

*Original Article***Effect of physical training on metabolic responses of pregnant rats submitted to swimming under thermal stress**

Rodrigo Alexis Lazo-Osório^{*a}, Rafael Pereira^b, Junia Scarlatelli Christofani^b, Adriana Kowalesky Russo^b, Marco Machado^c, Wellington Ribeiro^a, Ivan da Cruz Piçarro^b

Abstract

BACKGROUND: The aim of this study is to assess the effect of pre-pregnancy physical training on metabolic responses and its effects on offspring.

METHODS: Three groups of rats (n = 7 in each group): sedentary pregnant rats (PS), exercised during pregnancy (PE) and pregnant rats trained before and during pregnancy (PT) were compared. They were separated into three subgroups regarding water temperature: 28°C, 35°C or 39°C. Plasma triglycerides and glucose levels, weight gain during pregnancy and rectal temperature pre and post exercise (swim), as well as the offspring size and weight were analysed.

RESULTS: Rectal temperature post exercise was lower than pre exercise at 28°C and 35°C, and higher at 39°C. Weight gain was lower at 39°C for the PT group and at 35°C for the PT and PE groups compared to the PS group. Plasma glucose, at 28°C and 39°C for PS and PE groups, was higher than those obtained at 35°C, while triglycerides were lower. For trained rats, plasma glucose and triglycerides were similar at all water temperatures. Trained rats presented lower triglyceride values at 35°C, and higher triglyceride values at 39°C compared to PS group. Glucose presented inverse results. None of the groups presented fetal reabsorption. However, in the PS group, the offspring presented lower weight gain at 28°C than at 35°C and 39°C.

CONCLUSIONS: These results suggest that pre-pregnancy physical training induces steady values of triglycerides and glucose during exercise at all water temperatures.

KEYWORDS: Physical Training, Pregnant Rats, Glucose Metabolism, Triglycerides Metabolism, Thermal Stress.

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Pregnancy produces important metabolic responses in the mother, directing an adequate substrate delivery to the fetus in order to enable its development and also prepare the mother for lactation.¹⁻³ On the other hand, it is known that high-intensity exercise during pregnancy can increase core temperature and catecholamine release.^{3,4} These effects can hamper heat exchange between mother and fetus and constrain the delivery of nutrients due to a reduction of utero-placental flow.^{1,3}

Exercise in water is suggested to be best physical activity for pregnant women⁵ because water is a favourable medium for heat dissipa-

tion and the greater hydrostatic pressure leads to a fluid flow from extra-vascular to intra-vascular space, which increases blood volume and, consequently, the utero-placental flow. However, water temperature must be considered because some studies carried out on animals^{1,6,7} observed deleterious effects on the fetus whose mother was submitted to swimming in water at temperatures different from thermo-neutral.

A recent study⁸ showed that daily exercise at 80% of maximal work supported into water under high thermal stress (at 22°C and 40°C) for 10-15 minutes did not modify metabolic re-

^a Department of Physiology, Universidade do Vale do Paraíba, São José dos Campos, Brazil.

^b Department of Physiology, Universidade Federal de São Paulo, São Paulo, Brazil.

^c Department of Physiology, Universidade Iguazu, Itaperuna, Brazil.

* Corresponding Author
E-mail: ralo@univap.br

sponses for pregnant rats, but it reduced the litter size when exercise was performed at extreme temperatures, suggesting fetal reabsorbing.

On the other hand, it is also known that physical training can modify the physiological responses to exercise, such as the cardio respiratory and metabolic responses, which become more efficient regarding work performance. These alterations could minimize the effects of exercise performed during pregnancy. However, the interaction between these conditions is complex. For example, a smaller increase of catecholamine is observed during exercises carried out by trained individuals; this effect induces a smaller reduction of blood flow to the splanchnic area. However, other results showed that, in spite of the higher blood flow for trained rats, the flow to the reproductive area was not different between trained and sedentary rats.⁹ Another example is the alteration in metabolic predominance induced by physical training, increasing lipid utilization. During pregnancy, this effect can represent a safeguard for glucose utilization, since it is the preferential substrate for the fetus.^{1,10} Nevertheless, the results obtained for pregnant animals suggest that these effects could be attenuated by endocrine alterations produced during pregnancy.¹¹ Considering the complexity of the interaction between physical training and pregnancy, further research is necessary to clarify the factors involved.

The present study aims to analyse the influence of physical training and thermal stress on metabolic responses in pregnant rats as well as the effect on offspring. Pregnant sedentary rats, rats exercised during pregnancy, and rats trained before and during pregnancy, were compared. In order to evaluate metabolic responses, plasma levels of triglycerides and glucose were determined on the 20th day of pregnancy and the offspring were counted and weighed, after a caesarean section. Triglycerides and glucose was chosen to evaluate metabolic responses to the exercise because of their relationship with exercise and fetal development.^{12,13}

Methods

The sample consisted of sixty-three female Wistar rats (190 ± 1 g), kept in individual cages, receiving water and food (*ad libitum*) (Purina-Brazil), in an artificially illuminated ambience, with a 12 hour day-night cycle and mean temperature of 24°C.

Initially, all animals were submitted to an adaptation period (5 days) of swimming for 5 minutes in water at thermo-neutral temperature in a pool (50 cm x 45 cm). All the animals at the start of experimental procedure weighed between 180g and 220g and were in their reproductive maturity period.

The determination of maximal work load was performed in a swimming pool with water at thermo-neutral temperature. The work load was increased every 3 minutes by weights attached to the animal's tail and corresponding to 1%, 2%, 3%, etc. of the total body weight, until the maximal work load was reached by the animal's exhaustion (unable to surface for 10 s).

All rats were kept in cages (3-4 females for each male) during 12 hours (night period). The copulation was confirmed the following morning by the presence of sperm in vaginal smears.

The pregnant rats ($n = 63$) were then separated into three groups: sedentary rats (PS, $n = 21$; 188 ± 2 g) submitted to water immersion but did not perform exercise during pregnancy, exercised rats (PE, $n = 21$; 193 ± 2 g) submitted to swimming sessions at 80% of maximal work load supported for 30 minutes during 19 days of pregnancy, trained rats (PT, $n = 21$; 188 ± 2 g) submitted to swimming sessions during 45 days before and during pregnancy for 30 minutes, for 19 days of pregnancy, at 80% of maximal work load supported into water, which was calculated for each rat before pregnancy. Each group was divided into three subgroups ($n = 7$), regarding water temperature: 28°C, 35°C, and 39°C. All swimming sessions were carried out between 8:00 A.M. and 12:00 P.M.

In order to determine weight gain during pregnancy, the rats were weighed on the first and 20th day of the gestational period (Ohaus balance). Rectal temperature was measured dai-

ly by thermometer before and after water immersion or swimming.

The rats were decapitated on the 20th day of pregnancy in order to collect a blood sample (5ml) for determining plasma levels of triglycerides and glucose by the enzymatic method.⁸ Just after decapitation, a caesarean section was carried out and the offspring were counted and weighed.

Statistical analysis was performed by two-way ANOVA and post hoc Tukey test. The null hypothesis was set up at $p < 0.05$.

Results

Mean values \pm standard error for rectal temperatures are shown in table 1.

Comparing rectal temperatures, prior and post conditions, showed that, there was a decrease in rectal temperature for all experimental groups

at 35°C and 28°C, while an increase was found at 39°C, and temperature (post-prior) was significantly higher at 28°C than at 35°C and 39°C. Weight gain during pregnancy is presented in table 2.

At 28°C, there was no difference between PS, PE and PT groups, but at 35°C, PT and PE presented smaller weight gain than the PS group, and at 39°C, only PT presented a smaller gain.

Plasma glucose was higher and plasma triglycerides were lower at 28°C and 39°C than at 35°C for sedentary and exercised rats. Trained rats did not present differences in these variables at any of the water temperatures and had different values than sedentary rats at 35°C and 39°C (Table 3).

Offspring from PS28 presented smaller body weight than offspring from PS35 and PS39. The litter size was not different among the groups at any of the water temperatures (Table 4).

Table 1. Mean values \pm standard error of Rectal temperature (°C) determined during 19 days of pregnancy for sedentary rats (PS), rats exercised during pregnancy (PE) and trained rats exercised before and during pregnancy (PT), prior to and post-immersion or swimming (80% of maximal work load supported) into water at temperatures of 28°C; 35°C or 39°C. (n = 7 for each group)

Groups	Prior	Post	Δ (Post-Prior)
PS 28	37.60 \pm 0.05	33.65 \pm 0.11*	3.95 \pm 0.12
PE 28	37.47 \pm 0.15	30.30 \pm 0.24*	7.17 \pm 0.11
PT 28	37.42 \pm 0.17	30.58 \pm 0.43*	6.92 \pm 0.16
PS 35	37.37 \pm 0.32	36.98 \pm 0.20*	0.38 \pm 0.14
PE 35	37.37 \pm 0.15	36.83 \pm 0.12*	0.54 \pm 0.07†
PT 35	37.38 \pm 0.04	36.69 \pm 0.08*	0.68 \pm 0.06†
PS 39	37.54 \pm 0.05	39.79 \pm 0.06*	2.25 \pm 0.09†
PE 39	37.43 \pm 0.04	39.90 \pm 0.03*	2.47 \pm 0.03†
PT 39	37.37 \pm 0.05	39.84 \pm 0.06*	2.46 \pm 0.03†

* $p < 0.05$ significantly different than prior condition

† $p < 0.05$ significantly different than 28°C condition

Table 2. Mean values \pm standard error of Weight gain (g) for sedentary pregnant rats submitted to immersion (PS), rats exercised during pregnancy (PE) and trained rats, exercised before and during pregnancy (PT) at 80% of maximal work load supported into water at 28°C, 35°C or 39°C. (n = 7 for each group)

Groups	Weight gain
PS28	99.14 \pm 3.56
PE28	90.71 \pm 4.67
PT28	85.28 \pm 4.36
PS35	109.00 \pm 4.74
PE35	93.57 \pm 3.12*
PT35	89.85 \pm 3.65*
PS39	107.42 \pm 3.91
PE39	95.85 \pm 4.18
PT39	84.57 \pm 4.64*

* $p < 0.05$ significantly different than PS at the same temperature

Table 3. Mean values \pm standard error of Plasma glucose (mg/dl) and triglycerides (mg/dl) on the twentieth day of pregnancy, determined in blood samples collected 24 hours after the last immersion or swimming session (80% of maximal work load supported) of pregnant sedentary rats (PS), rats exercised during pregnancy (PE) and trained rats exercised before and during pregnancy (PT) in water at temperatures of 28°C, 35°C or 39°C. (n = 7 for each group)

Groups	Glucose	Triglycerides
PS28	84.28 \pm 2.57*	292.28 \pm 12.39*
PE28	88.71 \pm 2.50*	284.00 \pm 20.34*
PT28	80.28 \pm 1.97	317.57 \pm 17.75
PS35	76.00 \pm 2.39	458.57 \pm 18.52
PE35	78.57 \pm 3.91	410.57 \pm 18.52
PT35	88.00 \pm 2.14†	372.42 \pm 18.79†
PS39	101.57 \pm 3.75*	285.42 \pm 20.14*
PE39	108.28 \pm 4.39*	245.71 \pm 10.11*
PT39	84.14 \pm 2.60†	377.14 \pm 16.41†

* p < 0.05 significantly different than 35°C

† p < 0.05 significantly different than PS

Table 4. Mean values \pm standard error of Offspring weight (g) and size of litter in pregnant sedentary rats (PS) submitted to daily immersion, rats exercised during pregnancy (PE) and trained rats exercised before and during pregnancy (PT) at 80% of maximal work load supported into water at 28°C, 35°C or 39°C. (n = 7 for each group)

Groups	Weight	Number of individuals
PS28	3.30 \pm 0.07	11.28 \pm 0.68
PE28	3.36 \pm 0.08	9.71 \pm 0.68
PT28	3.44 \pm 0.07	10.57 \pm 0.71
PS35	3.74 \pm 0.04*	11.71 \pm 0.80
PE35	3.53 \pm 0.07	10.71 \pm 0.91
PT35	3.52 \pm 0.10	10.85 \pm 0.79
PS39	3.59 \pm 0.04*	11.57 \pm 0.80
PE39	3.32 \pm 0.08	9.28 \pm 0.80
PT39	3.31 \pm 0.07	10.42 \pm 0.75

* p < 0.05 significantly different than PS28

Discussion

The literature demonstrates that pregnant rats have lower glucose levels than non-pregnant ones submitted to the same exercise conditions, which means they are not influenced by water temperature.⁸ Our findings indicate that, under thermal stress (28°C or 39°C), sedentary and exercised pregnant rats present higher plasma glucose values than at 35°C. These results suggest that they have either a smaller uptake or an increase of glyconeogenesis from lactate, alanine and piruvate.^{14,15}

An increase in homeostasis alterations (exercises in high or low temperatures) induces higher increases in catecholamine and cortisol release.¹⁶⁻¹⁹ A raise in adrenalin induces pancreas beta cells to reduce the insulin release, which

induce alpha cells to increase glucagon.²⁰ Catecholamines, cortisol and glucagon are gluconeogenesis and hepatic glycogenolysis inducers, contributing to glicemic levels rise.^{16,19,21,22}

Yet, for trained animals, plasma glucose was kept steady, suggesting that daily thermal stress did not modify the regulatory mechanism of plasma glucose in these animals. The results obtained from pregnant sedentary rats and exercised rats showed alterations of plasma glucose according to the temperatures to which they were submitted daily. It suggests that the duration of immersion or swimming was more important than the temperature concerning the determination of plasma glucose levels, since in the previous study, which used more extreme

temperatures with a shorter duration of exposition, pregnant rats showed steady levels of glucose.⁸

By determining the glucose level during exercise performed by pregnant women, greater hypoglycemia was recorded compared to that observed in resting condition, but this hypoglycemia is smaller during water exercises than land exercises.^{2,23,24}

Glucose returned to resting level within 24 hours of the last session of exercise, indicating the efficiency of the glucose level adjustments.²⁵ This quick regulation is made mainly by the hyperglycemic hormones action (catecholamine, cortisol and glucagon) that have their serum concentrations increased by exercise.^{16,22}

In the present study, trained rats had lower values of plasma triglycerides at 35°C than sedentary rats, indicating an increase of lipid utilization in this condition. Plasma triglyceride levels were also lower at extreme temperatures rather than at 35°C for exercised and sedentary rats, but trained rats did not present differences in plasma triglycerides at any of the water temperatures. When the current results were compared to our previous study,⁸ in which pregnant rats were submitted to more extreme temperatures but for a smaller duration, it was observed that both glucose and triglycerides were more influenced by the duration of exposure (30 minutes) rather than by the temperature, which was warmer than in the previous research.

Increased serum concentrations of triglycerides during the exercise are also induced by the hormones cited above. Catecholamines, cortisol and glucagon act on adiposity cells rising the lipolysis and lipids release to blood. Concomitantly the decrease in insulin concentration increases the hormone-sensitive lipase, augmenting the fat acids availability too.²⁶

Increased triglyceride levels were observed in pregnant rats, when compared with non-pregnant animals, especially at the end of pregnancy.^{2,25} This increase was produced by the endogenous triglycerides from the blood stream.²⁷ Therefore, a lipid represents an alternative substrate for the mother, safeguarding glucose for the fetus. On the other hand, aerobic training

modifies the substrate utilization during exercise, increasing lipid mobilization and safeguarding glucose.¹

In a previous study carried out in our laboratory,⁸ we did not observe any alteration in weight gain of rats submitted to daily swimming sessions of 10-15 minutes, at different water temperatures (22°C, 35°C and 40°C). In the present study, using water at more agreeable temperatures for a longer exercise period, it was observed that exercised rats (PE) at 35°C and trained rats (at 35°C and 39°C) had smaller weight gain than sedentary pregnant rats, indicating that substrate mobilization for exercise interferes with total weight gain.

The effect of exercise on maternal weight gain is contradictory. A smaller weight gain was found by some authors.^{25,28-33} Nonetheless, other authors did not observe any alterations.³⁴⁻³⁶

In the present study, as in our previous paper,⁸ after immersion or swimming, pregnant rats modified their rectal temperature with relation to water temperature: decreasing at 28°C and 35°C and increasing at 39°C. In spite of these alterations, a reduction in the litter size was not observed at extreme temperatures, although the duration of immersion or exercise was greater than that employed in our previous study. Nevertheless, if the length of exposure is much longer, where the rats exercise daily for one hour at temperatures of 34.6°C and 37.6°C, fetal abnormalities and reabsorption can be found, probably due to an increase of maternal core temperature.³⁷

Regarding exposition to the cold and heat during pregnancy, some studies showed that hypothermia as well as hyperthermia could produce deleterious effects in fetus.^{6,7,38-42}

There is a temperature gradient between mother and fetus (the fetus presents a temperature 0.4 to 0.6°C greater than the mother), allowing heat transference from the fetus to the mother.⁴³ During high-intensity exercise, this gradient could be reversed, modifying the heat exchange between mother and fetus.^{6,7} This response could lead to fetal hyperthermia and consequent deleterious effects on offspring.⁴⁴

When the weight of offspring was investigated, it was verified that sedentary pregnant rats submitted daily to water immersion at 28°C presented litters with smaller weight in relation to offspring from mothers submitted to immersion at 35°C and 39°C. This result suggests that maternal hypothermia, leading to reduction in utero-placental flow, could induce an inadequate delivery of substrate to the fetus and, in consequence, decrease its weight. Besides, such an effect was not found in exercised and trained rats, suggesting that compensatory mechanisms to safeguard an adequate delivery of nutrients to the fetus were developed by training.

Artal et al⁴⁵ published a guideline for exercise during pregnancy of the American College of Obstetricians and Gynecologists and advocated the benefits of an exercise program to the pregnant and to the fetus. In a recent study Barakat et al⁴⁶ concluded that the regular practice of exercise during the pregnancy did not influence the gestational age, but this study included only sedentary participants. Our results demonstrated that an exercise program before and

during the pregnancy produced better results than an exercise program only during pregnancy. Further studies are necessary to find the effect of pre pregnancy training on other variables (hormonal responses, cardiovascular and respiratory responses, gestational age, etc.) in animal and human models, considering the great similar physiology between rat and human pregnancy.

Conclusions

We concluded that physical training before pregnancy induces a greater stability of plasma triglycerides and glucose, regardless of daily swimming sessions at different water temperatures. These exercise conditions before pregnancy interfere with total weight gain during pregnancy, without deleterious effects on offspring.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

DALO and IDCP carried out the design and coordinated the study, participated in most of the experiments and prepared the manuscript. JSC, AKR and WR carried out all the experiments and participated in manuscript preparation. MM and RP provided assistance for all experiments and participated in manuscript preparation specially the interpretation and description of the results and discussion.

All authors have read and approved the content of the manuscript.

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