Artigo Original

NEONATALLY-INDUCED DIABETES: LIPID PROFILE OUTCOMES AND OXIDATIVE STRESS STATUS IN ADULT RATS

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SUMMARY

Background. Experimental models are developed for the purpose of enhancing the understanding of the pathophysiological mechanisms involved in diabetes. Experimental findings lead to the development of treatment strategies to maintain metabolic conditions as close to normal as possible. There are several reports about streptozotocin induced mild diabetes to reproduce type 2 diabetes. However, studies about the interaction among glucose levels, lipid profile, and oxidative stress in these animals remain insufficient. Therefore, this study evaluated these parameters in blood samples from adult Wistar rats treated neonatally with streptozotocin.

Methods. Female newborn Wistar rats received streptozotocin (70 mg/kg, i.p.) on the 5th day of life (n5-STZ). Glycemia was measured in the 3rd and 4th month of life. At the end of the 4th month, blood samples were collected and processed for lipid profile and oxidative stress measurements.

Results. Glycemia of n5-STZ rats were significantly higher compared to those of control rats ($p<0.05$). There was no alteration in levels of total cholesterol, triglycerides, lipid peroxidation (TBARS), SOD activity and GSH-t determination ($p>0.05$) in the n5-STZ animals when compared to control group. However n5-STZ animals showed a significant decreased HDL-cholesterol rate ($p<0.05$).

Conclusion. This streptozotocin-induced diabetes model in rats caused hyperglycemia (120-360mg/dL), characterizing mild diabetes. This glycemic level led to HDL-lipoprotein alteration, which was not sufficient to impair antioxidant enzyme activities or determination of lipid peroxidation in adult life of rats. Further this experimental investigation contributed to the understanding of different results found in other models for mild/moderate diabetes induction in laboratory animals as well as to a better comprehension of the pathophysiological mechanisms of mild diabetes or hyperglycemia in humans.

Key words: Rat Streptozocin. Lipid. Oxidative stress. Diabetes. Experimental study.

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Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, secretion insufficiency and receptor insensitivity to endogenous insulin[1]. Its incidence is associated with high morbidity and mortality rates. Increased oxidative stress is believed to play an important role in the etiology and pathogenesis of chronic complications of diabetes[2,3], and is mainly characterized by imbalance between organism antioxidant defenses and oxidant molecules, known as free radicals. Free radicals are very reactive species, capable of inducing oxidation of the biological membrane of phospholipids and proteins, resulting in modifications of cell function and cellular death[4,5,6].

Many animal models have contributed to the elucidation of human diabetic syndromes and associated genetic factors. Among these animal models, there are those that reproduce human diabetes using streptozotocin (STZ). Probably, STZ induces diabetes by generating reactive oxygen radicals (ROS), which leads to islet cell destruction in experimental animals[7]. Models for rats presenting severe diabetes (fasting glycemic level higher than 200/360 mg/dL), which reproduce uncontrolled type-1 diabetes (DM1) in humans, are well established

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utilizing high doses of STZ during the adult life of animals[8,9,10,11]. Experimental mild diabetes (fasting glycemic level from 120 to 360 mg/dL) characterizes clinical status of type 2 *Diabetes mellitus* (DM2). Portha et al.[12] were the first to describe an animal model for mild diabetes using neonatal STZ. Some authors administered STZ at day of birth (n0-STZ)[13,14], 2 days after birth (n2-STZ)[15,16], after 5 days of life (n5-STZ)[16,17], and on the 2nd and 9th days of life[18]. These studies showed that, at 8 weeks and thereafter the animals presented impaired glucose tolerance and a 50% decrease in pancreatic insulin content with mild hypoinsulinemia. As reviewed by Portha et al, incompetence of the regenerated beta cells may be due to a reduced GLUT2 content, limiting glucose entry and metabolism and a decreased glucokinase affinity to glucose[19]. Experimental findings lead to the development of treatment strategies to maintain metabolic conditions as close as possible to normal. There are several reports about the use of streptozotocin to induce mild diabetes reproducing type 2 *Diabetes mellitus*. However, these investigations did not disclose any relation among glucose tolerance, insulin content and insulin resistance with lipid profile and oxidative stress status in adult life of rats. Therefore, this study aimed to analyze the model of mild diabetes induction in Wistar rats and to evaluate these parameters in the adult life of rats treated neonatally with streptozotocin.

Methods

Wistar male and female rats weighing about 180g (90 days of life) were adapted in our laboratory for seven days. The rats were kept in collective cages, in controlled conditions of temperature of (22 ± 3°C), light (12 h light/dark cycles) and relative humidity (60 ± 5%). The animals were fed with laboratory chow (Purina®) tap water *ad libitum* and cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals. The local Committee of Ethics in Animal Experimentation approved all experimental procedures of this study.

Female rats were mated overnight with normal male rats (parental generation). Sperm presence in vaginal wet smears in the following morning was considered as day zero (0) of pregnancy. Pregnant rats were kept in individual cages during the pregnancy period (21 days), including vaginal delivery and lactation periods (21 days). The female newborn (NB) received streptozotocin (STZ, 70 mg/kg body weight, intraperitoneally) dissolved in citrate buffer (0.1M, pH 4.5) at day 5 of life, as previously described by Murali & Goyal [17]. The terminology n5-STZ is used here to refer to this version of a model that characterizes clinical status of DM2. In the control group, NB received only citrate buffer, at day 5 of life.

In the month 3 and 4 of life, blood samples were obtained from a cut tail tip for non-fasting glycemic determinations (glucose oxidase) using a glucosimeter (One Touch Ultra - Johnson & Johnson®) in the morning. At the end of 4th month of life, the glycemia were obtained immediately before anesthesia. All animals were anesthetized with sodium pentobarbital and killed. Blood samples were collected from each animal and processed for lipid profile and oxidative stress measurements. The chemicals were purchased from Wiener (Rosario, Argentina) and Sigma Chemical (St. Louis, MO, USA). Blood samples were collected in anticoagulant-free test tubes and kept at low temperature for 30 min and then centrifuged at 3,500 rpm for 10 min at 4°C. The supernatant was collected as serum and stored at -80°C for lipid determination. Another blood fraction was placed in anticoagulant tubes and centrifuged at 1,200 rpm for 10 min at room temperature for assay of oxidative stress biomarkers, which were estimated in the washed erythrocytes[20].

Table 1 - Non-fasting glycemic levels of rats treated with citrate buffer solution (control group) and streptozotocin (n5-STZ group) in the neonatal period (mean ± SEM)

	Groups	
Glucose (mg/dL)	Control	n5-STZ
3rd month	78.6 ± 3.93	192.4 ± 30.7*
4th month	121.8 ± 6.4	216.9 ± 15.7*

*p<0.05 - statistically significant difference compared to control group

Serum concentrations of total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were determined by the enzymatic method[21]. The absorbance of these parameters was measured at 505 nm.

Oxidative stress biomarkers evaluated were superoxide dismutase (SOD), glutathione total (GSHt) and thiobarbituric acid reactive substances as the lipid peroxidation index (TBARS). For TBARS concentration, the absorbance was measured at a wavelength of 535 nm and results were expressed as nM of thiobarbituric acid reactive species (TBARS) per gram of hemoglobin (nM/g Hb). The SOD enzymatic activity unit was defined as SOD units able to produce 50% of pyrogallol oxidation inhibition. All data were expressed in units of SOD per milligram of hemoglobin. GSHt, which consists of reduced and oxidized glutathiones, was enzymatically determined using 5.5'- dithio-bis (2-nitrobenzoic acid) (DTNB) and glutathione reductase in the presence of a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), forming 2-nitro-5-thiobenzoic acid. GSHt activity was measured at 412 nm on a spectrophotometer. One unit of its activity was equal to the micromolar of substrate reduced per gram of hemoglobin[20].

Data are expressed as mean ± standard error of mean (SEM). The Student t test was used to determine differences between groups[22]. The limit of statistical significance was 5% ($p<0.05$).

Results

Glycemia

In the 3rd month of life, the glycemic means of rats in the n5-STZ group were significantly higher than those of rats in the control group ($p<0.05$). All rats in the control group exhibited glycemia under 100 mg/dL. Among the rats in the n5-STZ group, 50.0% showed glycemia under 120 mg/dL and 50.0% from 120 to 360 mg/dL. In the 4th month of life, glycemic means of rats in the n5-STZ group remained significantly higher in relation to the control group ($p<0.05$). When glycemic levels were individually analyzed, alteration was observed in the distribution of glycemic ranges of rats in the n5-STZ group, since all the animals presented glycemia between 120 and 360mg/dL. Nevertheless, in the control group, the rats maintained glycemic levels similar to those found in the 3rd month of life. Glucose levels at month 3 and 4 of life are presented in Table 1 and the glucose range distributions are presented in Table 2.

Table 2 - Distribution of rats in relation to non-fasting glycemic levels. Rats treated with citrate buffer solution (control group) and streptozotocin (n5-STZ group) in the neonatal period (mean ± SEM)

	Groups			
	3rd month		4th month	
Glycemia	Control	n5-STZ	Control	n5-STZ
< 120 mg/dL	78.6 ± 3.9 (100.0%[a])	91.1 ± 10.6 (50.0%[a])	108.5 ± 14.8* (40.0%[a])	0 *
120 - 360 mg/dL	0	293.6 ± 26.9 (50.0%[a])	130.7 ± 1.5* (60.0%[a])	216.9 ± 15.7* (100%[a])

[a] % of rats presenting glycemia with indicated values in the 3rd and 4th months of life.
*p<0.05 - statistically significant difference compared to month 3.

Biochemical parameters and oxidative stress biomarkers

The biochemical parameters evaluated in the 4th month of life are presented in Table 3. Both groups did not exhibit statistical differences (p>0.05) in serum levels of total cholesterol and triglycerides. However, n5-STZ animals showed a significant decrease in HDL-cholesterol levels as compared to the control group (p<0.05).

The evaluation of oxidative stress parameters is presented in Table 4. Streptozotocin-induced diabetic rats did not present changes in lipid peroxidation (TBARS). Alterations were not observed in SOD activity or GSH-t determination when compared to the control group (p>0.05).

Table 3 - Biological lipid profile in the 4th month of life in rats treated with citrate buffer solution (control group) and streptozotocin (n5-STZ group) in the neonatal period (mean ± SEM)

	Groups	
	Control	n5-STZ
Triglycerides (mg/dL)	87.7 ± 20.1	106.0 ± 17.9
Cholesterol (mg/dL)	130.0 ± 36.1	179.3 ± 28.5
HDL-cholesterol (mg/dL)	47.5 ± 6.3	32.4 ± 2.5 *

*p<0.05 - statistically significant difference compared to control group.

Table 4 - Determination of thiobarbituric acid reactive species (TBARS), enzymatic activity of antioxidant dismutase superoxide (SOD) and concentration of total glutathione (GSHt) in the 4th month of life in rats treated with citrate buffer solution (control group) and streptozotocin (n5-STZ group) in the neonatal period (mean ± SEM)

	Groups	
	Control	n5-STZ
TBARS (nM/g Hb)	226.1 ± 71.9	276.2 ± 22.3
SOD (U/mg Hb)	7.1 ± 0.4	7.2 ± 0.4
GSHt (uM/g Hb)	0.032 ± 0.003	0.034 ± 0.010

p>0.05 - non-significant

DISCUSSION

In this study, the control animals presented normoglycemia at months 3 and 4. Control animals presented slight glycemia above 120mg/dL at the 4th month of life however, these animals were not considered mild diabetic because glycemia was verified in the non-fasting state and our data corroborate those of other authors[17,23]. Our results showed that STZ administration on the 5th day of neonatal life caused onset of hyperglycemia in the 3rd month of life in rats, whose postprandial glycemic mean was of approximately 192.4 mg/dL. This model presented glycemic results similar to those found by Murali & Goyal[17]. The individual analysis of glycemia in rats showed that 50% did not present mild diabetes at the 3rd month. Nevertheless, after the 4th month of life, 100% of the n5-STZ rats showed glycemic levels between 120 and 360 mg/dL, similar to the glycemic mean observed by other researchers[24,25] thus ensuring the viability of the model for mild diabetes induction. Further, Weir et al.[26] demonstrated that n5-STZ rats presented lack of significant insulin re-accumulation in the pancreas, 2 weeks after the b-cell insult.

In patients, *Diabetes mellitus* increases the risk of developing atherosclerosis and coronary artery disease[27,28,29], and lipids are indicated as some of the major pathogenic biological markers in situations of metabolic dysfunction (such as in insulin resistance, associated type-2 *Diabetes mellitus* or not)[30]. This investigation showed that n5-STZ animals presented a non-significant increase in total cholesterol and triglyceride levels and decreased HDL levels. With regard to the absence of alterations in the determinations of total cholesterol and triglycerides, our findings are not in agreement with those described in literature. Insulin resistance and *Diabetes mellitus* affect virtually every lipid and lipoprotein and, therefore, dyslipidemia is present in most diabetic patients[27,31,32]. Dyslipidemia is reflected by elevated levels of triglycerides, VLDL and LDL-cholesterol and lower HDL-cholesterol levels[33]. Although our results do not reflect comments in clinical studies, the Brazilian Society of Cardiology acknowledges that decreased HDL (isolated or in association with increased levels of LDL and/or triglycerides) characterizes the laboratory classification of dyslipidemia[34]. HDL cholesterol and apo AI levels are characteristically reduced in insulin-resistant people. Much of this derives, as in the case of low dense LDL, from the action of CETP (cholesterol ester transferase) mediated transfer of cholesteryl ester from HDL to triglyceride rich lipoproteins (chylomicrons and VLDL). A consistent finding is the inverse relationship between plasma insulin (or C-peptide) concentrations, which are measures of insulin resistance and HDL-cholesterol levels[35].

There is increasing evidence, in both experimental and clinical studies, to suggest that oxidative stress plays a major role in the pathogenesis of *Diabetes mellitus*. In this study, TBARS levels in the n5-STZ rats remained unaltered. Our result suggests that the neonatal STZ-induced diabetes model was insufficient to increase glycemia and so, exacerbate oxidative stress. However, increase of glycemia to a moderate level was verified. With respect to SOD, the enzyme responsible for neutralizing

superoxide anion levels, this study showed no changes in SOD activity in the n5-STZ rats. These results corroborate with literature, which observed no significant changes in SOD activity in diabetic rats[36,37]. However, there are also conflicting findings in literature. There is evidence of reduced SOD activity in alloxan-induced diabetic rats[38], and higher activity was observed in DM1 and DM2 subjects[39].

The glutathione antioxidant system has a fundamental role in cellular defense against reactive free radicals and other oxidant species [40]. GSHt concentration also presented no change in the n5-STZ rats in this investigation. Damasceno et al.[6] observed reduction in GSH determination in STZ induced-severe diabetic rats. Furthermore, it has been suggested that there may be temporal changes in enzyme activity that are both transitory and biphasic in nature. For instance, after prolonged hyperglycemia in severe diabetes (glycemia>360 mg/dL), the induction of certain antioxidant enzymes or a return to normal values from previously decreased values may occur as a compensatory mechanism in response to constant exposure to increased stress. This could explain the decrease, increase or normality in SOD and GSH observed by different investigators. Thus, the present study showed that the neonatal streptozotocin-induced mild diabetes model in rats caused lipid profile alteration in the HDL-cholesterol level, but it was not sufficient to impair antioxidant enzyme activity or peroxidation lipid determination sampled from adult Wistar rats.

Conclusion

The present investigation confirmed a mild diabetes status since the 4th month of life in rats. This study contributed to understanding the relation between lipid profile and oxidative stress status in the mild/moderate diabetes model for laboratory animals as well as to a better comprehension of the pathophysiological mechanisms of mild diabetes or hyperglycemia.

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Conflict of interest: none

Resumo

Diabete induzido no período neonatal: repercussões no perfil lipídico e avaliação dos marcadores de estresse oxidativo na vida adulta de ratas

Introdução. Modelos experimentais são desenvolvidos com propósito de ampliar o entendimento dos mecanismos fisiopatológicos envolvidos no diabete. Os achados experimentais levam ao desenvolvimento de tratamentos alternativos para a manutenção das condições metabólicas normais. Existem vários estudos sobre o diabete induzido por streptozotocin mimetizando o quadro clínico do DM2. No entanto, a interação entre os níveis de glicose, perfil lipídico e estresse oxidativo nestes animais são escassos. Portanto, o objetivo do trabalho foi avaliar estes parâmetros em ratas Wistar adultas com diabete induzido com streptozotocin no período neonatal.

Métodos. Fêmeas recém-nascidas receberam streptozotocin (70mg/Kg, ip) no 5º dia de vida (n5-STZ). A glicemia foi medida no terceiro e quarto meses de vida dos animais. No final do quarto mês de vida, amostras de sangue foram coletadas e processadas para a dosagem de lipídios e marcadores de estresse oxidativo.

Resultados. A glicemia das ratas do grupo n5-STZ foi significativamente maior comparada às ratas do grupo controle (p<0,05). Não houve alteração nos níveis de colesterol total e triglicérides, peroxidação lipídica (TBARS), atividade da SOD e determinação da GSH-t (p>0,05) nas ratas n5-STZ em relação às ratas do grupo controle. No entanto, houve diminuição significativa no HDL-colesterol (p<0,05).

Conclusão. Este modelo de indução de diabete em ratas causou hiperglicemia (120-360mg/dL), caracterizando o diabete moderado. Essa glicemia levou a alterações no HDL-colesterol, a qual não foi suficiente para prejudicar a atividade das enzimas antioxidantes ou marcadores da peroxidação lipídica na vida adulta. Além disso, esta investigação experimental contribuiu para entender os diferentes resultados encontrados em outros modelos de indução do diabete moderado em animais de laboratório, como também para a melhor compreensão dos mecanismos fisiopatológicos do diabete moderado ou da hiperglicemia em humanos. [Rev Assoc Med Bras 2009; 55(4): 384-8]

Unitermos: Ratas Streptozotocin. Lipídeos. Estresse oxidativo. Diabetes. Estudo experimental.

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