EFFECTS OF PHYSICAL TRAINING DURING PREGNANCY ON BODY WEIGHT GAIN, BLOOD GLUCOSE AND CHOLESTEROL IN ADULT RATS SUBMITTED TO PERINATAL UNDERNUTRITION

Filipe Falcão-Tebas¹
Amanda Theresia Tobias¹
Adriano Bento-Santos²
José Antônio dos Santos³
Diogo Antônio Alves de Vasconcelos¹
Marco Antônio Fidalgo³
Raul Manhães-de-Castro¹
Carol Góis Leandro³

1. Nutrition Department, Federal University of Pernambuco – Recife, Pernambuco.
2. Neuropsychiatry and Behavior Sciences Department, Federal University of Pernambuco – Recife, Pernambuco.

Mailing address:
Rua Prof. Moraes Rego, 1.235 – Departamento de Nutrição – Cidade Universitária – 50670-901 – Recife, PE, Brasil.
E-mail: filippe_oliveira@hotmail.com

ABSTRACT

The incompatibility of perinatal undernutrition and adequate nutrition during development increases the risk of early onset of non-communicable diseases in adulthood. However, it has been considered that maternal physical activity may attenuate these effects. This study aimed to evaluate the effects of physical training during pregnancy on body weight gain, waist circumference, glycaemia and cholesterolemia in adult offspring submitted to perinatal undernutrition. Female Wistar rats (n = 12) were divided into four groups: control (C, n = 3), trained (T, n = 3), undernourished (U, n = 3) and undernourished and trained (T+U, n = 3). During gestation and lactation, U and T+U groups were fed a low protein diet (8% casein) and C and T groups fed a normal protein diet (17% casein). The protocol of moderate physical training was performed on a treadmill (5 days/week, 60 min/day, at 65% of VO2max) and began 4 weeks before pregnancy. At pregnancy, the duration and intensity of training were reduced (5 days/week, 20 min/day, at 30% VO2max) until the 19th prenatal day. At weaning, male pups (CP = 9, TP = 9, UP = 7, T+UP = 9) received standard diet and evaluations took place at 270 days old. Abdominal circumference (AC) was evaluated in relation to body weight. Enzymatic colorimetric method glucose-oxidase/peroxidase and cholesterol-oxidase was used to evaluate fasting glycaemia and cholesterolemia, respectively. Rats from UP group showed high body weight gain during growth, higher AC, glycaemia and cholesterolemia values when compared to CP. Concerning the T+UP group, body weight gain was attenuated, and the AC, glycaemia and cholesterolemia were normalized (p<0.05). These results demonstrate that physical training during pregnancy reduces the effects of perinatal undernutrition on some murinometric and biochemical indicators of adult offspring.

Keywords: phenotypic plasticity, low-protein diet, physical exercise.

INTRODUCTION

Fetal growth and development depend on genetic, hormone and placental factors, the maternal milieu as well as adequate supply of oxygen and nutrients. In particular, inadequate supply of nutrients during fetal life has been associated with low weight at birth and deficit during growth and maturation. Likewise, incompatibility between undernutrition during the perinatal period and nutrition throughout life may be related to the onset of metabolic diseases. Perinatal undernutrition negatively influences the nervous system development, causes delay in reflex ontogenesis in breast feeding rats and alters the eating behavior in adulthood. Moreover, there are alterations in the cardiac muscle morphology, delay in the acquisition of the normal patterns of locomotor activity and deficiency in the contractile elastic properties of the skeletal muscle of adult rats.

The term “programming” or “phenotypic plasticity”, has been used to explain that during ontogenesis, the development of each organ or system goes through a critical window of sensitivity or plasticity, in which the environmental factors may generate adjustments in the phenotype which remain throughout life. Maternal physical activity induces to physiological adaptations during pregnancy involving the fetal-placental growth and increase of availability of nutrients and oxygen for the fetus. However, such effects on the oxygenation and fetal-placental growth are directly correlated with the physical fitness level of the mother and in which moment during pregnancy the exercise program is performed.

According to the American College of Obstetricians and Gynecologists, women with low-risk pregnancy may practice moderate physical exercise (up to 70% of VO2max) and light exercise (up to 40% of VO2max) for about 30 minutes a day, all days of the week. However, it is still difficult to establish percentage of physical exertion recommendations during pregnancy since intensity, kind and duration of the physical exercise are determinant for the physiological adaptations in the mother and the reflections on the offspring. Epidemiological studies performed in a rural community in India with pregnant women demonstrated an inverse relationship between exertion intensity and weight at kids’ birth. In animals, female rats trained before pregnancy (five days/week, 60 min/day, at 65% of VO2max) with progressive decrease of exertion during pregnancy (five days/week, 30 min/day, at 40% of VO2max) presented less remarkable decrease in oxygen consumption at rest. This effect was determinant to attenuate the effects of perinatal protein undernutrition related to the maturation of the nervous system and somatic growth rate of the pups.
The studies which associate physical training during pregnancy with the phenotypic plasticity hypothesis are still scarce. In addition to that, little is known about the long term consequences of a physical training program during pregnancy. Thus, the present study had the aim to evaluate the effects of physical training during pregnancy in the ponderal evolution, abdominal circumference, glycaemia and cholesterolemia of adult offspring submitted to perinatal undernutrition. Our hypothesis is that physical training during pregnancy attenuates the effects of the programming induced by perinatal protein undernutrition.

MATERIAL AND METHODS

Animals

This study was approved by the Ethics in Studies with Animals Committee of the Center of Biological Sciences of UFPE (protocol # 23076.049077/2010-80). Animal manipulation and care followed the guidelines by the Brazilian Committee of Animals Experimentation (COBEA).

12 Wistar albino female rats (60 days old), body weight between 180 ± 11g, obtained in the Nutrition Department of the Federal University of Pernambuco were used. The rats were kept in an animal facility with standard conditions of temperature of 23°C ± 1 and light cycle from 18:00 to 6:00h, with free access to water and standard food from the animal facility (52% carbohydrates, 21% proteins, 4% lipids – Nuvilab CR1-Nuvital®, Curitiba, Paraná, Brazil). The rats were divided in two experimental groups: control (C, n = 6) and trained (T, n = 6). Group T performed a moderate physical training program on treadmill (EP-131®, Insight Equipments, SP, Brazil) (table 1). After the four-week physical training, the rats from the two groups were placed to mate (two females for one male). The pregnancy diagnosis was performed through vaginal smear assay for sperm presence. If pregnancy is detected, half of the rats of each group (C and T) was submitted to hypoprotein diet (8% casein), while the other rats received normoprotein diet (17% casein). The diets designed were isocaloric, with alteration only in the protein content, consisting of the following groups: control (C, n = 3, 17 % casein); trained (T, n = 3, 17% casein); undernourished (U, n = 3, 8% casein) and trained undernurtured (T+U, n = 3, 8% casein). The physical training program was kept during pregnancy until the 19th day, with progressive intensity and duration of the sessions decrease (table 2). During the entire experiment the maternal body weight was weekly followed. The mothers kept receiving the casein-based experimental diet and the litters were adjusted to six pups per mother. After weaning (at 22 days of life of the pups), three male pups of each litter were randomly used. The pups were fed with standard diet of the animal facility and divided in four groups according to the manipulation of their respective mothers: pups from control mothers (CP, n = 9), pups from trained mothers (TP, n = 9), pups from undernourished mothers (UP, n = 7) and pups from trained and undernourished mothers (T+UP, n = 9).

Physical training protocol

The animals were previously adapted to the animal facility inverted cycle (during 15 days) and to the treadmill for three days (10 min/day, with velocity of 0.3km.h⁻¹). The moderate physical training program was submitted to hypoprotein diet (8% casein), while the other rats received normoprotein diet (17% casein). The diets designed were isocaloric, with alteration only in the protein content, consisting of the following groups: control (C, n = 3, 17 % casein); trained (T, n = 3, 17% casein); undernourished (U, n = 3, 8% casein) and trained undernurtured (T+U, n = 3, 8% casein). The physical training program was kept during pregnancy until the 19th day, with progressive intensity and duration of the sessions decrease (table 2). During the entire experiment the maternal body weight was weekly followed. The mothers kept receiving the casein-based experimental diet and the litters were adjusted to six pups per mother. After weaning (at 22 days of life of the pups), three male pups of each litter were randomly used. The pups were fed with standard diet of the animal facility and divided in four groups according to the manipulation of their respective mothers: pups from control mothers (CP, n = 9), pups from trained mothers (TP, n = 9), pups from undernourished mothers (UP, n = 7) and pups from trained and undernourished mothers (T+UP, n = 9).

### Table 1. Physical training program for female rats according to velocity, duration and intensity of each session during a four-week period in the pre-gestational period(16)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Velocity (km.h⁻¹)</th>
<th>% of VO₂max</th>
<th>Duration of each stage (min)</th>
<th>Total duration of each session (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week (adaptation)</td>
<td>0.3</td>
<td>36.1 ± 2.7</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>38.7 ± 2.9</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>37.8 ± 1.9</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>35.7 ± 3.1</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>2nd week</td>
<td>0.5</td>
<td>47.8 ± 3.1</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>57.3 ± 5.0</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>54.4 ± 2.4</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>42.5 ± 1.7</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>3rd week</td>
<td>0.6</td>
<td>51.1 ± 2.8</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>64.0 ± 3.3</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>57.2 ± 3.4</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>4th week</td>
<td>0.6</td>
<td>42.9 ± 1.7</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>66.4 ± 4.9</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>65.3 ± 4.7</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>57.8 ± 3.9</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>

### Table 2. Physical training program for female rats according to velocity, duration and intensity of each session during the three-week period during pregnancy(16)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Velocity (km.h⁻¹)</th>
<th>% of VO₂max</th>
<th>Duration of each stage (min)</th>
<th>Total duration of each session (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>0.4</td>
<td>52.9 ± 3.4</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>58.7 ± 4.4</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>63.1 ± 1.4</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>66.4 ± 4.9</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>62.0 ± 2.6</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>2nd week</td>
<td>0.4</td>
<td>42.0 ± 1.3</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>47.8 ± 5.1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>43.5 ± 5.7</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>42.1 ± 3.2</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>3rd week</td>
<td>0.3</td>
<td>36.7 ± 1.8</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>32.2 ± 2.9</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>32.8 ± 1.7</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>29.9 ± 2.6</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>
used consisted of four training weeks, five times per week, 60 minutes per day at 65% of \( V_{O_2\text{max}} \). Adaptation to physical training took place on the first week, which consisted of 20-minute sessions, divided in four stages of five minutes, during five days. After the adaptation, the protocol was divided in progressive stages in each session: 1) warm-up (five minutes); 2) intermediate zone (10 minutes); 3) training zone (30 minutes); and 4) final period (five minutes) (table 1).

In the gestation period, progressive decrease in training intensity and duration has occurred. Training consisted of three weeks of training, five days per week, being reduced to 20 minutes per day at 30% of \( V_{O_2\text{max}} \) (table 2).

**Body weight and weight gain evaluation**

Body weight expressed in grams was weekly evaluated in the mothers and monthly in the pups on a digital electronic scale – Marte, model S-1000, with maximum capacity of 1,000g and sensitivity of 0.01g. The weight gain percentage was calculated using the weaning weight as reference according to the formula:

\[
\% \text{ weight gain} = \frac{\text{weight of the day (g) x 100}}{\text{weaning weight (g)}} - 100
\]

Abdominal circumference was determined by the highest circumference between the upper iliac crest and the last rib and expressed in centimeters.

**Glycaemia and cholesterolemia evaluation of the pups**

After six-hour fast, the animals had a cut in the tail tip for blood collection. The serum glucose and cholesterol (mg.dL\(^{-1}\)) of the pups at 270 days of age were assessed. The blood glucose concentrations were identified by glucose-oxidase/peroxidase colorimetric enzymatic analysis and the reading by a glucometer (AccuChek Performa®, Roche Diagnostics). The cholesterolemia amount was quantified by cholesterol-oxidase method and analyzed by photometry with the values being calculated in the monitor (Accutrend Cholesterol®, Roche Diagnostics).

**STATISTICAL ANALYSIS**

Initially, all variables were submitted to the normality test (Kolmogorov-Smirnov). When variances normality and homogeneity were revealed, Student’s test was used for comparison of parameters of the female rats during the pre-gestation period. Inter-group analysis during pregnancy, weight of the pups at birth and during their growth and evaluation of the biochemical parameters of the animals at adulthood was performed with two-way ANOVA followed by Bonferroni post hoc test. The values are expressed in mean and standard error of the mean (SEM). Significance level was set at 5% (\( p < 0.05 \)) in all cases. The entire statistical analysis was performed using the GraphPad Prism® program (GraphPad Software Inc., La Jolla, CA, USA; version 5.0 for Windows).

**RESULTS**

The rats submitted to the moderate physical training protocol presented lower body weight gain values in the pre-gestation period from the third week (table 3).

During pregnancy, the groups were subdivided according to the diet offered to the mothers. The mothers that received hypocaloric diet presented lower body weight gain from the second week of pregnancy. The trained mothers that were undernourished presented lower weight gain values in the last week of pregnancy (table 4).

The mean weight value at birth of the pups from undernourished mothers was lower when compared to the control (CP = 6.1 ± 0.3; UP = 4.9 ± 0.3; TP = 7.1 ± 0.4; T + UP = 5.9 ± 0.2). The pups’ growth was followed from weaning until 270 days of life. The animals from undernourished and trained undernourished mothers presented lower values of body weight during growth (figure 1A). However, weight gain concerning weaning of the undernourished animals was higher from the 30th day of life, when compared to the control (figure 1B). Although the animals from the trained undernourished mothers had presented higher body weight gain during growth, these values were lower than the undernourished group (figure 1B).

The mean values of the abdominal circumference concerning body weight of the pups from undernourished mothers were higher compared to the control. In the group of animals from trained undernourished mothers there was no difference compared to the control and it was lower compared to the undernourished group (figure 2).

**Table 3.** Mean ± SEM values of the body weight gain of the female rats submitted to a moderate physical training program in the pre-gestational period.

<table>
<thead>
<tr>
<th>Pre-gestational body weight gain</th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd week</td>
<td>99 ± 0.9</td>
<td>95 ± 0.7</td>
</tr>
<tr>
<td>3rd week</td>
<td>130 ± 1.1</td>
<td>112 ± 0.9*</td>
</tr>
<tr>
<td>4th week</td>
<td>170 ± 2.3</td>
<td>130 ± 1.1*</td>
</tr>
</tbody>
</table>

\*\( p < 0.05 \) vs. control, Student’s t test.

**Table 4.** Mean ± SEM values of body weight gain of the female rats submitted to a moderate physical training in the pre-gestational period and light one during pregnancy and/or a hypoprotein diet during pregnancy.

<table>
<thead>
<tr>
<th>Gestational body weight gain</th>
<th>Groups</th>
<th>Control</th>
<th>Undernourished</th>
<th>Trained</th>
<th>Trained + undernourished</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td></td>
<td>402 ± 11</td>
<td>39 ± 0.9</td>
<td>42 ± 0.9</td>
<td>39 ± 1.0</td>
</tr>
<tr>
<td>2nd week</td>
<td></td>
<td>160 ± 21</td>
<td>142 ± 3.0</td>
<td>178 ± 24</td>
<td>151 ± 2.9</td>
</tr>
<tr>
<td>3rd week</td>
<td></td>
<td>369 ± 53</td>
<td>30 ± 5.1</td>
<td>382 ± 55</td>
<td>315 ± 6.1*</td>
</tr>
</tbody>
</table>

\*\( p < 0.05 \) vs. control, two-way ANOVA followed by the Bonferroni test.

Figure 1. Body weight expressed in grams (A) and weekly weight gain percentage of the 30 to 270 days concerning weaning (B) of rats. Groups: C\(_P\) (n = 9); T\(_P\) (n = 9); U\(_P\) (n = 7) and T+U\(_P\) (n = 9). Data are represented in mean ± SEM.

\( ^* \)\( p < 0.05 \) vs. C\(_P\); \(^{##} \)p < 0.05 vs. U\(_P\). Two-way ANOVA followed by Bonferroni test.
Glycaemia and cholesterolemia of the pups in adulthood which were submitted to perinatal undernutrition were higher compared to the control group (figure 3). The animals from trained undernourished mothers did not present difference compared to the control and presented mean values lower than the undernourished group (figure 3).

**Figure 2.** Abdominal circumference concerning the body weight [(cm/g) x 100] of the rats at 270 days. Groups: C (n = 9); T (n = 9); U (n = 7) and T+U (n = 9). Data are represented in means ± SEM. *p < 0.01 vs. C, #p < 0.01 vs. U. One-way ANOVA followed by the Bonferroni test.

**Figure 3.** Glycaemia and cholesterol of the rats at 270 days. Groups: C (n = 9); T (n = 9); U (n = 7) and T+U (n = 9). Data are represented in mean ± SEM. *p < 0.05 vs. C; #p < 0.01 vs. U. Two-way ANOVA followed by the Bonferroni test.

**DISCUSSION**

The aim of the present study was to evaluate the moderate physical training effects in the body weight gain of the mothers submitted or not to perinatal undernutrition, as well as the consequences in the 270 day-old pups. Corroborating our hypothesis, maternal physical training during pregnancy attenuated the deleterious effects of perinatal undernutrition in the ponderal evolution and murinometric and biochemical indicators in adult rats.

Concerning the undernourished mothers, it was observed lower body weight gain and that their pups were born with low weight, corroborating previous studies.20,22,23 The pups from undernourished mothers remained with lower growth trajectory until adulthood even when the balanced diet was offered. It is interesting to observe that body weight gain (expressed in percentage from weaning) was higher in the pups from undernourished mothers. This phenomenon is named catch up growth, in which the body compensates in the long term for a slow growth rate by protein deficit in diet during the initial periods of life.24 This post-natal rapid growth has been associated with increase of the percentage of adipose tissue, blood pressure as well as risk of developing glucose intolerance in long-term.25

On the other hand, the alterations in the catch up of the undernourished animals were attenuated in the pups from trained undernourished mothers, demonstrating hence that physical training may attenuate the effects of hypoprotein undernutrition. In the present study, the rats initiated the physical training protocol four weeks before pregnancy, in order to normalize the physiological parameters in response to the acute stress of an exercise session. Moreover, physical exercises of light intensity during pregnancy, similar to the ones used in our investigation, have been recommended, since, besides contributing to the maintenance of weight gain, they increased the fetal-placental growth rate and weight at birth.11 The subjacent mechanisms seem to include: a) increase of uterine blood flow; b) redistribution of blood flow; c) alterations in the fetal and placental production of hormones which control growth; and d) lower decrease of oxygen consumption at rest in response to physical training.16,26 These physiological parameters may be contributing to a better predictive adaptation response (a theoretical model which supports the hypothesis of the phenotypic plasticity) generated by the early stimulus.6 This response has been associated with epigenetic mechanisms, in which an external factor may modulate the DNA structure with no change in its sequence.28 In rats exposed to intrauterine undernutrition, higher leptin, insulin and peptide-C concentrations, indicators of the metabolic syndrome in adulthood, due to alterations in the genotype of animals have been observed.27 Thus, it has been reported that early environmental stimuli may modulate the phenotype throughout life.

The abdominal circumference has been used as an indicator of obesity in rats.21 In population studies, the abdominal circumference has been used as an indicator of risk of cardiovascular diseases and obesity onset in adults. According to the phenotypic plasticity hypothesis, the animals submitted to perinatal undernutrition presented higher values of abdominal circumference and our results are similar to the ones in previous studies.28,29 In rats, it has been demonstrated that newborn pups from undernourished mothers (50% of restriction of the diet received by the control ad libitum) presented at nine months of age increase in the expression of lipogenic and adipogenic transcription factors, leading to adipocyte hypertrophy.30 Conversely, in the animals from trained undernourished mothers, these results were attenuated. It is probable that the lower catch up growth observed in these animals may justify lower abdominal circumference.

Increase in abdominal circumference may also indicate higher plasma concentration of triglycerides, fatty acids and cholesterol, which are indicators of atherogenic risk.31 Our results demonstrate that offspring of undernourished mothers presented increase in glucose and cholesterol serum concentrations when adults. These effects have been verified in previous studies and are classic indicators of the beginning of peripheral resistance to insulin and dyslipidemias associated with the developmental origin of the metabolic syndrome.32 However, the animals from trained undernourished mothers did not present this hyperglycaemia and hypercholesterolemia scenario.
Therefore, some mechanisms which can theoretically support the physical training attenuation activity can be suggested: increase in placental volume, increase in the blood flow to the placenta after the exercise, increase in the passage of nutrients and oxygen to the fetus, increase in the maternal body lean mass and increase in the oxygen consumption at rest. It is worth mentioning that all these effects are directly related with the effort magnitude. In the present study, a standard training protocol from the direct measurements of oxygen consumption was used. The intensity and duration were controlled so that the training sessions could be of moderate and light intensity.

In conclusion, moderate physical training before and during pregnancy attenuated the perinatal undernutrition effects on the somatic growth and glucose and cholesterol serum levels in the adult pups. Our data corroborate the studies which test the phenotypic plasticity hypothesis and open a scenario for the effects of a positive environmental stimulus, being able to attenuate the undernutrition effects.

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