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Treadmill walking differently affects body composition and metabolic parameters of female rats from normal or small litters

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This work assessed whether walking affects bodily development and metabolic parameters of female rats raised in small litters (three pups, group S) or control litters (nine pups, group C). After weaning, some of the rats had five sessions per week of a 30-min treadmill walking (CE and SE), while the others remained sedentary (CS and SS) until the age of 120 days. Exercise caused a reduction of body weight (CS/CE = 1.18), Lee index (CS/CE = 1.04), fasting blood glucose (CS/CE = 1.35), mesenteric (CS/CE = 1.23), and ovarian fat (CS/CE = 1.33) in CE, but only glucose was decreased in SE (SS/SE = 1.16). The diameter of adipocytes decreased to a half in the small-litter groups. Exercise increased subcutaneous (CS/CE = 0.88 and SS/SE = 0.71), but decreased retroperitoneal adipocytes (CS/CE = 1.2 and SS/SE = 1.3). Litter size reduction had little impact on females at the age of 120 days, but the light physical activity seemed insufficient to counteract all the effects of lactational overfeeding. On the other hand, pups from exercised mothers had a decrease in their biometric and glycemic indexes, demonstrating the transgenerational action of regular, although light, exercise.

Keywords: obesity, exercise, small litter, blood glucose, adipocyte

Introduction

Obesity is a chronic disease, often associated with a positive energy balance, where genetic and environmental factors are involved, and it is linked to the appearance and aggravation of several metabolic disorders. The prevalence of obesity continues to increase in many countries; in Brazil, data from the World Health Organization show that the prevalence of overweight/obesity [body mass indexes (BMIs) above 25 $kg \cdot m^{-2}$] is higher than 50% in the population older than 15 years. This high prevalence is due to factors involving lifestyle, such as large caloric intakes and low levels of physical activity (1, 27). Life quality and expectancy are affected by excessive body fat. Insulin resistance, cardiovascular diseases, diabetes, and hypercholesterolemia are some examples of the susceptibility to metabolic and chronic-degenerative impairments that follow obesity (7, 20, 36, 42).

In recent years, excess body weight gain in childhood is calling increasing attention. The nutritional experiences at early life lead to the changes of growth and metabolism in later stages. The research demonstrates that the development of adipose cellularity at this early

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stage can determine the body composition of the adult and thus the appearance and aggravation of obesity-related diseases (6, 14, 23, 35, 37, 38, 42, 45).

Physical inactivity is a significant adjuvant in the maintenance of obesity and its complications. Exercise, regardless of body weight loss, contributes to the preservation of the lean mass, appetite control, improvement of physical condition, increase of the resting metabolic rate, and has a positive interference on the individual's health (20, 24, 33, 39). Ciolac and Guimarães (8) state that an active life can prevent obesity, regardless of the kind of physical activity. To sustain this statement, they report that athletes, healthy individuals, non-diabetic obese, and diabetic subjects improve their insulin sensitivity when practicing exercise on a regular basis; the effects of exercise on this parameter are both acute and chronic (8, 24). Exercise beginning early in life has more persistent effects, and this probably occurs because exercise can alter the development of the central pathways regulating energy homeostasis (39). It is argued that, in humans, 30 min of moderate physical activity five times a week are adequate for body weight and fat control (32).

In rats, litter size reduction is a classical model of overweight/obesity and increased adiposity (13, 14, 45). It is based on the modification of the post-natal nutritional environment (i.e., changing the number of pups per litter during lactation) as a mean of "programming" the central control of appetite and energy balance, resulting in the obese phenotype of the adult animal (6, 35, 38, 41, 42). Common findings in small litters are greater body weight gain, increased levels of insulin and corticosterone, greater fat deposition, and hyperphagia (25, 41, 42).

There is a great variety of possible levels of physical activity, but with laboratory animals, it is possible to design exercise protocols of specific intensities. In this study, it was assessed whether treadmill walking, a light and regular exercise, affects biometric and physiologic parameters of female rats raised in normal and small litters and whether exercising would affect the females' offspring.

Materials and Methods

Animals

The pregnant Wistar dams were obtained from the colony of the Central Animal House of the State University of Maringá, Paraná, Brazil, and kept at the animal house of the Department of Physiological Sciences under controlled light/dark cycles (12 h light/12 h dark) and temperature (22 ± 2 °C). Food and chow (Nuvilab CR1; Nuvital, Curitiba, PR, Brazil) were supplied *ad libitum*. The experimental protocols were approved by the Ethics Committee of the Institution (statement 015/2013).

The dams were housed in individual boxes, where they gave birth. The newborn litters were organized, so that each dam had three or nine pups, preferably females. Male pups were kept only when needed to complete the desired litter. The nine-pup litters were the control litter group (C) and the three-pup litters were the small-litter group (S). The assignment of the litters to group C or S depended on the litter size and number of females per litter, so that mixing of litters was kept to a minimum.

The pups remained with their mothers until the age of 21 days (weaning), when the female pups were put into a collective of plastic boxes in groups of three according to their original litter (C or S), with free access to water and chow. The male offspring was not included in this investigation. The C and S females were randomly subdivided into sedentary

(S) and exercised (E), thus yielding four groups, CS, CE, SS, and SE. The exercise protocol of groups CE and SE is described in *Physical activity* section.

All the records and experimental procedures were carried out in the female rats. The animals were followed up for 120 days. The body weight and nasoanal length were recorded at the end of this period and were used to calculate the Lee index ([$\sqrt[3]{}$body weight (g) body length (cm)$^{-1}$]·1,000) of the experimental groups. The Lee index is used as an indicative of overweight/obesity in rats (3, 33). The BMI (g·cm^{-2}) for rats (34) was also calculated.

The *in vivo* experiments of glucose tolerance test (GTT), insulin tolerance test (ITT), and exhaustion test were carried out between the age of 110 and 120 days. Overnight fasting preceded some protocols, as indicated in the following, and was of about 14 h.

Physical activity

Soon after weaning, the female rats of the CE and SE groups were subjected to physical activity. This consisted of walking in a programmed treadmill (KT3000; Inbramed, Porto Alegre, RS, Brazil), adapted to the training of up to eight rats at the same time. Treadmill walking was carried out in five sessions per week, at different days and times, excluding the periods of 10 a.m.–16 p.m. and 18 p.m.–6 a.m. There was a period of adaptation to the treadmill and to the activity. For the first 10 days (females aging 21–30 days), each session lasted 5 min with treadmill speed increasing from 0.2 to 0.6 km·h^{-1} for each two sessions. For the next 10 days, treadmill speed was kept at 0.6 km·h^{-1} and session duration progressively increased from 5 to 30 min. After adaptation, each walking session lasted 30 min at the speed of 0.6 km·h^{-1} from the age of 40 to 120 days. The treadmill walking protocol of the CE and SE female rats did not change during pregnancy or lactation, except that it was interrupted during the third week of gestation and first week of lactation to prevent premature delivery and *post-partum* anxiety and cannibalism.

Glucose tolerance test

The female rats, after overnight fasting, were given oral glucose by gavage (1.5 g·kg^{-1} body weight). Blood samples were collected from a caudal incision at 0, 15, 30, 45, 60, 90, and 120 min, 0 min being immediately before glucose administration. Blood glucose was determined with test strips and glucose meter Optium-Exceed (Abbott, São Paulo, SP, Brazil) and expressed as mg·dL^{-1}.

Based on the obtained data, the area under the curve (AUC) of the blood glucose variation during the 120-min test was calculated. The index of blood glucose decay (kGTT, %·min^{-1}) from 30 to 120 min was also calculated.

Insulin tolerance test

After a 2-h fasting (from 8 a.m. to 10 a.m.), the rats were given an injection of regular insulin (1 U·kg^{-1} body weight, i.p.; Novolin; Novo Nordisk, Montes Claros, MG, Brazil) for the experiments of insulin tolerance. Blood samples were collected from a caudal incision at 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min, 0 min being immediately before insulin injection. Blood glucose was determined with test strips and glucose meter and expressed as mg·dL^{-1}. The index of blood glucose decay (kITT, %·min^{-1}) was calculated for the first 30 min of the test (31).

Exhaustion test

After overnight fasting, the exhaustion test was performed as follows: the treadmill speed increased by 0.2 km·h^{-1} every 2 min from an initial speed of 0.4 km·h^{-1}. Exhaustion was

established as the moment the animal could not keep running. No painful stimuli were used to make the animals run.

Biochemical assays

The blood samples for the biochemical assays were collected after overnight fasting from a caudal incision. Appropriate test strips and Accutrend Plus (Roche, São Paulo, SP, Brazil) were employed. Lactate measurements were made immediately after the exhaustion test and are shown as $mmol \cdot L^{-1}$. For cholesterol and triglycerides, concentrations are given as $mg \cdot dL^{-1}$.

Tissue removal

The female rats were euthanized at the age of 120 days through excess anesthetic (sodium pentobarbital, 120 $mg \cdot kg^{-1}$ body weight, i.p.), after overnight fasting. The visceral fats (retroperitoneal, ovarian, periuterine, and mesenteric), the subcutaneous fat, and the liver were removed and the weights were expressed as 100 g^{-1} body weight.

Liver fat content

The method of fat extraction with methanol and chloroform was employed (18). Samples of liver of about 1 g were dried at 40 °C until weight stabilization. To these dried samples, it was added 10 mL of chloroform:methanol solution (2:1, v/v), homogenized and left to rest for 24 h. Next, 8 mL of the resulting solution and 1.6 mL of saline were rested for 1 h, centrifuged for 5 min at 1,000 r/min and the supernatant (4 mL) placed in a Petri dish for 24–48 h to evaporate. The lipid content was expressed as $mg \cdot g^{-1}$ of liver wet weight.

Isolation and measurement of adipocytes

Adipocytes were isolated from the subcutaneous, ovarian, and retroperitoneal fats following the methodology established by Rodbell with some adaptations (19, 40). After being removed, the fat pads were fragmented and placed in 4 mL digestion buffer (DMEM/HEPES 25 mM, bovine serum albumin fraction V – BSA in 4% collagenase II 1.25 $mg \cdot mL^{-1}$, pH 7.4, 37 °C) for about 20 min under constant agitation (150 r/min) in an orbital water bath shaker. The digested tissue was filtered and washed three times with 25 mL glucose-free EARLE/HEPES/BSA (EHB) buffer (20 mM containing 1% BSA and 1 mM sodium pyruvate), pH 7.4, at 37 °C. The diameter of adipocytes was measured using an image analysis system (Image-Pro Plus 4.5; Media Cybernetics, Rockville, MD, USA). Fifty randomly chosen adipocytes per fat pad per animal of each group were measured.

Evaluation of the litters

Some 70-day-old female rats from each experimental group (CS, CE, SS, and SE) mated with non-treated males. Eight pups were left in each litter; after weaning, the male pups of these litters were kept in plastic boxes (four males per box), according to the original group of their dams. These animals were not subjected to physical activity and were given water and chow *ad libitum* until the age of 60 days. The naming of these groups of pups was made according to their dam's original group: CSp, CEp, and SEp. The mated SS females did not get pregnant, so there was no SSp group (see *Assessment of the pups* section and other sections of *Results*).

The final (60 day) body weight and nasoanal length were recorded. After overnight fasting, blood glucose from a caudal incision was determined with test strips and glucose meter and expressed as $mg \cdot dL^{-1}$. The animals were euthanized by excess anesthetic (sodium pentobarbital, 120 $mg \cdot kg^{-1}$ body weight, i.p.). The visceral fats (periepididymal, mesenteric, and retroperitoneal) and the liver were removed and their weights were expressed as 100 g^{-1} body weight.

Statistical analysis

Data are shown as mean ± standard deviation (SD). Normality was checked through Shapiro–Wilk's test, followed by the appropriate *t*-test. Groups CS and SS were compared to test the effects of litter size. Groups CS and SS were compared with their respective exercised groups (CE and SE) to test the effects of physical activity. The data from the pups of groups CE and SE (named CEp and SEp, see *Results* section) were compared with those of group CS (CSp). Significant values were those below 0.05. Statistical analysis used GraphPad Prism v5 (GraphPad, San Diego, CA, USA).

Results

Biometric data

Table I shows the biometric data of the studied groups. At the age of 120 days, the Lee index was the only biometric parameter that was significantly higher in the SS female rats compared with those of the CS. Treadmill walking decreased body weight, Lee index, and BMI of group CE compared with CS, but did not change these parameters ($p > 0.05$) between groups SS and SE.

Exhaustion test

The sedentary groups ran significantly less time (CS 9.58 ± 2.89 min, $n = 6$ and SS 10.39 ± 1.89 min, $n = 9$) and reached lower treadmill speeds than their corresponding exercised groups (CE 14.06 ± 1.71 min, $n = 6$ and SE 13.87 ± 1.75 min, $n = 6$) ($p < 0.05$). However, running time was not statistically different when sedentary (CS vs. SS) or exercised (CE vs. SE) groups were compared ($p > 0.05$).

Insulin tolerance test and glucose tolerance test

Table II shows the data obtained during the GTT. Fasting blood glucose (0 min, immediately before glucose administration) was significantly lower in the exercised groups (CE and SE)

Table I. Biometric parameters at the age of 120 days of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E)

	CS ($n = 6$)	**CE ($n = 6$)**	**SS ($n = 10$)**	**SE ($n = 6$)**
Body weight (g)	295.60 ± 13.22	250.30 ± 6.59*	287.60 ± 18.33	298.30 ± 36.40
Lee index	287.00 ± 8.93	275.50 ± 4.83*	299.80 ± 8.15*	292.10 ± 9.04
BMI ($g \cdot cm^{-2}$)	0.57 ± 0.04	0.50 ± 0.01*	0.61 ± 0.04	0.60 ± 0.06

*$p < 0.05$ compared with group CS

Table II. Glucose tolerance test (GTT) of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E) at the age of 120 days after overnight fasting

	CS ($n = 6–9$)	CE ($n = 5–8$)	SS ($n = 9$)	SE ($n = 6–9$)
Blood glucose at 0 min ($mg \cdot dL^{-1}$)	92.00 ± 3.74	68.40 ± 2.79**	100.8 ± 9.35	87.17 ± 9.48***
Blood glucose at 15 min ($mg \cdot dL^{-1}$)*	161.80 ± 24.05*	124.30 ± 13.69*	142.10 ± 15.99*	150.30 ± 22.63*
Blood glucose at 120 min ($mg \cdot dL^{-1}$)	90.66 ± 19.59	79.67 ± 5.72*	96.10 ± 13.21	89.50 ± 9.54
AUC of GTT	225.00 ± 68.43	191.20 ± 78.04	120.00 ± 20.63**	198.70 ± 71.78***
kGTT ($\%\cdot min^{-1}$)	0.49 ± 0.16	0.56 ± 0.20	0.47 ± 0.18	0.46 ± 0.15

*$p < 0.05$ compared with 0 min of the group, **$p < 0.05$ compared with CS, ***$p < 0.05$ compared with SS

than in their sedentary pairs (CS and SS, respectively). Approximately 15 min after glucose administration, all the groups had blood glucose significantly higher than at 0 min, but did not differ from one another ($p > 0.05$). At the end of the test (120 min), only group CE had significantly higher blood glucose than at 0 min, while there was no difference between these two moments in the other groups ($p > 0.05$). Blood glucose did not differ ($p > 0.05$) at 120 min between groups CS/SS, CS/CE, and SS/SE.

The AUC of blood glucose variation during the GTT was significantly lower in group SS than in groups CS and SE. Groups CS and CE were not statistically different ($p > 0.05$).

The index of blood glucose decay (kGTT) was calculated for the interval from 30 to 120 min, similar to what is often done with ITT (26). There was no difference ($p > 0.05$) between the groups for this index.

Table III shows the data from the ITT, after a 2-h fasting. In all the groups, blood glucose at 90 min (time of lower blood glucose) and at 120 min (final blood glucose) after insulin injection were significantly lower than at 0 min. Blood glucose at 90 min was not different between the groups ($p > 0.05$). Group CS had higher blood glucose at 120 min when compared to groups SS and CE; groups SS and SE did not differ at 120 min ($p > 0.05$). The index of blood glucose decay during the first 30 min of the test (kITT) was not different between the compared groups ($p > 0.05$).

Biochemical assays

Marked differences were found for fasting blood glucose (Table II) between groups CS and CE, and SS and SE. Lower values were recorded in the exercised groups (CE and SE) compared with their respective sedentary pairs (CS and SS). After the 2-h fasting, before the ITT, the difference remained only between CS and CE (Table III). Cholesterol and triglycerides were not different between the compared groups (Table IV, $p > 0.05$).

As for the blood lactate (Table IV), measured immediately after the exhaustion test, higher values were recorded in the CE and SS compared with CS. The groups SS and SE were similar ($p > 0.05$). There was no significant correlation in any of the groups ($p > 0.05$, Pearson) between running time and blood lactate.

Table III. Insulin tolerance test (ITT) of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E) at the age of 120 days after 2 h of fasting

	CS ($n = 6$–9)	**CE ($n = 5$–8)**	**SS ($n = 9$)**	**SE ($n = 6$–9)**
Blood glucose at 0 min ($mg \cdot dL^{-1}$)	99.56 ± 12.80	62.67 ± 9.34**	98.30 ± 13.30	91.22 ± 16.54
Blood glucose at 90 min ($mg \cdot dL^{-1}$)	37.56 ± 18.12*	31.25 ± 11.73*	28.44 ± 5.20*	27.33 ± 4.09*
Blood glucose at 120 min ($mg \cdot dL^{-1}$)	57.22 ± 6.14*	30.57 ± 2.85*·**	37.78 ± 4.36*·**	34.56 ± 3.25*
kITT ($\%\cdot min^{-1}$)	2.25 ± 0.63	2.51 ± 1.20	3.05 ± 0.64	3.00 ± 0.95

*$p < 0.05$ compared with 0 min of the group, **$p < 0.05$ compared with CS

Table IV. Biochemical assays of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E) at the age of 120 days after overnight fasting

	CS ($n = 6$)	**CE ($n = 4$–6)**	**SS ($n = 10$)**	**SE ($n = 5$–6)**
Cholesterol ($mg \cdot dL^{-1}$)	158.20 ± 7.44	153.00 ± 2.45	160.60 ± 6.06	158.30 ± 7.01
Triglycerides ($mg \cdot dL^{-1}$)	142.30 ± 27.29	170.60 ± 44.00	149.50 ± 18.96	133.50 ± 31.33
Lactate ($mmol \cdot L^{-1}$)	3.17 ± 0.55	4.38 ± 0.82*	4.51 ± 0.80*	3.96 ± 0.38

*$p < 0.05$ compared with CS

Tissues and organs

The relative weights of body fats and liver of the four experimental groups are shown in Table V. Group SS had a tendency of increase of all the fats in comparison with group CS, but this was significant only for the mesenteric and ovarian fats.

In both exercised groups, there was a tendency of decreased relative weight of almost all the fats, when compared with their sedentary counterparts. However, again only the mesenteric and ovarian fats of group CE were statistically different from those of CS. Groups SS and SE did not differ statistically ($p > 0.05$) in the relative weight of their fats.

The relative weight of the liver was not different between the groups (Table V, $p > 0.05$). Liver fat content, in $mg \cdot g^{-1}$ of wet weight, was 0.08 ± 0.02 in CS ($n = 8$), 0.08 ± 0.01 in CE ($n = 8$), 0.08 ± 0.01 in SS ($n = 7$), and 0.08 ± 0.02 in SE ($n = 7$). These values were statistically similar ($p > 0.05$).

Table VI shows the diameter of the adipocytes isolated from the subcutaneous, ovarian, and retroperitoneal fats of the four experimental groups. Litter size reduction (group SS) decreased adipocyte diameter by about half in all these fats compared with the control (group CS). Treadmill walking affected adipocyte diameter differently on the fats assessed. However, even when statistical significance was not attained, the exercised groups had a tendency of larger subcutaneous and ovarian adipocytes and smaller retroperitoneal adipocytes than their sedentary counterparts.

Table V. Relative weight of fats and liver of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E) at the age of 120 days

Relative weight (100^{-1} g body weight)	**CS ($n = 6$)**	**CE ($n = 6$)**	**SS ($n = 10$)**	**SE ($n = 5$–6)**
Retroperitoneal fat	1.23 ± 0.18	1.17 ± 0.28	1.52 ± 0.35	1.44 ± 0.42
Mesenteric fat	1.41 ± 0.13	1.15 ± 0.41*	1.82 ± 0.37*	1.54 ± 0.29
Periuterine fat	1.52 ± 0.23	1.34 ± 0.34	1.75 ± 0.39	1.76 ± 0.49
Ovarian fat	1.17 ± 0.26	0.88 ± 0.28*	1.53 ± 0.42*	1.18 ± 0.44
Total visceral fat	5.28 ± 0.50	4.37 ± 1.16	6.43 ± 1.18	5.81 ± 1.71
Subcutaneous fat	0.58 ± 0.17	0.89 ± 0.31	0.74 ± 0.16	0.59 ± 0.26
Liver	2.85 ± 0.35	2.76 ± 0.14	2.55 ± 0.21	2.52 ± 0.20

*$p < 0.05$ compared with CS

Table VI. Diameter of adipocytes isolated from the ovarian, subcutaneous, and retroperitoneal fats of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E) at the age of 120 days

Diameter (μm)	**CS ($n = 4$–5)**	**CE ($n = 4$–5)**	**SS ($n = 4$–5)**	**SE ($n = 4$–5)**
Subcutaneous fat	91.79 ± 21.24	103.95 ± 8.55	53.83 ± 0.62*	76.19 ± 12.32**
Ovarian fat	51.81 ± 3.00	117.93 ± 5.46*	25.94 ± 0.78*	34.52 ± 5.72
Retroperitoneal fat	170.73 ± 20.76	142.97 ± 4.26*	76.60 ± 12.48*	59.24 ± 3.51**

*$p < 0.05$ compared with CS, **$p < 0.05$ compared with SS

The calculation of cellularity for the retroperitoneal fat had a high correlation with the absolute weight of this fat ($r = 0.723$; $p < 0.01$, Pearson). Higher cellularities were predicted for the small-litter groups than for the controls.

Assessment of the pups

The mated females from group SS did not have pups. As the number of female pups in group SE was small, only the male pups from groups CS, CE, and SE were evaluated, and were named CSp ($n = 7$), CEp ($n = 12$), and SEp ($n = 14$), respectively.

Table VII shows the biometric data of groups CSp, CEp, and SEp at the age of 60 days. The pups from exercised females, either from control or small litters (groups CEp and SEp) had body weight, nasoanal length, and weight/length ratio (WL) significantly lower than those of sedentary females (CSp). The BMI of these three groups, however, did not differ statistically ($p > 0.05$).

The relative weight of the visceral fat (sum of the epididymal, mesenteric, and retroperitoneal fats, 100 g^{-1} body weight) was 1.98 ± 0.39 in CSp, 1.53 ± 0.25 in CEp, and 1.77 ± 0.37 in SEp. The reduced visceral adiposity in the pups of the exercised dams reached statistical significance between CSp and CEp.

Table VII. Biometric data of pups (p) of female rats raised in control (C) litters or small (S) litters, sedentary (S), or exercised (E), at the age of 60 days

	CSp ($n = 7$)	**CEp ($n = 12$)**	**SEp ($n = 14$)**
Body weight (g)	319.00 ± 32.06	273.70 ± 18.35*	282.60 ± 16.5*
Nasoanal length (cm)	22.67 ± 0.59	21.50 ± 0.44*	21.74 ± 0.54*
WL ratio ($g \cdot cm^{-1}$)	14.06 ± 1.22	12.73 ± 0.77*	13.00 ± 0.60*
BMI ($g \cdot cm^{-2}$)	0.62 ± 0.05	0.59 ± 0.04	0.60 ± 0.03

*$p < 0.05$ compared with CSp

The relative weight of the liver (100 g^{-1} body weight) was not significantly different ($p > 0.05$) between CSp (4.50 ± 1.07), CEp (3.50 ± 0.36), and SEp (3.68 ± 0.51).

The overnight fasting blood glucose of the pups was 78.57 ± 8.30 $mg \cdot dL^{-1}$ in CSp, 69.25 ± 5.94 $mg \cdot dL^{-1}$ in CEp, and 65.77 ± 9.98 $mg \cdot dL^{-1}$ in SEp. The blood glucose of the pups from exercised dams (CEp and SEp) was statistically lower than that of CSp.

Discussion

Several authors state that early post-natal overnutrition, such as that caused by reducing the litter size during lactation, is a causative agent of later obesity (i.e., in adulthood) and is linked to metabolic and cardiovascular diseases (12, 36, 42, 45). Disturbances in the hypothalamic circuitry controlling feeding behavior and body weight management during this critical period of development are involved (11, 13, 23, 38, 45, 50).

Surprisingly, the sedentary female rats from the small litters (group SS) did not show body weight significantly different from that of the controls (group CS) at the age of 120 days. Although an increased body weight is frequently found in rats from small litters (11, 12, 14, 25, 41, 42, 50), it is not a universal observation. Stefanidis and Spencer (46) and Velkoska et al. (48) note that the effects of the small litter on body weight and adiposity are more marked in young animals and tend to smooth out with age. In addition, gender differences are also observed (46). Both of these factors – i.e., female rats aging 120 days – may have prevented the overt obesity in the small litter that would be expected at adult age. In addition, weight control in the rat might be restored when they are fed with normal, instead of highly caloric, diet (46).

On the other hand, the female SS rats had greater Lee index than those of group CS, as well as greater relative weight of some visceral fats, indicating that the small litter size during lactation did promote an alteration on body composition of the adult SS females. Even more significant and consistent was adipocyte size: those of the SS had half the diameter of those of the CS, and calculated cellularity was much greater. Taken as a whole, these biometric and morphometric data indicate that the small litter had a long-term impact on the female SS rats (14, 17).

Regular physical activity since childhood helps in the control of body weight and adiposity, and a sedentary lifestyle, together with increased food ingestion, is a major cause of the epidemics of obesity (1, 27, 32). According to Matsudo and Matsudo (32), exercise in obese subjects alters the body composition even without changes in body weight or BMI. The

comparison of the control groups of this study (CS and CE) showed that group CE had: (1) decreased body weight, Lee index, and BMI at the age of 120 days, (2) decreased body fat (significant for the mesenteric and ovarian fats); and (3) increased ovarian and decreased retroperitoneal adipocyte size. On the other hand, the female SE rats did not have significant changes of these values when compared to the sedentary (SS), except for increased subcutaneous and retroperitoneal adipocyte size, despite having a better performance during the exercise sessions than group CE, as observed by Santos et al. (43). In this way, treadmill walking had some positive effects on group CE, while group SE was, for the major part, refractory to this intervention. In this context, it seems relevant to the observation that the adrenergic efferentation is reduced in overfed rats subjected to exercise (44), which could explain, at least partially, the absence of body weight and fat weight loss in the female SE rats. Nery et al. (33) report the opposite changes of body weight in rats from small and large litters subjected to swimming, compared with their sedentary counterparts, and argue that swimming was not efficient in promoting the changes of body composition.

There was a trend (or significant) decrease of fat pad weight in the exercised groups (CE and SE) compared with their sedentary counterparts (CS and SS), but this was not always matched by adipocyte size, which tended to behave in the opposite direction (larger adipocytes in the exercised vs. the sedentary groups). It has to be noted, however, that there was a consistent and significant decrease of the retroperitoneal adipocyte diameter in the exercised groups (CE and SE). As visceral fat is associated with the changes in peripheral and hepatic insulin sensitivity seen in obesity (5, 15, 29, 49), the reduced size of retroperitoneal adipocytes promoted by treadmill walking may be relevant.

In addition, although cellularity of the retroperitoneal fat was an estimation, the higher values for the small-litter females are in accordance with the literature data, that adipose tissue cellularity is established by nutritional conditions early in life (9); this feature was not reversed by exercise in the present investigation.

Obesity in human populations and several rodent models is associated with insulin resistance and glucose intolerance (7, 29, 42). In these instances, it is expected as follows: 1) higher fasting blood glucose, 2) greater blood glucose increase and lower rate of decay after oral administration of glucose, and 3) lower decrease of blood glucose after insulin administration. During the GTT, orally given glucose is absorbed by the gastrointestinal tract to the blood, increasing blood glucose, and stimulating the release of insulin by pancreatic beta cells (28). Next, insulin, especially on liver, adipose tissue and muscle, promote glucose uptake, oxidation, and storage, thus decreasing blood glucose. The kinetics of the change on blood glucose after its oral administration is an indirect indicative of the efficacy of endogenous insulin in restoring the blood glucose levels.

Groups CS and SS did not differ on their glycemic kinetics during the 120 min of the GTT, as can be seen by their similar blood glucose values at 0, 15, and 120 min and kGTT. However, the AUC was significantly lower in the SS. Probably, this was because, although the differences between CS and SS were not significant, in group SS blood glucose at 0 and 120 min was higher, and at 15 min lower, than in CS. Taken together, these small differences resulted in a mean AUC statistically smaller in group SS. Larger AUCs in rats from small litters are a more common finding (42), but not always (20). In this study, the GTT values are consistent with the biometric data discussed above, and do not indicate overt changes in glucose tolerance in the female SS rats.

Fasting blood glucose (0 min of GTT) was lower in the exercised than in the sedentary groups, and that could be the result of an increased basal sensitivity to endogenous insulin

caused by treadmill walking during 100 days in groups CE and SE. The return of the AUC of GTT of group SE to values similar to the control groups (CS and CE) resulted from the lower basal (0 min) and higher peak (15 min) blood glucose during the test compared with the SS; this combination increased the blood glucose variation (i.e., the AUC) during the 120 min of the test.

ITT follows the behavior of blood glucose after the administration of a "bolus" of insulin (30, 31). The kinetics of the blood glucose reflects the capacity of the exogenous insulin in promoting glucose uptake by the tissues. The return of blood glucose toward the initial values is an indicative of the: (1) action of the endogenous counterregulatory hormones – glucagon, catecholamines, growth hormone, and glucocorticoids – than counteract insulin to prevent blood glucose from falling below physiologically acceptable values and (2) vanishing of the exogenous insulin (10).

The SS female rats did not differ from those of CS during the ITT except for the lower blood glucose at 120 min. Therefore, insulin resistance, common in rodents from small litters, was not found in the females of this study. Despite the lower basal blood glucose in the exercised groups, there was no evidence of increased insulin sensitivity, as assessed by ITT.

Running times before exhaustion did not differ between groups CS and SS; however, blood lactate was higher in the SS. Although there is much debate about the physiological significance of lactate assays in exercise and sports physiology (2, 4, 16, 22), higher lactate levels with equal running times/speeds before exhaustion in incremental tests may indicate more marked anaerobic metabolism, and thus poorer aerobic conditioning, which would be plausible in this study considering the characteristics of group SS compared to group CS.

The exercised rats (CE and SE) had a better performance during the test of exhaustion than their sedentary counterparts (CS and SS), demonstrating that regular long-term physical activity improved aerobic conditioning, reflected in the greater time to exhaustion. Once again, there was no correlation between blood lactate and time to exhaustion in the incremental test. The post-test lactate levels were higher in CE than in CS, but did not differ significantly between SS and SE. Taken together with the greater time to exhaustion in group CE compared with CS, and considering that the final speed reached by CE at the end of the test was 60% higher than in group CS, it is possible to suggest that group CE could have a better anaerobic capacity and overall performance. It is worth stressing, once more, that lactate measurements and their metabolic meaning are still the focus of intense research in exercise physiology; a few studies employ treadmill incremental tests and these analyses do not have a standard methodology.

The inability of the female SS rats of giving birth was a surprising observation. A report on the reproductive function of these animals is presented by Santos et al. (43).

The male rats whose mothers were exercised (groups CEp and SEp) had biometric and adiposity measurements – body weight, nasoanal length, WL ratio, liver weight, and visceral fat – and fasting blood glucose lower than those from sedentary mothers (group CSp). The results described in this study seem to point to a beneficial effect of regular physical activity of the dams on their litters, which is independent of maternal adiposity, as both groups of litters from exercised mothers had lower biometric and blood glucose indexes than those of sedentary mothers. Experimental interventions on the mothers are often reflected on their litters, but the specific transgenerational outcome depends on the intervention (e.g., physical activity protocol, food manipulation, etc.) and the parameter under assessment (7, 21, 26, 37, 38, 47). It should be noted that treadmill walking in the pregnant dams from CE and SE was

interrupted only during the third week of gestation and first week of lactation, so that exercise was present during critical periods of the pre- and post-natal development of the offspring (7, 45).

Conclusions

The model of obesity in rats by litter size reduction had little impact on the females of this study, in light of the results obtained compared to those frequently found in the literature. Factors such as age (120 days rather than younger animals) and gender (female rats instead of males) may have influenced the results. It deserves to mention, though, that adipocytes were consistently smaller in the small-litter groups than in the control groups, regardless of exercise.

Treadmill walking had some different effects on the exercised control and small-litter groups, suggesting that the effects of overfeeding during lactation, even when subtle, might change the response to light physical activity later in life. It is possible that early overfeeding, even when adult obesity is not seen, demands higher levels of physical activity to positively change the body composition. Nevertheless, the response of the retroperitoneal (i.e., visceral) adipocytes was a positive observation in both exercised groups.

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