

Programmed changes in the adult rat offspring caused by maternal protein restriction during gestation and lactation are attenuated by maternal moderate–low physical training

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Abstract

The effects of maternal moderate–low physical training on postnatal development, glucose homeostasis and leptin concentration in adult offspring subjected to a low-protein diet during the perinatal period were investigated. Male Wistar rats (aged 150 d old) were divided into four groups according to maternal group: untrained (NT_p, *n* 8); trained (T_p, *n* 8); untrained with a low-protein diet (NT + LP_p, *n* 8); trained with a low-protein diet (T + LP_p, *n* 8). The trained mothers were subjected to a protocol of moderate physical training over a period of 4 weeks (treadmill, 5 d/week, 60 min/d, at 65% VO_{2max}) before mating. At pregnancy, the intensity and duration of exercise was progressively reduced (50–20 min/d, at 65–30% VO_{2max}). The low-protein diet groups received an 8% casein diet, and their peers received a 17% casein diet during gestation and lactation. The pups' birth weight and somatic growth were recorded weekly up to the 150th day. Fasting blood glucose, cholesterol, serum leptin concentration, glucose and insulin tolerance tests were evaluated. The T_p animals showed no changes in somatic and biochemical parameters, while the NT + LP_p group showed a greater abdominal circumference, hyperglycaemia, hypercholesterolaemia, glucose intolerance and lower plasma leptin. In the T + LP_p animals, all of those alterations were reversed except for plasma leptin concentration. In conclusion, the effects of a perinatal low-protein diet on growth and development, glucose homeostasis and serum leptin concentration in the offspring were attenuated in pups from trained mothers.

Key words: Glucose homeostasis; Gestational exercise; Developmental plasticity; Perinatal undernutrition

The peri- and preconception periods are now thought to be critical for the long-term effects on fetal development and postnatal growth and may predispose offspring to phenotypic changes and metabolic diseases later in life⁽¹⁾. Unbalanced nutrient intake during this critical period of development has been associated with subsequent health risks and disease in the offspring, according to epidemiological data and numerous experimental observations⁽²⁾. This phenomenon has been termed 'developmental plasticity'⁽³⁾. Developmental plasticity is the property of a given genotype to produce

different phenotypes in response to distinct environmental conditions⁽⁴⁾.

The maternal low-protein diet model is one of the most extensively studied models of early growth restriction⁽⁵⁾. A low-protein diet (8% casein) during gestation followed by a normal diet throughout the life course has been associated with growth restriction, slightly elevated systolic blood pressure and increased fasting plasma insulin concentrations compared with control offspring⁽⁵⁾. If maternal protein restriction is continued during lactation, there is lasting growth

Abbreviations: BWG, body-weight gain; GTT, glucose tolerance test; ITT, insulin tolerance test; LP, low-protein rats; NT, untrained rats; NT_p, pups of untrained rats; NT + LP, untrained rats with a low-protein diet; NT + LP_p, pups of untrained rats with a low-protein diet; T, trained rats; T_p, pups of trained rats; TBS-T, mixture of Tris-buffered saline and Tween 20; T + LP, trained rats with a low-protein diet; T + LP_p, pups of trained rats with a low-protein diet.

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restriction, age-dependent loss of glucose tolerance, insulin resistance, hypertension and hyperleptinaemia, even when the offspring are weaned onto a control diet⁽⁵⁾. In adult rats (110 d) subjected to a low-protein diet (10% casein) during pregnancy and lactation, Zambrano *et al.*⁽⁶⁾ observed a lower serum leptin concentration. Leptin is a hormone mainly produced by adipocytes; however, it is also produced by several tissues, including skeletal muscle⁽⁷⁾. The presence of leptin in the muscle could be indicative of adipocyte infiltration, or leptin may have been produced directly by muscle fibres⁽⁸⁾.

Recently, we demonstrated that controlled moderate- to low-intensity physical training before and during gestation attenuated the impact of the low-protein diet by improving the mothers' resting oxygen consumption and the growth rate of the offspring⁽⁹⁾. In addition, physical exercise training has been associated with a reduced risk of metabolic disease and enhances both cardiorespiratory and metabolic functions. During gestation, the beneficial effects of physical exercise are dependent on the volume of exercise⁽⁹⁾. There are different physiological responses according to the type and frequency of exercise, the physical fitness of the mother, the time point in pregnancy when the exercise is carried out and the duration and intensity of exercise^(10,11). In 2002, the American College of Obstetricians and Gynecologists published exercise guidelines for pregnancy⁽¹²⁾. In these recommendations, it was suggested that after medical approval, 30 min or more of moderate exercise a day on most, if not all, days of the week is recommended for pregnant women. Exercise is considered moderate when oxygen consumption is approximately 50–70% of VO_{2max} . Regular practice of moderate exercise has been associated with improved cardiorespiratory fitness, increased metabolic rate (reduction in body weight) and increased muscle mitochondrial biogenesis⁽¹³⁾. At rest, the rate of placental red blood flow increases, and more glucose and oxygen delivery to the placental site are observed in women subjected to a physical training regimen⁽¹¹⁾. An epidemiological study found that moderate physical exercise during pregnancy is associated with a 100–150 g increase in birth weight⁽¹⁴⁾.

Little is known about the long-term effects of maternal physical activity on adult offspring subjected to perinatal undernutrition. This is a topic of particular interest as a maternal lifestyle can be considered a therapeutic means of countering the effects of either maternal undernutrition or overnutrition. Thus, in the present study, the effects of a maternal moderate–low protocol of physical training on postnatal development, glucose homeostasis and leptin concentration in adult offspring whose mothers were subjected to a low-protein diet during the perinatal period were investigated. Our hypothesis is that exercise-induced physiological adaptations during gestation, as seen in our previous studies, attenuate or modulate the impact of a perinatal low-protein diet on glucose homeostasis and leptin concentrations in adult offspring.

Materials and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Centre (protocol no. 80

23 076.049077/2010-80), Federal University of Pernambuco, Brazil and followed the Guidelines for the Care and Use of Laboratory Animals⁽¹⁵⁾.

Animals

Virgin female albino Wistar rats (*Rattus norvegicus*) aged 60 d were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil. Female rats were maintained at a room temperature of $23 \pm 2^\circ\text{C}$ with a controlled light–dark cycle (dark 09.00–21.00 hours). Standard laboratory chow (52% carbohydrate, 21% protein and 4% lipids; Agribands-Purina Limited) and water were given *ad libitum*. The animals were randomly divided into two groups: untrained rats (n 8) and trained rats (n 8). The trained rats were subjected to a training programme of moderate running over a period of 4 weeks (5 d/week and 60 min/d) on a treadmill (EP-131; Insight Equipments) at a controlled intensity based on their VO_{2max} ⁽⁹⁾. After the 4-week training period, the rats were mated (two females for one male). The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, day 0 of pregnancy. Pregnant rats were then transferred to individual cages. Half of the rats from each group received a 17% casein diet, and the other half received an 8% casein isoenergetic diet (low-protein group, LP)⁽¹⁶⁾ *ad libitum*. Thus, two more groups were formed, which are as follows: untrained (NT, n 4); trained (T, n 4); untrained with a low-protein diet (NT + LP, n 4); trained with a low-protein diet (T + LP, n 4). The exercise programme was maintained during gestation, with a progressive reduction in duration and intensity until the 19th day of gestation. There was no physical exercise during the suckling period. During lactation, the offspring were kept in litters of six pups, and their mothers continued to be fed with the 8% casein or 17% casein diet. Only male offspring from each litter were used. In all subsequent experiments, two male offspring were randomly chosen from each litter. The pups were divided into four groups according to their mothers' assigned group: untrained (NT_p, n 8); trained (T_p, n 8); untrained with a low-protein diet (NT + LP_p, n 8); trained with a low-protein diet (T + LP_p, n 8). At 150 d of age, pups were killed by cardiac exsanguination.

Physical training protocol

Physical training was performed according to Amorim *et al.*⁽⁹⁾. Briefly, rats ran on a treadmill during the 4 weeks (5 d/week, 60 min/d, at 65% VO_{2max}) before pregnancy. The protocol was divided into four progressive stages in each session: (1) warm-up (5 min); (2) intermediary (10 min); (3) training (30 min); (4) cool-down (5 min). The percentage of VO_{2max} during the sessions of training before gestation was kept at approximately 55–65%. During pregnancy, rats ran (5 d/week) at a progressively reduced duration and intensity of effort (first week 50 min/d (approximately 65% of VO_{2max}), second week 30 min/d (approximately 45% of VO_{2max}) and third week 20 min/d (approximately 32% of VO_{2max})). There was no physical training during the lactation period.



Measurement of food intake and body weight during gestation

During gestation, dams were housed individually, and their daily food consumption was determined by the difference between the amount of food provided at the onset of the light cycle and the amount of food remaining 24 h later⁽¹⁷⁾. Body and food weights were recorded to the nearest 0.01 g. Body weight was recorded daily throughout the experiment by a Marte Scale (AS-1000; Marte Cientifica) approaching 0.01 g. Percentage of weight gain (%BWG) was calculated by the formula:

$$\% \text{ BWG} = (\text{body weight (g)} \times 100 / \text{weight at the first day of gestation (g)}) - 100.$$

Blood glucose measurements

Fasting glycaemia levels were evaluated weekly during gestation using blood samples from the tail vein of rats, using a glucometer (Accu Check Advantage and Roche) and the glucose oxidase method. The animals were fasted overnight.

Postnatal developmental patterns of offspring

The body weights of pups were recorded weekly throughout the experiment with a Marte Scale (AS-1000) approaching 0.01 g. Percentage of weight gain (%BWG) was calculated at different intervals of time until 150 d old (birth to 30th, 31st to 90th and 91st to 150th) by the formula:

$$\% \text{ BWG} = (\text{body weight (g)} \times 100 / \text{weight at the first day in the interval (g)}) - 100.$$

The body lengths of pups were recorded by measuring the external surfaces (nose-to-anus length) using a digital calliper (0.01 mm accuracy). Body weight and body length were used to determine BMI (weight (g)/length (cm)²)⁽¹⁸⁾. Abdominal circumference was recorded by measuring the midpoint between the lower rib margin and the iliac crest in the horizontal plane⁽¹⁸⁾.

Glucose tolerance test and insulin tolerance test of offspring

The glucose tolerance test (GTT) was performed at 145 d and the insulin tolerance test (ITT) at 147 d of age. In both tests, animals were fasted overnight. Blood sample collections were performed by cutting the tip of the tail to remove approximately 10 µl of blood. The first blood sample was collected (time zero) before the injection of glucose. In the GTT, a 50% glucose solution (Equiplerx Pharmaceutical Limited) at a dose of 1 mg/g body weight was administered intraperitoneally. Blood samples were then collected at 15, 30, 45, 60 and 120 min after administration. The area under the glucose curve was obtained by blood glucose values at 0, 30, 60 and

120 min using the trapezoidal method⁽¹⁹⁾. In the ITT, a solution of insulin (Eli Lilly do Brazil Limited) at a dose of 0.75 mU (34.125 ng)/g body weight was administered intraperitoneally, and additional blood samples were collected at 15,

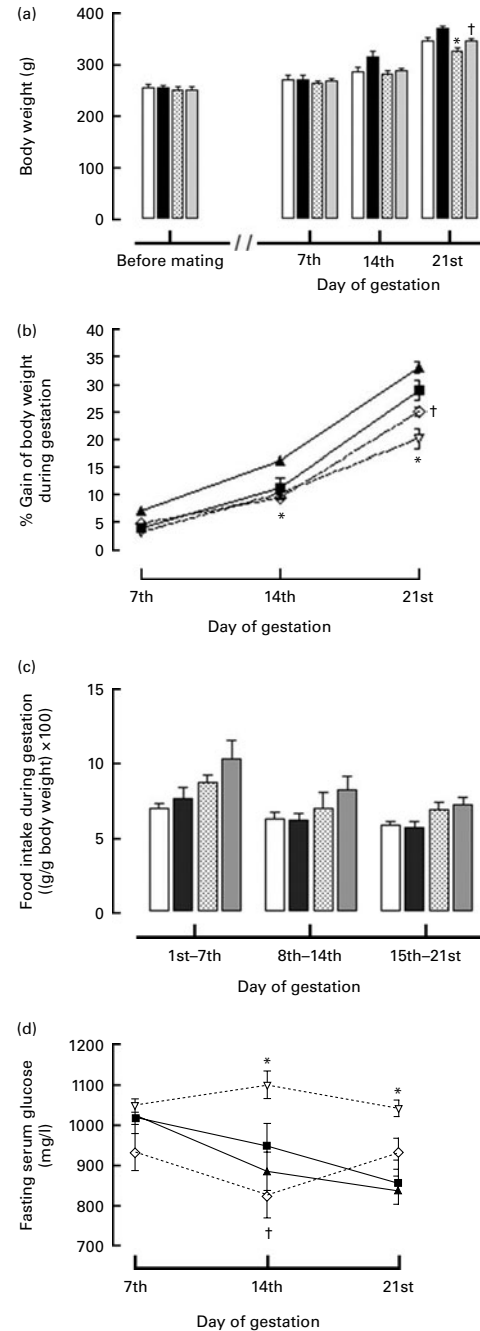


Fig. 1. (a) Body weight (g), (b) percentage of body-weight gain in each third week of gestation, relative to the body mass on the first day of pregnancy, (c) relative daily food intake expressed in g/d during gestation and (d) fasting blood glucose by non-trained (NT, n 4; (a, c) □ and (b, d) ■), trained (T, n 4; (a, c) ■ and (b, d) ▲), non-trained + low-protein (NT + LP, n 4; (a, c) ▨ and (b, d) ▩) and trained + low-protein (T + LP, n 4; (a, c) ▩ and (b, d) ◇) dams. Values are means with their standard errors represented by vertical bars. *Mean values were significantly different from the NT + LP group (P<0.05; two-way ANOVA). †Mean values were significantly different from the T + LP group (P<0.05; two-way ANOVA).

30, 45, 60 and 120 min. Glucose disappearance constant was calculated from the blood glucose values obtained at 0, 30 and 60 min (Kitt)⁽¹⁹⁾.

Resting blood glucose, cholesterol and leptin

At 150 d old, animals were anaesthetised with ketamine (0.25 ml/100 g body weight) and xylazine (0.25 ml/100 g body weight). Blood was sampled by cardiac puncture for the quantification of overnight fasting serum glucose, cholesterol and leptin concentrations. Glycaemia and cholesterolaemia were determined in blood samples using a glucometer and a cholesterol meter (Accu Check Advantage and Accutrend GCT; Roche Diagnostics Limited), respectively. Plasma leptin concentration was determined by a RIA kit (Linco Research, Inc.), with an assay sensitivity of 0.5 ng/ml and an intra-assay variation coefficient of 2.9%. The samples were analysed in one assay.

Western blotting for leptin content analysis

The soleus muscle (right) was weighed and homogenised in an ice-cold lysis buffer (50 mM-HEPES, 1 mM-MgCl₂, 10 mM-EDTA, Triton X-100 1%, pH 6.4) and protease inhibitor cocktail (1 mg/ml aprotinin, leupeptin and phenylmethylsulfonyl fluoride; Sigma-Aldrich, Inc.). Total protein content of the supernatants was determined by the bicinchoninic acid method (Protein Assay Kit; Thermo Fisher Scientific, Inc.).

Samples with 10 µg total protein were separated by SDS-PAGE (12%) and transferred to a nitrocellulose membrane (Hybond P; Amersham Pharmacia Biotech, Inc.). Membranes were blocked for 90 min with 5% non-fat dry milk in TBS-T (20 mM-Tris, 0.5 M-NaCl and 0.1% Tween 20). The membranes were then washed three times with T-TBS and incubated overnight with primary antibody anti-leptin (rabbit monoclonal – 1:1000; Sigma Chemical Company). They were then washed and incubated for 1 h with secondary antibody anti-rabbit (goat anti-rabbit conjugated with horseradish peroxidase 1:1000; Santa Cruz Biotechnology, Inc.). After the membranes had been washed three times with T-TBS, antibody binding was visualised using 3,3-diaminobenzidine tetrahydrochloride (10 mg in 15 ml Tris buffer, 0.1 M, pH 7.4). Images were scanned, and the bands were quantified by densitometry using Image J (Cibernetics Media, Inc.).

Statistical analysis

Results are presented as means with their standard errors. Intra-litter analyses were performed and found not to be significant. For statistical analysis, data were analysed by two-way repeated-measures ANOVA, with mothers' diet (NT and NT + LP) and physical training (T and T + LP) as factors. Pearson's correlation coefficient was used to correlate body-weight gain with the number of pups born per mother. Significance was set at *P* < 0.05. Data analysis was performed using the statistical program Graphpad Prism 5[®] (GraphPad Software, Inc.).

Table 1. Body weight, body length, BMI and abdominal circumference of the offspring at 30, 60, 90, 120 and 150 d old‡ (Mean values with their standard errors)

	Ages (d)	Groups							
		NT _p (n 8)		T _p (n 8)		NT + LP _p (n 8)		T + LP _p (n 8)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)	Birth	6.2	0.1	6.2	0.1	4.8*	0.08	5.1	0.06
	30	86.1	2.1	80.0	2.5	38.8*	2.1	48.6	2.5
	60	258.8	5.6	249.2	7.7	188.6*	3.4	232.9†	5.5
	90	351.4	8.8	342.5	7.3	284.9*	9.4	329.3†	11.7
	120	393.1	9.9	386.6	10.1	289.3*	5.3	372.4†	4.8
	150	421.8	8.6	449.3	3.5	343.2*	11.3	398.5†	1.5
% BWG	Birth–30	1388.7	102.1	1187.2	112.2	708.9*	41.9	852.2	51.1
	31–90	309.1	10.5	328.3	15.9	641.1*	19.9	494.5†	15.2
	91–150	20.9	3.7	30.3	4.4	20.5	4.4	21.0	4.4
Body length (cm)	30	14.7	0.2	14.1	0.12	12.3*	0.18	12.6	0.16
	60	20.3	0.2	19.7	0.23	18.3*	0.18	19.5†	0.22
	90	21.3	0.2	21.6	0.16	19.9*	0.21	21.8†	0.15
	120	23.2	0.3	22.8	0.28	21.1*	0.26	22.7†	0.15
	150	24.1	0.2	24.2	0.12	23.6*	0.14	23.9	0.21
	BMI (g/cm ²)	30	0.39	0.004	0.40	0.01	0.27*	0.01	0.31
60		0.63	0.01	0.67	0.01	0.56*	0.01	0.59	0.01
90		0.78	0.01	0.76	0.02	0.62*	0.02	0.74†	0.01
120		0.74	0.01	0.76	0.01	0.63*	0.01	0.70†	0.01
150		0.74	0.02	0.75	0.01	0.67*	0.01	0.70	0.01
Abdominal circumference (cm)		150	17.7	0.2	17.6	0.2	18.6*	0.5	17.5†

NT_p, pups of untrained rats; T_p, pups of trained rats; NT + LP_p, pups of untrained rats with a low-protein diet; T + LP_p, pups of trained rats with a low-protein diet; %BWG, percentage of body-weight gain.

* Mean values were significantly different from the NT_p group (*P* < 0.05; two-way ANOVA).

† Mean values were significantly different from the NT + LP_p group (*P* < 0.05; two-way ANOVA).

‡ During gestation, the dams were subjected to physical training and fed a low-protein diet. During lactation, the dams continued to receive a low-protein diet.

Results

Before mating, there was no difference between the groups in terms of body weight ($P > 0.05$). During gestation, moderate physical training attenuated the effects of a low-protein diet as reflected by the mother diet–training interaction for this analysis ($F_{6,36} = 4.757, P < 0.0001$). Actually, the effects of a low-protein diet on BWG during gestation were seen at the third week of gestation in the untrained dams (NT + LP), while in the T + LP dams, these effects were normalised (Fig. 1(a) and (b)). Data were adjusted for the number of pups born to each dam (NT, median 11.0 (minimum–maximum 9–13); T, median 11.5 (minimum–maximum 9–14); NT + LP, median 10.5 (minimum–maximum 8–11); T + LP, median 11.0 (minimum–maximum 9–12)). Pearson's correlation coefficient between the number of pups and the mother's BWG was not significant ($r^2 = 0.27, P = 0.452$). Relative daily food intake during gestation was not different between the groups ($F_{6,36} = 0.423, P = 0.85$; Fig. 1(c)). Fasting blood glucose only changed in the NT + LP mother group, which displayed greater values compared with the NT + LP group ($F_{6,36} = 2.316, P < 0.05$; Fig. 1(d)).

Pups from mothers subjected to physical training before and during gestation and/or to a low-protein diet during gestation were evaluated from birth to 150 d. Pups from mothers subjected to physical training showed a less pronounced reduction in body weight, body length and BMI especially in the interval between 60th and 120th day old, with a significant mother diet–physical training interaction ($F_{4,50} = 7.439, P < 0.001$; Table 1). In addition, at the 150th day of life, the NT + LP_p animals displayed a greater abdominal circumference than the NT_p animals that was attenuated in the T + LP pups (Table 1).

From 145–147 d old, pups were subjected to the GTT and ITT. There were no differences between the groups on the GTT and ITT curves (Fig. 2(a) and (c)); however, areas under the glycaemic curve were greater in the NT + LP_p group than in the NT_p group (Fig. 2(b)). The rate of disappearance of glucose was lower in the NT + LP_p animals (Fig. 2(d)). The effects of the maternal low-protein diet were attenuated in response to physical training (T + LP_p) (mother diet–physical training interaction: $F_{15,245} = 20.21, P < 0.001$).

At 150 d old, the NT + LP_p animals showed greater fasting glycaemia and cholesterolaemia when compared with the NT_p group. However, in the T + LP_p animals, glycaemia (NT_p: mean 91.1 (SEM 1.6); T_p: mean 98.0 (SEM 2.1); NT + LP_p: mean 108.4 (SEM 2.0); T + LP_p: mean 91.3 (SEM 3.8)) and cholesterolaemia (NT_p: mean 153.3.1 (SEM 0.6); T_p: mean 156.0 (SEM 1.4); NT + LP_p: mean 172.1 (SEM 1.3); T + LP_p: mean 164.1 (SEM 0.9)) were normalised ($F_{3,56} = 5.145, P = 0.003$; Fig. 3).

Leptin content in the soleus muscle was greater (+45%) in the NT + LP_p pups in comparison with the NT_p group. Conversely, leptin content was lower in pups from trained mothers (T + LP_p – 48%) in comparison with the NT + LP_p pups, with a significant mother diet–physical training interaction ($F_{3,58} = 3.967, P < 0.05$). Plasma leptin concentrations

were lower in the NT + LP_p animals when compared with the NT_p group, and physical training was not able to attenuate this effect (NT_p: mean 4.4 (SEM 1.3); NT + LP_p: mean 1.8 (SEM 0.1); T + LP_p: mean 1.3 (SEM 0.1); Fig. 4).

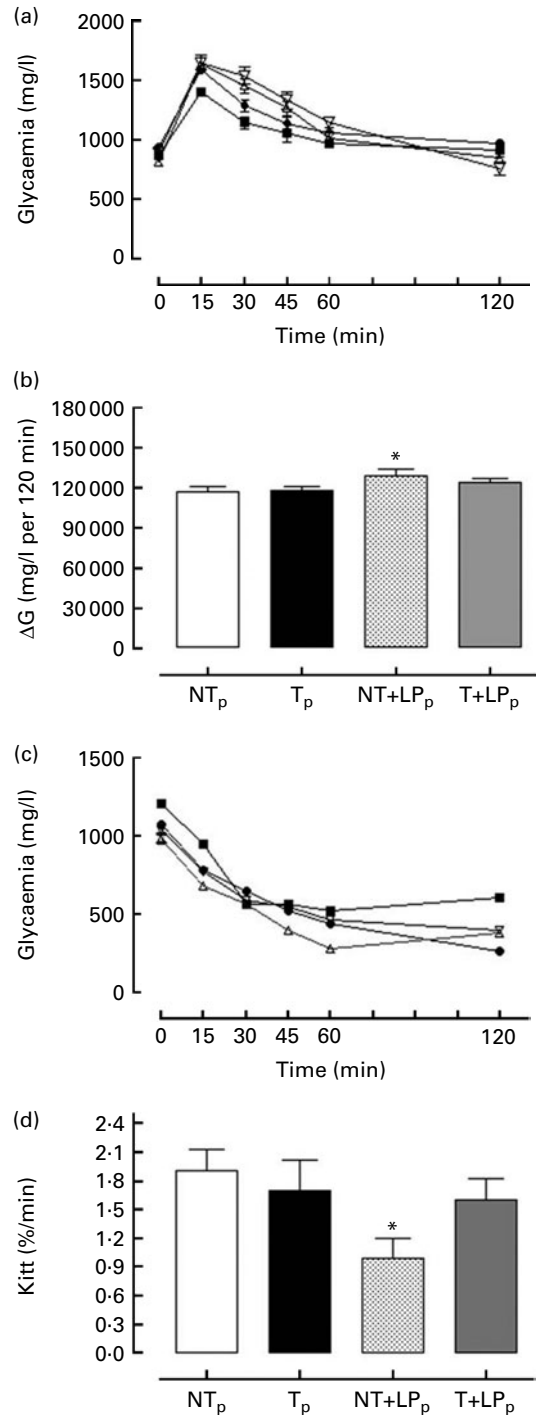


Fig. 2. (a) Glucose tolerance test, (b) areas under glycaemic curve, (c) insulin tolerance test and (d) rate of disappearance of glucose 'Kitt' of offspring at 145–147 d old from untrained (NT_p, n 8; (a, c) ●), trained (T_p, n 8; (a, c) ■), untrained + low-protein (NT + LP_p, n 8; (a, c) ▲) and trained + low-protein mothers (T + LP_p, n 8; (a, c) ▽). Values are means with their standard errors represented by vertical bars. * Mean values were significantly different from the NT_p group ($P < 0.05$; two-way ANOVA).

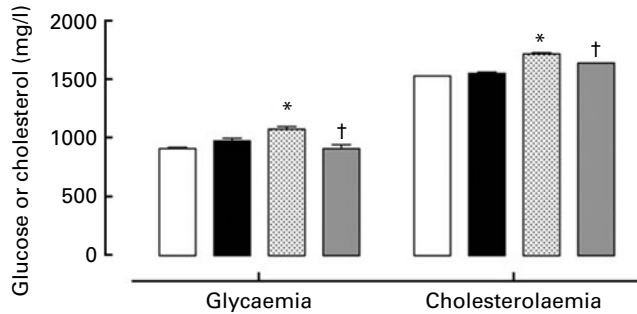


Fig. 3. Fasting blood glucose and cholesterol of the offspring at 150 d old from untrained (NT_p, *n* 8; □), trained (T_p, *n* 8; ■), untrained + low-protein (NT + LP_p, *n* 8; ▨) and trained + low-protein mothers (T + LP_p, *n* 8; ▩). Values are means with their standard errors represented by vertical bars. * Mean values were significantly different from the NT group ($P < 0.05$; two-way ANOVA). † Mean values were significantly different from the NT + LP group ($P < 0.05$; two-way ANOVA).

Discussion

An active maternal lifestyle, including regular physical activity and moderate physical training, improves aerobic fitness and the maternal–fetal physiological reserve and, thus, enhances nutrient and oxygen delivery to the fetus⁽¹¹⁾. In the mother, improved cardiovascular function, limited BWG and a reduced risk of gestational diabetes mellitus and gestational hypertension have been observed⁽²⁰⁾. For the fetus, reduced fat mass, high tolerance to stress and an advanced neuro-behavioural maturation have been observed⁽²⁰⁾. Our previous studies using animal models have shown that maternal physical training on a treadmill (5 d/week, progressive reduction in duration and intensity 50–20 min/d, 65–30% VO_{2max}) resulted in reduced BWG and high resting oxygen consumption (resting VO_2) and attenuated the delayed reflex ontogeny induced by undernutrition^(9,21). The present study has evaluated the long-term effects of a maternal moderate–low physical training protocol on BWG, glucose homeostasis and leptin concentration in adult offspring whose mothers were subjected to a low-protein diet during the perinatal period.

In the present study, although trained mothers demonstrated increased body-weight gain in the second and third weeks of gestation, the food intake did not differ, with the exception of the T + LP_p animals. The present results are in agreement with previous studies^(9,21). Because physical training can induce an increase in maternal lean body mass⁽¹³⁾, it may be inferred that body weight is increased by a mechanism that includes an increase in the synthesis of muscle proteins⁽⁹⁾. In dams, physical training was also able to normalise the effects of a low-protein diet on fasting serum glucose. This differential effect observed in previously active compared with undernourished dams may be related to reductions in the risk of gestational diabetes mellitus, as an exercise programme may improve insulin sensitivity and fasting plasma glucose concentrations of women at risk for gestational diabetes⁽²²⁾.

Low-protein diet offspring remained growth retarded throughout life and maintained a greater abdominal circumference, even when fed the control diet *ad libitum* from weaning to adult life. The present results are in agreement with

previous studies that have indicated that pups from undernourished mothers experienced a reduced postnatal growth trajectory^(9,23–25). Several hormonal changes, including greater leptin and T3 and lower serum insulin-like growth factor concentrations, are associated with malnutrition during gestation and lactation and can be possible imprinting factors in the growth and development programming of the progeny⁽²⁶⁾. Maternal physical training had beneficial effects on the postnatal growth rate in offspring from undernourished mothers. The underlying mechanisms of these effects may be related to metabolic changes, redistribution of blood flow and changes in the production of fetal and placental hormones that control growth⁽⁹⁾. The insulin-like growth factor and their associated binding proteins are thought to be an important mechanism underlying the long-term effects of maternal physical training⁽²⁷⁾. Treadmill exercise (20 m/min, 20 min/d, during 19 d) results in an increase in the plasma concentration of growth hormone, insulin-like growth factor I and insulin-like growth factor binding protein-3 in the late period of pregnancy⁽²⁷⁾. Nevertheless, these effects are directly dependent on the volume of effort (duration and intensity), and opposite

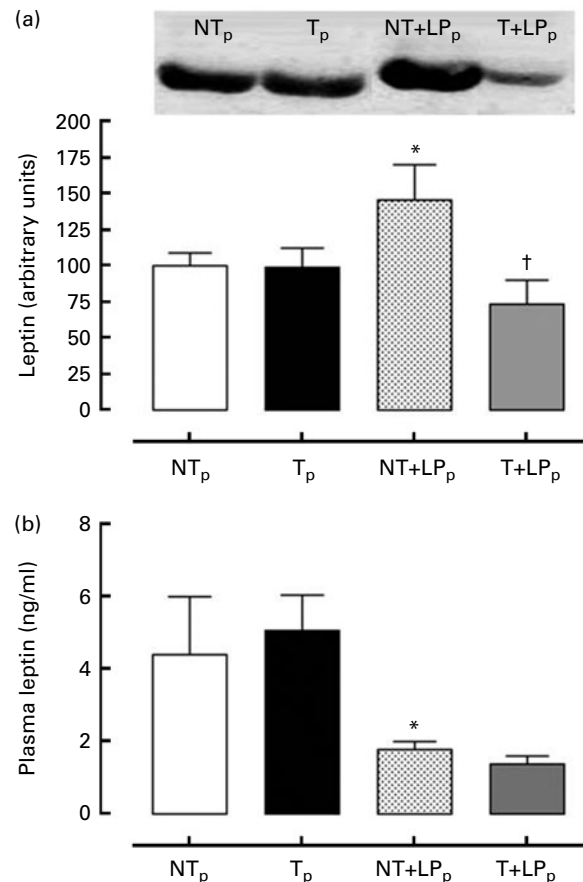


Fig. 4. (a) Leptin in the soleus muscle and (b) plasma leptin of the offspring at 150 d old from untrained (NT_p; *n* 8), trained (T_p; *n* 8), untrained + low-protein (NT + LP_p; *n* 8) and trained + low-protein mothers (T + LP_p; *n* 8). Values are means with their standard errors represented by vertical bars. * Mean values were significantly different from the NT group ($P < 0.05$; two-way ANOVA). † Mean values were significantly different from the NT + LP group ($P < 0.05$; two-way ANOVA).

effects are observed in high-intensity exercise during pregnancy⁽²⁸⁾. In the present study, the intensity and duration of each session of exercise was controlled in order to keep the effort approximately 65–30% of $\text{VO}_{2\text{max}}$. Thus, positive effects on offspring growth are observed when undernourished dams are subjected to moderate–low physical training.

In the present study, a perinatal low-protein diet induced greater glycaemia and cholesterolaemia, a greater area under the glucose curve and a reduced rate of disappearance of glucose than observed in their pairs. The present results confirm those presented in previous studies^(25,29). It is interesting to note that physical training attenuated the deleterious effects of perinatal undernutrition. These observations indicate that maternal physical exercise initiated in early pregnancy induces fetoplacental adaptations and can be considered as a therapeutic means of countering the effects of maternal undernutrition, which may provide a useful strategy for enhancing nutrient and oxygen availability to the fetus. The underlying mechanism can be related to epigenetic modulation induced by physical activity that regulates gene expression⁽³⁰⁾. For example, it has been found that physical exercise induces the DNA methylation of brain neurotrophic factors (BDNF-IV), increases the concentration of protein involved in DNA methylation and mRNA, and increases the acetylation of histones⁽³⁰⁾. In the case of these changes occurring during the critical period of fetal development, physical activity assumes an important role in the control of gene transcription in the context of the long-term effects of developmental plasticity. According to advances in the studies of the Developmental Origin of Health and Disease and epigenomic factors, physical activity during gestation opens new therapeutic possibilities for low-cost treatment for disorders associated with perinatal undernutrition.

In the present study, leptin concentrations were evaluated in the plasma and skeletal muscle. We have observed that protein restriction during gestation and lactation induced a reduced plasma leptin concentration at 150 d of age. In contrast, previous studies in which dams were subjected to undernutrition during lactation have indicated that adult offspring developed greater leptinaemia and central leptin resistance with increased total (39%) and visceral fat mass (2.3 times)^(31,32). Different periods early in life to induce undernutrition appear to be crucial in the long-term effects of plasma leptin concentrations. Maternal physical training was not able to normalise the effects of maternal low-protein diet. Thus, a maternal low-protein diet during gestation and lactation seems to affect the synthesis and metabolism of leptin, and also causes the effects on their receptors that persist long after the subsequent normalisation of nutrition⁽³³⁾.

Leptin is mainly produced by the adipocyte; however, it is also produced by several other tissues, including skeletal muscle⁽⁷⁾, where its expression may be different from that produced in the adipocyte. In the present study, the NT + LP_p animals experienced an increase in the expression of leptin in the skeletal muscle. Leptin in the muscle could be indicative of adipocyte infiltration or may be due to leptin produced directly by the muscle fibres⁽³⁴⁾. The dissociation of leptin serum levels and muscle leptin content in the NT + LP_p

group may be explained by the fact that leptin serum levels are associated with adipose tissue mass, and the muscle contribution to plasma leptin levels may not be important. The importance of leptin muscle content may be on the control of local glucose homeostasis, as it has already been shown that intramuscular fat is associated with insulin resistance⁽³⁴⁾. With training, this supposed adipocyte infiltration may decrease, thereby normalising leptin intramuscular production.

The primary aim of the present study was to test the hypothesis that moderate physical training before and during gestation attenuates the effects of perinatal low-protein undernutrition. Indeed, the effects of a perinatal low-protein diet on the development, glucose homeostasis and leptin concentrations in the skeletal muscle of the offspring were attenuated in pups from trained mothers.

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