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Diabetic Rats Exercised Prior to and During Pregnancy: Maternal Reproductive Outcome, Biochemical Profile, and Frequency of Fetal Anomalies

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Abstract

The aim of this study was to evaluate the effects of exercise prior to or during pregnancy on maternal reproductive outcome, biochemical profile, and on fetal anomaly frequency in a rat pregnancy model utilizing chemically induced diabetes. Wistar rats (minimum n = 11 animals/group) were randomly assigned the following groups: group 1 (G1), sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; and G6, diabetic, exercised prior to and during pregnancy. A swimming program was utilized for moderate exercise. On day 21 of pregnancy, all rats were anesthetized to obtain blood for biochemical measurements. The gravid uterus was weighed with its contents, and the fetuses were analyzed. The nondiabetic rats exercised prior to pregnancy presented a reduced maternal weight gain. Besides, G2 and G3 groups showed decreased fetal weights at term pregnancy, indicating slight intrauterine growth restriction (IUGR). In the diabetic dams, the swimming program did not have anti-hyperglycemic effects. The exercise applied only during pregnancy caused severe IUGR, as confirmed by reduced fetal weight mean, fetal weight classification, and ossification sites. Nevertheless, exercise was not a teratogenic factor and improved the rats' lipid profiles, demonstrating that the exercise presented possible benefits, but there are also risks prior and during pregnancy, especially in diabetic pregnant women.

Keywords

exercise, diabetes mellitus, pregnancy, reproductive outcome, anomaly

Introduction

Diabetes mellitus is the name given to a group of disorders with different etiologies. It is characterized by impairments in carbohydrate, protein, and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action.[1] Diabetes is a syndrome of great importance because it affects 6% of the world population, and by the year 2025, 300 million individuals will be affected.[2] In pregnant diabetic individuals, high rates of perinatal mortality are observed, and miscarriage rates are 3 times more common.[3,4] Maternal hyperglycemia is also associated with fetal macrosomia,[5] a result that is quite controversial in experimental models of diabetes and pregnancy. In rats, severe diabetes is a model for intrauterine growth restriction (IUGR), which is caused by maternal hyperglycemia associated with an increased insulin secretion and overstimulation of the insulin producing β-cells during fetal life. In later life, a fetal hypoinsulinemia is found, which is related to β-cell exhaustion

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leading to reduced fetal growth.[6-8] In addition, maternal hyperglycemia also causes high levels of triglycerides (TGs) and total cholesterol (CHO) and decreases in liver glycogen levels but no changes have been observed in protein metabolism.[9-11]

After the discovery of insulin, some researchers emphasized the interaction between this hormone and physical activity, with potential benefits for the treatment of diabetes.[12] Physical activity has long been known for its role in controlling glycemic levels by direct or indirect effects on insulin action, which involves muscle glucose uptake and glycogen synthesis.[13,14] However, a major question remains regarding the correlation between the potential benefits and risks of physical activity on fetal development during pregnancy.

Clinical research may not completely answer the questions regarding the isolated effects of exercise during pregnancy; thus, experimental models are required. Previous studies have demonstrated that diabetic rats that underwent exercise (swimming program) from day 7 to day 20 of pregnancy had improved lipid profiles[15] and decreased embryo death,[16] as compared to sedentary diabetic rats. However, the smaller offspring from exercised diabetic dams indicated that exercise led to exacerbated IUGR. Furthermore, this swimming program increased the fetal skeletal anomalies.

Higher levels of physical activity before or during early pregnancy are associated with a significantly lower risk of developing gestational diabetes mellitus.[17] Nevertheless, there is a lack of interdisciplinary scientific evidence on the effects of exercise during normal pregnancy or during pregnancy complicated by diabetes,[18] making it difficult to define the role of exercise as an adjunct therapy in diabetes during pregnancy. Moreover, little is known about the effects of exercise before pregnancy on the maternal–fetal organism. Therefore, the aim of this study was to evaluate the effects of exercise, beginning prior to pregnancy or during pregnancy, on maternal reproductive outcome, biochemical profile, and on the frequency of fetal anomalies in a rat pregnancy model utilizing chemically induced diabetes.

Materials and Methods

Experimental Animals

Wistar female and male rats, weighing approximately 200 g, were obtained from the Unesp Vivarium (São Paulo State, Brazil). During the acclimatization (2 weeks) and experimental periods, rats were maintained in an experimental room under conditions with controlled temperature ($22 \pm 2^{\circ}C$) and humidity ($50\% \pm 10\%$) and a 12-h light/dark cycle, with ad libitum access to commercial diet (Purina rat chow, Brazil) and water. The local Experimental Ethical Committee for Animal Research approved the protocols used in this study.

Experimental Diabetes Induction

Diabetes was induced by streptozotocin (STZ, Sigma Chemical Co St. Louis, Missouri), according to a previously described method.[19] Streptozotocin was dissolved in citrate buffer (0.1 mol/L, pH 6.5) and administered (intravenously [iv]) at a dose of 40 mg/kg body weight. Nondiabetic rats only received (iv) citrate buffer. For inclusion criteria, the diabetic state was confirmed by blood glucose levels $\geq$300 mg/dL, 7 days after STZ injection, using a One-Touch Ultra glucometer (Johnson & Johnson, HDI Home Diagnostics, Inc, Florida). For nondiabetic rats, the inclusion criteria used was blood glucose levels <120 mg/dL. Glycemic values were expressed in milligrams per deciliter (mg/dL).

Mating Procedure

All female rats were mated overnight with nondiabetic male rats. The day when sperm was found in the vaginal smear was designated gestational day 0. The mating procedure consisted of 15 consecutive days, a period comprising approximately 3 estral cycles, until a replicate number of groups were obtained. However, during this period, nonmated female rats were considered to be infertile and were discarded from the study.[20]

Experimental Groups

Pregnant diabetic rats were randomly distributed among 6 experimental groups (minimum $n = 11$ animals/group): group 1 (G1), sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; and G6, diabetic, exercised prior to and during pregnancy.

Exercise Program

For exercise, we utilized a swimming program, which is considered to be a program of moderate intensity, according to the procedure by Volpato et al.[16] To familiarize the rats with the swimming system (water), the rats were exposed to it daily for 15 minutes for 5 days in a cage ($100 \times 70 \times 60$ cm) containing water at a depth of 10 cm at 32°C. This period corresponded to the interval between diabetes induction and the mating period (G1, G2, G4, and G5 groups) or 1 month before the mating period (G3 and G6 groups). The pregnant rats that remained in water at a depth of 10 cm at 32°C were classified as sedentary. Afterward, the female rats that were familiarized with the swimming system were placed in a cage containing water at a depth of 40 cm. Exercise on the first day under these conditions was about 20 minutes, with progressive increases of 10 minutes each day until they completed 60 minutes. Following which the rats were trained to swim for 1 hour daily until the end of pregnancy. Swimming was always performed in water at a temperature of 32°C between 9 AM and 10 AM for 6 days a week.

Evaluation at Term of Pregnancy

Blood glucose levels were measured about every 7 days until the end of pregnancy, at approximately 9 AM. On day 21 of pregnancy, the rats were anesthetized by sodium pentobarbital

and humanely killed. The uterus was removed and weighed, and the ovary and uterine contents were examined to determine the number of corpora lutea, implantation sites, and resorptions (embryonic death), and the number of viable fetuses. The rate of embryonic loss before implantation was calculated as follows: (number of corpora lutea − number of implantations) × 100/number of corpora lutea. This calculation was used as a measurement of failed conception effects or preimplantation loss. The percentage of embryonic loss after implantation was calculated as follows: (number of implantations − number of live fetuses) × 100/number of implantations. This calculation was used as a measurement of the abortifacient effect or for identification of postimplantation loss.[21] If there was a lack of visible implantation sites, the uterine corns were stained with a preparation of 10% ammonium sulfate.[22] The fetuses and placentas were weighed to calculate the placental index as placental weight/fetal weight. The mean birth weight of the control pups (G1) was 5.3 ± 0.5 g. Newborns in the experimental groups whose birth weights did not diverge more than ± 1.7 standard deviations (SDs) from the G1 mean (ie, those weighing within the 4.5-6.1 g range) were classified as adequate for pregnancy age (APA). Those whose weights were at least 1.7 SDs greater than the G1 mean birth weight were classified as large for pregnancy age (LPA). Those whose birth weights were at least 1.7 SD lower than the G1 mean birth weight were classified as small for pregnancy age (SPA).[23] All fetuses were evaluated under a microscope with respect to incidence of external anomaly. After external analysis, half the fetuses of each dam were fixed in Bouin fluid and serial sections were prepared, as described by Wilson,[24] for visceral examination. The remaining fetuses were prepared for examination of the skeletons by the staining procedure described by Staples and Schnell.[25] The degree of ossification was evaluated using the parameters proposed by Aliverti et al.[26]

Biochemical Profile Analysis

Blood samples were collected from each rat, placed into anticoagulant-free test tubes, maintained in ice for 30 minutes and then centrifuged at 6300*g* for 10 minutes at 4°C. Supernatant was collected as serum and stored at −80°C for further determination of biochemical parameters. Soleus muscle samples were collected (200 mg), placed in 30% sodium hydroxide, and stored at −80°C for further determination of muscular glycogen.

Serum concentrations of total CHO, TGs, and high-density lipoprotein (HDL) were determined using the enzymatic method, and total protein (TP) concentrations were estimated by the colorimetric method[27]; both methods utilized Sigma assay kits. Very-low-density lipoprotein (VLDL) serum levels were calculated from the concentration of TGs.[28] Concentrations of muscular glycogen were determined by the method described by Nomura et al.[29]

Statistical Analysis

Analysis of variance followed by Student-Newman-Keuls test was used to compare mean values. The percentage values were calculated by Fisher exact test. Differences were considered statistically significant if $P < .05$.

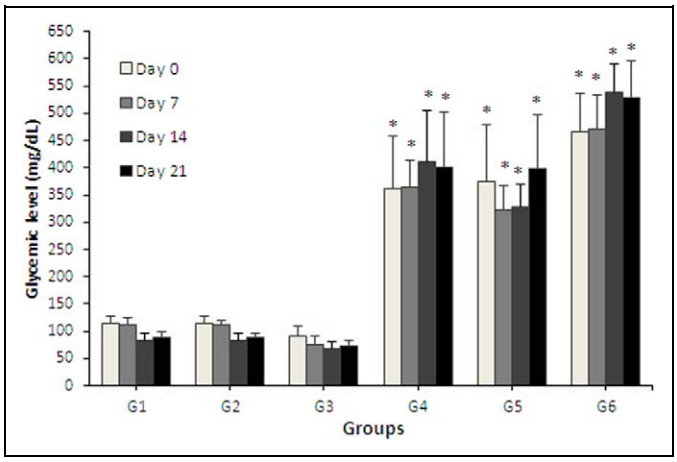

Figure 1. Glycemic levels in nondiabetic or diabetic rats not exercised (G1 and G4) or exercised during (G2 and G5) or prior to and during (G3 and G6) pregnancy. Data shown as means ± SDs (ANOVA − Student-Newman-Keuls pos hoc test). ANOVA indicates analysis of variance; G1, sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; G6, diabetic, exercised prior to and during pregnancy; SD, standard deviation. *$P < .05$—statistically significant difference compared to the G1 group.

Results

Maternal Glycemia

The blood glucose levels of nondiabetic dams were lower than 120 mg/dL during the entire pregnancy period. In diabetic groups, at the beginning of pregnancy, maternal blood glucose levels were higher than 300 mg/dL, and these levels were maintained for the entire pregnancy. The swimming program did not alter the blood glucose levels of the nondiabetic and diabetic rats, regardless of when they were measured (Figure 1).

Maternal Biochemical Measurements

Table 1 shows that the swimming program did not alter any biochemical parameters in nondiabetic dams. The G4 rats showed reduced levels of TP and HDL and increased TGs and VLDL concentrations compared to the G1 group. Rats from the G5 group were characterized by decreased TG and VLDL-c levels compared to the G4 group. Rats that exercised prior to and during pregnancy (G6) were characterized by increased muscular glycogen concentrations in relation to the other experimental groups.

Maternal Reproductive Outcomes

Rats in the G2 group had reduced fetal body weight at the end of pregnancy as compared to the G1 group. The rats of the G3

Table 1. Biochemical Analysis From Nondiabetic or Diabetic Rats Not Exercised (G1 and G4) or Exercised During (G2 and G5) or Prior to and During (G3 and G6) Pregnancy.[a]

	G1	G2	G3	G4	G5	G6
Muscular glycogen, mg/100 mg tissue	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.9 ± 0.4[b,c,d]
Total protein, g/dL	11.2 ± 5.6	8.0 ± 2.5	9.2 ± 2.8	5.7 ± 1.2[b]	4.6 ± 1.0[b]	8.3 ± 3.6
Triglycerides, mg/dL	248.3 ± 88.8	257.2 ± 149.3	397.3 ± 133.3	777.5 ± 333.5[b]	524.3 ± 148.5[b,c]	821.2 ± 269.1[b,d]
Total cholesterol, mg/dL	145.0 ± 46.2	124.9 ± 35.8	129.6 ± 44.9	175.3 ± 29.4	137.9 ± 18.5	169.2 ± 35.1
HDL, mg/dL	68.0 ± 19.6	76.0 ± 30.5	59.3 ± 22.4	41.3 ± 6.9[b]	52.7 ± 12.9	53.0 ± 18.5
VLDL, mg/dL	49.7 ± 17.8	51.5 ± 29.9	79.5 ± 26.7	155.5 ± 66.7[b]	104.9 ± 29.7[b,c]	164.3 ± 53.8[b,d]

Abbreviations: ANOVA, analysis of variance; G1, sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; G6, diabetic, exercised prior to and during pregnancy; HDL, high-density lipoprotein; SD, standard deviation; VLDL, very-low-density lipoprotein.

[a] Data shown as means ± SDs (ANOVA—Student-Newman-Keuls post hoc test).

[b] $P < .05$—statistically significant difference compared to the G1 group.

[c] $P < .05$—statistically significant difference compared to the G4 group.

[d] $P < .05$—statistically significant difference between the G5 and G6 groups.

group demonstrated a reduction in this parameter in relation to G1 and G2 groups. Rats in the G3 group presented increased rates of SPA and decreased APA percentages compared to G1 rats. The G3 rats also presented reductions in maternal weight gain compared to the G1 group. All diabetic groups presented reductions in maternal weight gains, with or without adding the uterine weight. The diabetic, exercised rats contained reduced placental weights and indexes in relation to G4 rats, and the rats in the G5 group showed reductions in fetal body weight and APA percentages and increases in SPA rates compared to G4 dams (Table 2).

Ossification Sites

Table 3 shows the mean number of ossification sites in fetuses from experimental groups. The G3 group presented reductions in the metatarsus and caudal vertebra compared to the G1 group. The diabetic groups presented reductions in the forepaw phalanx, metacarpus, metatarsus, caudal vertebra, and total ossification sites compared to the nondiabetic group (G1). The G5 group was characterized by intensified reductions in the forepaw phalanx, metacarpus, and total ossification sites.

Frequency of Fetal Anomalies

There were no alterations in the frequency of external anomalies among the experimental groups. No significant alterations were observed in the frequencies of visceral and skeletal anomalies in the fetuses from nondiabetic, exercised dams compared to G1 rats. All dams of diabetic groups (G4, G5, and G6) had increased rates of fetuses with visceral and skeletal anomalies compared to G1 rats. The swimming program promoted increases in skeletal anomalies (abnormally shaped sternebrae) in diabetic rats of the G5 group compared to G4 dams, and the G6 group showed increases in visceral anomalies (hydrourether) compared to G4 rats (Table 4).

Discussion

In the present study on nondiabetic and diabetic female rats, we showed that the swimming program did not interfere with blood glucose levels, independently of the time at which exercise was initiated. The literature already recognizes the potential benefits of this adjuvant therapy on maternal glycemic control and complications, such as gestational diabetes. However, the evidence of this impact on gestational diabetes has not been examined systematically.[17,30] Specifically, in individuals with type 1 diabetes, the exercise response is conditioned to the action of the exogen insulin and the quality of metabolic control. In type 1 diabetes with appropriate metabolic control, moderate intensity exercise causes falls in glycemic levels, which stimulates the use of glucose. Exercise also maintains the concentration of circulating insulin and stabilizes hepatic glucose production. These advantageous effects are not observed in the presence of severe hyperglycemia, which is usually associated with ketosis, a characteristic of uncontrolled diabetes. Under these conditions, there is impaired glucose consumption and hepatic and muscular glycogenesis, with activation of glycogenolysis, lipolysis, and ketogenesis, thereby worsening the hyperglycemia and the metabolic acidosis risk.[31] The interaction between exercise, uncontrolled diabetes, and pregnancy can intensify these complications through the action of placental hormones that favor insulin resistance, starting from week 24 of human gestation.[32] In diabetic pregnant rats, the refractivity of maternal hyperglycemia to exercise was already described by Uriu-Hare et al,[33] thus confirming the observations of this study. In the same manner, certain studies did not confirm the action of exercise on the control of glycemic levels in the clinical investigations with diabetic pregnant women[34,35] and experimental animals.[15]

Independently of the time at which swimming was initiated, nondiabetic dams presented no alterations in biochemical measurements. In particular, we showed that exercise did not impair glucose, protein, and lipid catabolism in the nondiabetic groups, confirming the maintenance of muscular glycogen, TP, TG, total CHO, VLDL-c, and HDL-c levels. These results

Table 2. Reproductive Outcomes From Nondiabetic or Diabetic Rats Not Exercised (G1 and G4) or Exercised During (G2 and G5) or Prior to and During (G3 and G6) Pregnancy.[a]

	G1	G2	G3	G4	G5	G6
Pregnant females, N	16	14	15	11	16	11
Corpora lutea						
Total, N	214	205	202	147	199	134
Mean ± SD	13.4 ± 1.7	14.6 ± 2.5	13.5 ± 1.3	13.4 ± 2.4	12.4 ± 1.4	12.2 ± 1.3
Implantation						
Total, N	192	183	169	134	186	126
Mean ± SD	12.0 ± 2.6	13.1 ± 2.9	11.3 ± 3.7	12.2 ± 1.9	11.6 ± 1.8	11.5 ± 1.8
Live fetuses						
Total, N	181	167	157	108	165	108
Mean ± SD	11.3 ± 3.1	11.9 ± 3.0	10.5 ± 3.8	9.8 ± 2.8	10.3 ± 2.2	9.8 ± 2.0
Per implantation sites, %	94.3	91.8	92.9	80.6[b]	88.7	85.7[b]
Dead fetuses						
Total, N	0	2	1	5	3	0
Mean ± SD	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.3	0.5 ± 0.9	0.2 ± 0.4	0.0 ± 0.0
Resorptions						
Total, N	12	14	11	23	18	18
% per litter, mean ± SD	7.9 ± 12.8	7.1 ± 8.6	5.7 ± 13.8	16.9 ± 19.1	9.3 ± 13.2	14.1 ± 12.6
% Preimplantation loss	10.3	10.7	16.3	8.8	6.5	6.0
% Postimplantation loss	5.7	8.7	7.1	19.4	11.3	14.3
Fetal body weight, g						
Mean ± SD	5.3 ± 0.5	5.1 ± 0.3[b]	4.9 ± 0.5[b,c]	4.2 ± 0.6[b]	3.7 ± 0.4[b,d]	4.1 ± 0.6[b,e]
% SPA	4.7	4.5	12.7[b]	72.5[b]	95.8[b,d]	78.7[b,e]
% APA	93.2	95.5	86.6[b]	27.5[b]	4.2[b,d]	21.3[b,e]
% LPA	2.1	0.0	0.6	0.0	0.0	0.0
Placental weight, g						
Mean ± SD	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2[b]	0.5 ± 0.1[d]	0.5 ± 0.1[d]
Placental index, ×100						
Mean ± SD	9.8 ± 1.4	9.2 ± 1.6	9.2 ± 1.2	16.0 ± 4.9[b]	14.7 ± 3.6[b,d]	12.8 ± 3.3[b,d,e]
Maternal weight gain (g) − MWG (day 21 − day 0)						
Mean ± SD	124.9 ± 29.4	131.2 ± 23.9	108.5 ± 27.7[c]	80.6 ± 23.2[b]	72.8 ± 28.6[b]	78.7 ± 17.4[b]
Gravid uterus weight, g						
Mean ± SD	77.2 ± 20.1	80.3 ± 20.3	68.6 ± 24.6	63.7 ± 13.7	60.2 ± 13.1	60.7 ± 13.0
MWG minus gravid uterus weight, g						
Mean ± SD	47.7 ± 20.9	51.1 ± 9.6	39.9 ± 10.1	16.8 ± 15.2[b]	12.6 ± 24.9[b]	19.9 ± 8.3[b]

Abbreviations: ANOVA, analysis of variance; APA, adequate for pregnancy age; G1, sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; G6, diabetic, exercised prior to and during pregnancy; LPA, large for pregnancy age; MWG, maternal weight gain; N, number; SD, standard deviation; SPA, small for pregnancy age.

[a] Data shown as means ± SDs (ANOVA—Student-Newman-Keuls post hoc test) and proportions (%) (Fisher exact test).

[b] $P < .05$—statistically significant difference compared to the G1 group.

[c] $P < .05$—statistically significant difference between the G2 and G3 groups.

[d] $P < .05$—statistically significant difference compared to the G4 group.

[e] $P < .05$—statistically significant difference between the G5 and G6 groups.

corroborate those of Bessinger and McMurray.[36] With regard to the diabetic female rats, we demonstrated that there was an increase in muscular glycogen and TP concentrations when the rats exercised prior to pregnancy. The increase in muscular power is capable of inducing the activation of several biochemical pathways that are essential for muscular hypertrophy. Diabetic female rats displayed mechanisms of muscular adaptation to the swimming program. Their metabolic response to the type of exercise also contributed to muscular hypertrophy, which explains their increased mitochondrial volume and number, glycogen accumulation, and protein synthesis. There were incremental changes in the HDL-c rates of diabetic dams, which underwent the exercise program, while rats that began the exercise at the beginning of the pregnancy were characterized by decreased TG and VLDL-c levels, indicating that swimming contributed to CHO transport, which can prevent the emergence of atherosclerotic diseases in these female rats, as confirmed by previous studies.[15]

Exercise performed by nondiabetic dams did not interfere with maternal reproductive outcome (number of ovulations, implantations, embryonic deaths, and number of live and dead fetuses). Among diabetic female rats that exercised only during pregnancy, there was an increased percentage of live fetuses in implantation sites. Maternal weight gain was smaller in the nondiabetic dams that exercised before and during pregnancy. In all the diabetic groups, the rats were characterized by

Table 3. Ossification Sites From Nondiabetic or Diabetic Rats Not Exercised (G1 and G4) or Exercised During (G2 and G5) or Prior to and During (G3 and G6) Pregnancy.[a]

	G1	G2	G3	G4	G5	G6
Forepaw phalanx	3.6 ± 0.5	3.3 ± 0.6	3.1 ± 0.7	2.5 ± 0.8[b]	1.5 ± 1.0[b,c]	2.0 ± 0.8[b]
Metacarpus	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.1	3.8 ± 0.2[b]	3.2 ± 0.5[b,c]	3.5 ± 0.4[b,c,d]
Hindpaw phalanx	0.9 ± 1.1	0.2 ± 0.5	0.6 ± 1.3	0.1 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Metatarsus	4.7 ± 0.2	4.8 ± 0.2	4.6 ± 0.4[b,e]	4.1 ± 0.1[b]	4.0 ± 0.1[b]	4.1 ± 0.2[b]
Caudal vertebra	4.9 ± 0.8	4.5 ± 0.8	4.2 ± 0.7[b]	4.0 ± 0.9[b]	3.4 ± 0.5[b]	3.7 ± 0.7[b]
Sternebrae	6.0 ± 0.1	6.0 ± 0.0	6.0 ± 0.0	5.9 ± 0.2	6.0 ± 0.4	6.0 ± 0.1
Total	24.1 ± 2.3	22.7 ± 1.1	22.4 ± 2.6	20.5 ± 2.3[b]	18.1 ± 1.9[b,c]	19.2 ± 1.9[b]

Abbreviations: ANOVA, analysis of variance; G1, sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; G6, diabetic, exercised prior to and during pregnancy; N, number; SD, standard deviation.

[a] Data shown as means ± SDs (ANOVA—Student-Newman-Keuls post hoc test).

[b] $P < .05$—statistically significant difference compared to the G1 group.

[c] $P < .05$—statistically significant difference compared to the G4 group.

[d] $P < .05$—statistically significant difference between the G5 and G6 groups.

[e] $P < .05$—statistically significant difference between the G2 and G3 groups.

decreased maternal weight gain (with or without the uterine content). Pregnancy is characterized by progressive increases in maternal weight gain as the fetus grows.[32] Therefore, the smallest fetal weight gain among these groups might be one of the causes to explain the reduced maternal weight. Other mechanism that might be involved in reduced maternal weight gain is the highest metabolic demand to practice the exercise.

In this study, nondiabetic and diabetic dams that underwent exercise presented decreased fetal weights, independently of the time at which exercise was initiated, thus indicating IUGR. The presence of IUGR can be related to fetal hypoxia caused by exercise.[37] The implications of exercise on fetal development are controversial, particularly when the intensity (light, moderate, or intense) is considered. Evidence suggests that there is no correlation between light or moderate intensity exercise and IUGR.[38-40] However, there are studies that have confirmed an association between intense exercise and IUGR,[38,41] while other studies have disagreed with such findings.[42,43] Conversely, others have reported that exercise is related to large birth weight.[44,45] Diabetic dams submitted to moderate exercise (treadmill) during the entire pregnancy resulted in offspring with larger weights.[33] These conflicting results are possibly related to the intensity of the maternal exercise, familiarity of the animal to the type of exercise, and differences in the type of species used.

Diabetic sedentary dams exhibited increased placental weights that are interpreted as a compensatory mechanism that attempts to favor the maternal–fetal exchanges and the nutritional supply to the fetus during development. In spite of this mechanism, the hyperglycemia of the intrauterine environment leads to the functional exhaustion of the fetal pancreas, resulting in reduced insulin secretion, causing impaired fetal growth, and increased rates of SPA fetuses as well as decreased rates of APA and LPA fetuses,[8] as observed in the present study. However, diabetic dams that underwent exercise presented reduced placental weights compared to diabetic sedentary rats. Consequently, the placental indexes of the diabetic groups were affected by the fetal and placental weights, confirming placental incapacity as nutrition and oxygenation organ of the fetus in development.

Moreover, the presence of IUGR verified in this study was confirmed by the reduced proportion of fetal ossification sites in diabetic rats. These results were impaired after diabetic rats underwent exercise, confirming the relationship of fetal immaturity with birth. Aliverti et al[26] proposed that the study of these ossification sites in fetuses would be the most affected points in immature fetuses.

A significant percentage of external and visceral anomalies was not observed in the fetuses from nondiabetic dams that were subjected to swimming. Although significant increases in wavy rib was verified in the groups that exercised at start of pregnancy, there was no alteration in the number of affected fetuses and dams (litters), which could characterize the teratogenic effect. Severe diabetes caused increased numbers of skeletal (sternebrae, ribs, and vertebral centers) and visceral (hydroureter and hydronephrosis) fetal anomalies. Maternal diabetes is one of the known causes of congenital malformations that is responsible for high morbidity and perinatal mortality.[4] Several etiological factors have been proposed to explain the congenital defects related to diabetes. The diabetic dams that exercised prior to and during pregnancy also presented increased numbers of fetuses with visceral anomalies, which are due to increased incidence of hydroureter. Hydroureter is recognized easily because the affected ureters are molded in an "S" shape or are twisted and completely transparent, thus easily identifiable, as seen in this study. Moreover, hydroureter may or may not be associated with congenital hydronephrosis, and any pelvic alterations in rodents could be considered transitory.[46] In agreement with the literature, we found that 10% to 20% of the offspring from control female rats presented this type of visceral anomaly.[7-9] However, there was no alteration in the number of affected dams (litters) among the different diabetic groups. This fact indicates that exercise (swimming) is not considered to be a teratogenic factor.

In conclusion, nondiabetic rats exercised prior to pregnancy presented a reduced maternal weight gain. Besides, both

Table 4. Frequency of External and Internal Anomalies From Nondiabetic or Diabetic Rats Not Exercised (G1 and G4) or Exercised During (G2 and G5) or Prior to and During (G3 and G6) Pregnancy.[a]

	G1	G2	G3	G4	G5	G6
External anomalies						
Fetuses examined (litter)	180 (16)	158 (13)	157 (15)	109 (16)	166 (13)	108 (15)
Fetuses with alteration (%)	0 (0.0)	1 (0.6)	0 (0.0)	2 (1.8)	3 (1.8)	1 (0.9)
Mean % fetuses with alteration per litter	0.0 ± 0.0	0.7 ± 2.5	0.0 ± 0.0	4.5 ± 15.1	2.1 ± 8.3	0.8 ± 2.7
Generalized edema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)
Macroglossia	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mandibular micrognathia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
Short tail	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.8)	0 (0.0)
Gastroschisis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
Visceral anomalies						
Fetuses examined, litter	84 (16)	69 (12)	74 (15)	54 (11)	78 (16)	51 (11)
Fetuses with alteration, %	18 (21.4)	10 (14.5)	13 (17.6)	22 (40.7)[b]	37 (47.4)[b]	32 (62.7)[b,c]
Mean % fetuses with alteration per litter	19.0 ± 17.9	15.9 ± 18.8	16.9 ± 19.9	44.7 ± 26.8[b]	48.1 ± 35.5[b]	67.4 ± 31.0[b]
Microphthalmia	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)
Anophthalmia	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)
Dilated esophagus	1 (1.2)	1 (1.4)	1 (1.4)	2 (3.7)	4 (5.1)	0 (0.0)
Ectopic esophagus	1 (1.2)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Distended bladder	1 (1.2)	0 (0.0)	2 (2.7)	1 (1.9)	0 (0.0)	0 (0.0)
Ectopic kidney	1 (1.2)	1 (1.4)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)
Hydroureter	11 (13.1)	5 (7.2)	9 (12.2)	22 (40.7)[b]	37 (47.4)[b]	32 (62.7)[b,c]
Dilated renal pelvis	0 (0.0)	2 (2.9)	0 (0.0)	1 (1.9)	0 (0.0)	1 (2.0)
Hydronephrosis	3 (3.6)	1 (1.4)	0 (0.0)	7 (13.0)[b]	6 (8.0)	9 (17.6)[b]
Skeletal anomalies						
Fetuses examined (litter)	89 (15)	66 (11)	82 (15)	56 (11)	86 (16)	57 (11)
Fetuses with alteration (%)	25 (28.1)	25 (37.9)	25 (30.5)	34 (60.7)[b]	74 (86.0)[b,c]	42 (73.7)[b]
Mean % fetuses with alteration per litter	26.5 ± 35.0	36.4 ± 29.2	28.3 ± 31.2	64.2 ± 33.7[b]	86.1 ± 21.9[b]	76.4 ± 21.8[b]
Craniofenestria	4 (4.5)	0 (0.0)	1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Cleft palate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bipartite ossification of vert. cent.	5 (5.6)	0 (0.0)	0 (0.0)	3 (5.4)	4 (4.7)	5 (8.8)
Dumbbell ossification of vert. cent.	4 (4.5)	2 (3.0)	0 (0.0)	18 (32.1)[b]	18 (20.9)[b]	15 (26.3)[b]
Supernumerary rib	14 (15.7)	10 (15.2)	9 (11.0)	19 (33.9)[b]	44 (51.2)[b]	17 (29.8)
Wavy rib	1 (1.1)	2 (3.0)	11 (13.4)[b]	0 (0.0)	3 (3.5)	0 (0.0)
Sternebra agenesis	2 (2.2)	0 (0.0)	0 (0.0)	1 (1.8)	10 (11.6)[b]	0 (0.0)
Incomplete ossification of sternebrae	6 (6.7)	9 (13.6)	4 (4.9)	20 (35.7)[b]	33 (38.4)[b]	41 (71.9)[b,c,d]
Unossified sternebrae	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	6 (7.0)	4 (7.0)
Bipartite sternebra	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	6 (7.0)	2 (3.5)
Abnormally shaped sternebrae	12 (13.5)	16 (24.2)	3 (3.7)[b]	20 (35.7)[b]	60 (69.8)[b,c]	49 (86.0)[b,c,d]

Abbreviations: ANOVA, analysis of variance; G1, sedentary, nondiabetic; G2, nondiabetic, exercised during pregnancy; G3, nondiabetic, exercised prior to and during pregnancy; G4, sedentary, diabetic; G5, diabetic, exercised during pregnancy; G6, diabetic, exercised prior to and during pregnancy; SD, standard deviation.
[a] Data shown as means ± SDs (ANOVA—Student-Newman-Keuls post hoc test) and proportions (%; Fisher exact test).
[b] $P < .05$—statistically significant difference compared to the G1 group.
[c] $P < .05$—statistically significant difference compared to the G4 group.
[d] $P < .05$—statistically significant difference between the G5 and G6 groups.

nondiabetic groups submitted to exercise showed decreased fetal weights at term pregnancy, indicating slight IUGR. However, other parameters demonstrated that exercise was safe. In the diabetic dams, the swimming program did not have antihyperglycemic effects. The exercise applied only during pregnancy caused severe IUGR, as confirmed by reduced fetal weight mean, fetal weight classification, and ossification sites. Nevertheless, exercise was not a teratogenic factor and improved the rats' lipid profiles, demonstrating that the exercise presented possible benefits, but there are also risks prior and during pregnancy. However, the possibility of extrapolating the findings of this study in relation to maternal–fetal implications of diabetic pregnant women should be done with more caution about the safety of exercise program.

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References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2012;35:S64-S71.
2. Kordowich S, Mansouri A, Collombat P. Reprogramming into pancreatic endocrine cells based on developmental cues. *Mol Cell Endocrinol*. 2010;315(1-2):11-18.
3. London MB, Gabbe SG. Fetal surveillance and timing of delivery in pregnancy complicated by diabetes mellitus. *Obstet Gynecol Clin North Am*. 1996;23(1):109-124.
4. Boulot P, Chabbert-Buffet N, d'Ercole C, et al. French multicentric survey of outcome of pregnancy in women with pregestational diabetes. *Diabetes Care*. 2003;26(11):2990-2993.
5. Reece EA, Coustan DR, Gabbe SG. *Diabetes in women: Adolescence, Pregnancy and Menopause*. 3rd ed. New York, NY: Lippincott Williams & Wilkins; 2004.
6. Rudge MVC, Damasceno DC, Volpato GT, Almeida FCG, Calderon IMP, Lemonica IP. Effect of *Ginkgo biloba* on the reproductive outcome and oxidative stress biomarkers of streptozotocin-induced diabetic rats. *Braz J Med Biol Res*. 2007;40(8):1095-1099.
7. Volpato GT, Calderon IM, Sinzato S, Campos KE, Rudge MV, Damasceno DC. Effect of Morus nigra aqueous extract treatment on the maternal-fetal outcome, oxidative stress status and lipid profile of streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 2011;138(3):691-696.
8. Holemans K, Aerts L, Van Assche FA. Fetal growth restriction and consequences for the offspring in animal models. *J Soc Gynecol Investig*. 2003;10(7):392-399.
9. Volpato GT, Damasceno DC, Rudge MV, Padovani CR, Calderon IM. Effect of *Bauhinia forficata* aqueous extract on the maternal–fetal outcome and oxidative stress biomarkers of streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 2008;116(1):131-137.
10. Damasceno DC, Volpato GT, Calderon Ide M, Rudge MV. Effect of Bauhinia forficata extract in diabetic pregnant rats: maternal repercussions. *Phytomedicine*. 2004;11(1-2):196-201.
11. Damasceno DC, Kiss AC, Sinzato YK, et al Maternal-fetal outcome, lipid profile and oxidative stress of diabetic rats neonatally exposed to streptozotocin. *Exp Clin Endocrinol Diabetes*. 2011;119(7):408-413.
12. Steppel JH, Horton ES. Exercise in patients with diabetes mellitus. In: Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ, eds. *Joslin's Diabetes mellitus*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:649-658.
13. Devlin JT. Effects of exercise on insulin sensitivity in humans. *Diabetes Care*. 1992;15(11):1690-1693.
14. Kim C. Gestational diabetes: risks, management, and treatment options. *Int J Womens Health*. 2010;2:339-351.
15. Volpato GT, Damasceno DC, Campos KE, Rocha R, Rudge MV, Calderon IM. Avaliação do efeito do exercício físico no metabolismo de ratas diabéticas prenhes. *Rev Bras Med Esp*. 2006;12:229-233.
16. Volpato GT, Damasceno DC, Kempinas WG, Rudge MV, Calderon IM. Effect of exercise on the reproductive outcome and fetal development of diabetic rats. *Reprod Biomed Online*. 2009;19(6):852-858.
17. Tobias DK, Zhang C, Van Dam RM, Bowers K, Hu FB. Physical activity before and during pregnancy and risk of gestational diabetes mellitus. *Diabetes Care*. 2011;34(1):223-229.
18. Ceysens G, Rouiller D, Boulvain M. Exercise for diabetic pregnant women. *Cochrane Database Syst Rev*. 2006;19(3):CD004225.
19. Damasceno DC, Volpato GT, Sinzato YK, et al Genotoxicity and fetal abnormality in streptozotocin-induced diabetic rats exposed to cigarette smoke prior to and during pregnancy. *Exp Clin Endocrinol Diabetes*. 2011;119(9):549-553.
20. Francia-Farje LA, Silva DS, Volpato GT, et al Sibutramine effects on the reproductive performance of pregnant overweight and non-overweight rats. *J Toxicol Environ Health A*. 2010;73(13-14):985-990.
21. Damasceno DC, Sinzato YK, Lima PH, et al Effects of exposure to cigarette smoke prior to pregnancy in diabetic rats. *Diabetol Metab Syndr*. 2011;3:20.
22. Salewski E. Farbemethode zum markroskopishen nachweis von implantatconsstellen an uterus der ratter naunyn schmuderbergs. *Arch Pharm (Weinheim)*. 1964;247:367.
23. Soulimane-Mokhtari NA, Guermouche B, Yessoufou A, et al Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. *Clin Sci (Lond)*. 2005;109(3):287-295.
24. Wilson JG. Methods for administering agents and detecting malformations in experimental animal. In: Wilson JG, Warkany J, eds. *Teratology: Principles and Techniques*. Chicago, IL: University of Chicago Press; 1965:47-74.
25. Staples RE, Schnell VL. Refinements in rapid clearing technic in the KOH-alizarin red s method for fetal bone. *Stain Technol*. 1964;39:61-63.
26. Aliverti V, Bonanomi L, Giavini E, Leone VG, Mariani L. The extent of fetal ossification as an index of delayed development in teratogenic studies on the rat. *Teratology*. 1979;20(2):237-242.
27. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. 5th ed. London, UK: AACC Press; 2000.
28. Friedwald WT, Levy RI, Fredickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative centrifuge. *Clin Chem*. 1972;18(6):499-502.
29. Nomura Y, Okamoto S, Sakamoto M, Feng Z., Nakamura T. Effect of cobalto n the liver glycogen content in the streptozotocin-induced diabetic rats. *Mol Cell Biochem*. 2005;277(1-2):127-130.
30. American Diabetes Association. Exercise and diabetes (position statement). *Diabetes Care*. 2004;27:S58-S62.
31. Vivolo MA, Ferreira SRG, Hidal JT. Exercício físico e diabete melito. *Rev Soc Cardiol Est São Paulo*. 1996;6:102-110.
32. Rudge MVC, Borges VTM, Calderon IMP. Adaptação do organismo materno à gravidez. In: Neme B, ed. *Obstetrícia básica*. São Paulo, Brazil: Sarvier; 2000:42-51.
33. Uriu-Hare JY, Keen CL, Applegate EA, Sterm JS. The influence of moderate exercise in diabetic and normal pregnancy of maternal and fetal outcome in the rat. *Life Sci*. 1999;45(7):647-654.

34. Lesser KB, Gruppuso PA, Terry RB, Carpenter NW. Exercise fails to improve postprandial glycemic excursion in women with gestational diabetes. *J Matern Fetal Med*. 1996;5(4):211-217.
35. Avery MD, Leon AS, Kopher RA. Effects of a partially home-based exercise program for women with gestacional diabetes. *Obstet Gynecol*. 1997;89(1):10-15.
36. Bessinger R, McMurray RG. Substrate utilization and hormonal responses to exercise in pregnancy. *Clin Obstet Gynecol*. 2003; 46(2):467-478.
37. Lima FR, Oliveira N. Pregnancy and Exercise. *Rev Bras Reumatol* 2005;45:188-190.
38. Spinillo A, Capuzzo E, Baltaro F, Piazzi G, Incola S, Iasci A. The effect of work activity in pregnancy on the risk of fetal growth retardation. *Acta Obstet Gynecol Scand*. 1996;75(6): 531-536.
39. Hjollund NH, Jensen TK, Bonde JP, et al Spontaneous abortion and physical strain around implantation: a follow-up study of first-pregnancy planners. *Epidemiology*. 2000;11(1):18-23.
40. Brow W. The benefits of physical activity during pregnancy. *J Sci Med Sport*. 2002;5(1):37-45.
41. Artal R, Posner MD. Respostas fetais ao exercício materno. In: Artal R, Wiswell RA, Drinkwater BL, eds. *O exercício na gravidez*. São Paulo, Brazil: Manole; 1999:213-224.
42. Sternfeld B, Quesenberry CP, Eskenazi B, Newman LA. Exercise during pregnancy and pregnancy outcome. *Med Sci Sports Exerc*. 1995;27(5):634-640.
43. Kardel KR, Kase T. Training in pregnant women: effects on fetal development and birth. *Am J Obstet Gynecol*. 1998;178(2):280-286.
44. Hall DC, Kaufmann DA. Effects of aerobic and strength conditioning on pregnancy outcomes. *Am J Obstet Gynecol*. 1987; 157(5):1199-1203.
45. Hatch MC, Levin B, Shu XO, Susser M. Maternal leisure-time exercise and timely delivery. *Am J Public Health*. 1998;88(10): 1528-1533.
46. Taylor P. *Practical Teratology.* 1st ed. New York, NY: Academic Press; 1986.