Effects of running wheel training on adult obese rats programmed by maternal prolactin inhibition

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Abstract

The inhibition of maternal prolactin production in late lactation leads to metabolic syndrome and hypothyroidism in adult offspring. Physical training is a therapeutic strategy that could prevent or reverse this condition. We evaluated the effects of a short-duration low-intensity running wheel training program on the metabolic and hormonal alterations in rats. Lactating Wistar rats were treated with bromocriptine (Bro, 1 mg twice a day) or saline on days 19, 20, and 21 of lactation, and the training of offspring began at 35 days of age. Offspring were divided into sedentary and trained controls (C-Sed and C-Ex) and sedentary and trained Bro-treated rats (Bro-Sed and Bro-Ex). Chronic exercise delayed the onset of weight gain in Bro-Ex offspring, and the food intake did not change during the experimental period. At 180 days, visceral fat mass was higher (+46%) in the Bro-Sed offspring than in C-Sed and Bro-Ex rats. As expected, running capacity was higher in trained animals. Most parameters observed in the Bro-Sed offspring were consistent with hypothyroidism and metabolic syndrome and were reversed in the Bro-Ex group. Chronic exercise did not influence the muscle glycogen in the C-Ex group; however, liver glycogen was higher (+30%) in C-Ex group and was unchanged in both Bro offspring groups. Bro-Ex animals had higher plasma lactate dehydrogenase levels, indicating skeletal muscle damage and intolerance of the training program. Low-intensity chronic training is able to normalize many clinical aspects in Bro animals; however, these animals might have had a lower threshold for exercise adaptation than the control rats.

Key Words
- programming
- running wheel
- lipid profile
- exercise
- prolactin
- early weaning

Introduction

Obesity is a health problem that is increasing at an astounding rate around the world. This condition is directly related to many health problems and results in a higher risk of other chronic non-communicable diseases and death (Beaglehole & Yach 2003). Some studies point to the quantity of visceral fat and its particular inflammatory profile to explain the adverse metabolic effects of obesity (Bosello & Zamboni 2000, Masuzaki et al. 2001).

Chronic exercise (physical training) is an interesting therapeutic strategy to counter the obesity epidemic. The American College of Sports Medicine recommends that most adults engage in moderate-intensity cardiorespiratory
exercise for a minimum of 30 min, 5 days/week (Garber et al. 2011). Studies showed that rats participating in training programs of short-duration (10–20 min) and low intensity had higher life expectancies (Retzlaff et al. 1966, Edington et al. 1972).

According to the WHO (2002), ‘exclusive breastfeeding is defined as no other food or drink, not even water, for 6 months of life’. Exclusive and prolonged breastfeeding has been associated with protection against long-term chronic diseases such as obesity and diabetes (Harder et al. 2005). However, only 35% of infants worldwide are exclusively breastfed during the first 4 months of life (WHO 2003).

Our group developed two experimental models for early weaning. In the first model, we used a pharmacological approach to inhibit the production of maternal prolactin (PRL) with the dopamine agonist bromo-α-ergocryptine (Bro). This method programs for obesity, hyperleptinemia, leptin resistance (Bonomo et al. 2007), and hypothyroidism (Bonomo et al. 2008), and symptoms (including insulin resistance and an altered lipid profile) are similar to those of metabolic syndrome (Moura et al. 2009). More recently, we obtained a similar profile in adult animals with a non-pharmacological model where a maternal bandage was used to cover the teats of the dam in the late lactation, thus also inducing an early weaning of offspring (Lima et al. 2011, 2013).

Using a well-established model of hypothalamic obesity in rodents (neonatal treatment with monosodium L-glutamate), Scamparin et al. (2006) observed significant decreases in fat mass with a low-intensity swimming training program of 15 min/day, 3 days a week. This result indicated that short-duration low-intensity training programs could be an interesting way of promoting physical activity. In addition, moderate physical training attenuates the effects of a low-protein maternal diet during lactation on the expression of leptin in the visceral adipose tissue of the adult offspring (de Melo Montenegro et al. 2012). Thus, the aim of this study was to investigate the effects of short-duration low-intensity chronic exercise on adult rats born to dams subjected to an inhibition of the production of PRL during the last 3 days of lactation (which is equivalent to an early weaning of 1 month in a human). Additionally, we evaluated parameters, such as fat mass, lipid profile, glycogen levels, and glucose homeostasis, that might be affected by exercise. These parameters were shown to be affected by this experimental model of early weaning and could be reverted or prevented by low-intensity moderate exercise.

Materials and methods
The study protocol was approved by the Ethics Committee of the Biology, Institute of State, University of Rio de Janeiro, Brazil (CEA/186/2007 based on the principles promulgated by Brazilian law no. 11.794/2008 (Marques et al. 2009)). Wistar rats at 120 days of age were kept at 25±1 °C on a dark–light cycle (0700 –1900 h). Adult virgin female rats were caged with male rats at a proportion of 3:1. During pregnancy and lactation, dams were housed in individual cages and provided with water ad libitum and a standard pellet diet. After birth, all litters were adjusted to six males for each dam to maximize lactational performance. Generally, pregnant rats produce 10–12 pups. Only mothers with a litter size of ten were used to avoid the influence of the litter size on the programming effect.

Model of programming for maternal hypoprolactinemia during late lactation
Lactating rats were separated into two groups. The control group (C, n=30) received a saline treatment for the last 3 days of lactation (19, 20, and 21 days) and the treated group (Bro, n=30) received 1 mg Bro (Novartis) twice a day for the same period (last 3 days of lactation). After weaning, two pups were randomly selected from each litter. These pups received the saline or Bro treatment.
during lactation, resulting in the following experimental groups: the sedentary control group (C-Sed, n = 10), maintained in a sedentary condition during the experiment; the control group subjected to exercise training on a running wheel (C-Ex, n = 10); the treated sedentary group (Bro-Sed, n = 11); and the treated exercising group (Bro-Ex, n = 10) (Fig. 1).

Running exercise training program
At 35 days of age, each rat was made to run until 180 days of age; the training lasted 15 min and was performed 5 days a week. The mean velocity of the treadmill was set at 17 m/min for all experimental periods, as this intensity is considered low and represents a level of oxygen consumption below 50–60% of the maximum oxygen uptake (VO₂ max; Brooks & White 1978). Additionally, this speed is slightly above the walk–trot gait transition (Lynn et al. 1998) and thus does not constitute an exhaustive training load. Preliminary acclimation was not necessary, as all animals were able to run for 15 min without exhaustion in the first attempt after water stimulation was applied. The training protocol was performed on a motorized running wheel (model EP 172, Insight, São Paulo, Brazil), and additional exercise parameters (volume and duration) were based on previous work of Scomparin et al. (2006). To stimulate continuous running behavior, we used a water flux with a controlled temperature (32 °C) positioned at the rear end of the running wheel. A recipient positioned below the running wheel provided the water, and an aquarium pump generated the flux with a flow capacity of 300 l/h.

Body weight and food intake
The body weight was evaluated from 35 days of age until 180 days of age. Food intake was monitored every 2 days until killing and was calculated according to the equation:

\[ F = \frac{(IQ - FQ)d}{n} \]

where \( F \), food intake; \( IQ \), initial quantity; \( FQ \), final quantity; \( n \), number of animals in cage; and \( d \), number of days between measurements.

Physical capacity
At 180 days of age, the last training session consisted of a non-exhaustive physical test to evaluate the exercise capacity of all animals. Forty-eight hours before killing, the rats were subjected to an acute bout of training consisting of running for 15 min at the same velocity as during training (17 m/min).

Anesthesia and killing procedures
Forty-eight hours after the physical capacity evaluation, all offspring were killed with a lethal dose of thiopental sodium (400 mg/kg, i.p.). This interval was chosen to avoid any residual effects of acute exercise. Blood samples were obtained by cardiac puncture, centrifuged to obtain plasma (2000 × g at 4 °C for 15 min), and kept at −20 °C until subsequent analysis. The skeletal muscle (soleus and extensor digitorum longus (EDL)) and liver were excised and kept frozen (−80 °C). The visceral fat mass (VFM) was dissected out and weighed to evaluate the central adiposity.

Tissue glycogen content
This measurement follows the procedure reported previously (Casimiro-Lopes et al. 2008) and is briefly detailed here. The soleus and EDL muscles were weighed and homogenized in a Turrax dispersing machine (IKA, Wilmington, NC, USA) with 1 ml buffer (50 mol/l Tris–HCl; 5 mol/l NaF, 5 mol/l EGTA, and 1 mol/l dithiothreitol, pH 7.2). After centrifugation (2000 × g at 4 °C for 20 min), 600 µl supernatant were removed and frozen. Subsequently, 100 µl supernatant were incubated with 2 U amyloglucosidase (Sigma A7420) and suspended in 0.2 M sodium acetate at pH 4.8 in a final volume of 200 µl for 4 h at 40 °C. The liver was weighed and homogenized with 4 ml trichloroacetic acid (TCA) 10% (w/v). After centrifugation (1000 × g at 4 °C for 10 min), 2 ml supernatant were added to 5 ml of absolute ethanol and frozen. After 24 h, the mixture was centrifuged (1000 × g at 4 °C for 10 min) and the supernatant was discarded. Glycogen was hydrolyzed by boiling the pellet for 30 min with HCl (1 M). To neutralize the mixture, 1 ml NaOH (1 M) was added. For both assays, a standard curve with increasing concentrations of glycogen was constructed using the same experimental conditions. The glucose produced by muscle and liver glycogen hydrolysis was measured using a commercial kit (Glucox, Doles, Goiás, Brazil). To ensure that the glucose originated only from glycogen, blanks without enzyme were processed and subtracted from the experimental samples. The values were expressed as milligrams per liter per gram.
Measurement of blood hormones and glycermia

Hormone levels were determined by a single RIA. The total triiodothyronine (T₃) and thyroxine (T₄) levels were determined using a commercial RIA Kit (ICN Pharmaceuticals, Inc., New York, NY, USA), as described by Moura et al. (1987), with assay sensitivities of 25 ng/ml and 2 μg/dl and intra-assay coefficients of variation (CV) of 3.8 and 4% respectively. Insulin levels were measured with a commercial RIA Kit (ICN Biomedicals, Inc., Aurora, OH, USA) with an assay sensitivity of 0.1 ng/ml and an intra-assay CV of 4.2%. The corticosteronemia was evaluated using a commercial kit for rats (ImmuChem 125 I, double antibody, ICN Biomedicals, Inc., Costa Mesa, CA, USA) with an assay sensitivity of 7.7 ng/ml and an intra-assay CV of 7.1%. Glycemia was determined in blood samples from the tail vein of fasting rats using a glucometer (Accu-Chek Advantage; Roche Diagnostics). Glucose homeostasis was evaluated using the insulin resistance index (IRI), the product of the fasting insulin level (μIU/ml), and the fasting glucose level (mol/l), as proposed by Ahren & Scheurink (1998).

Blood lipid profile

Total cholesterol, HDL cholesterol (HDL-c), and triglycerides were evaluated using commercial kits (Bioclin, Belo Horizonte, Minas Gerais, Brazil). LDL cholesterol (LDL-c) and very LDL-c (VLDL-c) were calculated with the Friedewald equation (Friedewald et al. 1972). The data were expressed as milligrams per deciliter. The atherogenicity indices Castelli I and II were calculated with the equation proposed by Castelli (1984).

Plasma cell injury

Cell damage was estimated by plasma lactate dehydrogenase (LDH) activity, which was evaluated with a commercial kit (Doles). The data were expressed as international units per liter.

Statistical analysis

The normality of the distribution of each data set was evaluated using the Kolmogorov–Smirnov test. The body mass and food intake were analyzed using two-way ANOVA followed by a Bonferroni’s post-test. The other parameters were analyzed using one-way ANOVA with the Newman–Keuls post-test. The data are presented as the mean ± S.E.M., with the level of significance set at P<0.05. All analyses were performed using GraphPad Prism 5 Software (GraphPad Software, Inc., La Jolla, CA, USA).
Results

Body mass values were higher in animals of the Bro-Sed group than in animals of the C-Sed group at 98 days of age; the animals in the Bro-Ex group had lower values of body mass at 147 days of age than those in the Bro-Sed group. All these alterations persisted throughout the entire experiment. No significant differences were observed between the animals of the C-Ex group and those of the C-Sed group. The food intake did not significantly differ among any groups during the experimental period. At 180 days, the VFM was higher in the Bro-Sed animals than in the C-Sed group. The food intake did not significantly differ among any groups during the experimental period.

Table 1 shows the lipid profile and atherogenicity indices in the offspring subjected to the experimental conditions. The rats in the Bro-Sed group had higher levels of total cholesterol (+11%) but similar serum insulin levels. Blood glucose levels were lowest in the Bro-Ex offspring (−11%); however, the serum insulin level was lower in both trained groups (C-Ex and Bro-Ex, −22%) than in their respective controls. As expected, the IRI was higher in the Bro-Sed group (+25%) and lower in the trained groups (C-Ex, −25% and Bro-Ex, −32%), as shown in Table 2.

The thyroid hormone levels indicated that the animals of the Bro-Sed group had experienced a hypothyroid-like behavior; the total T₃ (−20%) and T₄ (−19%) levels were lower in these animals than in their respective controls. Physical training stimulated higher levels of total T₃ (+29%) in the Bro-Ex group than in the Bro-Sed group. Higher levels of serum corticosterone (+52%) were also observed in the Bro-Sed group; these levels were attenuated by physical training in the Bro-Ex animals (−37%), producing levels similar to those observed in the C-Sed and C-Ex animals (Table 3).

Table 4 shows the tissue glycogen levels and the effect of physical training. Hepatic glycogen was higher in the C-Ex group (+30%) than in the C-Sed group. Chronic exercise did not influence the skeletal muscle glycogen content in any group for any muscle fiber type.
Physical training on the motorized running wheel promoted higher levels of LDH in the Bro-Ex animals than in the C-Sed (+57%) and Bro-Sed (+85%) animals, as shown in Fig. 3.

**Discussion**

Our results show that the Bro-Sed offspring had symptoms of metabolic syndrome, including visceral obesity, hypertriglyceridemia, lower HDL-c, hypertension, and insulin resistance, as similar results previously reported by our laboratory (Bonomo et al. 2007, Moura et al. 2009). Physical training consisting of low-intensity short-duration chronic exercise on motorized running wheels induced higher fitness levels in regular rats (C-Ex group). The absence of adaptive alterations in other parameters such as the lipid profile and corticosterone and thyroid hormone levels could be due to training load parameters that were not sufficient to stimulate beneficial responses in normal animals. However, glucose homeostasis significantly improved both in terms of the insulin sensitivity and the liver glycogen levels.

Low-intensity short-duration physical training in Bro-Ex animals normalized most parameters of visceral obesity, lipid profile, and glucose homeostasis. Similar modifications in fat mass were described previously (Dantas et al. 2010) in animals exposed to overfeeding after treadmill training for 60 min/day, 5 days a week. Scomparin et al. (2006) reported that mice that were previously treated with monosodium glutamate (MSG) during the neonatal period had lower levels of visceral fat after shorter sessions of swimming training (15 min/day, 3 days a week), suggesting that the beneficial effects of chronic exercise could be achieved with lower training parameters.

Early weaning changes serum leptin levels in weaned animals (Bonomo et al. 2005, Lima et al. 2011). This early change in the leptin level is critical for establishing neural circuitry in the hypothalamus (Bouret et al. 2004) at a different set point of regulation. The inhibition of the production of maternal PRL may cause obesity in progeny via this mechanism; consequently, the inhibition of the production of PRL may impair the physical capacity of the progeny, as this inhibition is associated with other metabolic and hormonal alterations (such as changes in leptin levels and insulin resistance). Furthermore, the inhibition of the production of maternal PRL at the end of lactation induces a hypothyroidism in 180-day-old adult offspring (Bonomo et al. 2008) that is related to low physical capacity (Zarzeczny et al. 1996, Casimiro-Lopes et al. 2008). However, previous work from our laboratory showed that animals in this model had higher exercise capacity at 90 days; this capacity returned to control levels at 180 days, even in animals with higher levels of VFM during both periods. These results appear to be mediated by higher liver glycogen levels in young animals that normalized with increasing age (Casimiro-Lopes et al. 2012). Thus, the alterations in leptin, insulin actions, and thyroid hormonal status may have contributed to lower glycogen liver accumulation when the Bro animals grew older.

The total T₃ levels, which were lower in the Bro-Sed group, reached values similar to those observed in the C-Sed and C-Ex groups, indicating a normalization effect of chronic exercise. The change in T₃ levels appears to be more pronounced than that of the T₄ levels, suggesting

**Table 3** Thyroid hormones and corticosterone in sedentary and trained adult offspring of Bro- or saline-treated (control) animals. Values are given as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>C-Sed (n = 10)</th>
<th>C-Ex (n = 10)</th>
<th>Bro-Sed (n = 11)</th>
<th>Bro-Ex (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T₃ (ng/dl)</td>
<td>59.2 ± 3.0</td>
<td>54.7 ± 2.6</td>
<td>47.4 ± 2.4*</td>
<td>60.9 ± 3.1†</td>
</tr>
<tr>
<td>Total T₄ (µg/dl)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.05*</td>
<td>1.1 ± 0.04</td>
</tr>
<tr>
<td>Corticosterone (ng/ml)</td>
<td>252.6 ± 19.5</td>
<td>244.1 ± 18.7</td>
<td>384.1 ± 38.2*</td>
<td>242.7 ± 12.4†</td>
</tr>
</tbody>
</table>

* vs C-Sed and † vs Bro-Sed (P < 0.05).

**Table 4** Glycogen content in muscle (EDL and soleus) and liver in sedentary and trained adult offspring of Bro- or saline-treated (control) animals. Values are given as mean ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>C-Sed (n = 10)</th>
<th>C-Ex (n = 10)</th>
<th>Bro-Sed (n = 11)</th>
<th>Bro-Ex (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus glycogen (mg/ml per g)</td>
<td>0.3 ± 0.02</td>
<td>0.4 ± 0.04</td>
<td>0.3 ± 0.02</td>
<td>0.4 ± 0.03</td>
</tr>
<tr>
<td>EDL glycogen (mg/ml per g)</td>
<td>0.4 ± 0.08</td>
<td>0.5 ± 0.08</td>
<td>0.4 ± 0.06</td>
<td>0.6 ± 0.10</td>
</tr>
<tr>
<td>Liver glycogen (mg/ml per g)</td>
<td>15.9 ± 2.0</td>
<td>20.7 ± 0.9*</td>
<td>17.4 ± 0.5</td>
<td>19.0 ± 0.9</td>
</tr>
</tbody>
</table>

* vs C-Sed (P < 0.05).
a higher component of low T₃ syndrome due to lower peripheral deiodination of T₄ to T₃. Corticosterone is known to decrease liver deiodinase (Aceves et al. 2003, Araujo et al. 2009), and the corticosterone levels were higher in the animals of the Bro-Sed group; exercise appeared to normalize corticosteronemia in these animals. An acute bout of exercise appears to influence thyroid hormone concentrations (Fortunato et al. 2008, Casimiro-Lopes et al. 2012), a finding that was also observed in chronically exercised mice (Katzeff et al. 1988). In our study, the C-Ex rats did not show any alterations in thyroid hormone concentrations. Ciloglu et al. (2005) observed that the thyroid hormone response depends on the exercise intensity (i.e. heart rate percentage). In this case, the absence of response in the control rats could be related to the low intensity of the training program.

Corticosterone levels were not affected by physical training in the C-Ex group; however, the Bro-Ex animals had lower levels of corticosterone than those of the Bro-Sed group, suggesting a lowering effect mediated by chronic exercise. Starzec et al. (1983) observed that chronically stressed rats had lower levels of corticosterone when physically trained. Some studies suggest that exercise could reduce circulating ACTH levels or promote a higher threshold for adrenal gland stimulation by ACTH (Tharp & Buuck 1974, Wittert et al. 1996).

Interestingly, the lipid profile was completely normalized in the Bro-Ex offspring, further demonstrating the effect of running wheel training. This result was previously observed in other studies with different training modalities (Burneliko et al. 2004, Guerra et al. 2007).

Physical training did not significantly change the glucose levels in the C-Ex group; however, both the insulin and IRI levels were influenced by chronic exercise. The same pattern was observed in the Bro-Ex offspring when compared with the animals of the Bro-Sed group. These results could be explained by the glucose-lowering effects of exercise through increased GLUT4 expression in skeletal muscle, leading to higher insulin sensitivity (James et al. 1985, Terada et al. 2001). This higher insulin sensitivity also explains the higher liver glycogen concentration in the C-Ex group. However, this change was not observed in the Bro-Ex group, possibly contributing to a worse prognosis for these animals and higher LDH levels.

Higher physical capacity is a normal adaptation to chronic exercise that was observed in both trained groups. Interestingly, the skeletal muscle glycogen levels were not affected by training in any group, though liver glycogen levels were higher in the C-Ex animals. Additionally, none of the deleterious features of the inhibition of the production of maternal PRL affected the running capacity of the Bro offspring. Tissue depots of glycogen are generally of fundamental importance to exercise performance in humans (Tsintzas & Williams 1998) and rats (Slentz et al. 1990). Specifically, in rats, skeletal muscle glycogen is not a factor that determines exercise capacity. Pederson et al. (2005) observed that physical capacity for strenuous or endurance exercise was not significantly affected in mice lacking the Gys1 gene, which encodes the muscle isoform of glycogen synthase. Additionally, skeletal muscle glycogen is not mobilized during low-intensity exercise in rats (Baldwin et al. 1973, Raja et al. 2003); however, liver glycogen is strongly mobilized during exercise, independent of the intensity of the exercise (Gleeson & Waring 1986). Thus, these studies indicate that the short-duration low-intensity training elicited by the running wheel could stimulate glycogen supercompensation in C-Ex animals but not in Bro-Ex animals. These conflicting results suggest that the training parameters for Bro-Ex offspring could be inadequate. This deficiency is possibly caused by a lowered threshold for exercise recuperation, as the elevated training volume and/or intensity could interfere with glycogen compensation in rats (Ghanbari-Niaki et al. 2010).

Blood levels of LDH are related to tissue damage to cell membranes, leading to leakage of cell constituents (Dawson et al. 2002). This feature is also closely related to exercise intensity (Loegering 1974, Ramos et al. 2013); however, these results are not observed in the C-Ex offspring indicating raised cellular stress resistance (Jeong et al. 2012). Higher levels of plasma LDH in the Bro-Ex rats suggest an impaired capacity of cell membrane adaptation to chronic exercise load besides other positive responses in adiposity, lipid profile, thyroid status, and insulin sensitivity. Deleterious adaptations include the potential for increased cellular stress, as indicated by the higher LDH levels.
from excessive/inadequate training were reported by Moraska et al. (2000) and included the following: thymic involution, adrenal hypertrophy, and impaired immune function. Interestingly, other responses such as lower body mass and higher citrate synthase activity were also detected.

This paper demonstrates a novel model of metabolic syndrome caused by early weaning in which hypothyroidism may explain changes in the lipid profile, visceral adiposity, and glucose homeostasis; these changes are partially or totally restored by low-intensity exercise. However, some alterations persist and can compromise the future physical capacity of these programmed animals.

In summary, short-duration long-term running wheel training could counterbalance the characteristics of metabolic syndrome and the hypothyroid profile observed in animals subjected to maternal PRL inhibition. However, higher plasma LDH levels suggest that these animals appear to be more sensitive to training than previously thought. These results suggest that programmed animals in this experimental model can achieve substantial benefits from chronic exercise but could have a lower threshold for exercise response than control rats. Less frequent training sessions could offer a possible way to induce positive exercise adaptations in Bro-Ex offspring.

Declarations of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


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