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Multigenerational effects of chronic maternal exposure to a high sugar/fat diet and physical training

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Original Article

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Abstract

Pregnant individuals who overeat are more likely to predispose their fetus to the development of metabolic disorders in adulthood. Physical training is a prevention and treatment interventional strategy that could treat these disorders, since it improves metabolism and body composition. This study assessed the protective effect of physical exercise against possible metabolic changes in generations F1 and F2, whose mothers were subjected to a high-sugar/high-fat (HS/HF) diet. Wistar rats belonging to generation F0 were distributed into four groups ($n = 10$): sedentary control (CSed), exercised control (CExe), sedentary HS/HF diet (DHSed) and exercised HS/HF diet (DHExe). From 21 to 120 days of age, maintained during pregnancy and lactation period, CSed/CExe animals received standard feed and DHSed/DHExe animals a HS/HF diet. Animals from the CExe/DHExe underwent physical training from 21 to 120 days of age. Male and female F1 and F2 received a normocaloric feed and did not perform any physical training, categorized into four groups ($n = 10$) according to the maternal group to which they belonged to. An increase in body weight, adiposity and glucose, and a change in lipid profile in F0 were observed, while exercise reduced the biochemical parameters comparing DHSed with DHExe. Maternal exercise had an effect on future generations, reducing adiposity, glucose and triglyceride concentrations, and preventing deleterious effects on glucose tolerance. Maternal overeating increased health risks both for mother and offspring, demonstrating that an HS/HF diet intake promotes metabolic alterations in the offspring. Importantly, the physical training performed by F0 proved to be protective against such effects.

Introduction

Epidemiologic and experimental evidence have demonstrated that maternal overeating during pregnancy and/or lactation is associated with the development of chronic diseases in future generations. This relation has been termed metabolic programming, defined as the biological phenomenon that occurs between nutritional experiences in early life and the emergence of adulthood diseases. Its origins are associated with epigenetic modifications, such as DNA methylation and histone acetylation during critical life periods.[1–3]

There is evidence that offspring are sensitive to nutritional, hormonal and behavioral changes of the mother during the pregnancy and lactation periods,[4–6] resulting in the metabolic programming that contributes to the emergence of obesity-associated diseases, such as hyperinsulinemia, insulin resistance and glucose intolerance, among others.[7,8] Maternal overeating through a hypercaloric diet is the main risk factor for metabolic changes in newborns, which may develop into obesity in adulthood.[9,10]

Studies have demonstrated that previously overeat mothers gave birth to overweight offspring, which also suffered from physiological and metabolic changes developed from the neonatal period to adulthood. These changes are possibly generated by epigenetic mechanisms and may be transmitted to subsequent generations.[10] Overweight and obesity originated through metabolic programming has become a worldwide epidemic, resulting in the need to create strategies to reverse this situation and improve global health parameters.[11] In addition to a healthy nutrition program and other therapeutic procedures, as some medicine to lose weight, physical exercise is clinically proven to be a low-cost primary intervention for the treatment of obesity and associated chronic diseases.[12–15]

Although physical exercise has many health benefits, the effect of maternal exercise on the offspring metabolic phenotype is not quite clear.[12] Regarding animal models, some studies have indicated that maternal exercise improves glucose tolerance as far as the control and obese litters are concerned.[9] The present study tests the hypothesis of whether maternal overweight can

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Table 1. Control diet and HS/HF diet composition

Contents	Normocaloric diet Nuvilab®	High-sugar/high-fat diet Pragsoluções®
Protein	22 %	19.17 %
Carbohydrate	46 % (starch)	35.73 % (22 % sucrose)
Lipids	4.0 %	45.10 %
Mineral/fiber/ humidity	9.0/7.0/12 %	-
Total kcal/g	3.7	4.7

cause metabolic changes in offspring of subsequent generations and if moderate exercise training protects the next two generations against the development of metabolic syndrome in adult life.

Material and methods

All experimental procedures were previously approved by the Juiz de Fora Federal University Ethics Committee on Animal Use/ CEUA (n. 067/2013).

Animal source

The animals were obtained from the Reproductive Biology Center (CBR) breeding facilities belonging to the Juiz de Fora Federal University (UFJF), CIAEP n. 01.0048.2013.

Freshly weaned female Wistar rats ($n = 40$) 21 days old (F0) were used, weighing 25–35 g. All animals were maintained in polypropylene cages with four individuals per cage, in ventilated cabinets with controlled airflow, temperature (22°C ± 2°C) and relative air humidity (60 % ± 10 %). The animals had free access to feed and non-sterilized filtered water under a 12:12-h light–dark cycle, from 6 AM to 6 PM.

Experiment outline

F0 generation

F0 generation females ($n = 40$) were distributed into four groups: sedentary control group (CSed; $n = 10$), exercised control group (CExe; $n = 10$), sedentary high-sugar/high-fat (HS/HF) diet group (DHSed; $n = 10$) and exercised HS/HF diet group (DHExe; $n = 10$). The control group received commercial Nuvital® standard feed (Nuvilab CR-1 autoclavable, Colombo-PR, Brazil) containing 19 % protein, 46 % carbohydrate, 4.0 % lipids, 5 % cellulose and 4.5 % vitamins and minerals, providing 3.7 kcal/kg of feed, considered normocaloric. The HS/HF diet group received a pelleted feed rich in sucrose and lipids, composed of 19.17 % protein, 35.73 % carbohydrate, 22 % being sucrose and 45.10 % lipids, providing 4.7 kcal/kg (Table 1), premanufactured by the PragSoluções Comércio e Serviços Ltda® company (Jaú-SP, Brazil). All groups received the specified diet from 21 to 120 days of age, maintained during the pregnancy and lactation periods (Fig. 1).

Physical training protocol

Physical training was developed according to the exercise protocol reported by previous study,[16,17] with adaptations. The training protocol consisted of running on an automated treadmill with six bays (Insight® São Paulo-Brazil) at a 5° slope.

The animals from the exercised groups (CExe/DHExe) began treadmill training at 21 days old. First, animals faced 2 weeks of adaptation composed by five sessions at an initial speed of 10 m/min for a 10-min period. Subsequently, training sessions took place three times a week and 1 m/min speed was increased weekly, at the 2–3 min mark. This training was maintained until the animals reached 120 days of age, closing the training as a whole at a speed of 23 m/min for 75 min.

Both animal groups, sedentary and exercised, also went through three physical maximum exercise stress tests (MES test) with the aid of a Havard Aparatus® gas analyzer and individual rodent treadmill (Panlab technology for bioresearch, Harvard Apparatus® Barcelona-Spain) at a 5° slope (Fig. 1). The MES test protocol began at a 10 m/min speed, with a 1 m/min increase every 3 min. The fatigue point was determined as the moment the animal could not keep up with the pace demanded by the test stage. When this condition was reached, the stimulus grid was immediately disconnected, and the animal removed from the treadmill. The physical performance of the animals was measured by calculating the work, expressed in kilograms per meter, using the following formula:

$$\text{Kilogram-meter (Kgm)} = \text{Body weight (Kg)} \times \text{running time (min)} \times \text{treadmill speed (m/min)} \times \text{treadmill inclination (deg)}.[18]$$

F1 generation

When F0 generation females ($n = 40$) were 120 days of age, the estrous cycle stage of all groups was verified through a vaginal smear. Proestrus females were housed in cages at a three females to one male ratio. The presence of spermatozoa in the vaginal smear, performed the morning after mating, was considered indicative of likely pregnancy. Close to the probable date of delivery (20th day post-insemination), the rats were placed in individual boxes to allow for nest building and birth to the offspring from which the next generation (F1) would be assembled. The HS/HF diet was maintained throughout pregnancy and lactation. Litter size was not standardized at birth. The mean litter size for each treatment group was 8–10 pups.

After offspring weaning, 21 days after birth, both F1 male and female pups ($n = 80$) were weighed and distributed into 4 groups of 10 animals each, according to the maternal F0 group to which they belonged to: sedentary control (CSed1), exercised control (CExe1), sedentary HS/HF diet (DHSed1) and exercised HS/HF diet (DHExe1). All F1 animals received standard feed (Nuvital® feed, Curitiba, Brazil) and did not perform any physical treadmill training.

F2 generation

F1 females were mated with control males from the vivarium to obtain the F2 generation and were maintained in the same aforementioned conditions until birth and weaning. Litter size was not standardized at birth. The mean litter size for each treatment group was 8–10 pups. All were fed the standard feed and did not perform any exercise.

After weaning, 21 days after birth, both F2 male and female pups were also weighed and distributed into 4 groups of 10 animals each according to the maternal F1 group to which they belonged to: sedentary control (CSed2), exercised control (CExe2), sedentary HS/HF diet (DHSed2) and exercised HS/HF diet (DHExe2).

All F2 animals also received the standard feed and were not subjected to physical treadmill training.

Oral glucose tolerance test (OGTT)

At 90 days of age, F0, F1 and F2 animals were subjected to a 6-h fasting (07:00–13:00) in order to undergo an OGTT, subsequently housed in individual cages. A very small cut was made on the tail of

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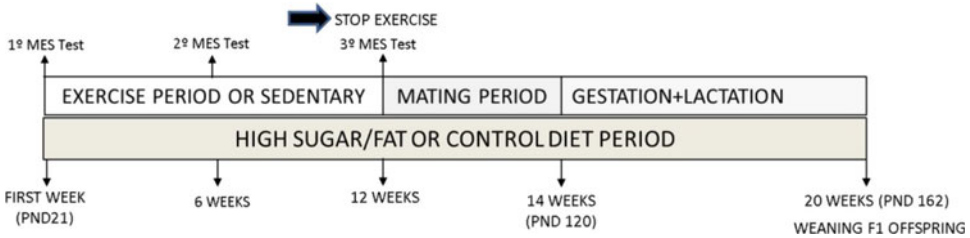

Fig. 1. Experimental design for the F0 generation. Maximum exercise stress test (MES test). Postnatal day (PND). During the mating period, all females were mated with control males from the vivarium.

each animal to obtain blood for glucose dosing. The first measure was taken at time zero (T0). The test proceeded by inserting a 50 % glucose solution via intragastric route at 2 g/kg of body weight, after the glucose injection blood samples were collected at times T1 (15′), T2 (30′), T3 (60′) and T4 (120′) and the total area under the OGTT curve was calculated. All samples were analyzed using a glucometer (Accu-ChekBlood Glucose Meters Roche® Germany). Serum glucose was also measured at the euthanasia moment using a commercial **Cobas c111- (Roche®** – Switzerland) kit.

Euthanasia

After a 6-h fasting, the animals were euthanized through total exsanguination under ketamine (90 mg/kg ip.) and xylazine (10 mg/kg ip.) anesthesia (König SA®, Avellaneda, Argentina), followed by diaphragm rupture. Perigonadal and retroperitoneal fats were removed and weighed in a fine scale (Bioprecisa®-specificity 0.0001 g-Brasil) and calculated the relative fat mass to their body mass. Serum glucose, triglyceride and total cholesterol levels were evaluated in F0, F1 and F2 animals, and determinations were performed using an automated equipment and commercial **Cobas c111- (Roche®** – Switzerland) kit.

Statistical analyses

The Shapiro–Wilk test was applied to verify normality and homoscedasticity assumptions. In the descriptive analysis, the studied variables were expressed in terms of their corresponding means and standard deviation. For the homocedastic data analysis a two-way ANOVA test was performed, followed by Bonferroni's *post hoc* test to identify possible differences between groups. Data sets were significantly different when considering the main effect of exercise (E), HF/HS Diet (D), their interaction (D × E; diet vs. exercise). The GraphPad Prism® version 8.0 for windows software (GraphPad Software Inc., San Diego, CA, USA) was used and $P < 0.05$ values were considered statistically significant.

Results

Maternal evaluation (F0)

Animals from the sedentary HS/HF diet group (DHSed) displayed weight gain (+8.66 %), increase in relative perigonadal (+50.79 %) and retroperitoneal (+64.48 %) fat weight, and high glucose and cholesterol levels when compared to the sedentary control group (CSed). DHSed compared to the exercised HS/HF diet group (DHExe), decreased body weight (−10.37 %) and serum glucose (−17 %), triglyceride (−32 %) and total cholesterol (12.32 %) levels were observed, as presented in Table 2. Statistically, no significant differences between control groups were observed. High-fat diet or exercise had no effect on the food intake (data not shown).

The OGTT indicated a 12.47 % increase in the area under the glycemic curve in the DHSed group when compared to the CSed group (Fig. 2).

Regarding physical training protocol efficiency, the work computing indicated a scenario where exercised CExe and DHExe groups displayed enhanced physical performance compared to the CSed and DHSed sedentary groups, as observed in Fig. 3.

Offspring evaluation

F1 females

Table 3 results indicated that the HS/HF diet consumed by the mother led to a body weight increase in DHSed1 pups at 90 days of age adulthood when compared to CSed1 pups from mothers that did not receive the HS/HF diet. A 7.36 % increase in body weight and a 6.69 % increase in glycemia were also noted. In turn, when comparing CSed1 and CExe1, 51.26 % and 106.75 % decreases in relative perigonadal and retroperitoneal fat mass were observed, respectively, as well as a decrease in fasting glucose (−8.73 %) and total cholesterol (−12.80 %) levels. Comparing DHSed1 and DHExe1, a 11.30 % decrease in body weight, relative perigonadal (−28.34 %) and retroperitoneal (−59.40 %) fat mass and fasting glucose (−9.62 %) were noted.

F1 males

Comparisons between DHSed1 and CSed1 groups indicate an increase in relative perigonadal (+15.78 %) and retroperitoneal (+9.71 %) fat mass in the adult F1 male offspring (Table 4). In turn, decreased body weight (−17.06 %), relative perigonadal (−35.71 %) and retroperitoneal (−45.83 %) fat mass and fasting glucose and triglycerides were observed when comparing CSed1 and CExe1. This decrease was also observed when comparing the DHSed1 and DHExe1 groups, as follows: body weight (−6.61 %), relative perigonadal (−18.56 %) and retroperitoneal (−25.59 %) fat mass, and fasting glucose and triglycerides.

The OGTT, represented by Fig. 4, indicates that effects of physical training can only be noticed due to a reduction of the glycemic curve in females. In males, however, the HS/HF diet consumed by F0 animals also contributed to the effect, leading to glucose intolerance in F1. Physical training led to effects only for the DHExe1 group.

F2 females

A decrease in relative retroperitoneal fat mass (−37.64 %) and total cholesterol levels (−12.04 %) was observed when comparing CExe2 and CSed2 F2 females. In turn, among HS/HF diet groups, a decrease in body weight (−9.76 %) was noted in the DHExe2 group when compared to DHSed2. These results are displayed in Table 5.

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Table 2. Body weight, perigonadal and retroperitoneal fat relative mass and maternal biochemical parameters (F0 generation)

Variables	CSed	CExe	DHSed	DHExe	Source of variation
Body weight at 120 days (g)	181.10 ± 9.93	182.10 ± 6.77	196.80 ± 11.71*	178.30 ± 10.22#	D/DE
Body weight after weaning (g)	212.20 ± 12.02	209.30 ± 14.42	210.70 ± 11.14	206.00 ± 14.86	NS
Perigonadal fat (g/relative)	1.89 ± 0.34	1.89 ± 0.16	2.85 ± 0.73*	2.77 ± 0.32	D
Retroperitoneal fat (g/relative)	1.07 ± 0.26	1.03 ± 0.21	1.76 ± 0.57*	1.40 ± 0.26	D
Glucose (mg/dl)	173.90 ± 2.86	162.70 ± 8.01	187.90 ± 7.05*	160.40 ± 15.35#	D/DE
Triglycerides (mg/dl)	31.33 ± 7.09	31.87 ± 4.71	32.12 ± 6.31	24.16 ± 3.43#	DE
Total cholesterol (mg/dl)	62.13 ± 5.21	60.44 ± 3.01	68.64 ± 5.53*	61.11 ± 4.55#	DE

Data are expressed as means ± standard deviation (MDP) of the analyzed parameters ($n = 8$–10). The symbols represent significant differences ($P < 0.05$) between groups.
Source of variation, D, Diet factor, DE interaction between the exercise factor and Diet, and NS no interaction.
*DHSed compared to CSed.
#DHSed compared to DHExe.

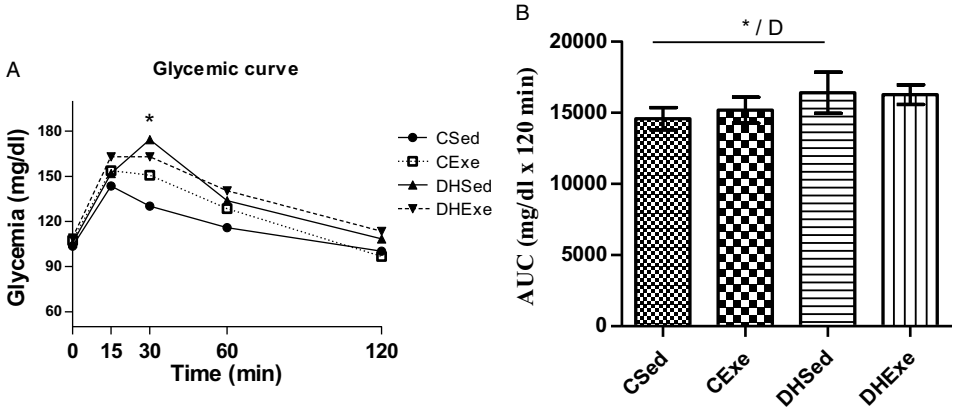

Fig. 2. The A graph represents the glycemic curve and the B graph represent the total area under the glycemic curve. The error range in the latter represents the standard deviation (SD) (n = 8 to 10). The symbols above the lines represent significant differences (p<0.05) between groups. *DHSed compared to CSed. Source of variation D, Diet factor.

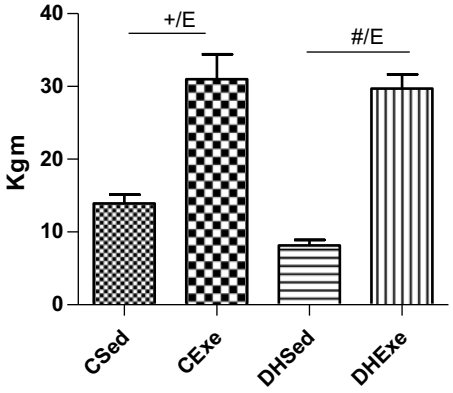

Fig. 3. The bar graph represents work during the third stress test. The error range in the latter represent the standard deviation (SD) (n = 6 to 8). The symbols above the lines represent significant differences (p<0.05) between groups. +CSed compared to CExe; #DHSed compared to DHExe. Source of variation, E, exercise factor.

F2 males

Increased body weight (+14.33 %) and relative perigonadal (+25.49 %) and retroperitoneal (+45.71 %) fat mass are observed when comparing DHSed2 to the control group CSed2. A reduction in relative perigonadal fat mass (−14.70 %), as well as in the fasting triglyceride (−37.96 %) and total cholesterol (−11.70 %) levels in the control group CExe2 compared to CSed2 was noted. Among HS/HF diet groups, the DHExe2 exercised group displayed decreased body weight (−15.63 %) and relative mass of perigonadal (−24.67 %) and retroperitoneal (−52.23 %) fat mass, as well as a reduction in triglyceride levels (−96.45 %). Results are presented in Table 6.

Regarding the OGTT, represented by Fig. 5, the protective effect of physical training along with a reduction of the glycemic curve is observed in females and males only when comparing the HS/F diet groups DHSed2 and DHExe2.

Discussion

Dietary and physical training effects on the F0 generation

The results reported in the present study confirm previous findings, indicating that animals exposed to a HS/HF diet tend to display weight gain and metabolic changes when compared to those on a standard diet. This confirms dietary efficiency, as it is rich in lipids and/or carbohydrates.[12,19–21] However, to date, no reports are available in the literature concerning the effects of the interaction between the sucrose- and lipid-rich diet applied in this study and physical treadmill training before pregnancy.

Our study corroborates literature data, proving that exercise displays an important translational implication for maternal obesity. Despite some the fact that some authors have applied

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Table 3. Body weight, perigonadal and retroperitoneal fat relative mass and biochemical parameters in F1 females

Variables	CSed1	CExe1	DHSed1	DHExe1	Source of variation
Body weight at 90 days (g)	166.90 ± 11.27	159.40 ± 5.63	179.20 ± 6.04*	161.00 ± 12.48#	E/D
Perigonadal fat (g/relative)	2.39 ± 0.32	1.58 ± 0.13+	2.40 ± 0.25	1.87 ± 0.26#	D
Retroperitoneal fat (g/relative)	1.53 ± 0.13	0.74 ± 0.13+	1.61 ± 0.10	1.01 ± 0.15#	E/DE
Glucose (mg/dl)	171.80 ± 8.09	158.00 ± 7.58+	183.30 ± 8.92*	167.20 ± 5.64#	E/D
Triglycerides (mg/dl)	45.77 ± 5.33	39.24 ± 6.07	42.44 ± 11.98	37.68 ± 8.08	NS
Total cholesterol (mg/dl)	69.26 ± 5.01	61.40 ± 5.03+	66.94 ± 6.90	58.33 ± 9.60	E

Data are expressed as means ± standard deviation (MDP) of the analyzed parameters ($n = 8$–10). The symbols represent significant differences ($P < 0.05$) between groups.
*DHSed compared to Csed; +CSed compared to CExe; #DHSed compared to DHExe. Source of variation, E, exercise factor, D, Diet factor, DE interaction between the exercise factor and Diet, and NS no interaction.

Table 4. Body weight, perigonadal and retroperitoneal fat relative mass and biochemical parameters in F1 males

Variables	CSed1	CExe1	DHSed1	DHExe1	Source of variation
Body weight at 90 days (g)	284.70 ± 9.24	243.20 ± 6.35+	280.50 ± 8.91	263.10 ± 14.11#	E/DE
Perigonadal fat (g/relative)	1.71 ± 0.07	1.26 ± 0.12+	1.98 ± 0.04*	1.67 ± 0.08#	E/D/DE
Retroperitoneal fat (g/relative)	1.75 ± 0.09	1.20 ± 0.14+	1.92 ± 0.11*	1.53 ± 0.11#	E/D
Glucose (mg/dl)	185.40 ± 2.80	171.70 ± 13.29+	191.70 ± 3.55	172.20 ± 7.43#	E
Triglycerides (mg/dl)	62.05 ± 11.46	37.03 ± 5.98+	64.08 ± 19.06	41.21 ± 7.40#	E
Total cholesterol (mg/dl)	70.23 ± 3.11	65.22 ± 5.39	72.18 ± 3.37	71.49 ± 4.34	NS

Data are expressed as means ± standard deviation (MDP) of the analyzed parameters ($n = 8$–10). The symbols represent significant differences ($P < 0.05$) between groups. *DHSed compared to CSed; +CSed compared to CExe; #DHSed compared to DHExe. Source of variation, E, exercise factor, D, Diet factor, DE interaction between the exercise factor and Diet, and NS no interaction.

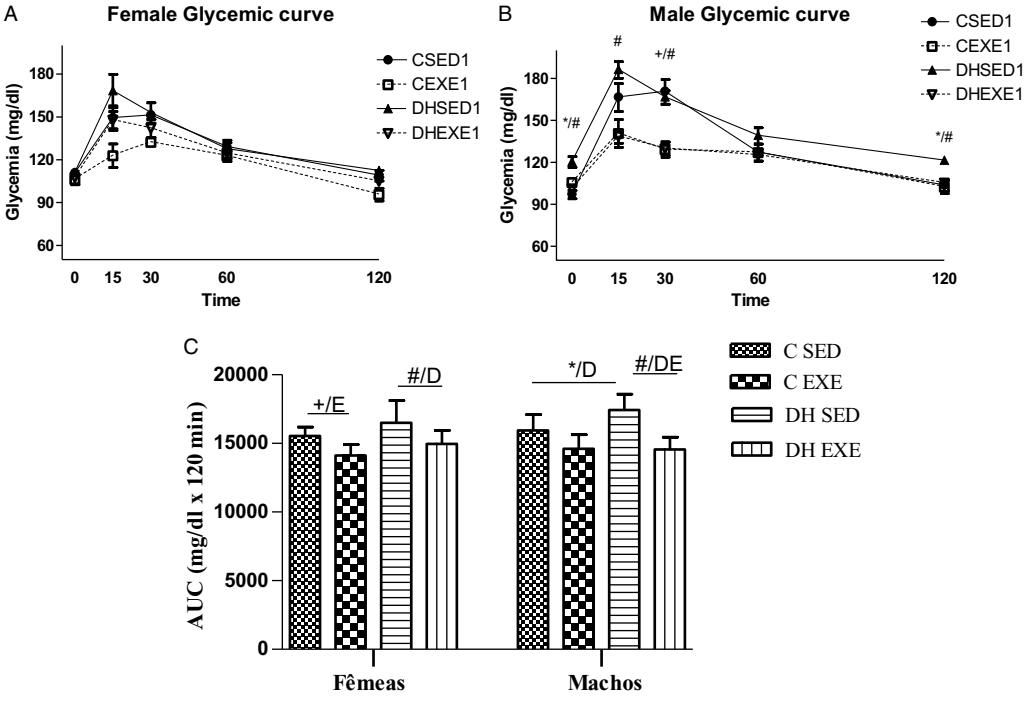

Fig. 4. The A and B graph represents the female and male glycemic curve respectively and the C graph represent the total area under the glycemic curve. The error range in the latter represents the standard deviation (SD) (n = 8 to 10). The symbols above the lines represent significant differences ($p < 0.05$) between groups. *DHSed compared to CSed; +CSed compared to CExe; #DHSed compared to DHExe. Source of variation, E, exercise factor, D, Diet factor and DE interaction between the exercise factor and Diet.

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Table 5. Body weight, perigonadal and retroperitoneal fat relative mass and biochemical parameters in F2 females

Variables	CSed2	CExe2	DHSed2	DHExe2	Source of variation
Body weight at 90 days (g)	167.20 ± 14.06	161.90 ± 10.39	179.80 ± 9.24	162.90 ± 10.90$^{\#}$	E
Perigonadal fat (g/relative)	1.92 ± 0.60	1.54 ± 0.32	2.37 ± 0.30	1.97 ± 0.60	NS
Retroperitonealfat (g/relative)	1.17 ± 0.38	0.85 ± 0.16^{+}	1.28 ± 0.17	1.02 ± 0.20	E
Glucose (mg/dl)	168.50 ± 14.07	170.00 ± 13.23	166.50 ± 5.24	168.50 ± 5.94	NS
Triglycerides (mg/dl)	38.13 ± 10.52	38.98 ± 3.52	43.50 ± 11.15	35.41 ± 5.10	NS
Total cholesterol (mg/dl)	64.35 ± 6.51	57.43 ± 4.76^{+}	67.00 ± 3.69	67.15 ± 2.52	E

Data are expressed as means ± standard deviation (MDP) of the analyzed parameters ($n = 8$–10). The symbols represent significant differences ($P < 0.05$) between groups. $^{+}$CSed compared to CExe; $^{\#}$DHSed compared to DHExe. Source of variation, E, exercise factor and NS no interaction.

Table 6. Body weight, perigonadal and retroperitoneal fat relative mass and biochemical parameters in F2 males

Variables	CSed2	CExe2	DHSed2	DHExe2	Source of variation
Body weight at 90 days (g)	267.20 ± 18.78	264.00 ± 10.37	305.50 ± 19.00*	264.20 ± 19.65$^{\#}$	D/DE
Perigonadal fat (g/relative)	1.53 ± 0.15	1.36 ± 0.13^{+}	1.92 ± 0.28*	1.54 ± 0.16$^{\#}$	E/D
Retroperitoneal fat (g/relative)	1.40 ± 0.26	1.38 ± 0.26	2.04 ± 0.45*	1.34 ± 0.18$^{\#}$	E/D/DE
Glucose (mg/dl)	183.30 ± 15.19	182.00 ± 14.27	184.20 ± 13.30	175.10 ± 8.08	NS
Triglycerides (mg/dl)	68.43 ± 14.37	49.60 ± 16.04^{+}	68.66 ± 12.51	34.95 ± 9.67$^{\#}$	E
Total cholesterol (mg/dl)	73.67 ± 5.58	65.95 ± 6.85^{+}	68.00 ± 4.77	61.34 ± 3.26	E

Data are expressed as means ± standard deviation (MDP) of the analyzed parameters ($n = 8$–10). The symbols represent significant differences ($P < 0.05$) between groups. *DHSed compared to CSed; $^{+}$CSed compared to CExe; $^{\#}$DHSed compared to DHExe. Source of variation, E, exercise factor, D, Diet factor, DE interaction between the exercise factor and Diet, and NS no interaction.

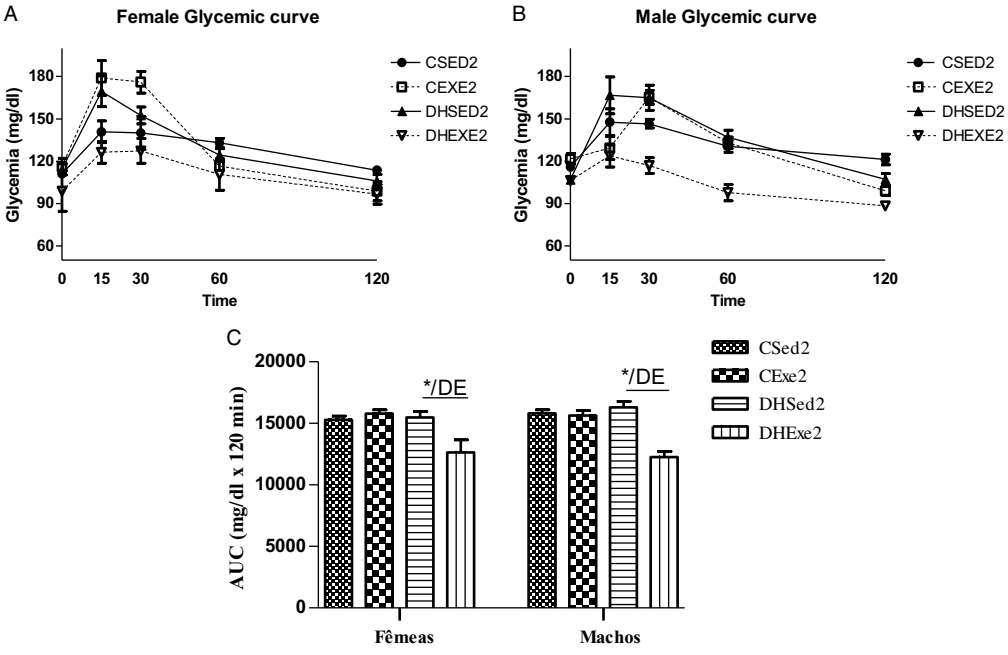

Fig. 5. The A and B graph represents the female and male glycemic curve respectively and the C graph represent the total area under the glycemic curve in F2. The error range in the latter represents the standard deviation (SD) (n = 6 to 8). The symbols above the lines represent significant differences ($p < 0.05$) between groups. $^{\#}$DHSed compared to DHExe. Source of variation, DE interaction between the exercise factor and Diet.

voluntary exercise, in order to control exercise speed and frequency, an established protocol was used, and the results confirm that the treadmill running training protocol was effective, since the work calculation results indicate improvements in the physical performance of trained animals, even when consuming an HS/HF diet. Physical training prior to pregnancy resulted in health benefits, such as decreased body weight and lipid and glycemic profile improvements in the DHExe group compared to the DHSed group. These results are aligned with studies that indicate that rats subjected to physical training when on a fat-rich diet present lower body weight gain[22] and glucose, triglyceride and total cholesterol level decreases.[20,23,24]

The importance of physical training on body weight maintenance and aerobic metabolism improvements has been shown[25]

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and is associated with positive physiological adaptations. These promote increased metabolic rate and stimulate the use of energetic substrates, such as glucose and fatty acids, which lead to direct effects mainly on serum glucose and lipid levels.[26,27] This, in turn, leads to improvements in glycemic and lipid profiles. Therefore, physical training can prevent and attenuate comorbidities, restoring normoglycemia and insulin sensitivity, as well as dyslipidemia, optimizing metabolic profile changes.[27–29] Regarding maternal generation, the HS/HF diet resulted in a slight glucose intolerance among sedentary groups and, corroborating literature assessments, decreased glucose uptake and subsequent cellular oxidation, along with increased gluconeogenesis via hepatic pathways, may result in hyperglycemia.[26,30] However, no significant changes among the exercised groups were observed. This may be due to the removal of the exercise stimulus during pregnancy and lactation in the CExe and DHExe groups.[30]

Consistent with findings of other studies applying diet and exercise prior or during pregnancy, it may be better to focus on interventions that improve metabolic fitness rather than only controlling maternal body weight, important in light of recent human studies that have indicated the possibility of modulating behavior in obese pregnant women as a potential long-term effect of these interventions in offspring.[31] These effects were observed in the present study, where exercise prior to pregnancy contributed to the improvement of metabolic parameters in the mother and/or the offspring.

According to literature, some mechanistic pathways may explain the beneficial effects of maternal exercise in obese mother glucose and triglyceride concentrations. In this case, maternal exercise effect may be linked to reduced circulating blood glucose due to increased glucose uptake rates in skeletal muscles and other peripheral tissues. In addition, some of the metabolic effects of maternal exercise during pregnancy in adult offspring, such as improvement of glucose tolerance, may be associated with enhanced insulin sensitivity, as physical activity is well known to improve both glucose tolerance and insulin sensitivity.[23,32–34]

Effects on descendants

Concerning the transgenerational metabolic programming model, the results of the present study demonstrate the model efficacy in inducing changes to offspring in future generations. This was more evident in males, especially those belonging to the F2 generation, which presented increased body weight, adiposity and cholesterol in the groups descending from the F0 generation that consumed a HS/HF diet (DHSed2). This was not observed in females. This study thus demonstrates the transgenerational effect of the sucrose- and lipid-rich diet and the physical treadmill training prior to pregnancy.

The HS/HF diet offered to sedentary mothers led to increased adiposity and body weight and glycemia in F1 males and females. Concerning the effects of exercise performed by mothers in both the CExe control group and DHExe diet group, decreased body weight, adiposity and glycemia were observed both in females and males. In addition, males also experienced improvements in triglyceride levels.

The results also indicate that a maternal high-fat and simple carbohydrates diet causes glucose intolerance in males and indicated that maternal physical training was effective in preventing these harmful effects in both males and females. This is corroborated by the study carried out by Stanford *et al.*,[12] who recently demonstrated that C57/Bl6 mice born from mothers consuming 60 % high-fat diet and placed in cages with running wheels before and during pregnancy presented improvements in the glucose tolerance test, suggesting that offspring were protected against maternal diet-induced glucose intolerance.

For F2, the results were more evident in males compared to females. Exercise effects on body weight and total cholesterol reduction were observed in females. In males, aside from a significant increase in weight, adiposity and cholesterol in the group whose F0 generation ingested a HS/HF diet, exercise was effective in improving both lipid and adiposity profiles.

A possible aerobic training-derived mechanism is ATP regeneration capacity, which increases mitochondrial enzymatic activity, mitochondrial density and oxygen consumption.[35,36] Epigenetic changes occur in skeletal muscle due to decreases in intracellular oxygen concentrations during exercise. In addition, physical training activates the intracellular peroxisome proliferator-activated receptor coactivator-1α (PGC-1 α) pathway, which is considered an energetic metabolism regulator in skeletal muscle and adipose tissue. It is also responsible for increasing mitochondria biogenesis,[37–39] protecting offspring from obesity, Type 2 diabetes mellitus and any harmful effects derived from a high-fat diet consumed by the maternal generation.[23,40]

One of the consequences of hypercaloric, hypolipemic or hyperglycemic diet consumption during pregnancy and lactation is a glycemic profile alteration in adult descendants.[6,41–43] Modulation of pancreatic β-cells may also occur, implying in glycemia alterations.[44]

Physical exercise performed by the maternal generation reduced plasma glucose levels of their progeny, possibly due to enhanced glucose uptake by skeletal muscles.[23] Other studies have also reported the same effect in F1 progeny, in which maternal exercise reduced plasma glucose concentrations, more effective in males than in females, and that such an effect was associated with increased glucose transporter type 4 (GLUT4) levels in skeletal muscle.[12,24]

Results from other studies have indicated that acute and chronic exercise effects in rats lead to increases in PGC1α expression in white adipose tissue,[23,45] capable of limiting visceral fat accumulation and reducing circulating triglyceride levels.[21,24,46] Thus, it has been demonstrated herein that chronic physical exercise performed leads to a protective effect in subsequent generations.

Conclusions

Maternal obesity in rats during pregnancy increases health risks both to the mother and the offspring, inducing increased body weight, adiposity, hyperglycemia and damaging the lipid profile. Light-to-moderate physical training performed by the F0 diet group prior to pregnancy resulted in benefits in normalizing blood glucose, triglyceride and cholesterol concentrations. Maternal exercise seems to lead to beneficial effects on limiting the impact of maternal metabolic programming in offspring, reducing adiposity and plasma glucose and lipid concentrations.

Although the harmful effects of a sucrose- and lipid-rich diet in individuals of a reproductive age can be transmitted to subsequent generations, the results of the present study suggest that it is possible to reverse these risks by means of chronic physical training, both in mothers and offspring. However, additional studies at the molecular and genetic levels are recommended to better

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understand the mechanisms responsible for the beneficial effects of physical training in subsequent generations.

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Conflicts of Interest. The authors declare no conflicts of interest.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (CONCEA) and have been approved by the institutional committee (CEUA-UFJF) protocol number – n. 067/2013. All experiments were performed at the Juiz de Fora Federal University (UFJF) Reproductive Biology Center (CBR) breeding facilities, which complies with the ethical standards of the relevant national guides on the care and use of laboratory animals (CONCEA), certification number: CIAEP n. 01.0048.2013.

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