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ORIGINAL ARTICLE

Mild energy restriction and physical swimming activity: biochemical effects and food preference in male rats

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Abstract

Background Caloric restriction at the beginning of life has been associated with the development of chronic diseases in adulthood. However, physical exercise can be advocated as a non-invasive intervention to minimize adverse effects.

Methods Female Wistar rats were fed either a normocaloric or hypocaloric diet from the third week of gestation to the end of lactation. The offspring were also submitted to either a normocaloric or hypocaloric diet and were allocated to groups with or without physical exercise. Thus, six groups were formed: normocaloric–normocaloric-inactive—NNI ($n=9$), normocaloric–normocaloric-active—NNA ($n=7$), hypocaloric–normocaloric-inactive—HNI ($n=8$), hypocaloric–normocaloric-active—NHA ($n=9$), hypocaloric–hypocaloric-inactive—HHI ($n=6$) and hypocaloric–hypocaloric-active—HHA ($n=6$). Body weight, food consumption and preference, biochemical variables, visceral fat and organ weight were evaluated.

Results Perinatal energy restriction led to lower body weight during the lactation period, but with recovery in all groups after weaning. No difference in food intake was found among the groups, but the food preference test revealed that the continual energy restriction and physical activity were associated with a preference for carbohydrates. Continuous energy restriction exerted a harmful effect on biochemical variables such as glucose, LDL-c and total cholesterol. Lipid recovery serum, however, was observed in the HNA group.

Conclusion Metabolic changes were more pronounced in animals submitted to a continual hypocaloric diet, but physical activity proved to be beneficial with regard to some of the analyzed variables.

Keywords Caloric restriction · Metabolism · Exercise · Diet · Rats

Introduction

Gestation, the lactation period, and infancy are critical periods for development and early environmental stimuli that can have an impact in adulthood [1, 2]. During ontogenesis, the development of each organ passes through a critical period of plasticity when environmental factors can lead to changes in the phenotype that remain throughout the remainder of one's life [1].

Phenotypic plasticity is associated with an adaptation that occurs in the organism in response to an altered environment [1–3], for example, nutritional adaptation in order to increase the odds of survival by protecting vital organs such as the brain, in detriment to peripheral organs [1]. This plasticity is consequently believed to be associated with a changed postnatal energy metabolism.

Adjustments in the energy metabolism emerge in an attempt to increase the efficiency of the organism regarding

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energy use and storage to maintain the body in a deficient environment, thereby benefiting survival if the postnatal environment remains deficient [1]. In an adequate or abundant nutritional environment, these adaptations are believed to increase the risk of disease development in adulthood, such as type 2 diabetes mellitus and cardiovascular disease [1–3]. But, if the nutritional environment is maintained, the offspring will have less risk of developing disease [4, 5].

The hypothesis of the predictive adaptive response is based on the theory that developing organisms receive information regarding the external environment and make "predictions" about future environmental conditions [4, 5]. If the prediction is correct, the phenotype is normal, but if a difference occurs between the predicted and actual environment, disease develops. Therefore, the adaptive hypothesis suggests that maintenance of the maternal diet by the offspring after weaning could attenuate the risk of future metabolic disorders [5].

Physical activity has been studied as an environmental stimulus that is beneficial to release neurotrophic factors and growth hormones, favoring neural plasticity, growth, and development [6, 7]. The effects of exercise on growth when combined with nutritional recovery have been documented in a previous study [8]. In 2013, Muniz et al. found an association between physical activity and the acceleration of somatic growth, neuromotor development and changes in biochemical variables [6].

The present study tested the hypothesis that continuing the maternal diet after weaning, with a mild caloric restriction, could be beneficial to the organism, reducing the risk of physio-metabolic disorders. Further, that submission to physical activity for an organism previously exposed to caloric restriction would reduce the adverse consequences. Thus, the aim of the present study was to evaluate how the change or continuity in the maternal diet combined or not with physical exercise; in this case swimming affects growth, food preference and metabolic changes in young offspring.

Materials and methods

This study received approval from the Animal Experimentation Ethics Committee of the Center for Biological Science of the *Universidade Federal de Pernambuco* (UFPE) (certificate no.: 23076.028444-2012-73) and followed the Guidelines for the Care and Use of Laboratory Animals [9].

Forty non-sibling, nulliparous female Wistar rats (*Rattus norvegicus*) and 20 males obtained from the Department of Nutrition University Federal of Pernambuco, Recife, PE, Brazil were used in this study. The animals were kept at a temperature of 22 ± 1 °C, 60% relative humidity with a controlled inverted 12 h dark cycle (8.00–20.00 h) and 12 h light cycle (20:00–8:00 h). Female virgins were mated with males (female to male proportion of 2:1) of the same strain and pregnancy was confirmed by visualizing sperm in the vaginal secretion and monitoring the increase in the body weight of the females. Water and experimental diet (control or low energy diet) were given *ad libitum* throughout the experimental period. After delivery, offspring were maintained in litters of eight pups (male to female proportion of 4:4 or 3:5). At weaning (21d old), only male offspring were used in the present study and the females were used in another study.

The control diet and low energy diet were casein based and adapted from AIN-93 [10]. Half of the female rats received the normocaloric diet and half received the hypocaloric diet beginning in the third week of gestation through to the end of the weaning period. After weaning, six groups were formed based on post-weaning diet (commercial diet or maintenance of hypocaloric diet) and degree of physical activity: normocaloric–normocaloric-inactive (NNI, $n = 9$), normocaloric–normocaloric-active (NNA, $n = 7$), hypocaloric–normocaloric-inactive (HNI, $n = 8$), hypocaloric–normocaloric-active (HNA, $n = 9$), hypocaloric–hypoocaloric-inactive (HHI, $n = 6$) and hypocaloric–hypocaloric-active (HHA, $n = 6$).

The control AIN-93 diet contained 18% protein, 63% carbohydrates and 19% lipids, providing 3.6 kcal/g. The post-weaning commercial diet contained 23% protein, 63% carbohydrates and 11% lipids, providing 3.6 kcal/g. The adapted low energy diet contained 18% protein, 64% carbohydrates and 18% lipids, providing 2.4 kcal/g. Further details on the hypocaloric diet can be found in Muniz et al. [6]. Figure 1 displays the distribution among the different groups.

The physical activity model was designed to replicate a recreational activity that can be introduced in early childhood and is based on the protocol described by Muniz et al. [6]. Beginning with the post-weaning period (Day 21), the animals exercised between 7:30 and 8:00 in the morning, five times a week, followed by a 2-day rest period. In the first week, there was an adaptation protocol; the animals were submitted to 10 min of swimming on the first day, with a 5-min increase on subsequent days until reaching 30 min on the fifth day. The animals then continued to swim 30 min a day until completing 62 ± 2 days of life.

The animals swam in a cylindrical recipient measuring 50.5 cm in diameter and 50.00 cm in depth, equipped with a thermostat to control the water temperature, which was maintained at 31 ± 2 °C. The water was changed daily to control aquatic stress and environmental manipulation. The control animals were placed in the same environment in polyethylene cages with water (sufficient amount for the paws and part of the body to become wet) without performing the swimming activity.

Body weight was measured between 7:00 and 7:30 am throughout the experimental period using a digital scale

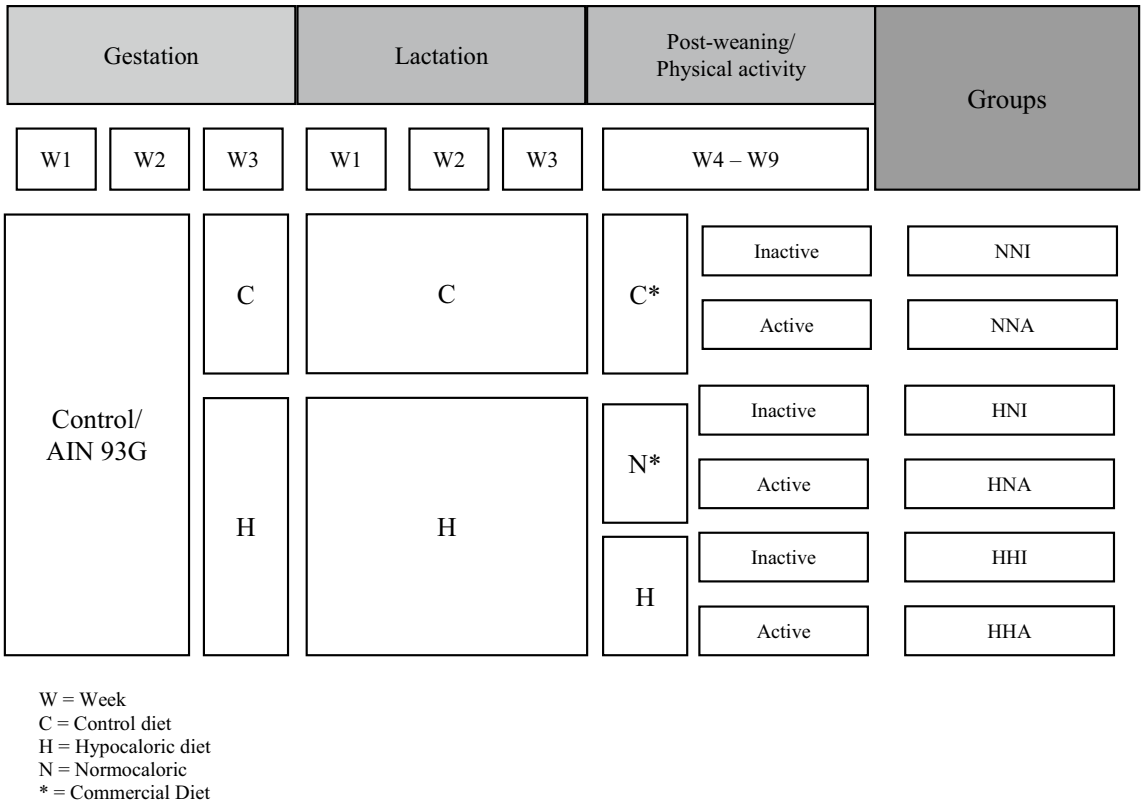

Fig 1 Distribution of experimental groups according to diet offered per period of life and level of physical activity

(Marte XL 500, class II) with a maximum capacity of 500 g and precision of 0.001 g. Weight was measured every three days from birth to weaning (21st day of life). After weaning, weight was measured once a week until the end of the study. Weight gain (WG) was determined using the following formula: WG% = [current weight (g) × 100/weight on first day (g)] − 100.

Food intake was evaluated weekly in the female rats (pregnant and nursing) and offspring [from weaning to the end of the experiment (Day 63)]. Rations and water were offered *ad libitum* and consumption was determined by subtracting the amount rejected from the amount offered. Mean consumption of the offspring was calculated per cage ($n = 2$ pups).

The acute food preference test (macronutrients) was performed in the final week of the experiment (±60 days of life) over three consecutive days. Three types of diets with similar ingredients to AIN-93 [10] and similar energy value were offered. The diets were classified as hyperproteic (34% protein, 53% carbohydrates, 13% lipids and 3.3 kcal/g), hiperlipidic (12% protein, 50% carbohydrates, 38% lipids and 3.9 kcal/g) and hyperglucidic (14% protein, 75% carbohydrates, 11% lipids and 3.4 kcal/g).

At 63 ± 2 days of life, after 10 to 12 h of fasting, the animals were anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg) and euthanized. A heart puncture was performed for blood collection. The abdominal, retroperitoneal and gonadal fat was removed after a single incision through the thoracic and abdominal regions and then weighed.

The blood samples were centrifuged at 1048 g for 20 min. The serum was transferred to a cryogenic tube and stored in a freezer at − 20 °C for subsequent biochemical analyses: glucose, total cholesterol, high-density lipoprotein fraction (HDL-c), low-density lipoprotein fraction (LDL-c), triglycerides, urea, creatinine, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The analyses were performed using reagent kits (BIOSYSTEMS) with the aid of automatic equipment (A15 Clinical Chemistry Analyzer, Biosystems®, Spain). The Friedewald equation [11] was employed to estimate the plasma level of LDL-c and very low-density lipoprotein fraction of cholesterol (VLDL-c).

The Shapiro–Wilk test was used to determine the distribution (normal or non-normal) of the data. One-way analysis of variance (ANOVA) was used for multiple comparisons among groups. Two-way or two-way repeated-measures ANOVA was used to measure interactions between two independent factors. The Bonferroni post hoc test was used when differences were found among groups. The data were

expressed as mean and standard error of the mean. GraphPad Prism 5.0 (version 2007) was used for all statistical analyses, with the level of significance set to 5% ($p < 0.05$).

Results

For the purposes of comparing the statistical tests, this study only compared groups with variables in common (HHI × HNI × NNI; HHA × HNA × NNA; NNA × NNI; HNA × HNI).

Figure 2 illustrates the weight progression (a), weight gain in the perinatal period (b), post-weaning weight (c) and post-weaning weight gain (d). The hypocaloric maternal diet did not affect fetal growth, as demonstrated by the lack of significant differences among groups with regard to birth weight (NNI: 6.42 ± 0.68 g; NNA: 6.19 ± 0.33 g; HNI: 6.69 ± 0.95 g; HNA: 6.21 ± 0.60 g; HHI: 7.15 ± 0.52 g; HHA: 6.79 ± 0.51 g). However, beginning at the 12th day of life, the offspring with the restricted diet had lower body weight (7% lower) than their normocaloric counterparts, with a 25% lower weight gain at the end of weaning (NNI: 51.8 ± 4.9 g; NNA: 50.9 ± 3.3 g; HNI: 40.1 ± 3.5 g; HNA: 37.1 ± 4.8 g; HHI: 37.8 ± 10.2 g; HHA: 36.7 ± 9.9 g; $p < 0.000$) (Fig. 2a). This lower weight was confirmed by the low perinatal weight gain among the animals from mothers fed the hypocaloric diet (NNI: 704.9 ± 26.9%; NNA: 712.2 ± 22.6%; HNI: 481.9 ± 23.1%; HNA: 490.3 ± 26.5%; HHI: 428.6 ± 19.2%; HHA: 439.2 ± 8.9%; $p < 0.000$) (Fig. 2b).

After weaning, the hypocaloric groups exhibited complete weight recovery, with no significant difference among

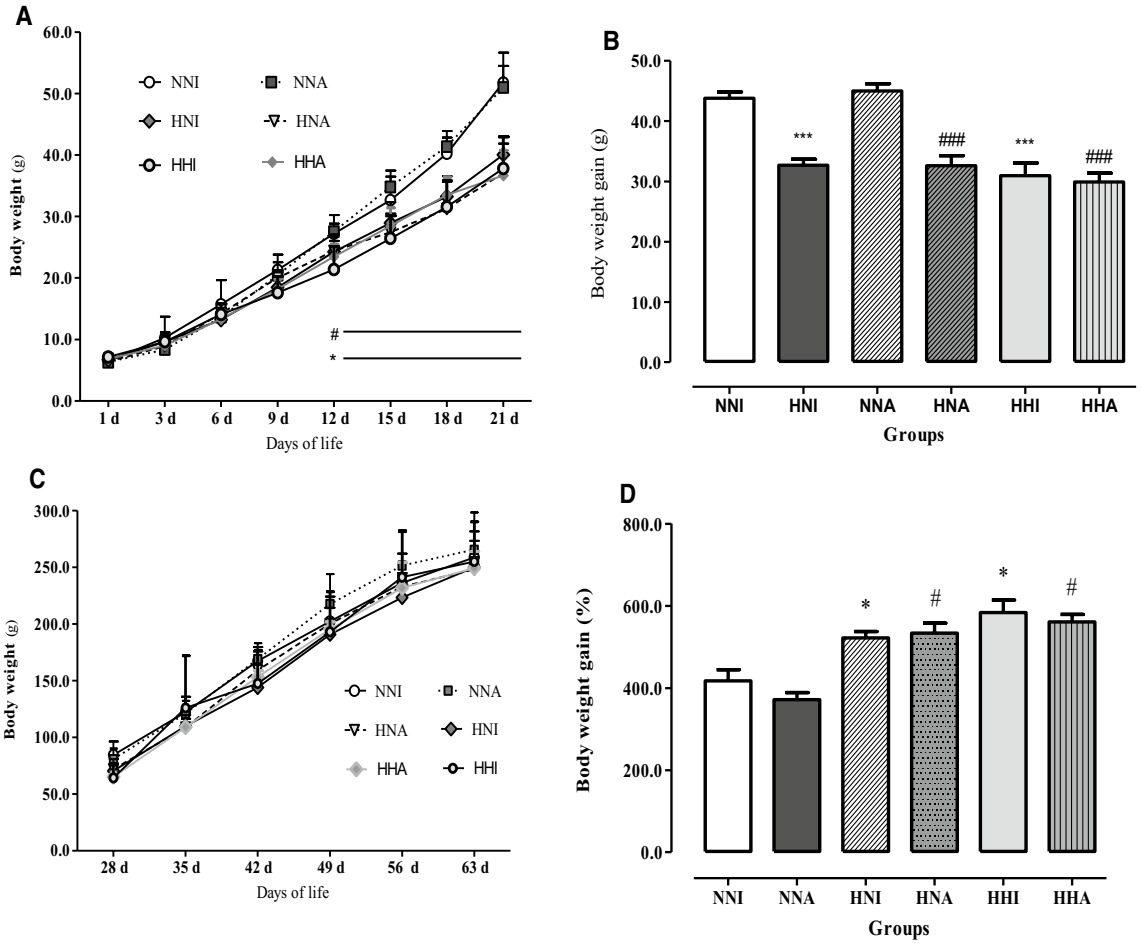

Fig. 2 Weight progression and body weight gain during perinatal period and after weaning of pups submitted to different dietary and swimming physical activity. [Body weight; Weight gain; Days of life; Groups]. Weight progression in perinatal period (**a**) and after weaning (**c**). Weight gain in perinatal period (**b**) and after weaning (**d**). Data expressed as mean and standard error. One-way ANOVA (**b, d**) and two-way repeated-measures ANOVA (**a, c**) followed by Bonferroni test (**a–d**). NNI: normocaloric–normocaloric-inactive ($n = 9$); NNA: normocaloric–normocaloric-active ($n = 7$); HNI: hypocaloric–normocaloric-inactive ($n = 8$); HNA: hypocaloric–normocaloric-active ($n = 9$); HHI: hypocaloric–hypocaloric-inactive ($n = 6$); HHA: hypocaloric–hypocaloric-active; ($n = 6$); ($p < 0.05$); *vs NNI; #vs NNA

the groups at the end of the period (NNI: 258 ± 22.9 g; NNA: 265.4 ± 32.8 g; HNI: 250.0 ± 8.8 g; HNA: 248.0 ± 25.5 g; HHI: 255.8 ± 35.2 g; HHA: 250.8 ± 15.8 g; $p = 0.65$) (Fig. 2c). Considering the percentage of weight gain, the hypocaloric groups had greater weight gain than the control animals, independent of physical activity, with similar body weights at 61–63 days of age (Fig. 2d). The mean absolute food intake (g) did not significantly differ among the groups (Fig. 3).

The preference for the hyperproteic diet varies with the period of dietary intervention and physical activity. In turn the preference for the hyperglycogen diet was not significant between the groups; and in relation to the hyperlipidic diet shows that its preference was reduced in the inactive continuous hypercaloric and in the active normocaloric (Fig. 4).

Figure 5 shows intragroup macronutrient feeding preference. In these results, the evaluation occurred among the animals of each experimental group. It was evidenced that the variable physical activity increased the consumption of glucose diets in the continuous hypercaloric groups (Fig. 5e, f). Among the inactive individuals, both the control group (NNI) (Fig. 5a) and the recovered group (HNI) (Fig. 5c) presented higher consumption of lipid diets, whereas the active groups with caloric restriction (HNA) (Fig. 5d) or not (NNA)(Fig. 5b), showed a distinct profile having a higher appreciation for glucose diet in relation to other diets.

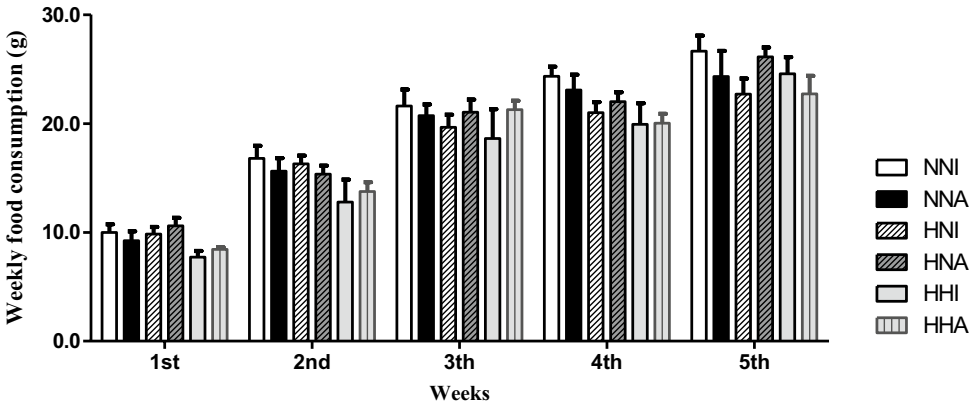

Fig. 3 Weekly food intake (g) of after weaning at 60 days old. [Mean weekly intake; Weeks; 1st, 2nd, 3rd, 4th, 5th]. Two-way repeated-measures ANOVA; NNI: normocaloric–normocaloric-inactive ($n = 9$); NNA: normocaloric–normocaloric-active ($n = 7$); HNI: hypocaloric–normocaloric-inactive ($n = 8$); HNA: hypocaloric–normocaloric-active ($n = 9$); HHI: hypocaloric–hypocaloric-inactive ($n = 6$); HHA: hypocaloric–hypocaloric-active; ($n = 6$); ($p < 0.05$)

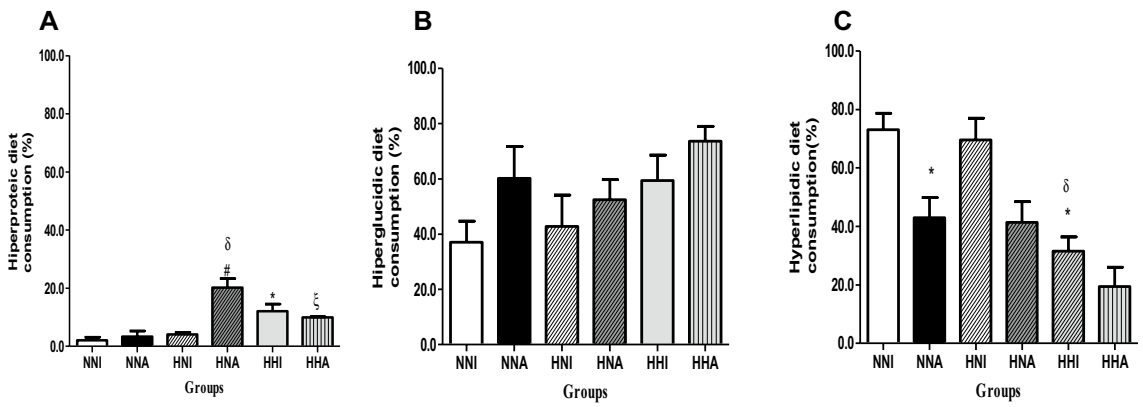

Fig. 4 Consumption (%) of diets used in food preference test. Percentage of hyperproteic diet consumption; Groups; Percentage hyperglicidic diet consumption; Groups; Hyperlipidic diet consumption; Groups]. (**a**) Hyperproteic; (**b**) Hyperglicidic; (**c**) Hyperlipidic. One-way ANOVA followed by Bonferroni post hoc test; NNI: normocaloric–normocaloric-inactive; NNA: normocaloric–normocaloric-active; NNI: normocaloric–normocaloric-inactive ($n = 9$); NNA: normocaloric–normocaloric-active ($n = 7$); HNI: hypocaloric–normocaloric-inactive ($n = 8$); HNA: hypocaloric–normocaloric-active ($n = 9$); HHI: hypocaloric–hypocaloric-inactive ($n = 6$); HHA: hypocaloric–hypocaloric-active; ($n = 6$); ($p < 0.05$); * *vs* NNI; $^{\#}$ *vs* NNA; $^{\delta}$ *vs* HNI; $^{\xi}$ *vs* HHI

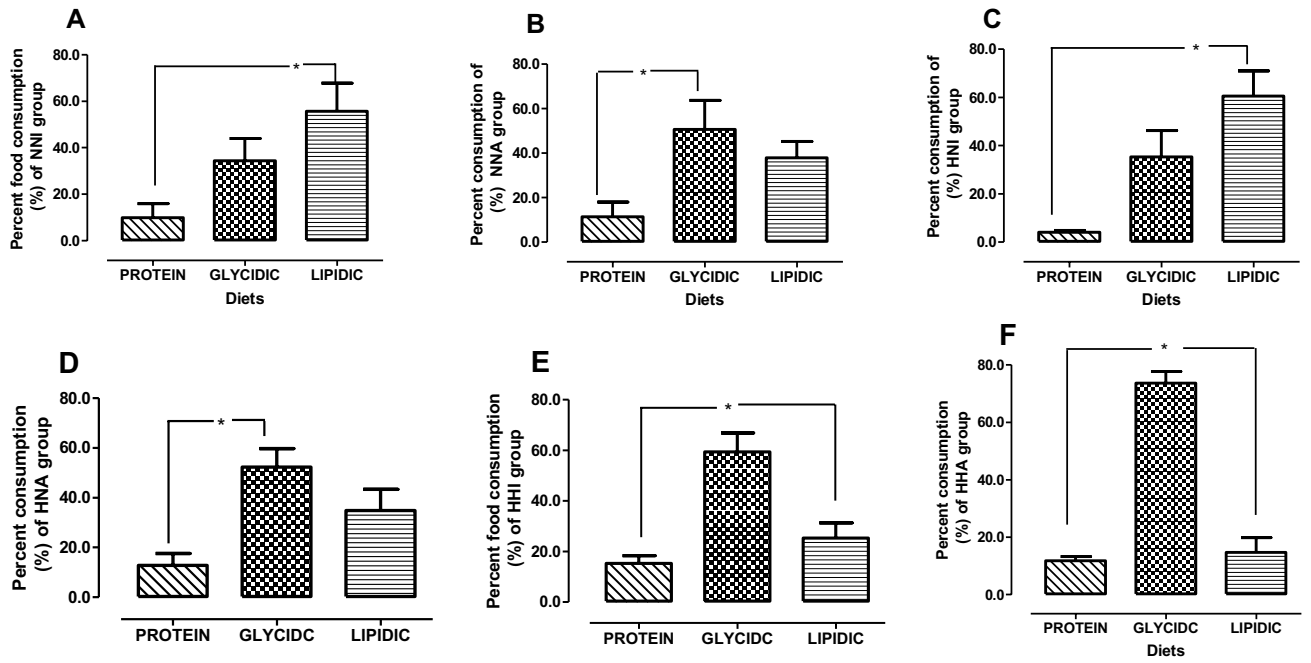

Fig. 5 Macronutrient preference intragroup of each group submitted to different diets and physical activity (swimming). **a** Percentage (%) of consumption in NNI group among the diets: Proteic; Glucidic; Lipidic; **b** Percentage (%) of consumption in NNA group; **c** Percentage (%) of consumption in HNI group among the diets: Proteic; Glucidic; Lipidic; **d** Percentage (%) of consumption in HNA among the diets: Proteic; Glucidic; Lipidic; **e** Percentage (%) of consumption in HHI among the diets: Proteic; Glucidic; Lipidic; **f** Percentage (%) of consumption in HHA group; diets; Proteic; Glucidic; Lipidic. **a** NNI; **b** NNA, **c** HNI, **d** HNA, **e** HHI, **f** HHA. One-way ANOVA followed by Bonferroni post hoc test; NNI: normocaloric–normocaloric-inactive ($n=9$); NNA: normocaloric–normocaloric-active ($n=7$); HNI: hypocaloric–normocaloric-inactive ($n=8$); HNA: hypocaloric–normocaloric-active ($n=9$); HHI: hypocaloric–hypocaloric-inactive ($n=6$); HHA: hypocaloric–hypocaloric-active; ($n=6$); ($p<0.05$)

Blood glucose levels were higher in the animals for which the hypocaloric diet was maintained in the post-weaning period in comparison to the control group and recovered groups (HNI and HNA), with no influence from the practice of physical activity. Triglyceride and VLDL-c levels were higher in the HNI and HNA groups compared to the control, but with an evident beneficial influence from performing physical activity.

Maintenance of the hypocaloric diet throughout the experimental period had a harmful effect on total cholesterol and LDL-c levels. Physical activity did not influence these variables. An increase in the concentration of HDL-c, especially in the active group (HHA), was a positive effect found in the groups maintained on the hypocaloric diet (Table 1).

Metabolites related to protein metabolism, such as creatinine, urea and uric acid, were increased in the groups maintained on the hypocaloric diet in comparison to their counterparts, independent of physical activity. Moreover, the HHA only differed from the group that received the hypocaloric diet during the perinatal period. Urea values also differed between the HHI and HNI groups (Table 1).

With regard to abdominal fat, the HNA was the only group to demonstrate a positive effect from physical activity, exhibiting less fat in this region compared to their counterparts (NNA and HHA) and inactive control (HNI). No significant effects were found in the other groups. The same results were found for retroperitoneal fat. No change was found regarding gonadal fat (Table 1).

Discussion

Perinatal caloric restriction (30%) did not affect birth weight, but led to lower body weight in the offspring after weaning. The lack of a difference in birth weight may be related to the low degree of aggression of caloric restriction on the nutritional status of the mother. Maternal nutrition prior to and during pregnancy is one of the most important variables to the prognosis of fetal growth and development [12]. Caloric restriction beginning at the third gestational week enabled the maternal reserves accumulated up to the second week to be mobilized for fetal growth. Muniz et al. report similar results using the resembling experimental model [6]. Alheiros et al. report similar results using the same dietary model offered either only during the lactation period or during pregnancy and lactation [13].

Caloric restriction of this study poses a 30% (2.4 cal/g) deficit of the calories recommended by the AIN-93G

Table 1 Effect of different dietetic treatment and physical activity (swimming) on biochemical variables and abdominal fat in young adult rats

GROUPS	Normocaloric Mean ± standard error		Recovered hypocaloric Mean ± standard error		Continual hypocaloric Mean ± standard error	
	NNI	NNA	HNI	HNA	HHI	HHA
Biochemical variables						
Glucose (mmol/L)	7.06 ± 0.15	7.53 ± 0.23	8.89 ± 0.69	7.83 ± 0.26	15.7 ± 1.6[a,c]	17.38 ± 1.55[b,e]
Triglycerides (mg/dL)	55.84 ± 5.37	73.56 ± 4.48	141.50 ± 8.46[a]	66.04 ± 5.70 [c]	73.30 ± 5.15[c]	81.14 ± 7.21
Total cholesterol (mg/dL)	43.11 ± 2.33	62.23 ± 1.56	71.42 ± 7.26	61.23 ± 2.84	86.24 ± 8.27[a]	119.3 ± 13.78[b,d,e]
HDL-c (mg/dL)	20.45 ± 3.67	34.15 ± 2.92	25.28 ± 3.35	34.04 ± 1.96	37.77 ± 3.17[a]	55.71 ± 4.94[b,d,e]
LDL-c (mg/dL)	38.55 ± 2.33	47.23 ± 4.37	33.38 ± 6.34	17.15 ± 3.31[b]	61.06 ± 8.17[a]	78.34 ± 3.79[b,e]
VLDL-c (mg/dL)	11.17 ± 1.07	14.70 ± 0.89	28.30 ± 1.69[a]	13.21 ± 1.14[c]	14.76 ± 1.01[c]	16.29 ± 1.37
AST (UI/L)	249.50 ± 14.31	294.60 ± 9.90	205.80 ± 17.20	265.60 ± 12.94	266.90 ± 30.07	230.70 ± 32.41
ALT (UI/L)	42.47 ± 3.49	59.40 ± 1.73	66.14 ± 7.79	78.17 ± 15.00	51.98 ± 5.94	59.93 ± 7.33
Creatinine (mg/dL)	0.55 ± 0.03	0.42 ± 0.01	0.42 ± 0.05	0.46 ± 0.01	0.77 ± 0.03[c]	0.86 ± 0.06[b,e]
Uric acid (mg/dL)	1.97 ± 0.17	1.90 ± 0.08	1.29 ± 0.17	2.20 ± 0.31	2.51 ± 0.54[a,c]	3.75 ± 0.57[b]
Urea (mg/dL)	29.58 ± 1.54	31.88 ± 0.86	34.25 ± 1.46	33.22 ± 1.64	44.16 ± 2.56[a]	46.83 ± 4.09[b,e]
Abdominal fat (g%)						
Retroperitoneal fat	1.46 ± 0.14	1.80 ± 0.14	1.49 ± 0.13	0.92 ± 0.05[b]	1.39 ± 0.15	1.78 ± 0.32[e]
Gonadal fat	1.17 ± 0.09	1.10 ± 0.08	0.98 ± 0.06	0.93 ± 0.08	1.25 ± 0.11	1.12 ± 0.13
Total abdominal fat	2.63 ± 0.17	2.90 ± 0.18	2.43 ± 0.18	1.80 ± 0.16[b]	2.54 ± 0.26	2.96 ± 0.43[e]

One-way ANOVA followed by Bonferroni post hoc test

NNI normocaloric–normocaloric-inactive ($n=9$), *NNA* normocaloric–normocaloric-active ($n=7$), *HNI* hypocaloric–normocaloric-inactive ($n=8$), *HNA* hypocaloric–normocaloric-active ($n=9$), *HHI* hypocaloric–hypocaloric-inactive ($n=6$), *HHA* hypocaloric–hypocaloric-active; ($n=6$); ($p<0.05$)

[a]vs NNI

[b]vs NNA

[c]vs HNI

[d]vs HHI

[e]vs HNA

(3.6 cal/g), without changing the macronutrient proportions. However, models of decreasing the amount of feed offered (30–50% decrease in the amount of food consumed by the animal) promote malnutrition, but also leads to greater stress in the animal. Hypoproteic diets (6–8% of proteins) restrict the amount of protein, but maintain adequate caloric content (3.6 cal/g); however, there are studies that show that maize reduces food intake during lactation by promoting an energy-protein malnutrition. Both models are already consolidated in the literature as promoting malnutrition.

In rats, a lactation peak occurs between 12 and 14 days following birth, with greater mobilization of maternal nutritional reserves for maintaining the high energy demand [12]. Beginning from the 12th day of life, the animals in the hypocaloric groups demonstrated a reduction in body weight, which was maintained until weaning. It is possible that the caloric restriction imposed at the end of gestation may have affected the composition/quantity of maternal milk, thereby reducing the growth rate of the offspring, as reported by Pine et al. [12].

After weaning, accelerated weight gain was found in the hypocaloric groups (catch-up), independent of physical activity, which agrees with the results of previous studies [14, 15]. When an organism is exposed to greater nutritional offer after deprivation, weight gain is accelerated due to metabolic adaptations to store energy more easily [1–3, 14, 15].

Curiously, the group maintained on the same hypocaloric maternal diet recovered body weight at a similar rate, which may stem from a metabolic change in favor of greater energy efficiency [16] and/or less thermogenesis [17] for the sake of preserving the species. This statement is based on the fact that food intake was not greater in the hypocaloric groups as a result of compensating for the imposed caloric reduction. Using similar caloric restriction, Muniz et al. [6], found no weight recovery in hypocaloric animals submitted to nutritional recovery. This difference in results from Muniz et al. [6] compared to the present findings may be a result of the period of physical activity and/or the different diet used after weaning (based on AIN-93 or standard chow diet).

The physical activity protocol employed in the present study did not result in a change in body weight. Divergent

results are found in the literature regarding the effect of exercise on body weight relative to the different employed protocols (variations in intensity, frequency, period, and volume of exercise). The absence of an effect of 9 weeks of swimming on body weight has also been reported in animals beginning exercise at 58 days of life (30 min a day, 5 days a week) [18]. However, endurance training (50 min a day, 5 days a week for 10 weeks) led to lower body weight in well-nourished rats and did not intensify weight loss in under-nourished rats, but altered the body composition, reducing body fat and promoting an increase in muscle mass [19].

An approximately 40% reduction in retroperitoneal and total abdominal fat occurred in the HNA group in comparison to its inactive counterpart, but not in the group maintained on the hypocaloric diet. The differences between the exercised hypocaloric groups demonstrate that the effects of the diet continuity were more determinant on outcomes in the litters than the effects of physical activity. Garg et al. found that animals submitted to caloric restriction in the perinatal period and receiving a standard diet after weaning exhibited twice as much fat tissue than the control group at 10 months of age [20]. One of the factors of this increase may precisely be the change in the thermogenesis of muscle tissue stemming from specific muscle transcription factors, thereby contributing to an increase in body fat [17]. In the present study, the recovered HNA group exhibited a 47% reduction in abdominal fat in comparison to its control counterpart. In a previous study, rats with induced metabolic syndrome submitted to moderate to high intensity aerobic exercise demonstrated a 55% reduction in retroperitoneal fat [21], which is similar to the value found in the present investigation.

The maintenance of litters with the diet offered to the mothers led to significant changes in the metabolism of lipids and glucose in the offspring. The results demonstrate that the hypocaloric diet in the perinatal period maintained after weaning was associated with an increase in glucose levels, indicating a greater risk for developing diabetes. A previous study demonstrated that caloric restriction of approximately 50% of the diet throughout the gestation and lactation periods reduces the mass of ß cells in the offspring after weaning, with a negative impact on insulin secretion [20]. Caloric restriction also seems to reduce insulin signaling in specific tissues, such as muscle, pancreas, liver and adipose tissue [22]. However, it is difficult to compare the present findings with data in the literature because of the few studies addressing the continuity of the maternal diet and the diversity in the composition of the diets offered.

The recovered groups (HNI and HNA) did not exhibit altered fasting glucose in comparison to the control groups or the animals maintained on the hypocaloric diet. In another study by our research group, hypoglycemia was found using the same diet throughout the entire gestation and lactation period or only in the lactation period, whereas no change was found when the diet was only used in the gestation period [13]. Muniz et al. also found hypoglycemia in recovered hypocaloric animals [6]. However, the diet employed by authors after weaning differed from that of the present investigation. The study by Alheiros et al. [13] did not use the same dietary intervention period. In another study, previously undernourished and recovered animals demonstrated hyperglycemia at 6 months of life [23], suggesting that insulin sensitivity seems to be modified over time due to the elevated sensitivity in the prenatal and immediate postnatal period.

Despite not demonstrating any glycemic change, the inactive recovered group (HNI) exhibited high triglyceride and VLDL-c serum values. Alheiros et al. [13] also found an increase in triglycerides and cholesterol in animals submitted to a hypocaloric diet only in the lactation period. These findings confirm that diet exerts a considerable influence on lipid metabolism, especially when offered in the lactation period. However, this increase was reversed in the exercised group, demonstrating the benefit of physical activity for this group. In submitting rodents to a mild to moderate intensity swimming exercise, Moura et al. found an increase in lipolysis and greater mobilization of stored fat for energy [24]. This greater mobilization may come from body stocks or even liver tissue, with no change in body weight, contributing to a reduction in serum levels of circulating lipids [25].

Exercise increases clearance and reduces the postprandial concentration of triglycerides and VLDL-c [26] reduction in the hepatic expression of the gene stearoyl-CoA desaturase-1, which is responsible for the biosynthesis of monounsaturated fatty acids (the main components of the particles of VLDL-c and triacylglycerol), and has been demonstrated in exercised rats [27]. Moreover, changes in the activity of the enzyme lipoprotein lipase, with the release of triglycerides from circulating lipoproteins or the low hepatic secretion of VLD-c, may also have contributed to the reduction in triglycerides [26] found in recovered exercised rats.

However, the effect of exercise was not equally extended to the animals maintained on the hypocaloric diet. Maintenance of the hypocaloric diet throughout the experimental period led to an increase in total cholesterol, LDL-c and HDL-c levels. The relationship between an adverse intrauterine and/or postnatal condition and hypercholesterolemia has been reported in the literature [18, 21]. Hyperglycemia triggers an increase in the production of LDL-c or an increase in the conversion of VLDL-c into LDL-c. There is also a tendency toward a reduction in the catabolism of LDL-c. This occurs due to the presence of apolipoprotein B on the surface of LDL-c particles, which undergo glycation when in contact with high glucose levels, and become less recognized by their receptors, thereby

reducing depuration and increasing the half-life of circulating LDL-c [28].

Continued caloric restriction also exhibited characteristics of kidney damage, as demonstrated by the increase in urea, creatinine and uric acid levels. One may also suggest that the maintenance of calorie restriction in young rats reflects a catabolic state, in which energy deprivation promotes a negative nitrogenated balance, leading to protein degradation in different organs, with skeletal muscle being the most affected [29].

The only biochemical benefit in the group maintained on the hypocaloric diet was the increase in HDL-c in the HHA group, which may have been the result of greater lipid mobilization for energy purposes due to the caloric restriction. An increase in the availability of lipids for energy purposes may have accelerated the biosynthesis of lipoproteins, thereby increasing the circulation of these substances [30].

The effects of exercise on the organism depend on the frequency, duration and intensity of effort, as well as diet in different periods of development [20]. Different physical activity protocols, dietary manipulations and intervention periods can result in different results with regard to metabolism, the use of energy substrates and body composition.

The food preference test can be an indicator of changes in controlling food intake. In the present study, the animals with continual caloric restriction demonstrated a significant preference for the hyperglucidic diet, especially in those that were subject to physical activity. Carbohydrate intake seems to involve the action of gamma aminobutyric acid, noradrenaline and neuropeptide Y in association with corticosterone and glucose circulating in the blood [31]. Exercise may modify this preference by changing the expression of hormones or hypothalamic neuropeptides [32] responsible for controlling ingestion and the metabolism of carbohydrates, which have been found in high levels in rats submitted to perinatal malnutrition [33].

However, the preference for carbohydrates in the hypocaloric groups may have increased the metabolic risk, since diets with high levels of simple carbohydrates are associated with cardiovascular disease, diabetes and obesity [34]. It should be pointed out that the preference of these animals may have been altered due to dietary habituation, as total food intake was three times lower than the amount ingested by the animals in the other groups. Additional studies are needed for further investigations and cellular analyses to test the predictive adaptive hypothesis and clarify the mechanisms underlying these initial findings.

Conclusion

The caloric restriction model promoted lower body weight in offspring upon weaning, which is similar to malnutrition models reported in the literature. Accelerated weight gain subsequent to perinatal caloric restriction seems to be associated with the development of metabolic disorders, such as hyperglycemia and dyslipidemia. However, the maintenance of caloric restriction in the present study did not support the hypothesis of a predictive adaptive response, as the HHA and HHI groups demonstrated accelerated weight gain and changes in the biochemical profile. Additionally, continuation of the hypocaloric diet and physical exercise led to a preference for carbohydrates. On the other hand, the inclusion of physical activity proved relevant to dyslipidemia and the abdominal fat concentration only in the animals with nutritional recovery after weaning.

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Compliance with ethical standards

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

Ethical approval This study received approval from the Animal Experimentation Ethics Committee of the Center for Biological Science of the Universidade Federal de Pernambuco (UFPE) (certificate no.: 23076.028444-2012-73) and followed the Guidelines for the Care and Use of Laboratory Animals.

Informed consent All participants provided informed consent prior to their participation.

References

1. Gluckman PD, Hanson MA (2007) Developmental plasticity and human disease: research directions. J Inter Med 261(5):461–471
2. Hales CN, Barker DIP (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetology 35:595–601
3. Wells JC (2011) The thrifty phenotype: an adaptation in growth or metabolism? Am J Hum Biol 23:65–75
4. Gluckman PD, Hanson MA, Spencer HG (2005) Predictive adaptive responses and human evolution. Trends Ecol Evol 20:527–533
5. Wells JC (2007) Flaws in the theory of predictive adaptive responses. Trends Endocrinol Metab 18:331–337
6. Muniz SG, Da Silva AM, Cavalcante TCF et al (2013) Early physical activity minimizes the adverse effects of a low-energy diet on growth and development parameters. Nutr Neurosci 16:113–124
7. Cotman CW, Berchtold NC (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 25:295–301
8. Torun B, Viteri FE (1994) Influence of exercise on linear growth. Eur J Clin Nutr 48:186–189
9. Bayne K (1996) Revised guide for the care and use of laboratory animals available. Am Physiol Soc Physiol 39:208–211
10. Reeves PG, Nielsen FH, Fahey GC (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of

Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939–1951
11. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
12. Pine AP, Jessop NS, Oldham JD (1994) Maternal protein reserves and their influence on lactational performance in rats. The effects of dietary protein restriction and stage of lactation on milk composition. Braz J Nutr 72:815–30
13. Alheiros-Lira MC, Araújo LL, Trindade NGV et al (2015) Short- and long-term effects of a maternal low-energy diet ad libitum during gestation and/or lactation on physiological parameters of mothers and male offspring. Eur J Nutr 54:793–802
14. Desai M, Babu J, Ross MG (2007) Programmed metabolic syndrome: prenatal undernutrition and postweaning overnutrition. Am J Physiol Regul Integr Comp Physiol 293:2306–2314
15. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-koelega A (2009) Timing and time of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 301:2234–2242
16. Qasem RJ, Yablonski E, Li J, Tang HM et al (2012) Elucidation of thrifty features in adult rats exposed to protein restriction during gestation and lactation. Physiol Behav 105:1182–1193
17. De Andrade PBM, Neff LA, Strosova MK et al (2015) Caloric restriction induces energy-sparing alterations in skeletal muscle contraction, fiber composition and local thyroid hormone metabolism that persist during catch-up fat upon refeeding. Front Physiol 6:254
18. Oliveira ECD (2007) Biochemical and nutritional evaluation of trained animals submitted to malnutrition and nutritional recovery. Dissertation. Federal University of Ouro Preto, Brazil
19. Giampietro MV (2007) Metabolic changes in malnourished rats in response to endurance training. Dissertation. University of São Paulo, São Paulo, Brazil
20. Garg M, Thamotharan M, Dai Y, Thamotharan S, Shin BC, Stout D (2012) Early postnatal caloric restriction protects adult male intrauterine growth–restricted offspring from obesity. Diabetes 61:1391–1398
21. Haram PM, Kemi OJ, Lee SJ et al (2009) Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity. Cardiovasc Res 81:723–732
22. Snoeck A, Remacle C, Reusens B, Hoet JJ (1990) Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. Biol Neonate 57:107–118
23. Cherrington AD, Edgerton D, Sindelar DK (1998) The direct and indirect effects of insulin on hepatic glucose production in vivo. Diabetology 41:987–996
24. Moura LP, Sponton ACS, Araújo MB et al (2013) Moderate physical activity from childhood contributes to metabolic health and reduces hepatic fat accumulation in adult rats. Lipids Health Dis 12:1–17
25. Golabi P, Locklear CT, Austin P, Afdhal S, Byrns M, Gerber L (2016) Effectiveness of exercise in hepatic fat mobilization in non-alcoholic fatty liver disease: systematic review. World J Gastroenterol 22:6318–6327
26. Garlick PJ, Millward DJ, James WPT, Waterlow JC (1975) The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. Biochem Biophys Acta 414:71–84
27. Gill JMR, Hardman AE (2003) Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). J Nutr Biochem 14:122–132
28. Després JP, Lemieux I, Prud'homme D (2001) Treatment of obesity: need to focus on high risk abdominally obese patients. BMJ 322:716
29. Seidel D (1987) Lipoproteins in liver disease. Clin Chem Lab Med 25:541–552
30. Fletcher B, Berra K, Ades P, Braun IT, Burke LE, Durstine JIL (2005) Managing abnormal blood lipids. Circulatory 112:3184–3209
31. Bernardes D, Manzoni MSJ, De Souza CP, Tenório N, Dâmaso AR (2004) Efeitos da dieta hiperlipídica e do treinamento de natação sobre o metabolismo de recuperação ao exercício em ratos. Revista Brasileira de Educação Física e Esporte 18:191–200
32. Johnston AL, File SE (1991) Sex differences in animal tests of anxiety. Physiol Behav 49:245–250
33. Cambraia RPB (2004) Psychobiological aspects of feeding behavior. Nutr Rev 17:217–225
34. Bell SJ, Sears B (2003) Low-glycemic-load diets: impact on obesity and chronic diseases. Crit Rev Food Sci Nutr 43:357–377