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Zinc and iron bioavailability in a powder or in-bottle-sterilized infant formula estimated by in vitro and in suckling rats

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Abstract

Maillard reaction and lactose isomerization may be induced during the processing involved in the manufacture of infant formulas. The effects of dehydratation and sterilization in an infant formula on iron and zinc bioavailability were studied. A powder (PIF), previously reconstituted, and an in-bottle-sterilized liquid infant formula (LIF), from the same manufacturer, were evaluated using an in vitro method and in suckling rats. After in vitro digestion the dialyzed and non-dialyzed soluble, and insoluble fractions of iron and zinc were separated. Two-week-old rat pups were fed PIF or LIF in a drinking bottle for 7 days. Infant formula intake (I), body weight and the fecal and urinary excretions were monitored and the following parameters calculated: apparent absorption (A), retention (R), and the coefficients %A/I, %R/A and %R/I. Soluble iron (dialyzed) and zinc (non-dialyzed) were higher ($p < 0.001$) in LIF than PIF after in vitro digestion. Insoluble iron was similar in both infant formulas but insoluble zinc was lower ($p < 0.05$) in LIF than PIF. Food intake ($p = 0.045$) and body weight on day 4 ($p < 0.05$) and on day 7 ($p < 0.001$) were lower in LIF compared to PIF. A, R ($p < 0.05$ for both minerals), %A/I, and %R/I ($p < 0.001$ and $p < 0.05$ for iron and zinc, respectively) were significantly lower in rats fed LIF. Similarly, the %R/A of iron was lower ($p < 0.001$) in this group. Hematocrit and hemoglobin did not show significant differences. Iron and zinc levels in liver, spleen and erythrocytes were similar in both groups, but skin iron concentration was higher in LIF. Therefore, in contrast with the in vitro results, consumption of the in-bottle-sterilized formula determines lower iron and zinc bioavailability compared to the reconstituted powder infant formula.

Keywords: Infant formula; Iron; Zinc; Bioavailability; Maillard reaction; Sterilization

1. Introduction

Food processing induces changes and interactions between food constituents that may affect their chemical properties and consequently nutrient bioavailability. Infant formulas are available in powder and liquid forms. Drying and sterilization are thermal processes commonly used in the manufacture of infant formulas. Several studies indicate that the heating involved in these processes induces the formation of Maillard reaction products (MRP) due to the reaction of lysine with lactose [1], the later being abundant in milk products. Lactose also participates in an isomerization reaction which can be detected by measuring the amount of lactulose formed. Recently, it was observed that UHT and in particular, in-bottle-sterilized infant formulas, contain higher levels of lactulose than the corresponding powdered infant formulas. Conventional sterilization produces proportionately greater protein denaturation, higher levels of MRP and a higher lactulose/furosine ratio when compared to UHT and dehydration processes [2,3]. MRP have the potential to interact with several minerals and trace elements including zinc [1] and iron [4] forming insoluble ligands which decrease bioavailability. In addition, proteins damaged by heating processes may lose properties associated with trace element absorption mechanisms [5,6].

Few studies have investigated the nutritional consequences of heat treatment used in the manufacture of infant formulas. Several authors have reported alteration in protein utilization by rats when consuming heat-processed infant formulas [7,3]. However, data concerning the influence of processing on infant formula trace element bioavailability are scarce. The importance of this area of research cannot be underestimated due to the frequency of iron [8] and zinc [9] deficiencies in infants.

Studies in babies are limited due to both ethical and methodological reasons. Alternatively in vivo studies can be supported by in vitro tests to estimate the availability of

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minerals and trace elements. The availability of an element is governed by its solubility which can be estimated by in vitro digestion methods, but it has been suggested that they are only useful to predict the absorptive direction, not its magnitude [10]. The method often reported and extensively used is that of Miller [11]. In this method the proportion of the compounds diffusing across a semipermeable membrane during a simulated intestinal digestion stage, is used as a measure of the element's availability. In Miller's original method, an equilibrium dialysis is reached on both sides of the semipermeable membrane which later has been replaced by continuous dialysis in-vitro methods that remove the dialysate components continuously, facilitating further dialysis [12,13]. It has been hypothesized that this method better simulates the in vivo situation in which the blood circulation continuously removes the digested substances. Nevertheless, according to Diepenmaat-Wolters and Schreuder [13] the results obtained by both methods are quite similar. Dialyzed iron and zinc from various meals have shown a good correlation with in vivo absorption in some studies [14], but not always [15,16]. Therefore, the analysis of the dialyzed as well as the non-dialyzed fractions are performed in our laboratory and compared with results obtained in rats.

Suckling rat pups have been extensively used as an infant model for evaluating mineral availability from infant formulas [17,18]. These animals may be separated from their dams because they are already capable of drinking from a bottle in their third week of life [18]. Moreover, it has been demonstrated that the pup is a better model than the adult rat since they are accustomed to a milk diet and presumably have a gastrointestinal physiology more similar to the human infant [17].

The aim of this study was to assess iron and zinc bioavailability from an infant formula subjected to dehydration or conventional sterilization using an in vitro method and to compare the results obtained with suckling rat pups.

2. Material and methods

2.1. Infant formula

A powder form (PIF) and an in-bottle-sterilized liquid (LIF) infant formula (both first age formulas) were provided by the same manufacturer (Nutricia, Zoetermeer, The Netherlands). The powder form was reconstituted with deonized water to 127 g/kg and used in liquid form for the in vitro and in vivo experiments. PIF and LIF contained respectively (g/L): lactose, 71.2 and 71.0; casein, 5.6 and 5.6; whey proteins, 8.4 and 8.4; fat, 36 and 36. Iron levels were: 4.59 ± 0.05 and 5.06 ± 0.03 μg/ml (mean $\pm$ standard error for five determinations), and zinc levels were: 3.95 ± 0.05 and 4.00 ± 0.05 μg/ml (mean $\pm$ standard error for five determinations), for the powder and liquid infant formula, respectively. Two heat markers were also analyzed [3] in PIF and LIF respectively (g/L): lactulose, 0.143 and 4.56; and furosine (index of early Maillard reaction products), 0.205 and 0.105. Lactulose/furosine ratios were 0.69 and 43.4 in PIF and LIF respectively.

2.2. In vitro digestion

Duplicate samples of 225 g of the reconstituted powder (PIF) and liquid (LIF) infant formula, were digested in three consecutive days using the method of Miller et al. [11] subsequently modified [20]. The pH of each sample was adjusted to 2.0 using 6 M HCl. Pepsin (Sigma Chemical, St. Louis, MO), 0.5 g/100 g homogenate was added and the samples were incubated at 37°C for 2 h in a shaking-water bath. At the end of the incubation, aliquots containing 40 g of the pepsin digests were transferred to 250 ml plastic bottles.

Segments of dialysis tubing, 12000 MW cut off (Medicell International LTD size: 9 36/32″) containing 50 ml of a solution of $NaHCO_3$ (Merck, Darmstadt, Germany) equivalent to the titratable acidity were placed in each bottle. This was followed by the addition of 10 ml of pancreatin-bile extract (Sigma Chemical Co., St. Louis, MO). Incubation continued for 2 hr. The dialysis tubes were removed and rinsed with deionized water (MilliQ plus, Millipore, Bedford, MA). The weights and the pH of the retentates and diffusates were measured. The retentate (non-dialyzed) fraction was centrifuged for 15 min at 1000 g (J.P. Selecta, S.A., Barcelona, Spain) in order to separate the soluble and insoluble non-dialyzed forms of iron and zinc.

Results are expressed as the percentage of the dialyzed, soluble non-dialyzed and insoluble non-dialyzed fractions from the total amount of each trace element obtained at the end of the gastric digestion stage.

2.3. In vivo assays

Rat assays were approved by the Ethical Committee of the Faculty of Pharmacy and the *Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT).* Twenty-four two-week-old suckling rats (initial body weight 30 ± 0.4 g, mean $\pm$ standard error) were housed in metabolic cages in an environmentally controlled room, maintained at 20–22°C, with a 12 h light-dark cycle and 55–70% humidity. The animals were randomly assigned to the PIF or LIF dietary treatments. They could freely access to the infant formulas in a drinking bottle during 7 days. Food intake and body weight were monitored. Feces were dried, weighed and homogenized and urine was collected in 0.5% v/v HCl solution, filtered (Whatman Filter Papers n°40, ashless, Whatman Ltd., England) and diluted. On the 7th day 6 rats from each group were anesthetized using sodium pentobarbital (Abbott Laboratories, S.A., Madrid, Spain), and blood was drawn from the carotid artery into acid-washed (HNO_3 10N) plastic vials and allowed to clot. Erythrocytes were obtained after centrifugation for 15 min at 1000 g (J.P. Selecta S.A., Barcelona, Spain). Liver, a segment of skin

Table 1
Iron and zinc distribution after the in vitro digestion (%)

	Dialyzed	Non-dialyzed		Recovery
		Soluble	Insoluble	
Iron				
Powder infant formula	23.08 ± 1.48	74.45 ± 0.98	9.66 ± 1.62	107.19 ± 1.58
Liquid infant formula	28.88 ± 2.26†	67.23 ± 2.37†	11.18 ± 1.48	107.31 ± 1.99
Zinc				
Powder infant formula	20.07 ± 2.65	64.93 ± 3.32	12.55 ± 3.36	97.54 ± 3.01
Liquid infant formula	17.43 ± 1.11*	72.94 ± 0.83†	8.58 ± 1.14*	98.95 ± 1.30

Values are means ± SD; N = 6.
Significant differences (Student's T test) at † $p < 0.001$; * $p < 0.05$.

and spleen were removed, weighed and stored at −20°C for analysis.

2.4. Analytical techniques

In fresh blood, hematocrit was determined by the microhematocrit method and hemoglobin by the cyanmethemoglobin method (Boehringer Mannheim, Mannheim, Germany). The infant formulas, the three fractions from the in vitro digestion, feces, livers, spleens, skins, and erythrocytes were dry-ashed in a muffle furnace at 500°C. Ashes were dissolved in an acid solution ($HCl/HNO_3/H_2O$: 1/1/1; Suprapur, Merck, Darmstad, Germany). Iron and zinc were determined in all samples by atomic absorption spectrophotometry (Perkin-Elmer 1100B Norwalk, CT, USA). Stock standard solutions of iron and zinc (1 g/L) were prepared from Tritrisol (Merck). Calibration solutions were prepared from the stock standard solutions by serial dilution with de-mineralized water (MilliQ plus), and a blank solution was also used.

A pool of feces was used as an internal control to assess precision. The interassay coefficient of variation was 4.19% for iron and 2.33% for zinc. Lyophilized Liver (certified reference material CRM 185; Community Bureau of Reference, Brussels, Belgium) yielded a value of 211 ± 2 μg/g for iron (mean ± SD of five determinations) (certified value 214 ± 5 μg/g) and 142 ± 4 μg/g for zinc (mean ± SD of five determinations) (certified value 142 ± 3 μg/g).

2.5. Indices

The following indices were calculated from the data obtained for the intake, fecal and urinary excretion of iron and zinc:

Apparent absorption = intake - fecal
% A/I = Apparent absorption/Intake x 100.
Apparent retention = apparent absorption −urinary loss
% R/A = Apparent retention/Apparent absorption x 100
% R/I = Apparent retention/Intake x 100

2.6. Statistical analysis

The data were analyzed by the unpaired Student-t test. The level of significance was established at $p < 0.05$. Data were processed with the Biomedical Statistical Package [21], running VMS-DEC Alpha 2100.

3. Results

3.1. In vitro assays

Table 1 shows the in vitro results for iron and zinc obtained with the liquid (LIF) and powder infant formula (PIF). Values of recovery obtained for iron and zinc were respectively above and below 100%, however the differences between infant formulas were not significant in either mineral.

Dialyzed iron was significantly higher in the liquid than the powder infant formula ($p < 0.001$), while the soluble non-dialyzed iron was lower in LIF compared to PIF ($p < 0.001$). There were no significant differences between the two groups in insoluble non-dialyzed iron. The percentage of dialyzed zinc was significantly lower in LIF than PIF ($p < 0.05$). The non-dialyzed soluble fraction was markedly higher ($p < 0.001$) and the insoluble zinc significantly lower ($p < 0.05$) in LIF compared to PIF.

3.2. In vivo assays

Food intake (I) was lower in the animals fed LIF compared with PIF ($p = 0.045$, Table 2). Accordingly, body weight in the former group was significantly lower from day 4 ($p < 0.05$) to the end of the assay, day 7 ($p < 0.001$).

There was a tendency for iron intake to be lower in LIF compared to PIF fed rat pups, but the differences did not reach statistical significance. Iron absorption (A) and retention (R) were significantly lower in LIF compared to PIF ($p < 0.05$, Figure 1) fed rat pups, due partly to the slightly lower iron intake but also due to the lower absorption and retention efficiencies, as shown by the very significant dif-

Table 2
Intake (ml/week) and body weight (g)

	Intake	Body weight		
		Day 1	Day 4	Day 7
Powder infant formula	82.0 ± 0.4	30.1 ± 0.4	28.6 ± 0.4	29.5 ± 0.3
Liquid infant formula	66.1 ± 6.5*	30.1 ± 0.4	26.8 ± 0.4*	26.7 ± 0.4†

Values are means ± SE; N = 12 rats/group.
Significant differences (Student's T test) at † $p < 0.001$; * $p < 0.05$.

ferences in %A/I, %R/A and % R/I ($p < 0.001$) between the two groups.

Table 3 shows that liver and spleen weights were lower in LIF animals, although the differences were only significant in the first organ ($p < 0.05$). The liver/body weight ratio was not significantly different between groups (3.7 ± 0.1 and 3.6 ± 0.2% in PIF and LIF, respectively). Iron concentrations in both organs were higher in LIF animals, without reaching the level of significance. Iron concentration in the skin was higher in LIF compared to PIF and the differences were very significant ($p < 0.001$). However, no significant differences were observed in erythrocytic iron, hematocrit or hemoglobin.

Zinc intake did not vary significantly between groups, but zinc absorption and retention were lower in LIF compared with PIF fed pups ($p < 0.05$, Figure 2). Similarly %A/I and %R/I, were significantly lower ($p < 0.05$) in LIF animals. In contrast the differences in %R/A were very small and did not reach the level of statistical significance.

There were no significant differences between groups in the concentrations of zinc in liver, spleen, skin and erythrocytes (Table 3), although the values, except those of the skin, tended to be lower in the rats fed the sterilized infant formula.

4. Discussion

4.1. In vitro assays

Analysis of iron in the two forms of infant formula were close to the published data provided by the manufacturer. It is probable that the difference observed for the soluble dialyzed iron between the two infant formulas tested could be due to factors such as the type of salt used and/or its addition before or after heat processing [22]. However, it is more likely that other factors have contributed to the differences found since it is feasible that the same iron compound was used for both formulas having been manufactured by the same company.

The sterilized infant formula was found to have a higher content of lactulose and lower content of furosine than the reconstituted powder infant formula, and hence a high lactulose/furosine ratio. These results indicate that sterilization of LIF was a more intensive heat treatment or involved more processing steps than the drying process used in PIF. The higher lactulose/furosine ratio seen in LIF could be attributed to degradation of early Maillard reaction products or Amadori compounds (precursors of furosine) giving rise to products of late stages of the Maillard reaction.

Sterilization induces protein changes such as hydrolysis, denaturation and the formation of higher molecular weight compounds including MRP. García et al. [23] observed the highest percentage of dialyzed iron in in vitro tests using infant formulas based on protein hydrolysates compared with other non-hydrolzed protein formulas. Heating of infant formulas must be high to cause pronounced protein denaturation and Maillard browning before the solubility of iron is affected and hence its dialysability [24,25]. In a study using egg white, the dialysability of iron was similar when the sample was treated for 1 week at temperatures of either 50°, 60° or 70°, but decreased significantly when heated at 80°C [26]. Accordingly, Lee and Cydesdale [27] reported that baking bread increased insoluble iron.

Previous analysis of heat markers, such as lactulose and furosine, and denatured proteins in these infant formulas showed that the content of hydrolyzed proteins was higher in LIF than in PIF [3]. This decrease in high molecular weight proteins may reduce the number of ligands formed with iron and be responsible for the observed increase in iron dialysis. LIF also contained more MRP and high molecular protein aggregates than PIF and exhibited a brown color. Therefore, a fraction of the iron was bound to the high molecular weight compounds accounting for the higher percentage of insoluble iron analyzed in LIF. This difference was not statistically significant when the powdered infant formula was compared with LIF. However, more pronounced effects of Maillard reaction were seen by Leahey and Thompson [26] and Lee and Cydesdale [27] working with other foods. In conclusion, the most predominant effect of infant formula sterilization in LIF observed in the in vitro tests, was the increase in iron dialysis. While the decrease in iron availability resulting from iron insolubility, was negligible.

The distribution of zinc found in the in vitro digestion fractions, contrasts with that obtained for iron. The difference could be attributed to a higher affinity of zinc for MRP [28,4]. In another in vitro study, Maillard browning products formed from amino acid/glucose solutions, bound zinc and decreased zinc availability compared with an unheated control. Moreover the degree of binding seemed to be re-

Table 3
Iron and zinc content in liver, spleen, skin and erythrocytes and hemoglobin levels in lactating rats fed the powder and in-bottle-sterilized infant formula

Infant formula	Liver			Spleen			Skin		Erythrocytes		Hc	Hb
	Weight g	Fe μg/g	Zn μg/g	Weight g	Fe μg/g	Zn μg/g	Fe μg/g	Zn μg/g	Fe μg/g	Zn μg/g	%	g/100ml
Powder	1.11 ± 0.03	43.0 ± 5.2	70.0 ± 4.5	0.09 ± 0.00	211.3 ± 30.3	295.6 ± 28.8	10.6 ± 0.2	65.6 ± 3.1	744.0 ± 68.1	15.3 ± 3.3	42.6 ± 1.0	10.2 ± 0.1
Liquid	0.98 ± 0.03*	55.1 ± 4.4	61.7 ± 2.6	0.08 ± 0.00	214.4 ± 27.5	229.3 ± 24.4	13.5 ± 1.1†	73.3 ± 4.1	804.8 ± 32.0	13.6 ± 0.6	43.3 ± 0.7	10.2 ± 0.4

Values are means ± SE; N = 12 rats/group.
Significant differences (Student's T test) at † $p < 0.001$; * $p < 0.05$.
Hc: hematocrit; Hb: hemoglobin.

lated to the extent of browning and nature of the proteins present [29]. In other studies, infant formulas based on protein hydrolysates, enhanced zinc dialysis [23]. In the present study, dialyzed zinc was not higher in LIF, although this formula contained a higher degree of protein hydrolysis than PIF and supports the observation that the metal was tightly bound to high molecular weight components, such as advanced MRP and other protein aggregates [1]. These compounds should be soluble, since the soluble non dialyzed zinc was found to be the most abundant fraction.

The results of the in vitro availability assay suggest that iron and zinc exhibited different affinities for the compounds formed during sterilization. Zinc favored ligands with high molecular weight soluble compounds while iron was predominantly bound to low molecular weight soluble compounds and readily dialyzed.

The presence of emulsifiers exclusively found in LIF may confound the mechanisms explained above since an increase in the soluble forms of zinc was observed by Sandström et al. [17] who indicated that the bipolar nature of emulsifiers added to liquid formulas, can attract divalent cations such as zinc, binding it to the lipid fraction.

4.2. In vivo assays

A previous study using the same animal model, demonstrated that 2-week old suckling rats can drink milk from a bottle and reach a final body weight (approximately 40 g) corresponding to the weaning stage after 1 week [19]. Differences in voluntary food intake and body weights were observed depending on the type of milk (cow's milk or infant formula) administered to the rat pups, there being a greater consumption of cow's milk leading to higher body weights, possibly due to the differences in composition and resemblance to rat's milk [19].

In the present study, the higher body weight corresponded to the animals that consumed the powder infant formula and must be attributed to the differences in food processing. The acceptability of flavor and smell decreased when the lactulose content of processed milk increased, as noted by Burton [30]. Nagendra et al.[31] observed that feeding growing rats with infant formulas containing up to 1% lactulose, did not produce changes in infant formula intake or growth. In our study, the experimental conditions were different because we used suckling rats and both infant formulas were administered in liquid form, thus the lactulose levels seem to be quite high, especially in LIF. MRP also affect food intake and body weight [32], although the response has not always been observed [33]. Therefore, both the higher levels of lactulose and MRP in LIF compared to PIF may have determined the lower food intake. Body weight differences were highly significant at the end of the study. In addition to lower intake, protein utilization was compromised [3], as was the nutritive utilization of micronutrients such as iron and zinc, as observed in this study (Figure 1 and 2).

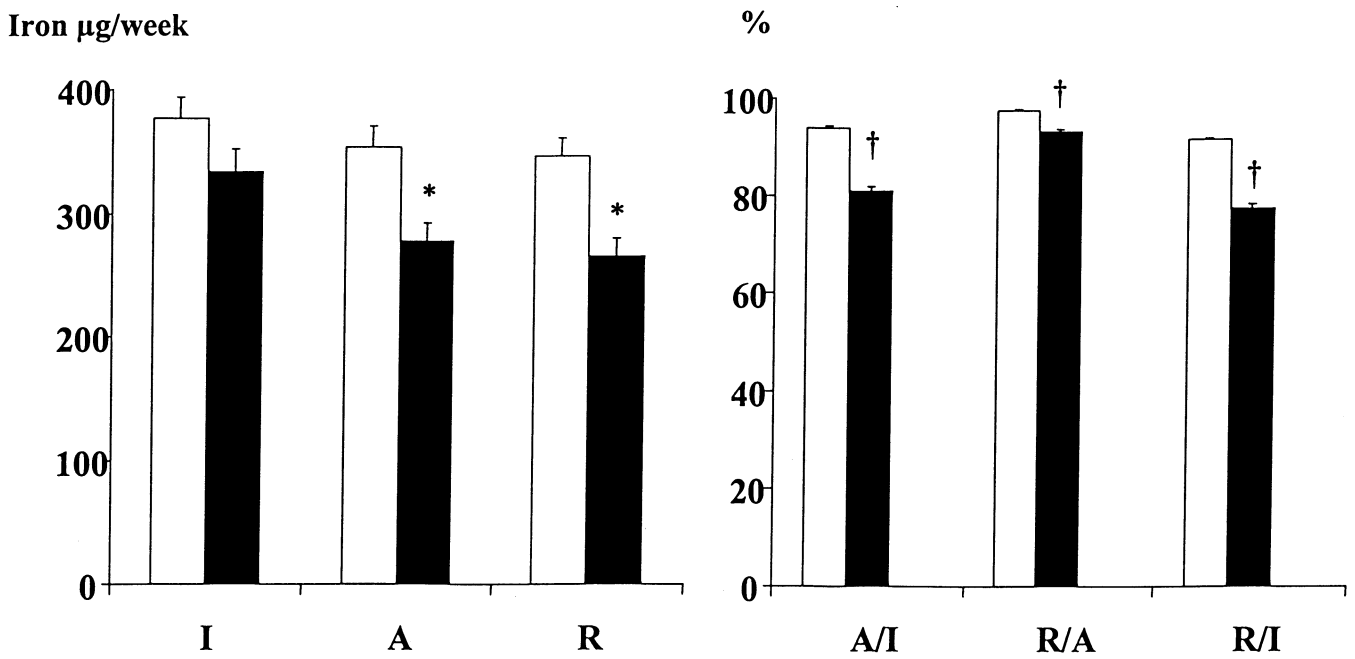

Fig. 1.Digestive and metabolic utilization of iron in lactating rats consuming: a powder infant formula (open bars) and a liquid infant formula (shaded bars). Values are mean ± SE. N = 12 rats/group. Intake (I), absorption (A) and retention (R). Significantly different at † $p < 0.001$; *$p < 0.05$.

Several factors shed light on the differences in digestion (A/I) and metabolism (R/I and R/A) for iron and zinc between LIF and PIF. The lower body weight observed in LIF may involve retarded maturity of digestive function and also under use of trace elements stores, accumulated during embryonic growth. Contrastingly, studies on bioavailability of calcium performed in these rats revealed an increase in calcium digestibility in LIF probably in an attempt to increase absorption [19]. The different responses seen for the trace elements compared with calcium, may be explained by an increased calcium need for osseous formation [34], at the early stages of growth and the observation that MRP has a higher affinity for iron and zinc than calcium. However, the second mechanism seems unlikely, since some soluble

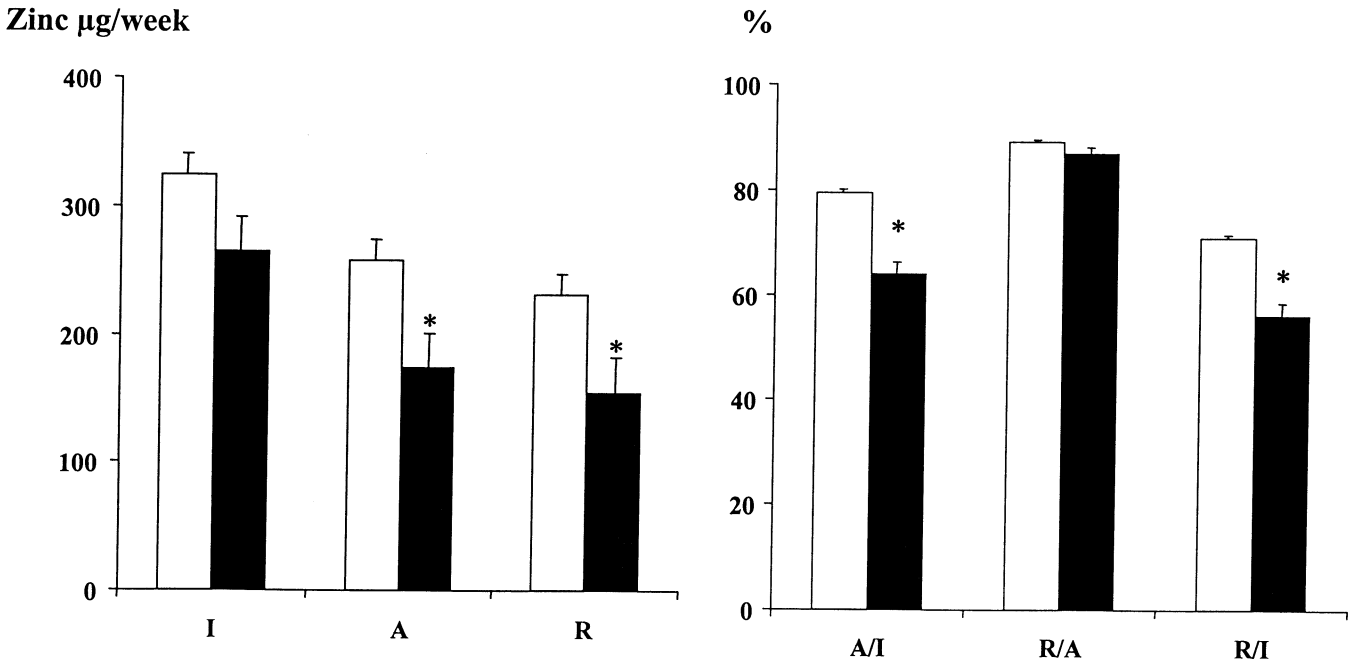

Fig. 2. Digestive and metabolic utilization of zinc in lactating rats consuming: a powder infant formula (open bars) and a liquid infant formula (shaded bars). Values are mean ± SE. N = 12 rats/group. Intake (I), absorption (A) and retention (R). Significantly different at * $p < 0.05$.

forms of iron and zinc were seen to increase after in vitro digestion of the sterilized infant formula.

The lower %R/A of iron obtained in LIF fed pups, indicates relatively higher iron urinary losses in this group, and is in agreement with several authors who have shown that MRP can increase urinary iron and decrease retention [4]. The possible complexes between MRP and iron were soluble but not utilized in the body. Consequently, the lower inefficient digestive (%A/I) and retention (%R/A) processes reflect reduced %R/I in LIF.

Although zinc %R/A values in LIF and PIF in the present work are similar, when a larger number of animals were tested by our group, we obtained a significant decrease in this parameter which was associated to the presence of MRP in the sterilized infant formula [19]. It is well known that these compounds are able to bind zinc and the ensuring complex excreted in urine [1]. Moreover, MRP can also affect zinc absorption [39]. Hurrell [4] concluded that non-absorbable MRP, are responsible for lower zinc absorption, while early MRP can be absorbed and later excreted in urine. The results obtained from the in vitro study, indicate that LIF favors the soluble non-dialyzed zinc at the expense of both dialyzed and insoluble zinc. With suckling rats, most of it remains unabsorbable, and thereby not bioavailable. The deleterious nutritional status associated with the consumption of the sterilized infant formula could also contribute to lower zinc needs and lower bioavailability.

In addition, other authors have observed that protein digestibility decreases due to sterilization of infant formulas [7,3]. Since zinc absorption depends on protein digestion products [5], it is suggested that protein damage in the LIF formula was another factor, besides MRP, and that nutritional status contributed to the observed zinc absorption decrease.

The observation that the rats consuming the sterilized formula, presented a lower body weight and reduced iron need, is also supported by the iron tissue content data (Table 3). Wharf et al. [35] found that birth weight affected the size of iron stores, and rate of growth which can affect the depletion of those stores. In agreement, the low body weight caused by LIF consumption may contribute to slightly higher liver iron level. Erythrocytic iron, hematocrit and hemoglobin values obtained in the present study were within the normal range [36,37]. Nevertheless, the skin iron concentration was elevated in LIF animals compared to PIF indicating that their nutritional status was not adequate [38].

In contrast to iron, zinc in liver, spleen, skin and erythrocytes (Table 3) were lower in the rats fed the sterilized infant formula. The higher zinc skin levels in LIF fed rats, resembles the skin iron results and once again point to malnutrition [40,38].

Therefore, we can conclude that the main cause of reduced body weight in suckling rats fed the in-bottle-sterilized infant formula was the low food intake due to its MRP and lactulose content. The amount of iron and zinc absorbed and retained were lower compared with the powder infant formula fed rats, but high enough in relation to body weight, since tissue iron and zinc levels, hematocrit and hemoglobin were similar in both groups except iron skin levels indicative of malnutrition.

It is suggested to use mild heat treatment conditions in the processing of infant formulas, and to investigate further the effects of lactulose and Maillard reaction products in infant formulas on food intake and bioavailability of iron and zinc, as well as other nutrients, in human infants.

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