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Availability of iron from milk-based formulas and fruit juices containing milk and cereals estimated by *in vitro* methods (solubility, dialysability) and uptake and transport by Caco-2 cells

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Abstract

Iron solubility, dialysability and transport and uptake (retention + transport) by Caco-2 cells as indicators of iron availability have been estimated in the *in vitro* gastrointestinal digests of infant foods (adapted, follow-up and toddler milk-based formulas and fruit juices containing milk and cereals (FMC)). Low correlation coefficients (in all cases *R*-squared $\leqslant$ 37.1%) were obtained between iron solubility or dialysability versus transport or uptake efficiency – a fact emphasizing the importance of incorporating Caco-2 cell cultures to *in vitro* systems in order to adapt the conditions to those found in *in vivo* assays. The highest uptake efficiency corresponded to FMC (25.6–26.1%) and toddler formulas (32.1–41.9%), the samples with the highest ascorbic acid contents and ascorbic acid/iron molar ratios. In addition, the toddler formulas contained caseinphosphopeptides with the cluster sequence SpSpSpEE, representing the binding site for minerals. In adapted formulas, greater iron uptake efficiency was obtained for the formulation containing ferrous lactate (22.7%) versus ferrous sulfate (4.7%).

Keywords: Iron bioavailability; Infant foods; Simulated digestion; Caco-2 cells

1. Introduction

Iron deficiency is the most common nutrient deficiency in the world, and anaemia is the most prevalent nutritional health problem among children in developing and developed countries. Iron supplementation of infant foods is one way of increasing the iron content in the diet of growing infants although, in many such children, iron deficiency anaemia develops mainly as the result of inappropriate supplies of absorbable iron. It is therefore important to know whether the iron in infant foods is bioaccessible and bioavailable (Sarriá & Vaquero, 2004).

The first step toward bioavailability is represented by solubility within the intestinal tract (bioaccessibility) for subsequent absorption (Salovaara, Sandberg, & Andlid, 2002). In infant foods, iron is mainly found the form of non-heme iron, which has a strong tendency to interact with other food or meal components. On average, only about 7% of ingested non-heme iron is absorbed (Carpenter & Mahoney, 1992). The iron of the common non-heme pool is subject to the interplay of promoter factors, such as mucin, organic acids (e.g., ascorbic acid) and certain amino acids, compounds that can form soluble complexes with iron ions, and inhibitory factors, such inositol hexaphosphate and polyphenols, yielding insoluble complexes with iron and thus rendering the latter non-absorbable (Salovaara et al., 2002).

For the evaluation of iron bioavailability in infant foods, *in vitro* methods are a good alternative to *in vivo* techniques, and generally consist of simulated gastrointestinal digestion, followed by determination of how much of the iron is soluble (Bermejo et al., 2002; Sarriá &

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Vaquero, 2001) or dialyses through a membrane of a certain pore size (Bosscher et al., 2001; De Souza, Colli, & Silverio, 2005; Drago & Valencia, 2004; García, Alegría, Barberá, Farré, & Lagarda, 1998; Sarriá & Vaquero, 2001). The element solubility or dialysability can be used to establish trends in the bioavailability or relative bioavailability values of iron. These *in vitro* methods have been improved by incorporating a human colon carcinoma cell line (Caco-2) that exhibits many of the functional and morphological properties of mature human enterocytes (Pinto et al., 1983). The system is thus able to mimic and estimate the uptake and/or transport of mineral elements, and has been used to assess iron uptake from infant formulas (Etcheverry, Wissler, Wortley, & Glahn, 2004a; Glahn, Lai, Hsu, Thompson, & Guo, 1998; Jovaní, Barberá, Farré, & Martín de Aguilera, 2001; Jovaní, Viadel, Laparra, Barberá, & Farré, 2004) and fruit juices (Boato, Wortley, Liu, & Glahn, 2002; Yeung, Glahn, & Miller, 2003). To our knowledge, the transport by Caco-2 cells of iron contained in infant foods has not been investigated to date.

The aim of the present study was to evaluate the availability of iron from different infant foods, based on three parameters: solubility, dialysability and a model that combines *in vitro* digestion and iron retention and transport by Caco-2 cells.

2. Materials and methods

2.1. Samples

Totally different samples have been studied:

- Two milk-based adapted infant formulas with the same composition except for the iron salt used for enrichment: ferrous sulphate (A^s) or ferrous lactate (A^l).
- Two follow-up infant formulas, one with added *Bifidobacterium bifidum* and *Bifidobacterium longum* (FB), and the other without such addition (F).
- Two toddler formulas, one likewise containing *Bifidobacterium bifidum* and *Bifidobacterium longum* (TB), and the other without added bacteria (T).
- Three fruit juices containing different juice proportions (51–55%), of skimmed milk (6%) and cereals (1%) (FMC), intended for infants and young children (FMC^1 = pineapple and banana, FMC^2 = peach and apple, and FMC^3 = grape, orange and banana).

Samples were kept in their unopened vacuum commercial package (N_2/CO_2, <3% O_2, modified atmosphere) at 25 °C and protected from exposure to light until analysis. The compositions of the aforementioned samples are shown in Table 1.

2.2. Material and reagents

Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis MO, USA): pepsin (Porcine: cat no. P-7000), pancreatin (Porcine: cat. no. P-1750) and bile extract (Porcine: cat. no. B-8631). The working dissolutions of these enzymes were prepared immediately before use.

Iron standard solutions were prepared immediately before use by dilution with distilled deionized water of a standard solution of 1000 mg/l ($FeCl_3$ in 15% HCl, Titrisol, Merck, Barcelona, Spain).

Transport buffer contained 130 mM NaCl (Merck), 10 mM KCl (Merck), 1 mM $MgSO_4$ (Sigma Chemical Co.), 50 mM HEPES (Gibco, Scotland), 5 mM glucose (Sigma Chemical Co.) and pH = 7. The transport buffer was incubated at 37 °C until starting the assay.

All reagents used were of reagent grade, and Millipore-Milli Q distilled-deionised water (Millipore Ibérica S.A.; Barcelona, Spain) was used throughout the experiments.

For iron determination, glass and polyethylene material were washed with detergent, soaked with concentrated nitric acid (sp. gr. = 1.41), and rinsed three times with distilled-deionised water before use.

For vitamin C determination, the reagents used were 2,6-dichloroindophenol, ascorbic acid, acetic acid and $NaHCO_3$ from Merck, and metaphosphoric acid from Sigma Chemical Co. To test interference of reducing ions (such as ferrous iron and cuprous copper), methylene blue (Merck) was used.

Table 1
Energy value and protein, fat, carbohydrate and iron contents of the analyzed samples, referred to 100 ml ready-to-eat sample (manufacturer supplied data)

	$A^{s,la}$	F, FB[a]	T	TB[a]	FMC^1	FMC^2	FMC^3
Energy (kcal)	68	65	65	61	60	57	56
Protein (g)	1.5	2.0	2.6	1.9	0.6	0.4	0.5
Casein (g)	0.7	1.0	1.7	1.2	0.5	0.3	0.4
Fat (g)	3.8	3.3	2.5	2.6	0.1	0.1	0.1
Carbohydrates (g)	7.1	7.0	8.0	7.7	14.3	13.7	13.5
Lactose (g)	7.1	3.5	8.0	2.5	1.3	1.2	1.2
Maltodextrins (g)	0	3.5	0	5.2	0	0	0
Fe (mg)	0.8	1.3	1.3	1.2	–	–	–

A: adapted formula (supplemented with [s]ferrous sulphate or [l]ferrous lactate), F: follow-up formula, FB: follow-up formula with bifidobacterium, FMC: juice + cereals + milk ([1]pineapple and banana, [2]peach and apple, [3]grape, orange and banana), T: toddler formula, TB: toddler formula with bifidobacterium.

[a] Powered samples were reconstituted according to the manufacturer instructions (13% w/v).