ELSEVIER

Food Chemistry 64 (1999) 403–409

Food Chemistry

Calcium dialysability as an estimation of bioavailability in human milk, cow milk and infant formulas

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Received 13 March 1998; received in revised form and accepted 28 May 1998

Abstract

The calcium dialysability of human milk, cow milk and infant formulas is estimated by applying the in vitro method (Miller, Schricker, Rasmussen, & Van Campen, (1981). *American Journal of Clinical Nutrition, 34,* 2248–2256) slightly modified, as a measure of its bioavailability. The influence of the protein composition of infant formula samples on calcium bioavailability is also studied. Whole cow milk, a pool of human milk and 18 commercial infant formulas of 6 different types are analysed. The highest values of calcium dialysability (mg Ca/100 ml infant formula) correspond to premature (13.6) and follow-up formulas (11.8) and the lowest ones to adapted formulas (3.7); the latter values are similar to that of human milk (4.0). The highest value corresponds to cow milk (18.9). In relation to the possible influence of the protein fraction on calcium dialysability, it is higher in formulas in which casein is the main protein fraction and in protein hydrolysates than in other formulas, although these formulas are also the ones with the highest calcium content. Content is the factor that most influences dialysability.

Keywords: Bioavailability; Calcium dialysability; Infant formula milk

1. Introduction

Calcium is an essential element for the growth and development of infants. (Hernández Rodríguez, 1994; Turnlund, 1994). It is mainly or exclusively provided to them by human milk or infant formulas.

Human milk, cow milk and infant formulas differ in their calcium content. The values corresponding to human milk range from 150 to 301 mg Ca/l (Fomon and Nelson, 1995). Individual differences and variations, depending on the lactation period, have been reported. Given that calcium is absorbed better from human milk than from infant formulas, higher calcium contents are used in making these formulas (Fomon, Owen, Jensen, and Thomas, 1963; Rudloff and Lönnerdal, 1990).

Several international organisms have established recommendations on the calcium content of different types of formulas (ESPGAN, 1977, 1981, 1987, 1990; Codex Alimentarius Commission, 1982, 1987). These contents and the Ca/P relationship are regulated by law (EEC, 1991).

Infants absorb the macronutrients of human milk and use them well (Lönnerdal, 1997). Therefore, its composition is the reference standard for the design of infant formulas. However, the utilization values of mineral elements are unknown because several physiological and dietary factors influence their bioavailability. Given the essentiality of calcium, it is necessary to know, not only its content in milk and infant formulas, but also the fraction that is absorbed and used, i.e. its bioavailability.

Factors affecting the bioavailability of calcium include physiological ones like the vitamin D, calcium and phosphorus status, growth, pregnancy and age, and dietetic factors such as lactose, proteins, calcium and phytic acid (Allen, 1982).

In human milk calcium is bound mainly to serum proteins or as part of low molecular weight complexes (Fransson and Lönnerdal, 1982, 1983), whereas in cow milk, it is bound mainly to casein (Fransson and Lönnerdal, 1983). This difference in the location of calcium in human and cow milk could partially explain the observed differences in calcium bioavailability in these two types of milk and also explain differences in infant formulas.

There is a different distribution of calcium between casein and serum proteins in human and cow milk. On the other hand, the ratio of serum proteins:casein is also

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0308-8146/98/$—see front matter
PII: S0308-8146(98)00151-4

different—60:40 in human milk and 20:80 in cow milk. In infant formulas this ratio is usually 60:40, similar to that of human milk. It should also be pointed out that: (1) the serum proteins:casein ratio is modified all through the lactation period, from 10:90 in colostrum to 50:50 during prolonged lactation in human milk (Kunz and Lönnerdal, 1992); (2) the caseins and serum proteins of human and cow milk are different. In the former, β-casein is the major one, whereas in cow's milk it is α-casein (Brostrom, 1985). In the case of serum protein, lactoferrin and β-lactoglobulin are the major ones in human milk and cow milk, respectively (Lönnerdal, 1997).

It should also be mentioned that the casein of cow milk forms big micelles and contains colloidal calcium phosphate, while in human milk the micelles are small (Kunz & Lönnerdal, 1989). This can affect precipitation and digestibility in the gastrointestinal tract (Lönnerdal, 1997).

There are also differences in the amino acid composition and degree phosphorylation between human and cow milk. Several authors have reported that the phosphopeptides formed during the digestion of caseins (CPP) have a positive effect on calcium absorption (Mellander, 1950; Mykkanen and Wasserman, 1980; Li et al., 1989; Naito et al., 1989; Zhang et al., 1994) because of their ability to bind calcium and maintain it in a soluble form in the intestinal lumen, and therefore available for absorption. The formation of CPP seems more difficult from bovine casein than from the human one (Brommage et al., 1991).

All the differences between human and cow milk proteins can also contribute to the differences in calcium bioavailability in human milk, cow milk and infant formulas, and for this reason it is important to take into account the proteic composition of the samples.

In vivo and in vitro methods have been proposed to estimate bioavailability. The in vitro ones are usually based on the simulation of a gastrointestinal digestion of food in conditions fixed beforehand. In fact, these methods measure the fraction of the element available for absorption. It is possible to differentiate between the following two types:

1. The methods based on the solubility of the mineral; these estimate the fraction of the ingested element available for absorption (Schwartz et al., 1982; Wien and Schwartz, 1983, 1985; Zemel, 1984; Kim and Zemel, 1986; Nadeau and Clydesdale, 1991; Michel et al., 1993). They measure the amount of the soluble element in the supernatant obtained after the gastrointestinal digestion of the food, centrifugation and filtration.

2. The methods based on element dialysability introduce a dialysis membrane during the intestinal digestion process, thereby simulating passive diffusion through the mucosa. Two types can be differentiated: methods based on a dialysis process in equilibrium (Miller et al., 1981; Keane et al., 1988; Reykdal and Lee, 1991; Shen et al., 1995) and methods based on continuous dialysability (Minihane et al., 1993; Wolters et al., 1993; Shen et al., 1994), which continuously remove the dialysed elements from the digestion media.

Several studies (Barltrop et al., 1977; Younoszai, 1981; Bronner, 1992) make it clear that, in infants, calcium is absorbed mainly by passive diffusion; the methods based on dialysability can therefore be useful in estimating the bioavailability of this element.

The aim of this work is to estimate the calcium dialysability of human milk, cow milk and infant formulas as a measure of its bioavailability and also to study the influence of the protein composition of these samples on calcium bioavailability.

2. Materials and methods

2.1. Samples

Whole cow milk, a pool of human milk provided by lactating women of the area (frozen immediately after the sampling) and 18 commercial infant formulas available in Spain, of six different types: adapted, follow-up, prematures, hypoallergenic, without lactose and with soy proteins) provided in their commercial packages by three manufacturers. The infant formulas studied can be classified as follows on the basis of their composition: (a) milk-based formulas ($n=15$) that, depending on the protein fraction, can be classified as serum proteins ($n=9$), casein-based ($n=3$), with the same proportion of serum and casein ($n=1$) and formulas based on protein hydrolysate ($n=2$); (b) formulas based on soy-proteins ($n=3$).

2.2. Material and reagents

The digestive enzymes and biliary salts were provided by Sigma Chemical Co (St. Louis MO, USA). The working dissolutions of these enzymes were prepared immediately before use. The 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 pancreatine and biliary salts was prepared by dissolving 0.2 g of pancreatine (P-1750, from porcine pancreas) and 1.25 g of salt biliary extract (B-8631 porcine) in 50 ml of 0.1 M $NaHCO_3$.

The dialysis membranes, with a pore size (MMCO) of 10000–12000 Da (Visking 3-20/32″, 15.9 mm, Medicell, London, UK) were boiled for 10 min in a solution of 0.01M EDTA-Na_2, 2% $NaHCO_3$ and 0.1% sodium dodecylsulphate to remove trace element impurities). Then the membranes were rinsed 5 times with deionized water and boiled for 5–10 min with deionized water. The ready-to-use membranes were

kept in a 20% ethanol solution in the refrigerator (4°C). They were rinsed several times with deionized water before use.

Calcium standard solutions were prepared immediately before use by dilution with deionized water of a standard solution of 1000 mg/L (Titrisol, Merck). Lanthanum solution (5 g/100 ml) was prepared with La_2O_3 (Merck).

All reagents used were reagent grade and Millipore-Milli Q distilled-deionized water was used throughout.

Glass and polyethylene material was soaked in HNO_3 (sp.gr.1.40) and then rinsed three times with deionized water.

2.3. In vitro digestion

The Miller et al. (1981) in vitro method with slight modifications (Concerted action no. 10—FLAIR Project) (Luten et al., 1996) was applied to human milk, cow milk and infant formulas.

2.4. Calcium determination

Calcium was measured by flame atomic absorption spectroscopy (FAAS) using a Perkin–Elmer 2380 atomic absorption spectrophotometer fitted with a calcium hollow cathode lamp under the following instrumental conditions: wavelength = 422.8 nm, slit = 0.7 nm; acetylene flow = 1.75 L/min, air flow = 14 L/min nebulizer = spoiler. Determinations were carried out directly in the dialysate obtained by applying the in vitro method, and also in human and cow milks after a mineralization of the organic matter (450°C). Calcium from infant formulas was directely determined in an aqueous dispersion of the formula (Ruiz et al., 1996).

In all cases, lanthanum was added to the solutions to obtain a content of 0.1% (w/v), except for infant formulas, where the content required is 0.4% (w/v).

2.4.1. Dialysability estimation

The bioavailability is expressed as dialysability (mg of Ca/100 ml of sample) or as dialysis percentage (%D), calculated as follows:

$$\%D = 100 \times \text{dialysability (mg/100ml)}/\text{total content (mg/100ml)}$$

3. Results and discussion

The values obtained for dialysability and the $\%D$ in human milk, cow milk and infant formulas are reported in Table 1. A relative index (RI) parameter indicating the bioavailability of calcium from cow milk and infant formulas with respect to human milk calculated as follows is included:

$$\text{RI} = \{\text{mg of calcium dialysate/100 ml of cow milk or infant formula}\} \times \{\text{mg of calcium dialysate/100 ml of human milk}\}^{-1}$$

A variance analysis (ANOVA) of one factor (dialysability or percentage of dialysis) and eight levels (adapted, follow up, prematures, hypoallergenic, without lactose, soy based, cow milk and human milk) was applied to the results (see Table 2).

Significant differences ($p < 0.05$) are indicated by the non-coincidence of the superindices in a column.

The highest values of dialysability correspond to prematures and follow up formulas and the lowest ones to adapted formulas. It is clear that calcium dialysability of the adapted formulas is similar to that of human milk, whereas the highest value corresponds to cow milk, as could be expected given its higher content.

The only significant differences detected in dialysis percentages relate to the adapted formulas.

The percentage of dialysis of calcium in human milk is similar to that reported by Shen et al. (1995), who applied an in vitro method similar to the one used in our study. Although the mentioned authors do not observe differences between the dialysability of calcium from human and cow milk, we found lower values for the former.

The possible influence of the composition of the protein fraction of infant formulas and milk on calcium bioavailability has been reported by several authors (Cooke and Nichoalds, 1985; Rudloff and Lönnerdal, 1990). The favourable effect of casein on calcium absorption has been ascribed to the inhibition of calcium precipitation by phosphate in the intestinal lumen by the action of phosphopeptides coming from the enzymatic hydrolysis of casein (Mellander, 1950; Patrick and Bacon, 1957; Mykkanen and Wasserman, 1980; Li et al., 1989; Naito et al., 1989; Berrocal et al., 1989), which maintains calcium in a soluble and absorbable form.

In order to study the possible influence of the protein fraction on calcium dialysability, infant formulas were classified according to the main protein fraction and the ANOVA of one factor (dialysability or dialysis percentage) and five levels (serum, casein, serum = casein, protein hydrolysates and soy) was applied. The results are reported in Table 3.

Calcium dialysability is higher in formulas in which casein is the main protein fraction and in protein hydrolysates than in the other formulas. This observation supports the above mentioned positive effect of casein on calcium absorption, although these formulas are also the ones with the highest calcium content. Content is the greatest determinant of dialysability as has been indicated by several authors (Khan et al., 1969; Greger, 1988; Cashman and Flynn, 1996).

Table 1
Calcium: total content, dialysability, % dialysis and relative index (IR) of infant formulas, cow and human milk

Samples		Ca total content (mg/100 ml)	Dialysability (mg/100 ml)	% *D*	Relative index (RI)
Adapted	A	51.5	4.0 ± 0.4	7.8 ± 0.7	1.00
	B	51.9	2.7 ± 0.3	5.2 ± 0.5	0.67
	C	53.9	4.3 ± 0.4	4.3 ± 0.4	1.08
Follow up	A	83.4	14.9 ± 0.8	17.9 ± 0.9	3.73
	B	63.4	11.9 ± 1.6	18.9 ± 2.6	2.97
	C	79.9	7.9 ± 0.9	9.9 ± 1.1	1.97
Prematures	A	92.4	14.8 ± 1.1	16.0 ± 1.2	3.70
	B	68.6	6.3 ± 0.8	9.2 ± 1.2	1.57
	C	64.2	18.1 ± 1.3	28.2 ± 1.9	4.52
Hypoallergenic	A	51.2	12.4 ± 1.4	24.2 ± 2.7	3.10
	B	74.3	10.3 ± 1.5	13.9 ± 1.9	2.57
	C	38.7	5.9 ± 0.6	15.4 ± 1.6	1.48
Without lactose	A	56.7	6.8 ± 0.9	11.5 ± 1.6	1.70
	B	41.6	5.1 ± 0.4	12.2 ± 1.1	1.28
	C	62.0	12.4 ± 0.8	19.9 ± 1.4	3.10
Soy-based	A	49.3	8.9 ± 0.3	18.3 ± 0.7	2.23
	B	52.1	6.6 ± 0.9	12.7 ± 1.6	1.65
	C	62.3	10.9 ± 0.6	17.6 ± 0.9	2.73
Cow milk		94.8	18.9 ± 0.8	20.0 ± 0.9	4.73
Human milk		29.3	4.0 ± 0.2	13.6 ± 0.8	1.00

A, B and C denote manufacturers.

Table 2
Calcium dialysability and % of dialysis in infant formulas

Samples	*n*	Dialysability (mg/100 ml)		% Dialysis	
		x	CI	*x*	CI
Adapted	25	3.7[a]	2.3–5.0	7.1[a]	5.0–9.1
Follow up	35	11.8[b]	10.7–12.9	16.0[b]	14.3–17.7
Prematures	27	13.6[b]	12.3–14.9	18.9[b]	17.0–20.9
Hypoallergenic	25	8.8[c]	7.5–10.2	16.8[b]	14.7–18.9
Without lactose	28	8.8[c]	7.6–10.1	15.3[b]	13.4–17.2
Soy based	28	8.8[c]	7.6–10.1	16.5[b]	14.6–18.4
Cow milk	6	18.9[d]	16.2–21.7	20.0[b]	15.9–24.1
Human milk	6	4.0[a]	1.3–6.7	13.6[b]	9.5–17.7

n = number of data; *x* = mean value; CI = mean confidence interval. Different superscript letters within a column indicate significant differences ($p < 0.05$).

The only significant differences detected in mean dialysis percentages correspond to formulas having the same proportion of casein and serum proteins.

In a study carried out on premature infants, Cooke and Nichoalds (1985) obtained calcium absorption values similar to the calcium dialysis percentages of infant formulas containing serum proteins or casein as the main protein. Therefore, the protein composition does not affect calcium absorption.

In a study carried out with monkeys, Rudloff and Lönnerdal (1990) compared the calcium absorption of different types of infant formulas (protein hydrolysates, formulas with different proportions of serum proteins and casein and soy-based formulas) with that corresponding to human milk. They only detected significant differences between human milk and the different types of infant formulas, but not between the infant formulas. The value obtained for the formulas with casein as the main protein fraction was, however, slightly higher than the values corresponding to the other formulas.

Lönnerdal et al. (1994) studied the bioavailability of Ca, Fe, Zn, Cu and Mn of lactating rats fed infant formulas differing in the main protein source (casein, serum, protein hydrolysates, soy), cow milk and human milk. They reported that calcium absorption from infant formulas is higher than 70% and similar to the

Table 3
Calcium dialysability and % of dialysis in infant formulas classified according to the proteic fraction

Samples	*n*	Dialysability (mg/100 ml)		% Dialysis	
		x	CI	*x*	CI
Serum	88	8.6[a]	7.7–9.5	14.6[a]	13.5–15.8
Casein	40	13.0[b]	11.7–14.3	16.8[a]	15.1–18.5
Casein = Serum	9	4.3[c]	3.9–4.6	8.0[b]	7.4–8.6
Hydrolysates	13	11.1[a,b]	8.9–13.4	17.8[a]	14.9–20.8
Soy	28	8.8[a]	7.3–10.3	16.5[a]	14.5–18.5

n = number of data; *x* = mean value; CI = mean confidence interval. Different superscript letters within a colum indicate significant differences ($p < 0.05$).

one corresponding to human milk. The only detected lower absorption corresponded to soy formulas.

In our study, human milk was taken as a reference standard for comparing the calcium dialysability of the different formulas and of cow milk. The relative index (RI), the parameter used to establish the comparison, was higher than or equal to 1, in all cases except one adapted formula. That means a higher calcium dialysis from cow milk and infant formulas than from human milk. The calcium content was, however, somewhat lower in human milk (29.3 ± 1.4 mg/100 ml) than in the analysed infant formulas (38.7–92.4 mg/100 ml) or cow milk (94.8 ± 5.5 mg/100 ml). This observation agrees with the fact mentioned above that the most determinant parameter of calcium dialysability is the content of this element in the samples.

One indicator of bioavailability of calcium is its effect on bone mineralization in infants. Several comparative studies of this mineralization in infants fed human milk, milk and soy-based infant formulas have been carried out with contradictory results. Steichen and Tsang (1987) reported that the best mineralization was achieved by infants fed milk-based formulas, whereas the values obtained for the children taking human milk or soy-based formulas were similar. Chan et al. (1987), however, reported that mineralization was worse in infants fed soy-based formulas that in those receiving human milk. Finally, Hillman et al. (1988) did not detect significant differences in the mineral content of infants fed with human milk, milk-based or soy-based formulas.

Finally, in an assay carried out on rats, Huang (1993) observed a higher absorption of calcium from milk-based, protein hydrolysate and premature formulas than from soy-based ones. In the case of milk-based formulas, absorption was similar to that of human milk.

In conclusion the protein composition of infant formulas influences calcium dialysability, and the effect is more pronounced when casein is the protein source. However, infant formulas with casein as the main protein source and formulas based on protein hydrolysates are the ones having the highest calcium contents, and this is the main factor affecting calcium dialysability.

Acknowledgements

This study is part of a project ALI 94-0467 financially supported by the CICYT, Spain.

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