Effect of Gastric Digestion pH on Iron, Zinc, and Calcium Dialyzability from Preterm and Term Starting Infant Formulas

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ABSTRACT: Iron, zinc, and calcium dialyzability from preterm and term starting infant formulas were determined after in vitro digestion, using special gastric conditions prevailing in preterm and newborn infants. Mineral dialyzability was studied using pH 2.0, 3.5, and 4.5 for gastric digestion. The effect of gastric pH was more important on iron dialyzability (FeD) and zinc dialyzability (ZnD) than on calcium dialyzability (CaD). The effect on iron dialyzability was remarkable in fluid formulations with high digestibility: FeD was 18% to 20% when gastric digestion was made at pH 2, decreasing to 3% when made at pH 4.5. In most powder infant formulas with the lowest digestibility, FeD remained close to 10% despite variations in gastric digestion pH. Percent zinc dialyzability (ZnD%) steadily decreased when gastric digestion pH increased. At each pH, percent iron dialyzability (FeD%) and ZnD% from human milk were higher than those from infant formulas. Evaluation of mineral dialyzability from these infant formulas, using a gastric digestion pH prevailing in preterm and newborn infants, can provide valuable information on mineral availability.

Keywords: iron, zinc, mineral dialyzability, gastric pH, infant formulas

Introduction

New infant formulas are regularly introduced in the market. It is assumed that they provide adequate amounts of minerals and trace elements but, in fact, information about their mineral bioavailability is scarce (Lönnerdal and others 1994). In preterm infants, gastric acid secretion is lower than in term infants (Armand and others 1996). Also, gastric pH in newborns increases after food ingestion and remains higher than in children and adults (Hamosh 1996). Because pH 2 is optimum for pepsin activity, low activity is shown in preterm and term infants. These gastric conditions could affect mineral bioavailability from infant formulas.

Skikne and others (1981) observed lower iron absorption in patients with achlorhydria, compared with subjects with normal stomach acid secretion. This led to the recognition that gastric acid is essential for optimal non-heme iron absorption.

Regarding the effect of gastric digestion pH on zinc absorption, Allen (1998) suggested that pH could be an important factor in zinc solubility because some insoluble zinc salts can be solubilized in an acid gastric environment. Henderson and others (1995) observed that a gastric pH 5 or higher reduced by 28% the absorption of zinc acetate and by 82% the absorption of zinc oxide, compared with zinc absorption at a gastric pH 3 or lower. However, to our knowledge, there is no information about the influence of gastric pH on zinc absorption from zinc fortified foods.

Sheikh and others (1987), working with several calcium supplements, showed that pH was critical for in vitro solubilization of several calcium salts, such as calcium carbonate, citrate, acetate, lactate, and gluconate. However, even though their in vitro solubility was very different, calcium from various salts was absorbed to the same extent by healthy fasting adults. Knox and others (1991) postulated that gastric acid may be necessary to solubilize the insoluble calcium salts in the fasting state, but it is not required for calcium absorption in the fed state.

Ideally, bioavailability should be evaluated in human studies. However, complexity and cost limit their applicability. Iron dialyzability has been shown to predict iron bioavailability (Luten and others 1996). Results of in vitro tests evaluate only the availability for absorption, meaning that the trace mineral is not insoluble and/or forming a large MW complex. Some authors have shown that Zn dialyzability also has a good correlation with in vivo results and could be useful to predict bioavailability (Shen and others 1994, 1995; García and others 1998). For both iron and zinc, conditions prevailing in the intestinal tract are major determinants of absorption. The assessment of Ca dialyzability has also been proposed to predict bioavailability (Shen and others 1994, 1995; Kennefick and Cashman 2000). For trace elements such as Ca, where complex homeostatic mechanisms regulate not only absorption but also retention, conditions in the intestinal lumen may not be the main factor to regulate bioavailability. However, information on calcium dialyzability is useful to assess the amount of soluble and potentially absorbable Ca.

Hence, a modified in vitro test was used to evaluate the influence of gastric digestion pH on iron, zinc, and calcium dialyzability as a measure of mineral availability from preterm and term starting formulas, and from human milk. This study introduces a gastric phase adapted to the gastrointestinal pH of preterm and newborn infants.

Materials and Methods

L-ascorbic acid, PIPES disodium buffer, dithiothreitol, digestive enzymes, and bile salts were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Metaphosphoric acid was acquired from J.T. Baker (Phillipsburg, N.J., U.S.A.). Spectra/Pore® I dialysis tub-

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ing (cut-off 6000 to 8000) was purchased from Fischer Scientific (Fairlawn, N.J., U.S.A.). Methanol (high-performance liquid chromatography [HPLC] grade) was purchased from Merck (Darmstadt, Germany). All other chemicals were reagent grade.

Cow's milk-based infant formulas (IF) were purchased from the market. Duplicates of the same formula, taken from packages with different lot number, were used for analysis. Three preterm formulas were evaluated: 2 powdered (PP1, PP2) and 1 fluid (PF3), and 3 term starting, 2 powdered (TSP1, TSP2) and 1 fluid (TSF3). Powdered infant formulas were reconstituted according to the manufacturers' instructions. Pooled samples of mature human milk (HM) were studied for comparison. Milk samples were collected during the 1st mo postpartum in the morning, by completely emptying 1 breast, either manually or mechanically. An aliquot was taken and kept in a plastic container at –20 °C until analyzed.

Determination of iron, zinc, and calcium dialyzability

A modification of the in vitro Miller's method (Miller and others 1981), introduced by Wolfgor and others (2002), was used, adjusting the pH of the 0.15 *M* PIPES buffer instead of changing buffer molarity to obtain intestinal pH. In these samples, adjustment of PIPES molarity would cause molarities to be lower than 0.1 *M*, with low buffer capacity. Apart from this, important differences in buffer molarity would occur when studying different gastric digestion pHs. Therefore, the following modified method was used. Aliquots of homogenized samples (50 g) were adjusted to pH 2.0, 3.5, or 4.5 with 6 *N* HCl and, after addition of 1.6 mL pepsin digestion mixture (16% pepsin solution in 0.1 *N* HCl), the samples were incubated at 37 °C during 2 h in a shaking water bath. At the end of pepsin digestion, 2 aliquots of digest (15 g) were weighed in 100-mL beakers. Dialysis bags containing 18.75-mL 0.15 *M* PIPES buffer were placed in each beaker. Buffer pH used for each particular formula was calculated to obtain a final pH of digest-dialysate 6.5 ± 0.2. The main factors taken into account to calculate buffer pH were: buffer capacity of the food matrix (HCl needed to reach pH 2.0, 3.5, or 4.5), HCl mEq incorporated with pepsin solution (0.048 mEq), and acid mEq generated through enzymatic hydrolysis during in vitro digestion. To calculate acid mEq generated by hydrolysis, triplicates of pepsin digest plus bile-pancreatin solution were adjusted to pH 6.5 with 0.1 *N* NaOH, incubated during 120 min at 37 °C, and subsequently titrated to pH 6.5 with 0.1 *N* NaOH. To calculate the buffer pH the following equations were used:

$$[H^+] = Ka \times Ca/Cb \quad (1)$$

where Ca = concentration or mEq of acid ; Cb = concentration or mEq of base; and Ka = constant of acidity
Considering that 18.75 mL of 0.15 *M* PIPES buffer will contribute 2.8125 mEq, and substituting Ca and Cb, it results that:

$$[H^+] = \text{Total mEq} \pm X \quad (2)$$

$$Ka\ (2.8125 - \text{Total mEq}) - X$$

where Total mEq = HCl mEq needed to reach pH 2.0, 3.5, or 4.5 + 0.048 mEq (added with pepsin solution) + acid mEq generated through enzymatic hydrolysis during in vitro digestion, and X = acid mEq that must be provided by the buffer.
Finding the value of X from Eq. 2:

$$X = [f \times 2.8125/(f + 1)] - \text{total mEq}$$

where $f = [10^{(\text{PIPES pKa} - \text{desired final pH})}] = 10^{(6.8 - 6.5)} = 1.995$.

Replacing X in Eq. 3, the pH of 0.15 *M* PIPES buffer is obtained:

$$pH = -\log [Ka.\ X/(2.8125 - X) \quad (3)$$

Aliquots of each pepsin digest with dialysis bags containing PIPES buffer were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin-bile mixture (3.75 mL of 2.5% bile, 0.4% pancreatin solution in 0.1 *N* $NaHCO_3$) was then added to each beaker, and the incubation continued for another 2 h. At the end of the pancreatin-bile incubation, the dialysis bags were removed and rinsed with water. Both digest and dialyzate pHs were checked to be 6.5 ± 0.2. Bag contents were transferred to tared flasks, weighed and analyzed for their iron, zinc, and calcium content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in pepsin digests was made by AAS after wet ashing with HNO_3-$HClO_4$ (50:50). Lanthanum was added to all samples and standards analyzed for Ca to reach a 0.5% final concentration to prevent possible phosphate interference (Varian 1979).

Mineral dialyzability (Fe, Zn, Ca) was calculated from the amount of each dialyzed mineral, expressed as a percentage of the total amount present in each pepsin digest:

$$\text{Dialyzable mineral (\%)} = [D/(W \times A)] \times 100$$

where D is the total amount of each dialyzed mineral (μg); W is the weight of pepsin digest (g); and A is the concentration of each mineral in the pepsin digest (μg/g).

Ascorbic acid

Aliquots of all samples were extracted with 0.85% meta-phosphoric acid (MPA), and evaluated by HPLC (Waters, Milford, Mass., U.S.A.). Assessment was made according to Behrens and Madére (1987) but using dithiothreitol to reduce dehydroascorbic acid. The chromatographic system used a Nucleosil 100-10 C18 reversed-phase column, 25 cm × 4.0-mm inner dia (Macherey-Nagel, Düren, Germany) and a mobile phase of 80 m*M* sodium acetate buffer, pH 4.8, containing 15% methanol and 0.015% MPA. The final pH of the mobile phase was 4.6. A flow rate of 0.9 mL/min was used. Measurements were made with a UV detector at 254 nm. A chromatography station (CSW 1.7, DataApex Ltd., Prague, Czech Republic) was used for measuring peak areas.

Total nitrogen, non-protein nitrogen, and true protein nitrogen

Total nitrogen (TN) was determined by micro-Kjeldahl (AOAC 2000). Non-protein nitrogen (NPN) was separated from soluble proteins by addition of 24% trichloroacetic acid (TCA) and centrifugation at 10000 × *g* for 20 min at 4 °C. NPN was determined by micro-Kjeldahl in TCA supernatant. True protein nitrogen (TPN) was calculated as the difference between TN and NPN. A conversion factor of 6.25 was used to obtain true protein (TP).

Protein digestibility

Protein digestibility was determined according to Rudloff and L önnerdal (1992). Aliquots of 10 or 20 mL of infant formulas were adjusted to pH 4.5, 3.5, or 2.0 using 1 *N* HCl, and pepsin was added at a concentration of 10 mg/sample, so that the enzyme/substrate ratio was between 1/15 and 1/20, depending on the protein content of the infant formula. The samples were kept in the dark on a shaking water bath at 37 °C for 30 min. The pH was then gradually increased to 7.0 (within 10 min) using 0.5 *M* $NaHCO_3$; and 2.5 mL of a pancreatin solution (0.4 g/100 mL 0.1 *M* $NaHCO_3$) were added to each sample and incubated for 1 h at 37 °C. The digested

Table 1—Mineral composition and ascorbic acid content of infant formulas and pooled human milk[a]

Samples[b]	Iron (mg/100 g powder) or (mg/1000 mL)[c]	Zinc (mg/100 g powder) or (mg/1000 mL)[c]	Calcium (mg/100 g powder) or (mg/1000 mL)[c]	Ascorbic acid (mg/100 g powder) or (mg/1000 mL)[c]
PP1	2.54 ± 0.46	4.19 ± 0.20	545.7 ± 16.4	122.92 ± 2.61
PP2	5.00 ± 0.13	5.27 ± 0.24	508.8 ± 14.1	77.28 ± 0.08
PF3	8.52 ± 0.14	6.22 ± 0.19	529.9 ± 8.5	212.30 ± 0.07
TSP1	6.87 ± 0.09	5.32 ± 0.14	304.2 ± 10.4	62.25 ± 0.02
TSP2	10.44 ± 0.32	4.65 ± 0.15	335.7 ± 8.2	84.46 ± 1.35
TSF3	11.18 ± 0.91	5.60 ± 0.16	392.2 ± 22.2	111.66 ± 16.0
HM	0.49 ± 0.09	1.90 ± 0.10	198.90 ± 4.4	25.85 ± 2.71

[a]Values are expressed as means ± standard deviation (n = 4).
[b]HM = human milk; PF = preterm fluid formulas; PP = preterm powdered formulas; TSF = term starting fluid formulas; TSP = term starting powdered formulas.
[c]PP1, PP2, TSP1, TSP2: mg/100 g powder; PF3, TSF3, HM: mg/1000 mL.

Table 2—Total nitrogen (TN), non-protein nitrogen (NPN), true protein (TP), and digestibility of infant formulas[a]

Samples[b]	TN (g/L)	NPN (g/L)	TP (g/L)	NPN/TN (%)	Digestibility (%)
PP1	4.17 ± 0.02	0.34 ± 0.02	23.89 ± 0.02	8.28 ± 0.11	45.0 ± 2.6
PP2	2.58 ± 0.13	0.38 ± 0.01	13.77 ± 0.33	14.71 ± 0.35	82.5 ± 1.6
PF3	3.19 ± 0.08	0.41 ± 0.03	17.40 ± 0.14	12.79 ± 0.38	87.5 ± 2.7
TSP1	3.25 ± 0.07	0.39 ± 0.01	17.89 ± 0.10	12.03 ± 0.10	68.8 ± 1.4
TSP2	3.15 ± 0.02	0.34 ± 0.02	17.56 ± 0.02	10.78 ± 0.31	67.7 ± 0.7
TSF3	2.75 ± 0.10	0.41 ± 0.03	14.66 ± 0.22	14.75 ± 0.35	86.0 ± 1.9

[a]Values are expressed as means ± standard deviation (n = 4).
[b]PP = preterm powdered formulas; PF = preterm fluid formulas; TSF = term starting fluid formulas; TSP = term starting powdered formulas.

samples were immediately placed in boiling water and heated for 4 min to inactivate enzymes. Protein digestibility at each pH was calculated as the increase in NPN (after TCA precipitation of remaining proteins), following pepsin/pancreatin digestion, regarding true protein nitrogen.

$$\text{Digestibility \%} = 100 \times \Delta\,\text{NPN}/(\text{NT} - \text{NPN})$$

Reference materials

Triplicate samples of SRM infant formula 1846 (NIST, Gaithersburg, Md., U.S.A.) were run with each set of unknown samples to validate each batch of analysis for iron, zinc, calcium, and ascorbic acid. Agreement of the triplicate mean for the reference material within 5% of the certified value and less than 5% relative standard deviation for the triplicates were required for acceptance of the data.

Statistical analyses

Each experiment was carried out at least twice, and all analyses were performed in duplicate. The data were analyzed by 1-way ANOVA. A multiple comparison procedure of the treatment means was performed using Fisher's least significant difference (LSD) test. Significance of the differences was defined at $P \leq 0.05$.

Results and Discussion

Mineral composition and ascorbic acid content

All formulas, except PP1, were iron fortified (Table 1). PP1 had an iron content corresponding to a low-iron formula (Raiten and others 1998). Ascorbic acid (AA) was present in a 2.6 to 3 and 5 to 15 molar ratio to Fe in term starting and preterm formulas, respectively. All are adequate levels to enhance iron bioavailability (Stekel and others 1986). Zn fortification of the formulas was quite uniform, ranging from 4.19 to 6.22 mg/L, although the Fe/Zn ratio varied from 0.6 to 2.25. Calcium levels were higher in premature than in term starting formulas because the premature requirements are higher than those of term infants (Kaup 1998; Davidsson 1994). Mineral composition of the pooled human milk was in accordance with the values reported by other investigators (Poiffait and Adrian 1993, 1994).

TN, NPN, TP, NPN/TN, and digestibility of infant formulas

Values of TN, NPN, TP, and NPN/TN (Table 2) were usual for these type of formulas, as informed by Donovan and Lönnerdal (1989). The proportion of NPN, as well as its composition, varies considerably according to the whey source and the level used (Donovan and Lönnerdal 1989). Digestibility of each formula at pH 4.5 was not significantly different from digestibility at pH 2.0 and 3.5 (results not shown). As shown in Table 2, fluid formulas showed higher digestibility than powdered formulas. Among powdered formulas, only PP2 showed high digestibility.

Effect of gastric digestion pH on iron dialyzability

Figure 1 shows that gastric pH influenced percent iron dialyzability (FeD%) in different ways, depending on the infant formula evaluated. In most powder infant formulas with the lowest digestibility (PP1, TSP1, and TSP2), FeD remained close to 10%, despite variations in gastric digestion pH. In PP2, with higher digestibility values, the increase of gastric digestion pH to 3.5 was enough to significantly reduce FeD%. The effect on iron availability was remarkable in fluid formulations with high digestibility. Both fluid formulations (PF3, TSF3) had the highest FeD% at pH 2 (18% to 20%). However, there was a remarkable decrease when gastric digestion was made at pH 3.5 (FeD% 5), and a minimum of 3% was found when made at pH 4.5. FeD% from human milk suffered a minor decrease when digested at pH 3.5, but when using a gastric digestion pH of 4.5, it was reduced to half. Nevertheless, FeD% from human milk remained high at this pH (15%). At each pH, FeD% was higher from human milk than from infant formulas.

Effect of gastric digestion pH on zinc dialyzability

The effect of gastric digestion pH on percent zinc dialyzability (ZnD%) was quite steady, decreasing when the pH increased (Figure 2). PF3 was the most sensitive to the increase of gastric pH, and TSP1 was the least sensitive. At each pH, most of the infant formulas showed lower ZnD% than human milk.

Effect of gastric digestion pH on calcium dialyzability

Figure 3 shows percent calcium dialyzability (CaD%) for different IF at the 3 levels of gastric pH. In PP1, PF3, TSP2, and TSF3, CaD% decreased only when the gastric digestion pH was higher than 3.5. In other infant formulas (PP2, TSP1), even when gastric digestion occurred at pH 4.5, CaD% was not modified. However, in human milk, CaD% decreased significantly with an increasing pH. The values of CaD% in human milk were in a range comparable to those in infant formulas.

Discussion

The major determinant of Fe and Zn bioavailability is the proportion of the nutrient that is absorbed from the gastrointestinal tract. This is greatly influenced by physicochemical and dietary factors in the lumen. The values of Fe and Zn dialyzability from infant formulas were similar to bioavailability data from human studies (Sandström and others 1983; Lönnerdal and others 1984; Hallberg and others 1992a, 1992b; Lönnerdal 1997; Hurrell and others 1998; Ekmekcioglu 2000), supporting the reliability of the procedure. In all cases, FeD% and ZnD% from infant formulas were lower than those from human milk at each considered pH. The lower mineral bioavailability from infant formulas as compared with that from human milk is well documented (Sandström and others 1983; Lönnerdal and others 1984; Hallberg and others 1992a; Ekmekcioglu 2000). In human studies, several authors have reported an inhibitory effect of cow's milk proteins and calcium on iron bioavailability (Hurrell and others 1989; Jackson 1992; Hallberg and others 1992a; Hurrell 1997) and of casein on zinc bioavailability (Sandström and others 1983; Lönnerdal and others 1984; Singh and others 1989; Ekmekcioglu 2000). The inhibitory effect of casein content on Fe and Zn dialyzability has also been reported (Drago and Valencia 2004).

FeD% and ZnD% varied significantly in infant formulas (Figure 1 and 2). This could imply that both food formulation and technological processes influence Fe and Zn availability. Regarding mineral formulation, all formulas had ferrous sulfate and zinc sulfate as iron and zinc sources, respectively. Therefore, the mineral form was not responsible for differences in iron and zinc dialyzability. The AA:Fe molar ratio has been proved to be an important factor to improve iron bioavailability (Gillooly and others 1984), and iron dialyzability (Drago and Valencia 2004). However, ascorbic acid concentration was variable in the assessed infant formulas, but was not related to iron dialyzability (data not shown). Differences in formulation and processing may mask the improving effect of ascorbic acid on iron dialyzability.

Food processing could also lead to changes in the equilibrium of the ions and a rearrangement of ligands with mineral species, thus affecting the interactions of minerals with other dietary components (Watzke 1998). Rudloff and L nnerdal (1992) found that the severity of the heat treatments used during the processing of infant formulas has an influence on lipid-protein and protein-protein interactions. These interactions may affect protein digestion.

The influence of gastric pH on FeD% depended on the infant formula evaluated. To analyze the effect of different pHs during gastric digestion, the diverse roles of HCl in assisting digestion must be considered. HCl denatures food proteins and disrupts intermolecular bonds. It also causes minerals to dissociate from complexes with proteins and other ligands, thereby releasing them

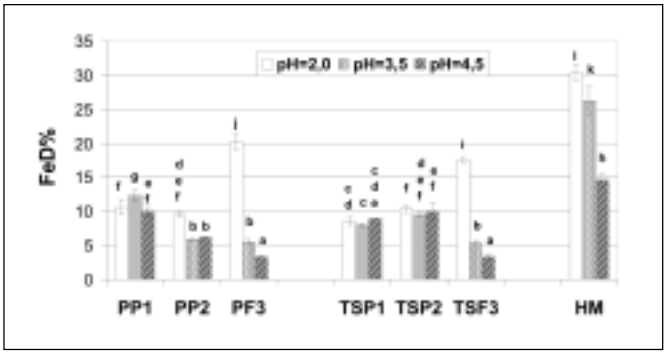

Figure 1—Influence of gastric digestion pH on percent iron dialyzability (FeD%) from infant formulas (PF = preterm fluid formulas; PP = preterm powder formulas; TSF = term starting fluid formulas; TSP = term starting powder formulas) and pooled human milk (HM). Bars with different letters are significantly different ($P < 0.05$).

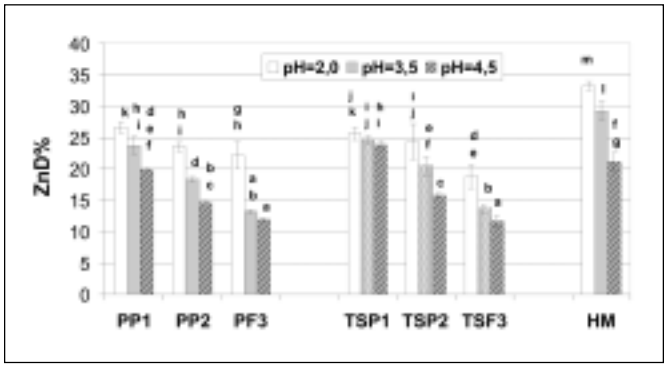

Figure 2—Influence of gastric digestion pH on zinc dialyzability (ZnD%) from infant formulas (PF = preterm fluid formulas; PP = preterm powder formulas; TSF = term starting fluid formulas; TSP = term starting powder formulas) and pooled human milk (HM). Bars with different letters are significantly different ($P < 0.05$).

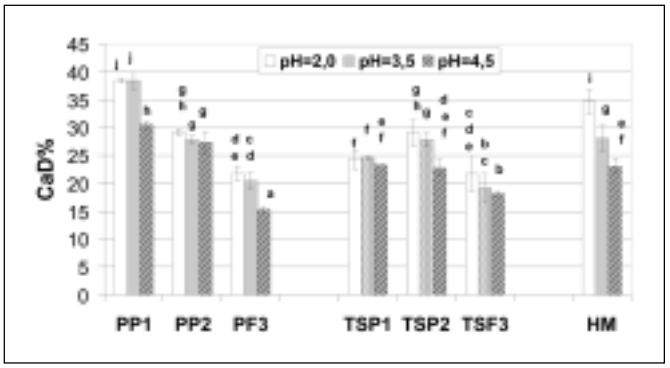

Figure 3—Influence of gastric digestion pH on percent calcium dialyzability (CaD%) from infant formulas (PF = preterm fluid formulas; PP = preterm powder formulas; TSF = term starting fluid formulas; TSP = term starting powder formulas) and pooled human milk (HM). Bars with different letters are significantly different ($P < 0.05$).

into solution. Thus, gastric pH could influence iron, zinc, and calcium solubility. A low pH (pH 1 to 2) also provides the optimal environment for pepsin activity. In our research, protein digestibility of each formula assessed after digestion at different pH levels, (2.0, 3.5, and 4.5) and estimated through the increase in NPN after TCA precipitation of remaining proteins did not differ significantly. However, the characteristics of the peptides obtained at each digestion condition may be quite different.

In 3 of the assessed infant formulas, iron availability was not modified by the increase of gastric digestion pH. The infant formulas less affected by different pHs of gastric digestion were those with the lowest digestibility (PP1, TSP1, TSP2). Even at pH 2, neither the solubilizing effect of acid pH nor the improved conditions for protein digestion, had any effect on iron released from the formulas with low digestibility. The formulas affected by the increase of gastric pH (both UHT-treated fluid formulas and PP2) had the highest digestibility.

Proteins would have different denaturation degree and peptides generated from non-denatured proteins digested at low gastric pH, could bind iron, rendering soluble low-molecular-weight complexes with high dialyzability. Peptides generated at higher gastric pH levels would render insoluble and/or higher MW iron complexes with low dialyzability.

With regard to Zn, the dissociating effect of gastric acidity could be more important than the effect on protein gastric digestion. In casein micelles, 1/3 of zinc is bound to phosphoserine residues, and 1/3 is more tightly bound to colloidal calcium phosphate; the latter is released when pH decreases to 4.6 (Singh and others 1989). In all assessed formulas, ZnD% was higher at pH 2, and decreased gradually with increasing pH, regardless of the processes used in formula elaboration (spray dried or UHT-sterilized). Therefore, it is possible to assume that, in these particular foods, acid pH favors ZnD% through zinc releasing from casein. In this study, we did not observe differences in the behavior of Zn dialyzability between UHT fluid and spray-dried infant formulas. Michel and others (1993) studied the influence of each step during the processing of infant formulas on soluble and lipid-bound calcium and zinc and observed that prewarming the blend at 101 °C causes much calcium and zinc to be bound to the lipid fraction, reducing the solubility of both minerals with respect to skim milk. Final sterilization or spray-drying did not cause additional changes in the solubility of both minerals. This could explain similar ZnD% values from UHT or spray-dried infant formulas.

Regarding calcium dialyzability from infant formulas, the values were similar to bioavailability data from human studies. However, calcium dialyzability levels from human milk were lower than those reported through in vivo studies (Armand and others 1996; L nnerdal 1997; Liu and others 1989). It is difficult to explain the influence of gastric digestion pH on CaD% because different calcium salts and various calcium levels are used in different infant formulas. However, there is some information about the effect of gastric pH on calcium bioavailability. Hirai and others (1992) reported a slow digestion of Ca(II)-α-lactalbumin by trypsin and chymotrypsin. They found that both an acid environment (pH 2.2) in which Ca (II) is released and a partial peptic digestion are necessary for rapid hydrolysis of protein in the gut lumen. In our in vitro studies, the effect of gastric pH was less marked on calcium dialyzability than on iron and zinc dialyzability. Complex homeostatic mechanisms involved in calcium absorption, excretion, and retention make it more difficult to use any in vitro result to estimate calcium bioavailability. Therefore, the small decrease in calcium dialyzability found in infant formulas when using pH 4.5 for gastric digestion may not be relevant from the nutritional point of view.

Conclusions

The influence of gastric digestion pH on mineral dialyzability depends on the infant formula evaluated. Both specific formulation and technological processes of infant formulas modify nutrient interactions, producing different responses to digestive processes, thus affecting mineral availability. The effect of gastric pH is more evident on iron and zinc dialyzability than on calcium dialyzability.

The effect of gastric digestion pH on iron availability is remarkable in fluid formulations. Iron dialyzability in spray-dried infant formulas was less susceptible to changes in gastric digestion pH. Nevertheless, in vitro evaluations of mineral dialyzability in formulas aimed at preterm infants should be performed using a gastric digestion pH similar to that present in these individuals.

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References

Allen LH. 1998. Zinc and micronutrient supplements for children. Am J Clin Nutr 68:495–8.
[AOAC] Assn. of Official Analytical Chemists. 2000. Official methods of analysis 960.52. In: 17th ed. Horwitz W, editor. Washington D.C.: Association of Official Analytical Chemists. I:7.
Armand M, Hamosh M, Mehia NR, çngelus PA, Philpott JR, Henderson TR, Dwyer NK, Lairon D, Hamosh P. 1996. Effect of human milk or formula on gastric function and fat digestion in the premature infant. Pediatr Res 40:429–37.
Behrens WA, Madére R. 1987. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic acid and dehydroascorbic acid in tissues, biological fluids and foods. Anal Biochem 15:102–7.
Davidsson L. 1994. Minerals and trace elements in infant nutrition. Acta Paediatr Suppl 395:38–42.
Donovan SM, Lönnerdal B. 1989. Non-protein nitrogen and true protein in infant formulas. Acta Pediatr Scand 78:497–504.
Drago SR, Valencia ME. 2004. Influence of components of infant formulas on in vitro iron, zinc and calcium availability. J Agric Food Chem 52:3202–7.
Ekmekcioglu C. 2000. Intestinal bioavailability of minerals and trace elements from milk and beverages in humans. Nahrung 44:S390–7.
García R, Alegría A, Barbera R, Farré R, Lagarda MJ. 1998. Dialyzability of iron, zinc and copper of different types of infant formula marketed in Spain. Biol Trace Elem Res 65:7–17.
Gillooly M, Torrance JD, Bothwell TH, MacPhail AP, Derman D, Mills W, Mayet F. 1984. The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. Am J Clin Nutr 40(3):522–7.
Hallberg L, Rossanser-Hultén L, Brune M, Gleerup A. 1992a. Bioavailability in man of iron in human milk and cow's milk in relation to their calcium contents. Pediatr Res 31:524–7.
Hallberg L, Rossanser-Hultén L, Brune M, Gleerup A. 1992b. Calcium and iron absorption: mechanism of action and nutritional importance. Eur J Clin Nutr 46:317–27.
Hamosh M. 1996. Digestión en el recién nacido. In: Clínicas de perinatología. Gastroenterología neonatal. Mexico; DF: Mcgraw-Hill Interamericana. p 171–88.
Henderson LM, Brewer GH, Dressman JB. 1995. Effect of intragastric pH on the absorption of oral zinc acetate and zinc oxide in young healthy volunteers. J Parenter Enteral Nutr 19:393–7.
Hirai Y, Pernyakov EA, Berliner LJ. 1992. Proteolytic digestion of alpha-lactalbumin: physiological implications. J Protein Chem 11:51–7.
Hurrell RF. 1997. Bioavailability of iron. In: Assessment of the bioavailability of micronutrients. Proceedings of an ILSI Europe Workshop. Eur J Clin Nutr 51:S4–8.
Hurrell RF, Davidsson L, Reddy M, Kastenmayer P, Cook JD. 1998. A comparison of iron absorption in adults and infants consuming identical infants formulas. Br J Nutr 79:31–6.
Hurrell RF, Lynch SR, Trinidad PT, Dassenko SA, Cook JD. 1989. Iron absorption in humans as influenced by bovine milk proteins. Am J Clin Nutr 49:546–52.
Jackson LS. 1992. The effect of dairy products on iron availability. CRC Crit Rev Food Sci Nutr 31:259–70.
Kaup SM. 1998. Aspects of mineral bioavailability in infant nutrition. Int Dairy J 8:435–41.
Kennefick S, Cashman KD. 2000. Investigation of an in vitro model for predicting the effect of food components on calcium availability from meals. Int J Food Sci Nutr 51:45–54.
Knox TA, Kassrjian Z, Dawson-Hughes B, Golner BB, Dallal GE, Arora S, Russell RM. 1991. Calcium absorption in elderly subjects on high- and low-fiber diets: effect of gastric acidity. Am J Clin Nutr 53:1480–6.
Liu YM, Neal P, Ernst J, Weaver C, Rickard K, Smith DL, Lemons J. 1989. Absorption of calcium and magnesium from fortified human milk by very low birth weight infants. Pediatr Res 25:496–502.
Lönnerdal B. 1997. Effects of milk and milk components on calcium, magnesium and trace elements absorption during infancy. Biol Rev 3:643–69.
Lönnerdal B, Cederblad A, Davidsson L, Sandstr m B. 1984. The effect of individual

components of soy formula and cows' milk formula on zinc bioavailability. Am J Clin Nutr 40:1064–70.
Lönnerdal B, Yuen M, Huang S. 1994. Calcium, iron, zinc, copper and manganese bioavailability from infant formulas and weaning diets assessed in rat pups. Nutr Res 14:1535–48.
Luten J, Crews H, Flynn A, Van Dael P, Kastenmayer P, Hurrell R, Deelstra H, Shen LH, Fairweather-Tait S, Hickson K, Farré R, Schemmer U, Frohlich W. 1996. Inter-laboratory trial on the determination of the in vitro iron dialysability from food. J Sci Food Agric 72:415–24.
Michel I, Lavigne C, Desrosiers T. 1993. Soluble and lipid-bound calcium and zinc during processing of infant milk formulas. J Food Sci 58:756–60.
Miller D, Schriker BR, Rassmussen RR. 1981. An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 34:248–56.
Poiffait A, Adrian J. 1993. Composition min rale du lait maternel: 1. Macro-éléments. Méd et Nut 29:163–71.
Poiffait A, Adrian J. 1994. Composition minérale du lait de femme: 2. Oligoéléments. Méd et Nut 30:63–71.
Raiten DJ, Talbot JM, Waters JH, editors. 1998. Assessment of nutrient requirements for infant formulas. LSRO Report. Life Sciences Research Office American Society for Nutritional Sciences. J Nutr 128 (11S):2140–81.
Rudloff S, Lönnerdal B. 1992. Solubility and digestibility of milk proteins in infant formulas exposed to different heat treatments. J Pediatr Gastroenterol Nutr 15:25–33.
Sandström B, Keen CL, Lönerdal B. 1983. An experimental model for studies of zinc bioavailability from milk and infant formulas using extrinsic labeling. Am J Clin Nutr 38:420–48.
Sheikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS. 1987. Gastrointestinal absorption of calcium from milk and calcium salts. New Engl J Med 27:532–6.
Shen L, Luten J, Robberecht H, Bindels J, Deelstra H. 1994. Modification of an in-vitro method for estimating the bioavailability of zinc and calcium from foods. Z Lebensm Forsch 199:442–5.
Shen L, Robberecht H, Van Dael P, Deelstra H. 1995. Estimation of the bioavailability of zinc and calcium from human, cow's, goat, and sheep milk by an in vitro method. Biol Trace Elem Res 49:107–18.
Singh H, Flynn A, Fox PF. 1989. Zinc binding in bovine milk. J Dairy Res 56:249–63.
Skikne BS, Lynch SR, Cook JD. 1981. Role of gastric acid in food iron absorption. Gastroenterology 81:1068–71.
Stekel A, Olivares M, Pizarro F, Chadud P, López I, Amar M. 1986. Absorption of fortification iron from milk formulas in infants. Am J Clin Nutr 43:917–22.
VARIAN. 1979. Analytical methods for flame spectroscopy. Varian Techtron Pty. Ltd. Springvale, Australia. Publication nr 85.
Watzke HJ. 1998. Impact of processing on bioavailability: examples of minerals in foods. Trends Food Sci Technol 9:320–7.
Wolfgor R, Drago SR, Rodr'guez V, Pellegrino N, Valencia ME. 2002. In vitro measurement of available iron in fortified foods. Food Res Int 35:85–90.