Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring

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Abstract

Maternal malnutrition during lactation reduces prolactin (PRL) and milk production, alters milk composition, and programs the body weight of the offspring. Our study aimed to evaluate the long-term effects of maternal hypoprolactinemia at the end of lactation on food ingestion, body weight, amount of retroperitoneal white adipose tissue (RPWAT), leptinemia, and anorectic leptin effect in the adult offspring. Lactating rats were treated with bromocriptine (BRO), a PRL inhibitor, 1 mg twice a day, or saline (C – control) for the last 3 days of lactation. The body weight and food intake were monitored, and after sacrifice at 180 days, the RPWAT was weighted. In a second experiment, the anorectic leptin effect was tested on 180-day-old animals. Adult offspring whose

mothers were BRO-treated showed higher body weight (10%), higher amount of RPWAT (2.3 times), higher total body fat (+39%), and hyperleptinemia (2.9 times) when compared with C, although food intake did not alter. After injection of leptin, the food ingestion at 2, 4 and 6 h was unaffected in BRO animals, confirming a resistance to the anorectic effect of leptin. Since the maternal PRL inhibition during lactation programs, a higher body weight with no alteration of food ingestion, we suggest a hypometabolic state. The leptin anorectic resistance can be due to the hyperleptinemia. We suggest that PRL changes during lactation can regulate body weight during adulthood.

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Introduction

Our group has shown that adverse situations during lactation, such as malnutrition and hormonal changes, could permanently affect the nutritional and hormonal status of the progeny (Passos *et al.* 2000, 2002, 2004, Cravo *et al.* 2002, Teixeira *et al.* 2002, 2003, Vicente *et al.* 2004, Lins *et al.* 2005). This association has been denominated as metabolic imprinting or programing, which is defined as a biological phenomenon that determines relationship between physical and chemical stimuli in early life and future functional status (Lucas 1994, Waterland & Garza 1999, Barker 2003, Moura & Passos 2005). Barker *et al.* (1993) has associated low birth weight with diseases related to the metabolic syndrome (diabetes, obesity, and hypertension) during adulthood.

The body-weight modulation is performed by many factors, including hormones, such as leptin. Leptin is a hormone secreted by adipose tissue, which reduces the food intake and increases the energy expenditure, maintaining the body-weight homeostasis (Zhang *et al.* 1994, Friedman & Halaas 1998). Maternal energy malnutrition during lactation programs overweight in the adult offspring, without change in food consumption (Passos *et al.* 2000). Those animals

presented a leptin resistance to their anorexigenic effect (Passos *et al.* 2004).

During lactation, in rats, malnutrition causes hypoprolactinemia (Lisboa *et al.* 2006), explaining the failure in milk synthesis and changes in their macronutrient, iodine, and thyroid hormone content (Passos *et al.* 2000, 2001a,b). Previously, we reported that the milk production suppression through the inhibition of prolactin (PRL) synthesis with administration of an agonist of dopaminergic receptor type 2, bromocriptine (BRO), causes malnutrition in neonatal pups and changes the transfer of leptin through the milk (Bonomo *et al.* 2005).

Therefore, in this work, we investigated the consequences of maternal hypoprolactinemia during lactation upon body weight, food intake, leptin, and leptin anorectic action in the adult life of the offspring.

Material and Methods

Three-month-old Wistar rats were maintained in a room under a 7 h light:19 h darkness cycle and controlled temperature (23/24 °C). Virgin female rats were caged with one male rat at a ratio of 2:1. After mating, each female was

placed in an individual cage with water and food available *ad libitum* until parturition. The use of the animals was according to the Animal Care and Use Committee of the Biology Institute of the 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 (Bayne 1996).

After birth, excess pups were removed, so that only six male pups were kept per dam, because it has been shown that this procedure maximizes lactation performance (Fishbeck & Rasmussen 1987).

Twelve lactating rats were separated into the following groups: BRO – treated with 1 mg bromo- α -ergocriptine (BRO; Novartis, SP, Brazil), twice a day, for the last 3 days of lactation before death and C – control group, which received saline treatment for the same time (Bonomo *et al.* 2005). We tested several days of PRL inhibition and 3 days was the maximum period that permitted the pups’ survival through to adulthood (data not shown). Table 1 shows the body weight of the pups before and during maternal BRO treatment.

After weaning (21 days of lactation), three pups of each dams’ group had their body weight and relative food intake (g/100 g BW) monitored every 4 days until 180 days, when the rats were killed by decapitation. Trunk blood was collected and the retroperitoneal white adipose tissue (RPWAT) was dissected out and immediately weighed.

Body fat determination

The rats were eviscerated and the carcass was weighed, autoclaved for 1 h, and homogenized on distilled water (1:1; Leshner & Litwin 1972). The homogenate was stored at –20 °C for analysis. Three grams of homogenates were used for determining the fat mass gravimetrically (Stansbie 1976, Toste *et al.* 2006). The samples were hydrolyzed on a shaking water bath at 70 °C for 2 h with 30% KOH and ethanol. The total fatty acids and free cholesterol were removed by washing three times with petroleum ether. After drying overnight in a vacuum, all tubes were weighed and data were expressed as grams of fat per 100 g carcass.

Leptin anorectic effect test

Peptide Recombinant mouse leptin (PeproTech, Rocky Hill, NJ, USA) was dissolved in saline (0.9% wt/vol) and

injected as a bolus at a dose of 0.5 mg/kg body weight intraperitoneally (Martin *et al.* 2000, Passos *et al.* 2004).

Feeding study C and BRO adult (180 days old) offspring were divided into the following groups: leptin (Clep and BROlep) or saline (Csal and BROsal). All groups were food-deprived for 24 h with free access to water before the test. After injection, the rats were returned individually to their cages and provided with the standard diet. Food intake was measured by weighing the food, 2, 4, and 6 h after leptin or saline injections. In order to check the effectiveness of the acute leptin injection, we measured leptinemia after 2 h and 24 h in both groups.

Serum leptin

Blood samples of 180-days old C and BRO offspring were centrifuged and serum was stored at –20 °C until assayed. Leptin was determined by RIA kit (Linco Research, St Charles, MO, USA). This kit has an assay sensitivity of 0.5 ng/ml and a range of detection from 0.5 to 50 ng/ml. All measures were done in one assay. The intra-assay variation was 6.9%.

Statistical analysis

Data are reported as means $\pm$ S.E.M. Two-way ANOVA and Newman–Keuls multiple comparison were used to analyze food intake in response to the leptin acute administration, and the Student’s *t*-test to analyze the other experimental observations, with significance level set at *P* < 0.05.

Results

During maternal BRO treatment, pups presented a significant low body weight gain (day 2, 6%; day 3, 8%), as demonstrated in Table 1.

The body weight of offspring whose mothers were injected with BRO for the last 3 days of lactation was significantly higher than the controls from day 133 until 180 days, reaching about 10% of overweight (*P* < 0.05; C, 397.4 $\pm$ 8.9 vs BRO, 438.2 $\pm$ 20.8; Fig. 1). However, these rats did not show changes in relative food intake since weaning until adult life (C, 4.4 $\pm$ 0.3 vs BRO, 4.0 $\pm$ 0.7; Fig. 2).

We also detected on 180 days-old BRO group, a higher amount of RPWAT (2.3 times, *P* < 0.05; C, 14.7 $\pm$ 2.8 vs BRO, 33.3 $\pm$ 2.6; Fig. 3) and total body fat (+39%, *P* < 0.05; C, 7.4 $\pm$ 0.5 vs BRO, 10.3 $\pm$ 0.7; Fig. 4), confirming that the overweight is due, at least in part, to an increase in adiposity.

The adult rats of BRO-treated dams presented hyperleptinemia (2.9 times, *P* < 0.05; C, 5.8 $\pm$ 1.3 vs BRO, 17.0 $\pm$ 1.7; Fig. 5). To check this high leptin serum levels in BRO group, we performed an anorectic test of the leptin effect. The test effectiveness of the anorectic effect of leptin is showed by the data of C group, where leptin injection inhibited more than

Table 1 Body weight of pups during maternal BRO or saline treatment. Values are given as the means $\pm$ S.E.M.

	C (g)	BRO (g)
Days of lactation		
19	50.4 $\pm$ 1.2	48.0 $\pm$ 0.8
20	53.1 $\pm$ 1.1	50.0 $\pm$ 0.7*
21	55.5 $\pm$ 1.3	51.2 $\pm$ 0.6*

n = 5 animals/group, **P* < 0.05.

Figure 1 Body-weight evolution, from weaning to adulthood, of offspring whose mothers were BRO-treated (BRO) or saline-treated (C) during lactation. Values are given as the mean $\pm$ S.E.M. $n=18$ animals/group, $*P<0.05$.

50% of the food intake when compared with group Csal, in all the three periods studied (Fig. 6A, Table 2). However, leptin-injected BRO group showed unaffected food intake after 2, 4, and 6 h (Fig. 6B, Table 2), which demonstrates a resistance to the leptin anorexigenic action. We also measured leptinemia after 2 h of acute saline or leptin administration and after 24 h in both groups, as shown in Table 3. Clep and BROlep presented high serum leptin after 2 h ($+1.24$ times and $+100\%$ respectively) and unchanged levels after 24 h.

Discussion

We showed previously that hypoprolactinemia at the end of lactation causes malnutrition (Bonomo *et al.* 2005), because there was a marked decrease in milk production. Other authors showed a different kind of programming by energy malnutrition during lactation (Waterland & Garza 1999, Godfrey & Barker 2000, Vickers *et al.* 2000, 2002, 2004, Breier *et al.* 2001, Teixeira *et al.* 2002, Vicente *et al.* 2004). Here, we showed that maternal hypoprolactinemia at the end of lactation caused overweight, higher visceral fat, hyperleptinemia, and hypothalamic leptin resistance in their adult offspring.

The higher body weight with normal food intake detected in BRO adult rats is similar to our previous data in adult animals whose mothers were submitted to energy restriction during lactation (Passos *et al.* 2002, Teixeira *et al.* 2002). In the present work, we showed that the overweight developed by the adult offspring of BRO-treated dams was due, at least partially, to a higher amount of central adiposity.

In obese individuals, higher leptin levels do not produce the expected satiety and increase in energetic expenditure. This unexpected leptin effect is explained through leptin resistance, caused by downregulation of their hypothalamic receptors (Considine *et al.* 1996, Martin *et al.* 2000), by a reduced blood-brain barrier transport (Burguera *et al.* 2000, Banks 2001) or by an impairment of the intracellular transduction pathway (Widdowson 1997, Wilson *et al.* 1999, Ahima & Flier 2000, Jéquier 2002).

Concerning the leptin anorectic test in adult BRO offspring, since we did not detect a decrease in food consumption after leptin acute injection, as observed for the adult control rats, it is reasonable to believe that adult BRO rats did have a hypothalamic leptin resistance. The leptin resistance observed in BRO adult rats may be explained by their hyperleptinemia. These programmed rats also presented higher serum leptin levels at weaning (Bonomo *et al.* 2005), similar to the changes observed in leptinemia of pups whose

Figure 2 Food intake evolution, from weaning to adulthood, of offspring whose mothers were BRO-treated (BRO) or saline-treated (C) during lactation. Values are given as the means $\pm$ S.E.M. $n=18$ animals/group.

Figure 3 Amount of retroperitoneal white adipose tissue (RPWAT) of adult offspring whose mothers were BRO-treated (black bars) or saline-treated (white bars) during lactation. Values are given as the mean $\pm$ S.E.M. $n=10$ animals/group, * $P<0.001$.

mothers were malnourished during lactation (Teixeira *et al.* 2002). When the pups of energy-malnourished mothers become adults, they also presented leptin resistance (Passos *et al.* 2004). In the same way, other models of neonatal leptin imprinting (Cravo *et al.* 2002, Lins *et al.* 2005, Toste *et al.* 2006) are associated with leptin resistance. It seems that in the present model, higher neonatal leptinemia also imprints for leptin resistance at adulthood. Our data support the hypothesis of a central role of leptin on the body-weight control mechanisms imprinted at the beginning of life, especially on lactation.

We detected an 8% decrement on body weight of BRO pups at 21 days-old, corroborating our previous findings (Bonomo *et al.* 2005), which characterize neonatal malnutrition. This early malnutrition may be the imprinting factor for adult overweight, as demonstrated by other authors (Waterland & Garza 1999, Godfrey & Barker 2000, Vickers *et al.* 2000, Passos *et al.* 2000, 2002, 2004, Breier *et al.* 2001, Teixeira *et al.* 2002, Vicente *et al.* 2004). Thus, we believe that maternal hypoprolactinemia early in a pup's life plays the relevant role for the nutritional imprinting phenomena detected in our study. Our hypothesis is supported by the fact that milk presents large amounts of PRL, which is probably transferred by the milk and absorbed by the immature newborn gastrointestinal tract (Ellis & Picciano

Figure 4 Total body fat mass of adult offspring whose mothers were BRO-treated (black bars) or saline-treated (white bars) during lactation. Values are given as the mean $\pm$ S.E.M. $n=10$ animals/group, * $P<0.007$.

Figure 5 Serum leptin levels of adult offspring whose mothers were BRO-treated (black bars) or saline-treated (white bars) during lactation. Values are given as the mean $\pm$ S.E.M. $n=10$ animals/group, * $P<0.01$.

1995, Ben-Jonathan *et al.* 2006). However, the function of this milk secreted PRL on the developing offspring is unknown.

The events that could link low maternal PRL imprinting with the higher body weight of the offspring were based on the fact that BRO offspring received higher leptin transfer through the milk (Bonomo *et al.* 2005) and that leptin administration to pups or to dams are related with the same kind of body weight programming (Lins *et al.* 2005, Toste *et al.* 2006).

Figure 6 (A) Leptin anorectic effect of C adult offspring: Csal (white bar) and Clep (gray bars). (B) Leptin anorectic effect of BRO adult offspring: BROsal (black bars) and BROlep (checkered bars). Values are given as the mean $\pm$ S.E.M. $n=8$ animals/group * $P<0.05$.

Table 2 Food intake (g) in leptin anorectic test of BRO and C adult offspring. Values are given as the means $\pm$ S.E.M.

	2 h	4 h	6 h
C sal	0.6 $\pm$ 0.2	1.0 $\pm$ 0.2	1.7 $\pm$ 0.2
C lep	0.2 $\pm$ 0.1*	0.4 $\pm$ 0.2*	0.7 $\pm$ 0.2*
BRO sal	0.8 $\pm$ 0.1	1.2 $\pm$ 0.2	1.8 $\pm$ 0.2
BRO lep	0.9 $\pm$ 0.1	1.4 $\pm$ 0.2	1.9 $\pm$ 0.2

$n=8$ animals/group, * $P<0.05$.

Table 3 Leptinemia (ng/ml)/2 h leptin anorectic test of BRO and C adult offspring. Values are given as the means $\pm$ S.E.M.

	2 h	24 h
C sal	5.8 $\pm$ 1.3	5.7 $\pm$ 1.4
C lep	13.0 $\pm$ 2.8*	8.0 $\pm$ 1.8
BRO sal	17.0 $\pm$ 1.7	17.4 $\pm$ 1.6
BRO lep	34.0 $\pm$ 3.3*	22.3 $\pm$ 2.1

$n=5$ animals/group, * $P<0.05$.

Another important issue is related to the model of PRL blockade. Here, we only used BRO with the aim of PRL inhibition, but it is difficult to distinguish the hypoprolactinemia effect and a possible direct BRO effect on the pups, as well as the short-term and moderate (8%) undernutrition, much more higher in the maternal malnourished models (20% for a longer period). BRO is a specific dopamine type 2 receptor agonist, mainly found in the pituitary gland (Ben-Jonathan & Hnasko 2001). Therefore, even if BRO is transferred through the milk to the pups, its putative effect is over pups' PRL itself. Then, we believe that maternal PRL inhibition by BRO is the main imprinting factor. Further studies are being carried out in order to test a possible direct BRO effect in pups.

Finally, our data make evident the crucial role of PRL, even when it is blocked for a short period at the end of lactation, as an important factor that programs body weight regulation and adiposity, suggesting the importance of the PRL effect on milk transfer of other possible imprinting factors, such as leptin.

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