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HEALTH SCIENCES

Impact of different exercise intensities on pregnant rats and on their offspring

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Abstract: This study aimed at evaluating the levels of different maternal exercise intensities on maternal and fetal outcomes. Wistar rats were mated and the pregnant rats were distributed into four experimental groups (n = 13 animals/group): Control (Not exercise group - 0% of the anaerobic threshold- AT), mild (20%), moderate (80%), and heavy-exercise intensity (140% of AT). These AT were matched to the load of 0, 1, 4 and 7% of the body weight of the animal related to swimming-induced physical intensity. In pregnancy, biomarkers related to maternal blood gases, oxidative stress, metabolism, and reproductive performance, and outcomes of their offspring were analyzed. The mild and moderate-swimming caused no change on implantation, live fetus numbers and oxidative stress status. However, the rats submitted to mild-exercise presented respiratory alkalosis and the heavy-exercise group showed respiratory acidosis. In addition, fetuses of the heavy-exercise dams were smaller for gestational age and lower serum adiponectin levels compared to those of other groups. In conclusion, the moderate-exercise intensity caused beneficial effects for maternal environment and the mild and moderate-exercise presented similar fetal repercussions. Nevertheless, the heavy-exercise intensity caused maternal metabolic alterations that damaged the fetal growth. Therefore, these findings confirm that physical intensity should be carefully conducted to avoid maternal complications and, consequently, compromised fetal repercussions.

Key words: exercise intensities, swimming, pregnancy, fetuses.

INTRODUCTION

Water immersion is one of the best resources to increase the aerobic capacity of pregnant women (Juhl et al. 2010). In the maternal organism, the application of physical exercise have showed an improvement of cardiovascular function, prevention of excessive body weight gain, reduction of fat retention and improvement of physical fitness (Clapp 2000). Considering the most recent ACOG guidelines (2015), all pregnant women, without obstetric and medical contraindications, should be encouraged to exercise in a manner similar to the guidelines that are given to non-pregnant women. They can practice aerobic and strength exercises with moderate intensity for at least 20 to 30 minutes a day on most days of the week (Perales et al. 2017). Furthermore, clarity regarding the definition of exercise intensity is still lacking.

Several biochemical markers of physical training provoked great interest and represent a significant advance in recent decades for a better understanding of the importance of physical exercise practice for the health of the population. Among these markers, enzymatic activity of citrate synthase is studied, as it is relevant for the evaluation of oxidative metabolism (Basset & Howley 2000). Citrate synthase catalyzes the first reaction of the Krebs cycle, where condensation of acetyl coenzyme A (Acetyl-CoA) with oxaloacetate occurs to form citrate and coenzyme A (CoA) (Halliwell & Gutteridge 1998). Several studies have used mitochondrial enzyme activity to confirm or not the influence of physical exercise on the oxidative adaptation of rat skeletal muscle (Goodyear et al. 1992, Powers et al. 1994, Silva et al. 1997, Bexfield et al. 2009, Moreira et al. 2013). The increased activity of oxidative enzymes present in mitochondria is indicative of the optimization of aerobic metabolism (Halliwell & Gutteridge 1998). Another marker used is creatine kinase (CK), an enzyme that plays a key role in energy formation in muscle cells, as it is responsible for maintaining adequate levels of adenosine triphosphate (ATP) during muscle contraction (Katirji & Al-Jaberi 2001). CK is as a marker of muscle damage since, is cytoplasmic; and has no ability to cross the sarcoplasmic membrane barrier (Melin et al. 1997, Nosaka & Newton 2002, Poprzęcki et al. 2004). As a result, increased serum CK concentrations are used to indicate muscle membrane damage (Smith et al. 1994, Brown et al. 1997, Close et al. 2005, Nosaka et al. 2005).

The effects of maternal physical activity on fetal four cannot be adequately investigated in human pregnancies for self-evident ethical reasons. Therefore, animal models are essential to fill in the gaps in our knowledge. Rat models, besides being time- and cost-efficient, are very well suited for mechanistic studies. In male rats, exercise intensity has been defined in terms of lactate levels, pH and anaerobic threshold. However, studies assessing the effect of physical activity on female rats do not provide clear definitions of exercise intensity levels (Uriu-Hare et al. 1989, Simsek et al. 2005, Volpato et al. 2009, Damasceno et al. 2011). In our research laboratory, rats submitted to swimming during pregnancy had conflicting maternal and fetal results (Volpato et al. 2009, Corvino et al. 2015a, b), as there are benefits after swimming only to rats-mothers or only to offspring. Controversial results regarding the effects on offspring can be explained due to the intensity of physical exercise. In strenuous exercise, a state of fetal hypoxia may occur, leading to risks for its development (Bennell 2011). According to Bell (2002), intense physical exercises with high frequency and maintained for long periods of gestation can result in low birth weight newborns. There are several explanations to justify the small fetuses for gestational age, such as altered secretion of adipocytokines, as adiponectin, which has been implicated in intrauterine growth (Gluckman et al. 1996). Netto et al. (2017) used the lactate minimum test to determine the anaerobic threshold (the point during exercise when aerobic metabolism is switched to anaerobic metabolism) in non-pregnant and pregnant Wistar rats during swimming program. In pregnant rats, the exercise with mild, moderate and heavy-intensity levels correspond to 20%, 80% and 140% of the anaerobic threshold, respectively. Herein, we hypothesized that among the three intensities tested the most adequate is moderate because it should represent a load that it is possible to practice physical exercise with stabilization of blood lactate for better maternal and perinatal outcomes. Based on these data, this study aimed at assessing the effects of different maternal exercise intensity levels on maternal outcomes and their offspring.

MATERIALS AND METHODS

Animals

Male and female adult Wistar rats weighing approximately 200-240 g were used. The animals were purchased from Biological Research Centre (CEMIB), State University of Campinas (UNICAMP), adapted and maintained at the Laboratory of Experimental Research of Obstetrics and Gynecology, Unesp, under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 10%), 12 hours light/dark cycle, and water and feed were provided *ad libitum*. The experimental design is showed in figure 1.

Procedures and animal handling were performed in accordance with the guidelines provided by the Brazilian College of Animal Experimentation in agreement with the International Guiding Principles for Biomedical Research Involving Animals promulgated by the Society for the Study of Reproduction. The local Ethics Committee for animal experiments approved all protocols described in this study (Protocol Number 1050/2013).

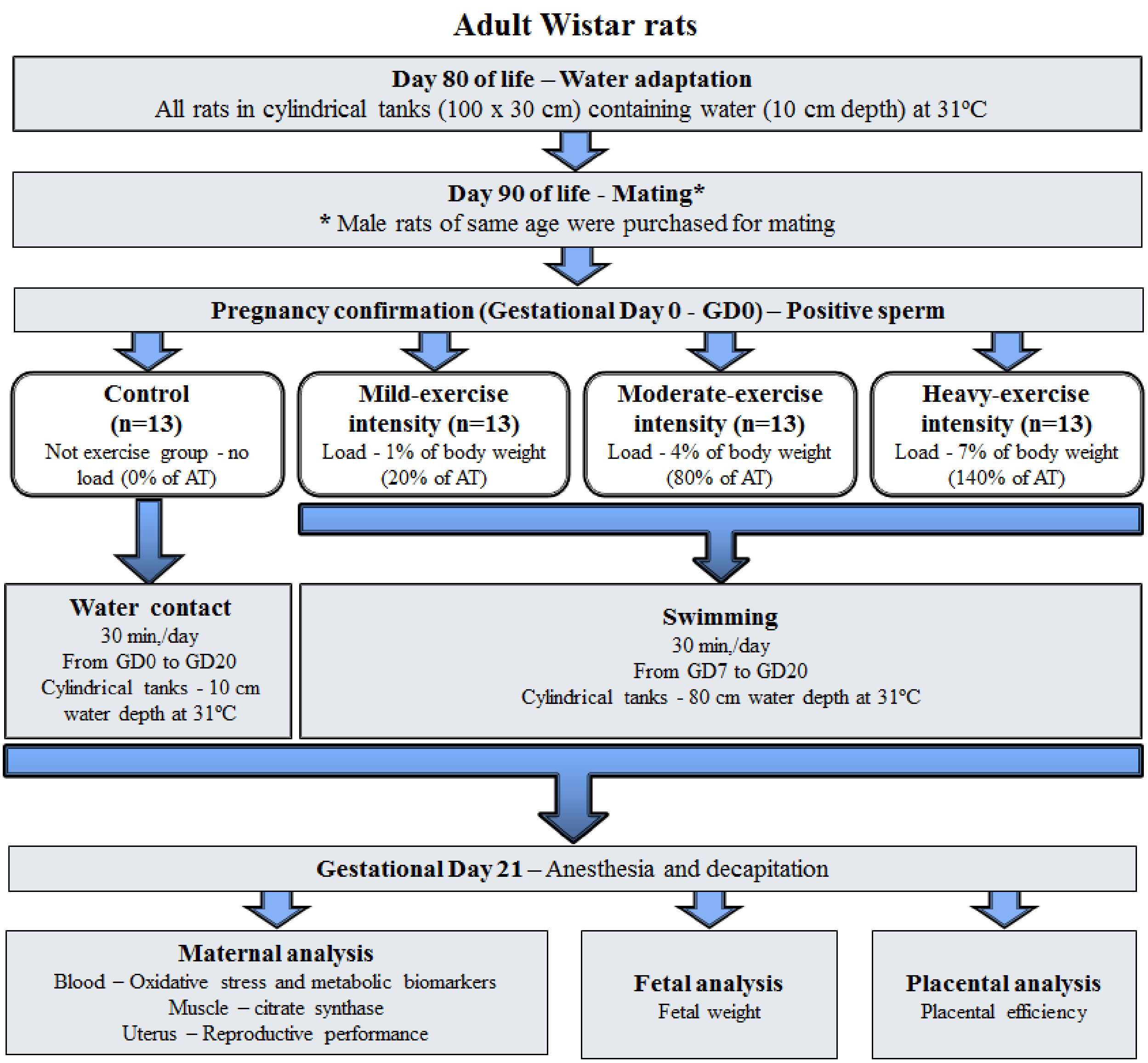

Figure 1. Experimental design.

Mating procedure and experimental groups

All female rats were mated overnight with male rats. The day when sperms were found in the vaginal smear was designated as gestational day 0 (GD0). The mating period consisted of 15 consecutive days until a replicate number of groups was obtained. However, during this period, nonmated female rats were considered infertile and were discarded from the study (Santos et al. 2015). The pregnant rats were submitted to 0%, 20%, 80% and 140% of the anaerobic threshold (AT) for the swimming practice. In previous study (Netto et al. 2017), the AT of the pregnant rats was identified in minimum load of 5% of the body weight. Then, for this investigation the pregnant rats were distributed into four experimental groups (n = 13 animals/group) - 1) Control: Not exercise group - no load (0% of AT); 2) Mild-exercise intensity: with 20% of AT, which corresponded to 1% of body weight of each rat; 3) Moderate-exercise intensity: with 80% of AT, which corresponded to 4% of body weight; 4) Heavy-exercise intensity: with 140% of AT, equivalent to 7% of body weight.

Physical exercise – swimming program

To familiarize the rats to the swimming system (water), the rats were daily exposed to water for 10 minutes in cylindrical tanks (100 x 30 cm) containing water at a depth of 10 cm at 31±1°C. The adaptation period corresponded to 80 days of life (10 days before the mating period) and after pregnancy positive diagnosis (the first week of pregnancy - from day 0 to day 6). The first day of exercise was carried out on the day 7 of pregnancy, corresponding to post-embryonic implantation period. The swimming was performed according to the exercise intensity and, therefore, each rat carried its respective load. Fishing pellets were used as loads and inserted into jackets, tied to the rat´s chest. The exercise was held in the morning for 30 minutes daily. The rats were subjected to swimming from day 7 to day 20 of pregnancy (Netto et al. 2017). An appropriate control with sedentary rats was made with the same time of exposure to water. However, the water was shallow enough for the rats to stand/walk to reduce the confusing variable of stress between treatment groups.

At term pregnancy – maternal analysis

On day 21 of pregnancy, the rats were anesthetized using isoflurane inhalation (1 mL/min dose) for collection of arterial blood through puncture of the aorta. When the animals were in anesthesia and with stable breathing, the laparotomy and the arterial blood collection were performed. A portion of the samples was collected in tubes containing anticoagulant (heparin) for measurement of analysis creatine kinase (CK) and oxidative stress markers [malondialdehyde (MDA) and reduced thiol groups (S-H)]. For oxidative stress analysis, the blood samples were processed according to a modified method of de Souza et al. (2010) and the samples were stored at -80°C and analyzed by spectrophotometry. CK measurements were performed using a commercial kit (EnzyChromTM® Creatine kinase Assay Kit, USA). Another part of the arterial blood samples was used for measurement of temperature, pH (normality limit: 7.35 -7.45 – compensated status), pCO_2 (CO_2 partial pressure), HCO_3, lactate and SO_2 (oxygen saturation) in the i-Stat System® apparatus (Abbott®, USA). SO_2 was used as a parameter to confirm arterial blood samples and as the inclusion criterion. Thus, rats with values above 95% were included in gasometry analyses.

The gravid uterus was dissected to count dead and alive fetuses, resorption (embryonic death), and implantation site numbers. The percentage of post-implantation loss was calculated (Volpato et al. 2015). The gastrocnemius muscle samples were withdrawn, stored at -80°C, and used for analysis of citrate synthase activity according to protocol modified by Alp et al. (1976)

At term pregnancy - fetal and placental analyses

Following the collection of fetuses from the uterine horns, each fetus was weighed for classification of fetal body weight in comparison to control group at same pregnancy time. The fetuses were classified was small (SGA), adequate (AGA) or large (LGA) for gestational (pregnancy) age to the mean ±1.7 x standard deviation (SD) of body weights obtained in the control group according to Afiune et al. (2017). The fetal blood pool samples were collected from trunk region for serum adiponectin levels according to manufacturer (Millipore® EZRADP-62K). The placentas were weighed to calculate the placental efficiency (fetal weight/placental weight) (Moraes-Souza et al. 2017).

Statistical analysis

The results of the maternal metabolic biomarkers and reproductive outcomes and fetal serum adiponectin levels were analyzed by comparison two-by-two using Student t test. The classification of fetal body weight was analyzed by affixing the categories (SGA, AGA, and LGA) for the comparison between the groups. Then, the analysis of the proportionate measures were performed by Fisher's exact test. For all statistical comparisons, $p<0.05$ was considered statistically significant.

RESULTS

The Table I shows the number of rats that began the experiment, completed full pregnancy, submitted or not to swimming during total period and number of dead rats. All rats of control, mild and moderate-exercise groups had at term pregnancy, were able to swim the complete program, and presented no death in these groups, except one rat submitted to moderate swimming program that bled before swimming and was removed from the group. The heavy-exercise group began the experiment with 13 animals, and three of them died during the experiment, totalizing 10 rats at term. Of these 10 rats, two rats completed the swimming program but eight did not.

Table I. **Outcomes of female rats submitted to mild, moderate and heavy-exercise intensity during pregnancy.**

	Control	Mild-Exercise	Moderate-Exercise	Heavy- Exercise
Pregnant rats	13	13	13	13
Beginning training	13	13	12	13
Complete swimming training	13	13	12	2
Incomplete swimming training	0	0	0	8*
Maternal death	0	0	0	3
At term pregnancy	13	13	12	10

***p<0.05 - compared to other groups (Fisher's Exact Test).**

The rats submitted to mild-exercise intensity presented pH > 7.5, characterizing alkalosis status, and heavy-exercise showed pH < 7.5, which confirmed acidosis. The pCO_2 and HCO_3 levels were greater in heavy-exercise compared to those of other groups. The activity of plasma creatine kinase was increased in the heavy-exercise group in comparison to control group. All of animals presented SO_2 greater than 95%. The muscular citrate synthase activity, blood lactate, blood MDA and reduced thiol (S-H) group concentrations showed no significant difference among the groups at term pregnancy (p>0.05) (Table II).

The data of maternal reproductive outcomes were shown in Table III. The rats submitted to heavy-exercise intensity during pregnancy gained less weight than control rats, and the litter weight was lower than the moderate-exercise group. The placental efficiency was reduced in all exercise groups in relation to control rats, but it was the lowest in heavily exercised rats (p<0.05).

The mild-exercise group presented greater percentage of fetuses classified as LGA in relation to control group, and heavy-exercise dams had SGA fetuses compared with those of control, mild and moderate-exercise groups (Figure 2a). The serum adiponectin levels of fetuses of heavy-exercise mothers were lower in relation to those of fetuses of other dams (Figure 2b).

Table II. **Maternal metabolic biomarkers of rats submitted to mild, moderate and heavy-exercise intensity during pregnancy.**

	Control (n=13)	**Mild-Exercise (n=13)**	**Moderate-Exercise (n=13)**	**Heavy- Exercise (n=10)**
Temperature (°C)	36.8±0.5	37.4±0.4	36.5±1.6	37.3±0.5
pH	7.38±0.01	7.50±0.09*	7.42±0.08	7.23±0.08*#$
pCO_2	34.0±5.3	21.9±4.5*	28.8±7.5	55.0±7.9*#$
HCO_3	20.6±2.5	17.5±4.5*	18.7±2.1*	24.1±1.5*#$
SO_2	96.5±3.6	98.8±0.8	97.2±2.3	95.2±5.0
Lactate (mmol/L)	5.5 ± 1.6	5.9 ± 3.0	5.2 ± 1.2	4.5±0.8
Creatine kinase (U/L)	206.4±38.5	231.9±35.0	218.6±18.6	263.9±10.9*#$
Citrate Synthase (µmol/min/mg protein)	0.64±0.06	0.78±0.29*	0.79±0.35*	0.49±0.16*#$
Blood MDA (nM/mg Hb)	43.6±9.3	37.8±10.1	47.2±14.9	36.0±10.0
Blood reduced thiol groups (mM/mg Hb)	2.7±0.3	2.6±0.2	2.7±0.2	2.7±0.1

Data are presented as the mean ± standard deviation.
Limit rate for pH (pH< 7.35 = acidosis; pH> 7.45 = alkalosis).
***p<0.05 - compared to Control group; #p<0.05 - compared to Mild-exercise group; $p<0.05 - compared to Moderate-exercise group (Student t test).**

Table III. Maternal reproductive performance of rats submitted to mild, moderate and heavy-exercise intensity during pregnancy.

	Control (n=13)	Mild- Exercise (n=13)	Moderate-Exercise (n=13)	Heavy- Exercise (n=10)
Maternal weight gain (g)	126.7±16.2	108.3±14.7*	115.1±13.1*	106.0±22.5*
Implantation sites	12.9±1.6	12.4±1.3	13.5±1.8	12.3±0.9
Resorption number (embryonic death)	0.4±0.7	0.9±1.4	0.4±0.7	1.3±1.8
Live fetus number	12.6±1.6	11.6±1.7	13.3±2.0	12.0±1.2
Post implantation loss (%)	2.4	6.7	3.1	5.2
Litter weight (g)	88.8±9.6	84.1±7.2	90.7±19.0	80.7±9.2*
Placental efficiency	12.2±1.9	11.6±1.9*	11.4±1.9*	10.8±1.7*[#$]

Data are presented as the mean ± standard deviation or percentage (%).
***p<0.05 - compared to Control group; [#]p<0.05 - compared to Mild-exercise group; [$]p<0.05 - compared to Moderate-exercise group (Student t test).**

DISCUSSION

The mild and moderate- intensity swimming applied to rats did not affect the number of rats that reached full term pregnancy, but the heavy-exercise led to the death of two rats before the end of pregnancy. Only intense-swimming caused changes in muscle damage, as confirmed by greater levels of creatine kinase. However, regardless of the exercise intensities applied to the rats during pregnancy, there is no compromise in the number of implantations or live fetuses. In addition, there were no changes in embryo loss rates. Regard to fetal growth, intense-swimming caused an increased number of small fetuses, which was confirmed by the increase in the percentages of SGA and levels of serum adiponectin of these offspring. The other swimming intensities did not damage the growth of the fetuses. The moderate-exercise protocol used in this study was the most adequately applied to maternal metabolic parameters and fetal growth. The heavy program of swimming caused impairment for both mothers and their offspring.

The rats exposed to mild-exercise showed higher pH values (pH> 7.45) and a lower level of pCO_2, indicating a respiratory alkalosis. Then, these pregnant rats performed a hyperventilation to compensate that alkalosis. In other studies, hyperventilation and respiratory alkalosis also occurred in different species submitted to in mild-exercise (Hastings et al. 1982, Smith et al. 1983, Chin et al. 2007). Our findings showed rats submitted to heavy-exercise had lower pH values (pH <7.35) and higher pCO_2, characterizing a respiratory acidosis. In an attempt to compensate for that acidosis, an increase in HCO_3 arisen. Nevertheless, it was not enough to normalize this acid level. According to Kato et al. (2005), the hypercapnia-induced respiratory acidosis directed to a lower blood pH due to lower blood lactate concentration at maximal exercise.

The enzymatic activity of creatine kinase (CK) in serum or plasma is useful for the evaluation of muscle damage from physical exercise (Foschini and Prestes 2007). In this study, there were no changes in the CK activities in mild and moderate-exercise intensity, suggesting these exercise intensities were appropriate. However, the rats submitted to heavy-exercise intensity presented increased CK activity, indicating muscle damage.

Several studies have used the activity of mitochondrial enzymes to analyze the influence of physical exercise on the oxidative adaptation of skeletal muscle in rats (Bexfield et al. 2009). An increase in the citrate synthase activity has been reported in swimming exercise (Medeiros et al. 2004). However, our findings showed the citrate synthase activities were not modified after swimming during pregnancy regardless of intensity. Previous studies have indicated that the increase in the enzyme citrate synthase activity correlates with the intensity (Bexfield

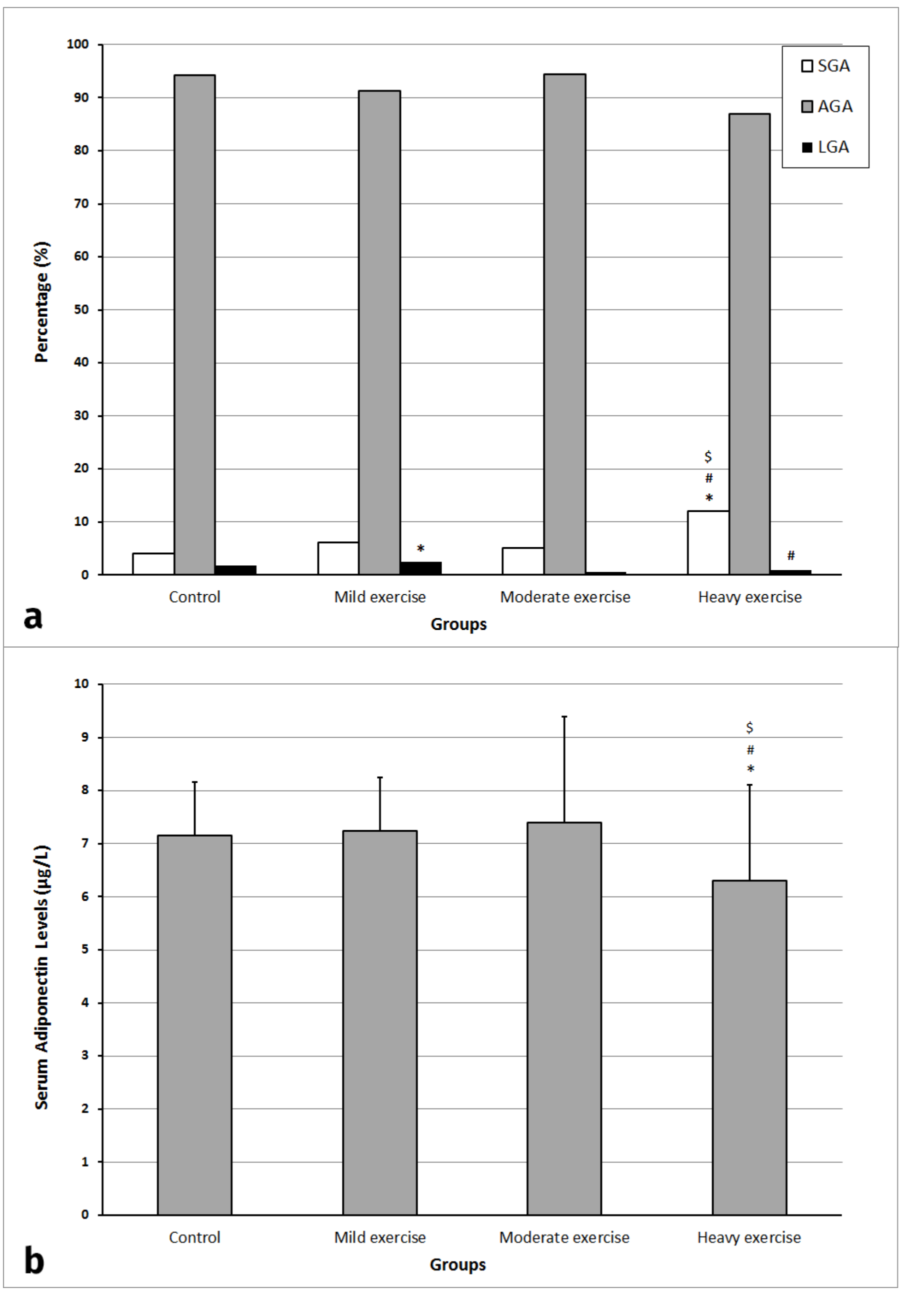

Figure 2. a - Classification of fetal body weight, b - Serum adiponectin levels of fetuses from rats submitted to mild, moderate and heavy-exercise intensity during pregnancy. Data are presented as percentage. SGA - Small for gestational age; AGA - Adequate for gestational age; LGA – Large for gestational age. *p<0.05 - compared to Control group; #p<0.05 -compared to Mild-exercise group; $p<0.05 - compared to Moderate-exercise group (a- Fisher's Exact Test; b- Student t test)

et al. 2009) or duration of applied exercise (Powers et al. 1994). Then, it was expected that the group of rats submitted to heavy-exercise showed an increased citrate synthase activity, but no difference in this marker has been found. This can be explained because 10 of the 13 rats in this group that achieved at term pregnancy performing the exercise program were unable to swim the time established in the protocol (30 min), making an average of 10 minutes from 7 to 14 days of pregnancy and approximately 3 minutes from 15 to 20 days de pregnancy. Therefore, the citrate synthase activity does not reflect actual impairment with the practice of heavy-exercise.

The oxidative stress status biomarkers presented no changes in the blood of exercised rats. Evidence shows there is an increase in reactive oxygen species during physical exercise. To protect organs from tissue damage, antioxidant enzymes respond adaptively, thereby increasing their activities (Reddy et al. 1999). However, this finding was not observed in this study.

The rats submitted to swimming at a heavy intensity gained less weight at term pregnancy. Our findings showed that the mild and moderate-exercise intensities had no measurable adverse effects on the number of implantation, embryo death and live fetuses and no appearance of external fetal malformations, indicating that our swimming protocol has been safe in pregnancy. It was verified mild to moderate-exercise during pregnancy did not harm the mother-fetus relationship. Physical exercise is a safe activity with beneficial effects for the mother and fetus during pregnancy (Evenson et al. 2014). Although the rats submitted to heavy-exercise presented no changes in maternal reproductive outcomes, three rats of this group died before at term pregnancy due to exercise overload, and the rats that reached full pregnancy presented an early exhaustion signs during training and were removed from the water to prevent their deaths. Then, these findings did not reflect the impaired performance of a complete exercise protocol. Thus, it is important that the exercise protocols are performed with professional guidance and moderation, i.e., the type of exercise, weekly frequency, duration of each session, and intensity must be well controlled (Department of Health and Human Services 2018). Regarding studies with experimental animals, the results remain inconsistent because of the variability in the species and experimental protocols used. It was verified that heavy-exercise was not adequate, but we suggested that might be due to no previous training to the mother. Further studies might be conducted with rats submitted to previous exercise to pregnancy to avoid damaging for mother and fetuses.

In our study, the classification of the fetal weight was predominantly considered as adequate for gestational age (AGA) in all groups, regardless of swimming intensity applied in their dams. However, the heavy-exercise group presented greater percentage of fetuses classified as small for gestational age (SGA). In addition, these fetuses presented lower levels of serum adiponectin. Adiponectin is a protein highly expressed in human adipose cells (Yannakoulia et al. 2003, Chan et al. 2004). This protein modulates insulin action and promotes anti-atherogenic and anti-inflammatory effects (Nishizawa et al. 2002, Combs et al. 2003). The adiponectin levels are inversely proportional to body fat content and presents negative correlation with the insulin resistance (Yang et al. 2001, Nemet et al. 2003). The adipocytokines have been associated with intrauterine growth (Lapillonne et al. 1997). Kamoda et al. (2004) compared adiponectin levels between female and male neonates but found no differences in adiponectin levels between the genders. These authors verified lower adiponectin levels in SGA than in AGA infants.

However, there are further studies in the literature showing that exercise during pregnancy cause no change (Gilbert et al. 2012), decrease (Damasceno et al. 2013) or increase (Uriu-Hare et al. 1989) on weights in birth of rats, showing that this result is contradictory. The exercised dams, regardless of intensity, exhibited increased placental weights as a compensatory mechanism to increase maternal-fetal exchanges and the nutritional supply to the fetus during development. However, the placental efficiency was decreased in exercised groups, but was more reduced in heavy-exercise group, causing lower blood flux to fetuses and, consequently leading to intrauterine growth restriction, confirmed by higher percentage of fetuses classified as SGA.

In conclusion, the moderate-swimming intensity caused beneficial effects for the maternal environment and the mild and moderate-exercise presented similar fetal repercussions. Nevertheless, the heavy-exercise intensity caused maternal metabolic alterations impairing fetal growth. Therefore, these findings confirm that exercise should be recommended for specialized professionals to avoid maternal complications and repercussions on their offspring.

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All authors wrote and reviewed the manuscript, agree with its contents, and consent to its publication. AO Netto & DC Damasceno - Conception and design, AO Netto, FQ Gallego & NCD Macedo - Data acquisition; AO Netto, FQ Gallego, YK Sinzato, GT Volpato, E Zambrabo & DC Damasceno - Data analysis and interpretation. All authors were responsible for critical revisions of the paper and approved the final version of the manuscript. The authors declare no conflict of interest.