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Calcium, iron, zinc and copper transport and uptake by Caco-2 cells in school meals: Influence of protein and mineral interactions

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Abstract

A combined in vitro digestion/Caco-2 cell culture system is used to estimate calcium, iron, zinc and copper transported and cell uptake (retention plus transport) corresponding to 8 dishes usually distributed to a Spanish school lunchroom, with an evaluation of the influence of proteins and mineral interactions. Mineral uptake percentages were as follows: Ca (3.3–56.3), Fe (7.8–67.4), Zn (5.6–54.9), Cu (14.6–96.6). The protein content of the menus analyzed (22.9–162.9 mg/g) exerts a positive influence upon iron uptake ($r = 0.938$), and a negative influence upon calcium uptake ($r = -0.755$) – with no influence upon the uptakes of either Zn or Cu. Mineral interactions are observed at dietary concentrations in the school menus studied. A negative and positive interaction is seen between soluble iron after in vitro digestion and Zn transported ($r = -0.733$) and Cu retention ($r = 0.800$), respectively. Solubilized Zn exerts a negative influence upon iron retained ($r = -0.831$).

Keywords: School meals; Bioavailability; Minerals; Caco-2

1. Introduction

Most school age children and adolescents have their main meal of the day in the school lunchroom, and this meal often constitutes the principal mineral contribution to the daily recommended intake (Cámara, Amaro, Barberá, & Clemente, 2005). The childhood diet must be adequate to support normal growth and development, and appropriate amounts of minerals are required since a deficient intake of certain minerals can produce diseases and lead to abnormal development. Mineral deficiency is usually caused by a low mineral content in the diet when rapid body growth is occurring and/or when there is poor absorption of minerals from the diet (Favier, 1993). In this way, not only must the absolute amounts of minerals be increased in the edible portions of foods, but these minerals must also be in forms that are bioavailable to the person consuming them.

As an alternative to human and animal studies, the availability of minerals from dishes and composite diets could be estimated, based on in vitro systems that usually consist of simulated gastrointestinal digestion, followed by the measurement of mineral solubility (Cámara et al., 2005). These procedures measure only the absorbed mineral, and are unable to assess subsequent mineral absorption and utilization. In vitro models of a cell culture (Caco-2) grown on solid or microporous supports, allowing the estimation of mineral uptake and transport, improve the system (Ekmekcioglu, 2002) and have been applied to different individual foods though not to composite dishes.

Mineral bioavailability is influenced by other food components which play an important role in the absorption within the intestinal lumen. Among them, the importance

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of proteins has been shown with in vivo studies (Baech et al., 2003; Sandström, 1992) as well as in vitro studies (Glahn, Wien, Van Campen, & Miller, 1996; Mulvihill & Morrisey, 1998) for improving iron and zinc bioavailability. Also of note are the interactions among the different minerals at the absorption site, since it is thought that some metallic ions, with similar physicochemical properties could compete for the same cellular transport systems. Thus, an excess in the concentration of one of them, could lead to a reduction in the absorption level of some other element. In this sense, calcium–zinc (Larsen & Sandstrom, 1992), calcium–iron (Hallberg, Rossander-Hulthen, Brune, & Gleerup, 1992; Yan et al., 1996), zinc–iron (O' Brien, Zavaleta, Caulfield, Yang, & Abrams, 1999) and zinc–copper mineral interactions (August, Janghorbani, & Young, 1989; Bertolo, Bettger, & Atkinson, 2001a) have been reported.

Given the importance of an adequate intake of minerals in childhood, and considering that school menus represent 30–35% of the total daily intake (Griffith, Sackin, & Bierbauer, 2000), the aim of the present study was to evaluate mineral retention, transport and uptake (retention plus transport) by Caco-2 cells of calcium, iron, zinc and copper of school meals, as well as the effect of proteins and mineral interactions. Finally, an attempt has been made to rank the mentioned dishes according to their values as dietary sources of calcium, iron, zinc and copper.

2. Materials and methods

2.1. Materials

Digestive enzymes and bile salts were supplied by Sigma Chemical Co. (St. Louis, MO, USA). The working solutions of these enzymes were prepared immediately before use.

Pepsin solution was obtained by dissolving 1.6 g of pepsin (P-7000 from porcine stomach) in 10 ml of HCl (0.1 M). The solution of pancreatin and bile salts was prepared by dissolving 0.4 g of pancreatin (P-170 from porcine pancreas) and 2.5 g of bile salt (B-8631 of porcine origin) in 100 ml of 0.1 M $NaHCO_3$.

Standard Ca, Fe, Zn and Cu solutions were prepared immediately before use by dilution (with distilled deionized water) of a 1000 mg/l standard solution (Titrisol, Merck, Darmstadt, Germany). The lanthanum solution (5 g/100 ml) was prepared from La_2O_3 (Merck).

All reagents used were of analytical grade, and Millipore-MilliQ distilled deionized water was used throughout. Glass and polyethylene material was soaked in HNO_3 (sp. gr. 1.38) for 15 min and then rinsed three times with distilled deionized water.

2.2. Samples

Eight dishes habitually included in the monthly programme of the school menu, with different formulations and forms of preparation were supplied by a catering establishment. Ingredients of dishes with protein contents are shown in Table 1.

Aliquots of dishes were frozen in a still-air freezer at −18 °C until required for analyses and processing.

2.3. Simulated digestion

The method described by Cámara et al. (2005) was applied. Briefly, 30 g of each dish were homogenized with 70 ml of deionized distilled water, and the pH was adjusted to 2.0 with 6 N HCl. After 15 min the pH value was checked, and if necessary was readjusted to 2. To carry out pepsin–HCl digestion, 0.5 g of pepsin solution per 100 g of sample was added. The mixture was then incubated for 2 h at 37 °C in a shaking water bath.

Prior to the intestinal digestion step, the pH of the gastric digests was raised to 5 by dropwise addition of 1 M $NaHCO_3$. Then 18.8 ml of the pancreatin–bile salt mixture was added and incubation continued for an additional 2 h. To stop intestinal digestion, the sample was maintained for 10 min in an ice bath. The pH was adjusted to 7.2 by dropwise addition of 0.5 M NaOH.

The intestinal digest was heated for 4 min at 100 °C to inhibit the sample proteases and was then immersed in an ice bath to cool. Aliquots of 20 g of the digested sample were transferred to polypropylene centrifuge tubes (50 ml,

Table 1
Description of the dishes and protein contents

Dish	Formulation/preparation	Protein content (mg/g)
Cuban style rice	Rice[a], chicken broth, fried tomato[a], sausage[a], sunflower oil, garlic and salt	22.9 ± 2.12
Macaroni with tuna	Macaroni[a], tuna, tomato, sunflower oil and salt	34.9 ± 2.50
Lentils with sausage	Lentils[a], sunflower oil, mashed tomato, salt, coloring, laurel, chicken broth, carrot, sausage[a], onion, pepper, garlic, potatoes[a]	44.8 ± 2.59
Stew	Chickpeas[a], green beans, carrot, pork bone, chicken, potato[a], veal ragout[a], salt pork, chicken broth and salt	51.6 ± 4.32
Chicken in breadcrumbs with vegetable stew	Chicken in breadcrumbs[a], vegetables and sunflower oil	80.2 ± 5.05
Chicken in sauce	Chicken breast[a], chicken broth, flour, onion, almonds, sunflower oil, potatoes, coloring matter and salt	102 ± 13.1
Fried hake	Hake filet and sunflower oil	163 ± 3.23
Spanish/potato omelet	Potatoes, egg, sunflower oil and salt	54.0 ± 2.44

[a] Main ingredients.

Costar Corning Europe, Badhoevedorp, The Netherlands) and centrifuged 3500*g* for 1 h at 4 °C. Then, the supernatant (soluble fraction) was collected and pooled.

Prior to addition of the soluble fraction to the cells, glucose (5 mM final concentration) and HEPES (50 mM final concentration) were added to make the soluble fraction similar to the culture media, and then water was added to adjust the osmolarity to 310 ± 10 mOsm/kg (Freezing point osmometer, Osmomat 030, Berlin, Germany).

2.4. Cell culture

Caco-2 cells were obtained from the European Collection of Cell Cultures (ECACC 86010202, Salisbury, UK) and used in assays at cell between passages 50–60. Cells were seeded at a density of 35×10^4 cells per filter in polyester membrane chamber inserts (24 mm diameter, 0.4 μm pore size; Transwell®, Costar Corp., NY, USA). The transwell filters were placed into 6 well plates dividing an apical or a donor-like compartment from a basal or acceptor compartment.

The cells were grown in minimum essential medium (MEM; Gibco BRL Life Technologies, Scotland) with 10% v/v fetal bovine serum (FBS), 1% v/v nonessential amino acids (Gibco BRL Life Technologies, Scotland), 1% v/v L-glutamine (Biowhittaker, Walkersville, USA), 1% v/v antibiotic solution (penicillin–streptomycin) (Biowhittaker, Walkersville, USA), and 0.1% v/v fungizone (Gibco BRL Life Technologies, Scotland) at pH 7.2–7.4. The cells were maintained at 37 °C in an incubator with 5% CO_2, and 95% atmospheric air at constant humidity. The medium was changed every two days.

2.5. Mineral retention and transport by Caco-2 cells

Mineral cell retention, transport and uptake (retention plus transport) from school meals were measured using the soluble fraction of the simulated digestion and Caco-2 cells.

Twenty-one days after initial seeding, spent culture medium was aspirated from the apical and basolateral chambers, and the apical and basolateral cell surfaces of the monolayer were washed three times with PBS at 37 °C. The transepithelial electrical resistance (TEER) was measured. Only those filters that had a TEER > 250 Ω cm^2 at the beginning and end of the experiment were included. The basolateral compartment was filled with 2.5 ml of buffer (blank) [130 mM NaCl, 10 mM KCl, 1 mM $MgSO_4$, 50 mM HEPES, and 5 mM glucose, pH 7.2] and the apical compartment was filled with 1.5 ml of soluble mineral fraction. Transport was measured after 2 h of incubation at 37 °C. Basolateral compartment contents were collected. Subsequently, the monolayer was washed twice with PBS (pH 7.4). TEER was measured again to monitor any damage to the monolayer during the assays. Then, the monolayer was washed twice with a solution containing 150 mM NaCl, 1 mM EDTA, 10 mM HEPES, pH 7.2 at 4 °C, to remove non-specifically-bound mineral and residual medium. The cells were lysed and harvested by the addition 1 ml of 2% (w/v) sodium dodecyl sulfate.

Cell viability after 2 h of exposure to the uptake solutions was assessed by trypan blue exclusion, and was typically 80–95%.

2.6. Protein determination

The protein contents of 8 dishes studied was determined by the AOAC method (2002).

2.7. Mineral determination

Calcium, iron, and zinc contents in blank, soluble mineral fraction, cell monolayer and basolateral content and copper content in blank and soluble mineral fraction were determined by flame atomic absorption spectrophotometry (AAS; Perkin–Elmer, Model 2380, Norwalk, USA). Copper content of cell monolayer and basolateral content were determined by graphite furnace-atomic absorption spectrophotometry with Zeeman effect (Perkin Elmer Analyst 600); prior dry organic matter destruction (450 °C) was applied.

Differences between the mineral contents of the monolayer incubated with soluble mineral fraction and the contents of monolayer incubated with blank yield an estimation of the cellular retention (μg) of minerals. Transport was evaluated by the difference between the mineral amount in the basal chamber and transport buffer (transport blank).

Retention percentages were calculated as follows: retention $(\%) = 100 \times R/C$, where R = mineral retention (μg mineral/well), and C = soluble mineral content added (μg).

Transport percentages were calculated as follows: transport $(\%) = 100 \times T/C$, where T = difference between mineral amounts in the basal chamber and transport buffer (blank) (μg mineral/well), and C = soluble mineral content added (μg).

2.8. Experimental design and data analysis

Each series of assays described in this study represents 6 replicates of the experimental protocol. Replicates of each assay were conducted on two different days.

Data on retention, transport and total mineral cell uptake were analyzed by one-way ANOVA (Statgraphics Plus 5.0). Tukey's multiple range test was used to determine significant differences among means ($p < 0.05$) (Box, Hunter, & Hunter, 1978).

3. Results and discussion

Mineral retention, transport and uptake (retention plus transport) are shown in Tables 2–5. Soluble mineral added values reflect the solubility of the elements, because in all

Table 2
Calcium: retention transport and uptake by Caco-2 from school meals

Dishes	Calcium						
	Ca (soluble) added[a] (μg)	Retention		Transport		Uptake	
		(μg)	(%)	(μg)	(%)	(μg)	(%)
Macaroni with tuna	35.6 ± 3.03	14.5 ± 2.06	40.6 ± 5.79	5.59 ± 1.68	15.7 ± 4.73	20.1 ± 1.86	56.3 ± 6.20
Stew	62.3 ± 5.12	6.20 ± 0.01	9.94 ± 0.01	21.1 ± 2.92	33.9 ± 4.68	27.3 ± 1.48	43.9 ± 2.90
Potato omelet	54.1 ± 5.08	5.45 ± 2.10	10.1 ± 3.88	15.4 ± 2.10	28.4 ± 3.88	20.8 ± 2.01	38.5 ± 3.96
Chicken in sauce	34.3 ± 5.72	1.49 ± 0.70	4.33 ± 2.04	7.92 ± 1.71	23.1 ± 5.00	9.41 ± 1.20	27.4 ± 2.10
Cuban style rice	50.4 ± 6.30	3.88 ± 1.46	7.70 ± 2.90	12.3 ± 0.99	24.5 ± 1.97	16.2 ± 1.25	32.2 ± 1.98
Lentils	48.7 ± 4.31	4.40 ± 0.73	9.04 ± 1.50	17.9 ± 4.67	36.9 ± 9.59	22.3 ± 2.81	45.9 ± 6.52
Chicken in breadcrumbs	54.2 ± 7.44	1.86 ± 1.50	3.43 ± 2.91	n.d.	n.d.	1.86 ± 1.50	3.43 ± 2.91
Fried hake	102 ± 5.90	1.11 ± 053	1.09 ± 0.51	2.23 ± 0.01	2.18 ± 0.01	3.34 ± 0.27	3.27 ± 0.46

Mean values ± standard deviation ($n = 6$).
Uptake = retention plus transport.
n.d., not detectable.
[a] Ca content in the aliquot of soluble fraction (1.5 ml) added to cell cultures.

Table 3
Iron: retention transport and uptake by Caco-2 from school meals

Dishes	Iron						
	Fe (soluble) added[a] (μg)	Retention		Transport		Uptake	
		(μg)	(%)	(μg)	(%)	(μg)	(%)
Macaroni with tuna	1.84 ± 0.43	1.10 ± 0.01	60.1 ± 0.01	0.14 ± 0.01	7.52 ± 0.01	1.24 ± 0.01	67.4 ± 0.01
Stew	3.37 ± 0.37	0.18 ± 0.01	5.46 ± 0.01	0.23 ± 0.12	6.83 ± 2.73	0.41 ± 0.06	12.2 ± 0.16
Potato omelet	4.11 ± 0.44	0.11 ± 0.07	2.79 ± 1.94	0.21 ± 0.01	5.03 ± 0.01	0.32 ± 0.04	7.79 ± 0.09
Chicken in sauce	2.47 ± 0.45	0.21 ± 0.08	8.42 ± 3.65	0.49 ± 0.11	20.00 ± 4.46	0.70 ± 0.09	28.3 ± 0.20
Cuban style rice	3.35 ± 0.22	0.24 ± 0.14	7.19 ± 4.06	0.26 ± 0.11	7.67 ± 3.32	0.50 ± 0.12	14.9 ± 0.54
Lentils	1.61 ± 0.44	n.d.	n.d.	0.13 ± 0.01	7.98 ± 0.01	0.13 ± 0.01	7.98 ± 0.01
Chicken in breadcrumbs	2.54 ± 0.43	0.37 ± 0.01	14.6 ± 0.01	0.25 ± 0.01	9.70 ± 0.01	0.62 ± 0.01	24.4 ± 0.01
Fried hake	0.99 ± 0.10	0.55 ± 0.01	55.7 ± 0.01	0.06 ± 0.01	6.19 ± 0.01	0.61 ± 0.01	61.6 ± 0.01

Mean values ± standard deviation ($n = 6$).
Uptake = retention plus transport.
n.d., not detectable.
[a] Fe content in the aliquot of soluble fraction (1.5 ml) added to cell cultures.

Table 4
Zinc: retention transport and uptake by Caco-2 from school meals

Dishes	Zinc						
	Zn (soluble) added[a] (μg)	Retention		Transport		Uptake	
		(μg)	(%)	(μg)	(%)	(μg)	(%)
Macaroni with tuna	3.40 ± 1.04	0.58 ± 0.01	16.9 ± 0.01	1.04 ± 0.17	30.7 ± 4.95	1.62 ± 0.09	47.7 ± 2.45
Stew	2.88 ± 0.31	0.98 ± 0.14	34.0 ± 5.12	0.60 ± 0.08	20.7 ± 2.93	1.58 ± 0.11	54.9 ± 4.50
Potato omelet	3.31 ± 0.37	0.06 ± 0.01	1.70 ± 0.01	0.18 ± 0.11	5.52 ± 3.60	0.24 ± 0.06	7.25 ± 1.70
Chicken in sauce	4.11 ± 0.59	0.12 ± 0.02	2.96 ± 0.59	0.17 ± 0.09	4.23 ± 2.39	0.29 ± 0.08	7.06 ± 1.40
Cuban style rice	2.84 ± 0.78	0.30 ± 0.12	10.6 ± 4.49	0.08 ± 0.04	2.83 ± 1.83	0.38 ± 0.09	13.4 ± 3.10
Lentils	3.80 ± 0.43	0.29 ± 0.10	7.53 ± 2.80	0.90 ± 0.10	23.8 ± 2.78	1.19 ± 0.10	31.3 ± 2.80
Chicken in breadcrumbs	3.28 ± 0.43	0.40 ± 0.05	12.1 ± 1.64	n.d.	n.d.	0.40 ± 0.05	12.1 ± 1.64
Fried hake	1.51 ± 0.26	0.09 ± 0.01	5.65 ± 0.01	n.d.	n.d.	0.09 ± 0.01	5.65 ± 0.01

Mean values ± standard deviation ($n = 6$).
Uptake = retention plus transport.
n.d., not detectable.
[a] Zn content in the aliquot of soluble fraction (1.5 ml) added to cell cultures.

cases the initial amount of dishes, the digestion procedure applied, and the volumes of digest taken were the same.

No correlation was obtained between soluble mineral added to cells and retention, transport or cell uptake. This suggests that other factors, in addition to solubility, can play a role in mineral uptake and transport by cells.

The highest percentages of calcium retained correspond to macaroni with tuna (40.6%) and potato omelet (10.1%)

Table 5
Copper: retention transport and uptake by Caco-2 from school meals

Dishes	Copper						
	Cu (soluble) added[a] (μg)	Retention		Transport		Uptake	
		(μg)	(%)	(μg)	(%)	(μg)	(%)
Macaroni with tuna	106 ± 2.79	9.09 ± 0.96	8.62 ± 0.91	21.7 ± 4.75	20.6 ± 4.50	30.8 ± 2.85	29.2 ± 2.70
Stew	46.7 ± 6.46	37.41 ± 0.81	80.2 ± 1.73	n.d.	n.d.	37.4 ± 0.81	80.1 ± 1.73
Potato omelet	69.2 ± 4.65	n.d.	n.d.	16.3 ± 8.34	24.7 ± 0.77	16.3 ± 8.34	24.7 ± 0.77
Chicken in sauce	36.5 ± 15.27	35.06 ± 1.52	96.0 ± 4.16	0.25 ± 0.01	0.54 ± 0.01	35.3 ± 0.76	96.6 ± 3.54
Cuban style rice	114 ± 21.81	69.0 ± 11.05	60.7 ± 9.71	13.5 ± 4.50	11.9 ± 3.96	82.6 ± 7.50	72.6 ± 9.32
Lentils	257 ± 3.95	31.9 ± 9.29	12.4 ± 3.61	26.6 ± 7.19	10.3 ± 2.80	58.5 ± 8.10	22.8 ± 6.21
Chicken in breadcrumbs	74.0 ± 13.41	24.1 ± 3.86	32.6 ± 5.22	n.d.	n.d.	24.1 ± 3.86	32.6 ± 5.22
Fried hake	17.2 ± 2.90	2.50 ± 1.07	14.6 ± 6.25	n.d.	n.d.	2.50 ± 1.07	14.6 ± 6.25

Mean values ± standard deviation ($n = 6$).
Uptake = retention plus transport.
n.d., not detectable.
[a] Cu content in the aliquot of soluble fraction (1.5 ml) added to cell cultures.

while the highest percentages of transported mineral to legumes were in lentils (36.9%) and stew (33.9%). The percentages of calcium uptake ranged between 56.3% for macaroni with tuna and 3.27% for fried hake.

The legumes and cereal meals presented the highest percentages of calcium uptake (retention plus transport), while the meat and fish plates showed lower uptakes for this mineral. With the exception of macaroni with tuna, which presented the highest percentage retention of calcium, a significant correlation was established between this percentage and the protein content ($r = -0.847$, $R^2 = 71.85$, $p < 0.05$), and a trend was observed when decreasing the quantity of retained calcium with the increase in protein content. In the same way, a significant negative correlation was established for the percentage of calcium uptake and proteins ($r = -0.755$, $R^2 = 56.93$, $p < 0.05$) for the eight meals studied.

These results differ from those reported in a study performed in healthy women, where a diet low in proteins resulted in lower intestinal calcium absorption. Intestinal calcium absorption in the low protein diet (0.7 g protein/kg) was 19 ± 3% which was significantly lower than in the high diet (2.1 g protein/kg) which averaged 26 ± 3% (Kerstetter, O' Brien, & Insogna, 1998). However, Heany and Recker (1982) found no association between intestinal calcium absorption and protein intake in women consuming their habitual amount of dietary protein (averaging 1 g protein/kg). Other authors (Gueguen & Pointillart, 2000; Whiting, Anderson, & Weeks, 1997) point out that excess protein generally leads to an increase in the amount of calcium lost in the urine. This is especially true for proteins with high contents of sulfur-containing amino acids (cysteine, methionine), the breakdown of which releases sulfur oxidized as sulfate, causing moderate acidosis and increasing the excretion of calcium in the urine. Sulfate ions also bind calcium, preventing its tubular reabsorption and even its incorporation into bone.

No statistically significant interaction has yet been established between retained, transported or uptaken calcium and the content of iron, zinc and copper present in the soluble fraction. This is particularly relevant for calcium–zinc interaction, since some authors report competition between the two minerals for a common metallic multidivalent transporter with greater affinity for zinc (Bertolo, Bettger, & Atkinson, 2001b). However, Spencer, Norris, and Osis (1992) reported that, in humans, while the supplementation with zinc sulphate can inhibit calcium absorption when it is found in low quantities in the diet, in the presence of Ca:Zn weight proportions of <1.64, such inhibition does not occur above a Ca:Zn weight proportion of >2.3. In our study, the weight proportion of calcium:zinc is found in the soluble fraction added to cellular culture is between 67.5 for fried hake and 8.3 for chicken in sauce, the results are well justified.

The meals with increased protein content, namely, chicken with vegetable stew (80.2 mg/g), chicken in sauce (102 mg/g) and fried hake (163 mg/g) present some of the highest percentages of retained, transported and uptaken iron (see Table 3). Together with these meals, macaroni with tuna also presents high iron retention and uptake percentages, despite the lower protein content (34.9 mg/g). The potential effect of proteins on iron bioavailability has been well documented by others authors (Engelmann et al., 1998; Hallberg, Hoppe, Andersson, & Hulthen, 2003; South, Lei, & Miller, 2000). This is referred to as the "meat factor", and although it still remains unclear, it seems that peptides released during the digestion of proteins bind iron to form complexes within the intestinal lumen, and would increase its solubility (Berner & Miller, 1985). Other authors have attributed this effect to the ability of sulfhydryl groups of amino acids, such as cysteine, to reduce Fe(III) to Fe(II) (Mulvihill & Morrisey, 1998; Mulvihill, Kirwan, Morrisey, & Flynn, 1998). In fact, according to the data obtained, a linear positive correlation can be established between the percentage of iron retained and protein content ($r = 0.879$, $R^2 = 77.23$, $p < 0.01$) and between iron uptake percentage and protein content ($r = 0.938$, $R^2 = 88.07$, $p < 0.01$) for all the meals studied,

excepting macaroni with tuna, where the iron uptake percentage may be attributed to other factors.

As regards the amount of mineral transported, and although it was not possible to establish a significant positive correlation between transported iron and protein contents, the highest percentages of transported iron corresponded to chicken in sauce (20.0%) and chicken with vegetables (9.70%), while lower percentages were recorded for fried hake (6.19%). This once again underscores the enhancing effect of meat proteins upon iron bioavailability.

In Caco-2 cells (Glahn et al., 1996) an increase in iron uptake has been reported in the presence of meat proteins in the form of beef (1.39%), chicken (1.17%) and fish (1.33%) versus other proteins, such as caseins (0.35%). Similarly, Kapsokefalou and Miller (1995), in rat intestinal contents, have reported a greater iron absorption percentage with beef proteins than with other proteins sources such as egg and milk.

On the other hand, a statistically significant correlation ($r = -0.831$, $R^2 = 69.04$, $p < 0.05$) has also been established between the percentage of iron retained and the amount of zinc present in the soluble fraction. Iron–zinc interaction occurs at the absorption site, probably due to the presence of a common transporter (Pérès et al., 1999). Zinc-supplementing can adversely affect iron status in pregnant women with hemoglobin concentrations of <85 g/l (Christian et al., 2001). Pérès et al. (1999) described (in rats) that when both ions were present as inorganic salts, this interaction takes place at very low Fe: Zn molar rations (1:1.5). In the present study, the Fe:Zn molar ratios, of the soluble fractions of the menus analyzed, in most cases ranged between 1:1.1 and 1:2 – an observation that supports the negative interaction of zinc with iron absorption. In humans, Aggett, Crofton, Khin, and Gvozdanovic (1983) have shown that excess zinc exerts an inhibitory effect upon iron absorption. In turn, Merzenich et al. (1994) reported similar findings in preschool children receiving either a zinc–iron supplement (30 mg iron; 15 mg zinc) or 30 mg of iron alone. Both treatments produced a significant response in hemoglobin and zinc protoporphyrin, but ferritin only rose in the iron alone group. Despite the 1:2 ratio of zinc to iron, the results suggest a negative effect of zinc on the uptake of iron or its storage.

The highest percentages of zinc retained correspond to stew (34.0%) and macaroni (16.9%). The highest percentages of zinc transported were obtained for macaroni (30.7%) and lentils (23.8%). In the same manner as iron, the presence of animal proteins can improve zinc absorption (Sandström, 1992; Solomons, 1982). The effect would be similar to that observed for iron kept soluble within the intestinal lumen. However, in our study, no correlation was found between protein contents and zinc retention, transport and uptake. In fact, the two menus with the greatest protein contents showed the lowest percentages of zinc uptake: chicken in sauce (7.06%) and fried hake (5.65%).

Jalla, Steirn, Miller, and Krebs (1998), using stable isotopes, found no difference in zinc absorption by breast-fed infants fed with an assigned complementary food (beef or cereal). Absorption for both groups averaged ~40%. Although zinc absorption was not significantly greater in beef than in cereal, the greater zinc content of the meat resulted in significantly more absorbed zinc from beef. The results obtained point to the need for reassessment of whether protein action upon zinc bioavailability is attributable to a marked protein-mediated enhancing effect or simply to the fact that proteins are a good dietary source of this element.

The high zinc uptakes recorded for stew (54.9%), macaroni (47.7%) and lentils (31.3%) suggest a possible enhancing effect mediated by vegetable proteins upon zinc bioavailability. In beans, Lombardi-Boccia, Carbonaro, Lullo, and Carnovale (1994) reported a positive influence of proteins, and specifically the globulin fraction (which presents the highest cysteine content), on zinc bioavailability.

With reference to the interaction between zinc and other minerals, in all dishes in which transport of zinc was detected, the results showed a negative correlation between the transported zinc content and the quantity of iron present ($r = -0.733$, $R^2 = 53.78$, $p < 0.1$), thus again illustrating iron–zinc interaction. Decreased serum zinc concentrations have been reported after iron supplementation with doses >60 mg/d (Hambidge, Krebs, Sibley, & English, 1987; O' Brien et al., 1999), but also at a level of supplementation of only 18 mg/d in pregnant teenagers (Dawson, Albers, & McGanity, 1989).

Finally, no relationship was noted between protein content and copper uptake, retention and transport. The highest percentages of retained copper were obtained for chicken in sauce (96%), with a high content in protein, while fried hake, with the highest protein content, yielded one of the lowest copper uptake percentages (14.6%). The highest copper transport percentages corresponded to potato omelet (24.7%) and macaroni (20.6%).

A positive correlation can be established between copper retention and iron content ($r = 0.800$, $R^2 = 63.98$, $p < 0.05$) for all menus except potato omelet. Copper is essential for iron metabolism and hemoglobin biosynthesis. In this context, Cu-supplementing accelerates hemoglobin synthesis in children with hypochromic anemia treated with iron salts (Harris, 2001). In rats, Ramírez-Cárdenas, Brunoro Costa, and Pinheiro Reis (2005) have observed increases in hemoglobin, hematocrit and serum iron levels, when for one same amount of iron (24 ppm), the copper content in diet is increased from 0 to 6 ppm. This positive interaction between the two minerals could explain why a high iron content can favour Cu absorption.

4. Conclusions

Solubility and cell uptake (cell retention plus transport) do not always show parallel trends. As a result, these parameters cannot easily be used as indicators of mineral bioavailability from foods. The affinity of a compound

for a given mineral and the type of complex formed may be as important as solubility for determining the effect on mineral absorption. If bound tightly, donation of element to mucosal cell may not occur. In this context, assays in Caco-2 cells, comprising simulation/estimation of the absorption process, may offer a better idea of bioavailability than solubility.

Although the results obtained show the amount of bioavailable calcium supplied by the studied menus to be important, the principal amounts are contributed by milk and dairy products. This fact must be taken into account, particularly during the growth period of children. Likewise, a possible negative influence of proteins on calcium absorption has been observed, with the absence of interactions with other minerals at dietary levels.

When considering iron bioavailability, the main dietary iron sources were found to be meat dishes. The latter yielded some of the highest iron uptake, retention and transport values – a correlation being observed between food protein content and iron uptake and retention. A marked positive protein effect on iron uptake was noted, together with a negative effect of zinc on iron retention.

The highest zinc bioavailability percentages corresponded to legume- and cereal-based dishes. Taking into account the contents of this mineral, evaluated in an earlier study (lentils: 7.81 μg Zn/g; stew: 7.11 μg Zn/g) (Cámara et al., 2005), and the enormous importance of zinc in childhood nutrition, the inclusion, at least once a week, of foods of this kind in school menus is advisable. In this context, the negative effect of iron on zinc transport is underlined.

A positive copper–iron interaction has been observed, possibly attributable to the metabolic interrelations between the two minerals.

Despite the limitations posed by in vitro studies, the model used in the present work allows us to establish comparisons between menus based on mineral bioavailability, and to assess the amount of mineral amenable to absorption. It should be pointed out that the observed mineral interactions have been recorded for dietary amounts of the studied minerals, i.e., they correspond to the true-life situations of school menus.

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