Original Article

Reproductive Sciences
2015, Vol. 22(8) 991-999

DOI: 10.1177/1933719115570905
rs.sagepub.com
SAGE

Intrauterine Growth Restricted Rats Exercised at Pregnancy: Maternal–Fetal Repercussions

S. B. Corvino, MSc[1], A. O. Netto, MSc[1], Y. K. Sinzato, PhD[1], K. E. Campos, PhD[1,2], I. M. P. Calderon, PhD[1], M. V. C. Rudge, PhD[1], G. T. Volpato[1,2], E. Zambrano, PhD[3], and D. C. Damasceno, PhD[1]

Abstract

To evaluate the effect of swimming in pregnant rats born with intrauterine growth restriction (IUGR) and their offspring, IUGR rats were obtained using the streptozotocin-induced severe diabetic (SD) rats. In this study, the nondiabetic parental generation presented 10 rats and diabetic parental generation presented 116 rats. Of these, the mated nondiabetic female rats were 10 and the number of diabetic rats was 45. In relation to term pregnancy, there were 10 animals in the nondiabetic group and 15 rats in the diabetic group. In the offspring of SD rats (IUGR group), 43 females were classified as small for pregnancy age, 19 rats were classified as appropriate for pregnancy age, and 0 female was classified as large for pregnancy age. The nondiabetic and SD pregnant rats generated offspring with appropriate (control [C]) and small (IUGR) weight for pregnancy age, respectively. At adult life, the C group was maintained as nonexercised C group and IUGR rats were distributed into 2 subgroups, namely, nonexercised (IUGR) and exercised (IUGRex). The rate of mated rats in the IUGR group was reduced compared to the C group. During pregnancy, the IUGR rats presented hyperinsulinemia, impaired reproductive outcomes, decreased body weight, hypertriglyceridemia, and hyperlactacidemia. The IUGRex presented reduced insulin and triglyceride levels. Thus, swimming improved lipid metabolism and increased insulin sensitivity. However, the offspring showed retarded growth, reinforcing the need to stimulate the exercise practice in women under supervision with different professional expertise to promote appropriate gestational conditions and improve perinatal outcomes.

Keywords

diabetes, exercise, rat, pregnancy, IUGR

Introduction

Human epidemiological[1,2] and experimental animal studies[3-5] have shown that a suboptimal environment either in the uterus or early in the neonatal life alters growth and predisposes individuals to lifelong health problems. Health conditions throughout life result from the combination of genetic predisposition and environmental influences. Although the genotype is determined at conception, the phenotype is permanently modulated by external factors, which begins in the prenatal period. This effect is known as "fetal origins of adult diseases."[6-8] There is evidence that the nature of fetal programming is such that it is involved in many disease phenotypes, including those of successive generations.[9-11] Laboratory animal models have been developed in an attempt to understand the pathophysiological mechanisms involved in an unfavorable intrauterine environment, among them the use of glucocorticoids,[12] reduced blood flow by bilateral uterine artery ligation,[13,14] chronic hypertension,[15] protein malnutrition,[11,16] and β-cytotoxic drug for severe diabetes induction.[17-20]

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 (> 300 mg/dL—severe diabetes) and their offspring was born small for pregnancy age (SPA) due to an intrauterine growth

[1] Laboratory of Experimental Research on Gynecology and Obstetrics and Graduate Program on Gynecology, Obstetrics and Mastology, Botucatu Medical School, Univ Estadual Paulista_Unesp, Botucatu, São Paulo, Brazil
[2] Institute of Biological and Health Sciences, University Center of Araguaia, Mato Grosso Federal University, Barra do Garças, Mato Grosso, Brazil
[3] Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador, Zubirán, Mexico

Corresponding Author:
D. C. Damasceno, Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina de Botucatu, Univ Estadual Paulista_Unesp, Distrito de Rubião Júnior, s/n, CEP: 18618-970, Botucatu, Estado de São Paulo, Brazil.
Email: damascenofmb@gmail.com

restriction (IUGR).[18,19,21,22] 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.[23,24]

Traditionally, the association between diet and insulin is the therapeutic resource most used to control blood glucose concentrations. Physical exercise has been known for its role in controlling glycemic levels.[25,26] The major question remains regarding the correlation between the potential benefits and the risks of physical exercise on fetal development during pregnancy. However, studies that investigate the effect of exercise on animals with IUGR were not developed. Therefore, rats that developed in an unfavorable intrauterine environment and born with IUGR may present alterations during adulthood, including pregnancy, and compromise embryo–fetal development. We hypothesized that the exercise program applied to IUGR rats during pregnancy may positively interfere with the maternal organism, contributing to improved embryo, fetal, and perinatal development. In this context, the objective of the present study was to evaluate the effect of exercise (swimming) considering the transgenerational effects on pregnant IUGR rats born in hyperglycemic intrauterine environment.

Material and Method

Experimental Animals

Female and male Wistar rats (CEMIB—UNICAMP, Campinas, São Paulo State, Brazil) weighing approximately 200 g were housed in a certified animal care. Food and water were provided ad libitum. All experimental procedures were approved by the Ethics Committee on Animal Experiments of the Botucatu Medical School—Universidad Estadual Paulista (UNESP). The rats were maintained in Laboratory of Experimental Research on Gynecology and Obstetrics under controlled conditions (temperature 22°C $\pm$ 2°C, humidity 55% $\pm$ 5%, and 12-hour light–dark cycle).

Diabetes Induction: To Create an Uncontrolled Intrauterine Environment for Obtaining IUGR Offspring

Diabetes was induced in adult life of female rats (approximately at 90 days of age) by beta-cytotoxic agent (STZ; Sigma-Aldrich Corporation, St. Louis, Missouri).[22] The STZ was dissolved in a citrate buffer (0.1 mol/L, pH 4.5) and administered intravenously (iv) at a dose of 40 mg/kg body weight. Nondiabetic (ND) rats received (iv) only citrate buffer using similar route and administration period. Seven days after STZ injection, the diabetic state was confirmed by blood glucose levels $\geq$ 300 mg/dL using a conventional glucometer. For nondiabetic rats, the inclusion criteria used was blood glucose levels $\leq$120 mg/dL. Glycemic values were expressed in mg/dL.[22] After 1 week of diabetes induction or buffer administration (ND), all adult female rats were mated overnight with nondiabetic adult male rats. The morning on which spermatozoa were found in the vaginal smear was designated pregnancy day 0.[18] The offspring were born by spontaneous delivery, following which the female offspring were separated and classified according to the mean values of fetal weights of the ND group (mean $\pm$ 1.0 $\times$ standard deviation [SD]). The newborns were classified as small for pregnancy age (SPA) when weight was smaller than mean of the ND group (mean$-$ 1.0 $\times$ SD), appropriate for pregnancy age when weight was similar to the mean values of the ND group (mean $\pm$ 1.0 $\times$ SD), and large for pregnancy age when weight was superior to mean of ND group (mean $+$ 1.0 $\times$ SD). The data were presented as percentual values.[22] The female newborns of the nondiabetic dams and classified as APA were included and denominated as the C group, and the female offspring born of the severe diabetic dams and classified as SPA were included and named as IUGR group (IUGR). After newborn classification, only 8 newborns (4 male and 4 female) were maintained with their mother during lactation up to weaning period (day 21 postnatal). After weaning, these offspring were maintained until adulthood (approximately 90 days of life).

Experimental Design of C and IUGR Rats

At day 90 of life, C and IUGR rats were submitted for mating using similar proceedings to mother rats. During pregnancy, the pregnant rats in the C group were maintained in the individual cages from day 7 to 20 of pregnancy and in the IUGR group distributed into 2 experimental groups, namely, IUGR not exercised (IUGR) and IUGR exercised (IUGRex) and too were maintained in the individual cages from day 7 to 20 of pregnancy.

Physical Exercise (Swimming Program)

The swimming program was applied in IUGRex rats from day 7 to 20 of pregnancy according to standard protocol established in our laboratory.[19]

Body Weight and Biochemical Determinations During Pregnancy in C, IUGR, and IUGRex Dams

At morning of days 14 and 20 of pregnancy, maternal body weights, postprandial glycemia, and triglyceride were determined for evaluation of exercise 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. For evaluation of triglycerides levels, we used Accutrend Plus (Roche Diagnostics GmbH, Mannheim, Germany), and the values were expressed as mg/dL. At day 14 of pregnancy, blood samples were collected to determine insulin concentration (Crystal Chemical—ELISA Kit 90010 - Rat Insulin Elisa kit; Crystal Chemical Company, Harris County, Texas) for evaluation of insulin resistance during pregnancy. At days 0 and 20 of pregnancy, lactate determination (Accutrend Plus) was performed to verify the exercise effect before and after application of swimming program in these dams.

Table 1. Outcomes of Female Rats From Parental and First Generation.

	Parental Generation	
	Nondiabetic	Severe Diabetic
Number of rats	10	116
Number of mated female rat	10/10 (100%)	45/116 (38.8%)[a]
Number of rat with at-term pregnancy	10/10 (100%)	15/45 (33.3%)[a]
Littler size/rat	11.2	8.0[a]
	First generation	
	APA/control (from nondiabetic dam)	SPA/IUGR (from diabetic dam)
Female offspring at birth	38	43
Number of alive female offspring at 3 months	35/38 (92.1%)	40/43 (93.0%)
Number of selected female rat for experiment	12/35 (34.3%)	40/40 (100%)
Number of mated female rat	12/12 (100%)	23/40 (57.5%)[a]

	Control	IUGR	IUGRex
Number of rat with at-term pregnancy	12/12 (100%)	10/10 (100%)	12/13 (92.3%)

Abbreviations: APA, appropriate for pregnancy age; IUGR, intrauterine growth restriction; SPA, small for pregnancy age (SPA); LPA, large for pregnancy age.
[a]$P < .05$—statistically significant difference from nondiabetic/control group (Fisher's exact test).

Fetal Classification of Offspring From C, IUGR, and IUGRex Dams

The offspring were born by spontaneous delivery, following which the offspring were classified according to the mean values (mean $\pm$ 1.0 $\times$ SD) of weights of the newborn in the C group.[22]

Statistical Analyses

The results were presented as mean $\pm$ SD. Analysis of variance followed by Tukey Multiple Comparison test were used for all parameters analyzed during pregnancy. The Fisher exact test was used for percentual data. Statistical significance was considered as $P < .05$.

Results

Outcomes of Parental Generation (Nondiabetic and Severe Diabetic Rats) and First Generation (APA or C and SPA or IUGR)

In relation to parental generation, 116 female rats were injected with STZ and of these, 100% presented blood glucose concentration above 300 mg/dL. In the nondiabetic group, 10 rats received citrate buffer and 100% of them presented blood glucose concentration below 120 mg/dL. At adult age, all nondiabetic female rats and 45 diabetic female rats mated. Of these, all nondiabetic rats (100%) and 15 severe diabetic rats reached at-term pregnancy. The severe diabetic dams presented lower alive fetuses compared to nondiabetic rats (Table 1).

Maternal Reproductive Performance of C, IUGR, and IUGRex Dams

The ND dams (n = 10) gave birth to 38 newborn and the severe diabetic dams (n = 15) gave birth to 43 newborn. Only the female rats were maintained until adult life and both experimental groups had female offspring from different litters. To continue the experiment, for the C group, the minimum number of female rats was included (n = 12) and the remaining rats were excluded. In the IUGR group, a higher number of female rats (n = 40) was used because this experimental group was subdivided (IUGR and IUGRex). A total of 100% of C rats presented positive pregnancy diagnosis and at-term pregnancy with offspring. In the IUGR group, 23 rats presented positive pregnancy diagnosis and were randomly subdistributed in 2 groups, IUGR (n = 10) and IUGRex (n = 13), which showed only 1 rat did not reach at-term pregnancy (Table 1).

Maternal Body Weight During Pregnancy in C, IUGR, and IUGRex Dams

The IUGR rats whether or not subjected to physical exercise showed significantly decreased maternal body weight ($P < .05$) compared to the C group at day 14 of pregnancy. At day 20 of pregnancy, only the IUGRex group continued presenting a decreased mean body weight in relation to the C group (Figure 1).

Biochemical Determinations in C, IUGR, and IUGRex Dams

At day 14 of pregnancy, there was no significant difference ($P > .05$) in the mean glycemia in the experimental groups.

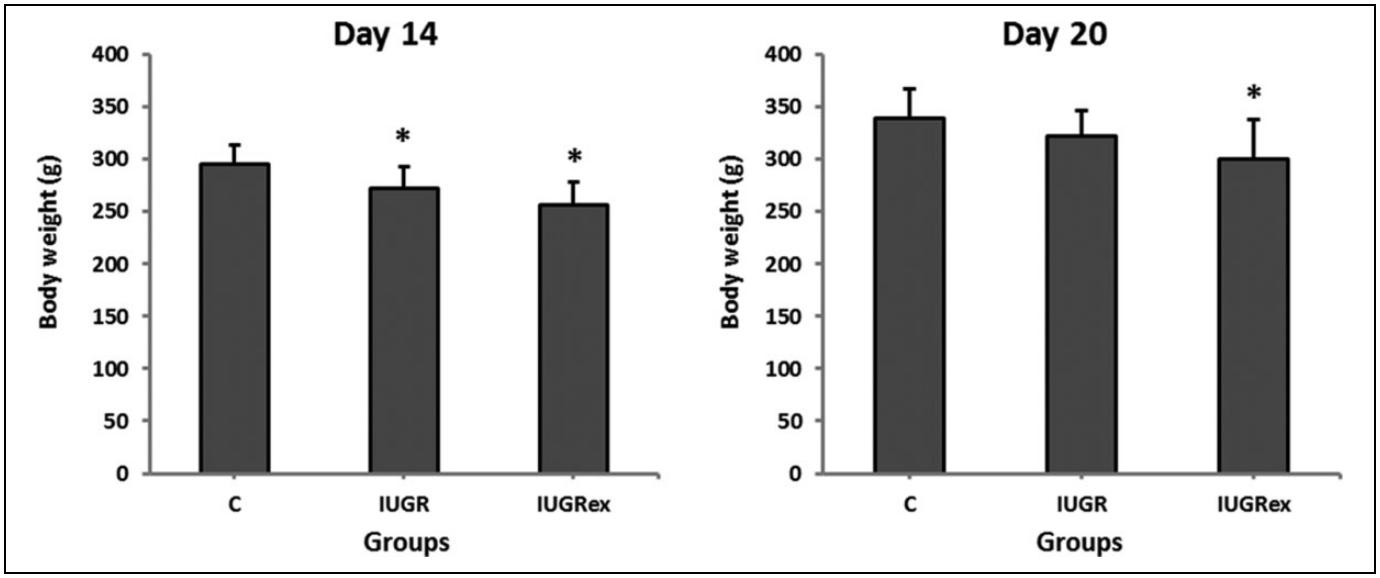

Figure 1. Maternal body weight at days 14 and 20 of pregnancy (Control group, n = 12; intrauterine growth restriction [IUGR] group, n = 10; IUGRex group, n = 12). Data presented as mean ± standard deviation (SD). *$P < .05$—statistically significant difference from the control group (Tukey Multiple Comparison Test).

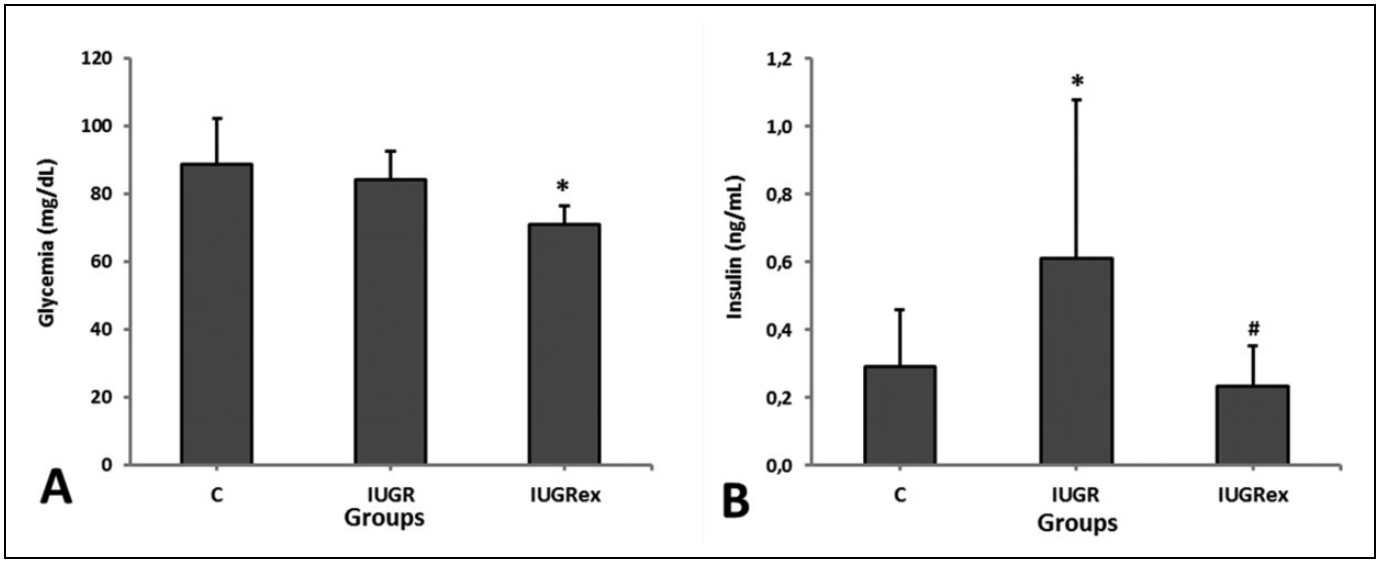

Figure 2. Maternal glycemia (A) and insulin (B) at day 14 of pregnancy (control group, n = 12; intrauterine growth restriction [IUGR] group, n = 10; IUGRex group, n = 12). Data presented as mean ± standard deviation (SD). *$P < .05$—statistically significant difference from the control group (Tukey Multiple Comparison Test). #$P < .05$—statistically significant difference from the IUGR group (Tukey Multiple Comparison Test).

In the same day, the insulin concentration increased ($P < .05$) in the IUGR group compared to C and IUGRex groups (Figure 2). The cholesterol levels showed no significant difference ($P > .05$) among the experimental groups (data not shown). The mean triglyceride level at day 14 of pregnancy showed no alteration among groups. At day 20 of pregnancy, there was increase ($P < .05$) in the triglyceride level in the IUGR group compared to C and IUGRex groups. (Figure 3). In relation to lactate concentration, at days 0 and 20 of pregnancy, there was a significant increase ($P < .05$) in the IUGR and IUGRex groups compared with the C group (Figure 4).

Classification of Body Weights of the Offspring as SPA, APA, and LPA

There was a reduced percentage of APA fetuses in the IUGR and IUGRex groups in relation to the C group. It was observed an increase in the proportion of SPA fetuses in the IUGRex compared to the C group (Figure 5).

Discussion

In the present study, it was verified a decreased rate of at-term pregnancy in rats from severe diabetes and IUGR groups.

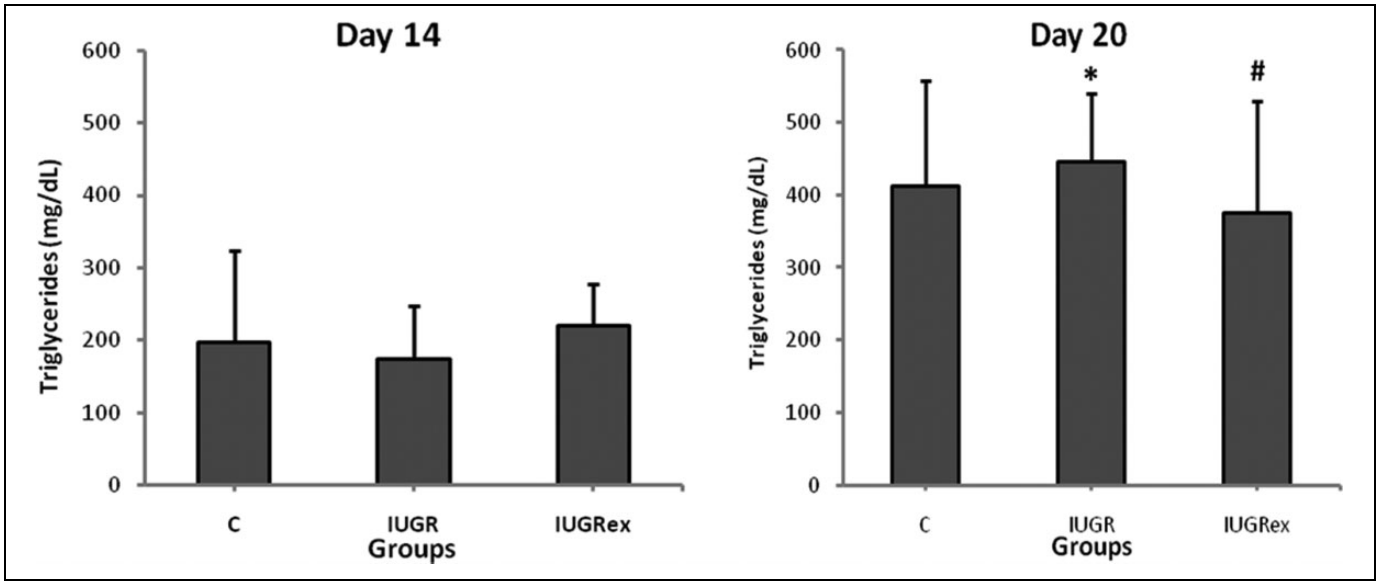

Figure 3. Maternal triglyceride levels on days 14 and 20 of pregnancy. (control group, n = 12; intrauterine growth restriction [IUGR] group, n = 10; IUGRex group, n = 12). Data presented as mean ± standard deviation (SD). *P < .05—statistically significant difference from the control group (Tukey Multiple Comparison Test). #P <.05—statistically significant difference from the IUGR group (Tukey Multiple Comparison Test).

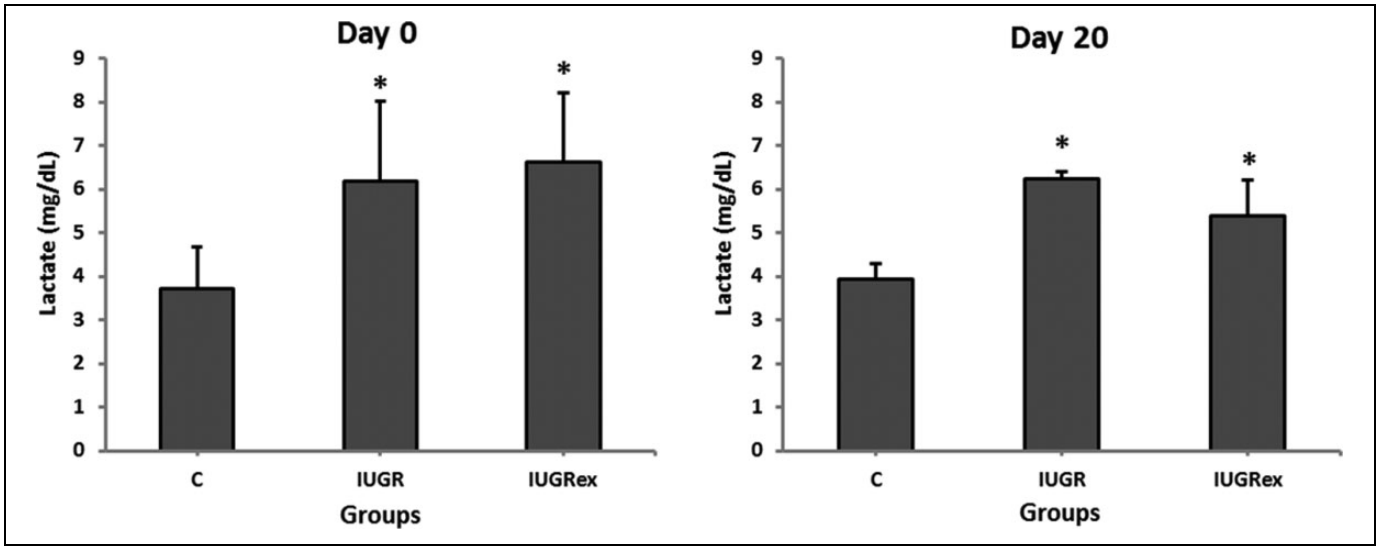

Figure 4. Maternal lactate levels before (day 0 of pregnancy) and after exercise (swimming; day 20 of pregnancy; control group, n = 12; intrauterine growth restriction [IUGR] group, n = 10; IUGRex group, n = 12). Data presented as mean ± standard deviation (SD). *P < .05—statistically significant difference from control group (Tukey Multiple Comparison Test).

Hyperglycemia (severe diabetes) in an unfavorable maternal intrauterine environment (IUGR) caused a disturbed fetal programming, which affected at adulthood of these animals. These disturbances led to a prejudicial effect on fecundation, embryonic implantation, and embryofetal development. This may be explained because diabetes is associated with increased risk of reproductive problems such as impaired folliculogenesis and steroidogenesis, anovulation, and spontaneous abortions.[27] A study performed in our laboratory showed that the STZ-induced diabetic state in rats caused a larger proportion of fetuses classified as SPA and a lower number of those classified as APA.[22] Maternal hyperglycemia causes a functional exhaustion of fetal pancreas with a reduced insulin synthesis and secretion (fetal hypoinsulinemia), leading to fetal hyperglycemia. This hormone is similar to growth hormone and it is necessary for proper growth in children. Then, its lack leads to an IUGR. Fetal decreased insulinemia has also been suggested in the etiology of fetal growth restriction, showing that severe diabetes in rats is a model for IUGR.[23,24,28] The terms IUGR, SGA, and low body weight (LBW) are often used interchangeably. However, while IUGR means a fetal phenotype resulting from the failure of the fetus to reach its

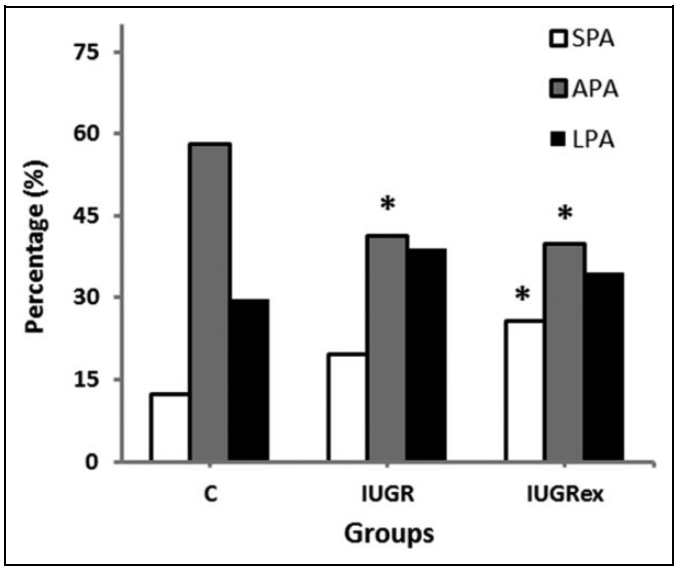

Figure 5. Fetal body weight classification as appropriate (APA), small (SPA), and large (LPA) for pregnancy age at birth day of offspring from control and intrauterine growth restricted dams. Data presented as percentage. *$P < .05$—statistically significant difference from the control group (Fisher's exact test).

growth potential (leading or not to LBW), SGA is defined as a birth weight below a certain cutoff for the respective gestational age. In the present study, the IUGR rats continued presenting LBW at until adulthood (data not shown) and during pregnancy (day 14). However, at day 20 of pregnancy, the body weight of IUGR rats was similar to C rats, showing that IUGR rats presented no catch-up growth. In relation to swimming program, IUGR dams showed lower body weight gain at late pregnancy. This fact maybe explained because the prolonged reduction in availability of substrates leads to a slowing in growth. This enhances the ability of the fetus to survive by reducing the use of substrates and lowering the metabolic rate. This fact contributes for slowing of growth at-term pregnancy and discrepancy in organ size.[29]

Normal pregnancy is associated with hyperinsulinemia and a progressive decline in insulin sensitivity.[30,31] In response to the increased demand for insulin, the maternal pancreas adapts by increasing insulin synthesis, secretion, and cell proliferation.[32-34] In the rat, these adaptations peak around day 14 of pregnancy, and by the end of gestation, the maternal cell mass has nearly doubled.[35,36] In the present study, the IUGR dams presented higher levels of insulin at day 14 of pregnancy compared to the C group (normal pregnancy). In another investigation it was also verified that IUGR rats presented increased levels of insulin at day 14 of pregnancy.[37] The additional stress of pregnancy may result in a further deterioration of these processes in the IUGR dams, leading to the adverse effect on glucose homeostasis.[38] Retarded growth of fetuses and newborns has a reduced population of pancreatic β cells.[39] The adaptations to IUGR that limit insulin secretion during fetal period are not fully known, and studies in humans demonstrated contradictory results in relation to β cell mass.[40,41] However, the normal compensation to insulin resistance in adults is an increase in β cell mass and insulin secretion,[42,43] and, any restriction of this compensation as an individual ages and develops obesity and insulin resistance will result in hyperglycemia and type 2 diabetes.[44] In humans, measures of basal and glucose-stimulated insulin secretion are not consistently related to birth weight. A positive relationship between birth weight and insulin secretion may reflect compensatory increases in insulin secretion in response to developing insulin resistance, whereas in others a negative relationship may reflect late stages of β cell failure.[45] Thus, in our model of IUGR, we hypothesized that these dams present defects in insulin action that precede defects in insulin secretion leading to development of insulin resistance, which was reverted by exercise during pregnancy. The regular practice of exercise increases insulin sensitivity on the muscle and other tissues in rodents and, promotes the translocation of glucose transporter 4 (GLUT-4) stimulating the PI3K, which is involved in glycogen synthesis in liver and muscle and lipogenesis.[46,47] Similar to the effects of physical exercise, insulin also causes GLUT-4 translocation in rat skeletal muscle.[48,49] Because blood flow is increased during exercise, it could be hypothesized that the exercise-induced recruitment of GLUT-4 to the plasma membrane is secondary to increased delivery of insulin to the working muscles. The contraction can recruit GLUT-4 to the plasma membrane in rat skeletal muscle independent of insulin, showing that insulin is not required for muscle contraction to increase glucose uptake in skeletal muscle.[50-52] Goodyear et al[52] verified that insulin-resistant patients can increase rates of muscle glucose uptake with exercise because the muscle is able to bypass defects in the insulin-signaling molecules and activate the exercise-specific signaling mechanisms.

In the present study, it was verified a hypertriglyceridemia at late pregnancy (day 20 of pregnancy) in C rats, corroborating to other studies.[53,54] However, the IUGR dams presented hypertriglyceridemia more pronounced compared to the C group, and exercise contributed to reduce the levels of this parameter in IUGR dams similar to levels observed in the C group. Boloker et al.[37] also verified increased triglyceride levels in IUGR rats at late pregnancy when compared to C rats. However, these authors observed no hypertriglyceridemia in C rats.

The IUGR dams presented hyperlactatemia, and the swimming program was not efficient to normalize this parameter in pregnancy. The literature evidences that placental insufficiency is present in severe diabetic rats.[18,55,56] The placental insufficiency leads to fetal hypoxemia, hypoglycemia, and therefore hypercatecholaminemia, leading to a continuous suppression of glucose oxidation. Therefore, endocrine and metabolic adaptations are necessary to conserve fetal nutrients by lowering skeletal muscle energy requirements for protein synthesis and fetal growth in IUGR condition.[57-59] We propose that IUGR animals from diabetic mothers maintain these phenotypes and continue using lactate produced by anaerobic glycolysis in skeletal muscle as a substrate for glucose,[60,61] and the liver clearance stabilizes plasma lactate concentrations leading

to the mild hyperlactatemia[60] confirming the fact that the IUGR rats presented higher lactate levels during pregnancy.

The IUGR dams presented lower number of fetuses classified as APA. This finding shows the impaired repercussions of an unfavorable intrauterine environment from severe diabetes (grandmother) up to offspring of IUGR dams, confirming altered fetal programming. A more prolonged reduction in availability of substrates leads to a slowing in growth. This enhances the ability of the fetus to survive by reducing the use of substrates and lowering the metabolic rate. Slowing of growth in late gestation leads to disproportion in organ size because organs and tissues that are growing rapidly at that time are affected the most.[29] This study showed an increased number of small fetuses for pregnancy age in exercised dams. Thus, we suggested that the exercise did not contribute for nutrient delivery in complicated pregnancies like IUGR, but it did not aggravate the status found in IUGR dams.

Conclusion

Thus, the swimming program applied during pregnancy of IUGR rats improved lipid metabolism and higher insulin sensitivity of these rats. However, the resources to produce an adequate fetal development were limited, contributing to retarded growth of their offspring. These data reinforce the need to stimulate the exercise practice in women under supervision with different professional expertise to promote appropriate gestational conditions and to improve perinatal outcomes.

Acknowledgments

The authors are grateful to CAPES, to Talisia C Moreto for the care of the animals, and to Research Support Center (RSC) of the Botucatu Medical School, Universidad Estadual Paulista (UNESP), for their valuable statistical contribution. The authors also thank students Isabela Lovizutto Iessi and Aline Bueno for the care of the animals.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by grants from FAPESP, Brazil (04/01077–8) under the co-ordination of Dr Débora C. Damasceno.

References

1. Ravelli AC, Van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr*. 1999;70(5):811-816.
2. Roseboom TJ, Van der Meulen JH, Ravelli AC, et al. Blood pressure in adults after prenatal exposure to famine. *J Hypertens*. 1999;17(3):325-330.
3. Guzman C, Cabrera R, Cardenas M, Larrea F, Nathanielsz PW, Zambrano E. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. *J Physiol*. 2006;572(pt1):97-108.
4. Nathanielsz PW. Animal models that elucidate basic principles of the developmental origins of adult diseases. *ILAR J*. 2006;47(1):73-82.
5. Ozanne SE, Hales CN. Lifespan: catch-up growth and obesity in male mice. *Nature*. 2004;427(6973):411-412.
6. Barker DJP. Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition*. 1997;13(9):807-813.
7. Kanaka-Gantenbein C. Fetal origins of adult diabetes. *Ann N Y Acad Sci*. 2010;1205:99-105.
8. Hanson MA, Gluckman PD. Developmental origins of health and disease: moving from biological concepts to interventions and policy. *Int J Gynaecol Obstet*. 2011;7292(11):60003-60009.
9. Fernandez-Twinn DS, Ozanne SE. Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. *Phys Behav*. 2006;88(3):234-243.
10. Aerts L, Van Assche FA. Animal evidence for the transgenerational development of diabetes mellitus. *Int J Biochem Cell Biol*. 2006;38(5-6):894-903.
11. Zambrano E. The transgenerational mechanisms in developmental programming of metabolic diseases. *Rev Invest Clin*. 2009;1(1):41-52.
12. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest*. 1998;101(10):2174-2181.
13. Jansson T, Lambert GW. Effect of intrauterine growth restriction on blood pressure, glucose tolerance and sympathetic nervous system activity in rat at 3-4 months of age. *J Hypertens*. 1999;17(9):1239-1248.
14. Simmons RA, Templeton LJ, Gertz S J. Intrauterine growth retardation leads to the development of Type 2 diabetes in the rat. *Diabetes*. 2001;50(10):2279-2286.
15. Bassan H, Bassan M, Pinhasov A, et al. The pregnant spontaneously hypertensive rat as a model of asymmetric intrauterine growth retardation and neurodevelopmental delay. *Hypertens Pregnancy*. 2005;24(3):201-211.
16. Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ. Islet function in offspring of mothers on low-protein dietduring gestation. *Diabetes*. 1991;40(suppl 2):115-120.
17. Volpato GT, Damasceno DC, Campos KE, Rocha R, Rudge MV, Calderon IMP. Avaliação do efeito do exercício físico no metabolismo de ratas diabéticas prenhes. *Rev Bras Med Esp*. 2006;12(5):229-233.
18. 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.
19. Volpato GT, Damasceno DC, Kempinas WG, et al. Effect of exercise on the reproductive outcome and fetal development of diabetic rats. *Reprod Biomed Online*. 2009;19(6):852-858.
20. Damasceno DC, Silva HP, Vaz GF, et al. Diabetic rats exercised prior to and during pregnancy: maternal reproductive outcome, biochemical profile, and frequency of fetal anomalies. *Reprod Sci*. 2013;20(7):730-738.

21. 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.
22. de Souza M da S, Lima PH, Sinzato YK, Rudge MV, Pereira OC, Damasceno DC. Effects of cigarette smoke exposure on pregnancy outcome and offspring of diabetic rats. *Reprod Biomed Online*. 2009;18(4):562-567.
23. Pedersen J. Weight and length at birth of infants of diabetic mothers. *ActaEndocrinol*. 1954;16(4):330-342.
24. Holemans K, Aerts L, Van Assche FA. Fetal growth restriction and consequences for the offspring in animal models. *J SocGynecol Invest*. 2003;10(7):392-399.
25. Devlin JT. Effects of exercise on insulin sensitivity in humans. *Diabetes Care*. 1992;15(11):1690-1693.
26. Kim C. Gestational diabetes: risks, management, and treatment options. *Int J Womens Health*. 2010;2:339-351.
27. Chabrolle C, Jeanpierre E, Tosca L Ramé C, Dupont J. Effects of high levels of glucose on the steroidogenesis and the expression of adiponectin receptors in rat ovarian cells. *Reprod Biol Endocrinol*. 2008;19:6-11.
28. Calderon IMP, Rudge MVC, Ramos MD, Peraçoli JC. Estudo longitudinal, bioquímico e histoquímico de placentas de ratas diabéticas - relação com a macrossomia e o retardo do crescimento intra-uterino. *Rev Bras Ginecol Obstet*. 1999;21(2):91-98.
29. Simmons R. Developmental origins of adult metabolic disease: concepts and controversies. *Trends Endocrinol Metab*. 2005; 16(8):390-394.
30. Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA. Longitudinal changes in insulin resistance and insulin release in non-obese pregnant women. *Am J Obstet Gynecol*. 1991;165(6 pt 1):1667-1672.
31. Catalano PM, Tyzbir ED, Wolfe RR, et al. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. *Am J Physiol*. 1993;264(1 pt 1):E60-E67.
32. Parsons JA, Brelje TC, Sorenson RL. Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology*. 1992;130(3):1459-1466.
33. Bone A, Taylor K. Metabolic adaptation to pregnancy shown by increased biosynthesis of insulin in islets of Langerhans isolated from pregnant rats. *Nature*. 1976;262(5568):501-502.
34. Kwai M, Kishi K. In vitro studies of the stimulation of insulin secretion and β-cell proliferation by rat placental lactogen-II during pregnancy in rats. *J Reprod Fertility*. 1997;109(1): 145-152.
35. Nieuwenhuizen A, Schuiling G, Moes, et al. Role of increased insulin demand in the adaptation of the endocrine pancreas to pregnancy. *Acta Physiol Scand*. 1997;159(4):303-312.
36. Marynissen G, Aerts L, Assche FV. The endocrine pancreas during pregnancy and lactation. *J Dev Physiol*. 1983;5(6):373-381.
37. Boloker J, Gertz SJ, Simmons RA. Gestational diabetes leads to the development of diabetes in adulthood in the rat. *Diabetes*. 2002;51(5):1499-1506.
38. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull*. 2001;60:5-20.
39. Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. *BMJ*. 1998;317(7171): 1481-1487.
40. Van Assche FA, De Prins F, Aerts L, Verjans M. The endocrine pancreas in small-for-dates infants. *Br J Obstet Gynaecol*. 1977; 84(10):751-753.
41. Béringue F, Blondeau B, Castellotti MC, Bréant B, Czernichow P, Polak M. Endocrine pâncreas development in growth-retarded human fetuses. *Diabetes*. 2002;51(2):385-391.
42. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*. 2003;52(1):102-110.
43. Polonsky KS, Sturis J, Bell GI. Seminars in Medicine of the Beth Israel Hospital, Boston. Noninsulin- dependent diabetes mellitus - a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med*. 1996;334(12):777-783.
44. Thorn SR, Rozance PJ, Brown LD, Hay WW Jr. The intrauterine growth restriction phenotype: fetal adaptations and potential implications for later life insulin resistance and diabetes. *Semin Reprod Med*. 2011;29(3):225-236.
45. Gatford KL, Simmons RA. Prenatal programming of insulin secretion in intrauterine growth restriction. *Clin Obstet Gynecol*. 2013;56(3):520-528.
46. Teran-Garcia M, Rankinen T, Koza RA, Rao DC, Bouchard C. Endurance training-induced changes in insulin sensitivity and gene expression. *Am J Physiol Endocrinol Metab*. 2005;288(6): E1168-1178.
47. Pessin JE, Saltiel AR. Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest*. 2000;106(2): 165-169.
48. Douen AG, Ramlal T, Cartee GD, Klip A. Exercise modulates the insulin-induced translocation of glucose transporters in rat skeletal muscle. *FEBS Lett*. 1990;261(2):256-260.
49. Goodyear LJ, Hirshman MF, Horton ES. Exercise-induced translocation of skeletal muscle glucose transporters. *Am J Physiol*. 1991;261(6 pt 1):E795-E799.
50. Dela F, Ploug T, Handberg A, et al. Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM. *Diabetes*. 1994;43(7):862-865.
51. Richter EA, Ploug T, Galbo H. Increased muscle glucose uptake after exercise. No need for insulin during exercise. *Diabetes*. 1985;34(10):1041-1048.
52. Goodyear LJ, Hirshman MF, Napoli R, et al. Glucose ingestion causes GLUT4 translocation in human skeletal muscle. *Diabetes*. 1996;45(8):1051-1056.
53. Munilla MA, Herrera E. Maternal hypertriglyceridemia during late pregnancy does not affect the increase in circulating triglycerides caused by the long-term consumption of a sucrose-rich diet by rats. *J Nutr*. 2000;130(12):2883-2888.
54. Herrera E, Lasunción MA, Gomez-Coronado D, Aranda P, López-Luna P, Maier I. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am J Obstet Gynecol*. 1988;158(6 pt 2): 1575-1583.
55. Kanter M, Yoruk M, Koc A, Meral I, Karaca T. Effects of cadmium exposure on morphological aspects of pancreas, weights

of fetus and placenta in streptozotocin-induced diabetic pregnant rats. *Biol Trace Elem Res*. 2003;93(1-3):189-200.

56. 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.
57. Carver TD, Quick AA, Teng CC, Pike AW, Fennessey PV, Hay WW Jr. Leucine metabolism in chronically hypoglycemic hypoinsulinemic growth-restricted fetal sheep. *Am J Physiol*. 1997;272(1 Pt 1):E107-117.
58. Galan H. L., Anthony R. V., Rigano S., et al. Fetal hypertension and abnormal Doppler velocimetry in an ovine model of intrauterine growth restriction. *Am J Obstet Gynecol*. 2005;192(1):272-279.
59. Regnault TR, de Vrijer B, Galan HL, Wilkening RB, Battaglia FC, Meschia G. Development and mechanisms of fetal hypoxia in severe fetal growth restriction. *Placenta*. 2007; 28(7):714-723.
60. Limesand SW, Rozance PJ, Smith D, Hay WW Jr. Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. *Am J Physiol Endocrinol Metab*. 2007;293(6): E1716-E1725.
61. Thorn SR, Regnault TR, Brown LD, et al. Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. *Endocrinology*. 2009;150(7):3021-3030.