Evaluation of the effect of physical exercise in the metabolism of pregnant diabetic rats

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ABSTRACT

The aim of the present study was to evaluate if physical exercise (swimming program), begun in different periods of pregnancy of diabetic rats, promotes changes in the maternal metabolism. Severe diabetes was induced in female rats using Streptozotocin. The rats were mated and randomly assigned to three groups (n = 13 rats/group): sedentary (G1) or exercised from day zero (G2) or day seven (G3) to day 21 of pregnancy. The exercise consisted of a moderate swimming program. During pregnancy, the body weight and glycemic level were weekly evaluated. All the female rats were killed on day 21 of pregnancy to carry out laparotomy. The blood samples were collected to determine total protein, triglycerides, total cholesterol and VLDL-cholesterol. Liver and muscle samples were collected to determine hepatic and muscular glycogen, respectively. Regardless the initial moment, the exercise did not alter glycemic level, body weight evolution and total protein, hepatic and muscular glycogen concentrations. However, the swimming program begun on the 7th day of the pregnancy decreased the triglyceride rate (G1 = 369.10 ± 31.91 mg/dL; G2 = 343.32 ± 162.12 mg/dL; G3 = 212.35 ± 70.32 mg/dL), total (G1 = 176.48 ± 28.25 mg/dL; G2 = 141.33 ± 19.77 mg/dL; G3 = 129.86 ± 33.16 mg/dL), and VLDL (G1 = 64.92 ± 24.41 mg/dL; G2 = 63.54 ± 28.31 mg/dL; G3 = 42.53 ± 14.12 mg/dL) cholesterol concentrations compared to G1 group. Physical exercise did not interfere on the maternal glycemic levels. Thus, the swimming program began on the day seven of the pregnancy was a beneficial treatment for the lipidic profile of the diabetic rats. This result validates an association of regular physical activity to diet and insulin treatment in pregnancy complicated by diabetes.

Keywords: Exercise. *Diabetes mellitus*. Pregnancy. Metabolism. Rats.

INTRODUCTION

The therapeutic use of physical exercise for the diabetes treatment has been promoted since 600 B.C. After the insulin discovery in 1922, some researchers highlighted interaction between this hormone and regular physical activity, with possible benefic results in the diabetes treatment [1-2]. From that moment on, the triad diet, insulin and physical exercise became the treatment principle for diabetes[2]. The current trend is that exercising should be also prescribed to pregnant diabetic women, despite the insufficient data that attribute risks or benefits to the mother or newborn[3].

The American Diabetes Association (ADA) and the American College of Obstetrics and Gynecology (ACOG) agree on the opinion that despite the neonatal exercising later effects are not defined yet, its practice should be encouraged for the majority of pregnant women[4-5].

One of the physical exercise benefits during pregnancy is to promote maternal weight control gain[6]. The maintenance of a suitable body weight for the diabetics is also important, however, few articles relate exercise to weight change during pregnancy. Cunha[7] did not observe difference in the weight gain in diabetic pregnant women submitted to walking. Uriu-Hare *et al.*[8] did not verify alteration in the body weight of diabetic female rats submitted to exercise either.

Pregnancy brings several physiological alterations and all the energetic resources are channeled to the fetus without harm to the mother. Therefore, the mother needs of carbohydrates, proteins and lipids increase in this period[9]. The metabolic alterations in the mother's body caused by exercise are dependent on its type, intensity and duration, as well as the gestational period in which it is practiced[10]. In low intensity, the glucose plasmatic concentrations are kept at a constant level in pregnancy. In moderate exercise, the glycemia regulation is balanced between the liver's debt and peripheral capitation; as a consequence, the plasmatic glucose also remains constant. However, if this exercise duration is extended, glycemia decrease occurs[11], increasing such decrease with the pregnancy time[12-13]. In the diabetes and pregnancy binary, besides the exercise characteristics, one should take the type of diabetes and the metabolic control into consideration[14].

The physical conditioning effects on the lipid profile of the type 1 diabetes patients are variable and evaluated in small non-controlled studies. A randomized clinical essay, including adult males submitted to an aerobic physical exercise program for 12 to 16 weeks, confirmed that, although the exercise being related to physical conditioning, its practice has greater relevance on the lipid metabolism, straightly related to the body relative fat indices[15].

Exercising during pregnancy increases the triglycerides and free fatty acids indices in the blood, removing a higher energetic amount, although it does not seem to increase the proteins use[10]. Little has been reported on the metabolic and hormonal responses of exercise practiced during pregnancy. Moreover, no data evaluating the exercise responses in pregnancy complicated with diabetes in the literature are available. Clinical research may not completely answer the issues on the isolated effect of physical exercise during pregnancy, and specific experimental results of this practice in pregnancy associated with diabetes are scarce. The aim of this study was to verify whether physical exercise practice (swimming), initiated at different times of pregnancy in diabetic rats, promotes alterations in maternal metabolism, in order to diminish the questioning on the results of exercise practiced during pregnancy complicated with diabetes.

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METHOD

Animals

Wistar female rats weighting around 240 g, at reproductive age (three months) were used. The animals were adapted to the Gynecology and Obstetrics Laboratory of Experimental Research – Unesp conditions, with controlled environment (temperature between 21º and 25ºC and photo period of 12 hours) for seven days. Water and Purina® food (26,6% protein, 3,5% fat, 9,6% fibers and 8,6% minerals, according to the manufacturer's specification) were offered *ad libtum*. This work was approved by the Ethics Committee in Animal Experimentation of the Medicine University of Botucatu – Unesp.

Diabetes induction, adaptation to the water environment and pregnancy diagnosis

After the animals' adaptation period, the diabetes was induced by *Streptozotocin* (STZ – SIGMA *Chemical Company,* St. Louis, MO), dissolved in citrate buffer (0,1 M; pH 6,5), in the 40 mg/kg weight dose and applied in the rat's tail vein. The glycemia was evaluated seven days after the STZ administration, through puncture in the rat's tail in order to obtain a drop of blood, which was placed on glycol tape (One Touch Ultra, Lifescan Johnson & Johnson, UK) for reading in specific glucometer (One Touch Ultra, Lifescan Johnson & Johnson, UK). Severe diabetes characterized by glycemic indices higher than 200 mg/dL was considered for this study's inclusion[16-17].

After diabetes confirmation, mating was initiated. The female rats were kept with non-diabetic males during the night. The next morning, vaginal rubbing was performed in order to analyze its content and diagnose pregnancy. The presence of sperm and the estro phase characteristics confirmed the diagnosis and that day was considered day zero of pregnancy[16-17].

In the period between the diabetes induction and the mating, all the rats were daily placed in water tanks (100 cm of length x 70 cm of width x 60 cm of depth), with maximum level of 10 cm of warm water at 31ºC, for ten minutes. Such procedure allowed animals' adaptation to the water environment, without determining physical conditioning through swimming practice[18].

Experimental groups

The pregnant diabetic female rats were randomly distributed in three experimental groups according to the physical exercise practice and beginning: rats not submitted to swimming (group G1, n = 13), rats submitted to swimming from day zero of pregnancy (group G2, n = 13) and rats submitted to swimming from day seven of pregnancy (group G3, n = 13).

Swimming program

The swimming program, developed for moderate intensity practice of physical exercise (figure 1), was modified from the one by Lancha *et al.*[18], since the rats did not receive additional overload to their bodies due to progressive weight gain caused by their pregnancy. The rats that suffered intervention were placed in the same adaptation to the water environment tanks under the same temperature, at this time with 40 cm of water, which was sufficient to stimulate them to swim. The activity was conducted in the morning, from Monday to Saturday and resting on Sundays. The initial duration was of 20 minutes, with progressive increase of 10 minutes per day up to the maximal time of 60 minutes, with a total of five days of adaptation to the physical exercise. The 60 minutes time was kept until the end of the pregnancy, allowing thus an aerobic physical effort around 60 to 70%[18]. Depending on the experimental group, the program was implemented at day zero (G2) or day seven (G3) of pregnancy. During this period, the rats from group G1 were placed in the water environment, in the same conditions of the adaptation phase, without swimming practice, though.

Data collection and pregnancy resolution

During pregnancy, the female rats were kept in individual cages and daily weighted. The glycemia, also performed through glycol tape, and the animals' weighting was performed at day zero, 7, 14 and 21 of pregnancy. The average percentage increase of the glycemic indices and of the rats weight at day 21 (end) in relation to day zero of pregnancy (beginning) was calculated. At day 21 of pregnancy, all rats from the different experimental groups (G1, G2 and G3) were anesthetized with sodium pentobarbital (Hypnol®) at 3%, and submitted to bleeding so that the blood samples were processed in order to obtain biochemical determinations. Liver (500 mg) and muscle (200 mg) samples were also removed, placed in sodium hydroxide solution at 30% and kept in freezer at –80ºC for hepatic and muscular glycogen dosing. Total proteins were determined through colorimetric method and well as triglycerides and total cholesterol through enzymatic method using WIENER kits for all serum samples[19]. Very low density lipoprotein (VLDL) was determined through formula proposed by Friedwald[20]. The determination of the hepatic and muscular glycogen concentrations was performed through the technique proposed by Dubois *et al.*[21].

Statistical analysis

The variance analysis (ANOVA) was applied considering the treatment effects. The F test was performed in order to verify the data normality, and when the average values of the variables did not adequate to the normality test, they suffered statistical transformation (log) for application of parametrical statistical test. The Student-Newman-Keuls test was applied in order to compare all the

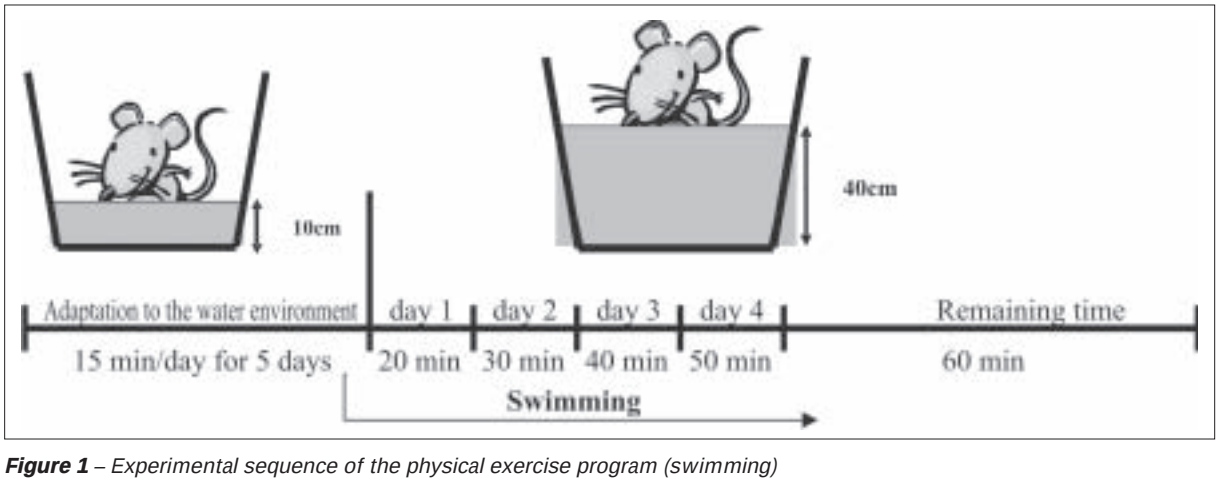

Figure 1 *– Experimental sequence of the physical exercise program (swimming)*

parameters[22]. A significance level of 5% was considered as limit of statistical significance ($p < 0,05$).

RESULTS

Mother glycemia. Independently of the moment in which it was initiated, the swimming program did not alter the glycemia of diabetic rats in pregnancy. In the beginning of the pregnancy, the maternal glycemia was higher than 300 mg/dL. These levels were kept throughout the pregnancy (figure 2), glycemia severely high in relation to non-diabetic animals (90-120 mg/dL)[23]. The calculation of the average percentage increase of the glycemia at day 21 of pregnancy in relation to day zero did not significantly differ between the experimental groups (table 1).

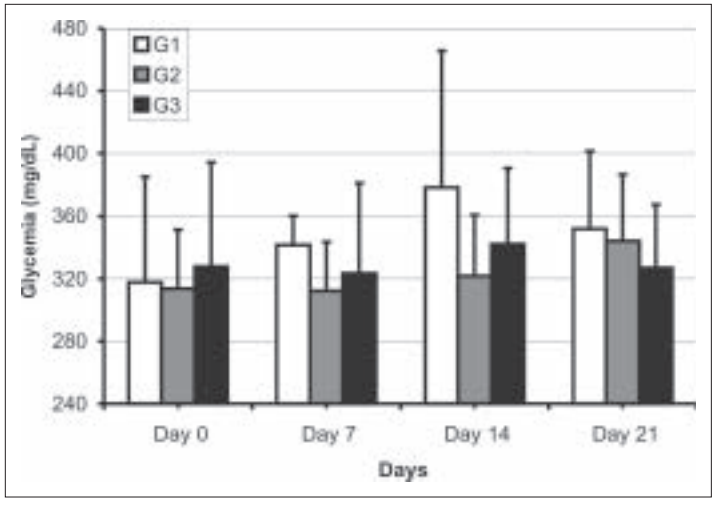

Figure 2 *– Averages and Standard deviations of the glycemia (mg/dL) of diabetic rats non-practitioners (G1) or physical exercise practitioners from day zero (G2) or day seven (G3) of pregnancy*

Values do not differ between groups at the 5% probability index – Student-Newman-Keuls test

TABLE 1
Average percentage increase (%) during pregnancy, of the glycemia and the body weight of diabetic rats non-practitioners (G1) or physical exercise practitioners from day zero (G2) or day seven (G3) of pregnancy

	G1	G2	G3
Glycemia	7.88	8.08	2.60
Body weight	34.83	35.05	39.91

Values do not differ between groups to 5% of the probability index – Student-Newman-Keuls test.

Maternal body weight. All the rats had the same initial weight, around 230 g, similar to non-diabetic animals. The maternal weight gain was progressive and similar between the groups, reaching the end of pregnancy with approximate increase of 36% of the initial weight. The exercise begun at day zero or day seven of pregnancy did not alter this parameter in diabetic rats (figure 3). The analysis of the average percentage increases of the body weight of the diabetic rats did not show significant differences in groups G2 and G3 in relation to G1 (table 1).

Biochemical parameters. The hepatic and muscular glycogen concentration in the end of pregnancy was similar in all groups of pregnant rats, average of 30 mg/Kg and 4 mg/Kg, respectively. The exercise did not alter the total proteins concentrations either. The animals submitted to exercise from day seven of pregnancy presented decrease of triglycerides blood concentration (G1 = 369,10 ± 31,91 mg/dL; G2 = 343,32 ± 162,12 mg/dL; G3 = 212,35 ± 70,32 mg/dL), total cholesterol (G1 = 176,48 ± 28,25 mg/dL; G2 = 141,33 ± 19,77 mg/dL; G3 = 129,86 ± 33,16 mg/dL) and of VLDL-cholesterol (G1 = 64,92 ± 24,41 mg/dL; G2 = 63,54 ± 28,31 mg/dL; G3 = 42,53 ± 14,12 mg/dL) compared to the diabetic exercise non-practitioner (table 2). However, these values did not reach the normality parameters of non-diabetic animals (Triglycerides 177,0 mg/dL; cholesterol 119,1 mg/dL and VLDL 35,4 mg/dL)[23].

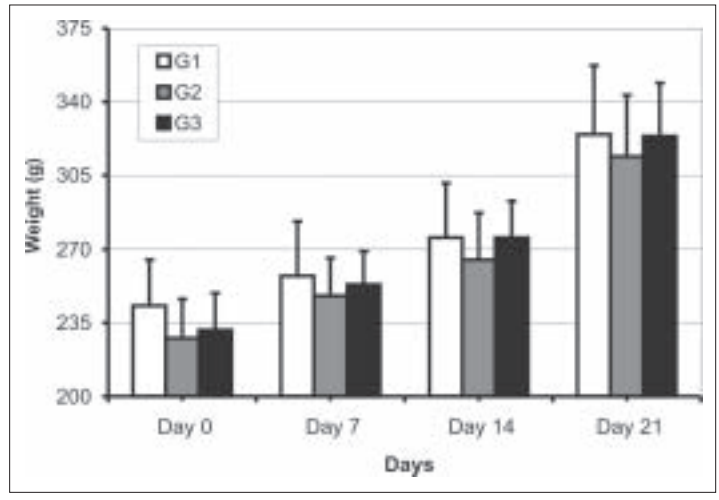

Figure 3 *– Averages and Standard deviations of the body weight (g) of diabetic rats non-practitioners (G1) or physical exercise practitioners from day zero (G2) or day seven (G3) of pregnancy*

Values do not differ between groups to the 5% probability index – Student-Newman-Keuls test

TABLE 2
Averages and Standard deviations of the biochemical parameters obtained at the end of pregnancy of diabetic rats non-practitioners (G1) or physical exercise practitioners from day zero (G2) or day seven (G3) of pregnancy

Variables	G1	G2	G3
Hepatic glycogen (mg/100 mg tec. hep.)	2.83 ± 1.06	2.81 ± 0.86	3.27 ± 0.96
Muscular glycogen (mg/100 mg tec. musc.)	0.38 ± 0.11	0.45 ± 0.13	0.43 ± 0.17
Total proteins (g/dL)	5.59 ± 1.13	5.09 ± 1.25	5.21 ± 1.03
Triglycerides (mg/dL)	369.10 ± 31.91	343.32 ± 162.12	212.35 ± 70.32*
Total cholesterol (mg/dL)	176.48 ± 28.25	141.33 ± 19.77	129.86 ± 33.16*
VLDL (mg/dL)	64.92 ± 24.41	63.54 ± 28.31	42.53 ± 14.12*

* Statistically significant difference in relation to the G1 group at 5% probability index – Student-Newman-Keuls test.

DISCUSSION

In the type 1 diabetes, with suitable metabolic control, regular physical exercise of moderate intensity determines decrease in the glycemia indices through the stimulation of glucose peripheral use, keeps the circulating insulin concentration and stabilizes the glucose hepatic production. These desirable effects are not observed in the presence of severe hyperglycemia and damage to the use of glucose and hepatic and muscular glucogenesis occurs with the glycogenolysis, lipolysis and cytogenesis activation, increasing hence, the hyperglycemia and the risk of metabolic acidosis[2,24]. The interaction between physical exercise, badly controlled diabetes and pregnancy may intensify these complications through the placental hormones activity that favors by itself peripheral resistance to the insulin activity from the 24th week of pregnancy[25].

The desired response, such as glycemia decrease and hepatic glycogen stabilization, would depend on possible physical conditioning, also difficult due to maternal metabolic alterations[2,24]. In this context, it was expected that the swimming program applied to severely diabetic pregnant rats, pregnancy experimental model

and badly controlled type 1 diabetes[26-27], would worsen the maternal situation, with increase of glycemic indices and decrease of the hepatic glycogen concentrations. However, the results showed that the intervention begun at days zero or seven of pregnancy did not influence the analyzed lipidic parameters.

In diabetic pregnant rats, the maternal hyperglycemia refractoriness to physical exercise has been reported by Uriu-Hare *et al.*[8], confirming the observations of our work. Thereby, some authors could not confirm the physical exercise action in the glycemic indices control of diabetic pregnant women either[28-29]. Damasceno *et al.*[23] associated the depletion of the hepatic glycogen supplies to the severe hyperglycemia extended action worsened by the oxidative stress in rats' pregnancy. The evaluation of oxidative stress biomarkers in shorter intervals in pregnancy could differentiate the acute and chronic effects of physical exercise in rats's pregnancy with severe diabetes and will clarify the apparent divergences between the found results in different articles under clinical and experimental concerns.

Pregnancy is characterized by progressive increase of the maternal weight gain, derived from the fetus and her annexes growth (around 40%) and by adaptations proper of the body (the remaining 60%), characterized by anabolism at the beginning and catabolism at the end of pregnancy[25]. In this work, the maternal weight progress was progressive and similar between the groups of diabetic rats, submitted or not to the swimming program. Such evidence could indicate at least non-harmful effect of this intervention. The evaluation of weight gain in diabetic pregnant women treated with diet and insulin, submitted or not to an oriented walk program, did not show direct effect of physical exercise either. Nevertheless, the results of this controlled clinical essay confirmed indirect benefit of regular physical activity on the maternal weight gain, through the increase of lean mass and restriction of fat mass indices increase[7]. The maternal weight does not seem to be a suitable parameter to confirm the effects of any kind of intervention, in pregnant women and in diabetic pregnant rats as well, if considered isolated[16,30-32]. Clinically speaking, the differentiation of the maternal weight composition could confirm this positive effect of regular physical activity in diabetic rats' pregnancy.

Besides the mentioned alterations, the metabolism of proteins and lipids is also compromised in type 1 diabetic individuals. The lack of the insulin action causes interruption of the synthesis and increase of the protein catabolism, with total use of such supplies and liberation of large quantities of amino acids in the plasma, used for energy production or as substrate for the gluconeogenesis[33]. The stimulated lipolysis results in liberation of fatty acids and glycerol in the blood stream, facilitating the hepatic conversion in phospholipids and cholesterol. These two substances, associated to the hepatic glycerides, are also liberated in the blood stream by specific carrier proteins, the lipoproteins, also quite increased due to the lack of insulin[34]. The regular practice of physical exercise prevents and controls these undesired consequences of the diabetes[5,15,24].

This experimental model reproduces the clinical results Regular physical activity prevented the protein catabolism from happening, confirmed by the maintenance of the total protein indices in the two groups of diabetic pregnant rats. The swimming program practiced from the seventh day of pregnancy improved the maternal lipid profile, decreasing the triglycerides, total cholesterol and VLDL-cholesterol concentrations in relation to the diabetic group exercise non-practitioner. Such decrease may be derived from the supply of significant quantities of energy, needed for the light to moderate exercise practice[10]. The VLDL-cholesterol is the main lipoprotein triglycerides carrier from the liver to the remaining tissues[35], which justifies the similar behavior in the evolution of its concentrations after two weeks from the beginning of the swimming program.

The difference in results related to time and pregnancy period of the program application is remarkable. Apparently, the swimming practice two times a week was better for the lipoprotein metabolism of the diabetic pregnant rats. The best evaluations were confirmed when the physical exercise was practiced in the period corresponding to the second and the third trimesters of women's pregnancy. In this phase, the resistance to the insulin action and the energetic needs for the fetal development are increased in all pregnancies[25]. The easiness of metabolic discompensation, characteristic of type 1 diabetes pregnant women, is also more common in this period[36-37]. The association of these factors could justify the best response to physical exercise in the second half of pregnancy.

Therefore, physical exercise of moderate intensity was not able to promote the normoglycemia in diabetic pregnant rats. Yet, the exercise presented positive effects on the triglycerides, cholesterol and VLDL profile in these animals, even in controlled diabetes. However, further studies are necessary in order to evaluate the exercise effects in models of moderate diabetes, being able to show that exercise would be a therapeutic factor even more efficient.

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