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Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation

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Abstract

We had shown that adult animals, whose mothers were submitted to protein or energy restriction during lactation, differ from controls in their body weight and thyroid function. The aim of this study was to evaluate, from birth through six months of age, leptin serum concentration, body weight and food intake in animals whose mothers received protein or energy restricted-diet during lactation as follows: control (C)—23% protein; protein-restricted (PR)—8% protein; energy-restricted (ER)—23% protein, in restricted quantity, according to the mean ingestion of the PR group. After weaning (day 21) all pups had free access the control diet. Body weight of pups from PR mothers were always lower than those from controls ($p < 0.05$), while body weight of pups from ER mothers surpassed that of the C group significantly at 140 days of age. The food intake was lower in both offspring from PR and ER mothers, normalizing on the 32th day in pups from ER mothers and on the 52th day in pups from PR mothers. Leptin serum concentration in both offspring from PR and ER mothers were significantly decreased on the 12th day ($p < 0.05$) and increased on the 21st day ($p < 0.05$) compared to control. After weaning there was no differences among the groups. It is possible that changes in leptin concentration during lactation in the offspring of malnourished groups could permanently modify the setpoint for body weight control.

Keywords: Protein malnutrition; Energy malnutrition; Leptin; Rats; Lactation

1. Introduction

The most prevalent form of nutritional disorder of children in developing countries is still the malnutrition. However, in adults, contrary to a condition that has prevailed until recently, overweight is now the most prevalent disorder in these countries. The reasons of this transition are still not well understood. Epidemiological [1,2] and experimental [3,4] reports correlate obesity in adulthood with malnutrition in the first days of life, a relationship that has been termed metabolic imprinting [5].

We had shown that protein malnutrition in lactating rats was associated with thyroid dysfunction in the dams [6], in 60 days and 180 days old offspring [7,8]. We also had demonstrated that mother's nutrition during lactation can determine the body weight of their offspring in the adult life, and this can be mainly associated with protein and lipid milk concentration [9]. The offspring of energy-restricted mothers had lower body weight until weaning. However, after weaning, those animals were heavier than the offspring of controls and protein-restricted mothers. In contrast, the offspring of protein-restricted mothers presented lower body weight from birth to 6 months of age.

These data suggest that body weight regulation in the adult life is determined in early stages of pregnancy and/or lactation and could be mediated by changes in the serum concentrations of several weight-regulatory hormones, such as leptin, MSH, thyroid hormones, that could regulate in a long run hypothalamic set-point for energy intake or metabolic rate.

Leptin is produced, mainly, by adipose tissue [10], but recent studies showed that it is also produced by the pituitary, skeletal muscle, placenta, stomach and epithelial cells of mammary gland [11–16]. Leptin is a protein that regu-

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lates energy disposable in the peripheral adipose tissue by specific hypothalamic signals and affects many functions as body weight, food intake, body temperature and metabolic rate [17–19]. These effects depend partly on the inhibition of Neuropeptide Y and stimulation of CRH synthesis [10].

Some reports had shown that energy restriction leads to a reduction in leptin serum concentration both in mice [20] and humans [21]. Ahima et al [20] had shown that preventing the starvation-induced fall in leptin, injecting exogenous leptin, substantially blunts the changes in gonadal, adrenal and thyroid axes in male mice, and prevents the starvation-induced delay in ovulation in female mice. Legradi et al [22] had demonstrated that the decrease in pro-TRH mRNA observed during fasting was also normalized with leptin administration. In the same way, Carro et al [23] had shown that leptin administration to fasted young rats normalized the starvation-induced decrease in GH secretion and that leptin antibody administration was associated with GH reduction.

To the best of our knowledge, no study has been specifically designed to evaluate the short and long term effects of protein and energy restriction only during lactation period on the leptin serum concentrations in the offspring. So, in this paper we analyzed the effects of protein- and energy-restricted diet during lactation on the leptin serum concentration from birth until six months of age aiming to identify whether mother nutritional condition had a short and long term consequences on the offspring leptin serum concentration.

2. Material and methods

Wistar rats were kept in a room with controlled temperature (25 ± 1°C) and with artificial dark-light cycle (lights on from 7:00 a.m. to 7:00 p.m.). Three-month old, virgin female rats were caged with one male rat at a proportion of 2:1. After mating, each female was placed in an individual cage with free access to water and food until delivery. The experimental design was approved by the Animal Care and Use Committee of the Biology Institute of State University of Rio de Janeiro, which based their analysis on the principles described in the Guide for the Care and Use of Laboratory Animals [24].

Forty two dams each were randomly assigned to one of the following groups: control group, with free access to a standard laboratory diet containing 23% protein; protein-restricted (PR) group, with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted diet (ER) group, receiving a standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PR group. Therefore, the amount of food consumed in both ER and PR groups was about the same. Table 1 shows the composition of the diets, which follows recommended standards [25]

The protein-restricted diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of starch was calculated so as to make up for the decrease in energy content due to protein reduction.

Table 1
Composition of the control and low-protein diets

	Control§	Protein-Restricted*
Ingredients (g/Kg)		
Soybean + Wheat	230.0	80.0
Corn starch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mix†	4.0	4.0
Mineral mix†	40.0	40.0
Macronutrient composition (%)		
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0
Total enetgy (KJ/Kg)	17038.7	17038.7

§ Standard diet for rats (Nuvilab-NUVITAL Nutrientes LTDA, Paraná, Brazil)

* The low-protein diet was prepared in our laboratory using the control diet and replacing part of its protein with corn starch. The amount of the latter was calculated so as to make up for the decrease in energy content due to protein reduction.

† Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (25)

Within 24 hr of birth, excess pups were removed, so that only 6 male pups were kept per dam, because it has been shown that this procedure maximizes lactation performance [26]. Malnutrition was started at birth, which was defined as day 0 (d0) of lactation, and was ended at weaning (d21).

After weaning, 3 animals of each litter were randomly chosen and placed together in the cage with free access to water and to standard diet with 23% of protein until sacrifice, in the same room conditions described above for the mothers.

We performed seven sets of experiments for the following periods: 4, 12, 21, 30, 60, 120 and 180 days of litter age. For each time point we sacrificed 18 animals per group, with a lethal dose of pentobarbital and blood was obtained by cardiac puncture, in a total of 378 animals.

Body weight and food intake were only studied in rats that were sacrificed at day 180. Body weight was monitored once every four days, from birth until 180 days of age.

The food intake was measured from weaning until 180 days of age. The amount of diet ingested was the difference between the weight of food that rested in the food bin (Da) and the amount placed four days before (Db). These data were then used to calculate food intake according to the formula:

$$\text{Food intake (g)} = \left(\frac{\text{Db} - \text{Da}}{3}\right) : 4$$

where, 3 correspond to the number of animals in each cage.

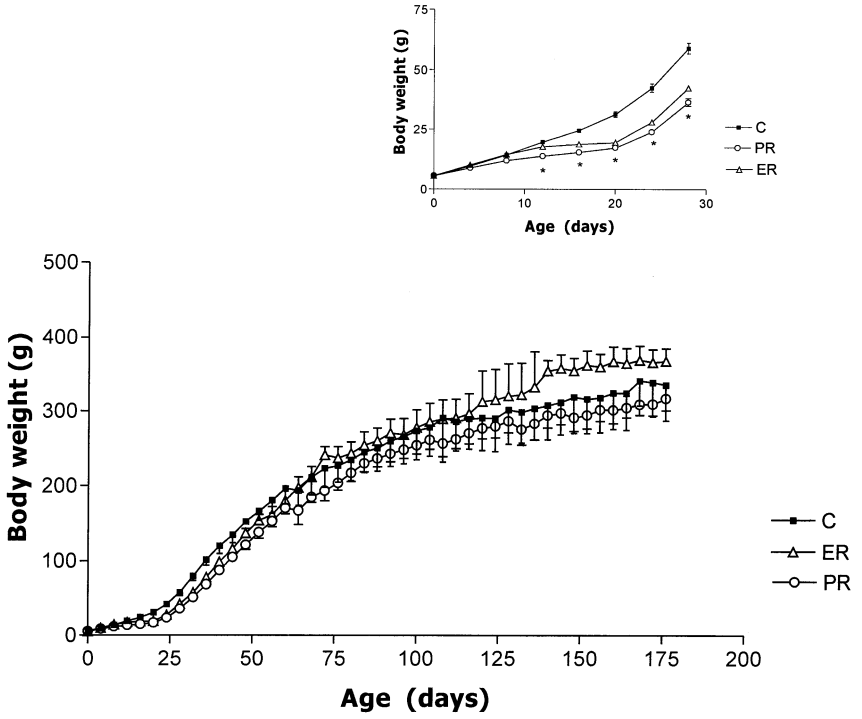

Fig. 1. Body weight, from weaning to adulthood, of offspring which were only sacrificed at day 180, whose mothers fed a normal (■), protein-restricted (○) and energy-restricted (△) diet during lactation. Values are given as the mean ± SEM of 18 animals per group at each time point. Differences between control and protein-restricted groups are significant ($p < 0.05$) from day 21 until 80, and from day 164 onward. Differences between control and energy-restricted groups are significant ($p < 0.05$) from day 21 until 52 and from 140 days.

We did test the similarity of the food intake and body weight curves between each set of experiment with the complete curve of the 180 days study. As we did not find any differences among the curves, we only analyzed and presented the complete curve of the 180 days experiment.

Blood samples were centrifuged to obtain serum, which was individually kept at −20°C until assay. Leptin was measured using commercial kit (Murine Leptin Elisa-DSL-10–24100).

2.1. Statistical analysis

The data are reported as mean ± SEM. Two -way analysis of variance (ANOVA) were used to assess the effect of time and diet treatment on the offspring body weight and food intake, considering the longitudinal character of data. As the interaction was significant we performed the one-way ANOVA followed by the Newman-Keuls as post-hoc test to identify when the groups were significantly different. The level of significance for these analyses was set at $p < 0.05$. We also tested in the same way if there were significant differences for each group with time. The effects of mother nutritional condition on serum leptin concentration of the offspring were analyzed by the one-Way ANOVA followed by Newman Keuls test, but as we performed seven different ANOVAS, we correct the level of significant p value dividing 0.05 by 7, making the level of significance 0.007.

3. Results

The body weight of pups whose mothers were submitted to protein or energy restriction during lactation was significantly ($p < 0.001$) lower than that of controls from day 8 until the end of lactation (Fig. 1). After weaning, offspring from PR mothers continued to have a lower (approximately 10%, $p < 0.05$) body weight until 180 days of age. The body weight of offspring of ER mothers surpassed that of controls by approximately 10% ($p < 0.01$), since 140 days through 180th days of age (Fig. 1). The two-way ANOVA showed that both treatment and time affected the result and were considered highly significant ($F(2,2346) = 108.2$, $p < 0.0001$; $F(45,2346) = 404.2$, $p < 0.0001$, respectively).

Fig. 2 shows the effects of dietary treatment on the food intake of offspring. Animals from PR mothers consumed less diet ($p < 0,01$) from weaning until 52th day when compared with controls, normalizing thereafter. The pups from ER mothers had a similar eating behavior, but they normalized their ingestion earlier, at day 32 (Fig. 2). The two-way ANOVA showed that both treatment and time affected the result and were considered also highly signifi-

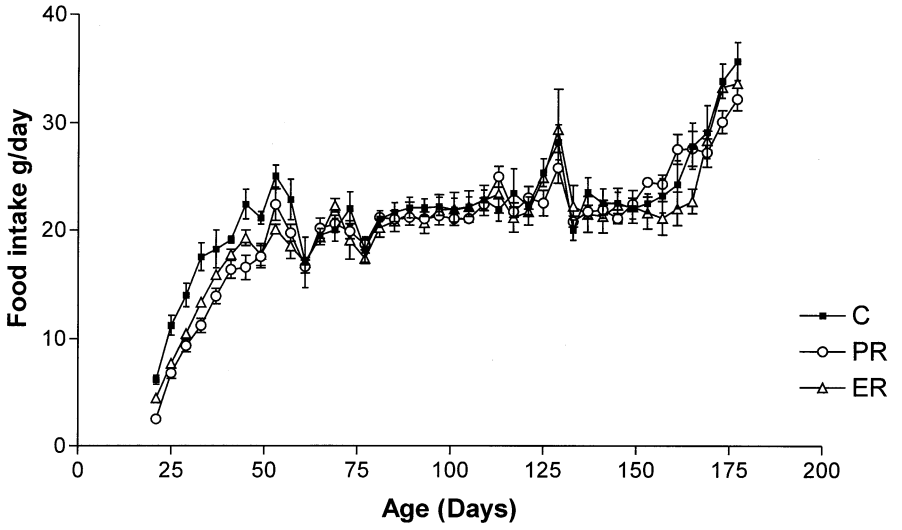

Fig. 2. Food intake, from weaning to adulthood, of offspring which were only sacrificed at day 180, whose mothers fed a normal (■), protein-restricted (○) and energy-restricted (△) diet during lactation. Values are given as the mean ± SEM of 18 animals per group in each time point. Differences between control and protein-restricted groups are significant ($p < 0.01$) from weaning until 52 days of age. Differences between control and energy-restricted groups are significant ($p < 0.01$) from weaning until 32 days of age. Both diet-restricted groups normalized their ingestion until 180 days of age.

cant ($F(2,2091) = 19.85$, $p < 0.0001$; $F(40,2091) = 69.77$, $p < 0.0001$, respectively).

The effect of protein and energy restriction diet during lactation on leptin serum concentration of offspring is demonstrated in Fig. 3. Both groups from PR and ER mothers had a significant decrease in leptin concentrations on the 12th day ($F(2,53) = 21.24$, $p < 0.0001$) and increase on the 21st day ($F(2,53) = 5.86$, $p < 0.0051$) when compared to the C group. After weaning there was no significant differences among the groups.

4. Discussion

These data reinforce previous findings from our laboratory [9], where the offspring of ER mothers had lower body weight until weaning. However, after weaning, those animals were heavier than the offspring of controls. In contrast, the offspring of PR mothers presented lower body weight from birth to 6 months of age. These data reinforce the concept of metabolic imprinting, intended to describe the basic biological phenomena that putatively underlie relations among nutritional experiences of early life and later disease [2,5].

Since the food intake was similar in the 3 groups the changes in body weight may be caused by others factors, besides food intake, especially those involved with the regulation of the metabolic rate.

Leptin is one of the determining factor of food intake and energy expenditure and serum leptin changes in the direct reason of adipose tissue mass [10,27–29].

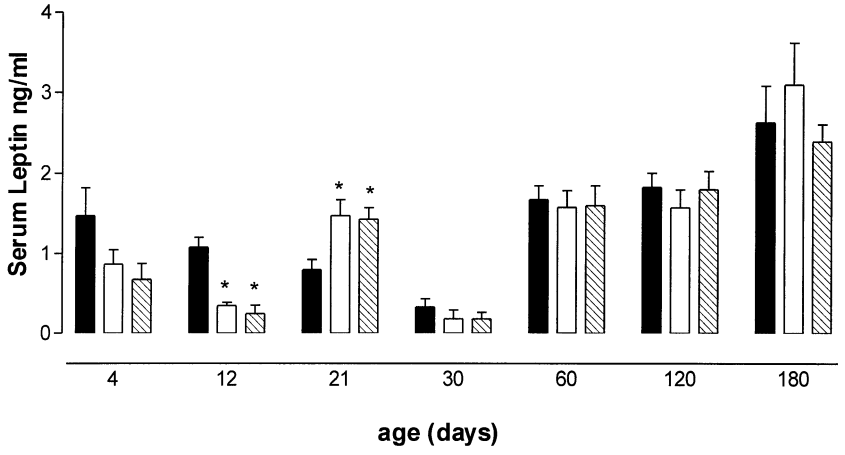

Fig. 3. Leptin serum concentrations in pups whose mothers were fed a control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diets during lactation. Values represent the mean ± SEM. Were used different animals for each time point and were sacrificed 18 animals in each group at each time point. Significant differences between either of the diet-restricted groups and controls (*), were determined by a multiple comparison of means test.

Malnutrition in adult animals is associated with lower levels of serum leptin, due, mainly, to low fat mass [20]. Therefore, our data in the first period of lactation are consistent with the literature, independently of the kind of malnutrition. The data of higher serum leptin at weaning, in spite of the offspring underweight, are not in agreement with those shown in offspring of protein-restricted mothers during gestation and lactation [30], where despite offspring underweight, serum leptin at weaning was unchanged. It is possible that lactation could be a more critical period to determine the leptin levels in the offspring.

Some studies had shown that leptin is present in the milk [31,32]. Therefore, we cannot discard the possibility that a higher transfer of this hormone through the milk of malnourished mothers had occurred, leading to a further reduction in offspring body weight. Since leptin is also produced by the epithelial cells of mammary gland [32], its regulation in this organ could be different from that in the adipose tissue. We had shown a similar higher transfer of T_3 and iodine through the milk of malnourished mothers during lactation [33,34], which may be of adaptive importance.

Diet with a higher lipid concentration increases the leptin serum levels [35,36]. We had shown recently that the milk of energy restricted dams have a higher lipid concentration [9] and, it could stimulate, at least in the offspring of ER mothers, the higher leptin serum concentration in these animals at weaning. We also had demonstrated that the milk of PR mother had lower protein concentration. However, there was no report about the effect of low protein in the milk upon leptin serum concentration in the offspring.

Another explanation for the higher serum leptin at weaning in the pups of malnourished mothers during lactation is the changes on the regulatory mechanism of leptin synthesis rates in other tissues, such as the brain, pituitary, brown adipose tissue, and skeletal muscle [13,15,16].

It is possible that changes in leptin concentration during lactation in the malnourished groups could affect the regulation of others factors which contribute to a long-term body weight control.

The fact that leptin serum concentration after weaning was unchanged demonstrates that maternal malnutrition during lactation did not affect long-term leptin serum concentration, differently from the metabolic imprinting that we had observed for body weight, which was determined by maternal nutritional condition during lactation. Therefore, we conclude that in adult animals whose mothers were malnourished during lactation the relation between body mass and serum leptin concentration is lost.

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