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Beneficial effects of maternal swimming during pregnancy on offspring metabolism when the father is obese

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Brief Report

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Abstract

We aimed to evaluate the impact of maternal exercise training on the offspring metabolism and body size caused by father obesity. C57BL/6 male 4-week-old mice were fed a high-fat diet (HF father) or control diet (C father), while equal age female mice were fed only a C diet and were separated into two groups: trained (T mother) and non-trained (NT mother), and at 12 weeks of age mice were mated. A continuous swimming protocol was applied for 10 weeks (before and during gestation), and offspring were followed since weaning until sacrifice (at 12 weeks of age). HF father, compared to C father, showed obesity, elevated total cholesterol (TC) and triglycerides (TG), and glucose intolerance. Both sexes HF/NT offspring showed hyperglycemia, glucose intolerance and high levels of TC and TG, without obesity. However, HF/T offspring showed data close to C/NT, demonstrating the beneficial effect of maternal exercise in the offspring of obese fathers.

Introduction

The negative impact of maternal obesity on adiposity and offspring metabolism is already well established in the literature.[1,2] However, recent experimental findings have challenged conventional ideas about metabolic programming, suggesting that something else might be relevant through paternal programming.[3,4] The contribution of fathers to offspring programming is pertinent to Western society because about 70% of men of reproductive age are obese or overweight.[5]

Exercise training is an essential factor in reducing risk factors for obesity and diabetes, such as unbalanced energy metabolism, glucose utilization and insulin sensitivity.[6] The results on the progeny of exercise training during pregnancy have been well investigated, mainly focusing on fetal growth and metabolism indicating that the influence on male and female offspring is different, denoting sex dimorphism.[7]

We have the hypothesis of having a benefit for offspring that are programmed metabolically because the father is obese when the mother is not obese and does some physical training during pregnancy. Therefore, the study aimed to evaluate the impact of the maternal exercise training (swimming) in the offspring metabolically programmed by an obese father. In this short communication, we demonstrated the change in the body growth and metabolism of the puppies due to the obese father. The auspicious information is that the swimmer mother brings benefits to offspring.

Material and methods

Animals and diets

All procedures were made to minimize suffering and protocols respect the current guidelines for experimentation with animals (National Institutes of Health Publication No. 85–23 revised, 1996) and ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines). The Committee on the Ethics of Animal Experiments of the University of the State of Rio de Janeiro approved the protocol (Protocol Number CEUA/003/2018).

Male and female C57BL/6 mice were kept under controlled temperature conditions in the NexGen system (Allentown Inc., PA, USA, 21 ± 2°C and 12 h/12 h dark/light cycle) with free access to food and water. Four-week-old male mice were randomly divided into two groups and fed a high-fat diet (HF, designated as a HF father group, HF-Fa, $n = 20$) or a control diet (C, identified as a C father group, C-Fa, $n = 20$). Moreover, 4-week-old female mice were separated into a trained group (T, designated as a T mother group, T-Mo, $n = 20$) and a non-trained group (NT, identified as a NT mother group, NT-Mo, $n = 20$). Female mice were only fed with the C diet.

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The total energy of the HF diet was 32% from carbohydrates, 19% from protein and 49% from lipids. The total energy of the C diet was 64% from carbohydrates, 19% from protein and 17% from lipids. The HF diet had an energy availability of 20.7 KJ/g, while the C diet had it of 16.5 KJ/g. The diets C and HF were formulated with purified ingredients having the content of vitamins and minerals identical according to the standards recommended by the American Institute of Nutrition for a rodent to support growth during pregnancy, lactation and post-weaning periods of life (AIN-93G)[8] and were manufactured by PragSolucoes (Jau, SP, Brazil) (Supplemental Table S1).

The male mice (fathers) received their respective diets (C or HF) pre-mating (from 4-weeks old until12-weeks old). Again, the female mice (mothers) were only fed the control diet the during gestation and lactation (the difference was the exercise training). We separated only one male and one female from each group at 12-weeks old, which crossed for mating, thus obtaining four groups of progenitors: C-Fa/NT-Mo ($n = 10$ of mothers and fathers), C-Fa/T-Mo ($n = 10$ of mothers and fathers), HF-Fa/NT-Mo ($n = 10$ of mothers and fathers) and HF-Fa/T-Mo ($n = 10$ of mothers and fathers) (Supplemental Figure S1).

Litters were standardized to six pups immediately after birth (three males and three females, when possible) and stayed with the respective mother until weaning. Only one male and one female offspring of each litter were kept forming the experimental groups. The pups were identified according to sex and designed for the origin of the father (first capital letter) and mother (second capital letter) and were fed the control diet until 12-weeks of age when they were sacrificed.

Exercise training

Four-week-old female mice were randomly separated into two groups and followed by 2 weeks of adaptation and 8 weeks of training (6 weeks before mating and an additional period of 2 weeks during pregnancy): (a) swimming training (T, $n = 20$), and (b) non-training (NT, $n = 20$). The exercise protocol has been defined.[9] Briefly, the first 2 weeks referred to adaptation with a progressive adjustment of time (seven min/day until 60 min/day, three times/week) without an increase of weight in the tail, and then swimming sessions five times/week for 8 weeks. The animals were left free to swim and were gently stimulated to swim when necessary, to ensure that the animals swam throughout the experiment.

The training protocol corresponded to approximately 40–60% of VO_2 max, considering the subject sedentary, emphasizing low-to-moderate intensity and long duration of the exercise. A swimming apparatus was specially planned for mice, measuring 90 cm length, 30 cm width and 30 cm height, and divided into 12 lanes (surface area of 15 × 15 cm per lane). The temperature of the water was maintained between 30 and 32°C. The presence of a vaginal plug was used to indicate copula and was considered the 1st day of gestation. The mothers were trained swimming for 2 weeks in the pregnancy period, 3 days/week with 30 min/day.

Body mass (BM) and food intake (FI) and energy intake (EI)

The BM was measured weekly (Monday, 12 am). FI was measured daily (11 am, fresh pellets were provided daily and any remaining pellet from the previous day was discarded) as the amount of food left in the cage deducted of the food supplied. EI was the product of food consumption and energy content of the diet.

Oral glucose tolerance test (OGTT)

The OGTT of fathers and mothers was measured 2 days before mating and in 12-weeks old offspring (2 days before the euthanasia). After 6-h of food deprivation (1–7 am), the animals received 25% glucose in sterile saline (0.9% NaCl, 1 g/kg), administered by orogastric gavage. The glycemia was measured before and after the glucose administration at the 15, 30, 60 and 120 min in blood from the tail vein (glucose value at time zero was considered fasting glucose, glucometer Accu-Chek Active; Roche Applied Science, Brazil). The 'area under the curve' of blood curves was calculated from 0 to 120 min using the trapezoid rule to assess glucose tolerance[10] (GraphPad Prism 7.04 for Windows; GraphPad Software, La Jolla, CA, USA).

Sacrifice

We sacrificed parents after offspring weaning and offspring at 12 weeks old. The animals were food-deprived (from 1–7) and then profoundly anesthetized (sodium pentobarbital, 150 mg/kg intraperitoneal). Blood samples (exsanguination by the cut of cervical vessels) was centrifuged separating the plasma (1200 ***g*** for 15 min), and the genital fat pad (in the abdominal caudal portion in connection with the epididymis in males and the ovaries and oviducts in females) was dissected and weighed.

Plasma analyses

Total cholesterol (TC) and triglycerides (TG) were measured (automatic spectrophotometer and commercial kit, Bioclin System II; Quibasa, Belo Horizonte, MG, Brazil).

Data analysis

The data passed for normality and homoscedasticity of the variance analyses, therefore, were expressed as the mean and standard deviation. The frequencies of liveborn pups between C and NT mother were analyzed with the χ^2 test. We tested the differences in the groups with one-way analysis of variance followed by the *post-hoc* test of Holm–Sidak (GraphPad Prism 7.04 for Windows). The P-value of < 0.05 was considered statistically significant.

Results and discussion

Parents' data

Effects of the HF diet on fathers and swimming in mothers

a) Fathers

The HF diet consumed by fathers had as a consequence a greater EI by +20% and greater BM in the HF-Fa groups than in the C-Fa group (Fig. 1). Moreover, fasting glucose and OGTT ($P < 0.001$, +40%), TC ($P = 0.008$, +38%) and TG ($P < 0.001$, +55%) plasmatic levels were higher in the HF-Fa group compared with the C-Fa group.

b) Mothers

The OGTT in the mothers was also lower in the T-Mo group compared to the NT-Mo group ($P = 0.013$, −18%, Table 1).

Offspring's data

The number of liveborn pups and stillborn were not significantly difference comparing the NT mother and T mother ($\chi^2 = 0.852$, $P = 0.356$).

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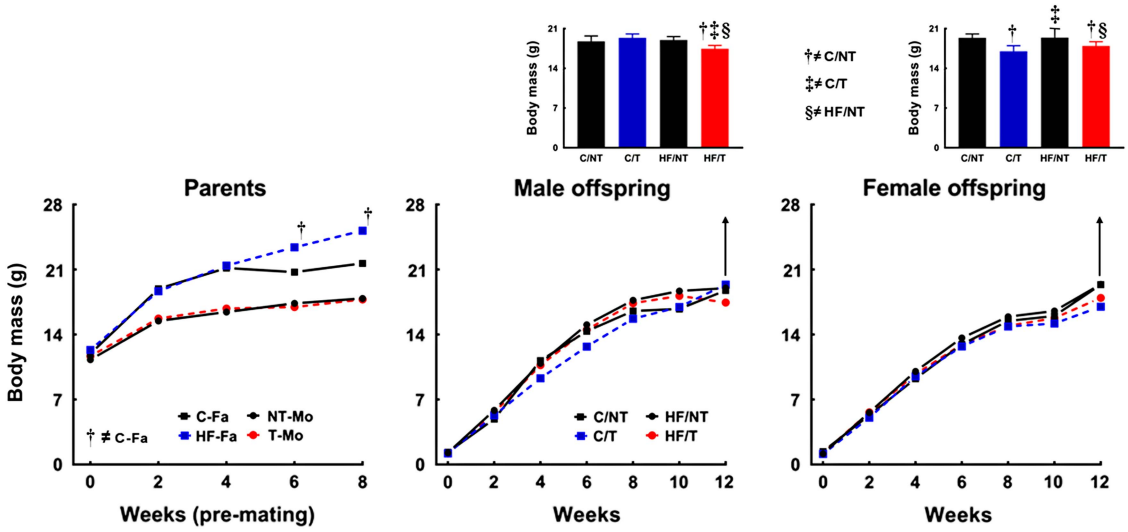

Fig. 1. Body mass evolution. Mean and SD (one-way analysis of variance and *post-hoc* test of Holm–Sidak). C, control diet; Fa, father; HF, high-fat diet; Mo, mother; NT, non-trained; T, trained.

BM and genital fat pad mass

The BM was not significantly different among the groups of males and the groups of females. The genital fat mass was weightier in the HF/NT group than in the C/NT group (male, +100%; female, +110%), but lighter in the HF/T offspring compared to the HF/NT offspring (male, −27%; female, −48%; Table 1).

Fasting glucose, OGTT, TC and TG

a) Males

The FI and the EI were not different in the male offspring. However, fasting glucose (+27%), OGTT (+20%), TC (+125%) and TG (+50%) were all augmented in the groups HF/NT *v.* C/NT, and fasting glucose remained higher in HF/NT *v.* C/T (+30%). OGTT, TC and TG showed low levels in the groups HF/T *v.* HF/NT (Table 1).

b) Females

Moreover, the FI and the EI were not different in the female offspring. However, fasting glucose was higher in the groups HF/NT *v.* C/NT (+18%), but lower in the groups HF/T *v.* HF/NT (−17%). Moreover, OGTT (+23%), TC (+80%) and TG (+38%) showed higher levels comparing HF/NT *v.* C/NT offspring, and lower levels in HF/T *v.* HF/NT offspring (OGTT, −14%; TC, −40%; TG, −22%; Table 1).

Our current knowledge of father transmitting adverse metabolic effects to offspring, primarily because of his obesity condition, has changed our conception about the care we should have with our children.[3,11] Therefore, the goal of the study was to demonstrate that mother can attenuate the adverse metabolism and higher BM effects transmitted by obese/overweight fathers to the offspring by swimming, even when pups are sedentary and fed a control diet.

Our findings on fathers fed a HF diet agrees with literature reporting that a diet rich in saturated fatty acids can change the insulin action leading to hyperglycemia and increased BM associated with a pro-inflammatory framework, which can be transmitted to other generations.[12] Moreover, studies in rodents demonstrated an influence of diet-induced paternal obesity in the health programming of offspring with effects that can be extended to the second generation.[13,14] However, the mechanisms are still unclear and subject to more investigation.[15] The epigenetics should be considered as a mechanism involved in the transmission father-to-offspring of father's obesity on health and pup characteristics, which is an essential issue for the health care of the population.[4]

We did not see offspring obesity at the age they were sacrificed, which is relevant because of the BM maintenance can ensure that all changes observed were independent of the increase of BM, a direct result of the father programming[16] and that the maternal swimming was linked with the benefits reported. The alterations in lipid metabolism observed here are not unexpected as an epigenetic change associated with parental nutrition.[17] Therefore, we assumed that in the long term the offspring would become obese because of paternal hyperglycemia usually predisposes offspring to develop obesity associated with an impaired hypothalamic leptin signaling.[18]

Swimming is a moderate exercise for the entire body during pregnancy since it puts less weight on the bones and ankles than other sports, and it can be continued during pregnancy.[19] Moreover, it is recommended in pregnancy for sedentary women considering that it contributes to an improvement of the cardiorespiratory condition,[20] and safer for the fetus than the terrestrial exercises.[21]

It is relevant to notice that submaximal intensity training before and during gestation in mice is associated with a reduction in BM and a change in carbohydrate and lipid metabolism in adult offspring, depending on their nutritional status regardless of diet.[22] Moreover, exercise training during pregnancy may be considered an alternative treatment for hyperglycemia in this period, even though exercise has positive results about offspring.[23] The exercise performed by the mother can also affect offspring in the metabolism mechanism and basal glucose suggesting that physical activity before and during gestation could protect against obesity and insulin resistance of progeny.[24] No sexual dimorphism was observed in the offspring, considering their metabolism and biochemistry.

We hypothesize that maternal physical activity improves placental perfusion and uterine vessel complacence[15] and has beneficial effects on blood pressure and glucose metabolism.

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Table 1. Biometry and biochemistry (offspring, first capital letter = father; second capital letter = mother)

	Father		Mother	
Parents	C	HF	NT	T
EI (kJ/mouse/day)	34.3 ± 2.11	41.0 ± 1.92*	28.7 ± 2.01	28.5 ± 1.74
FG (mmol/l)	5.9 ± 1.39	8.2 ± 2.21*	5.6 ± 0.31	6.7 ± 0.69
FI (g/mouse/day)	2.1 ± 0.36	2.0 ± 0.37	1.7 ± 0.47	1.7 ± 0.47
OGTT (AUC, mmol/l/min)	806.9 ± 127.4	1133.0 ± 120.4*	961.1 ± 60.2	788.1 ± 56.8**
TC (mg/dl)	61.0 ± 13.66	84.4 ± 7.26*	67.1 ± 4.36	71.9 ± 5.96
TG (mg/dl)	33.3 ± 4.4	51.8 ± 6.67*	30.2 ± 5.32	30.3 ± 2.87
	C/NT	**C/T**	**HF/NT**	**HF/T**
Male offspring				
BM (birth, g)	1.3 ± 0.16	1.2 ± 0.06	1.3 ± 0.14	1.2 ± 0.11
BM (weaning, g)	6.1 ± 1.08	6.4 ± 0.89	7.2 ± 0.79	7.0 ± 1.38
BM (12-weeks, g)	18.8 ± 0.97	19.0 ± 1.33	22.1 ± 0.60	17.5 ± 0.60
EI (kJ/day/mouse)	32.8 ± 2.56	31.2 ± 1.99	32.8 ± 2.09	33.0 ± 2.61
FG (mmol/l)	4.8 ± 0.71	4.7 ± 0.53	6.0 ± 0.43†‡	5.2 ± 0.29§
FI (g/day/mouse)	2.0 ± 0.39	1.9 ± 0.44	2.0 ± 0.22	2.0 ± 0.56
Genital fat pad (g)	0.13 ± 0.02	0.16 ± 0.04	0.26 ± 0.04†	0.19 ± 0.04§
OGTT (AUC, mmol/l/min)	777.3 ± 40.1	765.7 ± 33.8	938.1 ± 26.8†‡	725.1 ± 35.3§
TC (mg/dl)	1.4 ± 0.46	2.1 ± 0.44	3.1 ± 0.06†	1.8 ± 0.52§
TG (mg/dl)	0.6 ± 0.06	0.7 ± 0.10	0.9 ± 0.09†	0.7 ± 0.06§
Female offspring	**C/NT**	**C/T**	**HF/NT**	**HF/T**
BM (birth, g)	1.4 ± 0.15	1.1 ± 0.11	1.2 ± 0.12	1.2 ± 0.10
BM (weaning, g)	6.2 ± 1.14	6.3 ± 1.10	7.2 ± 0.72	6.8 ± 1.15
BM (12-weeks, g)	19.4 ± 0.67	17.2 ± 1.31	19.4 ± 1.61	18.0 ± 0.74
EI (kJ/day/mouse)	32.8 ± 2.42	30.5 ± 2.09	33.0 ± 2.22	34.0 ± 1.99
FG (mmol/l)	5.3 ± 0.31	4.6 ± 0.44	6.2 ± 0.52†‡	5.2 ± 0.39§
FI (g/day/mouse)	2.0 ± 0.48	1.8 ± 0.32	2.0 ± 0.26	2.0 ± 0.57
Genital fat pad (g)	0.10 ± 0.04	0.08 ± 0.04	0.21 ± 0.03†	0.11 ± 0.05§
OGTT (AUC, mmol/l/min)	747.3 ± 28.5	744.4 ± 26.3	917.4 ± 22.2†‡	786.1 ± 38.7§
TC (mg/dl)	1.8 ± 0.17	1.8 ± 0.11	3.2 ± 0.24†	1.9 ± 0.22§
TG (mg/dl)	0.6 ± 0.11	0.6 ± 0.18	0.9 ± 0.06†	0.7 ± 0.15§

C, control diet; HF, high-fat diet; NT, non-trained; T, trained; EI, energy intake; FG, fasting glycemia; FI, food intake; OGTT, oral glucose tolerance test; AUC, area under the curve; BM, body mass; TC, total cholesterol; TG, triglycerides.
Mean ± SD, one-way analysis of variance and *post-hoc* test of Holm–Sidak.
$P < 0.05$ when: *≠C father; **≠NT mother; †≠C/NT; ‡≠C/T; §≠HF/NT.

Taken together, these effects would tend to counterbalance the epigenetic effects caused by the obese father in the offspring.

Parents play a significant role in the metabolic calendar of offspring, particularly in non-genetic factors in the causal pathway. Our results determine the benefits of maternal swimming, even in the presence of the epigenetic impact of paternal obesity, resulting in beneficial effects to the pups.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174418001046

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Conflicts of Interest. None.

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