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Infant formula iron dialysability related to other nutrients

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Abstract

The dialysable iron in relation to total iron, to protein sources, to calcium, magnesium, copper, zinc, and vitamin C of 17 infant formulas, from four different multinational companies were analyzed. Total iron, calcium, magnesium, copper, and zinc were determined by atomic absorption spectrophotometry. The dialysable iron was determined. The considered vitamin C concentration was that declared on the labels. There was no significant statistical relationship between total iron and dialysable iron ($P=0.54$, $r=0.09$) when considered separately. When analyzed considering protein sources, this relationship was shown to be inversely proportional to protein hydrolysate ($P=0.03$, $r=-0.72$), and soy protein ($P=0.02$, $r=-0.93$). The percentage of dialysable iron was significantly greater from the protein hydrolysate ($P<0.01$). The dialysable iron showed a negative correlation with calcium ($P<0.05$, $r=-0.61$). In conclusion, the infant formulas whose protein sources are casein and casein plus whey protein should present Ca:Fe and Fe:vitamin C ratios that allow considerable iron dialysability.

Keywords: Iron; Infant formulas; Nutrients; Available

1. Introduction

Breastfeeding is considered to be ideal for newborns, especially in their first month, because of its nutritional, immunological and psychosocial benefits. Most of the authors report the neglect of breastfeeding practice as the principal cause of anemia in infants below six months old (Lost, Name, Jeppsen, & Ashmead, 1998), because despite its low and variable iron concentration, 0.04–1.92 μg/ml, (Dorea, 2000), the iron bioavailability in breast milk is high (Mc Millan, Landaw, & Oski, 1976). When human milk is prematurely replaced by cow's milk in infant nourishment, there is an increased possibility of iron deficiency due to the low bioavailability of the mineral (Oski & Stockman, 1980) and to the intestinal blood loss (Ziegler, Fomon, & Nelson, 1990).

From the beginning of the industrial revolution to the production of powder milk, we can observe increasing progress in infant formula composition. In order to supply infants with all the required nutrients for their growth and development, these milk-based formulas increasingly resemble breast milk composition (Martinez & Krieger, 1985).

Iron absorption occurs in the small intestine, duodenum and proximal jejunum, and it depends on the interaction that may occur in the intestinal lumen with other compounds. Thus, for instance, other mineral elements, phenolic compounds (Suítor & Bailey, 2000), and albumin (Lynch, 1997) can restrain Fe absorption by competing for absorption sites (in the case of copper) or by forming insoluble Fe compounds, which are therefore non-absorbable. On the other hand, fermented products, meat cysteine (Layrisse, 1984), ethanol, citric

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and lactic acids (House, 1999) and vitamins A (Layrisse et al., 1997) and C are considered to be stimulating factors for non-heme iron absorption (Lynch, 1997), for they exert a reducing effect on the ferric form (Fe3+), keeping iron in its ferrous form (Fe2+) which is best absorbed. During digestion, protein foods turn into peptides that may complex iron in the intestinal lumen and influence its absorption. Peptides may either restrain or facilitate iron absorption depending on the carbonic chain size (Vannucchi, Menezes, Campana, & Lajolo, 1990). It is important to mention that individual physiological conditions also alter iron bioavailability, which reflects the balance between dietetic and physiological factors on the absorption, transport, cellular organization, storage and excretion of nutrients (Fairweather-Tait, 2001).

Considering the worldwide occurrence of iron deficiency anemia, the consequent need of adequate iron intake, especially by the infant population, and the interference of other nutrients in dietetic iron bioavailability, this present paper has the purpose of evaluating, in infant formulas, the dialysable iron in relation to total iron, to protein sources, to calcium, magnesium, copper, zinc and vitamin C.

2. Materials and methods

2.1. Materials

The sample comprised 17 types of infant formulas, from four different multinational companies. As a protein source, seven formulas had casein, five had casein plus whey protein, three had the hydrolysate one, and two the soy protein. The analyses were always performed according to the product's expiring date. Each type of infant formula was analysed at least twice (each time in triplicate), this is the reason why the number of samples for each type of infant formula is different.

Acid-washed glassware was used throughout the study (Instituto Adolfo Lutz, 1985). The water went through weekly quality control, by means of conductivity reading, ($\leqslant$1.0 μs at 25 °C). All chemicals used were of analytical grade.

2.2. Concentrations of total iron, copper, zinc, calcium and magnesium

After wet digestion (HNO_3:$HClO_4$3:1) (Marks, Moore, Kanabrocki, Oester, & Kaplan, 1971) of the samples, the minerals were determined by atomic absorption spectrophotometry, in Polarized Zieman AAS Hitachi Z-5000 equipment, according to the Association of Official Analytical Chemists criteria (Association of Official Analytical Chemists, 1980), under the following conditions: hollow cathode lamp, wavelength 284.3 nm (Fe), 324.8 nm (Cu), 213.9 nm (Zn), 422.7nm (Ca), 285.2 nm (Mg), slit 0.2 nm, 0.7 nm (Ca), air/acetylene oxidant flame. To determine calcium and magnesium, 0.5% of lanthanum oxide solution was added to each sample. The working standard solutions were prepared by using appropriate solutions of each mineral, from Tritisol line – Merck. The mineral concentrations were calculated from the linear regression straight-lines obtained.

For quality control determinations of total Fe, the casein diet AIN93G/160497 was used, which is a secondary reference standard (Ramos, 1999) according to the National Institute of Standards & Technology Certificate.

2.3. Concentration of vitamin C

Vitamin C concentration was that declared on the labels.

2.4. Determination of dialysable iron (DFe)

At first, the samples, in triplicate, were reconstituted according to the recommendations of each manufacturer to obtain 120 ml of milk. Therefore, the weight of samples was diversified. The dialysable iron was determined by the in vitro method described by Miller, Schricker, Rasmussen, and Van Campen (1981). The samples were acidified, to pH 2.0, with HCl, put into beakers, divided into aliquots of 20 ml, to which pepsin was added, and left for 2 h at 37 °C. Afterwards, the dialysis tubes were made up with $NaHCO_3$ and placed in their respective beakers in order to alkalize the solution. The second digestion was performed by adding pancreatin/bile to each beaker, and they were left for 2 h at 37 °C. The quantity of pancreatin/bile was calculated in accordance with the content of protein/g of each sample. After complete digestion, deionized water was added, making up to 25 ml. The determination method for DFe had a detection limit of 0.02 μg Fe/ml and quantification of 0.30 μg Fe/ml. The average variation coefficient was 4.57% (2.03–8.39%), demonstrating the adequate accuracy of the method.

2.5. Statistical analysis

Multiple linear regression analysis (Neter, Kutner, Nachtsheim, & Wasserman, 1996) was used to verify the influence of total iron on iron dialysability, of protein source on the total iron dialysable iron ratio and of independent variables Ca, Mg, Cu, Zn and vitamin C on the dependent variable dialysable Fe.

The Kruskal Wallis Test (Sokal & Rohlf, 1969) was used to compare the percentages of DFe among protein sources. An α confidence level of 5% was adopted.

Table 1
Multiple linear regression of the relationship between total iron and dialysable iron of infant formulas

Variable	Constant	SE	P	r	R^2 (%)
Total iron	1.346	0.05	0.54	0.09	0.7

Dependent variable: Ln dialysable Fe.

3. Results and discussion

There was no statistically significant relationship between total Fe and DFe ($P=0.54$, $r=0.09$) of infant formulas, when other nutrients were not considered (Table 1). These results confirm literature reports that the quantity of total iron present in food does not influence the content of dialysable iron (Cook, 1983; Lynch, 1997), demonstrating that a larger content of total iron is not responsible for a higher absorption of this mineral. The amount of absorbed non-heme iron decreases when the quantity of iron in the meals increases, underlining the existence of regulatory mechanisms for non-heme iron absorption in the intestinal mucosa (Bezwoda et al., 1983), which is significantly affected by dietetic composition and by iron status in the organism (Cook, 1990; Hallberg, Hultén, & Gramatkovski, 1997).

The quantity of total Fe did not influence the quantity of DFe. However, this relationship underwent influence from the protein type, as Table 2 shows, in which the percentage of DFe was significantly higher, from the protein hydrolysate ($P<0.001$) in relation to the other protein sources, which did not show significant difference from each other. Also, the multiple linear regression analysis (Table 3) demonstrated that the relationship between total Fe and DFe was not significant when protein sources were casein ($P=0.55$, $r=0.15$) or casein plus whey protein ($P=0.24$, $r=0.31$); but was significantly and inversely proportional when the variables were the protein hydrolysate ($P=0.03$, $r=-0.72$) or soy protein ($P=0.02$, $r=-0.93$).

The casein in infant formulas is presented as calcium or sodium caseinate. The iron bound to casein plays an important role in reducing and inhibiting its dialysability, since the in vitro intact casein test reveals that most of the iron does not cross the dialysis membrane, indicating the occurrence of an iron–casein complex in insoluble form. Casein contains great amounts of calcium that compete for absorption sites, and amounts of phosphorus, which form compounds that precipitate, making iron solubility difficult and diminishing its absorption (Hurrell, Lynch, Trinidad, Dassenko, & Cook, 1989).

Table 2
Total iron (mg/100 g) and dialysable iron (%) of infant formulas according to protein source

Source protein	Iron	
	Total ((mg/100 g)×SD)	Dialysate (%)
Casein ($n=19$)	6.58 ± 1.07	3.56[b]
Casein + whey protein($n=16$)	6.53 ± 2.51	5.55[b]
Protein hydrolysate ($n=9$)	6.32 ± 1.09	32.21[a]
Soy protein ($n=8$)	6.49 ± 1.01	5.26[b]
Value of P	0.607[A]	<0.001[B]

[A] P descriptive level of Kruskal Wallis Test.
[B] P descriptive level of Kruskal Wallis Test and Dunn's Multiple Comparison Test; figures in columns with different superscript letters have with significant difference.

Some studies show that casein, whey protein and egg-albumin are not capable of oxy-reduction (Kapsokefalou & Miller, 1991). Thus, excluding the capacity of oxy-reduction, we can infer that adding whey protein has reduced the proportion of casein, decreasing the complex of insoluble polymers, as well as the proportion of calcium and phosphorus contents, diminishing the competition for absorption receptors and, explaining the rise of 1.99% of dialysable iron in the casein plus whey protein, in relation to casein, achieved in this study.

We could observe 3.56% of dialysable iron in the presence of casein and 32.2% in the presence of protein hydrolysate. A similar result was described in the presence of casein, 4%; but in the presence of protein hydrolysate, a value of, 16.8% (Garcia, Alegria, Barberá, Farre, & Lagarda, 1998) that is about 50% of that obtained in this study. Such a difference is probably due to the different degree of protein hydrolysis.

The infant formula with protein hydrolysate presented the lowest quantity of total iron (6.32 mg) and the highest iron dialysability, 32.2%. These results confirm that total iron does not have any direct relationship with dialysable iron, as discussed before. The results also confirm literature reports that show a significant increase of dialysable iron with protein hydrolysate (Garcia et al., 1998). This increase probably occurs because certain components, such as amino acids, peptides and other chelating agents are able to keep iron in its soluble form, preventing its precipitation at the neutral or alkaline pH existing in the duodenum (Jackson, 1997). Another hypothesis is that the degree of hydrolysis these proteins, in to amino acids and peptides, shows a positive relationship with dialysable iron, as described by Hurrell et al. (1989).

The total Fe showed an inverse relationship with the DFe when the protein sources were the hydrolysate on soy. In these sources, the more iron added, the lower is the dialysable percentage. Such an inverse relationship

Table 3
Multiple linear regression of the relationship between total iron and dialysable iron and protein sources of infant formulas

Protein source	Constant	SE	P	r	R^2 (%)
Casein ($n=19$)	0.230	0.12	0.55	0.15	2.2
Casein + whey protein ($n=16$)	0.980	0.008	0.24	0.31	9.6
Hydrolysate protein ($n=9$)	4.17	−0.11	0.03	−0.72	51.8
Soy protein ($n=8$)	10.86	−1.33	0.02	−0.93	86.4

Table 4
Multiple linear regression of the relationship between dialysable iron and the variables calcium, magnesium, copper, zinc and vitamin C of infant formulas

Variables	Coefficient	SE	P
Constant	19.96	3.856	
Calcium	−2.852	0.599	<0.001

Dependent variable: Ln dialysable Fe ($r=-0.61$, $R^2=37.2\%$).
Mg, Cu, Zn and vitamin C contents had no significant effect on dialysed iron.

was described by Garcia et al. (1998) and Saarinen and Siimes (1977) who verify absorption of 9% and 7% in formulas with 6.8 and 12.8 mg Fe/l, respectively. A possible mechanism involves receptor saturation. Thus, while all receptors are already linked to the mineral, the increase of total iron would only give a proportionally - lower value of absorbed iron. In this way, an optimal ratio between total iron and absorbed iron probably exists.

The phytate forms a soluble complex with iron at pH 7.0, and precipitates in the presence of calcium and magnesium (Subba Rao & Narasinga Rao, 1983), reducing its bioavailability. Studies conducted with cereals have also proven that phytic acid can reduce mineral bioavailability and change protein functional characteristics (Champagne et al., 1985). This study revealed 5.26% of DFe in the presence of soy protein and 3.56% in the presence of casein. Two hypotheses may explain this difference. First, the decrease in content of phytic acid, since the soy protein extract is purified. Second, the decrease of insoluble complexes (phytate/mineral) is due to the lower proportion of calcium and magnesium from soy, compared to casein.

The multiple linear regression of the relationship between DFe and variables calcium, magnesium, copper, zinc and vitamin C demonstrated that only calcium had a significant inverse relationship with DFe ($P<0.001$, $r=-0.61$); that is, for each calcium logarithm unit, the logarithm of DFe was lowered by 2.85 (Table 4). Cook, Dassenko, and Wittaker (1991) described reduction in iron supplement absorption ($FeSO_4$) in humans when, at the same time, a calcium carbonate supplement ($CaCO_3$) is supplied in a proportion of 33:1 (Ca:Fe). The Ca:Fe ratio of infant formulas analyzed in this present paper has shown variation from 50:1 to ≥100:1. These values are much higher than those reported by Cook et al. (1991) and they can harm iron bioavailability even more.

It is noticeable that vitamin C did not significantly correlate with dialysable iron (Table 4), despite its promoting action in non-heme iron bioavailability (House, 1999). This has probably occurred on account of the micronutrient ratios. Cook and Monsen (1977) described a direct proportion between vitamin C and absorbable iron when the vitamin content ranged from 25 to 1000 mg, giving an average rise of 1.65–9.57 times in iron absorption. Further research shows that ascorbic acid intakes from 50 to 500 mg, together with meals, increase non-heme iron absorption from 1.12 up to 6.52 times (Hallberg, 1986). Infant formulas fortified with 15 mg of ferrous sulfate per litre present 3% of absorbed iron in the absence of vitamin C but, after adding 100 or 200 mg of this vitamin, there is an increase in iron absorption of 5% or 8%, respectively, (Hurrell, 1997).

In infant formulas, the qualitative choice of macronutrients, especially protein source, depends on its final purpose, either to wean healthy infants or for specific physio-pathological conditions, not excluding, in both cases, the need to provide other nutrients in appropriate amounts. This is particularly true if the protein source is casein, due to the greater amount of calcium. Therefore, other calcium or iron sources and/or adjustments of their proportions should be tested in order to minimize negative effects on iron dialysability without compromising calcium supply (Kennefick & Cashman, 2000). Likewise, because of the vitamin C action mechanism on bioavailable iron, it would be sensible for infant formulas to have better ratios among these micronutrients even though it is difficult to determine such contents, besides other difficulties related to food technology. The results allow us to conclude that infant formulas in which protein sources are casein are casein plus whey protein should present Ca:Fe and Fe:Vitamin C ratios that allow greater iron dialysability.

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