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Rapid communication

Perinatal malnutrition programs gene expression of leptin receptors isoforms in testis and prostate of adult rats

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ABSTRACT

The aim of this paper was to evaluate if maternal malnutrition during lactation programs the expression of leptin receptor isoforms in the testes and prostate ventral lobe of adult rats. At delivery, Wistar rats were separated into 3 groups: control group (C) with free access to a standard laboratory diet containing 22% protein; protein-energy restricted group (PER) with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted group (ER) receiving standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PER group. All animals were sacrificed at 90 days of age. Both PER and ER groups presented low body weight from the first days after birth, however, while the ER group reached the control weight around day 80, the body weight of PER group was significantly lower compared to controls until the day the animals were killed. In relation to tissue weight, only the relative testis weight of the ER group presented an alteration compared to the control group ($p < 0.03$). There was also no alteration in the leptin serum levels among the groups. The main leptin receptors isoforms, OBRa and OBRb were significantly increased in the testis (OBRa: $C = 0.71 \pm 0.10$; $PER = 1.14 \pm 0.17$; $ER = 1.92 \pm 0.70$, $p < 0.0007$, OBRb: $C = 0.87 \pm 0.04$; $PER = 1.20 \pm 0.05$; $ER = 1.44 \pm 0.17$, $p < 0.001$) and prostate (OBRa: $C = 0.70 \pm 0.18$; $PER = 1.30 \pm 0.14$; $ER = 1.65 \pm 0.22$, $p < 0.014$, OBRb: $C = 0.77 \pm 0.14$; $PER = 1.16 \pm 0.04$; $ER = 1.30 \pm 0.13$, $p < 0.027$) of both malnourished groups. However, the testis OBRc ($C = 1.52 \pm 0.06$; $PER = 1.35 \pm 0.23$; $ER = 3.50 \pm 0.72$, $p < 0.023$) and OBRf ($C = 1.31 \pm 0.12$; $PER = 1.66 \pm 0.27$; $ER = 3.47 \pm 0.55$, $p < 0.009$) and prostate OBRc ($C = 0.48 \pm 0.13$; $ER = 1.18 \pm 0.34$, $p < 0.01$) and OBRf ($C = 0.73 \pm 0.15$; $PER = 0.99 \pm 0.11$; $ER = 1.83 \pm 0.30$, $p < 0.016$) isoforms were significantly increased only in the ER group. The results presented here show for the first time that both testis and prostate leptin receptor isoforms gene expression are programmed by perinatal malnutrition. These data further stress the importance of monitoring maternal and neonatal status, as well as other pathophysiological situations, to combat the appearance of long-term diseases.
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1. Introduction

Leptin is an adipose tissue-secreted hormone, which decreases caloric intake and increases energy expenditure [1]. Subsequent studies showed that leptin is also involved in the reproductive function regulation [2]. Leptin exerts its biological effects through the activation of specific receptors (Ob-R), of which six isoforms are at present recognized (from Ob-Ra to Ob-Rf). Of these isoforms only Ob-Rb contains all intracellular parts being responsible for leptin signal transduction [3]. As the hormone, leptin receptors have also been detected in the prostate [4] and in the testis [5].

Nutritional deprivation during the perinatal period, which is one of the most prevalent conditions affecting children in the world, plays an important role in the pathogenesis of diseases during adulthood, including type 2 diabetes [6] and cardiovascular disease [7]. From these observations, the concept of fetal programming has been advanced.

The exact processes through which maternal nutrition or maternal environment affects reproductive function in the offspring remain unclear [8]. There is evidence that maternal restriction affects sexual maturation, testosterone and luteinizing hormone serum levels, testicular function and fertility rates in adult male rats. However some effects emerge in later life [9]. We have described recently, that maternal malnutrition during lactation programs the expression of leptin and their receptors in the ovary [10].

Despite our previous papers showing that maternal malnutrition can alter the testes and prostate structure and morphology [11,12] of weaned pups we have failed to find papers about how maternal malnutrition during lactation programs the prostate and testes gland in the adult rats in relation to leptin expression.

Obesity is associated with alterations in spermatogenesis [13] and also with prostate neoplasms, such as BPH and prostate cancer [14,15]. Concerned about leptin and its relation with nutrition and reproductive diseases raised the question whether a metabolic programming of testes

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and prostate by perinatal malnutrition could be involved in some dysplasia of the urogenital system. So, the goal of this paper was to evaluate if maternal malnutrition during lactation programs the expression of leptin receptor isoforms in the testes and prostate ventral lobe of adult rats.

2. Material and methods

2.1. Animals

Wistar rats were kept in a room with controlled temperature (25 ± 1 °C) and a dark–light cycle (lights on from 0700 to 1900 h). Virgin female rats of 3 months of age were caged with one male rat at a proportion of 2:1. After mating, determined by the presence of a vaginal plug, each female was placed in an individual cage with free access to water and food until delivery. The handling of the animals 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 Guide for the Care and Use of Laboratory Animals [16], and the study design was approved by the local Ethical Committee for the care and use of laboratory animals.

2.2. Experimental design

Nine pregnant Wistar rats were separated after delivery into 3 groups: the control group (C), 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 (PER), with free access to an isoenergy and protein-restricted diet containing 8% protein; and the energy-restricted group (ER), receiving standard laboratory diet in restricted quantities. The protein-restricted diet was prepared as described previously [17].

In spite of having free access to an isoenergy and protein-restricted diet, the dams of PER group consumed about 60% of the amount eaten by those dams receiving standard laboratory diet throughout the lactation period. The food ingestion of both C and PER groups was calculated every day to guarantee that the amount of food received daily by both PER and ER groups would be the same. Dams body weight was evaluated at parturition and at weaning to confirm the weight loss that follows this diet protocol [18]. In response to the reduction in food intake, dams of both restricted groups presented a 20% weight loss at the end of the lactation period.

Within 24 h of birth, excess pups were removed so that only 6 pups were kept per dam, as it has been shown that this procedure maximizes lactation performance [19]. Malnutrition of the studied rats was started at birth, which was defined as day 0 of lactation (d0), and was ended at weaning (d21). After weaning, six male 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 when they were killed with a lethal dose of pentobarbital. The blood was collected by cardiac puncture and the serum kept at −20 °C for subsequent determination of hormonal parameters. Testis and ventral prostate lobes were excised, dissected, weighted and kept at −80 °C for subsequent measurements of leptin receptor isoforms transcripts by RT-PCR. To evaluate the nutritional state, food consumption of the offspring was monitored each 5 days from weaning onward, while body mass were monitored each 5 days from birth until the end of experiment.

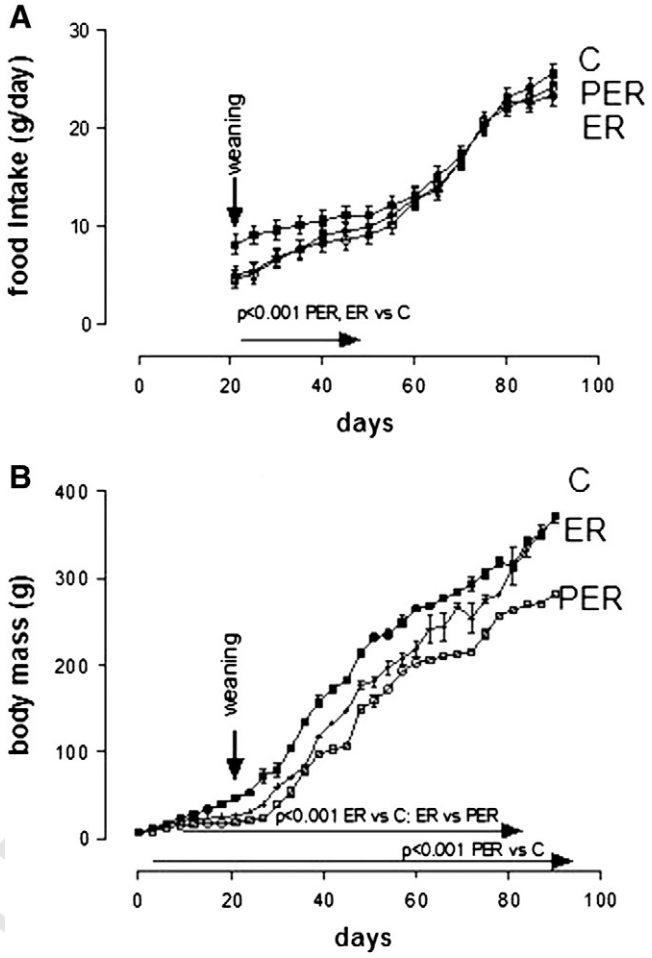

Fig. 1. Body weight (g) [A] and food consumption (g/day) [B] in control group (C), protein-energy restricted group (PER) and energy-restricted group (ER). Values are given as mean ± standard deviation of six animals obtained from 3 different dams.

2.3. RNA extractions

Total RNA was extracted using TRIZOL reagent (Invitrogen) according to the manufacturer's protocol. Briefly, tissues were homogenized in

Table 1
Oligonucleotide sequences used for amplification of reverse transcriptase–polymerase chain reactions and cycling conditions for the different sets of pairs.

Gene	Sequence (5′–3′)	Cycle profile	Cycles
GAPDH	accacagtccatgccatcac tccaccaccctgttgctgta	94 °C/3 min,94 °C/30 s 58 °C/2 min,72 °C/2 min	30
OBRa	cactgttaatttcacaccagag gtcattcaaaccatagtttagg	97 °C/5 min,96 °C/1:30 min 55 °C/1:30 min,72 °C/3 min	35
OBRb	tgctcggaacactgttaat gaagaagagcaaatatca	94 °C/2 min,94 °C/1 min 55 °C/1 min,72 °C/15 min	34
OBRc	tgctcggaacactgttaat atagagtatctaacctgcacccctt	97 °C/5 min,96 °C/1:30 s 55 °C/1:30 s,72°C/3 min	36
OBRe	tcctggacactgtcacctaa atcaggattgccaat ttaca	97 °C/5 min,96 °C/1:30 s 55 °C/1:30 s,72°C/3 min	39
OBRf	gctgctcggaacactgttaat acggcatccactctatatcct	97 °C/5 min,96 °C/1:30 s 55 °C/1:30 s,72°C/3 min	30

Table 2
Testis and prostate ventral lobe weight and serum leptin levels in control group (C), protein-energy restricted group (PER) and energy-restricted group (ER). Values are given as mean ± standard deviation of six animals obtained from 3 different dams.

	C	PER	ER	p
Testis weight				
Absolute	1.38 ± 0.04	1.35 ± 0.04	1.46 ± 0.07	p = 0.35
Relative	4.05 ± 0.11	4.42 ± 0.15	4.72 ± 0.22	p = 0.03
Prostate weight				
Absolute	0.27 ± 0.03	0.28 ± 0.03	0.25 ± 0.03	p = 0.76
Relative	0.79 ± 0.07	0.92 ± 0.09	0.81 ± 0.08	p = 0.47
Serum leptin	3.09 ± 0.24	3.17 ± 0.62	2.53 ± 0.33	p = 0.43

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