Ratas nascidas com restrição de crescimento intrauterino submetidas à natação antes e durante a prenhez: Repercussões maternas e perinatais

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Botucatu
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Dissertação apresentada à Faculdade de Medicina de Botucatu – Unesp, Programa de Pós-Graduação em Ginecologia, Obstetrícia e Mastologia. Área de concentração: Tocoginecologia, para obtenção do título de doutora.

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Palavras-chave: Exercicio; Hiperglicemia; Prenhez; Ratas; Restrição de crescimento intrauterino.

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“Todos os dias Deus nos dá um momento em que é possível mudar tudo que nos deixa infelizes. O instante mágico é o momento em que um 'sim' ou um 'não' pode mudar toda a nossa existência.”

(Paulo Coelho)

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daquele que me confere poder”

Filipenses 4:13

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"Dê a quem você ama: asas para voar, raízes para voltar e motivos para ficar."

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"As coisas mais simples da vida são as mais extraordinárias, e só os sábios conseguem vê-las."

(Paulo Coelho)

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Artigo

INTRAUTERINE GROWTH RESTRICTED RATS EXERCISED BEFORE AND DURING PREGNANCY: MATERNAL-FETAL REPERCUSSIONS

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Resumo

Avaliar o efeito do exercício de natação antes e durante a prenhez em ratas que nasceram com restrição de crescimento intrauterino (RCIU) e na sua prole. As ratas RCIU foram obtidas usando um modelo de indução de diabete grave (DG) por *streptozotocin*. As ratas não-diabéticas e DG geraram descendentes apropriados (C) e pequenos (RCIU) para a idade de prenhez, respectivamente. Na vida adulta, as ratas C e RCIU foram distribuídas em 4 subgrupos: controle não-exercitado (C); controle exercitado (Cex); restrição de crescimento intrauterino não-exercitado (RCIU) e restrição de crescimento intrauterino exercitado (RCIUex). A taxa de acasalamento do grupo RCIU foi reduzido comparado ao grupo controle. As ratas RCIU apresentaram diminuição do peso corpóreo desde o nascimento até o período de lactação independentemente do exercício físico. No 90º dia de vida, as ratas RCIU apresentaram intolerância à glicose comparada ao grupo C. Os pesos dos orgãos maternos (coração, pâncreas e pulmão) foram maiores, os tecidos adiposos (pancreático, peritoneal, esternal e periovariano) e a adiposidade total e relativa das ratas RCIUex foram menores em relação ao grupo Cex. Na prole, o peso corpóreo de fêmeas e machos foram reduzidos nos grupos RCIU e RCIUex comparado aos dos grupos C and Cex, respectivamente. No entanto, foi observado aumento nas taxas de filhotes classificados como apropriados para a idade de prenhez no grupo RCIUex. O peso relativo do coração dos descendentes machos e do cerebro e do pulmão dos desecendentes fêmeas e machos foram maiores no grupo RCIUex comparado aos do Cex. Com isso, o programa de natação aplicado antes e durante a prenhez previniu a intolerância à glucose, reduziu a adiposidade em geral e aumentou os pesos dos orgãos na mãe e nos descendentes, mostrando o efeito benéfico do exercicio físico para ratas RCIU.

Palavras-chave: Restrição de crescimento intrauterino (RCIU), ratas, prenhez, exercício, hiperglicemia.

Abstract

To evaluate the swimming effect before and during pregnancy of rats born with intrauterine growth restriction (IUGR) and on their offspring. For this, IUGR rat offspring were obtained from the streptozotocin-induced severely diabetic (SD) dams. The nondiabetic and SD pregnant rats generated offspring with appropriate (Control, C) and small (IUGR) weight for pregnancy age, respectively. At adult life, C and IUGR groups were distributed into four subgroups: non-exercised control (C): exercised control (Cex), non-exercised IUGR (IUGR) and exercised IUGR (IUGRex). The rate of mated IUGR rats was reduced compared to control group. The IUGR rats presented decreased body weight from birth to lactation regardless of physical exercise. At day 90 of life, IUGR rats presented glucose intolerance compared to C group. The maternal heart, pancreas and lung weights were increased, and the adipose tissues (pancreatic, peritoneal, esternal and periovarian fat), total and relative adiposity of IUGRex rats were reduced compared to Cex. In the offspring, female and male body weights were reduced in the IUGR and IUGRex groups in relation to those of C and Cex, respectively. There was increase of the newborn classified as appropriate for pregnancy age in IUGRex group. The relative weights of heart of male offspring and of brain and lung of female and male offspring were increased in the IUGEex group than Cex. Thus, the swimming applied before and during rat pregnancy prevented glucose intolerance, reduced general adiposity and increased maternal and offspring´s organ weight, showing benefit effect of physical exercise for IUGR rats.

Key words: intra-uterine growth restriction (IUGR), rats, pregnancy, exercise, hyperglycemia.

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I. Introduction

Evidence that maternal body composition has important effects on the offspring came from studies showing that extremes of maternal body composition in pregnancy are associated with adverse long-term outcomes in the offspring (Godfred & Barker, 2000). Human epidemiological and animal laboratory studies show that suboptimal environments in the womb and during early neonatal life alter development and predispose the individual to lifelong health problems. The concept of the developmental origins of adult diseases (DOHaD) has become well accepted because of the compelling animal studies that have precisely defined the outcomes of specific exposures such as nutrient restriction, overfeeding during pregnancy (Nathanielsz, 2006). Therefore, the nature of fetal programming is extremely important and involves many diseases remains in successive generations (Fernandez-Twinn & Ozanne, 2006; Aerts &Van Assche, 2006; Zambrano, 2009).

Health research improves the quality and health care, influencing health policy and ensuring patient safety. Translational research is an important tool that allows researchers to expand knowledge and to aplly the results from the bench to the bed and the bed to bench (Pechhold *et al*., 2009). However, human studies are limited not only for ethical reasons but also by the many uncontrollable variables that modify the intrauterine environment. Therefore, it is necessary to develop adequate experimental models (Lopez-Soldado & Herrera 2003). The experimental models using laboratory animals are relevant to expand and improve the understanding of the pathophysiological mechanisms as in the inappropriate intrauterine environment. The literature describes experimental models to generate an inappropriate maternal environment, including corticosteroids (Nyirenda *et al*., 1998), decreased uterine blood flow through the uterine arteries bilaterally ligament (Jansson & Lambert, 1999; Simmons *et al*. 2001, Gallo *et al*, 2012; Tran *et al,* 2013), chronic hypertension (Bassan *et al*, 2005), protein malnutrition (Dahri *et al*., 1991; Zambrano, 2009), uncontrolled Type 1 *Diabetes mellitus* (Damasceno *et al*., 2002, 2011).

The maternal hyperglycemia during pregnancy causes complications for both mother and their offspring. Previous studies showed that adult female rats with diabetes chemically induced by streptozotocin (STZ) present high glycemia (> 300mg/dL - severe diabetes) and their offspring was born small for pregnancy age (SPA) due to an intrauterine growth restriction (IUGR) (Volpato *et al*., 2008; 2009; Damasceno *et al*., 2011; de Souza *et al*., 2009). This fact may be explained because maternal

hyperglycemia led to fetal hyperglycemia, causing exhaustion in the beta pancreatic cell and consequently hypoinsulinemia and the decreased insulin level causes IUGR (Pedersen, 1954; Holemans *et al*., 2003).

There is several procedures to control maternal glycemia and to prevent impairments on the embryofetal development. Traditionally, the association between diet and insulin is the therapeutic resource most used to control blood glucose concentrations. However, alternative therapies are being tested as adjuvants, such as exercise. (Devlin, 1992; Kim, 2010). In general, the benefits of regular physical activity are: improvement of cardiac *performance* , reducing body fat index and water retention, providing better glycemic control and better perinatal outcome (Melzer *et al*., 2010). Physical activity has long been known for its role in controlling glycemic levels by direct or indirect effects on insulin action (Uriu-Hare *et al*., 1989). However, a major question remains regarding the correlation between the potential benefits and risks of physical exercise on fetal development during human pregnancy. A previous study performed in our laboratory demonstrated that swimming applied to diabetic rats from day 7 (after embryo implantation) to day 20 of pregnancy led to an improvement in maternal lipid metabolism, showing beneficial results Besides, these rats presented reduced embryonic death rates (resorption) compared to diabetic nonexercised dams (Zaidise *et al*., 1999). However, these rats showed fetuses presenting small weight for pregnancy age (Bessinger & McMurray, 2003). Another study performed by Corvino et al. (2015 – in press) showed streptozotocin-induced diabetic adult female rats (severe diabetes) presented intrauterine growth restricted offspring (IUGR). These adult IUGR rats were submitted to a swimming program during pregnancy similar to Volpato et al. (2006, 2009). The Corvino´s finding showed that these rats presented vaginal delivery and, at day 10 post partum, it was observed unchanged maternal weight gain and blood glucose level. Besides, there was maternal improved lipid profile and increased insulin sensitivity, showing beneficial results of this exercise type for maternal organismo. However, it was verified the offspring of IUGR rats submmited to swimming program presented small weight for pregnancy age, suggesting a intrauterine growth restriction. This result suggests that intensity, type, period of swimming application may be factors that interfere inadequately on the embryofetal development (Corvino *et al*., 2015 – in press).

Considering the negative results in the offspring of IUGR rats using the Volpato et al. (2006) protocol and given the results from Vega *et al*. (2013), who applied another model of exercise with lesser

duration a day and fewer times a week, to improve maternal metabolism and perinatal outcomes, we hypothesize that the development of a new swimming model applied to IUGR rat in adulthood generates an appropriate intrauterine environment and to promote fetal programming in their offspring, thus preventing the appearance of diseases in adulthood. Therefore, the objective of this study was to evaluate the swimming effect before and during pregnancy of rats born with intrauterine growth restriction and on their offspring.

II. Materials and Method

1. Animals

Female and male Wistar rats (CEMIB - UNICAMP, Campinas - São Paulo State - Brazil) weighing approximately 200 grams (g) were housed in a certified animal care. Food and water were provided *ad libitum*. The rats were maintained on Laboratory of Experimental Research on Gynecology and Obstetrics under controlled conditions (temperature 22±2°C, humidity 55±5% and 12h light/dark cycle).

2. Diabetes induction: to create an uncontrolled intrauterine environment for obtaining intrauterine growth restricted (IUGR) offspring

2.1. Severe diabetes induction

Severe diabetes was induced at adult life of female rats (approximately at 90 days of age) by beta cytotoxic drug (Streptozotocin- STZ; Sigma Chemical Company, USA). STZ was dissolved in a citrate buffer (0.1 mol/L, pH 4.5) and intravenously (i.v.) administered at a dose of 40 mg/kg body weight (de Souza *et al*., 2009). Control rats received only citrate buffer using similar route and administration period. Seven days after STZ injection, the diabetic state was confirmed by blood glucose levels ≥ 300 mg/dL using a conventional glucometer. For nondiabetic rats, the inclusion criteria used was blood glucose levels ≤ 120 mg/dL. Glycemic values were expressed in milligrams per deciliter (mg/dL). After one week of diabetes confirmation or buffer administration (control), all adult female rats were mated overnight with

non-diabetic adult male rats. The morning on which spermatozoa were found in the vaginal smear was designated pregnancy day 0 (Volpato *et al*., 2008). The offspring was born by spontaneous delivery.

2.2. Sexing and body weight classification for offspring

All newborns (NB) were examined fresh for sex determination by the anogenital distance, which is about twice larger in the male than in the female (Damasceno *et al.,* 2008). Following, the female offspring were separated and classified by the mean ± 1.0 x standard deviation (SD) according to the mean values of fetal weights of the control group: as small for pregnancy age (SPA) when weight was smaller than mean of the control group - 1.0 x SD; appropriate for pregnancy age when weight was into of the mean values of control group (mean ± 1.0 x SD), and large for pregnancy age when weight was superior to mean of control group + 1.0 x SD (de Souza *et al*., 2009). The data were presented as percentual values. The female newborns born of the non-diabetic dams and classified as appropriate for pregnancy age (APA) were included and denominated as control group, and the female offspring born to severe diabetic dams and classified as small for pregnancy age were included and named as intrauterine growth restriction group (IUGR). After fetal classification, only eight newborns (rather female) were maintained with their dam for lactation up to weaning period (day 21 postnatal). Following, these offspring after weaning were maintained until adulthood (approximately 90 days of life). All non-diabetic and diabetic rats dams were anesthetized, killed and discarded the experiment.

3. Body weight, Oral Glucose Tolerance Test (OGTT) and biochemical determinations before pregnancy of control (C) and intrauterine growth restricted (IUGR) dams.

At mornings of days 90 and 120 of life, the maternal body weights and oral glucose tolerance test (OGTT) was performed. For OGTT, after fasting for 6 hours, a glucose solution (200 g/L) was administered by *gavage* at a final dose of 2 g/kg body weight. Following, the blood samples were obtained from a cut tip tail for glycemic determinations using a specific glucosemeter (OneTouch Ultra—Johnson & Johnson®) at 0, 30, 60, and 120 minutes (min) (de Campos *et al.,* 2007).

4. Experimental groups

For distribution of control and IUGR rats, submitted or not to swimming, the experimental groups were denominated:

- **C (control):** non-exercised APA female rats,
- **Cex (exercised control):** APA female rats exercised prior to and during pregnancy;
- **IUGR (intratuerine growth restriction)**: non-exercised SPA female rats,
- **IUGRex (intratuerine growth restriction)**: SPA female rats exercised prior to and during pregnancy.

5. Physical exercise (swimming program) of control (C) and intrauterine growth restricted (IUGR) rats: prior to and during pregnancy

At day 90 of life, one month before the mating period, C and IUGR rats were randomly selected to begin swimming program modified of other two exercise protocols: as the swimming Volpato *et al*. (2006) and as the exercise time of Vega *et al*. (2013), who used the wheel system for the rats. The Cex and IUGRex rats (Generation F1) were exposed to swimming program three times per week in a cage (100 x 70 x 60 cm) containing water at a depth of 40 cm (sufficient for them to be encouraged to swim) at 32±C, and without additional overhead to the body during 15 min, followed by 15-min rest and a second 15 min run between 9 hours AM and 10 AM. Throughout the study, the rats were allowed two non consecutive rest days weekly.

6. Obtaining pregnant rats

At day 120 of life, all groups (control – C; C exercised - Cex; IUGR and IUGR exercised - IUGRex) rats were submitted for mating using similar proceedings to mother rats. During pregnancy, the pregnant rats were maintained in the individual cages. The exercised groups (Cex and IUGRex) continued swimming program during this period until day 20 of pregnancy.

7. Body weight and biochemical determinations during pregnancy of control (C), exercised control (Cex), IUGR and exercised IUGR (IUGRex) dams

At mornings of days zero (early pregnancy), 7 (embryonic period), 14 (fetal period) and 20 of pregnancy (end of pregnancy – at term pregnancy), the maternal body weights and post prandial glycemia were determined for evaluation of swimming effect. All blood samples were obtained by venous puncture of the tail. Blood glucose concentrations were measured by conventional glucometer and these values were expressed in mg/dL.

At the day 17 of pregenacy, OGTT was performed in all these rats to evaluate the presence or absence of glucose intolerance following the methodology described in item 3.

8. Body weight and biochemical determinations after delivery of control (C), exercised control (Cex), IUGR and exercised IUGR (IUGRex) dams

On the day 1 after vaginal delivery, the mother rats and their offspring were weighed. Then, the sexing and body weight classification of these newborns were performed as described in item 2.2 of this experiment.

At day 5 (half of lactation period) and 9 (end of experiment), the dams and their offspring were again weighed, the milk production was calculated and blood samples were obtained by venous puncture of the tail for blood glucose concentrations were measured by conventional glucometer (mg/dL), and the maternal triglyceride and cholesterol concentrations by Accutrend® Plus (mg/dL).

At day 10 after delivery, the dams and their offspring were weighed and anesthetized with sodium pentobarbital (Hypnol® - 50 mg/kg body weight) and maternal heart, lung, pancreas, liver, adipose tissues (peritoritoneal, periovariano, periuterino, pancreatic and sternal - only mothers) and mammary gland were collected. These organs were dissected and weighed to obtain the relative weight (absolute weight / body weight x 100). Regarding adipose tissues, the calculation of total fat (sum of all adipose tissues) and relative weight (total body fat / body weight x 100).

9. Statistical Analyses

The data were presented as mean ± standard deviation. To avoid overinfluence of data from a single mother in study, the females for each group came from different litters. For comparison only two groups were analyzed by Student's unpaired t-test for normal distribution and Mann-Whitney for abnormal

distribution of data. The proportion data were analyzed by Fisher´s exact test. $P<0.05$ was considered as statistical significance limit.

10. Ethical aspects

The Ethics Committee on Animal Experiments of the Botucatu Medical School – UNESP, approved all experimental procedures performed in this study (Protocol Number: 938/2012).

III. RESULTS

Maternal data

Oral glucose tolerance test (OGTT)

Figure 1 shows the OGTT. At 90 days old, it was oberved that the blood glucose levels were increased in the timepoints 30 and 60 minutes (min) in the IUGR group compared to those of control group (Figure 1A). With 120 days of age, there was glycemic increase in the Cex group compared control group (C) and decreased blood glucose levels in IUGRex group compared to Cex group only in timepoint 30 min (Figure 1B). On the day 17 of pregnancy, at the beginning of OGTT (timepoint zero before the glucose overload), the Cex and IUGRex groups presented reduced blood glucose levels compared to their respective control groups (C and IUGR), respectively. At the end of OGTT (timepoint 120 min), the IUGRex group presented reduced glycemia in relation to Cex and IUGR groups (Figure 1C).

Body weght

Figure 2 shows the evolution of body weight. With 90 days of age, the rats of the IUGR group presented lower body weights compared to those of control group (Figure 2A). In the day 120 of life, the IUGR and IUGRex groups also showed reduced weight ($p < 0.05$) compared to their respective control groups (C and Cex) (Figure 2B). During pregnancy, there was an increase of maternal body weight in all groups, but IUGRex and IUGR groups on days 0, 7 and 14 of pregnancy had lower body weights in relation to their respective C and IUGR groups, respectively. The IUGRex rats also showed decreased weight on the day 20 of pregnancy compared to Cex group (Figure 2C). During the lactation period (days 1, 5 and 9), the IUGR IUGRex groups showed decreased body weights compared to C and Cex groups, respectively (Figure D).

Litter size

It was verified no change on the litter size ando n the number of newborns among experimental groups (C = 9.80; Cex = 11.37; IUGR = 10.80 and IUGRex = 11.25 newborns).

Milk production

The experimental groups showed an milk mean production on the day 5 of lactation in the control group (3.33 ± 1.12 g); Cex (4.93 ± 3.12 g); IUGR (4.40 ± 0.80 g) and IUGRex (3.91 ± 12.02 g) and on the day 9 in the control group (3.83 ± 3.18 g); Cex (2.50 ± 7.20 g); IUGR (3.90 ± 3.92 g) and IUGRex (5.48 ± 2.55 g). There was no statistically significant difference in milk production among experimental groups.

Reproductive outcomes

In relation to parental generation, 64 female rats were injected with streptozotocin and of these 100% presented blood glucose concentration above 300 mg/dL. In the group nondiabetic, 7 rats received citrate buffer and 100% of them presented glycemia below 120 mg/dL. At adult life, all nondiabetic female rats and 27 diabetic female rats mated. Of these, all non-diabetic rats (100%) and 13 severe diabetic rats reached at term pregnancy. The severe diabetic dams presented lower alive fetuses compared to non-diabetic rats (Table 1).

Organ and adipose tissues relative weights

The relative weight of heart of Cex rats was reduced compared to C group. In IUGRex group, there is an increase compared to Cex group ($p < 0.05$). The relative weight of pancreas and lung were increased in IUGRex group compared to the group Cex. There was no statistically significant difference in the relation to relative weights of liver and mammary gland among the different experimental groups ($p > 0.05$) (Table 2).

The relative weight of the adipose tissues (pancreatic, peritoneal, periovariano and sternal) were reduced in IUGRex rats compared to Cex rats. Regarding and peritoneal and periovariane adipose tissue, it was verified a reduction in relative weight in IUGRex rats compared to the IUGR group. The relative weight of sternal adipose tissue was also reduced in IUGR group compared to the C group (Table 2).

Total and relative adiposity/fat

In relation to total fat, the IUGR group showed a decrease compared to control group (C) and the IUGRex group also showed reduced adiposity compared to Cex and IUGR groups. The relative fat was reduced in IUGRex rats in relation to Cex and IUGR rats (Table 2).

Newborn data

Body weight

The females and males newborns showed reduced body weights (days 1, 5 and 9 of postnatal life) in IUGR and IUGRex groups in relation to C and Cex groups, respectively (Figure 3).

Blood glucose levels

The glycemic mean of female newborns at day 5 (C group = 117.65±9.20 mg/dL; Cex group = 113.84±10.45 mg/dL; IUGR group = 114.50±10.59 and IUGRex groups = 117.43±10.30) and day 9 (C group = 119.00±21.64 mg/dL; Cex group = 117.52±13.57 mg/dL; IUGR group = 113.69±8.81 and IUGRex groups = 107.61±13.32) presented no difference compared to day 5 and day 9. The glycemic mean of male newborns at day 5 (C group = 110.71±8.52 mg/dL; Cex group = 115.93±11.01 mg/dL; IUGR group = 108.16±14.13 and IUGRex groups = 116.65±12.65) and day 9 (C group = 114.00±12.12 mg/dL; Cex group = 114.58±12.60 mg/dL; IUGR group = 104.82±13.59 and IUGRex groups = 109.93±17.61) presented no difference compared to day 5 and day 9, regardless of the groups that mothers were inserted.

The maternal blood glucose levels were not correlated ($p > 0.05$) with the blood glucose levels of their newborns (data not shown).

Organ relative weights

The newborns females and males showed relative weights of brain and lung increased in IUGR and IUGRex groups compared to C and Cex groups, respectively. Only female newborns showed increased relative weight of the lung in Cex group compared to the control group (C). The male offspring showed an increase in heart weight in IUGRex group compared to those of Cex rats (Table 3).

IV. DISCUSSION

In the present study, the rats born with intrauterine growth restriction (IUGR) presented glucose intolerance (blood glucose levels about 140 mg/dL) with 90 days of life. These experimental data are equivalent to the inclusion criterion for patients with impaired glucose human and are compatible findings of Dallaqua et al. (2012). Corroborating our results, a model of uteroplacental insufficiency in rats showed that the first generation (F0) originated restricted female newborns (F1). On the day 18 of pregnancy, there was no reduction in plasma glucose of the same manner compared to those of control group during the oral glucose tolerance test, indicating a glucose intolerance in all timepoints of this test. (Gallo *et al.,* 2012).

After 30 and 50 days of swimming application, the rats of IUGRex group showed reduction in blood glucose levels in OGTT at 120 day and 17 day of pregnancy, showing that exercise was beneficial for these rats. Physical exercise and insulin physiologically stimulate glucose transport in skeletal muscle (Hayashi et al., 1997; Goodyear & Kahn, 1998). Exercise helps positively in fetal weight and morphological development of the organs of fetuses, controls the *Diabetes mellitus* appearance and regulates lipid metabolism (Uriu-Hare et al.,1989; Volpato et al., 2006).

In this study, regardless of whether or not to practice swimming, the rats of IUGR group were born and continued with lower weights throughout the experiment (day 90 and day 120 of life, pregnancy and lactation period), showing that these rats showed no catch up growth. The catch-up growth can be defined as a realignment of an individuals genetic growth potential after-intrauterine growth retardation (IUGR) Simmons (2005). According Holemans et al. (1997; 2003), IUGR rats, obtained from the diabetes induction in rats on the 11th day of pregnancy, presented lower body weight during their entire postnatal life corroborating our findings. Furthermore, it was found that a uteroplacental insufficiency in F0 females led to a reduced body weight of F1 female newborns at postnatal day 1 and these restricted females remained with lower weights at all ages studied (Tran *et al*., 2013; Gallo *et al*.,2012). Contradictorily, other authors found increased body weight, featuring catch up on the model of uteroplacental insufficiency in F0 females. These adult rats had newborns with low weights at postnatal day 1, but caught up to controls at mating (4 months) (Gallo et al, 2012;. Mazzuca et al, 2012). (Gallo *et al*., 2012; Mazzuca *et al*., 2012).

The absence of catch up growth may play important role in protecting them from adverse metabolic outcomes in the long term and to prevent the deterioration of in vivo insulin action that occurs

with age and as a result, glucose levels are more easily maintained. (Tran *et al*.,2013), corroborating with our results realted to glycemia during pregnancy and lactation periods. Besides, the catch-up growth in IUGR might differently influence the type 2 diabetes pathogenesis. The insulin resistance might play a major role in the subjects who show catch-up growth while insulin secretion defect or impaired β-cell development play a major role in the subjects who fail to undergo catch-up growth. Corvino et al. (2015) also verified no catch up growth in IUGR rats, suggesting that the IUGR groups presented defects in insulin action that precede defects in insulin secretion leading to development of insulin resistance. However, the swimming program apllied to IUGR pregnant rats caused increase in insulin sensitivity.

In our study, the rats born with IUGR who practiced swimming presented weight gain during pregnancy and reduced total and relative adiposity, showing beneficial effect of exercise for these rats. This can lead to prevention of future obesity that can develop in adulthood of offspring born of IUGR (Ravelli et al 1999; Valdez et al., 1994). However, another study using voluntary exercise in wheel before and during pregnancy of obese rats (MO) observed that these mothers gained less weight during pregnancy without changes in offspring weight at birth, MEx improved maternal carbohydrate metabolism but the signs of dysfunctional carbohydrate metabolism remained, suggesting the level of exercise, beginning even before pregnancy, could not completely suppress the effects of MO. Importantly, short periods (30 min a day) of exercise for 1 month clearly provides benefits to both mother and offspring (Vega et al., 2013). Care is necessary drawing conclusions from this interesting finding as exercise may have different effects on different fat depots. We would pose the testable hypothesis that exercise induced mechanisms change some adipocyte metabolic pathways leaving others A different exercise regimen may be required to modify them. (Veja *et al*.,, 2013). In contrast, regular exercise is known to reduce body fat (Pate *et al*., 1995), with the majority of research focused on exercise-induced fatty acid oxidation (Karzel &Friedman, 1991). Besides, these results reinforce the importance of the type and intensity of exercise as well as to the duration and frequency of exercise sessions to carefully balance between potential benefits and potential harmful effects. Additional attention should be given to progression in intensity over time.

Regarding the relative weights of maternal organs, our results showed that the relative weights of heart, pancreas and lung of exercised IUGR rats were similar to the control group. In a model of uteroplacental insufficiency in F0 females generated restricted females (F1) and it was verified that organ

relative weights (heart and pancreas) were also not different between control and restricted pregnant groups at day 19 of pregnancy (Gallo *et al*., 2012).

In this study, it was found no changes in litter size. Similarly, in others investigations it was demonstrated that the total (male and female) F2 litter size was not different between control and restricted rats (Gallo *et al*., 2012; Tran *et al*., 2013). However, in another study it was verified that uteroplacental insufficiency in F0 females caused reduction in number of F1 male and female newborns (5–6 restricted pups vs. 8–9 control pups, respectively) (Tran *et al*., 2013).

In relation to F2 male and female newborns from mothers restricted (IUGR) (regardless of the swimming practice), in this study it was verified higher percentage of newborns classified as adequate for pregnancy age [IUGR (68.3%) and IUGRex (61.5%) vs control (68.0%), Cex (45.6%)] (data not shown), showing that the exercise was not harmful for offspring growth of exercised rats, unlike the results of Damasceno et al. (2012) and Volpato et al (2014), who showed that swimming (60 min/day, 6 times/week) in non-diabetic rats increased the rate of newborns classified as small for age of pregnancy, confirming the intrauterine growth restriction. Our results indicate that the unfavorable intrauterine environment is modified positively by the practice of maternal exercise.

The glycemia of female and male newborn showed no changes in the perinatal period. It has been shown in our study that the male and female newborns of IUGRex mothers group showed an increase in the relative weight of the heart, brain and lung and it was also observed increased realative weight of brain and lung (females and males) in the IUGR mothers group. The female newborns of Cex dams had increased relative weight of the lung, demonstrating once again that exercise apllied to IUGR rats was not hoarmful to their offspring.

In summary, there is ample evidence that an abnormal intrauterine environment can induce alterations in fetal metabolism with persisting consequences in late life and successive generations. Our data show that the newborns from diabetic rats were born with IUGR and developed glucose intolerance at adulthood. However, when these rats were subjected to a swimming program before and during pregnancy, the intolerance glucose was prevented. Besides, there was a reduced adiposity general preventing the possibilities of developing an obesity status, an increased organ weight in maternal organism and in

offspring, and increased rate of newborn classified as adequate for pregnancy age, showing benefit effect of physical exercise is IUGR rats in two successive generations.

V. ACKNOWLEDGEMENTS

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Table 1. Outcomes of female rats from parental and first generation.

	Parental Generation	
	Non-diabetic	**Severe diabetic**
Number of rats	7	64*
Number of mated female rat	7/7 (100%)	27/64 (42.24%)*
Number of rat with at term pregnancy	7/7 (100%)	13/27 (48.10%)*
Littler size / rat	13.14	7.84*

	First Generation	
	APA/Control (from non-diabetic dam)	**SPA/IUGR (from diabetic dam)**
Female offspring at birth	29	37
Number of alive female offspring at 3 month	23/29 (79.31%)	18/37 (51.35%)
Number of mated female rat	15/23 (65.21%)	10/18 (55.55%)

	C	**Cex**	**IUGR**	**IUGRex**
Number of rat with at term pregnancy	6	8	5	5

*$p<0.05$ – significant statistically difference from nondiabetic/control groups (Fisher´s exact test).

Table 2. Relative weight of maternal (%) and weight of different adiposes tissues (%) at day 10 of lactation of control not exercised (C), exercised control (Cex), intrauterine growth restricted not exercised (IUGR) and exercised IUGR (IUGRex) dams.

Organs	Groups			
	C (n=7)	Cex (n=8)	IUGR (n=5)	IUGRex (n=5)
Heart[a]	0.35±0.04	0.28±0.01[$]	0.33±0.04	0.34±0.01[#]
Pancreas[b]	0.24 ±0.03	0.22 ±0.03	0.30 ±0.05	0.25 ±0.01[#]
Lung[a]	0.50±0.06	0.44±0.08	0.51±00.15	0.62±0.11[#]
Liver[b]	3.45±0.121	3.47±0.19	3.42±0.58	3.78±0.48
Mammary gland[b]	6.32±0.89	6.43±0.75	6.01±2.44	5.51±1.11
Adiposes tissues				
Peritoneal (g)[b]	1.34±0.59	1.51±0.265	0.92±0.33	0.42±0.10[#&]
Periovariano (g)[b]	0.28±0.12	0.36±0.09	0.36±0.18	0.15±0.03[#&]
Periuterino (g)[a]	0.70±0.22	0.60±0.21	0.51±0.25	0.41±0.17
Pancretic(g)[a]	0.19±0.10	0.22±0.66	0.21±0.10	0.12±0.03[#]
Sternal (g)[b]	0.07±0.03	0.06±0.02	0.02±0.007[*]	0.03±0.007[#]
Total fat (g)[b]	8.41±2.52	9.92±4.11	5.15±2.39[*]	2.50±1.39[#&]
Relative fat (%)[b]	0.85±0.29	0.79±0.15	0.67±0.18	0.43±0.08[#&]

Values are expressed as mean ± standard deviation (SD).

[$] $p<0.05$ - statistically significant difference between C and Cex

*$p<0.05$ - statistically significant difference between C and IUGR

# $p<0.05$ - statistically significant difference between Cex and IUGRex

[&] $p<0.05$ - statistically significant difference between IUGR and IUGRex

([a]– t test; [b]– Mann Whitney test)

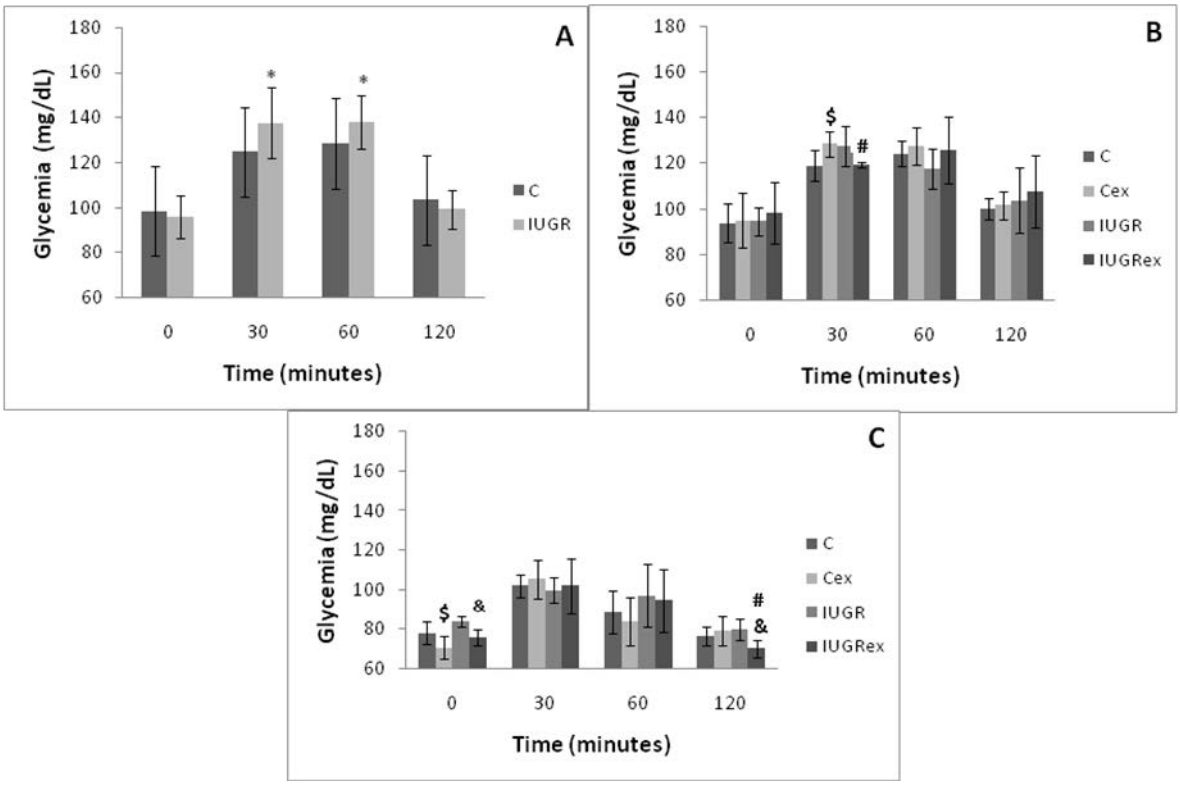

Figure 1: Oral glucose tolerance test at day 90 of life (A), at day 120 of life (B) and at day 17 of pregnancy (C) of control not exercised (C, n=7), exercised control (Cex, n=8), intrauterine growth restricted not exercised (IUGR, n=5) and exercised IUGR (IUGRex, n=5) dams.

Values are expressed as mean ± SD.

$^{\$}$ $p<0.05$ - statistically significant difference between C and Cex

*$p<0.05$ - statistically significant difference between C and IUGR

# $p<0.05$ - statistically significant difference between Cex and IUGRex

$^{\&}$ $p<0.05$ - statistically significant difference between IUGR and IUGRex

(t test for data at days 90 and pregnancy; and Mann-Whitney test for data at day 120 of life).

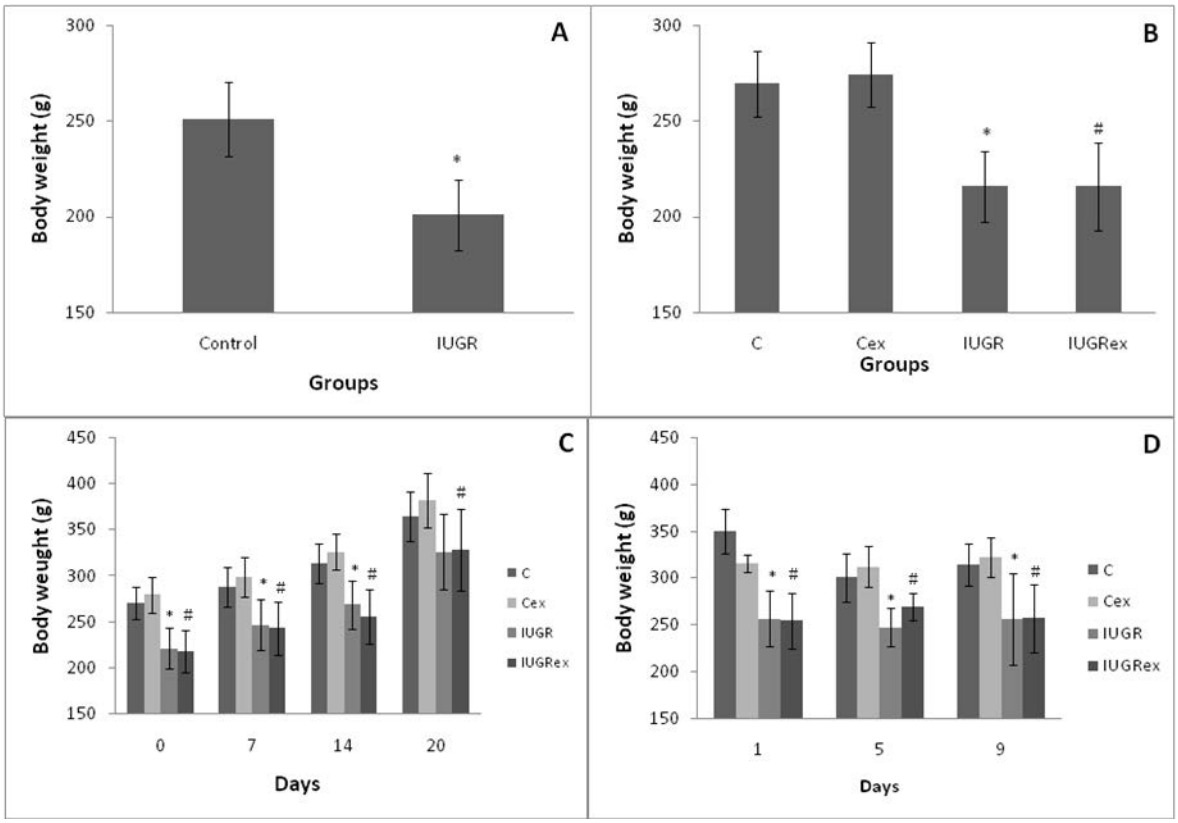

Figure 2: Body weight at day 90 (A), at day 120 of life (B), during pregnancy (C) and during lactation (D) of control not exercised (C, n=7), exercised control (Cex, n=8), intrauterine growth restricted not exercised (IUGR, n=5) and exercised IUGR (IUGRex, n=5) dams.

Values are expressed as mean ± SD.

* $p<0.05$ - statistically significant difference between C and IUGR

# $p<0.05$ - statistically significant difference between Cex and IUGRex

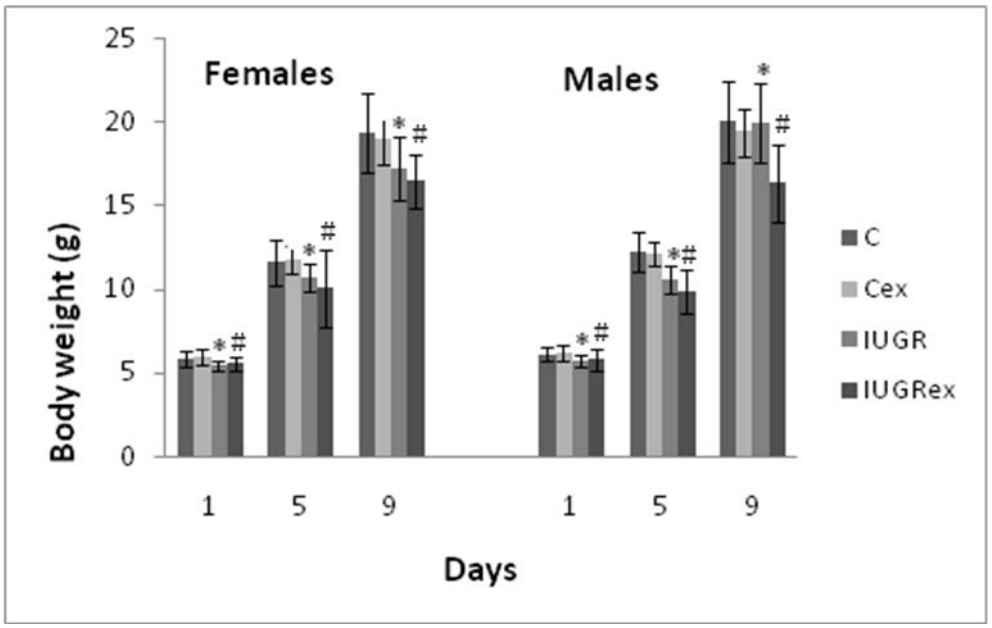

Figure 3: Body weight of females from control not exercised (C, n= 21), exercised control (Cex, n= 33), intrauterine growth restricted not exercised (IUGR, n= 21) and exercised IUGR (IUGRex, n= 22). Body weight of males from control not exercised (C, n= 22), exercised control (Cex, n= 30), intrauterine growth restricted not exercised (IUGR, n= 19) and exercised IUGR (IUGRex, n= 18).

Values are expressed as mean ± SD.

* $p<0.05$ - statistically significant difference between C and IUGR

# $p<0.05$ - statistically significant difference between Cex and IUGRex

(t test for data at days 1 and 9 of life, and Mann-Whitney Test for data at day 5 - females; t test for data at days 1 and 5 of life, and Mann-Whitney Test for data at day 9 0 male).

Table 3. Relative weight of organs of newborns from control not exercised (C), exercised control (Cex), intrauterine growth restricted not exercised (IUGR) and exercised IUGR (IUGRex) dams at day 10 of lactation.

	Groups			
	C	Cex	IUGR	IUGRex
Females	n = 21	n = 33	n = 21	n = 22
Brain[a]	3.61±0.56	3.56±0.57	4.09±0.43[*]	4.17±0.46[#]
Pancreas[a]	0.19±0.05	0.19±0.05	0.19±0.05	0.18±0.04
Lung[b]	1.76±0.09	1.94±0.50[$]	1.98±0.20[*]	1.96±0.13[#]
Heart[a]	0.57±0.07	0.54±0.07	0.58±0.10	0.54±0.07
Liver[a]	2.23±0.26	2.30±0.25	2.16±0.34	2.28±0.25
Males	n = 22	n = 30	n = 19	n = 18
Brain	3.15±0.72	3.51±0.76	4.13±0.51[*]	3.98±0.55[#]
Pancreas[b]	0.16±0.04	0.19±0.04	0.18±0.08	0.19±0.06
Lung	1.81±0.16	1.83±0.17	2.04±0.22[*]	2.04±0.19[#]
Heart[a]	0.52±0.05	0.53±0.06	0.56±0.11	0.58±0.08[#]
Liver	2.00±0.75	2.32±0.26	2.17±0.35	2.30±0.27

Values are expressed as mean ± SD.

[$] $p<0.05$ statistically significant difference between C and Cex

[*] $p<0.05$ statistically significant difference between C and IUGR

# $p<0.05$ statistically significant difference between Cex and IUGRex

([a]– t test, [b]–Mann-Whitney test)

Anexos

UNIVERSIDADE ESTADUAL PAULISTA
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FMB

CEEA Ética

Comissão de Ética em Experimentação Animal

Criada através da Portaria DFM nº 30 de 26/04/99

Certificado

Certificamos que o **(Protocolo CEEA 938/2012 "Marcadores de crescimento perinatal e impriting genético nos descendentes advindos de um ambiente intrauterino desfavorável; Estudo experimental"**, a ser conduzido por **Silvana Barroso Corvino**, orientada pela Profª. Drª. Débora Cristina Damasceno, Co-orientada por Felipe Perecin, com a colaboração de Aline Bueno, Aline de Oliveira Netto, Bruna Dallaqua, Franciane Quintanilha Gallego, Gustavo Tadeu Volpato, Profª Iracema de Mattos Paranhos Calderon, Isabela Lovizutto Iesse, Kleber Eduardo de Campos, Profª Marilza Vieira Cunha Rudge e Yuri Karen Sinzato , está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA), com a ressalva de que os **"ratos"** são provenientes de Biotério convencional, sem condições de atestar a Sanidade dos mesmos.

Projeto de Pesquisa aprovado em reunião da CEEA em 26/04/2012

Profª Drª Maria Rosa Bet Moraes Silva
Presidente da CEEA

Alberto Santos Capelluppi
Secretário da CEEA

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

MUDANÇA DE TÍTULO EM PROJETO DE PESQUISA

Objetivo Acadêmico:

( ) Pós Doutorado
(X) Tese Doutorado
( ) Dissertação de Mestrado
( ) Trabalho científico
( ) Outros: Especificar

Título Inicial:

Marcadores de crescimento perinatal e impriting genético nos descendentes advindos de um ambiente intrauterino desfavorável: Estudo experimental

Título Final:

Ratas nascidas com restrição de crescimento intrauterino submetidas à natação antes e durante a prenhez: repercussões maternas e perinatais

Data da reunião do CEP que aprovou o parecer inicial: 26/04/2012.

Declaro que o trabalho não sofreu alterações nos objetivos e/ou conteúdo metodológico da época de apresentação para análise do CEP.

~~Nome e Assinatura do Orientador(a)~~
Débora C. Damasceno

~~Nome e Assinatura do Orientado(a)~~
Silvana Barroso Corvino

- **Projetos submetidos via Plataforma Brasil:** Preencher o formulário, digitalizar e postar no sistema Plataforma Brasil.
- **Projetos submetidos anteriormente à Plataforma Brasil:** Preencher o formulário em duas vias e protocolar no CEP que emitiu o parecer inicial de aprovação.

Experimental Physiology

Manuscript #	EP-RP-2015-085099
Current Revision #	0
Submission Date	22-Jan-2015 10:10
Current Stage	Under Review
Title	INTRAUTERINE GROWTH RESTRICTED RATS EXERCISED BEFORE AND DURING PREGNANCY: MATERNAL-FETAL REPERCUSSIONS
Running Title	RATS EXERCISED BEFORE AND DURING PREGNANCY
Manuscript Type	Research Paper
Special Issue	N/A
Corresponding Author	Ms. Silvana Barroso Corvino (Univ. Estadual Paulista)
Contributing Authors	Gustavo Tadeu Volpato , Marilza Vieira Cunha Rudge , Débora Cristina Damasceno
Abstract	To evaluate the swimming effect before and during pregnancy of rats born with intrauterine growth restriction (IUGR) and on their offspring. For this, IUGR rat offspring were obtained from the streptozotocin-induced severely diabetic (SD) dams. The nondiabetic and SD pregnant rats generated offspring with appropriate (Control, C) and small (IUGR) weight for pregnancy age, respectively. At adult life, C and IUGR groups were distributed into four subgroups: non-exercised control (C): exercised control (Cex), non-exercised IUGR (IUGR) and exercised IUGR (IUGRex). The rate of mated IUGR rats was reduced compared to control group. The IUGR rats presented decreased body weight from birth to lactation regardless of physical exercise. At day 90 of life, IUGR rats presented glucose intolerance compared to C group. The maternal heart, pancreas and lung weights were increased, and the adipose tissues (pancreatic, peritoneal, esternal and periovarian fat), total and relative adiposity of IUGRex rats were reduced compared to Cex. In the offspring, female and male body weights were reduced in the IUGR and IUGRex groups in relation to those of C and Cex, respectively. There was increase of the newborn classified as appropriate for pregnancy age in IUGRex group. The relative weights of heart of male offspring and of brain and lung of female and male offspring were increased in the IUGEex group than Cex. Thus, the swimming applied before and during rat pregnancy prevented glucose intolerance, reduced general adiposity and increased maternal and offspring´s organ weight, showing benefit effect of physical exercise for IUGR rats. What is the central question of this study? Development of a new swimming model applied to IUGR rat in adulthood generates an appropriate intrauterine environment and to promote fetal programming in their offspring, thus preventing the appearance of diseases in adulthood.
New Findings	What is the main finding and its importance? The swimming applied before and during rat pregnancy prevented glucose intolerance, reduced general adiposity and increased maternal and offspring´s organ weight, showing benefit effect of physical exercise for IUGR rats.
Editor	Not Assigned
Suggested Referees List by Author	Elena Zambrano, Yuri Sinzato, Kleber Campos
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   a. Article File 1 (last updated: 01/22/2015 09:56:12) PDF (248KB) Source File (PDF) 281KB
   b. Figure 6 (last updated: 01/22/2015 09:58:45) PDF (188KB) Source File (PDF) 360KB
2. Cover Art (last updated: 01/22/2015 10:09:24) PDF (172KB) Source File (PDF) 481KB

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UNIVERSIDADE ESTADUAL PAULISTA
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Botucatu, 23 de Janeiro de 2015.

Prezados Professores,

Gostaria de ressaltar que, além do manuscrito apresentado na forma escrita e em aula, foram preparados outros dois manuscritos:

Title: Evaluation of body weight and biochemical aprameters before and after delivery of control animals.

Authors: Silvana Barroso Corvino, Gustavo Tadeu Volpato, Nathália Cristine Dias de Macedo, Yuri Karen Sinzato, Débora Cristina Damasceno.

Objective: To analyze the body weight and biochemical parameters before and after delivery of control animals.

Major findings: In adulthood, the animals showed increased body weight, serum insulin and ingestion of water and food. At day 17 of pregnancy, the control rats responded adequately to oral glucose tolerance test (OGTT), i.e., it was verified a glycemic increase after the glucose overload with blood glucose levels <140 mg/dL and glycemic return at end of test. At term pregnancy, there was also weight gain due to fetal growth. In the postpartum period studied, we observed that control rats presented reduced blood glucose levels at day 9 of lactation. This fact suggests that their newborns are in progressive growth and require higher nutrient for its development, since in this period are not yet able to feed alone. This manuscript was prepared to assist researchers with regard to the comparison of the findings of experimental groups versus a control group, as these data are difficult to obtain.

Journal: *Scandinavian Journal of Medicine & Science in Sports* (Fator de impacto: 3,17).

Title: Comparative analysis of different models of swimming applied to pregnant rats born with intrauterine growth restriction

Laboratório de Pesquisa Experimental de Ginecologia e Obstetrícia

UNIVERSIDADE ESTADUAL PAULISTA
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Campus de Botucatu

Authors: Silvana Barroso Corvino, Gustavo Tadeu Volpato, Aline Netto, Iracema Mattos Paranhos Calderon, Marilza Vieira Cunha Rudge, Débora Cristina Damasceno

Objective: To perform a comparative analysis of different models of swimming applied to pregnant rats born with intrauterine growth restriction

Major findings: The exercise program 1 applied to rats born with intrauterine growth restriction (IUGR_1) was swimming with 60 minutes of exercise a day, 6 days a week during pregnancy (from day 7 to day 20 of pregnancy). In this exercise program, the rats showed no altered blood glucose levels, but there was a concentration of serum insulin decreased at day 14 of pregnancy, showing beneficial effect on the resistance to insulin of these rats. The body weight of female and male newborns (day 5 of postnatal life) in IUGRex_1 dams were reduced and there was a change in the classification of body weights of the newborns, since the rate of newborns classified as small for gestational age was greater, confirming a intrauterine growth restriction. We suggest that the swimming program applied was high intensity leading to increased rate of IUGR newborns and and reduction of newborns classified as adequate or large for pregnancy age. As for the exercise program 2, the IUGR rats (IUGRex_2) were submitted to swimming for 15 minutes, followed by 15 minutes of rest and another 15 minutes of swimming, before and during pregnancy (from day 7 to day 20 of pregnancy). The IUGRex_2 rats presented positive change in OGTT, decrease in total and relative adiposity, increase in the proportion of newborns classified as appropriate for pregnancy age, showing beneficial effects of this type of program. Thus, the swimming program 2 applied for rats was better because led to improvements for maternal organism and their offspring.

Journal: *Journal of Science in Sports* (Fator de impacto: 3,07)

Atenciosamente,

Silvana Barroso Corvino

Ciente e de acordo,

Profa. Dra. Débora Cristina Damasceno

Orientadora