Maternal protein-energy and energy-restricted diets during lactation possibly could program folliculogenesis and the ovarian expression of leptin and its different isoform receptors in rats

Both protein-energy and energy-restricted diets during lactation program the ovarian function of the rat offspring, leading to a reduction of folliculogenesis, possibly as the consequence of the altered expression of leptin and its isoform receptor genes. (Fertil Steril® 2009;92:1755–7. ©2009 by American Society for Reproductive Medicine.)

Leptin, the product of the obese (*ob*) gene, is an important satiety and reproductive hormone classically secreted by adipose tissue (1). The relationship between nutrition and reproduction has been investigated extensively, but the exact mechanism connecting these processes still is not known fully.

There are six known splice variants of the leptin receptor (OBR), all with the same extracellular domain, but with differing intracellular domains. The isoforms can be classified into three classes: short (OBRa, OBRc, OBRd, OBRf), long (OBRb), and secreted (OBRe). Only the isoform OBRb is considered to be capable of signal transduction across the cell membrane (2). Leptin could be a regulator of hypothalamic-pituitary-ovarian function affecting growth of ovarian follicles and steroidogenesis (3).

Lactation could be a critical period in determining the future endocrine status of the progeny (4). Recently, we showed that maternal protein and energy malnutrition during lactation leads to growth retardation and delayed onset of puberty (5) and atrophy of the uterine endometrial glands and affects folliculogenesis in pubertal female rats (6, 7). The goal of this study was to evaluate the effect of maternal malnutrition during lactation on folliculogenesis, leptin, and the different isoforms of leptin receptor expression in the ovary of the adult rat offspring.

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Wistar rats were kept in a room with controlled temperature (25°C ± 1°C) and artificial dark-light cycle. The handling of the animals and the study design were approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro. After delivery, nine pregnant Wistar rats were separated into three groups: the control group, with free access to a standard laboratory diet containing (in grams per 100 g) 23 protein, 66 carbohydrate, 11 fat, and 17,038.7 kJ/kg total energy; the protein-energy–restricted group, with free access to an isoenergy and protein-restricted diet containing 8% protein; and the energy-restricted group, receiving standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the protein-energy–restricted group. The protein-restricted diet was prepared as described previously (5).

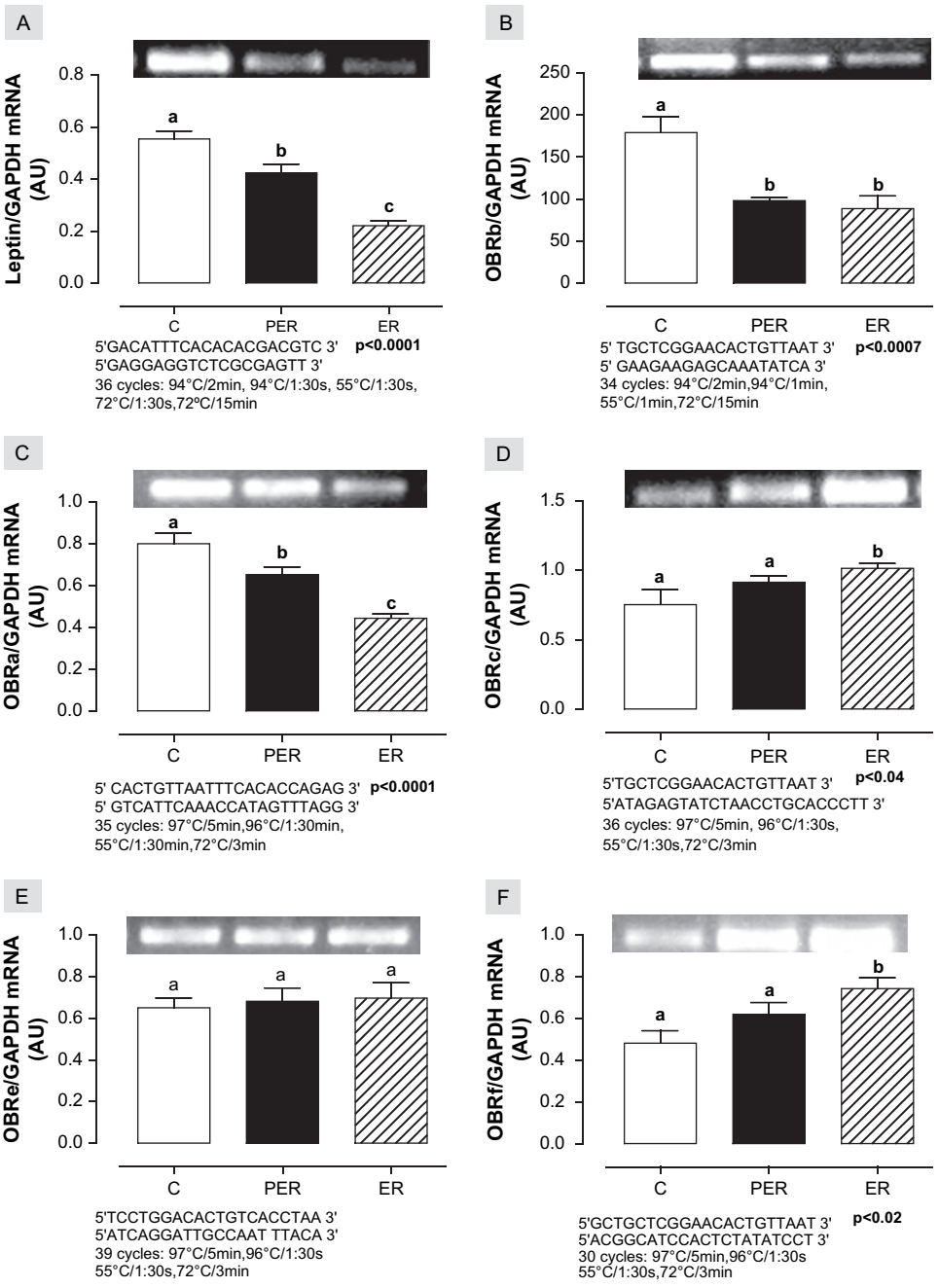
Malnutrition of the studied rats started at birth and was ended at weaning, when female pups of the same treatment group were housed in groups of three animals per cage and given unlimited access to food and water until 90 days of age. Then, only the animals in the proestrum stage were killed with a lethal dose of pentobarbital, and the following parameters were evaluated: food consumption, body weight, linear growth, adipose tissue mass, E_2, and T and leptin serum concentrations. The abdominal, ovarian, and uterus adipose tissue depots were extracted and weighed, then considered as adipose tissue mass. The left ovary was processed and stained with hematoxylin and eosin for histologic examination of ovarian follicles as described previously (7). Ribonucleic acid was extracted from the right ovary with use of Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Then leptin and leptin isoform receptors were evaluated by reverse transcriptase–polymerase chain reaction.

The data were reported as mean ± SEM. Statistical significance of experimental observations was determined by the one-way analysis of variance followed by the Newman-Keuls test. The level of significance was set at $P<.05$.

There was no difference in the body weight (control = 240 ± 3.7 g; protein-energy–restricted = 235 ± 3.6 g; energy-restricted = 221 ± 9.2 g), linear growth (control = 21 ± 0.08 cm; protein-energy–restricted = 20 ± 0.4 cm; energy-restricted = 19 ± 0.2 cm), food consumption (control = 33 ± 0.3 g; protein-energy–restricted

FIGURE 1

Expression of leptin (**A**) and the isoform receptor OBRb (**B**), OBRa (**C**), OBRc (**D**), OBRe (**E**), OBRf (**F**) genes in ovaries of control group (*C*), protein-energy–restricted group (*PER*), and energy-restricted group (*ER*). After reverse transcriptase–polymerase chain reactions, the amplified fragments were run on a 1.5% agarose gel and visualized by ultraviolet transillumination. Above each graph, a representative ethidium bromide–stained gel depicts products for expression of each gene. Below each graph are the primer sequence and cycle profile. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. The ratios between the signal intensities (arbitrary units) of each gene are represented as means ± SEM of five animals. Different letters mean statistical significance. mRNA = messenger RNA.



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= 28 ± 0.2 g; energy-restricted = 27 ± 0.2 g), or the amount of abdominal adipose tissue related to body weight (control = 13.7 ± 1.1 g/mg body weight; protein-energy–restricted = 14.1 ± 0.6 g/mg body weight; energy-restricted = 11.6 ± 0.4 g/mg body weight) among the groups. Both protein-energy–restricted and energy-restricted groups presented a significant reduction in the number of primordial (control = 6.6 ± 0.2; protein-energy–restricted = 5.2 ± 0.2; energy-restricted = 5.4 ± 0.4, $P<.01$), primary (control

= 5.8 ± 0.7; protein-energy–restricted = 4.0 ± 0.3; energy-restricted = 2.2 ± 0.5, $P<.001$), and Graaf follicles (control = 2.2 ± 0.3; protein-energy–restricted = 1.2 ± 0.3; energy-restricted = 0.6 ± 0.2, $P<.006$).

Serum T concentration was below the limit of sensitivity of the standard assay preparation and therefore could not be measured. Estradiol (control = 125.4 ± 20.4 pg/mL; protein-energy–restricted = 116.6 ± 16.2 pg/mL; energy-restricted = 102.1 ± 9.3 pg/mL) and leptin related to body weight (control = 9.77 ± 0.88 ng/mL/mg body weight; protein-energy–restricted = 9.73 ± 0.53 ng/mL/mg body weight; energy-restricted = 10.8 ± 0.98 ng/mL/mg body weight) did not differ significantly among the groups. As shown in Figure 1, both maternal diets led to a significant reduction in the leptin gene (Fig. 1A), in OBRb (Fig. 1B), and in OBRa (Fig. 1C) when compared with the control group. However, there was a significant increase in the energy-restricted group when compared with controls, in relation to the OBRc (Fig. 1D) and OBRf (Fig. 1F) expression, whereas in the protein-energy–restricted group this increment was not statistically different. The OBRe (Fig. 1E) did not show statistical difference among the groups.

Leptin has been proposed as a link between the nutritional status and reproductive processes (1). Leptin secretion is related primarily to body adipose tissue size and caloric intake (3). Our present results show that there is no difference in food consumption or in the abdominal adipose mass among the groups that could explain the normal leptin levels.

There is controversy concerning the interaction between leptin and steroid hormones. Unlike the situation in humans, leptin concentrations remain constant throughout the estrous cycle in rats (8). However, a more recent study shows that leptin concentrations are at a maximum at proestrus, a time when the E_2 is at its peak (9). In this study we show that both leptin and E_2 serum levels are unaltered by maternal malnutrition during lactation. Although we cannot confirm that the secretion of both hormones is regulated by each other, this result suggests an interaction between both hormones, which is in agreement with previous reports (9).

The decrement observed in the number of primordial follicles could result from a direct action of malnutrition in the ovary of the pups in the first days of life when primordial follicles are being formed (10). We have published previously (11) that the milk composition was altered by maternal malnutrition. The milk composition of the protein-restricted group presented a significant lower protein concentration, whereas the energy-restricted group presented a significant higher lipid and protein concentration. Total milk energy was always significantly lower in the protein-energy–restricted group.

These data also reinforce the reduced fertility rate in 1-year-old rats showed by Guzman et al. (12) suggesting that the reduction of follicular reserve, which serves as a ticking clock to the onset of senescence, could be responsible for the decrease in the fertility rate.

Leptin deficiency in mice is associated with suppression of ovarian folliculogenesis and increase in ovarian granulosa cell apoptosis (3). Because OBRb is the functional leptin receptor and OBRa seems to be involved with the leptin transport into the oocyte (13), we can hypothesize that the changes observed in the gene expression of leptin, OBRa, and OBRb receptor isoforms after maternal malnutrition during lactation could have contributed to the reduction of folliculogenesis in these animals. Despite no specific function having been described for the other short isoforms yet, the increase in the OBRc and OBRf gene expression could have been important to maintain the growth and development of some follicles, as well as the E_2 serum concentration.

The maternal malnutrition did not change the soluble isoform of leptin receptor, OBRe. In mice, it has been reported that the ObRe isoform is produced at a sufficiently high level to act as a buffering system for free circulating leptin (14). Therefore, we can hypothesize that normal OBRe expression was important to keep the leptin serum levels unaltered.

The notion that nutrition during the early phases of human development can predispose or program individuals to adult disease has aroused considerable interest, particularly since the last decade. We suggest that both protein-energy and energy-restricted diets during lactation would program the ovarian function of the offspring, leading to a reduction of folliculogenesis, probably in consequence of the altered expression of leptin and its isoform receptor genes. On the basis that the adipose tissue can increase in number or size at any developmental time, it should not be under a metabolic programming control. This fact could explain the maintenance of its function, such as leptin synthesis and secretion.

REFERENCES

1. Magni P, Motta M, Martini L. Leptin: a possible link between food intake, energy expenditure, and reproductive function. Regul Pept 2000;92:51–6.
2. Hegyi K, Fulop K, Kovacs K, Toth S, Falus A. Leptin-induced signal transduction pathways. Cell Biol Int 2004;28:159–69.
3. Gonzalez RR, Simon C, Caballero-Campo P, Norman R, Chardonnens D, Devoto L, et al. Leptin and reproduction. Hum Reprod Update 2000;6:290–300.
4. Lucas A. Programming by early nutrition: an experimental approach. J Nutr 1998;128:401S–6S.
5. Faria TS, da Fonte Ramos C, Sampaio FJ. Puberty onset in the female offspring of rats submitted to protein or energy restricted diet during lactation. J Nutr Biochem 2004;15:123–7.
6. Brasil FB, Faria TS, Costa WS, Sampaio FJ, Ramos CF. The pups' endometrium morphology is affected by maternal malnutrition during suckling. Maturitas 2005;51:405–12.
7. Faria TS, Brasil FB, Sampaio FJ, Ramos CF. Maternal malnutrition during lactation alters the folliculogenesis and gonadotropins and estrogen isoforms ovarian receptors in the offspring at puberty. J Endocrinol 2008;198:625–34.
8. Bennett PA, Lindell K, Wilson C, Carlsson LM, Carlsson B, Robinson IC. Cyclical variations in the abundance of leptin receptors, but not in circulating leptin, correlate with NPY expression during the oestrous cycle. Neuroendocrinology 1999;69:417–23.
9. Duggal PS, Weitsman SR, Magoffin DA, Norman RJ. Expression of the long (OB-RB) and short (OB-RA) forms of the leptin receptor throughout the oestrous cycle in the mature rat ovary. Reproduction 2002;123:899–905.
10. Rajah R, Glaser EM, Hirshfield AN. The changing architecture of the neonatal rat ovary during histogenesis. Dev Dyn 1992;194:177–92.
11. Passos MC, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. Nutrition Research 2000;20. 106312.
12. Guzman C, Cabrera R, Cardenas M, Larrea F, Nathanielsz PW, Zambrano E. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. J Physiol 2006;572:97–108.
13. Chehab FF, Qiu J, Ogus S. The use of animal models to dissect the biology of leptin. Recent Prog Horm Res 2004;59:245–66.
14. Lollmann B, Gruninger S, Stricker-Krongrad A, Chiesi M. Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and, e in different mouse tissues. Biochem Biophys Res Commun 1997;238:648–52.