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Nutrition

Assessing the dietary intake of calcium, magnesium, iron, zinc and copper in institutionalised children and adolescents from Guatemala. Contribution of nutritional supplements

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ABSTRACT

In childhood and adolescence an adequate mineral intake is essential for normal growth and immune function, and to prevent chronic diseases in adulthood. The aim of this study was to analyse the dietary intake of Ca, Mg, Fe, Zn and Cu in children and adolescents from an orphanage-school in Guatemala and to assess the mineral contribution of nutritional supplements used by this population. Mineral content was analysed in nutritional supplements, bioaccesible fractions obtained after an *in vitro* gastrointestinal method, and diets sampled by a 7-day duplicate diet study. The average mineral content in the duplicate diets, including supplements, was (mg/d): Ca 452, Mg 230, Fe 25, Zn 8 and Cu 0.22. Especially Ca and Cu values were below recommended daily intakes. The content of these minerals in the nutritional supplements (mg/serving) was 49–112 for Ca, 1.2–3.8 for Fe, 24–47 for Mg, 0.7–4.16 for Zn and 0.08-0.23 for Cu. A great proportion of dietary minerals was provided by supplements, the contribution of which was: Ca 53.1%, Mg 58.4%, Fe 27.6%. Zn 82.2% and Cu 98.5%. Mineral bioaccessibility in supplements was particularly low for Ca and, to a lesser extent, for Fe and Zn. In spite of the high supplement consumption (up to 4 servings/d) important mineral deficiencies were detected in the diet of institutionalised children. Findings suggest that formulation and nutritional values of supplements used in Latin America should be carefully reviewed, in order to be a useful tool in the prevention and treatment of hidden hunger.

1. Introduction

Adequate mineral intake during infancy and adolescence is essential for normal growth and immune function, and to prevent chronic diseases in adulthood. Micronutrient deficiency, which may be due to low mineral content in the diet and/or low bioavailability, is often referred to as “hidden hunger”, and is prevalent in underdeveloped countries such as Guatemala, [1]. Nutritional interventions targeting reduced hidden hunger should include education, fortification [2] and supplementation, with supplements enriched with minerals being considered as the most appropriate intervention among children at risk of malnutrition [3].

Enriched mineral nutritional supplements contain minerals in different concentrations, and both the chemical form of the element and the composition of the supplements play an important role in determining their bioaccessibility [4]. Mineral bioaccessibility has been defined as the fraction of a mineral that is released from the food matrix in the gastro-intestinal tract, and thus the proportion of the mineral that becomes available for intestinal absorption [4]. Estimating the total mineral content in foods may be insufficient and mineral bioaccessibility should also be considered [4,5]. Several *in vitro* approaches have been developed in attempt to mimic the effects of the human digestion process; these methods are simple, rapid, inexpensive and easy to control [4,6].

Mineral status is essential in infant populations and micronutrient deficiencies are mainly related with impaired cognitive and physical performance [7]. According to the Food and Agriculture Organization/World Health Organization (FAO-WHO) recommendations [8,9], three basic approaches may be employed to assess the total dietary mineral intake: a) total diet studies, b) duplicate diet studies, and c) diary studies. The implementation of a duplicate diet study offers the advantage of providing more realistic exposure data for a particular population group since all foods are directly analysed “as-consumed”; in addition, its economic cost is adequate [10].

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The aim of this study was to determine the content of Ca, Mg, Fe, Zn and Cu and their bioaccessible fractions using an *in vitro* digestion method, in nutritional supplements widely consumed in Latin America. The contribution of these supplements to the total dietary mineral intake in Guatemalan institutionalised children, assessed by a 7-day duplicate diet study, was also evaluated.

2. Materials and methods

2.1. Chemicals

Standard solutions for Ca, Mg, Fe, Zn and Cu (Tritisol grade, from Merck, Darmstadt, Germany) were used in calibration. Bidistilled deionised water (Milli-RO 12 plus Milli-Q, Millipore, Bedford, MA) was used. All the chemicals were of analytical reagent grade. Nitric acid (65% v/v) and perchloric acid (37% v/v) (Merck) were used for sample mineralization. Lanthanum chloride (Merck) was used for Ca determination and ammonium molybdate (Merck) to precondition furnace tubes before Cu analysis. α amylase (A1031-5KU), pepsin (P7000), pancreatin (P1750) and bile salts (B8756) (Sigma-Aldrich, St. Louis, MO, USA) were used to simulate oral, gastric and intestinal digestion.

2.2. Instrumentation

A Perkin-Elmer 2100 atomic absorption spectrometer, an AS-800 autosampler and hollow cathode lamps (Perkin-Elmer, Norwalk, CT, USA) were used. Air-acetylene flame atomization was used to determine Ca, Fe, Mg and Zn, and electrothermal atomization to determine Cu. Pyrolytically coated graphite tubes (ref. B013-5653) and platforms (ref. B012-1092) were from Perkin-Elmer. Argon was used as internal gas. An Ultra Turrax homogenizer (Ika Labortechnik, China) was used for sample homogenization. All samples were subjected to a microwave-assisted mineralization procedure (Multiwave 3000, Anton Paar GmbH, Graz, Austria). A thermostatic water bath (Selecta, Barcelona, Spain), a pHmeter (Radiometer, Copenhagen N.V., Denmark) and a 5810-R centrifuge (Eppendorf AG, Hamburg, Germany) were used for the *in vitro* digestion method. An isoperbolic bomb calorimeter (PARR 1356, Biometa, IL, USA) was used for total energy determination in nutritional supplements.

2.3. Sampling strategies

Five nutritional supplements (S1-S5) enriched in minerals and made in Latin America were analysed. The S1-S4 are commercially prepared from soy flour and corn flour and consumed as "atol", adding water and sugar. The name "atol" refers to a pre-Hispanic beverage originally brewed with boiled corn and water. The S5 is made from texturised soy protein and is added to rice during food preparation as a substitute for meat.

For the duplicate diet study, a duplicate serving of all foods and beverages served for breakfast, mid-morning snack, lunch, afternoon snack and dinner, was obtained on seven consecutive days at a Guatemalan orphanage-school that provides full board to over 250 children and adolescents aged 4–18 years. These meals and supplements, described in Table 1, were the only foods and beverages consumed. Due to the impossibility of quantifying water consumption, this was not included. The food samples were subjected to a simulated eating procedure using normal knives and forks. The food items were sliced and the inedible parts discarded. The remaining parts were weighed and then homogenised.

All foods and beverages making up the diet were recorded and weighed, and transformed into energy and nutrient values using the Spanish Food Composition Tables and the Dietsource 3.0® software (Healthcare Nutrition S. A., Esplugues de Llobregat, Spain). Local products were included in the software program using the Food Composition Tables published by the Institute of Nutrition for Central

Table 1
Composition of the 7-day analysed diets. Nutritional supplements are indicated as S1-S5.

Meals	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Breakfast	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5	atol of S1, corn tortillas, black beans and rice cooked with margarine	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5	atol of S1, corn tortillas, black beans and rice cooked with margarine and S5.
Mid-morning	atol of S2	atol of S4	papaya	atol of S4	atol of S2	atol of S1	atol of S4
Lunch	corn tortillas, black beans and rice cooked with margarine	corn tortillas, black beans and rice cooked with margarine and S5	corn tortillas, black beans and rice cooked with margarine	cauliflower and broccoli cooked with margarine, corn tortillas, fried banana	corn tortillas, black beans and rice cooked with margarine	corn tortillas, roast beef and cooked rice with beans and margarine	corn tortillas, black beans and rice cooked with margarine and fried banana
Afternoon	watermelon	banana and lime juice	watermelon	atol of S3	watermelon	papaya	watermelon
Dinner	corn tortillas, black beans and rice cooked with margarine	corn tortillas, cucumber with lime and cooked rice with tomato sauce	corn tortillas, black beans and rice cooked with margarine	baked potato breaded with egg, rice cooked with tomato sauce and corn tortillas	corn tortillas, chicken fried in soybean oil and pasta with tomato sauce and cilantro	corn tortillas, potato and rice cooked with black beans and margarine	corn tortillas, grilled fish* and rice cooked with margarine

* Tilapia (river fish).

Table 2
Characteristics of the children and adolescents of the school-orphanage.

	% from total	Body mass (kg)	Height (cm)	BMI (kg/m^2)
Females	59			
Males	63			
4-8 y	29	18.8 ± 3.4	110.4 ± 9.0	15.5 ± 0.7
9-13 y	48	31.9 ± 6.7	135.0 ± 8.9	17.2 ± 1.4
14-18 y	23	50.9 ± 9.7	143.1 ± 45.4	20.3 ± 2.5

Data are means of 240 subjects ± SD; BMI: Body Mass Index.

America and Panama [11]. Nutritional composition of supplements was entered into the software program using the results obtained in the present assay.

Description of the main anthropometric characteristics of the institutionalised participants and distribution according to age are shown in Table 2. Prior permission was obtained from the orphanage-school authorities to conduct this study and details of the procedures involved were explained. The study protocol was approved by the Ethics Committee of the University of Granada (Spain).

2.4. In vitro digestion

The *in vitro* gastrointestinal digestion of supplements was carried out as reported by Mesías et al. [12] modified to include a previous oral step. Shortly before use, an α-amylase solution was prepared by dissolving 32.5 mg of α-amylase in 25 mL of 1 mM KCl (pH = 7).

For gastric digestion, 0.8 g of pepsin was dissolved in 5 mL of 0.1 mol/L HCl and for the intestinal step, 0.1 g of pancreatin and 78 mg of bile salts were dissolved in 25 mL of 0.1 mol/L $NaHCO_3$. One gram of each sample was suspended in 10 mL of doubly distilled deionised water and 250 μL of the α-amylase were added. The mixture was incubated at 37 °C for 30 min. Then, pH was adjusted to 2 with 6 N HCl, the pepsin solution was added at a proportion of 0.05 g of pepsin/g of sample, and samples were incubated at 37 °C in a shaking water bath at 110 oscillations/min for 2 h for the gastric digestion. For the intestinal digestion, the pH of the digest was raised to 6 with 1 mol/L $NaHCO_3$, and 2.50 mL of pancreatin/bile salts mixture was added. The pH was then adjusted to 7.5 with 1 mol/L $NaHCO_3$, and samples were incubated for 2 h at 37 °C and 110 oscillations/min. Samples were centrifuged at 3200 *g* for 60 min at 4 °C to separate the soluble fraction, which was reserved until analysis.

2.5. Chemical analysis

All analyses were performed in triplicate. Mineral analyses were carried out in supplements, bioaccessible fractions and diets. Previously, aliquots of 0.4 g of homogenised samples were microwave-mineralised with HNO_3 and $HClO_4$. The microwave oven was programmed at 1400 W and 80 bar as power and pressure limits. The mineralised solutions were diluted with doubly distilled deionised water, and Ca, Mg, Fe, Zn and Cu determined according to analytical conditions which had been previously optimised [13–15]. Accuracy of the method was corroborated using external reference certified standards: skimmed milk powder (certified reference material CRM 063R, Community Bureau of Reference, Brussels, Belgium) for Ca and Mg; typical diet (standard reference material SRM 1548a, National Institute of Standards and Technology, Gaithersburg, MD, USA) for Cu, Fe and Zn. Certified values were (mean ± SD): Ca 13.49 ± 0.10 mg/g; Mg 1.263 ± 0.024 mg/g; Cu 2.32 ± 0.16 mg/kg; Fe 35.3 ± 3.77 mg/kg; Zn 24.6 ± 1.79 mg/kg. Measured values (mean ± SD, n = 10) were: Ca 13.47 ± 0.07 mg/g; Mg 1.28 ± 0.020 mg/g; Cu 2.30 ± 0.08 mg/kg; Fe 36.9 ± 2.12 mg/kg; Zn 25.1 ± 0.99 mg/kg. Paired *t* tests showed good agreement between the certified and the obtained values at a significance level of 0.05%. Sample pools of supplements and diets were used as an internal control to assess analytical precision. The inter-assay coefficients of variation (CV%) were: Ca 5.02, Mg 4.73, Fe 3.21, Cu 6.90, Zn 2.03.

Analysis of energy and nutrients of nutritional supplements followed standard procedures described by AOAC [16]. Total energy was measured in an isoperbolic bomb calorimeter, total N by the Kjeldahl procedure, total fat by the Soxhlet method [16] and carbohydrate content by difference.

2.6. Calculations

In the nutritional supplements, using the data obtained for total mineral content (TC) and soluble fraction (S), the bioaccessible fraction was calculated as follows: bioaccessibility (%) = S/TC × 100. Taking into account the average daily mineral content of the diet (MD), and the mineral provided by the supplements consumed each day (MS), we calculated the contribution of nutritional supplements to the total intake of each mineral (TDI), expressed as percentage, as follows: TDI (%) = MS/MD × 100.

2.7. Statistical analysis

Data were interpreted using the statistical software package SPSS version 22.0 (SPSS, Inc., Chicago, Illinois, USA) for Windows. Results are expressed as the arithmetic mean and standard deviation, or median and range.

3. Results

3.1. Content and bioaccessibility of Ca, Mg, Fe, Zn and Cu in nutritional supplements

Mineral content of supplements (according to manufacturer notes and as analysed in the present study) and percentage of bioaccessibility calculated using vitro digestion are summarised in Table 3.

Information on mineral content was not completely listed in the label of supplements with only Ca, Fe and Zn being provided. In general, analysis suggested a higher or similar content of minerals than those labelled, with the only exceptions being Fe and Zn in S4 and in S5.

Mean Ca concentration ranged from 325 mg/100 g to 600 mg/100 g (S5 and S4 respectively). Following recommended servings, a contribution of ≈ 100 mg Ca/serving of supplement was derived, except for S4 (49 mg Ca/serving). Ca bioaccesibility of the supplements varied from 14% to 21.5%. Mg concentration was between 128 mg/100 g and 315 mg/100 g, contributing 24 mg /serving - 59 mg /serving. The bioaccessible Mg fraction ranged from 43% to 57% from the initial total content. Large variations were observed for the content of trace elements in supplements and were as follows: Fe 8 mg/100 g - 20.2 mg/100 g, Zn 4.6 mg/100 g - 22.2 mg/100 g and Cu 410 μg/100 g - 1515 μg/100 g. Moreover, the bioaccessibility after in vitro digestion greatly differed between the supplements analysed (from 5% to 38% for Fe, 10%–46% for Zn, and 41%–82% for Cu). Average values for bioaccessibility were lowest for Fe (14%), rising in Ca (18%), through Zn (22%), to Mg (50%) and highest in Cu (64%).

3.2. Energy and nutrient content of nutritional supplements

Results from analysis of supplement content are depicted in Table 4. All the supplements presented high values for energy content, around 430 kcal/100 g, mainly due to their elevated concentration of carbohydrates (between 60 g/100 g and 81.6 g/100 g). The supplements were also good sources of protein, especially the S5 (54.1 g/100 g), and showed low content of fat.

3.3. Ca, Mg, Fe, Zn and Cu in the diets. Contribution of supplements

Composition of the diet (Table 1) was in accordance with the typical

Table 3
Mineral content in the nutritional supplements and in bioaccessible fractions (BF) obtained after *in vitro* digestion.

Mineral	Supplement				
	S1	S2	S3	S4	S5
Labelled[a] (mg/100 g)					
Calcium	373	305	305	554	333
Iron	14.9	14.9	14.9	17	7
Zinc	16	16	16	8.5	5
Analysed					
Calcium					
mg/100 g	519 ± 12.0	493 ± 5.9	489 ± 2.5	600 ± 11.0	325 ± 12.0
mg/serving[b]	97.0 ± 2.25	92.0 ± 1.1	91.0 ± 0.46	112.0 ± 2.0	49.0 ± 2.25
BF (%)[c]	19.0 ± 0.4	21.5 ± 1.8	18.0 ± 1.2	14.0 ± 1.4	15.0 ± 1.5
Magnesium					
mg/100 g	248 ± 19.0	132 ± 3.1	128 ± 2.6	246 ± 5.8	315 ± 25.0
mg/serving	46.4 ± 3.6	24.7 ± 0.7	23.9 ± 0.5	46.2 ± 1.1	59.1 ± 4.7
BF (%)	43.0 ± 1.2	57.0 ± 1.9	55.0 ± 2.3	45.0 ± 0.8	49.0 ± 1.0
Iron					
mg/100 g	17.1 ± 0.3	20.2 ± 1.02	19.0 ± 0.46	9.0 ± 0.18	8.0 ± 0.05
mg/serving	3.19 ± 0.05			1.68 ± 0.33	1.2 ± 0.05
BF (%)	13.0 ± 0.09	3.78 ± 0.22	3.56 ± 0.08		38.0 ± 0.5
		5.0 ± 0.1	5.2 ± 0.3	8.0 ± 0.4	
Zinc					
mg/100 g	18.7 ± 1.6	22.2 ± 3.5	22.2 ± 1.3	6.9 ± 0.3	4.6 ± 0.3
mg/serving	3.48 ± 0.3	4.16 ± 0.6	4.15 ± 0.2	1.3 ± 0.06	0.7 ± 0.06
BF (%)	18.0 ± 0.8	12.0 ± 0.3	10.0 ± 0.4	22.0 ± 0.13	46.0 ± 0.3
Copper					
µg/100 g	718 ± 40	421 ± 25	410 ± 15	729 ± 11	1505 ± 10
µg/serving	131 ± 7.3	79 ± 4.8	77 ± 2.8	134 ± 2.1	281 ± 2.1
BF (%)	68.0 ± 0.9	41.0 ± 1.8	57.0 ± 1.5	73.0 ± 2.4	82.0 ± 3.0

[a] Only values of Ca, Fe and Zn content were given in the label by the manufacturer.
[b] Recommended serving for nutritional supplements 18.75 g for S1-S4 and 15 g for S5.
[c] BF was calculated as the soluble fraction (%) from the total mineral content obtained after *in vitro* digestion.

diet of the population group studied, i.e. rice, black beans and "tortillas" made with corn meal. The consumption of eggs, meat or fish (river fish) was very limited (1–2 times per week), and the intake of dairy products was negligible. The nutritional supplements were consumed at a rate of 2–4 servings/d. The overall daily contribution of energy in the 7-day diet was 2012 kcal. The energy distribution of macronutrients was 69% carbohydrates, 12% proteins and 18% fat. Taking into account the g/serving of each supplement (18.75 g for S1-S4 and 15 g for S5) and the serving/day consumed (Table 1), it was calculated that supplements provided on average 11% energy, 25.2% protein, 3.6% fat and 10.6% carbohydrates of the total daily intake.

Table 5 shows the content of Ca, Mg, Fe, Zn and Cu in the menus analysed daily from the duplicated diet. For comparative purposes, the dietary reference intakes (DRI) according to the Food and Nutrition Board of the American Institute of Medicine [17] are also provided in the Table. The most serious deficiency was observed for Ca, with a mean dietary daily content of 452.4 mg, which is well below nutritional recommendations. The Mg diet content, 300 mg/d, was within recommended levels for children of less than 13 years of age. With regards to microelement content, Fe content was typically higher than levels recommended (25 mg/d), levels of Zn were adequate (ranging between 5.7 mg/d and 12.4 mg/d) and Cu content was low, though results depended on the age of the child being considered (from 117.8 µg/d to 460.5 µg/d).

With regards to the aim of studying the role of nutritional supplements in the mineral content of the diets, contribution of each mineral to the total content was calculated as a percentage. Results are shown in Fig. 1. The supplements contributed in a similar manner to the total Ca and Mg intakes (53.1% and 58.4%, respectively), providing more than half of the dietary content. Fe provided through supplementation represented 27.6% on average of the total intake, whereas the majority of Zn and Cu of the total diet was found to be supplemental (82.5% and 98.5% of Zn and Cu, respectively).

4. Discussion

The Guatemalan diet has been characterised as being low in density, content and bioavailability of micronutrients [2]. This is worrying, particularly among Guatemalan children and adolescents [1] as inadequate nutrition at this stage may have adverse effects on health, both at present and in the future [18]. To address this problem, food fortification and nutritional supplements of vitamins and minerals are

Table 4
Energy and nutrient content of the nutritional supplements.

	Supplement				
	S1[a]	S2[a]	S3[a]	S4[a]	S5[b]
Energy (kcal/100 g)	440 ± 2	414 ± 3	415 ± 7	446 ± 2	457 ± 1.5
Protein (g/100 g)	24.0 ± 0.1	14.7 ± 0.7	15.3 ± 0.3	24.0 ± 0.8	54.1 ± 0.2
Fat (g/100 g)	3.73 ± 0.06	3.24 ± 0.05	3.43 ± 0.01	4.18 ± 0.08	0.26 ± 0.01
Carbohydrates (g/100 g)	77.1 ± 1.5	81.6 ± 2.1	81.0 ± 0.6	76.5 ± 1.0	60.0 ± 0.9

[a] Recommended serving according to the label: 18.75 g.
[b] Recommended serving according to the label: 15 g.

Table 5
Daily contents of calcium, magnesium, iron, zinc and copper in the diet of institutionalized children and adolescents from Guatemala[a]. Comparison with recommended intakes.

Mineral	Median	Range	Reference dietary intake[b] *vs* age (years)				
			M/F[c] 4-8	M 9-13	F 9-13	M 14-18	F 14-18
Calcium (mg)	448.4	387.1–505.7	800	1100	1100	1100	1100
Magnesium (mg)	220.1	135.9–300.2	110	200	200	340	300
Iron (mg)	24.2	19.5–27.1	4.1	5.9	5.7	7.7	7.9
Zinc (mg)	8.5	5.7–12.4	4	7	8.9	7	7.3
Copper (μg)	249.8	117.8–460.5	340	540	685	540	685

[a] Data from a7-days duplicated diet sampled for 24 h periods, and referred to fresh matter.
[b] Source: Food and Nutrition Board of the American Institute of Medicine (FNB-IOM, 2018).
[c] M, male; F, female.

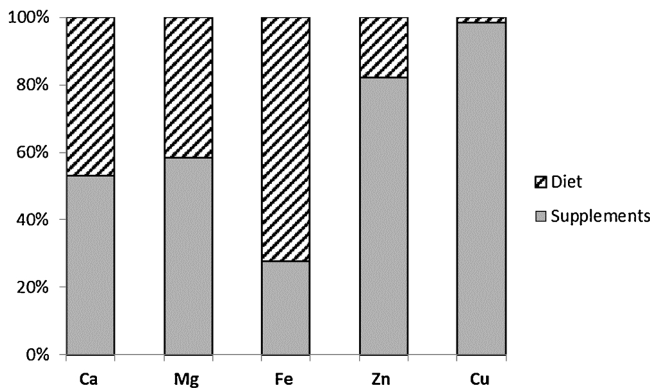

Fig. 1. Mean contribution of diet and supplements to the total daily intake of Ca, Mg, Fe, Zn and Cu in Guatemalan institutionalised children, expressed as a percentage (%).

strongly recommended [2]. Since quality assessments of the whole diet can be difficult, particularly in children [19], in this study we analysed the mineral content of the diet of an orphanage-school of Guatemala using the duplicate diet method. This provides a more accurate analysis of diet content as consumed foods are directly analysed [1]. In addition, the contribution and bioaccessibility of nutritional supplements widely used within the population of interest was also assessed.

The 2015–2020 Dietary Guidelines Advisory Committee (DGAC) confirmed that several nutrients, such as magnesium, iron and calcium, were “shortfall nutrients”, i.e. under consumed relative to recommendations of the Institute of Medicine, and that deficiencies were of a public health concern since they are linked to adverse health outcomes [20].

Calcium intake during childhood and adolescence is critical for developing good bone density which is necessary for skeletal consolidation and preventing fractures and osteoporosis in old age [21]. The Ca content reported in the analysed diets (Table 5) was 41%–57% of the DRI, thus indicating severe dietary deficiency. Ca consumption lower than that recorded in the present study has been observed among children and adolescents in developing countries, such as the Dominican Republic [22] and India [23]. In Morocco, calcium deficiency has also been reported for 85.5% of school children, with a mean intake of 522 mg/d [24]. The situation in developed countries appears to be completely different. Data from the NHANES program in the USA reports a mean Ca intake of between 971 mg/d to 1057 mg/d in children aged 2–18 years [25]. Adequate values of Ca consumption have also been found amongst Spanish, French and English children and adolescents [19,21]. Low Ca dietary content in the present study may be due to the lack of dairy products, which usually contribute up to 70% of the daily Ca intake [21]. According to O’Neil et al. [24], milk is the top food source of Ca in children of all age groups, followed by cheese, flavoured milk and pizza. In our study, however, 53% of Ca intake was provided by supplements, which in turn, had low bioaccessibility (Table 3), especially in comparison to other foods such as dairy products (63.5%) [5]. The high content of corn meal and soybean meal in the supplements, rich in fibre and phytic acid, could adversely affect the Ca availability. Bioaccessibility of foods typically found in Spanish school meals can range from 1.7% to 96.2% [26], while, mean Ca solubility of whole diets after in vitro digestion is 10%–15% [27]. These authors also highlighted the negative effect of fibre and phytates on Ca bioaccessibility. We therefore consider that an intervention focused on increasing Ca intake through dairy products should be promoted in this population group. Dairy foods, moreover, could contribute to greater intake of other nutrients whose low consumption currently presents a public health concern, for example vitamin D and potassium [25].

Average daily intake of Mg consumed through the diet (230 mg) met recommended intake for children aged 13 years and younger, and was 68%–87% of the DRI for those aged 14–18 years. Whole grains, nuts, legumes and dark green vegetables are some of the best sources of dietary Mg, and need to be consumed on a regular basis to ensure an adequate intake is achieved. Nuts do tend to form part of the regular diet of the population studied but insufficient quantities are consumed to meet Mg needs. Similar Mg intakes to those identified in the present study have been reported among 12–14 year old children in Turkey (221 mg/d -248 mg/d) [28] and children and adolescents aged 2–18 years from the USA (237 mg/d - 281 mg/d), although intakes were increased to 377 mg/d when supplements were used [29]. Mediterranean dietary patterns have been found to increase magnesium intake among Spanish adolescents compared with diets distinct from the Mediterranean diet [30]. According to data from the present study, adequate intake of Mg was achieved through the consumption of supplements, which contributed 58.4% to the total dietary daily content (Fig. 1). In addition, Mg bioaccessibility of supplements (43%–57%) could be considered as good, as was similar to that found in dairy products [15] and within the range found for nutritional supplements used in the UK (33%–95%) [4]. Other studies [31] reported Mg bioaccessibility from dairy-based infant formulas in the range of 24.5%–26.6 % and a mean bioaccessibility of 35% from infant formulas with hydrolysed proteins.

Iron deficiency is considered the most common and widespread nutritional deficiency in the world, affecting most seriously women and children [32]. The bioavailability of Fe, rather than the total dietary quantity, appears to be an important determinant of iron status [33]. The Fe content of the menus evaluated (mean of 25.3 mg/d) was higher than the DRI (4.1 mg/d - 7.9 mg/d) (Table 5) and the RDA (8 mg/d - 15 mg/d) for all age groups and for both sexes. Studies performed among similar population groups from India [23], Vietnam [34] and Morocco [35] have reported lower Fe intakes. The menus analysed by Zimmermann et al. [35] in Morocco, based almost exclusively on cereals and legumes, showed a suitable content of 10.8 mg Fe/d, 97% of which was non-heme Fe and with a low bioavailability, estimated at

1.0%–4.3% of total Fe. The low Fe bioavailability from legume and cereal-based diets has been proposed as the main cause of Fe anaemia in rural Africa and India [23,35]. A study performed among Guatemalan children from public and private schools [2] detected Fe intakes of between 11.4 mg/d and 18 mg/d, of which 21.1% was provided by ready-to-eat cereals which are completely absent in the diet of the children at the orphanage school in the present study. Thus, strong differences seem to exist across socioeconomic classes, in spite of the rapid food culture change that is occurring in Guatemala [2]. Three dietary factors have been proposed to limit iron absorption: quantity of iron, quality of iron, and the composition of the diet [32]. In the present diet, the majority of the dietary Fe was non-heme, which is known to be absorbed less efficiently (3%–8%) than heme iron (23%) [36,37], although the absorption of non-heme iron may vary up to 10-fold depending on enhancing or inhibiting factors such as ascorbic acid or tea consumption [38]. Fe bioaccessibility in supplements varied between 5% and 13% in S1-S4, this is similar to that found in European nutritional supplements (3%–14%) [4] but, surprisingly, increased to 38% in S5. The S5 supplement has higher in protein content (54 g/100 g) than the other supplements (15 g/100 g - 24 g/100 g) and, according to the manufacturer, is formulated with texturised soy protein, which significantly reduces phytic acid [39], thus enhancing Fe availability. In a Western-style diet including vegetables, fruits, meat and fish, Fe bioavailability is expected to be about 15% [10]; in a diet based on cereals and legumes that are high in phytic acid, which is the main inhibitor of Zn and Fe absorption, the Fe bioavailability Fe could be reduced to 5%–10% [40].

Zinc and copper are two important trace elements in human health, as they are cofactors for more than 100 enzymes, including those involved in the synthesis of connective tissue and sexual maturation [41]. Adequate Zn dietary content (8.1 mg/d) was observed in the present study (Table 5), although this represents lower than RDA for males aged 14–18years (11 mg/d) [17]. However, a large proportion (82.5%) was provided by supplements, since the main food sources of Zn, namely seafood, red meat, dairy products, eggs and cereals, were very scarce in the diet provided at the orphanage school. The fish occasionally included in the meals was tilapia, which has a Zn content of 5 mg/kg - 10 mg/kg [40]. It has been reported that children and adolescents from developing countries, such as India or Iran, may be at risk of Zn deficiency because of unwholesome food habits and the poor bioavailability of Zn from plant-based diets [23,41]. In the same sense, Goshima et al. [42] have reported that many Japanese children are deficient in Fe and Zn compared with dietary reference intakes. These authors used the duplicate-portion technique (3 days) to evaluate the intakes of Fe, Zn and Cu in Japanese preschool children and obtained average values of 3.1 mg/d, 4.0 mg/d and 0.45 mg/d, respectively. Laillou et al. [34] described a high prevalence of Zn deficiency in young children in Vietnam and remarked that the poorest groups among the population with a rice-based diet were at a higher risk of Zn deficiency. In Guatemala, a Zn fortification program based in fortified milk and cereal-based milk was implemented among school children, who increased Zn intakes from 6.2 mg/d up to 12.2 mg/d after 6 months of supplementation [43]. Other trials have demonstrated the efficacy of food fortification and dietary interventions in reducing Zn deficiency in children from Vietnam and Kenya [34,44].

In the present study, Zn bioaccessibility in nutritional supplements (10%–46%) was comparable to values of 3%–35% found by Tokalıoğlu et al. [4], although higher values (22.6%–93%) have been found after in vitro digestion of Spanish meals [26]. The highest percentage of Zn solubility observed in S5 may be related with its low Fe content, as it is known that there is a competitive effect between both minerals [45]. The negative effect of high dietary phytate content on Zn bioaccessibility has also been discussed [26]. According to Kawade [23] inadequate dietary intake of absorbable Zn is the primary cause of Zn deficiency in Indian children.

Low dietary content was observed for Cu, especially when compared with RDA values of 440–890 μg/d for children and adolescents [17]. Higher values of Cu intake are usually observed in several countries, such as Iran (1530 μg/d) [40], Spain (1200 μg/d) [46] or Italy (980 μg/d) [47]. In the USA, where dietary supplements are widely consumed, Cu intake increased from 900 μg/d in non-users to 2900 μg/d in users, thus exceeding the tolerable upper intake [29]. In Western diets, cereals contribute the majority of dietary Cu, followed by meat, fruit and dairy products [46], but these foods were barely consumed by the population of the present study. In the present study, the majority of the dietary Cu was provided by supplements, which was found to be highly available (47%–82%). Tokalıoğlu et al. [4] also reported a high percentage of Cu bioaccessibility in nutritional supplements (27%–66 %). Cámara et al. [26] reported a Cu soluble fraction ranging from 35.7% to 92.3% in Spanish school meals. In spite of the good bioaccessibility of the supplemental Cu, the low content observed in the analysed diet may lead to Cu deficiency, which has been related with anaemia, neutropenia, osteoporosis, skeletal abnormalities, cardiac disorders and impaired growth and development [26,41,46].

5. Conclusions

The results obtained from our duplicate diet study, the most reliable method to evaluate dietary mineral intake, reveal mineral deficiencies, particularly concerning Ca and Cu, in the diet of the children boarding at orphanage schools and participating in the present study. Nutritional supplements were widely consumed and contributed to more than half of the total mineral daily intake. In addition, the mineral bioaccessibility after in vitro digestion of supplements was especially low for Ca and Fe. Therefore, the provision of minerals by these supplements may be questioned and their formulation should be reviewed. The present findings help to evaluate the effectiveness of these supplements in children at risk of malnutrition or hidden hunger, and may be useful to design new nutritional strategies for similar population groups. Finally, children should receive appropriate nutritional education which teaches them to follow healthy balanced diets throughout their development and into adult life. Further, it is recommended that high dependence on supplements should be avoided.

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Conflict of interest

The authors declare no conflict of interest.

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