Availabilities of Calcium, Iron, and Zinc From Dairy Infant Formulas Is Affected by Soluble Dietary Fibers and Modified Starch Fractions

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OBJECTIVE: Insoluble dietary fiber is a known inhibitor of mineral absorption, whereas the effects of soluble dietary fibers (including prebiotics) are less known. The aim was to study calcium, iron, and zinc availabilities from dairy infant formulas supplemented with soluble dietary fibers and modified starches in vitro.

METHODS: Dairy infant formulas were supplemented with soluble dietary fibers (3%, dry wt) and modified starches (16% pregelatinized rice starch and 1.9% maltodextrin, dry wt) and kept in a well-controlled and defined environment in vitro. Pooled mature human milk was used as the reference standard.

RESULTS: Calcium availability from standard formula was elevated by 30% after inulin supplementation (17.2%), whereas locust bean gum (11.9%) and high esterified pectin (11.7%) reduced availability by approximately 10%. Iron availability from standard formula was increased by pregelatinized rice starch (3.8%), whereas availability was reduced in the following order: high esterified pectin (2.3%), oligofructose (2.2%), and low esterified pectin (2.1%). Zinc availability was highest after the addition of pregelatinized rice starch (13.5%) but lowest with the addition of locust bean gum (6.8%) and maltodextrin (5.4%).

CONCLUSIONS: This study showed that addition of soluble dietary fiber affects calcium, iron, and zinc availabilities in positive (inulin) and negative ways, depending on the type of the dietary fiber used. *Nutrition* 2003;19:641–645. ©Elsevier Inc. 2003

KEY WORDS: bioavailability, calcium, iron, soluble dietary fiber, zinc

INTRODUCTION

Supplementing dietary fiber fractions to foods is gaining increasing popularity because of their thickening, gelling, and stabilizing properties, which make them particularly interesting for technologic reasons. Their potential health benefits may prevent some major typical Western diseases. Evidence is indeed increasing concerning the beneficial effects of fructooligosaccharides (fructans of the chicory root), which may selective stimulate bifidobacterial growth, thus supporting their recognition as prebiotics.[1] However, non-digestible carbohydrates have been shown to impair the absorption of minerals and trace elements in the small intestine because of their binding and/or sequestering effects.[2,3] Therefore, concerns that dietary fiber may be detrimental to infants have risen. Dietary fiber fractions differ largely in their abilities to affect mineral and trace element bioavailability. The major determining factors are the number of ionizable functional groups, such as free hydroxyl groups and carboxyl groups (uronic acid content), and their physical structures during small intestinal digestion. Pectin is primarily a polymer of $\beta 1 \rightarrow 4$–linked D-galacturonic acid monomers that are usually esterified to various degrees with methanol. In solutions, the carboxyl groups of the unesterified units can form complexes with polyvalent metals. Inulin-type fructans are composed of D-fructofuranoses attached by $\beta 2 \rightarrow 1$ linkages. The product, with a degree of polymerization from 2 to 60, is labeled as inulin, whereas oligofructoses have a degree of polymerization below 10 and are produced by partial enzymatic hydrolysis of inulin. Dietary fiber, including dietary fructans, escapes digestion in the small intestine and is degraded by enzymes of the ileal colonic bacteria to produce by fermentation volatile fatty acids, the gases CO_2, H_2, and CH_4. Approximately 86% to 88% of the ingested doses of inulin and oligofructose were found in the effluent of ileostomy patients,[4] which was in the same order as the recovery of pectin.[5] During fermentation, elements may be released from the fiber molecule; as a result, one might consider the lower part of the digestive tract as a putative site for mineral absorption.[6] However, clear evidence from human studies is lacking, with the exception of calcium.[7]

By use of an optimized and in vitro–validated continuous flow dialysis model,[8] the availabilities of calcium, iron, and zinc from infant formulas supplemented with soluble dietary fiber fractions, such as inulin, oligofructose, high and low esterified pectins, locust bean gum, xanthan gum, and modified starches (rice and corn starches, maltodextrin), was studied in a well-controlled and defined environment. To our knowledge, this was the first time the effect of xanthan gum on calcium, iron, and zinc availabilities was investigated, although its use is legally allowed in Europe (Bel-

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gium) for products based on amino acids and proteins for infants with maldigestion.

TABLE I.

CALCIUM, IRON, AND ZINC CONTENTS OF HUMAN MILK AND INFANT FORMULAS*

Feeding	Ca (mg/dL)	Fe (mg/dL)	Zn (mg/dL)
Human milk	36 ± 0	0.03 ± 0.00	0.3 ± 0.0
Standard infant formula	88 ± 2	1.33 ± 0.02	0.99 ± 0.01
MRS (2.3 g/dL)	75 ± 2	1.41 ± 0.10	1.05 ± 0.05
MLD (0.3 g/dL)	77 ± 1	1.27 ± 0.04	0.75 ± 0.01
MLD (0.3 g/dL) + OF (0.4 g/dL)	73 ± 1	1.23 ± 0.04	0.73 ± 0.02
LBG (0.4 g/dL)	110 ± 1	1.36 ± 0.06	0.90 ± 0.01

* Data are expressed as content/dL (calculations based on normal reconstitution of infant formula powder with water: 3 spoons/85 mL water = 1 dL).

Values are given as mean ± standard deviation ($n = 4$).

LBG, locust bean gum; MLD, maltodextrin; MRS, modified rice starch; OF, oligofructose

MATERIALS AND METHODS

Dietary Fiber Fractions

Highly esterified (>65%) citrus pectin (>74% galacturonic acid; Unipectine BRUN NF, E 440) and low esterified (26% to 31%) pectin (>65% galacturonic acid; Unipectine OF 605, E440) were obtained from SKW Biosystems (Brussels, Belgium). Purified xanthan gum (Satiaxane CX 91, E415) was kindly provided by the same manufacturer. Highly purified inulin (>99.5% on dry solids; degree of polymerization ≥23, Raftiline) and oligofructose (≥93.2% on dry matter; <6.8% glucose, fructose, and sucrose; >99.5% carbohydrates; Raftilose P95) were purchased from Orafti Active Food Ingredients (Tienen, Belgium). Nutilis contained 90 g of polysaccharides (modified corn starch, E1442), less than 0.5 g of proteins, less than 0.15 g of lipids, and 0.2 g of sodium/100 g of formula (Nutricia, Zoetermeer, The Netherlands).

Human Milk and Infant Formulas

Pooled mature human milk was obtained. The standard infant formula was cow's milk based on and purchased from the manufacturer (Nutricia, Bornem, Belgium). Infant formulas thickened with locust bean gum (E410; Nutricia), pregelatinized rice starch (amylopectin; Mead Johnson, Brussels, Belgium), or maltodextrin and maltodextrin with oligofructose (Orafti Active Food Ingredients) were provided by the suppliers. The calcium, iron, and zinc contents of the human milk and infant formulas are shown in Table I. Calcium, iron, and zinc concentrations were measured with flame atomic absorption spectrometry in our laboratory.

Sample Preparation

Isolated dietary fiber fractions were added to the standard infant formula, and the mixed samples were homogenized. All samples were formulated to contain 3% dietary fiber on a dry weight basis. The formulas thickened with starch fractions contained 16% rice starch and 1.9% maltodextrin on a dry weight basis.

Chemicals and Reagents

Deionized water (MilliQ, Millipore, Bedford, MA, USA) was used throughout the study. All chemicals and reagents (Merck, Darmstadt, Germany) were of analytical grade, except for nitric acid and H_2O_2, which were suprapure. Pepsin (P-7000, porcine stomach mucosa) and bile salts (B-8631, porcine) were purchased from Sigma (St. Louis, MO, USA), and pancreatin (107133 0500, porcine) was obtained from Merck. According to the manufacturer's information, the activity of the pepsin powder was 1015 ΔA_{208}-U/mg of protein. The pancreatic preparation consisted of a mixture of 1 400 FIP-U protease/g, 300 000 FIP-U amylase/g, and 24 000 FIP-U lipase/g. Twenty grams of pepsin powder was dissolved in 100 mL of 0.1 M HCl. The pancreatic and bile mixture contained 5.6 g of pancreatin and 2.1 g of bile in 100 mL of 0.1 M $NaHCO_3$.

Equipment

Dialysis tubing with a molecular weight cutoff of 10 to 12 kDa (Visking 9 to 36/32) was obtained from Medicell Ltd. (London, UK). Stirred cells (model 8200; 200 mL) and related equipment were purchased from Amicon (Beverly, MA, USA). All cells were washed with detergents, soaked in 10% (v/v) HNO_3, and throughout the study rinsed with deionized water before use. Dialysis membranes (molecular weight cutoff, 1000 Da; Amicon) were soaked in 0.1 M NaOH and washed three times with deionized water before use.

Experimental Methods

Samples were subjected to acid destruction (HNO_3) under high pressure and temperature to analyze calcium, iron, and zinc contents. Approximately 0.4 g of the sample (dry weight) was weighed in a 50-mL Teflon vial (polytetrafluoroethylene) of a polypropylene vessel. Bidistilled water (1 mL), H_2O_2 (suprapure, 30%, 500 μL) and HNO_3 (suprapure, 65%, 2 mL) were added, and the destruction was performed in a microwave digestion oven.[9] Four replicates were taken throughout the entire destruction process for every mixed sample. Calcium, iron, and zinc contents of the fibers and starch fractions were measured in duplicate.

The calcium, iron, and zinc availabilities were evaluated in vitro with the use of a continuous-flow dialysis method with a preliminary intraluminal digestive phase adapted to the upper gastrointestinal tract of infants; the procedure is described in detail elsewhere.[8] Briefly, the method consisted of two phases: gastric and intestinal. Before gastric digestion with pepsin, the sample pH was adjusted to 2.0 with HCl (6.0 M). The sample was then incubated in a shaking water bath (37°C, 120 strokes/min) for 2 h to allow pepsin digestion. Titratable acidity was measured in an aliquot of the gastric digest (4 × 12.5 g), to which 15 g of freshly prepared pancreatin–bile mixture was added. The number of equivalents of NaOH required to titrate the amount of digest to pH 7.5 was calculated. Intestinal digestion was performed in an Amicon cell equipped with a dialysis membrane (molecular weight cutoff, 1000 Da). A dialysis bag containing an amount of $NaHCO_3$ (1 M) equivalent to the titratable acidity was added to the cell. Pressure inside the cell was adjusted to 50 psi (3.5 bar at 37°C) by using oxygen-free nitrogen. Initial dialysis was set for 30 min, after which 15 mL of the pancreatic–bile mixture was added to the digest. The dialysis was continued for an additional 2 h, during which dialysate fractions were collected. Reagent blanks were analyzed for calcium, iron, and zinc contents to correct for contamination from reagents, equipment, or enzymes.

Analytical Techniques

Calcium, iron, and zinc contents of the solutions were analyzed by flame atomic absorption spectrometry (AAnalyst 300, Perkin-

Elmer, Norwalk, CT, USA). Standard stock solutions (1000 ± 2 mg/L) were used for preparing standard calibration solutions of calcium, iron, and zinc. Calibration curves ranged from 0 to 2 mg/L of Ca, 0 to 1.0 mg/L of Fe, and 0 to 0.4 mg/L of Zn. Calcium concentrations in the solutions were measured by using the addition of calibrates. Iron and zinc were determined by the method of addition. Due to low iron concentrations in the dialysates, electrothermal atomic absorption spectrometry was used (4100 ZL, Perkin-Elmer). A 4-point calibration curve in the calibration range of 0 to 20 μg/L of Fe was constructed in 20% HNO_3. Concentrations were measured by the addition of calibrate after dilution of the samples in 20% HNO_3.

Calculations

The availability of calcium, iron and zinc was calculated from the amount in the dialysate (corrected for blank) in proportion to the total elemental content of the mixed sample, according to the following equation:

$$\text{availability (\%)} = [(D - Bl)/(W \times A)] \times 100\%$$

where D is the amount (μg) of micronutrient in the dialysate after intraluminal digestion, Bl is the amount (μg) of micronutrient in the blank dialysate after digestion, W is the dry weight (g) of the food sample used for intestinal digestion, and A is the micronutrient concentration (μg/g) in the mixed sample.

Validation Criteria of the Availability Procedure

Verification of the analytical methods was determined by recovery tests on standard infant formula and aqueous solutions, which contained 25 mM of Ca, 0.27 mM of Fe, and 0.15 mM of Zn. Recovery was calculated by the amounts of calcium, iron, and zinc found in the dialysate and retentate fractions after digestion. Recovery from the standard formula consisted of 95% ± 3 Ca, 117% ± 5 Fe, and 85% ± 2 Zn. From the aqueous solutions, recovery consisted of 94% ± 10 Ca, 114% ± 6 Fe, and 107% ± 5 Zn. Repeatability of the technique was tested on standard infant formula from four replicates over 24 h (intrabatch precision). Repeatability (coefficient of variation) values were 2.43% for Ca, 2.22% for Fe, and 5.01% for Zn.

Statistical Analysis

One-way analysis of variance procedures were applied by using Sigma Stat (SPSS, Cary, NC, USA). All values are presented as means ± standard deviations. A probability level of $P \leq 0.05$ indicated statistical significance.

RESULTS

Calcium, iron, and zinc contents of human milk and the standard infant formula are shown in Table I. Table II lists the calcium, iron, and zinc contents of the dietary fiber fractions. Calcium, iron, and zinc concentrations of the mixed samples (25% to 75% percentiles) ranged from 86 to 90 mg of Ca/dL, 1.41 to 1.69 mg of Fe/dL, and 0.91 to 1.10 mg of Zn/dL.

The availabilities of calcium, iron, and zinc from human milk, standard infant formula, and mixed samples are summarized in Table III. The availabilities in human milk were 19.6% ± 1.1 Ca, 30.2% ± 2.0 Fe, and 48.2% ± 4.1 Zn. Availabilities from the standard formula were 13.5% Ca, 3.4% Fe, and 9.3% Zn.

Calcium availability from inulin supplemented formula (17.2% ± 0.8) was significant higher than that from formulas thickened with locust bean gum (11.9% ± 0.8) or high esterified pectin (11.7% ± 0.7; $P < 0.05$). No differences were found between any of the other formulas.

TABLE II.

CALCIUM, IRON, AND ZINC CONTENTS OF DIETARY FIBER FRACTIONS*

Dietary fiber fraction	Ca (mg/g)	Fe (μg/g)	Zn (mg/g)
MCS (E1442, Nutilis)	0.15 ± 0.01	0.79 ± 0.02	0.01 ± 0.00
OF (Raftilose)	0.10 ± 0.00	1.65 ± 0.07	<LOQ
INU (Raftiline HP)	0.06 ± 0.00	1.40 ± 0.14	<LOQ
XG (E415, Satiaxane)	1.74 ± 0.08	19.0 ± 1.51	0.49 ± 0.02
LE pectin (E440, Unipectine)	1.27 ± 0.03	109 ± 1	0.71 ± 0.07
HE pectin (E440, Unipectine)	2.25 ± 0.09	31.6 ± 1.9	0.77 ± 0.10

* Values are given as mean ± standard deviation ($n = 2$).

LOQ, limit of quantification; HE, high esterified; INU, inulin; LE, low esterified; MCS, modified corn starch; OF, oligofructose; XG, xanthan gum.

Iron availability from rice starch formula (3.8% ± 0.4) was significantly higher than that from formulas supplemented with corn starch (2.7% ± 0.4), inulin (2.5% ± 0.5), maltodextrin (2.5% ± 0.2), maltodextrin plus oligofructose (2.5% ± 0.3), high esterified pectin (2.3% ± 0.5), oligofructose (2.2% ± 0.4), or low esterified pectin (2.1% ± 0.1; $P < 0.05$). Iron availability from rice starch formula was similar to that from the xanthan gum formula (2.9% ± 0.6) and the standard formula (3.4% ± 0.1; $P > 0.05$). The availability of iron from the standard formula was significantly reduced by indigestible carbohydrates in the following order ($P < 0.05$): high esterified pectin, oligofructose, and low esterified pectin.

Zinc availability was highest from the rice starch formula (13.5% ± 1.2) and significantly lower from formulas with locust

TABLE III.

CALCIUM, IRON, AND ZINC AVAILABILITIES*

	Ca (%)	Fe (%)	Zn (%)
Human milk	19.6 ± 1.1	30.2 ± 2.0	48.2 ± 4.1
Standard infant formula	13.5 ± 0.3	3.4 ± 0.1^{B}	9.3 ± 0.5
Thickened formulas			
MRS (2.3 g/dL)	14.6 ± 0.6	3.8 ± 0.4^{A}	13.5 ± 1.2^{A}
MLD (0.3 g/dL)	14.1 ± 0.7	2.5 ± 0.2^{a}	5.4 ± 0.7$^{a, b}$
MLD (0.3 g/dL) + OF (0.4 g/dL)	12.9 ± 0.8†	2.5 ± 0.3†$^{a, b}$	5.9 ± 1.1†^{a}
LBG (0.4 g/dL)	11.9 ± 0.8^{a}	3.0 ± 0.4^{C}	6.8 ± 0.4^{a}
Standard formula + dietary fiber fractions			
MCS (0.4 g/dL)	15.9 ± 1.9	2.7 ± 0.4^{a}	9.1 ± 1.0
OF (0.4 g/dL)	16.7 ± 1.3	2.2 ± 0.4^{a}	9.4 ± 1.2
INU (0.4 g/dL)	17.2 ± 0.8^{A}	2.5 ± 0.5†^{a}	12.2 ± 1.1^{B}
XG (0.4 g/dL)	16.2 ± 2.3	2.9 ± 0.6†	11.3 ± 2.1†
LE pectin (0.4 g/dL)	14.2 ± 1.4	2.1 ± 0.1$^{a, b}$	7.0 ± 0.7
HE pectin (0.4 g/dL)	11.7 ± 0.7^{a}	2.3 ± 0.5†$^{a, b, c}$	8.4 ± 0.9†

* Values are presented as mean ± standard deviation ($n = 4$). The same superscript letters in each column indicate statistically significant differences ($P < 0.05$, multiple comparisons with one-way analysis of variance).

† $n = 3$.

HE, high esterified; INU, inulin; LBG, locust bean gum; LE, low esterified; MCS, modified corn starch; MLD, maltodextrin; MRS, modified rice starch; OF, oligofructose; XG, xanthan gum bean gum (6.8% ± 0.4), maltodextrin plus oligofructose (5.9% ± 1.1), and maltodextrin (5.4% ± 0.7; $P < 0.05$). No differences were found between any of the other formulas.

DISCUSSION

From the results in this in vitro model, it appears that the conditions provided by mature human milk are optimal for the availabilities of calcium, iron, and zinc. Soluble dietary fiber affects calcium, iron, and zinc availabilities in positive and negative ways, depending on the type of dietary fiber used.

The use of isolated dietary fiber fractions rather than of whole foods was based on several considerations: First, the composition of unrefined fiber varies greatly from food to food. The different proportions of the fiber fractions may influence availability differently. Moreover, the properties of fiber can change markedly with time. Second, high-fiber foods often contain large amounts of micronutrients or are currently supplemented when manufactured for consumption by infants (e.g., infant cereals). The increased micronutrient content may account for conflicting results across studies.[3,10,11] Third, foods with high-fiber content often contain enhancers and inhibitors of micronutrient absorption (phytic acid) whose effects may exceed those of dietary fiber.[12] Using infant formula partly excludes those uncontrolled interferences because the nutrients that are present are well characterized.

Bioavailability can be described as that portion of a nutrient that can be used. This means that any potentially available part of a nutrient after gastrointestinal digestion should be attributed to its availability. However, measuring the release and solubilization of micronutrients in vitro does not necessarily represent the efficiency with which they will be translocated by the intestinal mucosa and used for metabolic functions. Both measurements are also partly dependent on physiologic factors. Therefore, in vitro techniques are particularly useful to predict the release of micronutrients from meals and to identify underlying food factors that affect bioavailability without considering host-related influences.

The present study showed that addition of inulin to standard formula increases the availability of calcium from 13.5% (±0.3) to 17.2% (±0.8). Although this represented an increase of 27%, it was not significant from the one-way analysis of variance on rank (Dunn's post test). Inulin did not alter iron and zinc availabilities, but oligofructose significantly lowered iron availability. The findings with regard to inulin correspond well with in vivo data. Coudray et al.[13] found that 40 g/d of inulin as a dietary supplement increases the apparent absorption and balance of calcium in men, without adversely affecting iron and zinc. Ellegärd et al.[14] reported that supplementation of the diet with 17 g/d of fructooligosaccharides does not impair mineral absorption in ileostomy patients. Similarly, van den Heuvel et al.,[15] using dual stable isotopes, demonstrated that ingestion of 15 g/d of inulin or oligofructose does not interfere with absorption of calcium or iron. In a subsequent study, the researchers demonstrated increased calcium absorption after oligofructose supplementation in adolescent boys.[7] The in vitro methodology provides conditions (e.g., pH, enzymatic, and mechanical) that closely simulate the intraluminal conditions of the upper part of the gastrointestinal tract. From the data obtained in this in vitro study, we may assume that the enhancing effect of inulin on calcium bioavailability in vivo, which takes place during the fermentation process (colonic phase), may already start during the initial phase of calcium absorption (upper intestine).

The present study showed that, in the presence of high esterified pectin, the availability of calcium is lowest when compared with the other formulas, but this was not significant. Other in vitro studies showed that pectin reduces calcium release after enzymatic digestion of skimmed milk. After fermentation, however, pectin does not affect total calcium release, which may indicate that colonic fermentation of dietary fiber releases calcium for potential absorption in the colon.[16] Addition of 36 g/d of pectin to the diet of adults does not affect calcium balance.[17] It is a well-known phenomenon that non-digestible carbohydrates (e.g., pectins) are fermented in the colon, thereby releasing their bound mineral and trace elements. Because colonic absorption of minerals and trace elements is possible, with the only scientifically proven element actually absorbed being calcium, colonic fermentation may be associated with enhanced calcium absorption. Consequently, the in vitro results on calcium availability in the presence of pectin may underestimate actual calcium bioavailability in vivo. However, they provide insights about the influence of pectin on calcium absorption in the upper part of the intestine.

The present study showed that both types of pectin appreciably reduce iron availability, whereas the effect of low esterified pectin was more pronounced. Addition of 0.42 g of low esterified pectin to 1 dL of formula reduced availability by 37%, from 3.4% (±0.7) to 2.1% (±0.1). These findings correspond with those of in vitro data from Platt and Clydesdale[18] and in vivo studies. Monnier et al.[19] found a significant decrease (46%) in fractional iron absorption after ingestion of 9 g of pectin per square meter of body surface in patients with idiopathic hemochromatosis when using double radiotracer techniques. Moreover, they demonstrated that about 83.6% to 92.3% of the iron is bound by pectin. In this study, pectin did not influence zinc availability, which was similar with in vivo data. Drews et al.[20] reported that addition of 14.2 g/d of pectin does not affect zinc retention in adolescent boys.

The present study showed that addition of locust bean gum decreases zinc availability by 27%, from 9.3% (±0.5) to 6.8% (±0.4), although this was not significant. Identically, locust bean gum reduced calcium and iron availabilities, but not significantly. Previous studies by our research group demonstrated that locust bean gum is a potent binder of calcium and, especially iron and zinc, under conditions approximating those in the small intestine,[21] and therefore may reduce calcium, iron, and zinc availabilities.[2,8] Formula thickened with xanthan gum showed no effect on the availabilities of calcium, iron, and zinc.

This study showed that addition of modified starch fractions increases calcium, iron, and zinc availabilities. This was the subject of a previous study performed in our laboratory.[8] The low zinc availability from the maltodextrin-supplemented formulas was unexpected and might be due in part to their low zinc content.

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