g [17]. Mungbean is mostly consumed with chapatti (unleavened bread) or boiled rice, or in the form of khichadi, a mixed dish of rice and mungbean. The nutritional potential of mungbean is limited by the presence of inherent antinutritional factors known to reduce the bioavailability of iron [18,19]. Both mungbean and guava are locally produced and consumed food crops in Haryana, India. We hypothesized that the simultaneous consumption of mungbean and guava by school children would improve their iron status and reduce anemia. Therefore, our primary objective was to assess the effect of complementing a daily mungbean-based meal with guava fruit on body iron stores, hemoglobin (Hb) concentrations, and anemia of school age children in a 7-mo randomized controlled trial.

Methods

Study site

The study was conducted in the Hisar district of Haryana State, northern India. Haryana is a small state in North India that shares borders with 4 states (Punjab, Haryana, Rajasthan, and Uttar Pradesh) and 2 Union Territories (Delhi and Chandigarh). It therefore has a mixed population that is representative of the majority of the population in North India. Hisar in Haryana was selected for practical reasons, because the State Agriculture University is situated here and the production of large batches of the same varieties of mungbean and guava was therefore possible. The climate is hot (46°C) in summer (May–June) and markedly cold (−1°C) in winter (January) with scanty rainfall in most areas. At the time of the study, Haryana accounted a total population of ∼25 million (2011 census), having 27.2% of households below the poverty line. Approximately 70% of the state's population is employed in agriculture. The typical diet in rural areas is mainly vegetarian with wheat, rice, and pearl millet being staples supplemented with moderate amounts of legumes, dairy products, tea, nuts, vegetables, and fruits [8,10]. A school-based study conducted in Haryana State found that the prevalence of soil-transmitted helminth infections was 28.7%–75.6% [20]. Two government primary schools were selected for this study, both located in 1 community, that is, Mangali village, in the immediate surroundings (∼12 km) of Hisar, Haryana State, India. Both schools were beneficiaries of the governmental lunch feeding program, where the children were entitled to receive a meal containing ∼400 kcal of energy and ∼8–12 g of protein during school days. Both schools were located at an altitude <500 m above sea level.

Study design

The study was a randomized controlled trial. The primary outcome of the study was body iron stores, whereas concentrations of Hb and other iron indicators were secondary outcomes. After screening, a total of 200 children from the 2 schools were enrolled in the study and randomly assigned to either receive a daily portion of mungbean dal only (control group; $n = 101$), or mungbean dal served with fresh guava (guava group; $n = 99$). Randomization was performed using a blocked randomization procedure with a block size of 6, and performed by a member of the coordinating team who was not present during the screening and enrolment in India.

Subjects

Children were selected by the members of the investigating team if they were aged between 6 and 10 y; were apparently healthy; without pre-existing medical conditions or regularly taking medication. Five weeks before the start of the trial, eligible children were screened by the study physician using a general medical health questionnaire. Exclusion criteria were severe anemia (<70 g/L); allergy or intolerances to any components of the meals; consumption of iron and vitamin C supplements during screening; and unwillingness to continue. A sample size of 100 children per treatment arm was estimated to be sufficient to detect a difference in increase in body iron stores of 1.7 mg Fe/kg body weight (BW) between the control and guava group, assuming a standard deviation of 4.0 mg Fe/kg BW [21], with 80% statistical power and assuming an attrition rate of 12% (Power and Sample Size Calculation, Version 3.1.6, Vanderbilt University School of Medicine).

Ethical aspects

Written informed consent was obtained from parents or legal guardians of children, and oral consent was obtained from the children who participated in the study. The protocol was approved by the Institutional Ethical Committee of Chaudhary Charan Singh Haryana Agricultural University, Hisar, India, and by the Medical Ethical Committee of Wageningen University, The Netherlands. The trial was registered at www.clinicaltrials.gov, with identifier NCT01191463. The study period was September 2010 to March 2011.

Preparation and serving of the lunch meals

Before the start of the study, the cooks preparing the meals were trained for the preparation of the mungbean dal, which was then freshly prepared daily in a standardized manner in both the schools. For this, mungbean was boiled with water and salt, seasoned with oil, cumin seeds, onion, tomato, and turmeric powder, and garnished with coriander leaves before serving. The mungbean variety MH 1-25, containing 6.0 mg iron per 100 g (on dry weight basis, having ~8% of moisture) was provided by the pulses section, Department of Plant Breeding, Chaudhary Charan Singh Haryana Agriculture University, Hisar, in a single lot. Other ingredients used in the preparation of mungbean dal along with fresh guava fruit were purchased from the local market every other day. Weighed amounts of mungbean dal (mean, 250 g corresponding to ~50 g raw mungbean) and fresh guava (mean, 75 g) were distributed to the children under strict supervision of the investigating team.

Participating children were identified by using a color-coded personal badge. Meals were served during mid-morning (11.00 am to 11.30 am and 11.30 am to 12.00 am, depending on the weather) 6 d/wk (except for school holidays). Children who had received a meal were asked to sit according to group allocation under the direct supervision of the investigating team. Eating bowls were color-matched with the color of the personal badge, and weighed amounts of mungbean dal were distributed to the children by the study team. Plastic zipper bags with weighed amounts of guava were distributed to the children of the guava group. Bowls with leftovers of dal were collected and weighed, and data were recorded on a daily basis. The served guava was always finished completely by all.

Assuming a high prevalence of parasitic infection among school children in North India, all children were treated against intestinal parasites with a single dose of 400 mg albendazole (Mann Pharmaceuticals, Mehsana, Gujarat, India) both at baseline and at study midpoint (3.5 mo).

Sample collection and laboratory analyses

Anthropometric data were collected at baseline and endpoint using standardized procedures and equipment [22]. Age was calculated from the date of birth record registered in schools. BW was measured using a SECA platform spring balance fixed on a wooden board, with a precision of 0.1 kg (Seca 890). Standing height was measured to the nearest 1 mm using a stadiometer. All measurements were done in duplicate and the average was calculated and used in data analyses.

Blood samples were collected at baseline and endpoint by a phlebotomist. A non-fasting 7-mL venous blood sample was collected in EDTA (1 mL) and serum (6 mL) collection tubes. EDTA tubes were kept on ice pads and transported to the Khetarpaul Hospital (Hisar, Haryana) for further processing. Hb concentration was analyzed on the day of collection with an hematology analyzer (KX-21, Sysmex Corporation) using manufacturer quality control material. Serum was separated from cells by centrifugation at 5000 × *g* for 10 min at 4°C, divided into aliquots, and transported frozen to the Division of Nutrition, Institute of Population Health and Clinical Research, St. John's Research Institute, Bangalore, India. Serum samples were stored at −80°C until analysis for concentrations of serum ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α1-acid glycoprotein (AGP). Baseline and endpoint serum samples were analyzed at the same time to avoid inter-analyzed variability. AGP concentrations were measured by immunoturbidimetry (Cobas Tina-quant; Roche Diagnostics, intermediate precision Coefficient of Variation (CV): 1.1%). sTfR and SF concentrations were measured by using commercial immunoassays and control materials (sTfR: CobasIntegra; Roche Diagnostics, intermediate precision CV: 5.4%; SF: Cobas E, Elecsys; Roche Diagnostics, intermediate precision CV: 4.4%). The Roche assay values were converted to the Ramco assay values using the regression equation suitable for body iron calculation [23]. CRP concentrations were measured with an automated chemiluminescent immunoassay system (Cobas Tina-quant; Roche Diagnostics; intermediate precision CV: 3.3%). Anemia was defined as Hb <115 g/L in children aged 5–11 y [24]. Iron deficiency (ID) was defined as either SF <15 μg/L or sTfR >8.3 mg/L, after correction for inflammation [25,26]. IDA was defined as combined ID and anemia. Total body iron was calculated from the ratio of sTfR to SF by using the method of Cook et al. [27,28].

Samples of mungdal and guava were collected at an interval of 15 d for nutritional analysis (mungdal: $n = 13$; guava: $n = 15$), which was carried out at the Department of Foods and Nutrition, Chaudhary Charan Singh Haryana Agriculture University, Hisar, India. Two samples of mungdal and 5 samples of guava were re-analyzed by the Division of Human Nutrition, Wageningen University, the Netherlands for quality control. Finely ground and moisture-free samples were used for nutritional evaluation. Moisture, crude fat, ash, total nitrogen, crude fiber, and total carbohydrate (by difference) contents were determined by Association of Official Agricultural Chemists (AOAC) methods [29]. A factor of 6.25 was applied to convert nitrogen to crude protein. Energy (kcal) content was calculated by the factorial method using factors of 4, 4, and 9 kcal/g for protein, carbohydrate and fat, respectively. Phytic acid was determined using the spectrophotometry method of Davies and Reid [30], with absorption being read at 465 nm against the standard blank. Polyphenols were extracted with methanol and estimated as tannic acid equivalents using Folin–Ciocalteu reagent [31]. Acid-digested samples were used for the determination of iron and calcium by atomic absorption spectrophotometry [32]. The vitamin C content of the samples was measured by HPLC with a reversed-phase column and photometric detection [33]. The molar ratio of vitamin C to iron was calculated by a weight quotient of 3.15 (1 mole of vitamin C equals 176.12 g, whereas 1 mole of iron equals 55.85 g).

Statistical methods

Data were analyzed by intention-to-treat using SPSS software (version 19.0; SPSS Inc.) and SAS software (version 9.20; SAS Inc.). Data were assessed for normality by visual examination of distribution plots and were normalized as appropriate by log transformation. Descriptive statistics were expressed as means, geometric means, with SD or confidence intervals (CIs) for continuous variables, and as counts and percentages for categorical variables. SF and sTfR were adjusted for inflammation (AGP and CRP) using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia regression correction approach [26]. Analysis of covariance was used to determine differences in Hb, SF, and sTfR concentrations, and in body iron stores between treatment groups. To estimate the treatment

effect sizes and 95% CIs, we adjusted each outcome, that is, concentrations of Hb, SF, sTfR, and body iron for their corresponding baseline values. Adjusted estimates were assumed more relevant than crude estimates because of minor differences in variables at baseline. Poisson regression with robust error variance and constant time at risk was used to estimate treatment effects relative to the control group for binary outcomes with the covsandwich syntax in SAS [34]. Z scores for weight-for-age, height-for-age, and BMI-for-age were calculated (WHO Anthro plus Software package, version 3.2.2). An exploratory subgroup analysis was done to compare the treatment effects between children who were either iron deficient or sufficient at baseline.

Results

At baseline, 101 and 99 children were randomly assigned to the control and guava groups, respectively. During the study, there were 4 dropouts in each of the groups; thus, 97 (control group) and 95 (guava group) children completed the study (Figure 1). One child in the guava group had an SF concentration >700 μg/L and was therefore excluded from statistical analysis. Children received the study meals for 139 d over a period of 7 mo. On average, 89.7% of children in the mungbean dal group and 90.4% of those in the guava group consumed the test meals daily. The consumption of mungbean dal (mean ± SD) was 242 ± 47 g/d, which corresponded to 46 ± 8.7 g of raw mungbean. The proportional amount consumed was 98% of what was served in both groups. The iron and vitamin C content (mean ± SD) per portion (242 g) of cooked mungbean dal were 3.0 ± 0.3 and 4.4 ± 1.3 mg, respectively (Table 1), corresponding to a vitamin C:iron molar ratio of 0.5:1 of meals in the control group. The iron and vitamin C content per portion (73 g) of guava were 0.2 ± 0.04 and 171 ± 34 mg, respectively, resulting in a molar ratio of 18:1 of meals in the guava group.

Baseline characteristics of children in both treatment groups are presented in Table 2 and 3. Children in the control group appeared to be slightly more undernourished and had poorer iron status indicators than children in the guava group. Mean Hb concentration was 114 g/L, with 46% of children being anemic. Median SF and sTfR concentrations were 23.7 μg/L and 8.6 mg/L, respectively, with 71% of children being iron deficient. Approximately 5.3% of children had elevated serum CRP concentrations (>5 mg/L), and 23.2% had elevated serum AGP concentrations (>1 g/L) at baseline. The baseline characteristics of the 8 children who dropped out did not differ from those of other participants.

Irrespective of treatment, Hb concentration increased in both groups over the course of the study, resulting in an overall decrease in the prevalence of anemia (17.2%) and IDA (9.9%) at endpoint. At the end of intervention, body iron stores did not differ markedly between groups (mean difference of guava compared with control group, 0.65 mg/kg BW; 95% CI: −0.34, 1.63 mg/kg BW; $P = 0.197$), nor did SF concentration [12.0% (−14, 42%, $P = 0.353$; Table 3]. In crude analysis, a reduction in sTfR was observed (guava compared with control group, −15%; 95% CI: −27, −2%; $P = 0.019$), but this effect was blunted after adjustment for baseline values. We found a treatment effect of 3.7 g/L (95% CIs: 1.6, 5.6 g/L; $P = 0.001$) in Hb concentration

TABLE 1
Composition of the mungbean dal and guava servings, as analyzed.

	Mungbean dal[1]	Guava[2]
Energy (kcal)	250 ± 5.2	36 ± 3.3
Protein (g)	14 ± 0.7	0.50 ± 0.2
Fat (g)	7.4 ± 0.2	0.3 ± 0.7
Carbohydrate (g)	52 ± 1.0	7.8 ± 0.1
Iron (mg)	3.0 ± 0.3	0.2 ± .04
Vitamin C (mg)	4.4 ± 1.3	171 ± 34
Phytic acid (mg)	234 ± 13.9	62 ± 5.9
Polyphenols (mg)	215 ± 11.4	20 ± 5.5
Calcium (mg)	142 ± 1.2	11 ± 1.1
Zinc (mg)	2.6 ± 0.25	0.2 ± 0.0

[1] Values represent means ± SD ($n = 13$), expressed per average weight of consumed mungbean dal of ~250 g, cooked with seasoning ingredients, corresponding to ~50 g of raw mungbean.
[2] Values represent means ± SD ($n = 15$), expressed per average weight of consumed guava fruit of ~75 g.

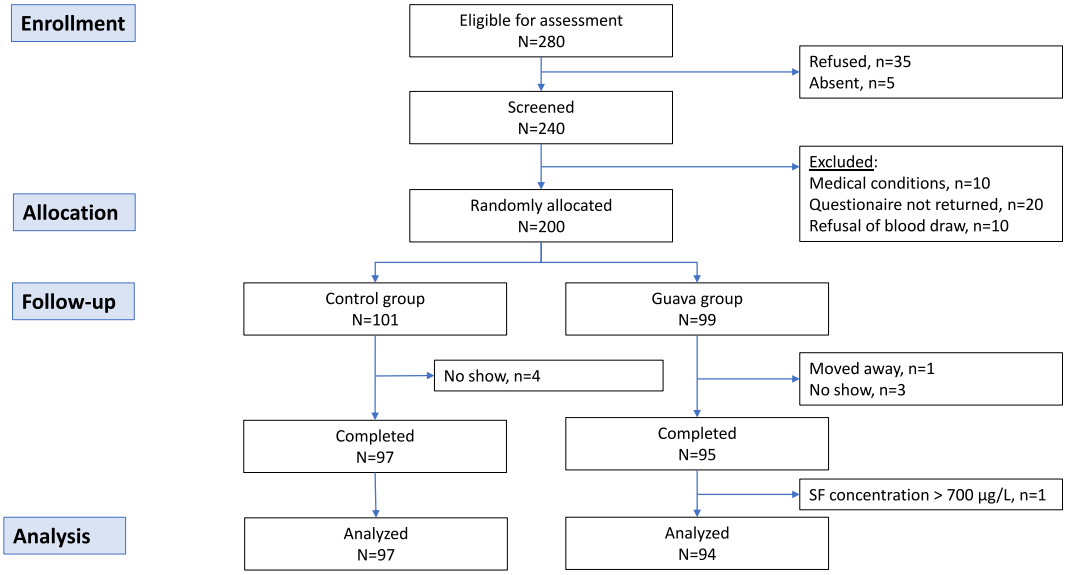

FIGURE 1. Flow diagram of recruitment, screening, enrolment, allocation, follow-up, and analysis of study participants.

TABLE 2
Baseline characteristics of school children, aged 6–10 y ($N = 193$), who were randomly allocated to receive a daily serving of mungbean dal (control group) or mungbean dal with guava fruit (guava group) for ~7 mo, 6 d/wk, in Hisar, India[1].

	Control group $N = 97$	Guava group $N = 94$
Age (y)	7.8 ± 1.0	7.7 ± 1.0
Female, *n* (%)	45 (46.3)	45 (47.9)
Height-for-age, Z-score	–1.24 ± 1.1	–1.15 ± 1.1
BMI-for-age, Z-score	–1.66 ± 1.0	–1.26 ± 0.8
Inflammation markers		
α_1-acid glycoprotein (g/L)	0.81 ± 0.2	0.83 ± 0.3
C-reactive protein (mg/L)	0.94 ± 2.8	1.10 ± 3.3
α_1-acid glycoprotein >1 g/L, *n* (%)	19 (19.6)	22 (23.2)
C-reactive protein >5 mg/L, *n* (%)	5 (5.2)	5 (5.3)

[1] Values are means ± SD, counts (%), or medians with 25th and 75th percentiles in parentheses.

after intervention. In line with this, the guava group showed a larger drop in the prevalence of anemia (−51%, 95% CI: −74, −10%; $P = 0.022$) and IDA (−56%, 95% CI: −83, 13%; $P = 0.087$) compared with control. No treatment effect on ID was observed.

A subgroup analysis was conducted by stratifying subjects for baseline ID or sufficiency (Table 4). Overall, treatment effects were larger in iron-deficient children compared with those who were iron sufficient at baseline. The mean difference in body iron stores between treatment groups among iron-deficient children was +0.93 mg/kg BW (95% CI: −0.17, 2.03 mg/kg BW; $P = 0.096$), whereas the treatment effect was negligible among children who were iron sufficient at study start (−0.26 mg/kg BW, 95% CI: −2.42, 1.91 mg/kg BW, $P = 0.813$). Iron-deficient children in the guava group also had 10% lower sTfR concentrations and a more pronounced increase in Hb concentrations compared with control, whereas no such effects were found for iron-sufficient children.

Discussion

We examined the efficacy of vitamin C (~171 mg per daily serving) from fresh guava consumed with a legume-based meal (containing ~3 mg iron per daily serving) in improving iron status of children in India. We found that consuming guava along with mungbean dal did not improve body iron stores and SF concentrations. However and perhaps more importantly, it did increase the concentration of Hb, and halved the prevalence of anemia and IDA in our study population. Treatment effects were more pronounced in children who were iron deficient at study start.

Enterocytes of the proximal small intestine absorb dietary non-heme iron in its reduced form via the divalent metal-iron transporter 1. Iron is subsequently carried into the circulation via ferroportin under the regulation of hepcidin, the systemic iron regulatory hormone, which in turn is increased by a negative feedback mechanism through transferrin iron saturation, inflammatory signaling, and iron stores [35]. Vitamin C supports iron dissolution in the gastric juice and enhances absorption by reducing Fe to the ferrous form, and by forming soluble Fe chelates that prevent it to precipitate [36–38]. Vitamin C is known to enhance the absorption of either native or fortified iron from foods in a dose-dependent way, specifically if consumed with meals containing polyphenols, phytic acid, and calcium [13,15,38]. The mungbean dal meal served in our study contained significant amounts of phytic acid (234 mg), polyphenols (215 mg), and calcium (142 mg), and only a moderate content of iron (~3 mg), which is therefore likely to be poorly absorbed [39,40]. Even though the amount of iron in the mungbean meals was moderate, we showed that consuming guava fruit along with it led to marked reductions in anemia and IDA in our study population.

Only few studies have experimentally investigated the effect of long-term consumption of vitamin C from natural sources on iron status indicators in children [41–43]. The results of these long-term intervention studies are largely consistent. Monárrez-Espino et al. [41] reported that consumption of 300 mL of natural guava juice (200 mg vitamin C) compared with placebo alongside the usual main meal for 10 wk resulted in increased concentrations of both Hb and plasma ferritin among anemic and iron-deficient Mexican children ($N = 28$) aged 6–9 y old [41]. A 5-mo cluster-randomized controlled trial with 25 g of either guava (56 mg vitamin C), banana, or cucumber added to a supplementary meal among 24–48 mo old Indian preschoolers ($N = 399$) with a high initial prevalence of anemia and ID also showed improvements both in Hb and iron status indicators in the guava group [42]. In both of these studies, the vitamin C to iron molar ratio was well above 4:1. Also, Egbi et al. [43] observed that Hb concentrations of school-aged Ghanaian children ($N = 150$), with moderate prevalence of anemia and ID, increased more when served a daily cowpea meal containing 3% of fish powder as a source of iron (13.3 mg) along with a drink rich in vitamin C (66 mg; vitamin C to iron ratio 1.6:1) for 6 mo compared with control; however, no difference in change of SF and iron stores was found between groups. Also, no improvement in Hb or iron status was seen when meals without fish powder (vitamin C to iron ratio 2.1:1) were served [40].

Studies in adult women showed less consistency [44–47]. Kandiah [44] showed a greater increase in Hb concentrations when daily serving tofu (6.73 mg iron) either with or without 250 mL of orange juice (303 mg vitamin C; vitamin C:iron ratio 14:1) for 1 mo to 14 nonanemic premenopausal, lacto-ovo vegetarian women in a crossover design, whereas no difference in SF concentration was found between treatments. In contrast, 16 wk of daily consumption of an iron-fortified breakfast cereal (16.6 mg iron) along with either vitamin C-rich kiwi fruit (164 mg vitamin C; vitamin C:Fe molar ratio, 3.7:1) or banana (no vitamin C) by nonanemic women with low Fe stores ($N = 69$) improved their SF and sTfR concentrations, whereas it did not notably impact their Hb concentrations [46]. Garcia et al. [45] served 500 mL of limeade (25 mg vitamin C) or placebo twice daily with usual meals (vitamin C:iron ratio ~10:1) for 8 mo to iron-deficient, mostly nonanemic Mexican women ($N = 36$), but did not observe any notable increases in Hb or in plasma ferritin compared with placebo. This may be attributed to a low amount of iron present in the meals, but this was not specified [43,44,46]. Lastly, a study in Indonesia ($N = 252$) showed that weekly supply to pregnant women of 600 g of fermented soybean (tempeh), 30 g of meat, 350 g of guava, 300 g of papaya, and 100

TABLE 3
Iron status indicators at baseline and at the end of study, and treatment effects (mean difference, 95% confidence intervals) after consuming daily meals (~7 mo, 6 d/wk) of mungbean dal with vitamin C-rich guava (guava group) compared with mungbean dal only (control group) by school children aged 6–10 y in Hisar, India.

	Control group $N = 97$	Guava group $N = 94$	*P* value
Body iron stores[1] (mg/kg BW)			
Baseline	1.12 ± 4.54	2.17 ± 4.33	
End of study	1.02 (0.26, 176)	2.01 (1.26, 2.76)	
Crude effect (mg/kg BW)	Reference	0.99 (–0.07, 2.04)	0.067
Adjusted effect[2] (mg/kg BW)	Reference	0.65 (–0.34, 1.63)	0.197
Serum ferritin[3] (μg/L)			
Baseline	22.2 (10.0, 38.1)	25.3 (13.7, 41.0)	
End of study	16.5 (14.0, 19.5)	18.8 (15.9, 22.3)	
Crude effect[4] (%)	Reference	14 (–12, 45)	0.282
Adjusted effect[2,4] (%)	Reference	12 (–14, 42)	0.353
Soluble transferrin receptor[3] (mg/L)			
Baseline	9.4 (7.0, 12.7)	7.8 (5.9, 11.3)	
End of study	8.3 (7.6, 9.0)	7.2 (6.6, 7.8)	
Crude effect[4] (%)	Reference	–15 (–27, –2)	0.019
Adjusted effect[2,4] (%)	Reference	–5 (–14, 2)	0.180
Hemoglobin[1] (g/L)			
Baseline	113 ± 11.4	115 ± 11.9	
End of study	121 (118, 123)	126 (124, 128)	
Crude effect (g/L)	Reference	5.1 (1.9, 8.2)	0.001
Adjusted effect[2] (g/L)	Reference	3.7 (1.6, 5.6)	0.001
Anemia[5], *n* (%)			
Baseline	46 (47.4)	42 (44.2)	
End of study	23 (23.7)	10 (10.5)	
Crude effect[6] (%)	Reference	–55 (–77, –11)	0.022
Adjusted effect[2,6] (%)	Reference	–51 (–74, –10)	0.022
Iron deficiency[7], *n* (%)			
Baseline	70 (72.2)	71 (75.5)	
End of study	83 (85.6)	82 (87.2)	
Crude effect[6] (%)	Reference	2 (–9, 14)	0.737
Adjusted effect[2,6] (%)	Reference	2 (–9, 14)	0.739
Iron-deficiency anemia[8], *n* (%)			
Baseline	39 (40.2)	29 (30.8)	
End of study	14 (14.4)	5 (5.3)	
Crude effect[6] (%)	Reference	–63 (–86, –2)	0.046
Adjusted effect[2,6] (%)	Reference	–56 (–83, 13)	0.087

Abbreviation: BW, body weight.
[1] Mean with 95% CI in parentheses.
[2] Effect of intervention adjusted for corresponding baseline values.
[3] Inflammation-corrected geometric mean with 95% CI in parentheses.
[4] Values indicate difference between groups, expressed as a percentage relative to the mungbean dal group, obtained by exponentiation of effect estimates from log-transformed data (all such values).
[5] Hemoglobin concentration <115 g/L.
[6] Values indicate percentage difference in prevalence as compared with mungbean dal (95% CI), obtained by conversion of prevalence ratios from robust Poisson regression.
[7] Inflammation-corrected serum ferritin <15 μg/L or soluble transferrin receptor >8.3 mg/L.
[8] Inflammation-corrected serum ferritin <15 μg/L or soluble transferrin receptor >8.3 mg/L, and hemoglobin concentration <115 g/L.

g of orange (vitamin C:iron ratio ~15:1) had no overall effects on Hb, SF, and body iron compared with control at the end of pregnancy, although improvements were seen in women who were iron deficient at study start [47].

Although all of these studies differed in study populations, study design, and method of analysis, a few aspects stand out. All studies in children were conducted in populations with a high prevalence of anemia and showed improvements in Hb, and sometimes also in iron status. Studies in adult women were mostly conducted in nonanemic women with more variable outcomes. A low vitamin C:iron ratio may however not be effective [43], as well as when usual iron intake was low and no extra iron was provided [45]. Also, iron stores take time to build up; hence, studies of short duration may not show improvements in iron status [44]. Collectively, however, these studies do support the hypothesis that, under circumstances of iron-deficient erythropoiesis and functional Fe deficit (low Hb), newly absorbed iron is preferentially used for erythropoiesis. This may be explained by the hypoxia-induced release of erythropoietin (EPO) by the kidneys, which stimulates erythropoiesis, and consequently iron demand [35]. Therefore, consuming vitamin C along with meals containing low or moderate amounts of iron, like in our case, would only improve iron stores (SF) when erythropoietic function (that is, Hb concentration) is restored. In

TABLE 4
Iron status indicators at end of study and treatment effects (mean difference, 95% confidence intervals) after consuming daily meals (~7 mo, 6 d/wk) of mungbean dal with vitamin C-rich guava (guava group) compared with mungbean dal only (control group) by school children aged 6–10 y in Hisar, India, stratified for ID status at baseline.

	Iron deficient at baseline[1]			Iron sufficient at baseline		
	Control group ($N = 70$)	Guava group ($N = 71$)	P value	Control group ($N = 27$)	Guava group ($N = 23$)	P value
Body iron stores[2] (mg/kg BW)	0.94 ± 3.96	2.35 ± 3.27		1.23 ± 3.28	0.94 ± 4.47	
Crude effect (mg/kg BW)	Reference	1.42 (0.21, 2.62)	0.022	Reference	–0.30 (–2.51, 1.91)	0.788
Adjusted effect[3] (mg/kg BW)	Reference	0.93 (–0.17, 2.03)	0.096	Reference	–0.26 (–2.42, 1.91)	0.813
Serum ferritin[4] (μg/L)	16.5 (13.5, 20.1)	19.4 (16.0, 23.5)		16.5 (11.8, 23.2)	17.2 (11.9, 24.9)	
Crude effect[5] (%)	Reference	17 (–12, 55)	0.251	Reference	4 (–59, 72)	0.879
Adjusted effect[3,5] (%)	Reference	14 (–15, 51)	0.336	Reference	3 (–61, 70)	0.918
Soluble transferrin receptor[4] (mg/L)	8.4 (7.4, 9.0)	6.7 (6.0, 7.4)		8.2 (6.7, 9.0)	8.8 (7.4, 9.9)	
Crude effect[5] (%)	Reference	–26 (–46, –9.1)	0.002	Reference	13 (–8, 37)	0.222
Adjusted effect[3,5] (%)	Reference	–10 (–21, –1)	0.029	Reference	3 (–12, 18)	0.717
Hemoglobin[2] (g/L)	119 ± 12.0[6]	126 ± 10.2		126 ± 8.35	126 ± 10.9	
Crude effect (g/L)	Reference	7.2 (3.5, 10.9)	<0.001	Reference	–0.7 (–6.2, 4.7)	0.793
Adjusted effect[3] (g/L)	Reference	3.8 (1.4, 6.2)	0.002	Reference	2.7 (–0.6, 5.9)	0.106

Abbreviations: BW, body weight; ID, iron deficiency.
[1] Inflammation-corrected serum ferritin <15 μg/L or soluble transferrin receptor >8.3 mg/L.
[2] Values are mean ± SD.
[3] Effect of intervention adjusted for corresponding baseline values.
[4] Values are inflammation-corrected geometric means (95% CI).
[5] Values indicate the difference between groups, expressed as a percentage relative to the mungbean dal group, obtained by exponentiation of effect estimates from log-transformed data.

our study, ID was driven more by high serum concentration of sTfR (56% of iron-deficient cases) than by low concentration of SF, indicating iron-deficient erythropoiesis. In line with this, a clear treatment effect on serum concentration of sTfR was shown for children who were iron deficient at study start (Table 4). Hence, adding vitamin C to the daily mungbean dal meal likely helped them to restore iron-dependent erythropoiesis [48].

In this study, fresh guava was chosen as source of vitamin C [13]. The vitamin C:Fe molar ratio in the guava group was 18:1, which is more than sufficient to exert a significant effect on iron absorption. Although green leafy vegetables, such as leaves of amaranth, drumstick, parsley, and cauliflower, contain considerable amounts of vitamin C and are available and consumed in North India, more than half of vitamin C is destroyed during ~15 min of cooking. Freshly consumed fruits are therefore more effective as a source of vitamin C and can easily be added to school meal programs on a cyclic basis. Other than guava, also aonla, karonda, ziziphus, strawberry, and papaya are fruits high in vitamin C that are available in North India, albeit taste, texture, price, and availability are constraints to their use in school meal plans. As an all-season fruit that is widely available and affordable, has a good storage life and a well-appreciated taste and texture, guava stands out in the North-Indian context.

In contrast to earlier suggestions [49,50], the current evidence shows that naturally derived vitamin C added to meals during prolonged periods of time can overcome food-related inhibition of iron absorption and improve functional iron status. This said, it cannot be excluded that compounds other than vitamin C in natural products may also exert effects on iron absorption (for example, non-provitamin A carotenoids, organic acids [16,51]) or hemoglobin formation (for example, pro-vitamin A carotenoids, B-vitamins [52,53]). Our finding of halving the prevalence of anemia among Indian children with a relatively simple measure as adding guava fruit to school meals is of utmost public health relevance and warrants follow-up.

The strength of the present research is that we measured the effect of long-term addition of vitamin C from a natural food source (guava) to a basic school meal on iron status in a study population with a high prevalence of anemia and ID. The large sample size, highly controlled intervention, comprehensive set of outcome measures, and robust data analysis allow strong inferences to be made. Although we did not measure serum ascorbic acid concentration, strict feeding supervision ensured compliance to the test meals throughout the study. The measurement of the vitamin C content of guava throughout the study showed natural seasonal variation in vitamin C content that may have had minor impact in view of the high overall vitamin C:Fe ratio of study meals. Hb concentration increased in both treatment groups over the course of the study, which may be attributable to provision of antihelminthic treatment to all children both at baseline and at midpoint. Intestinal helminths can have a strong impact on Hb concentrations and Fe status through occult gastrointestinal blood loss and interference with iron absorption [54]. Although we did not quantify parasitic infections in this study, children in India are reported to have a high prevalence of parasitic infections [9,11,12].

We conclude that daily consumption of a vitamin C-rich food source alongside a meal with a relatively moderate amount of native iron did not improve body iron stores of Indian school children but had a major impact on the occurrence of anemia. This underlines the importance of including vitamin C-rich foods in school meals. On the basis of this study and previous work, our findings can be generalized to school children living in similar settings of low dietary nonheme-iron intake, with diets high in iron absorption inhibitors, and a high prevalence of ID, anemia, and inflammation, such as in other rural areas in India, as well as large parts of South-East Asia and Africa.

Acknowledgments

We are grateful to the teachers of both schools: Pankaj, P. Sharma, Neelam, Rajbala, and Surekha. We thank I. de Jager, A. Nijhuis, K.E. Chagonda, S.M. Battung, A. Kueter, and A. Olie for their contribution during field work. We thank N.K. Khetarpaul, Khetarpaul Hospital, Hisar, India, for supervising and assisting biochemical analysis during the trial. We also thank the funding agencies: Department of Biotechnology, Ministry of Science and Technology, Government of India; Nutricia Research Foundation (grant no. 2010-17), The Netherlands; The Interdisciplinary Research and Education Fund (INREF) of Wageningen University through the Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition Project (TELFUN).

Author contributions

The authors' responsibilities were as follows – VR, IDB, DM, MBZ: designed the research; VR: conducted the research; VR, DM, NK, IDB: supervised fieldwork; PT: analyzed biochemical samples; VR, AMB: analyzed the data; VR, DM, PT, MBZ, AMB, IDB: interpreted the data; VR: wrote the first draft; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Funding

Department of Biotechnology, Ministry of Science and Technology, Government of India; Nutricia Research Foundation (grant no. 2010-17), The Netherlands; The Interdisciplinary Research and Education Fund (INREF) of Wageningen University through the Tailoring Food Sciences to Endogenous Patterns of Local Food Supply for Future Nutrition Project (TELFUN). The funding sources had no involvement in study design; collection, analysis, and interpretation of data; and writing of the manuscript; nor have they posed any restrictions regarding publication of study results.

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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