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Effects of short-term mild calorie restriction diet and renutrition with ruminant milks on leptin levels and other metabolic parameters in mice

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Abstract

Objective: The adaptation of an organism to a calorie-restricted diet is characterized by metabolic, endocrine, and immunologic changes. The objective of this study was to determine, in a mouse model, the changes in serum leptin levels in response to short-term mild calorie-restricted and renutrition diets using different ruminant milks.

Methods: Weaned Swiss albino mice were fed with a mild calorie-restricted diet for 12 d, after which they were renourished with cow, goat, sheep, or buffalo milk for 7 d. Body, thymus, and spleen weights and biochemical, hematologic, and endocrine parameters were evaluated.

Results: The mild calorie restriction did not significantly modify insulin and leptin levels. The renutrition diets increased insulin levels, being significant ($P < 0.05$) only when buffalo and sheep milks were used. Leptin concentrations increased in the control ad libitum (AD) group during the assayed period. After the administration of cow and goat milks, lower leptin levels were observed compared with the control AD group. All repletion diets significantly increased body, thymus, and spleen weights; however, spleen weight did not reach the values observed in the control AD group. Serum glucose and triacylglycerol levels increased after feeding with the renutrition diets. However, serum cholesterol did not increase after the renutrition period. We observed a significant decrease ($P < 0.05$) in the leukocyte counts in calorie-restricted mice in comparison with AD mice; after the renutrition period, the leukocyte count did not reach the values for the AD mice.

Conclusion: This study suggests that a short-term change in diet with a relatively low body weight loss does not significantly affect leptin concentrations in our mouse model. However, the assayed milks could be effectively used as alternative milk sources for weight gain and for the improvement of other metabolic parameters.

Keywords: Mild calorie restriction; Ruminant milk; Leptin; Insulin; Mice

Introduction

The ability of an organism to survive in a nutritionally scarce environment depends on its capacity to make appropriate metabolic adjustments [1]. Understanding the physiologic effects of a calorie-restricted diet or a malnutrition state may contribute to minimize its adverse consequences. In addition, some researchers have suggested that mild calorie restriction might provide multiple health benefits such as extending the lifespan of diverse organisms including mammals [1]. In rodents and primates, calorie restriction lowers plasma insulin, cholesterol, triacylglycerol, and insulin-like growth factor-1 levels and elevates plasma high-density lipoprotein levels [2].

The adaptation to a calorie-restricted diet is characterized by metabolic, endocrine, and immunologic changes. It is

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known that leptin and insulin levels are crucial neuroendocrine signals for the brain and initiate the adaptive response to starvation [3]. Leptin is a cytokine-like 16-kDa peptide mainly produced by adipose tissue and is secreted in the blood [4]. Leptin is also synthesized in other tissues and has many functions; it has been shown to play an important role in the regulation of neuroendocrine function and energy homeostasis and other energy-demanding physiologic processes, such as reproduction, hemopoiesis, and angiogenesis [5,6]. Serum leptin concentrations are positively correlated with the fat mass of the body [7]. However, short-term severe energy restriction can cause circulating leptin concentrations to decrease to a larger extent than would be expected from the loss of fat mass alone [8–10]. Leptin deficiency is well known to cause obesity through increased eating and decreased energy expenditure in *ob/ob* mice [4]. The link between leptin deficiency and starvation has been proposed by other investigators who observed that the hormonal profile of *ob/ob* mice resembled that observed in a starvation state [11] and that starvation is also a state of leptin deficiency. Leptin treatment rapidly reverses these changes in *ob/ob* mice [11]. It has been suggested that leptin administration reverses the neuroendocrine and immunologic changes of starved mice and of *ob/ob* mice, indicating that leptin deficiency mediates hormonal and immune abnormalities in both [11].

There is evidence that leptin could be a sensitive marker of nutritional status because it is directly correlated with several biochemical and anthropometric parameters [12]. Leptin could be used as an important signal to reflect the metabolic adaptations, especially in severe and moderate malnutrition.

The best treatment for malnutrition is nourishment. Nevertheless, successful nutritional rehabilitation requires the understanding of the effect of calorie restriction on the organism. The ideal diet has been the subject of debate and milk has been extensively accepted as an appropriate treatment for malnutrition status [13]. Although the main function of milk is nutrition, some of its components also have hormonal and immunologic functions. The nutritive quality of milk is determined by its composition. Indeed, milks from different ruminants differ in lipid, protein, carbohydrate, vitamin, and mineral contents and therefore in their nutritional properties. In this field, there is limited scientific evidence available on the importance of goat, sheep, or buffalo milk in human nutrition.

Although an increasing amount of evidence indicates that leptin may play an important role as the link between energy homeostasis and the immune system, accounting for several neuroimmunoendocrine abnormalities during nutrition-deficiency states, many questions remain unanswered. According to Faggioni et al. [14], even though leptin has shown limited efficacy as an antiobesity strategy, it may have therapeutic potential in diseases/conditions characterized by low leptin levels, such as malnutrition and lipodystrophy.

Renutrition diets supplemented with milk could increase leptin levels in malnourished hosts because leptin is one of the bioactive components present in milk [15]. Previous studies have demonstrated that the oral administration of leptin to rats produces an increase in serum leptin levels [15]. In our present study, we used milk from different ruminants to renourish mildly calorie-restricted mice because we hypothesized that feeding with milks with different nutrient compositions could have different effects on leptin secretion. Furthermore, the use of these milks would be more appropriate than exogenous leptin administration during calorie restriction because they could also protect mice from several consequences associated with starvation.

In the present work we studied the effects of short-term mild calorie restriction and different milk renutrition diets on serum leptin levels using a mouse model. Additionally, changes in insulin concentrations and other nutritional and metabolic parameters were investigated.

Materials and methods

Animals and feeding protocols

The overall experimental protocol is summarized in Figure 1. Weaned male Swiss albino mice were supplied and maintained in the Centro de Referencia para Lactobacilos (CERELA)-Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET) (Tucumán, Argentina). The experimental protocol was approved by the ethical committee for animal care at CERELA, and experimental procedures were carried out in accordance with institutional guidelines.

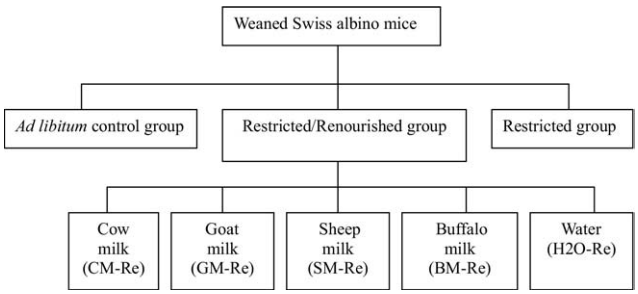

Fig. 1. Calorie restriction/renutrition experimental protocol in mice. After a 2-d acclimatation period, mice were matched by weight and assigned to one of three main groups: the control ad libitum group was fed with an ad libitum chow diet for 12 d, the calorie-restricted group was fed with a restricted diet, and the restricted/renourished groups were fed with a calorie-restricted diet for 12 d (calorie-restriction period) followed by 7 d (renutrition period) in which animals were fed with the same diet (without restriction) supplemented with cow (CM-Re), goat (GM-Re), sheep (SM-Re), or buffalo (BM-Re) milk. In this last group we added another subgroup in which mice received an ad libitum chow diet and water. BM-Re, renourished with buffalo milk; CM-Re, renourished with cow milk; GM-Re, renourished with goat milk; H_2O-Re, ad libitum chow diet and water; SM-Re, renourished with sheep milk.

All animals were housed in individual metabolic cages and acclimated to 22°C with a 12-h light/12-h dark cycle. Mice were adapted for 2 d with a nutritionally complete pellet diet providing 21% of calories as protein (casein), 66% as carbohydrates (corn starch) 13% as fat (corn oil), vitamin mixture (2.2%; ICN Biomedicals, Inc., Aurora, OH, USA), and salt mixture (4%; ICN Biomedicals, Inc.).

After a period of 2 d of diet acclimation, mice were matched by weight and assigned to one of three main groups.

Ad libitum control group

Eighteen animals formed the ad libitum (AD) control group. Six mice were sacrificed at day 0 (baseline values). The remaining 12 mice were provided with ad libitum access to chow diet for 20 consecutive days. Six mice were sacrificed at day 13 (which was the end of the calorie-restricted period), and the remaining six mice were sacrificed at day 20 (which was the end of the renutrition period).

Calorie-restricted group

In the calorie-restricted (CR) group, 12 animals were fed for 12 d with 75% of their normal diet intake to achieve a 10–25% weight loss compared with their appropriate control AD group. Six of these mice were sacrificed at day 13 at the end of CR period. The other six mice were kept under calorie restriction for the entire duration of the experiment (additional 7 d).

Restricted/renourished group

In the restricted/renourished (Re) group, 30 mice were fed with the CR diet for 12 d (CR period) followed by a 7-d renutrition period when animals were fed with the same diet (without restriction) supplemented with different ruminant milks. Animals were renourished with fresh, pasteurized, non-fortified, whole cow, goat, sheep, or buffalo milks. The nutrient composition of the different milks is presented in Table 1. In this last group a subgroup was added in which mice received ad libitum chow diet and water (H_2O-Re). The animals were sacrificed at day 20 (at the end of the renutrition period).

Table 1
Composition of cow, goat, sheep, and buffalo milks administered to calorie-restricted/renourished groups*

Components	Cow milk	Goat milk	Sheep milk	Buffalo milk
Proteins (%; p/v)	3.26	3.28	5.35	4.55
Total lipid (%; p/v)	3.50	4.16	7.26	8.90
Carbohydrate (%; w/v)	4.58	4.45	4.83	4.82
Calories (kJ)	263.18	286.21	443.97	492.19

w/v, weight per volume

* Mice were fed with a calorie restricted diet for 12 d (calorie-restriction period) followed by a 7-d renutrition period in which animals were fed with the same diet (without restriction) supplemented with fresh, pasteurized, non-fortified, whole cow, goat, sheep, or buffalo milk.

We used a 12-d CR period based on a previous study [16]. By analogy with the classification of human malnutrition by Gomez et al. [17], we developed a murine scale of malnutrition based on weight for age (WA), using the well-nourished control group as the standard. WA was calculated as weight of the animals in the diet groups divided by the expected weight based on the mice in the control group multiplied by 100%. The classification of Gomez et al. [17] uses the following categories for human malnutrition: 75–90% WA, mild malnutrition; 60–75% WA, moderate malnutrition; and 60% WA, severe malnutrition [17]. In the same way, we used a 7-d renutriton period, based in our previous investigations, in which we showed that the administration of a proper diet for 7 d was able to restore the CR mice to a nutritional and immunologic status similar to that of the control group [18,19].

Baseline values were obtained by sacrificing six mice in the AD group at the beginning of the experiment (day 0, after the 2-d acclimation period). Mice were sacrificed at day 0 (baseline day), at the end of the CR period (day 13), and at the end of the renutrition period (day 20) by cervical dislocation 3 h after the last scheduled feeding from 0800 to 1000 h. Blood was recovered by cardiac puncture. Blood samples for determination of total leukocyte count were collected in tubes containing ethylene-diaminetetra-acetic acid solution. Blood samples for serum biochemical measurements were collected in plastic centrifuge tubes and kept on ice until they were centrifuged; the serum was then separated and frozen at −20°C. The thymus, spleen, and liver were rapidly removed and kept in closed, saline-saturated dishes for subsequent cleaning and weighing.

Body, thymus, spleen, and liver weights

Body weights were measured and recorded daily and expressed in grams starting the day before the experiment began until the end of the treatment period. The thymus and spleen were weighed on day 0 (baseline day), at the end of the CR period, and at the end of the renutrition period. These results were expressed as milligrams of organ weight per 100 mg of body weight.

Biochemical measurements

Serum glucose, cholesterol, and triacylglycerol levels were determined by enzymatic methods, using commercial kits for oxidase/peroxidase method according to Trinder [20] and according to standard procedures (GT Laboratory, Rosario, Argentina) [20]. The number of leukocytes was determined by hematocytometric methods. Serum leptin levels were determined in triplicate using a mouse leptin enzyme-linked immunosorbent assay kit (Linco Research, St. Charles, MO, USA) and serum insulin levels were determined in triplicate using a rat/mouse insulin enzyme-linked immunosorbent assay kit (Linco Research).

Statistics

The results were obtained from three independent experiments. Experimental data were expressed as mean ± standard deviation. Single comparisons between groups were assessed by Student's *t* test. Multiple comparisons were assessed by two-way analysis of variance (MINITAB 14.1, Minitab Inc., State College, PA, USA). Tukey's test (for pairwise comparisons of the mean of different groups) was used to test for differences between groups. Differences were considered statistically significant at $P < 0.05$.

Results

Body and organ (thymus and spleen) weights

Table 2 presents the effectiveness of different milk diets in promoting body and organ weight gains in CR compared with AD mice. All groups weighed the same at the time of weaning (21 d of age). The CR diet was administered after the adaptation period. The growth of the AD group was followed throughout the trials. A 98.7% body weight increase was observed compared with baseline values (because the animals were in their active growth period). Although the CR group continued to grow, their weight gain was significantly lower ($P < 0.05$) than that of the AD group at the end of the depletion period. All CR mice showed a 15–23% body weight loss in comparison with the control AD group. In accord with the results shown in our previous work [16], these mice reached a mild CR status. All repletion diets fed to CR mice significantly increased body weight to the same extent. By the end of the renutrition treatments, the mice reached weights similar to those of the AD mice. When we compared the influence of the different renutrition diets on body weight, no significant differences were observed.

In CR mice, the thymus and spleen weight ratios were significantly lower ($P < 0.05$) than those of the control AD groups of the same age. The renutrition diets with different ruminant milks induced a significant increase ($P < 0.05$) in these parameters compared with the H_2O-Re groups, but spleen weights did not reach the values of the AD group after the renutrition period. No significant differences were observed among the various renutrition treatments (Table 2).

Table 2
Effect of a CR diet and renutrition with different ruminant milks on body, thymus, and spleen weights*

Groups	Thymus (mg/100 mg BW)	Spleen (mg/100 mg BW)	BW (g)
Refeeding group			
Cow milk	0.42 ± 0.09†	0.42 ± 0.09‡	23.62 ± 1.79
Goat milk	0.35 ± 0.10†	0.44 ± 0.05‡	22.15 ± 1.18
Sheep milk	0.38 ± 0.05†	0.42 ± 0,14‡	24.69 ± 1.32
Buffalo milk	0.40 ± 0.06†	0.40 ± 0.05‡	23.58 ± 1.04
Water	0.29 ± 0.03§	0.34 ± 0.04§	23.33 ± 1.01
AD group			
Renutrition period	0.46 ± 0.04	0.75 ± 0.04	26.83 ± 1.51
CR period	0.35 ± 0.05	0.47 ± 0.04	23.98 ± 1.51
CR group			
Renutrition period	0.21 ± 0.05§	0.29 ± 0.03§	17.98 ± 2.0
CR period	0.27 ± 0.03§	0.34 ± 0.01§	18.52 ± 2.53§

AD, ad libitum, control; BW, body weight; CR, calorie-restricted

* Body, thymus, and spleen weights were measured on the baseline day, at the end of the CR period, and at the end of the renutrition period with cow, goat, sheep, and buffalo milks and water. Values of body and organ weights on day 0 in all groups (AD and CR groups) were similar: BW 13.5 ± 0.98 g, thymus weight 0.12 ± 0.03 mg/100 mg of BW, and spleen weight 0.18 ± 0.01 mg/100 mg of BW. Data are represented as mean ± SD for 10 mice for BW and for 6 mice for thymus and spleen weights.

† Significantly different from water in refeeding group ($P < 0.05$, Student's *t* test).

‡ Significantly different from AD group after renutrition period ($P < 0.05$, Student's *t* test).

§ Significantly different from AD group after CR period ($P < 0.05$, Student's *t* test). No significant difference between milks was observed (two-way analysis of variance).

Determinations of serum glucose, cholesterol, and triacylglycerol levels and total leukocyte counts

The results of serum glucose, cholesterol, and triacylglycerol levels and total leukocyte count determinations are listed in Table 3. A significant increase ($P < 0.05$) in serum glucose levels was observed after all renutrition diets compared with the H_2O-Re group, reaching values similar to those of the control AD group of the same age. The highest ($P < 0.05$) serum glucose levels were observed in the sheep-Re groups. Caloric restriction only significantly modified the cholesterol levels in the CR mice that were kept under calorie restriction for the entire duration of the experiment. All milk-based renutrition diets induced a slight (non-significant) decrease in blood cholesterol concentration, whereas the H_2O-Re diet induced a significant decrease ($P < 0.05$) in serum cholesterol values compared with control AD mice of the same age. Renutrition caused a non-significant increase in serum triacylglycerol levels with respect to H_2O-Re animals. When mice were renourished with the goat milk diet, the values for serum triacylglycerol were slightly lower than those obtained with the other milk diets, whereas buffalo milk induced a significant increase ($P < 0.05$) compared with the control AD diet. When we analyzed the total leukocyte count for CR mice, we observed a significant decrease ($P < 0.05$) compared with those of the AD mice. After the renutrition period the number of leukocytes did not reach the values observed in AD mice. No significant differences between renutrition groups were observed.

Table 3
Effect of a CR diet and renutrition with different ruminant milks on biochemical determinations*

Groups	Biochemical determinations			
	Glucose (g/L)	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	Leukocyte count (10^3/mm^3)
Refeeding group				
Cow milk	1.87 ± 0.18	108.0 ± 16.90†	167.60 ± 15.760	3.70 ± 1.50‡
Goat milk	1.54 ± 0.27	112.4 ± 15.60†	135.60 ± 21.00	4.10 ± 0.50‡
Sheep milk	2.18 ± 0.71§	113.25 ± 18.80†	155.00 ± 33.70	4.00 ± 1.02‡
Buffalo milk	1.91 ± 0.14	116.0 ± 11.40†	178.80 ± 8.70‡	4.00 ± 0.78‡
Water	1.67 ± 0.31	79.5 ± 20.60§	149.50 ± 23.90	4.50 ± 0.70‡
AD group				
Renutrition period	1.78 ± 0.12	139.5 ± 14.10	128.00 ± 36.00	8.00 ± 0.40
CR period	1.71 ± 0.15	144.5 ± 11.10	129.00 ± 25.00	7.90 ± 0.30
CR group				
Renutrition period	0.79 ± 0.09‡	93.09 ± 0.13‡	57.03 ± 9.00	3.3 ± 0.50‡
CR period	0.81 ± 0.06§	133.8 ± 14.60	58.00 ± 0.05§	3.50 ± 0.60§

AD, ad libitum, control; CR, calorie-restricted

* Serum glucose, cholesterol, triacylglycerol, and leukocyte count determinations were measured on the baseline day and at the end of the CR period and the end of the renutrition period with cow, goat, sheep, and buffalo milks and water. Data are represented as mean ± SD for six mice. Values of biochemical determinations on day 0 in all groups (AD and CR groups) were similar: glucose 1.73 ± 0.06 g/L, cholesterol 149.00 ± 14.60 mg/dL, and triacylglycerol 127.00 ± 0.05 mg/dL.

† Significantly different from water in refeeding group ($P < 0.05$, Student's *t* test).

‡ Significantly different from AD group during renutrition period ($P < 0.05$, Student's *t* test).

§ Significantly different from AD group during CR period ($P < 0.05$). Significant differences ($P < 0.05$) between milks were observed (two-way analysis of variance) in glucose determination when sheep milk was used.

Leptin and insulin determinations

The effects of calorie restriction and renutrition using different ruminant milks on insulin and leptin levels are shown in Figures 2 and 3, respectively. In our model we observed no significant changes in serum insulin concentration at the end of the CR period compared with control AD mice of the same age. As expected, after different milk refeeding periods, we observed an increase in insulin levels in the Re groups when compared with the AD group after the renutrition period; however, this difference was only significant ($P < 0.05$) when buffalo and sheep milks were used.

Serum leptin concentration increased in the control AD group during the assayed period. The level of leptin was increased during calorie restriction (CR group) in a similar way as the control (AD mice during calorie restriction period). After the renutrition period, the cow- and goat-Re groups showed significantly lower values ($P < 0.05$) compared with sheep-, buffalo-, and H_2O-Re groups and compared with control AD mice of the same age.

Discussion

The results of the present study demonstrate that a reduction between 10% and 25% in body weight due to a mild CR diet in mice was not associated with a significant change in serum leptin and insulin over a period of 12 d. We also studied a renutrition protocol using different ruminant milks over the course of 7 d. Although leptin levels increased for the CR group in a similar way as the controls (AD mice during the CR period), at the end of the renutrition period with the cow and goat milk diets, leptin levels did not reach those of the control groups of the same age. The observed

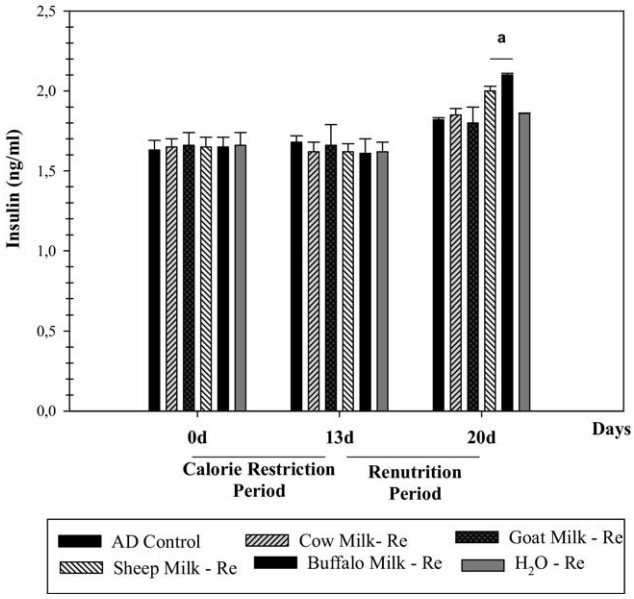

Fig. 2. Influence of a calorie-restricted diet and renutritition with different ruminant milks on serum insulin levels. Insulin concentrations were assayed on the baseline day, at the end of the calorie-restricted period, and at the end of the renutrition period with cow, goat, sheep, and buffalo milks and water. Data are represented as mean ± SD for six mice. [a]Significantly different from the control AD group after the renutrition period ($P < 0.05$, two-way analysis of variance). AD, ad libitum; Re, refeeding.

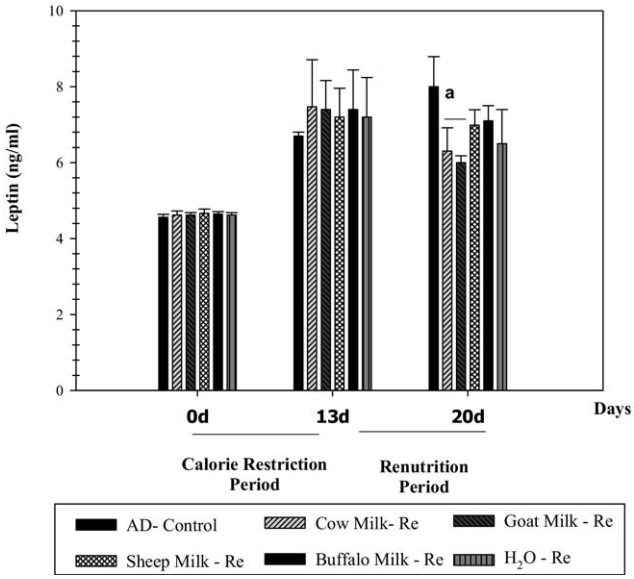

Fig. 3. Influence of a calorie-restricted diet and renutrition with different ruminant milks on serum leptin levels. Leptin concentrations were assayed on the baseline day, at the end of the calorie-restricted period, and at the end of the renutrition period with cow, goat, sheep, and buffalo milks and water. Data are represented as mean ± SD for six mice. [a]Significantly different from the control AD group after the renutrition period ($P < 0.05$, Student's t test, two-way analysis of variance). AD, ad libitum; Re, refeeding.

leptin levels, given the relatively small changes in body weight observed, might suggest that leptin secretion could be regulated by other factors, in addition to the amount of adipose tissue deposited. Similarly to the no significant increase of leptin levels during calorie restriction, we had also observed a significant increase on leptin levels after 45 d of a mild CR diet (data not shown). Therefore more studies are needed to confirm that these increases are due to a starvation response.

As expected, in agreement with previous studies, the mild CR period led to a decrease in serum insulin levels. Other in vivo and in vitro studies have suggested that insulin stimulates leptin production and release, thereby contributing to the regulation of starvation- and meal-induced modulations of leptin levels [21]. However, the effect of mild caloric restriction on leptin levels observed in the present work would be at least in part independent of insulin secretion. In this context, Cusin et al. [22] showed that in lean rats, insulin can be seen as an upregulator and downregulator of *ob* gene expression.

In addition, although no significant changes in leptin levels were observed, we noted a general tendency of increase due to mild calorie restriction compared with the control AD group. However, this result is not in agreement with the existing literature [23,24]. Possibly, according to Schrauwen et al. [25], greater changes in fat mass or changes in energy balance are necessary to produce significant changes in leptin levels. The slight increase of leptin levels could thus be due to inappropriate signals directed to the hypothalamus, suggesting that fat deposits are much larger. According to Schwartz and Seeley [26], when food is consumed in amounts that maintain reduced weight, a state of neutral energy balance is restored, and leptin concentrations may increase to values appropriate to body fat levels. Also, Gat-Yablonsky et al. [27] reported a significant stimulatory effect of exogenous leptin on longitudinal growth in a mouse model, even in the presence of low calorie intake. For this reason, the tendency of leptin levels to rise could be the result of an adaptive mechanism necessary for growth in weaned mice.

According to several researchers, serum leptin concentrations increase after renutrition [28]. However, the leptin values observed at the end of the renutrition period were lower than those in control AD mice of the same age. Moreover, leptin values observed with cow and goat milks were significant lower than with sheep and buffalo milks. This fact could be attributed to the lower lipid content of the former milks.

In the present study, we showed that CR mice recovered similar body weight with milk and water renutrition. No significant differences in body weight gain between different milk diets were observed; therefore, our results suggest that goat, sheep, and buffalo milks have a similar effect on growth as cow milk. Some studies have demonstrated that, at lower rates of weight gain, balanced tissue can be readily deposited. At high rates of weight gain, the body tended to conserve excessive adipose tissue, hindering, in short periods of renutrition, the recovery of lean tissue and muscle mass [29,30]. Therefore, the rehabilitation of CR mice might be more effectively achieved with cow and goat milk–based diets due to their lower lipid content compared with sheep or buffalo milk.

The type of response and the vulnerability of an organ to the effects of nutritional imbalance depend on the speed of cellular replacement. The kinetics of cell proliferation in the lymphoid organs, in particular the thymus, is highly sensitive to the effects of undernutrition [31]. The most frequently observed consequence is atrophy of the thymus and spleen, and this can be used as an early and critical marker for the organism's compromised nutritional state and immunologic deficiency.

The available information relating to the nutritional status of the thymus supports the view that nutrient composition, in particular the protein composition of the diet, is a factor capable of modifying the cellular development of the thymus [32–34]. Our previous results showed that a renutrition diet maintained over 7 d is able to reverse nutritional and immunologic changes in moderately malnourished mice [18,19]. Despite these previous data, the results obtained in this work allow us to conclude that renutrition for 7 d with milk-based diets, in a mild CR model, was not sufficient to reach normal values for spleen weight or total leukocyte count. Therefore, it will be necessary to determine if the functionality of the leukocytes is really affected. All the different milk-based renutrition diets induced an increase in thymus weight. No significant differences were observed in

the response of the mice among all the different milks assayed, despite their different compositions. These observations reaffirm the hypothesis that when CR animals (during active growth, as is the case for weaned mice) are renourished, compensatory but insufficient growth occurs [35,36]. The speed and magnitude of this growth are directly related to the initial degree of malnutrition [35–37].

In contrast, the re-establishment (during the renutrition period) of normal metabolic values for such parameters as serum glucose and triacylglycerol reflected the increased efficiency of energy utilization when the nutritional deficiency was corrected. Also, calorie restriction has been recognized as a natural therapy that improves health and extends longevity in humans and rodents. One health benefit of calorie restriction is that it lowers cholesterol and triacylglycerol levels; however, some studies have shown slight modifications in serum cholesterol in humans and animals [2]. We demonstrated a tendency to lower cholesterol levels with calorie restriction and a renutrition diet; this was significant in the H_2O-Re group. These findings could suggest that the Re mice could have established a new, more efficient homeostatic state that is maintained, with renutrition being significant only with water because the other groups received more calories with milks.

In conclusion, goat, sheep, and buffalo milks could be effectively used as alternatives to cow milk for weight gain and for partly improving the altered metabolic parameters. In our model, serum leptin secretion was slightly modulated by mild calorie restriction and by the renutrition diet. The cellular mechanisms responsible for the increased leptin concentrations in mild CR mice are still poorly defined; they might be correlated with the positive influence of leptin on longitudinal growth even in the presence of low calorie intake [27].

Further studies on treatment protocols for mild calorie restriction are required, and it would be appropriate to work with a chronic mild CR state, which would allow analysis of its influence on leptin levels and their regulation.

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