https://doi.org/10.1590/1678-98652017000100002

Intestinal absorption of iron and calcium from soy and cow's milk-based infant formulas in weanling rats pups

Absorção intestinal de ferro e cálcio de fórmulas infantis à base de leite de vaca e de soja em filhotes de ratos recém-desmamados

Maisa de Lima Correia SILVA[1]
Patrícia da Graça Leite SPERIDIÃO[1]
Renata MARCIANO[1]
Olga Maria Silvério AMÂNCIO[1]
Tânia Beninga de MORAIS[2]
Mauro Batista de MORAIS[1]

ABSTRACT

Objective

This study aimed to compare the intestinal absorption of iron and calcium between soy-based and cow's milk-based infant formulas in weanling rats.

Methods

Twenty male Wistar rats, twenty-one days old on the first day of weaning, were used in this experiment, divided in two Groups, one Group was fed soy protein-based infant formula the other, cow's milk protein-based infant formula. During the study period (ten consecutive days) the animals received food and water *ad libitum*. Hematocrit and hemoglobin were evaluated on the first, fifth, and tenth days by the Wintrobe and cyanomethemoglobin methods. Feces and urine were collected, beginning on the fifth day, for three consecutive days. On the tenth day, hepatic iron content was also analyzed. Hepatic iron as well as fecal and urinary iron and calcium analyses were performed using an atomic absorption spectrophotometer. At thirty-one days of age, the animals were anesthetized with ketamine and xylazine and sacrificed by exsanguination via the vena cava.

[1] Universidade Federal de São Paulo, Escola Paulista de Medicina, Departamento de Pediatria. R. Coronel Lisboa, 826, Vila Clementino, 04020-041, São Paulo, SP, Brasil. *Correspondência para*/Correspondence to: MB MORAIS. E-mail: <mbmorais@osite.com.br>.

[2] Universidade Federal de São Paulo, Escola Paulista de Medicina, Laboratório de Bromotologia e Microbiologia de Alimentos. São Paulo, SP, Brasil.

Support: *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.*

Results

The final concentration of hemoglobin in the group soy-based infant formula and milk-based infant formula were: 10.3±1.3g/dL and 10.9±1.0g/dL (p=0.310). The apparent absorption of iron and calcium, in that order, were: 73.4±10.2% and 70.2±9.5%; 97.2±0.7% and 97.6±1.0% (p=0.501; p=0.290). The apparent calcium retention was: 88.4% ±2.2 and 88.6±2.6% (p=0.848). Hepatic iron content was: 522.0±121.1mg/g and 527.8±80.5mg/g (p=0.907).

Conclusion

Intestinal iron and calcium absorption from soy-based infant formula is similar to that from milk-based infant formula in weanling rats.

Keywords: Calcium. Intestinal absorption. Iron. Milk proteins. Soy proteins.

RESUMO

Objetivo

Este estudo objetivou comparar a absorção intestinal de ferro e cálcio entre fórmulas infantis à base de leite de vaca e de soja em ratos recém-desmamados.

Métodos

Vinte ratos machos Wistar, com 21 dias de vida e no primeiro dia do desmame foram utilizados neste experimento, divididos em dois grupos: um alimentado com fórmula infantil à base de proteína de soja e o outro com fórmula infantil à base de proteína do leite de vaca. Durante o período de estudo (10 dias consecutivos), os ratos receberam dieta e água ad libitum. Hematócrito e hemoglobina foram mensurados no primeiro, quinto e décimo dia pelo método de Wintrobe e da cianometaemoglobina. As fezes e a urina foram recolhidas a partir do quinto dia durante durante três dias consecutivos. No décimo dia, o conteúdo de ferro hepático também foi analisado. O conteúdo de ferro hepático e a análise de ferro e cálcio nas fezes e na urina foram realizados utilizando espectrofotômetro de absorção atômica. Aos 31 dias de vida, os animais foram anestesiados com ketamina e xilasina e sacrificados por exsanguinação da veia vaca.

Resultados

A concentração final de hemoglobina no grupo fórmula infantil à base de soja e fórmula infantil à base de leite de vaca foram 10,3±1,3g/dL e 10,9± 1,0g/dL (p=0,310), respectivamente. A absorção aparente de ferro e cálcio foi de: 73,4±10,2% e 70,2±9,5%, para o primeiro grupo; e 97,2±0,7% e 97,6±1,0%, para o segundo (p=0,501; p=0,290). A retenção aparente de cálcio foi: 88,4±2,2% e 88,6±2,6% (p=0,848). O teor de ferro hepático foi: 522,0±121,1µg/g e 527,8±80,5µg/g (p=0,907), respectivamente.

Conclusão

A absorção intestinal de ferro e cálcio da fórmula infantil à base de soja é similar à da fórmula infantil à base de leite em ratos recém-desmamados.

Palavras-chave: *Cálcio. Absorção intestinal. Ferro. Proteínas do leite. Proteínas de soja.*

INTRODUCTION

Soy-based formulas have been used in infant nutrition because of their relatively low cost and their acceptance by infants[1]. Despite very limited indications for these formulas, they are used for a large number of infants worldwide[2]. Soy-based formulas are one type of alternative to milk-based formulas, and they are thus often introduced at a very early age or during the neonatal period. The indications for the use of a soy formula are restricted to several cases: proven serious and persistent intolerance to lactose; galactosemia; children from vegetarian families who cannot be breastfeed and whose parents wish to avoid animal-derived protein formulas for religious, philosophical or ethical reasons; and immunoglobulin-mediated allergy to cow's milk protein in children who are not sensitized to soy[1,2].

The composition of soy proteins is very complex and differs from the cow's milk proteins used in infant formulas[3]. In recent years, several publications have reviewed the potential adverse effects of the use of soy formulas, especially regarding phytate, aluminum, manganese, and phytoestrogen content. Data for other outcome variables are limited, but they suggest no change in visual acuity, cognitive development, response to vaccination, or number of immune cells[1,2,4-8].

Recent position statements issued by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)[1] Committee on Nutrition and the American Academy of Pediatrics (AAP)[2] note that soy formulas have nutritional disadvantages because their phytate content may reduce the absorption of minerals and trace elements[1,2,9]. This position is based on several studies that analyzed the interactions among different nutrients and the bioavailability of iron, calcium and other minerals. Negative effects on iron and calcium absorption have been observed in studies conducted on rat pups[10] weaned monkeys[11], infants[12,13], and adults[13-15]. The negative effects were attributed to phytate[10] and to the types of peptides released during protein digestion[11,14,16]. However, other studies have shown no negative effect on iron[17-19] or calcium[20,21] absorption when comparing soy-based infant formula to milk-based infant formula. In fact, some studies have reported that the bioavailability of calcium from soy-based infant formula is higher with than from milk-based infant formula[19,22,23].

This study was conceived because there is scant information on the effects of soy-based formulas on iron and calcium absorption over periods during which significant growth occurs. This study was undertaken because our laboratory employs an experimental model that allows the bioavailability of iron and calcium provided exclusively by infant formulas to be evaluated over a period during which the weight of recently weaned rats increased by approximately 50%. The difficulties of and ethical constraints in achieving this type of clinical trial in human infants were also considered. The objective of this study was therefore to compare the intestinal absorption of iron and calcium from soy- and cow's milk-based infant formulas in weanling rats.

METHODS

Study design and experimental diets

Twenty male Wistar rats aged twenty-one days (on the first day of weaning) were used in this experiment conducted by the Research Laboratory of the Department of Pediatrics of the *Universidade Federal de São Paulo* (Unifesp), *Paulista* School of Medicine, *São Paulo*, Brazil. Throughout the study period (ten consecutive days), the rats received deionized water via the MilliQ Plus system *ad libitum,* and a diet consisting of the same volume of fluid milk (150mL) was administered three times per day (either soy- or cow's milk-based infant formulas). All of the rats were kept in individual metabolic cages under a twelve-hour light cycle at a temperature of 23±1°C. Each cage was fitted with two troughs previously treated with nitric acid and rinsed with deionized water.

The animals allocated in the group soy-based infant formula (n=10 animals) were fed soy protein-based infant formula; the other group milk-based infant formula (n=10 animals) were fed lactose-free cow's milk protein-based infant formula consisting of 100% casein. All formulas were reconstituted according to the manufacturers' instructions, and the nutritional composition of each formula is shown in Table 1. Regarding the nutritional content, the values declared by the manufacturers on the product labels were considered. Laboratory analyses were not conducted to confirm the values listed on the product labels.

The experiment was performed in two stages. On the first day of the study, the rats were divided into two groups. The animals were anesthetized with ketamine and xylazine (66.6 and 13.3mg/kg, respectively), and the

 https://doi.org/10.1590/1678-98652017000100002

Table 1. Nutritional composition of the diets provided during the experiment according to the information contained on the label of each product.

Levels per 100mL of reconstituted formula	Soy-based infant formula	Milk-based infant formula
Energy (kcal)	74.10	73.70
Carbohydrates (g)	7.50	8.20
Lipids (g)	4.00	3.90
Protein (g)	2.00	1.50
Vitamin C (mg)	9.30*	9.30
Vitamin D (mg)	1.56	1.33
Calcium (mg)	60.00**	61.00***
Iron (mg)	0.89****	0.87*****
Fiber (g)	0.00	0.00

Note: *Addition of 0.4mg of vitamin C in the form of ascorbic acid; **Calcium in the form of calcium carbonate and tricalcium phosphate; ***Calcium in the form of calcium phosphate and calcium carbonate; ****Iron in the form of ferrous sulfate; *****Iron in the form of ferrous sulfate.

hemoglobin and hematocrit concentrations were then determined. Blood was collected from the tail of the animal. Hematocrit and hemoglobin were determined by the Wintrobe[24] and cyanomethemoglobin methods, respectively.

The volumes consumed by each animal were measured every time the formula was replaced and the troughs were cleaned. Each trough contained a bracket so that any leakage could be considered. This feature also allowed the calculation of total feed efficiency[25], which indicates how much of the infant formula is responsible for the observed weight gain in the rats (only the powder formula consumed was considered; the water was not included). Feed efficiency was calculated using the following formula: Feed efficiency = (weight gain (g)/total amount of diet consumed (g) over the 10-days period).

The study Protocol was approved by the Research Ethics Committee of the Unifesp. The study was conducted in accordance with institutional guidelines for experiments with animals (CEP nº 0659/10).

Blood sample analysis and experimental procedures

On the fifth day, hematocrit and hemoglobin were measured, and fecal occult blood testing was performed for each animal using the Hemoplus kit (Newprov – *Produtos para Laboratórios Ltda*., *Pinhais*, *Paraná*, Brazil). These procedures were repeated on the tenth day.

After five days of start of supply of the experimental diets, 0.1g of carmine-pink dye (Merck®, Frankfurt, Hesse, Germany) was added to the meals, and for three consecutive days, eliminated feces were collected beginning at the occurrence of the (reddish) color change. Approximately seventy-two hours after the addition of carmine-pink dye, Evans blue dye was added to the meals (Inlab, water-soluble). Fecal collection was stopped when the elimination of bluish colored feces began. The feces collected over these three days were properly labeled, weighed on an analytical electronic scale (Mettler Toledo – Model AB204, *Barueri*, *São Paulo*, Brazil) with a sensitivity of 0.0001g, and stored in a freezer (-20ºC).

During the fecal collection period, all eliminated urine was also collected. The urine samples collected over these three consecutive days were properly labeled, measured using a glass graduated cylinder, and stored at -20°C.

At thirty-one days of age, the animals were again anesthetized with ketamine and xylazine (66.6 and 13.3mg/kg) and sacrificed by exsanguination via the vena cava (Figure 1). Finally, the liver was surgically removed. The liver was weighed fresh and stored at -20°C. The liver was subsequently oven dried for 22 hours at 120°C until a constant dry weight was obtained. Determination of hepatic iron was performed after acid digestion of the dry tissue using atomic absorption spectrophotometry[26].

Apparent absorption and retention of iron and calcium

To assess the absorption of iron and calcium, the feces collected were then dried at 105°C. After 22 hours, the feces were weighed at 30 minute intervals until two successive weights were obtained with a difference of less than 1.0mg. A total of 500mg of dried feces from each animal was weighed, divided into two samples of 250mg (duplicate) each, and subjected to acid digestion using nitric acid and perchloric acid (volumes: 4mL nitric acid and 2mL perchloric acid; digester temperature: 200ºC; wavelengths: Iron=248.3nm, Calcium=422.7nm; slit: 0.7nm; flame air/acetylene; energy: Iron=60, Calcium=67). The iron and calcium analysis was performed using the Perkin-Elmer Model 5100 PC Uberlingen, Baden-Württemberg, Germany), an atomic absorption spectrophotometer[26]. To analyze iron, deionized water was added; to analyze calcium, lanthanum chloride (Merck®, Darmstadt, Germany) was added to the samples (final concentration: 1% lanthanum). Intakes of iron and calcium intake over the same period were also calculated. The percentage of iron absorbed was calculated using the following formula:

Percentage of iron absorbed = quantity of iron ingested (µg) – quantity of iron excreted (µg)/amount of iron ingested (µg) X 100. The total quantity of iron excreted was calculated based on the relation between the iron content of the dry feces and the total dry fecal weight using the following formula: Iron excreted (µg) = (Iron [µg] X total dry fecal weight [g])/1g of dry feces (g). Calcium absorption was calculated in the same manner.

To assess iron and calcium retention, the collected urine was filtered through filter paper (Whatman Filter Papers 40, ashless, circles, 90mmØ; Whatman Corporation, Clifton, New Jersey, United States of America) and diluted, enabling evaluation by atomic absorption spectrophotometry (Perkin Elmer model 5100 PC)[27]. Lanthanum chloride (Merck®) was added to the samples (final concentration: 0.5% lanthanum) for calcium analysis. The following formula was used to calculate the percentage of retained iron: Percentage of retained iron = (quantity of iron ingested [µg] – quantity of iron excreted fecal [µg] – quantity of iron excreted urine [µg])/quantity of iron ingested [µg]x100. Calcium retention was calculated in the same manner.

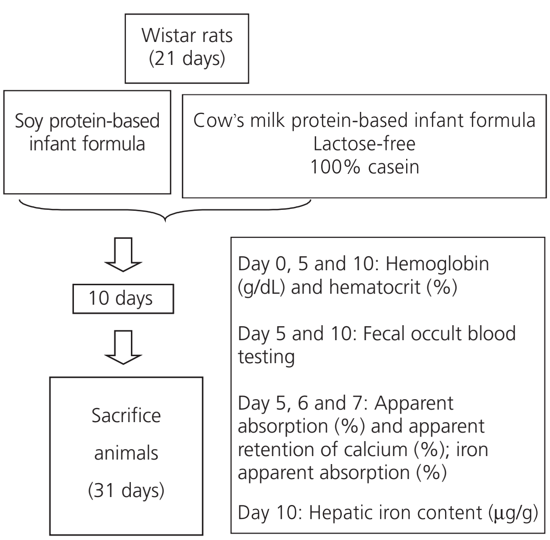

Figure 1. Study design.

Statistical analysis

Parametric variables were expressed as the means and standard deviations. For variables with normal distributions, Student's *t*-tests were used to compare independent groups. Jandel Sigma Stat® (Richmond, California, United States of America) version 3.5 software was used to

https://doi.org/10.1590/1678-98652017000100002

perform the statistical tests, and a significance level of 0.05 or 5% was adopted for rejection of the null hypothesis.

RESULTS

Baseline data

Before starting the experimental diets, there was no statistically significant difference between groups with respect to hemoglobin or hematocrit ($p>0,05$). This similarity between groups was maintained after the exclusion of one animal in each group that did not complete the study by reason of death after anesthesia without apparent cause. This information is presented in conjunction with other results in Table 2.

Iron and calcium intakes and feed efficiency

Iron and calcium intakes and feed efficiency are shown in Table 3. The iron and calcium intakes were similar in both groups ($p>0,05$). Feed efficiency was significantly higher in the group fed milk-based infant formula ($p<0,05$).

Hemoglobin, hematocrit, apparent absorption of iron, and hepatic iron

Table 2 presents the hemoglobin, hematocrit, apparent absorption of iron and hepatic iron values. There were no differences in the concentrations of hemoglobin or hematocrit at any time during the experiment ($p>0,05$). The apparent absorption of iron was similar in both groups ($p>0,05$). The amount of iron excreted in the urine was negligible; thus, calculation of the apparent retention of iron was unnecessary. The fresh weight of the liver in the group receiving soy-based infant formula (3.6±0.5g) was significantly lower ($p=0.002$) than the group fed milk-based infant formula (4.5±0.7g). However, this difference disappeared when analyzing the fresh weights of the livers relative to the total weight of the animals (Hepatosomatic Index [HIS]= liver weight (g)/animal weight (g)) ($p=0.156$), which were 0.05±0.01g/g and 0.05±0.01g/g, respectively. There were no statistically significant differences in hepatic iron levels ($p>0,05$).

Fecal occult blood testing was negative in all samples (based on the feces of all animals on the fifth and tenth days).

Table 4 presents apparent absorption and apparent retention of calcium. No significant

Table 2. Hb (g/dL) and Hct (%) on days 0, 5, and 10 of the experiment in rats; iron apparent absorption (%); and hepatic iron content (µg/g) in rats.

Variables	Soy-based infant formula (n=9)			Milk-based infant formula (n=9)			*p*
	M	±	SD	M	±	SD	
Hemoglobin (g/dL)							
Initial	7.5	±	1.2	7.7	±	1.1	0.697
Day 5	8.6	±	1.5	8.6	±	1.7	0.959
Day 10	10.3	±	1.3	10.9	±	1.0	0.310
Hematocrit (%)							
Initial	24.1	±	3.5	26.0	±	3.1	0.246
Day 5	28.2	±	4.3	28.8	±	5.9	0.822
Day 10	31.0	±	5.6	33.3	±	3.5	0.307
Apparent absorption of iron (%)	73.4	±	10.2	70.2	±	9.5	0.501
Hepatic iron content (µg/g)	522.0	±	121.1	527.8	±	80.5	0.907

Note: Results are express as Mean (M) ± Standard Deviation (SD), Student's *t*-test.

Table 3. Calcium and Iron intake (mg) and feed efficiency (g/g) during the 10 days of the experiment in rats.

Variables	Soy-based infant formula (n=9)			Milk-based infant formula (n=9)			*p*
	M	±	SD	M	±	SD	
Calcium intake (mg)	350.1	±	35.1	328.6	±	38.8	0.235
Iron intake (mg)	5.2	±	0.5	4.7	±	0.6	0.062
Feed efficiency (g/g)	0.2	±	0.1	0.4	±	0.1	<0.001

Note: Results are express as Mean (M) ± Standard Deviations (SD), Student's *t*-test.

Table 4. Apparent absorption (%) and apparent retention of calcium (%) in rats.

Variables	Soy-based infant formula (n=9)			Milk-based infant formula (n=9)			*p*
	M	±	SD	M	±	SD	
Apparent absorption of calcium (%)	97.2	±	0.7	97.6	±	1.0	0.290
Apparent retention of calcium (%)	88.4	±	2.2	88.6	±	2.6	0.848

Note: Results are express as Mean (M) ± Standard Deviations (SD), Student's *t*-test.

difference in apparent absorption or apparent retention calcium was observed between the groups ($p>0,05$).

DISCUSSION

This experimental model is used to improve knowledge and provide possible benefits to humanity; however, it does not accurately reflect what happens in humans. The objective of this study could not be met by performing this experiment on human infants. Thus, the interpretation of the results should account for differences between these species; however, certain common characteristics justify the use of experimental models to improve knowledge of issues that are of interest to human health.

This experimental model allows us to evaluate the effects of a single fluid food, as occurs in the feeding of infants before the introduction of complementary foods. In this study, one group received a lactose-free cow's milk-based infant formula, as previous studies have found that weaned mice fed formula with higher amounts of lactose experienced loose stools, suggesting lactose intolerance[28]. Thus, a lactose-free reference product was used to compare to the soy-based infant formula.

This is the first study that compares iron and calcium absorption from a soy-based infant formula and a milk-based infant formula over a continuous period (10 days), which is sufficient to cause significant changes in the physical development of the animal. This study used an experimental model previously developed in our laboratory that allowed us to evaluate the interactions of nutrients supplied to recently weaned rats fed exclusively with different types of fluid formulas used to feed infants[28-30]. Throughout the experiment, the animals in both groups did not receive rations other than their liquid foods.

We made no changes to the environment or to the diets over the study period; therefore, rats aged twenty-one days were used, which allowed an adequate amount of time for the rats to be nursed by their mothers. We chose not to deprive them of a breastfeeding period, respecting their physiological needs.

The ten-day period was selected based on previous studies conducted on our service, which proved satisfactory to meet our goals[28-30].

In addition, during the experiment, the rats maintained growth similar to that of animals fed with feed in other experiments[31]. To use of research resources rationally and to limit the number of animals used in experiments, it was considered unnecessary to set up a group fed a conventional diet (*e.g.,* American institute of Nutrition-93G [AIN-93G]). That is, the objective was to analyze the tested formulas (which are used for human infants) rather than to meet the needs of experimental animals during the growth stage[28-30].

We used a volume of 150mL, which is enough to maintain the animals' satiety. The intention was to ensure that the animals had more than enough food available to meet their needs. Based on previous experience and nutritional content of the formulas, it was known that these volumes were sufficient, although this was confirmed during the experiment[28]. As the desire was to evaluate the absorption of minerals from the products analyzed, no adjustments for animal growth compared to rats fed an AIN-93G diet were made.

As for iron absorption, our results are consistent with other *in vitro* assays[19-21]. The high ascorbic acid and citric acid contents of soy-based infant formulas compared to milk-based infant formulas may explain the similar amounts of iron absorbed from both infant formulas[19].

Some studies observed lower iron bioavailability from soy-based infant formulas than from milk-based infant formulas. This effect has been observed in rats[10], infants,[12,13] and adults[13-15]. This difference is attributed to phytate content[10]. Moreover, some polypeptides are able to bind to iron and prevent its absorption[14], as occurs with the 7S (β-conglicinina) protein fraction released during soy protein digestion[16].

Some studies[12,13,15,] find that the inhibitory effect of soy-based infant formula on iron absorption can be nullified by the addition of ascorbic acid or the removal of phytic acid. However, another study[13] reported decreased iron bioavailability from soy-based infant formula even if its extract is purified or reduced and ascorbic acid is added.

With respect to the intestinal absorption calcium, our results are similar to those of previous studies in infants[17] and weaned monkeys[18]. However, other *in vitro* studies[19,22,23] have reported greater calcium bioavailability from soy-based infant formulas than from milk-based infant formulas. Studies of rat pups[10] and weaned monkeys[11] have also reported lower bioavailability of calcium from soy-based infant formula than from milk-based infant formula. This reduced calcium bioavailability from soy-based infant formula has also been attributed to phytate[10] and to the types of peptides released during protein digestion[11]. However, phytate appears to be less relevant, as new preparations of soy-based formulas have low phytate content[32].

Over the years, soy-based infant formulas have been changed to improve digestibility, stability, mineral availability, and protein quality[32], for example, by reducing phytate content. Our results can therefore be explained by the reduction of the antinutrients (phytic acid) and insoluble complexes (phytate/mineral) during the industrial processing of soy.

Emphasis should be given to the significant difference in feed efficiency, with the lowest value in the group fed with soy-based infant formula. Soy formula contains anti-nutritional factors that could affect the availability of nutrients, which may explain this result.

Soy-based infant formulas have been developed to meet the nutritional requirements of full-term newborns, and several human studies have demonstrated that these formulas promote growth and development similar to that of infants fed milk-based infant formulas. However, these formulas exhibit no nutritional advantages over milk-based infant formulas[1,2]. In addition, they contain high concentrations of phytate and aluminum phytoestrogens (isoflavones) that can have undesirable effects. Indications for the use of soy-based formulas include persistent lactose intolerance, galactosemia, and ethical considerations (*e.g.,* a vegetarian diet)[1,2,5]. It is noteworthy that soy-based formulas may play a role in the prevention of allergic diseases, although they should not be used in children with food allergies during the first six months of life[1].

CONCLUSION

This experimental study demonstrated similar intestinal absorption of iron and calcium from soy- and cow's milk-based infant formulas in weanling rats. It is noteworthy that the literature does not include recent references on this subject.

CONTRIBUTORS

MLC SILVA and R MARCIANO participated in the data collection. MLC SILVA, PGL SPERIDIÃO, and MB MORAIS participated in the conception, study design, analysis and/or interpretation of data, the drafting the article or revising it critically for important intellectual content. OMS AMÂNCIO, and TB MORAIS participated in the laboratory analysis, analysis and/or interpretation of data.

REFERENCES

1. ESPGHAN Committee on Nutrition, Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KF, *et al*. Soy protein infant formulae and follow-on formulae: A commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2006;42(4):352-61.
2. Bhatia J, Greer F. Use of soy protein-based formulas in infant feeding. Pediatrics. 2008;121(5):1062-8.
3. Lönnerdal B. Nutritional aspects of soy formula. Acta Paediatr. 1994;402(s402):105-8.
4. Muraro MA. Soy and other protein sources. Pediatr Allergy Immunol. 2001;12(Suppl.14):85-90.
5. Badger TM, Gilchrist JM, Pivik RT, Andres A, Shankar K, Chen JR, *et al*. The health implications of soy infant formula. Am J Clin Nutr. 2009;89(5):1668S-72S.
6. Bennetts HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterrean clover pastures in Western Australia. Aust Vet J. 1946;22:2-12.
7. Naaz A, Szewczykowski MA, Sato T, Woods JA, Chang J, *et al*. The phytoestrogen genistein indusces thymic and immune changes: A human health concern? Proc Natl Acad Sci USA. 2002;99(11):7616-21.
8. Turck D. Soy protein for infant feeding: What do we know? Curr Opin Clin Nutr Metab Care. 2007;10(3):360-5.
9. Scientific Committee on Food. Report on the revision of essential requirements of infant formulae and follow-on formulae. *Brussels*: European Commission; 2003. SCF/CS/NUT/IF/65.
10. Lönnerdal B, Yuen M, Huang S. Calcium, iron, zinc, copper and manganese bioavailability from infant formulas and weaning diets assessed in rat pups. Nutr Res. 1994;14(10):1535-48.
11. Rudloff S, Lönnerdal B. Calcium and zinc retention from protein hydrolysate formulas in suckling rhesus monkeys. Am J Dis Child. 1992;146(5):588-91.
12. Davidsson L, Galan P, Kastenmayer P, Cherouvrier F, Juillerat MA, Hercberg S, *et al*. Iron bioavailability studied in infants: The influence of phytic acid and ascorbic acid in formulas based on soy isolate. Pediatric Res. 1994;36(6):816-22.
13. Hurrell RF, Davidsson L, Reddy M, Kastenmayer P, Cook JD. A comparison of iron absorption in adults and infants consuming identical infant formulas. Br J Nutr. 1998;79(1):31-6.
14. Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. Am J Clin Nutr. 1992;56(3):573-8.
15. Gillooly M, Torrance JD, Bothwell TH, MacPhail AP, Derman D, Mills W, *et al*. The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. Am J Clin Nutr. 1984;40(3):522-7.
16. Lynch SR, Dassenko SA, Cook JD, Juillerat MA, Hurrell RF. Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. Am J Clin Nutr. 1994;60(4):567-72.
17. Jovaní M, Barberá R, Farré R, Martín de Aguilera E. Calcium, iron, and zinc uptake from digests of infant formulas by Caco-2 cells. J Agric Food Chem. 2001;49(7):3480-5.
18. García R, Alegría A, Barberá R, Farré R, Lagarda MJ.Dialyzability of iron, zinc, and copper of different types of infant formulas marketed in Spain. Biol Trace Elem Res. 1998;65(1):7-17.
19. Nogueira CSN, Colli C, Amancio OMS. Infant formula iron dialysability related to other nutrients. Food Chem. 2005;90(4):779-83.

 https://doi.org/10.1590/1678-98652017000100002

20. Shenai JP, Jhaveri BM, Reynolds JW, Huston RK, Babson SG. Nutritional balance studies in very low-birth-weight infants: Role of soy formula. Pediatrics. 1981;67(5):631-7.

21. Rudloff S, Lönnerdal B. Calcium retention from milk-based infant formulas, whey-hydrolysate formula, and human milk in weanling rhesus monkeys. Am J Dis Child. 1990;144(3):360-3.

22. Bosscher D, Van Dyck K, Robberecht H, Van Caillie-Bertrand M, Deelstra H. Bioavailability of calcium and zinc from cow's milk-based versus soya-based infant food. Inter J Food Sci Nutr. 1998;49:277-83.

23. Roig MJ, Alegría A, Barberá R, Farré R, Lagarda MJ. Calcium bioavailability in human milk, cow milk and infant formulas: Comparison between dialysis and solubility methods. Food Chem. 1999;65(3):353-7.

24. Wintrobe M, Mollin D, Herbert V, Blanc B, Finch C, Halberg L, *et al*. Anemias nutricionales. Org Mund Salud Ser Inf Técn. 1968;405:5-39.

25. Campbell JA. Method for determination of PER and NPR. In: Evaluation of protein quality. Whasington (DC): National Academy of Sciences; 1963. p.31-2.

26. Marks GE, Moore CE, Kanabrocki EL, Oester YT, Kaplan E. Determination of trace elements in human tissue. I, Cd, Fe, Zn, Mg and Ca. Appl Spectrosc. 1971;26:523-7.

27. Fernandez FJ, Kahn HL. Clinical methods for atomic absorption spectroscopy. Clin Chem Newsl. 1971;3:24.

28. Costa ML, Freitas KC, Amancio OM, Paes AT, Silva SM, Luz J, *et al*. Iron absorption from infant formula and iron-fortified cow's milk: Experimental model in weanling rats. J Pediatr. 2009;85(5):449-54.

29. Silva ML, Speridião PG, Marciano R, Amâncio OM, Morais TB, Morais MB. Effects of soy beverage and soy-based formula on growth, weight, and fecal moisture: Experimental study in rats. J Pediatr. 2015;91(3):306-12.

30. Farjalla, LB. Absorção intestinal do ferro de fórmulas infantis anti-regurgitação espessadas com fibra alimentar goma jataí e amido de milho em ratos recém-desmamados [tese]. São Paulo: Universidade Federal de São Paulo; 2011.

31. Lopes, LA. Efeitos da restrição de macronutrientes sobre o crescimento corporal e a histopatologia do sistema nervoso central de ratos jovens desnutridos intra-útero [tese]. São Paulo: Universidade Federal de São Paulo; 1999.

32. Mendez MA, Anthony MS, Arab L. Soy-based formulae and infant growth and development: A review. J Nutr. 2002;132(8):2127-30.

Received: March 21, 2016
Final version: August 29, 2016
Approved: September 13, 2016