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ORIGINAL CONTRIBUTION

Moderate physical training attenuates muscle-specific effects on fibre type composition in adult rats submitted to a perinatal maternal low-protein diet

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Abstract
Aim To verify whether moderate physical training affects the muscle fibre composition of adult rats subjected to a low protein diet during the perinatal period.
Methods Male Wistar rats were divided into two groups according to their mother's diet during gestation and lactation: control (17% casein, C) and low-protein (8% casein, LP). On postnatal day 60, half of each group was submitted to moderate physical training (8 weeks, 5 days/week^{-1}, 60 min/day^{-1}, at 70% of VO_{2max}, T) or not. After the physical training period, *soleus* and *extensor digitorum longus* (EDL) muscles were removed. Myofibrillar ATPase staining was used to classify muscle fibres as type I, IIa, IIb, and intermediate.
Results In the EDL muscle, LP rats showed no changes in the fibre type proportion. Both the C + T and LP + T groups showed a higher percentage of fibres of type IIa, and a lower proportion of fibres of type IIb. In the *soleus* muscle, LP animals showed a reduction in the proportion of fibre types I and intermediate. C + T rats showed an increase in the fibre type I and IIa. In the LP + T rats, the proportions of the fibre types remained similar to control rats.
Conclusions Moderate physical training acts as a positive environmental stimulus that reverts the effects of a perinatal low-protein diet on the proportion of fibre types in skeletal muscle.

Keywords Perinatal undernutrition · Physical exercise · Programming · Muscle type fibre · Rats

Introduction

In humans, epidemiological studies have shown that there is an association between perinatal poor nutrition and increased fat mass, abdominal fatness, and reduced lean mass (muscle mass) in adult life [1, 2]. This reduced muscle mass can be related to early undernutrition-induced impaired development of muscle fibres [3]. In rats, two classical studies showed an association between perinatal undernutrition and the number of muscle fibres in different muscles [4, 5]. In the study of Bedi et al. [4], it was verified that there is a reduction in the counting of *soleus* and *extensor digitorium longus* (EDL) fibres (19 and 21% reductions, respectively) in adult offspring that suffered undernutrition during gestation and lactation. Wilson et al. [5] showed that severe undernutrition (restricted to 30% of normal food intake) during gestation induced a 19–34% deficit in fibre numbers in the *soleus*, as determined by counting every fibre in a muscle cross-section.

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Because alterations in skeletal muscle morphology may also play a role in the pathogenesis of insulin resistance (the muscle fibre type is shifted toward faster, more glycolytic fibres) [6], several studies have been conducted to understand the mechanism by which early environmental insults can be related to long-lasting effects in adult life [3, 6, 7]. Pups at 90 days of age whose mothers were submitted to gestational undernutrition (7.8% casein) showed an increase in the proportion of type IIa fibres (19.7%) in soleus muscles and in the proportion of type IIb fibres (63%) in EDL muscles [6].

Recently, we demonstrated in rats that a maternal low-protein diet (8% casein) reduced the size and number of myoblasts in culture [8]. In addition, myotubes from undernourished 90 day-old offspring were less ramified compared to offspring from the controls [8]. In fact, peri- and preconceptional periods have been considered to be critical for producing long-term effects on foetal development and postnatal growth and may predispose offspring to phenotypic changes and metabolic diseases later in life [9]. These phenomena have been termed “programming” or “predictive adaptive responses” [9].

However, skeletal muscle is highly plastic, adapting to environmental challenges by regulating the composition of slow- and fast-twitch myofibers [10]. Interventions including endurance exercise can induce the *trans*-differentiation of myofibers [10]. Indeed, physical exercise is a well-known inducer of positive organic adaptations, but its beneficial effects depend directly on the intensity of effort [11]. The intensity of exercise is widely expressed as a percentage of an individual’s maximal oxygen consumption (VO_{2max}). VO_{2max} is the maximum capacity of an organism to transport and utilise oxygen during incremental exercise [11]. Exercise is considered moderate when oxygen consumption is approximately 50–70% of VO_{2max}, and it has been associated with improved cardiorespiratory fitness, increased metabolic rate (reduction of body weight), mitochondrial biogenesis, and improved peripheral responses to insulin [11, 12]. In rats, it has been shown that an endurance running program (30 m/min on a 7% grade for 2 h/day for 5 weeks) induces an increase in the relative concentration of types I, IIa and IIx isoforms and a reduction in the proportion of type IIb in EDL muscle [13]. In response to a treadmill running program of 6 weeks, there was an increase in type I fibre in the *soleus* and types I and IIa in the *plantaris* as well as a concomitant decrease in type IIa fibre in the *soleus* and IIb in the *plantaris* [14]. Similarly, a protocol of physical training (1.6 km/day; 10% incline, for ~1 h, 5 days/week, 10 weeks) showed an increase of 40% of the type I fibres and a decrease in the type IIb fibres of the rat *plantaris* [15].

Previous studies have published results on the long-term effects of maternal undernutrition in the ages limited to foetal, neonatal or relatively young offspring [5, 7]. The novelty of the present study is that undernourished (low-protein diet) offspring were studied up to 125 days of life either without any environmental stimulus (sedentary animals) or in response to moderate physical training. To test the hypothesis that physical training attenuates the effects of a perinatal low-protein diet on the proportion of fibre types in skeletal muscle, we examined the effects of moderate physical training on the muscle fibre composition of adult rats submitted to a low protein diet during gestation and lactation.

Materials and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Center (protocol no 008676/2007-48), the Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals [16].

Animals and diet

Male Wistar rats (*Rattus norvegicus*) were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil. The rats were maintained at a room temperature of 23 ± 1 °C and in a light–dark cycle (light 6:00 a.m.–6:00 p.m.). Ten virgin female rats (aged 110 days and weighing 258.8 ± 5.7 g) were mated with male breeders (2 × 1). The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, i.e., day 0 of pregnancy. Pregnant rats were then transferred to individual cages. Control rats ($n = 5$) received a 17% casein diet, while low-protein diet mothers ($n = 5$) received an 8% casein (low-protein group, LP) isocaloric diet ad libitum (Table 1).

At the time of delivery, the litter size and pups’ birth weights were recorded. On the first day after birth (24 h after delivery), litters were standardised to 6 pups, and during the suckling period, their mothers continued to be provided with a diet of either 8% casein or 17% casein. After weaning (on the 22nd day of age), only male offspring (two or three randomly chosen male pups from each mother) were used. Male pups were divided into two groups according to their mother’s manipulations (C, $n = 14$; pups from control mothers; and LP, $n = 15$; pups from mothers fed a low-protein diet). The offspring were housed in a collective cage and received animals’ standard laboratory chow (52% carbohydrate, 21% protein, and 4% lipids—Nuvilab CR1-Nuvital®) [17] ad libitum.

Table 1 Composition of the diets (control 17% and low-protein 8%)

Ingredients	Amount for 1 kg of diet	
	Low-protein	Control
Casein	79.3 g	179.3 g
Vitamin mix[a]	10 g	10 g
Mineral mixture[b]	35 g	35 g
Cellulose	50 g	50 g
Bitartarato of choline	2.5 g	2.5 g
DL-metionine	3.0 g	3.0 g
Soya oil	70 mL	70 mL
Corn starch	750.2 g	650.2 g
Energy (kcal/g)	3.89	3.89

[a] Mineral mixture contained the following (mg/kg of diet): $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_2, 120; $FeSO_4 \cdot 7H_2O$, 200; trace elements, 400 ($MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g [per kg of diet])

[b] Vitamin mixture contained the following (mg/kg of diet): retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-aminobenzoic acid, 100; biotin, 0.6

On the 60th day after birth, animals were divided into four groups according to their physical training: control (C, $n = 7$), low-protein diet (LP, $n = 8$), control and submitted to physical training (C + T, $n = 7$), and low-protein diet and submitted to physical training (LP + T, $n = 7$). Trained rats ran in a treadmill over a period of 8 weeks (5 days/week^{-1}, 60 min/day^{-1}, at 70% VO_{2max}) [11].

Protocol of physical training

A protocol of physical training was performed according to Leandro et al. [11]. Briefly, rats ran in a treadmill (EP-131®, Insight Equipments, SP, Brazil) for a period of 8 weeks (5 days.week^{-1}, 60 min.day^{-1}). The protocol was divided into four progressive stages for each session of exercise: (1) warm-up (5 min, 0.3–0.5 km/h, at 25% VO_{2max}); (2) intermediary (10 min, 0.5–0.9 km/h, at 45% VO_{2max}); (3) training (30 min, 0.9–1.1 km/h, at 65–70% VO_{2max}); and (4) cool-down (5 min, 0.5 km/h, at 35% VO_{2max}) periods. Because the percentage of VO_{2max} during the sessions of training was maintained at approximately 65–70%, the exercise was classified as aerobic with a moderate intensity of effort [11]. In response to this protocol of physical training, our previous study has reported an increase of 42% in citrate synthase activity, an important metabolic marker for assessing the oxidative and respiratory capacity of skeletal muscle [11]. The non-trained group remained in their cages. The animals were not submitted to any type of reinforcement during exercise.

Offspring body weight and growth rate

The body weight of pups was recorded daily throughout the experiment with a Marte Scale, AS-1000, approaching 0.01 g. Percentage weight gain = [body weight (g) × 100/weight at first day of life (g)] − 100 [7]. The growth rate was calculated by the number of grams of body weight gained per day [7]. During the protocol of physical training, the percentage of body weight gain was calculated by body weight (g) × 100/weight at 60th day of life (g) − 100.

Muscle preparation

Forty-eight hours after the last session of physical exercise, animals were anesthetised *v.ip.* with urethane (12.5 mL/100 g body weight) and chloralose (0.5 mL/100 g body weight) [Sigma Chemical Co. (St. Louis, USA)]. Animals were sacrificed by an overdose of anaesthetics. The *soleus* and EDL muscles were dissected and immersed in n-hexane (C_6H_{14}) at a low temperature and were frozen in liquid nitrogen.

Histochemical analysis

Serial cross sections (10 μm) were cut with a cryostat (Jung Frigocut CM1100-Leica; Leica Microsystems Gmbh, Wetzlar, Germany) maintained at −20 °C and stained for myofibrillar ATPase [18]. The sections were left at room temperature and were incubated for 10 min in 400 mL pre-incubation solution (containing 280 mL of 0.2 M acetic acid and 120 mL of 0.2 M sodium acetate) in pH 4.3 or 4.55. The sections were then washed in distilled water and incubated at 37 °C in a solution containing ATP (pH 9.4) for 40 min. Next, sections were washed in distilled water, rinsed for 5 min in a 2% $CoCl_2$ solution and rinsed four times in distilled water. For revelation, we used 5% ammonium sulphate. The sections were then dehydrated in crescent ethanol baths (70°–100°) and immersed in toluene solution. After drying, the sections were mounted using a New Entellan® (Merck, Germany) [18].

The sections were analysed with a light microscope (Olympus Optical U-CMAD-2, Tokyo, Japan; 10× objective lens) connected to a computer (TV TUNER APPLICATION—TelSignal Company Limited, Taiwan, image capture software). The images of the histological cross-sections of the soleus and the EDL were captured for further analysis. Muscle fibres were labelled with respect to the four major types (I, IIa, IIb, intermediate) of fibres on the basis of differences in the staining intensity for ATPase after acid pre-incubation (pH 4.3 and 4.55) [19]. According to the different staining intensities, the following classification was used for EDL: pH 4.3 (type I, darkest and type II, lightest) and pH 4.55 (type I, darkest; type IIa, lightest and type IIb, grey). The classification of fibre type in soleus

Table 2 Mother's gain of body weight and food intake during gestation, and growth rate (grams per day) of the offspring whose mothers were submitted to either a control (C) or a low-protein diet (LP) during perinatal period

	C ($n = 5$)		LP ($n = 5$)		p
	Mean	SEM	Mean	SEM	
Mother's gain of body weight (g)					
Week 1 of gestation	4.06	1.10	2.70	0.9	0.113
Week 2 of gestation	11.23	0.98	8.97	0.8	0.370
Week 3 of gestation	29.76	1.01	20.9	0.7*	0.000
Food intake during gestation (g)	18.1	0.8	20.8	0.4	0.486
Number of pups per litter	11.2	1.6	9.3	2.0	0.479
Litter's birth weight (g)	66.2	5.8	49.3	4.4*	0.048
Pup's birth weight (g)	6.6	0.03	5.1	0.3*	0.007
Pup's growth rate (g/day)	C ($n = 14$)		LP ($n = 15$)		p
	Mean	SEM	Mean	SEM	
Lactation (0–21st day)	1.76	0.09	0.76	0.03*	0.001
22nd–30th day	3.96	0.06	2.42	0.03*	0.000
31st–60th day	6.06	0.1	4.96	0.1*	0.000

Pups were evaluated at different intervals during development. The values are presented as mean ± SEM

* $p < 0.05$. *t* test student

was: pH 4.3 (type I, darkest and type II, lightest) and pH 4.55 (type I, darkest; type IIa, lightest and Intermediate, grey) [19]. Histochemical analysis was performed using computerised image analysis from Mesurim PRO 3.2 software (developed by Jean-François Madre-Amiens, France). The muscle fibre type composition was determined by counting approximately 1,500 fibres in fields that were equally distributed over the sample.

Statistical analysis

Results are presented as the mean ± the standard error of the mean. Comparisons between the control and the low-protein groups were performed using Student's *t* test. Pearson's correlation coefficient was used to correlate the number of pups that were born and the mother's body weight gain during gestation. For statistical analysis, intra-litter analyses were performed and found not to be significant. Data were analysed by two-way repeated measure ANOVA, with the mother's diet (C, LP) and physical training (T, T + LP) as factors. Bonferroni's post hoc test was used. Significance was set at $p < 0.05$. Data analysis was performed using the statistical program Graphpad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA).

Results

A gain in body weight was lower in the last third of the gestational period in the mothers that were subjected to a low-protein diet (Table 2). Data were adjusted for the number of pups born for each dam [C = 12.0 (8–12) and LP = 9.0 (7–11); the values were expressed as the median (minimal and maximal)], and the correlation coefficient between the number of pups and the mother's body weight gain was not significant ($r^2 = 0.41$, $p = 0.112$). Daily food intake during gestation was not different between the groups. The litters and the pups' birth weights were lower in the LP pups than in the C pups. In addition, the growth rate of the pups from weaning up to the 60th days was lower in LP pups than that in control pups (Table 2).

At 60 days old, half of the number of pups in each group was submitted to a protocol of moderate physical training for 8 weeks. All of the groups showed an increase in body weight gain throughout the weeks, and LP pups remained with a lower body weight than C pups (Fig. 1a). However, in terms of the percentage of body weight gain, LP pups showed higher values when compared to controls (C = 30.6% and LP = 45.2%, $p = 0.021$). Moderate physical training attenuated the higher body weight gain in LP pups (C + T = 27%; LP + T = 30%, $p = 0.009$) (Fig. 1). During the protocol with moderate physical training, starting from the fifth week on, C + T showed a reduction in the gain of body weight. Similarly, LP + T animals showed a lower gain of body weight starting from the third week on (Fig. 1b).

As expected, there were more type I (oxidative) than type II (non-oxidative) fibres in the soleus muscles and more type II than type I fibres in the EDL muscles (Fig. 2a, b). There were no diet effects on the numbers of type II fibres in EDL muscle (Fig. 2a). However, the total number of the

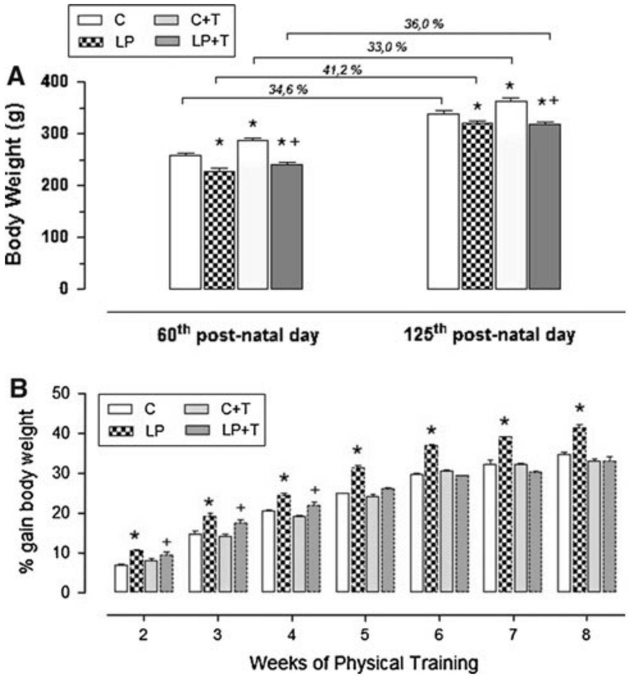

Fig. 1 Body weight of the offspring (**a**) and the percentage of gain of body weight (**b**) during the protocol of moderate physical training. Control (C. $n = 7$); low-protein diet (LP. $n = 8$); control and submitted to training (C + T. $n = 7$) and low-protein diet and submitted to training (LP + T. $n = 7$). The measurements were performed weekly up to the 8th week of physical training. Data are presented as means + S.E.M. $^*p < 0.05$ versus C. $^+p < 0.05$ versus LP by using two-way ANOVA and Bonferroni's post hoc test

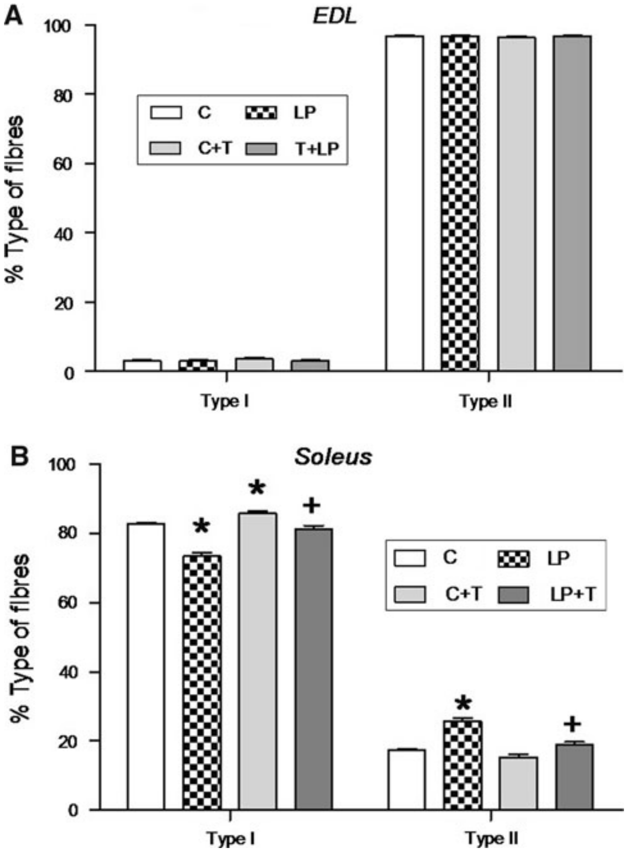

Fig. 2 The percentage of fibre type I and type II of EDL muscles (**a**) and soleus muscles (**b**) after a protocol of moderate physical training. Control (C. $n = 7$); perinatal low-protein diet (LP. $n = 8$); control and submitted to training (C + T. $n = 7$); and perinatal low-protein diet and submitted to training (LP + T. $n = 7$). The measurements were performed 48 h after the last section of physical training. Data are presented as means + S.E.M. $^*p < 0.05$ versus C. $^+ p < 0.05$ versus LP by using two-way ANOVA and Bonferroni's post hoc test

different fibre types within the soleus muscle was affected by the maternal dietary treatment: type I (C = 82.5 ± 0.4; LP = 78.2 ± 0.9, $p < 0.001$); and type II (C = 17.4 ± 0.4; LP = 21.7 ± 0.9, $p = 0.001$). The effects of a maternal low-protein diet on the different fibre types within the soleus muscle were attenuated in animals submitted to a moderate physical training when compared to LP (LP + T; type II = 18.8 ± 0.9, and type I = 81.1 ± 0.9, $p < 0.001$) (Fig. 2b).

At pH 4.55, it was possible to evaluate the types of fibres (purely oxidative, type I; purely glycolytic type IIb; oxidative-glycolytic type IIa and intermediate type) from soleus and EDL muscles. A perinatal low-protein diet did not affect the percentage of fibres in EDL muscle. Trained animals showed a higher percentage of fibre type IIa (C + T = 68.8 ± 0.7; C = 59.5 ± 1.1; $p < 0.001$) and a lower percentage of fibre type IIb when compared to C (C + T = 32.2 ± 0.6; C = 36.7 ± 1.1; $p < 0.001$) (Fig. 3a).

In soleus muscle, a maternal low-protein diet induced a reduction in the percentage of fibre type I (LP = 77.8 ± 0.5; C = 83.1 ± 0.5; $p < 0.001$) and intermediate (LP = 2.1 ± 0.1; C = 3.9 ± 0.2; $p < 0.001$). In addition, there was an increase in the percentage of fibre type IIa (LP = 19.0 ± 0.4; C = 12.9 ± 0.4; $p = 0.001$) (Fig. 3b). In response to physical training, there was an increase in fibre type I (C + T = 84.7 ± 0.2) and IIa (C + T = 14.8 ± 0.2). In LP + T animals, the percentage of fibres of type I was higher (LP + T = 80.3 ± 0.7; $p < 0.001$) and there was a reduction in the percentage of fibres of type IIa (LP + T = 17.2 ± 0.7; $p < 0.001$) when compared to LP animals (Fig. 3b).

Discussion

Maternal protein restriction is associated with lower stores of maternal nutrients, less transfer of nutrients to the offspring, and consequently reduced postnatal growth [20]. Our data showed a reduction in the gain of body weight during the last third of gestation. It has been found previously that an undernutrition-induced reduction of maternal

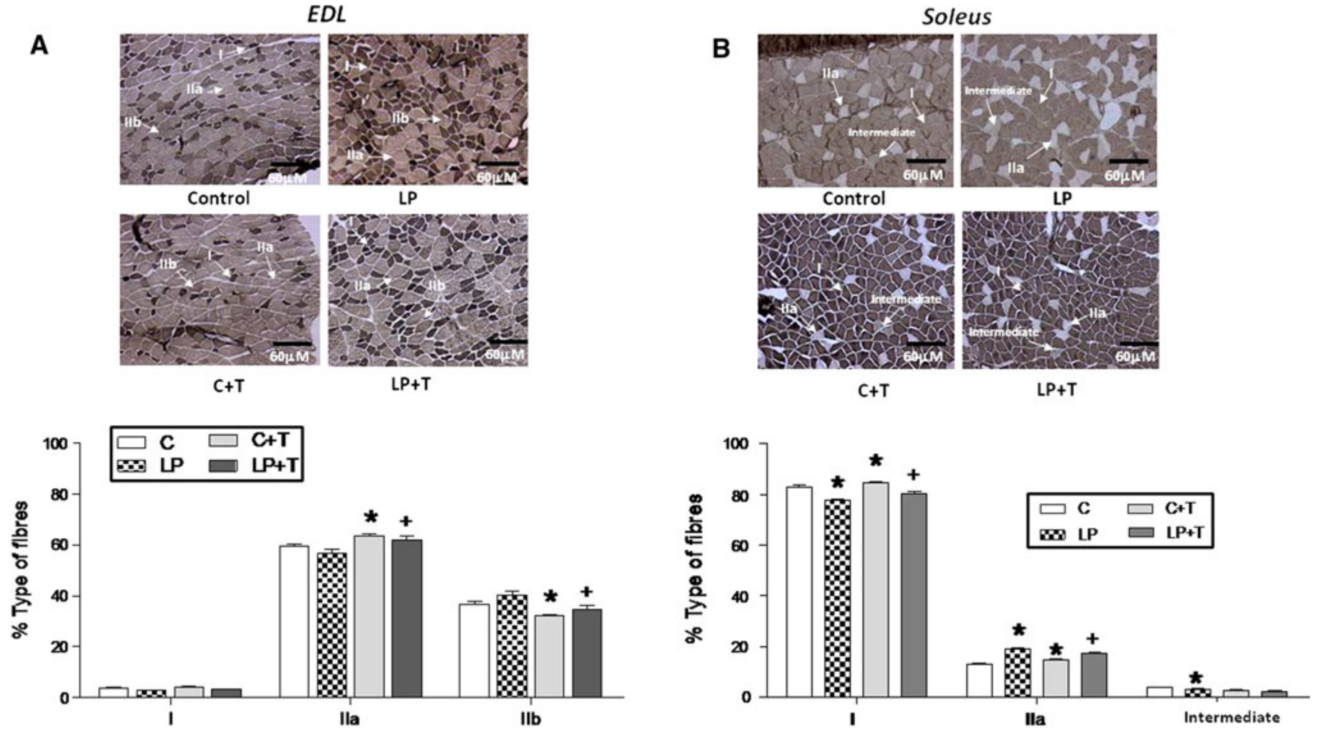

Fig. 3 Images of the ATPase-stained cross-sections of EDL (**a**) and soleus (**b**) muscles of 125-day-old rats. Muscle fibres classification on the basis of differences in staining intensity for ATPase was used for EDL: pH 4.3 (type I, *darkest* and type II, *lightest*) and pH 4.55 (type I, *darkest*; type IIa, *lightest* and type IIb, *grey*). The classification for fibre type in soleus was: pH 4.3 (type I, *darkest* and type II, *lightest*) and pH 4.55 (type I, *darkest*; type IIa, *lightest* and Intermediate, *grey*). Four experimental groups: Control (C); submitted to a perinatal low-protein diet (LP); submitted to a protocol of moderate physical training (C + T); and low-protein diet and trained (LP + T). *Graphics* indicate the percentage of fibres of type I, type IIa, type IIb and intermediate of the EDL muscle (**a**) and the soleus muscle (**b**) after the protocol of moderate physical training: control (C. $n = 7$); perinatal low-protein diet (LP. $n = 8$); control and submitted to training (C + T. $n = 7$); and perinatal low-protein diet and submitted to training (LP + T. $n = 7$). The measurements were performed 48 h after the last section of physical training. Data are presented as means + S.E.M. $^{*}p < 0.05$ versus C. $^{+}p < 0.05$ versus LP by using two-way ANOVA and Bonferroni's post hoc test

gain of body weight is positively correlated with a low birth weight, an impaired offspring growth rate and a loss of lean mass during development [21]. Indeed, pups from LP mothers showed a reduced body weight and a reduced growth rate. However, there was a catch-up during post-natal growth, as observed by the 45.2% of body weight gain in offspring following the perinatal growth restraint. Our results are in accordance with previous studies [22]. It has been reported that perinatal undernutrition affects the development of adipocytes by modulating adipogenesis and lipogenesis in adult life [23]. In rats, perinatal undernutrition (8% protein restriction) resulted in increased body weight that was linked to elevated adipose tissue weight and adipocyte hypertrophy [24]. In addition, epidemiological evidence has shown an association of perinatal undernutrition with early origins of both obesity and cardiovascular disease [2, 25].

In trained animals, a gain in body weight was lower in comparison to the pair's non-trained matching counterpart. It is well established that regular physical exercise increases lean body mass and induces a higher utilisation of fatty acids as fuel by skeletal muscles because the intensity of the exercise is maintained at 65–70% of VO_{2max} [12]. In rats, moderate physical training (treadmill for 2 h day^{-1}, 5 days week^{-1}, during 10 weeks) reduced fatty acid synthase activity, the rate-limiting enzyme for hepatic lipogenesis, and the accumulation of body fat [26]. In humans, resistance training and aerobic exercise (12 weeks, 65–85% maximum heart rate) enhanced lean muscle mass and induced physiological benefits in the management of obesity in male adolescents, including the prevention of body weight gain [27]. Our findings demonstrated that moderate physical training plays an important role as a positive environmental stimulus to revert the effects of perinatal undernutrition on the excessive gain of body weight. Thus, moderate physical training can be a possible strategy to attenuate long-term effects, to reduce the maternal environmental insult associated with obesity and cardiovascular disease.

Before birth, muscle fibres do not show any differentiation due the co-expression of slow (type I) and fast (type II) isoforms in all fibres in rats [28]. Previous studies have pointed out that the differentiation of muscle fibres into subtypes occurs during postnatal development [5, 29]. The suckling period was identified as the time of occurrence of subtypes; for example, at weaning, the soleus muscle presents at least 70–80% of fibre type I, 27–15% of fibre type IIa and 2.5–4.0% of intermediate fibre type. EDL presents 6.0% of fibre type I, 68–56% of fibre type IIa, and 25.5–40% of fibre type IIb [28]. The profile of the proportion of fibre types remains constant from weaning to adult animals, as seen in the present study and in previous studies [6, 28, 29].

Our results showed that perinatal LP diet shifted fibre types towards reduced slow fibres and elevated fast fibre characteristics. This result is in accordance with previous studies, which used the same experimental model to induce malnutrition (protein restriction) either during the gestational and suckling periods [4] or during only the gestational period [6]. Previous studies have demonstrated that different muscles may respond differently to perinatal insults [5, 21]. In fact, slow fibre is more sensitive to perinatal malnutrition once insulin-sensitive tissues undergo the long-lasting effects of perinatal protein restriction [30]. In addition, the relationship between protein synthesis and protein degradation in soleus muscle can accelerate muscle function loss and can initiate insulin resistance [6]. In contrast, by using another model of malnutrition (restricting 50% or less of the ad libitum control diet), it has been reported that there is an increase in the proportion of type I fibres and a reduction in the proportion of type II fibres in young offspring, due to a reduction in the number of fast fibres formed [5, 21]. The different protocols that induce malnutrition can justify the contrasting results. Moreover, our results indicate that perinatal LP diet programs the proportions of skeletal muscle fibre types. In addition, a shift in fibre distribution, from type I to type II, alters the activity of key oxidative and glycolytic enzymes and the ratio between glycolytic and oxidative enzyme activities in skeletal muscle [31]. All of these effects are strictly associated with type 2 diabetes, obesity and insulin resistance [30].

An evident feature of skeletal muscle is the ability to alter its phenotypic profile in response to specific stimuli. For moderate intensity training (60–70% of VO_{2max}), the increase in type I and IIa fibres usually contributes to an increase in oxidative metabolism and mitochondrial biogenesis [11, 12]. In the present study, trained animals showed an increase in the proportion of type IIa fibres in both soleus and EDL muscles. Similarly, previous studies have shown that endurance exercise training induces fibre type transitions characterised by increases in type I and IIa fibres and corresponding decreases in the fastest type IIb fibres in rats [32, 33]. A 13-weeks endurance running protocol on activity wheels increased the proportion of fibres that expressed the myosin heavy chain (MHC) IIa in the *plantaris* muscle [33]. In humans, three times a week for 12 weeks of strength training, endurance training or combined training induced an increase in the myosin heavy chain (MHC) type IIa content and was related to fast-to-slow fibre-type transitions [34]. These alterations can be explained by the adaptations that occur in the Ca^{+2} regulatory system [35], in the efficiency of protein related to metabolism, and in the proportions of fast motor units to slow motor units following a program of physical training [36].

The classical physical exercise-induced response in skeletal muscle is an initial protein breakdown, followed by an increase in muscle protein synthesis during recovery and following feeding [37]. In response to a program of moderate physical training, the alterations that were seen in pups from LP mothers were attenuated. Herein, we propose two mechanisms that include the role of the insulin-like growth factor I (IGF-I) and the stimulation of the nerve. IGF-1 is known to promote both muscle-cell proliferation and differentiation [7]. Early foetal malnutrition (40% of restriction ad libitum) followed by 3 weeks of postnatal nutritional rehabilitation resulted in a reduced local muscle IGF-1 mRNA [7]. The second mechanism includes the innervations of muscle that can influence fibre switching. Our previous study demonstrated that rats submitted to early malnutrition present long-term alterations in reflex latency and nervous conduction velocity [38]. However, one well-established effect of moderate physical training is the improved activation (stimulation of the nerve) of the muscle. Type IIa fibres are known be more sensible to hypertrophy in response to a program of physical training [37]. Thus, the present study showed for the first time that moderate physical training attenuates skeletal muscle muscle-specific effects on the fibre type composition of adult rats. Thus, it can be suggested that perinatal programming can be modulated by positive environmental stimuli in adult life, such as moderate physical training. Additional experiments on studying the underlying mechanisms that include oxidative and glycolytic enzyme activity, reactive oxygen species production, AMP-activated protein kinase, and mitochondrial biogenesis would be worthwhile to perform.

Conclusions

The present study showed that perinatal low protein diet affects a gain in body weight during growth and affects the proportion of muscle fibre in adult rats. In contrast, moderate physical training acts as a positive environmental stimulus that reverts the effects of perinatal undernutrition on the excessive gain of body weight and that attenuates the effects of a perinatal low-protein diet on the proportion of fibre types in skeletal muscle.

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Conflict of interest The authors declare that there are no conflicts of interest.

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