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Neonatal streptozotocin-induced diabetes mellitus: a model of insulin resistance associated with loss of adipose mass

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Abstract

The use of experimental models of diabetes mellitus (DM) has been useful in understanding the complex pathogenesis of DM. Streptozotocin (STZ) injected in rats during the neonatal period has usually led to the major features described in diabetic patients (hyperglycemia, polyphagia, polydipsia, polyuria, and abnormal glucose tolerance) in a short period. Diabetes mellitus is a product of low insulin sensibility and pancreatic beta-cell dysfunction. Its process is characterized by a symptomless prediabetic phase before the development of the disease. In this study, we investigated the long-term effects of diabetes induction regarding the cellular metabolic aspects of this model and its similarities with diabetes found in humans. Male Wistar rats (5-day old) were intraperitoneally injected with STZ (150 mg/kg) and followed up for 12 weeks. On the 12th week, animals were decapitated and peri-epididymal fat pads were excised for adipocyte isolation. The following studies were performed: insulin-stimulated 2-deoxy-D-[^{3}H]glucose uptake; incorporation of D-[U-^{14}C]-glucose into lipids and conversion into $^{14}CO_2$; and insulin binding. The weight gain rate of the STZ-treated group became significantly lower by the eighth week. These rats developed polyphagia, polydipsia, polyuria, and glycosuria, and impaired glucose tolerance. Biological tests with isolated adipocytes revealed a reduction in the insulin receptor number and an impairment in their ability to oxidize glucose as well as to incorporate it into lipids. Interestingly, parallel to reduced body weight, the adipocyte size of STZ rats was significantly small. We concluded that apart of a decrease in pancreatic insulin content, this experimental model of DM promotes a remarkable and sustained picture of insulin resistance in adulthood that is strongly related to a loss in adipose mass.

1. Introduction

Diabetes mellitus (DM) is a chronic heterogeneous disease characterized by hyperglycemia resulting from both insulin resistance and insulin deficiency secondary to pancreatic beta-cell failure [1].

Streptozotocin (STZ) is a glucose analog (2-deoxy-2-[3-methyl-*e*-nitrosourido]-D-glucopyranose) that was firstly extracted as an antibiotic from *Streptomyces achromogenes* in 1960 [2]. It possesses a diabetogenic effect due to its specific damaging action to pancreatic beta cells [3-5]. Although the mechanism of its cytotoxicity has not been completely elucidated, there are evidences suggesting the involvement of the major beta-cell glucose transporter (GLUT2) that allows the drug access to intracellular compartments. The drug causes DNA fragmentation through the formation of free alkylating radicals and reduction in cellular levels of nucleotides and related compounds, particularly the oxidized form of nicotinamide adenine dinucleotide, causing a rapid cellular necrosis [6,7].

Streptozotocin-induced diabetes mellitus has been widely used to study the pathophysiology of diabetes. The induction during adulthood produces severe diabetes that often needs insulin administration to ensure survival for longer periods. In the neonatally STZ-treated rats instead, the developing hyperglycemia, abnormal glucose tolerance, and mild hypoinsulinemia is an insidious process that is almost asymptomatic at the beginning and becomes manifested at

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the adult age, features that can resemble the natural course of diabetes in humans [8,9].

An explanation for this evolution is that during the neonatal period, the destruction of beta-cell population is not massive enough and could be compensated by the remaining differentiating cells that could increase the insulin secretion to keep glucose levels within a nearly reference range, avoiding the establishment of hyperglycemia. However, after a period, exhaustion of these cells ensues, and the resulting decreased pancreatic beta-cell function leads to the elevation of blood glucose levels [10].

There are many reports on hyperglycemia, altered glucose tolerance, and reduced insulin secretion a few days or even weeks right after neonatal injection of STZ, showing that this model is very useful for a better understanding of DM pathophysiology, but there is little information about the long-lasting diabetogenic effects on these animals, especially regarding insulin resistance. Diabetes mellitus in humans is characterized by a variable period of prediabetes until it gets developed. Even in type 1 DM, its course is characterized by a symptomless prediabetic phase, when the beta-cell loss is accelerated [11]. Neonatal induction of diabetes might mimic the progressive but not abrupt beta-cell destruction seen in diabetic patients, and the study of these animals during adulthood might be useful for a better understanding of DM pathophysiology if the insulin resistance persists for long periods.

Thus, considering the already established damage to the pancreatic beta cells directing to a reduction in the insulin content as well as the altered plasma insulin concentration previously described in this model, our work investigated cellular metabolic aspects of this model 12 weeks after diabetes induction.

2. Materials and methods

2.1. Animals

Five-day-old male Wistar rats were fasted (separated from their mother) for 8 hours. One group of animals (diabetic, STZ) was injected with STZ (150 mg/kg, IP) freshly diluted in citrate buffer (10 mmol/L, Na citrate, pH 4.5). The control (CO) group received only the vehicle solution in an equivalent volume. High mortality (75.4%) occurred in the STZ-treated group during the first days after the diabetes induction (data not shown). Therefore, the data here refer to the surviving animals. After weaning (day 21), the animals were kept in groups of 4 in collective cages under a 12-hour light-dark cycle (lights on at 7:00 AM) at 23°C and with a full access to food (Nuvilab balanced chow pellets, Nuvital, Columbo, Brazil) and water for the following 9 weeks.

Body weight and glycemia were weekly measured from weaning to sacrifice (12 weeks old). Tail blood was collected for glucose determination using a glucometer (Accutrend Glucose, Roche Diagnostics, Mannheim, Germany). During the 10th week of age, the animals were kept in metabolic individual cages to determine water and food intake, urinary volume, and glycosuria (glucose oxidase/peroxidase method, using a commercial kit—Glicose SL-e, CELM, Sao Paulo, Brazil).

2.2. Intravenous glucose tolerance test

Intravenous glucose tolerance test (75 mg glucose per 100 g body weight) was performed at 8:00 AM after a 12-hour fasting period. The glucose load was injected as a bolus via dorsal vein of the penis, and the blood glucose levels were obtained through caudal blood at the following times: 0, 5, 20, and 60 minutes after glucose infusion. The glucose concentration was determined using a glucometer (Accutrend Glucose, Roche Diagnostics).

2.3. Sacrifice

On the 12th week, the animals were decapitated under anesthetic (Na pentobarbital 4 mg per 100 g body weight, Hypnol, Cristalia, Itapira, Brazil) at 8:00 AM after 12 hours fasting. Trunk blood was collected in heparinized tubes, and the plasma obtained by centrifugation was used for the determination of plasma insulin, leptin, and glucose. After abdominal wall opening, the peri-epididymal fat pads were excised and processed for adipocyte isolation and the following studies were performed: insulin-stimulated 2-deoxy-D-[^{3}H]glucose uptake (2DGU); incorporation of D-[U-^{14}C]-glucose into lipids; conversion of D-[U-^{14}C]-glucose into $^{14}CO_2$; and insulin binding. The described procedures followed the institutionally approved protocol in accordance with the Ethical Principles in Animal Research adopted by the Institute of Biomedical Sciences Ethical Committee for Animal Research (no. 032/99).

2.4. Adipocyte isolation

After mincing the epididymal fat pads (±1.0 g) with fine scissors, they were added to a flask containing 4.0 mL of EHB digestive buffer (Earle's salts, 25 mmol/L HEPES, 4% bovine serum albumin [BSA]), 5 mmol/L glucose, and 1.25 mg/mL collagenase type II, pH 7,4, at 37°C [12]. The mixture was incubated for 30 minutes at 37°C in an orbital shaker (Gyrotory water bath shaker, G76D, New Brunswick Scientic, Edison, NJ) at 150 rpm. The isolated adipocytes were filtered through a fine plastic mesh, washed 3 times with 25.0 mL EHB incubation buffer (Earle's salts, 20 mmol/L HEPES, 1% BSA, no glucose, 2 mmol/L Na pyruvate, and 4.8 mmol/L $NaHCO_3$), pH 7.4, at 37°C, and resuspended to a final cell concentration of 20% (vol/vol, corresponding to $\sim 10^6$ cells/mL). These cell suspensions were maintained in a water bath for 30 minutes before initiating the biological tests described below. Adipocyte viability was tested with trypan blue, and cell number was determined as previously described [13].

2.5. Insulin-stimulated 2DGU uptake

Isolated adipocytes (20% cell suspension in EHB buffer) were incubated in the presence or absence of a maximally

insulin-stimulating concentration (10 nmol/L). At the end of the incubation period (30 minutes), the basal and maximal rates of 2DGU (in triplicates) were evaluated according to protocols described elsewhere [14].

2.6. Insulin binding to receptors

From the same 20% adipocyte suspension, 450-μL aliquots in EHB (pH 7.8) were transferred to 12 × 75 mm polypropylene test tubes prepared with a 10-μL mixture of A_{14}-monoiodo-^{125}I–labeled insulin (10 000 cpm per tube; Amersham Biosciences, Sao Paulo, Brazil) in the absence or presence of "cold" insulin in increasing concentration (0, 10, 25, 100, 250, 1000, 2500, 10 000 pmol/L or 1 μmol/L), in a 500-μL final reaction volume. This mixture was incubated for 180 minutes in a water bath at 16°C. The assay was interrupted by the centrifugation of 200-μL aliquots (in duplicate) through silicone oil. The radioactivity trapped in the cell pellets on the top of the oil layer was measured as described elsewhere [14]. The percentage of binding measured in the tubes containing 1 μmol/L cold insulin was discounted from the binding measurements obtained from the remaining tubes, and the difference was considered the amount of hormone specifically bound to its receptors. The insulin receptor number was determined using the Scatchard [15] method. The results are expressed as femtomoles per 10^6 cells.

2.7. Incorporation of D-[U-^{14}C]-glucose into lipids and conversion of D-[U-^{14}C]-glucose into $^{14}CO_2$

From a 20% adipocyte suspension in Krebs/Ringer/phosphate buffer (pH 7.4), with 1% BSA and 2 mmol/L glucose (at 37°C) and saturated with a gas mixture of CO_2 5%/O_2 95%, 450-μL aliquots were transferred to polypropylene test tubes (17 × 100 mm), containing D-[U-^{14}C]-glucose (2 mmol/L and 3700 Bq per tube), in the presence or absence of insulin (10 nmol/L). These samples were then incubated (500-μL final volumes) for 1 hour at 37°C in orbital shaker water bath (150 rpm). The tubes had an upper-isolated well containing a loosely folded piece of filter paper moistened with 0.2 mL of ethanolamine. After the incubation, the medium was acidified with 0.2 mL of H_2SO_4 (8N) and incubated for additional 30 minutes. At the end of the incubation, the filter paper was removed and placed into scintillation vials for the measurement of the adsorbed radioactivity. The remaining reaction mixture was treated with 2.5 mL of Dole's reagent (isopropanol, *n*-heptane, and H_2SO_4, 4:1:0.25 vol/vol/vol) for lipid extraction [12]. The results were expressed as nanomoles of glucose incorporated into lipids per (10^6 cells × hour).

2.8. Plasma hormones and glucose measurements

Plasma glucose determination was performed using the enzymatic glucose oxidase/peroxidase method [16] available as a commercial kit (Glicose SL-e, CELMSao Paulo, Brazil). Plasma leptin and insulin levels were quantified using rat leptin and insulin radioimmunoassay kits (Linco Research, St Charles, MO).

2.9. Estimated pancreatic insulin content (per 100 islets)

Rat pancreatic islets were isolated by collagenase digestion as described by Lacy and Kostianovsky [17]. The isolated islets were free from blood vessels, fat, and lymph nodes. The mixture was incubated at 37°C for 60 minutes in Krebs-Henseleit buffer (139 mmol/L Na^+, 5 mmol/L K^+, 1 mmol/L Ca^{2+}, 1 mmol/L Mg^{2+}, 124 mmol/L Cl, 24 mmol/L HCO_3) and 0.2% albumin, equilibrated in a mixture of CO_2 (5%) and O_2 (95%). The isolation procedure was ended by the addition of Hanks solution, and the acinar tissue was separated from Langerhans islets. The islets were collected one by one and kept in ice-cold bath. One hundred islets per pancreas were collected, homogenized, and insulin content was quantified using a rat insulin radioimmunoassay kit (Linco Research).

2.10. Statistical analysis

Results were expressed as mean ± SD. Student *t* test for unpaired samples was used to compare groups. The analysis was performed using the statistical software package GraphPad Prism version 3.0 for Windows (GraphPad Software, San Diego, CA). Differences were considered significant when $P < .05$.

3. Results

3.1. General characteristics of diabetic and CO groups

Body weight was significantly different between STZ and CO groups (256.8 ± 25.5 g, n = 10 [STZ] vs 311.8 ± 18.0 g, n = 10 [CO], $P < .05$), and interestingly, there was a tendency

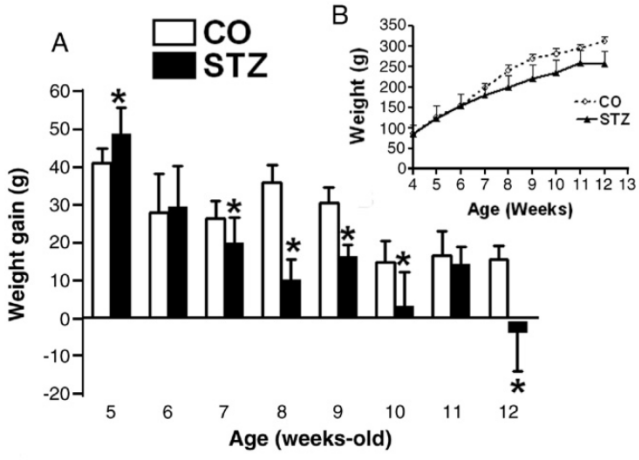

Fig. 1. A, Weight gain in CO and STZ rats. Weight gain was obtained by the difference between the present weight and that measured in the previous week. B, Weight was weekly determined in CO and STZ rats. Black and white bars represent STZ and CO groups, respectively. Bars represent mean ± SD (n = 15). *$P < .05$, STZ group vs CO group.

Table 1
Body weight, food and water intake, urinary excretion, glucose in urine, estimated pancreatic insulin content, glucose levels, and adipocyte size of 12-week-old STZ and CO rats

	CO	STZ
Body weight (g)	311.8 ± 18.0	256.8 ± 25.5 *
Food intake (g/24 h)	24.2 ± 1.3	47.2 ± 4.2 *
Water intake (mL/24 h)	40.8 ± 4.7	195.7 ± 16.3 *
Urinary excretion (mL/24 h)	17.5 ± 2.5	115.3 ± 11.0 *
Glucose in urine (mg/dL)	0	260 ± 20.2 *
Estimated pancreatic insulin content in isolated islets (μU/100 islets)	1625.7 ± 574.1	95.5 ± 166.3 *
Glucose levels (mg/dL)	99.6 ± 24.3	175.9 ± 61.8 *
Adipocyte size (μm)	81.4 ± 7.3	60.2 ± 12.1 *

Values are expressed as mean ± SD (n = 10).
* $P < .05$, STZ group vs CO group.

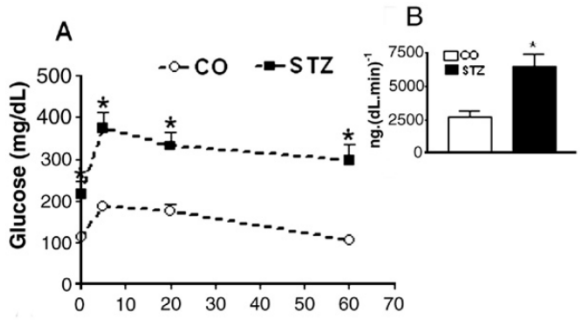

Fig. 3. Intravenous glucose tolerance test. The test was performed under anesthesia in 12-hour fasted rats. A glucose load (75 mg/100 g body weight) was injected as a "bolus" through the dorsal vein of the penis. The glycemic curve is shown in A and the area under curve is shown in B (incremental area) (n = 5). *$P < .05$, STZ group vs CO group.

of the CO rats to grow faster from the 8th to the 12th week of age. In fact, the weight gain rate, from the seventh week to the end of the study, became significantly lower in the diabetic rats (Fig. 1), and some weight loss was also observed in the last week.

Table 1 also displays differences in food and water intake and urinary volume that were significantly higher in the STZ group. The high plasma glucose and the low insulin concentrations presented by this group, as well as the presence of glycosuria, confirmed the diabetic condition of the STZ-treated group. This group also showed a significantly lower estimated pancreatic insulin content (Table 1).

The weekly blood glucose levels collected at 9:00 AM from fed animals were consistently higher in the STZ group (Fig. 2).

3.2. Intravenous glucose tolerance test

Fig. 3A shows distinct glycemic curves in the STZ and CO groups. Blood glucose levels as well as incremental area under the curve (Fig. 3B) of the STZ-treated animals were significantly higher throughout the test, indicating that the STZ rats were indeed glucose intolerant.

3.3. Insulin-stimulated 2DGU

There was no significant difference in the basal 2DG uptake rate in isolated adipocytes. The maximally insulin-stimulated rates, however, were significantly different (Fig. 4), suggesting that a defect in the insulin responsiveness has developed in STZ adipocytes.

3.4. Incorporation of D-[U-^{14}C]-glucose into lipids and conversion of D-[U-^{14}C]-glucose into $^{14}CO_2$

The ability of isolated adipocytes from STZ and CO rats to completely oxidize glucose to carbon dioxide is depicted in Fig. 5A. Both the basal and the maximally insulin-stimulated rates of glucose oxidation in STZ were

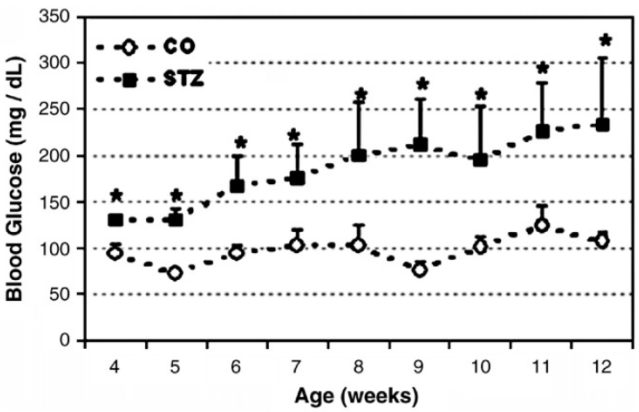

Fig. 2. Blood glucose profile in the STZ and CO rats. Blood samples were weekly collected from the prewarmed tails of awaken rats in fed state at 9:00 AM. Values are expressed as mean ± SD (n = 11). *$P < .05$, STZ group vs CO group.

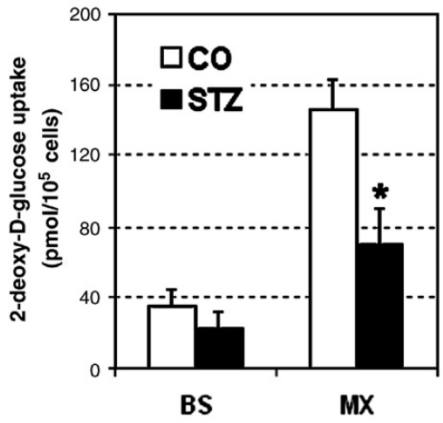

Fig. 4. 2-Deoxy-glucose uptake in isolated adipocytes from STZ and CO rats. Isolated cells were incubated with (MX) or without (BS) 10 nmol/L insulin for 30 minutes at 37°C after which a pulse of [^{3}H]-2-DG (1850 Bq, 0.4 mmol/L) was added. Uptake reaction was allowed for exactly 3 minutes. Values are expressed as mean ± SD (n = 7). *$P < .05$, STZ group vs CO group.

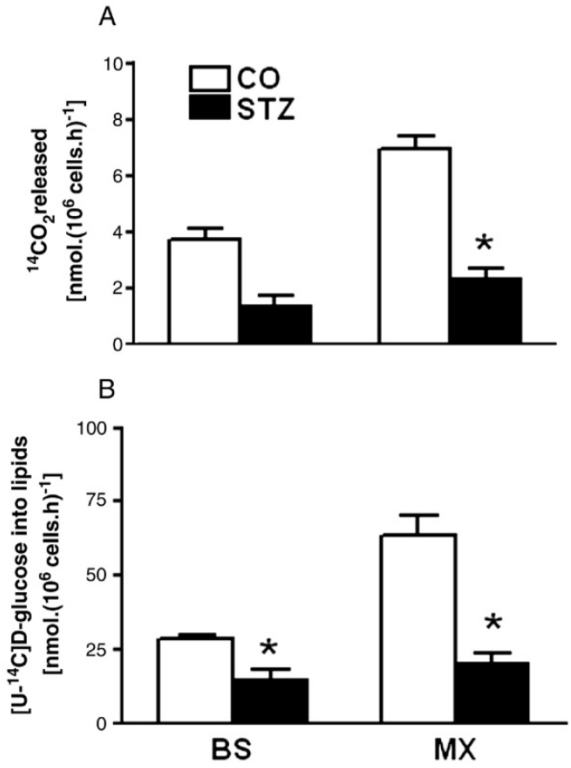

Fig. 5. [U-^{14}C]-glucose conversion to $^{14}CO_2$ and incorporation into lipids. A, Adipocytes were isolated and incubated with D-[U-^{14}C]-glucose (3700 Bq, 2 mmol/L) with or without insulin for 60 minutes at 37°C. The $^{14}CO_2$ released was collected in an ethanolamine-embedded filter paper after addition of H_2SO_4 8N. The radioactivity was determined by a scintillation counter. B, Dole's reagent was added to the remaining mixture for lipid extraction. Values are expressed as mean ± SD (n = 7). *P < .05, STZ group vs CO group.

more than 60% below those in CO rats. The basal and maximal rates obtained by the STZ group indicate low insulin responsiveness.

The rates of glucose incorporation into lipids (Fig. 5B) showed a similar picture with significant differences in both basal and insulin-stimulated conditions, demonstrating again

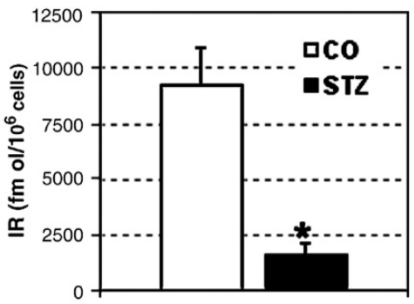

Fig. 6. Insulin binding to receptors. Isolated adipocytes were incubated with A^{14}-[^{125}I]monoiodo-insulin (10000 cpm per tube) and with or without cold insulin (increasing concentrations: 0-0.025-0.1-0.25-1.0-10 × 10^6 nmol/L) for 180 minutes at 16°C. Insulin receptors (IR) number was calculated according to Scatchard [15], and data are expressed as femtomoles per 10^6 adipocytes (n = 7). *P < .05, STZ group vs CO group.

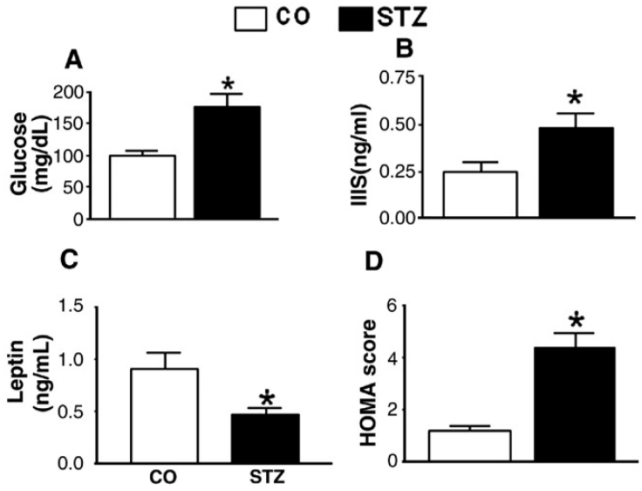

Fig. 7. Plasma (A) glucose, (B) insulin, and (C) leptin levels in 12-hour fasted CO and STZ rats. D, $HOMA_{ir}$ score [insulin (μU/mL) × glycemia (mmol/L)/22.5] [18]. Animals were decapitated and trunk blood was collected for the determination of plasma glucose and hormones. Bars represent the mean ± SD (n = 7). *P < .05, STZ group vs CO group.

the widespread deficit of insulin responsiveness in adipocytes from the diabetic group.

3.5. Insulin binding to receptors

To verify whether insulin receptor number was altered by STZ-induced diabetes and, therefore, was playing a role in lowering the adipocyte response to insulin, we measured the capacity of insulin binding to cell surface receptors determined according to Scatchard [15]. Fig. 6 depicts the results. As can be seen, an 84% reduction in binding capacity was observed in STZ rats.

3.6. Plasma hormones and glucose measurements

The glucose concentration in capillary blood from the tail was consistently higher in diabetic animals. The plasma obtained from 12-hour fasted adult animals was used to determine insulin and leptin levels as well as glucose concentration (Fig. 7). In the STZ group, a pronounced elevation in insulin levels was observed. Based on these values, homeostasis model assessment of insulin resistance ($HOMA_{ir}$) was calculated to determine the insulin resistance level in the STZ group, which was found to be significantly higher {$HOMA_{ir}$ = [$INS_{fasting}$ (μU/mL) × $GLU_{fasting}$ (mmol/L)]/22.5} [18]. Leptin levels were similarly reduced in the STZ group.

4. Discussion

The purpose of this study was to demonstrate the long-term effects of STZ-induced diabetes during neonatal period in rats. The results showed an insulin-resistant state together with damage to islets, indicating that the picture of the syndrome, already described by other authors [19], withstands for longer periods.

Despite of the presence of high glucose levels and low insulin sensitivity presented by the surviving animals of our diabetic group, they did not need insulin therapy throughout the study period (from weaning to the 12th week of age). The follow-up of body weight, glycemia, and food and water consumption clearly shows that these animals presented a progressive worsening of these parameters, indicating the increasing severity of the diabetic state. Direct measurements of food and water consumption, diuresis, and glycosuria made in rats maintained in metabolic cages confirmed the classic signs of polyphagia, polydipsia, polyuria, and glycosuria.

A clinical picture of glucose intolerance, altered insulin levels, and diminished pancreatic insulin content, as described by others [8,10], was reproduced here. Our observations were extended to cover the 8 week-period postweaning. The weight gain rate showed some distinct characteristics: (1) it was more accentuated in CO than in STZ rats, particularly after the seventh week of age; (2) the blood glucose levels were significantly higher in the STZ group from the fourth week to the end of the study and the difference became even more pronounced on the sixth week. Interestingly, differences in the water consumption, that was evaluated weekly (data not shown), became visibly increased in the STZ group only after the eighth week of age. Taken together, these observations clearly reveal that the intensity of diabetes was aggravated during the seventh to eighth week of age coinciding with the period of puberty. The role of sex hormones on the glucose metabolism is well established in addition to the effect of high circulating levels of steroids in generating insulin resistance [20]. Interestingly, the effect of castration has already been studied in STZ-treated rats, and it was shown that the removal of testicular androgen did not prevent the rise in plasma glucose levels, indicating that the peripubertal glucose rise might be due to the increasing body mass [19].

The insulin and glucose profiles during the glucose tolerance tests performed in 10-week-old animals (Fig. 3) showed differences between the groups. Fig. 3A and B complemented the data already obtained from weekly glycemia measurements and demonstrated the presence of glucose intolerance in STZ rats because, after 60 minutes postintravenous *bolus* injection of glucose, the glycemia did not return to basal levels. Moreover, our diabetic animals seemed to present a pronounced reduction in pancreatic insulin content (Table 1). It has been postulated that STZ generates a transient hyperglycemia that triggers a process to recover a minimum beta-cell mass enough to secrete insulin necessary to repair the diabetic state. On the other hand, if this partial recovery of beta-cell mass response to glucose-stimulated insulin secretion is not enough to overcome the supervening hormone resistance, a damage in secretion ensues leading the remaining beta-cell population to exhaustion [21,10]. In addition, the diabetic animals presented higher insulin levels than the CO group. Hence, this hyperinsulinemia would be a useless attempt to overcome the insulin resistance and then to normalize the glucose homeostasis. This picture of high insulin levels is certainly transitory as can be predicted by the low estimated pancreatic beta-cell insulin content presented by these animals at the 12th week of age, indicating that the remaining beta cells are near to exhaustion and sooner or later will not be able to sustain this hyperinsulinemia and, consequently, the integrity of these animals.

Regarding the leptin plasma levels, there was a significant reduction in the STZ group. Similar to our results, Sindelar and colleagues [22] demonstrated that the subcutaneous administration of recombinant leptin in these rats normalized plasma leptin levels and food intake as measured in the CO group, indicating that the hypoleptinemia might contribute to high food consumption presented by diabetic rats.

The biological assays with isolated adipocytes revealed an overt picture of insulin resistance. The STZ-treated group showed a marked down-regulation of insulin receptors and consequently low insulin binding to receptors together with hyperinsulinemia in the fed state. Several researchers [23-25] have shown that hyperinsulinemia could induce down-regulation of insulin receptors.

Insulin stimulates glucose transport into the adipocyte through glucose transporters translocated to the plasma membrane. The low response in the presence of insulin obtained in 2DG uptake assay confirms the presence of insulin resistance in the STZ group. To some researchers a reduced amount of glucose transporters could explain this low response in this model [26].

The capacity of adipocytes to oxidize glucose was remarkably reduced in the STZ group. Comparing the percentage of incremental values, the maximal response rose visibly in both groups (66% in the SZT group vs 85% in the CO group); however, it is important to notice that the basal rates were very distinct, indicating that insulin was unable to correct the resistance in the diabetic group, confirming that there is not only an impairment in insulin secretion, but the responsiveness to this hormone was somehow damaged in the STZ rats. In agreement to these results, we also obtained the same pattern of response in the rate of the incorporation of glucose into lipid. The maximal response (in the presence of insulin) was even more impaired in the diabetic group (increment above the basal response: 32% in the STZ group vs 200% in CO group). Some studies have shown that the ability to synthesize lipids from glucose is impaired in the diabetic population, and this defect has been related to alterations in enzyme activities of lipid biosynthesis. Interestingly, insulin therapy was able to correct this deficiency, indicating that this abnormality is reversible [27-32].

The $HOMA_{ir}$ score also strengthens the idea of insulin resistance in this diabetic model. This index estimates insulin sensitivity using fasting insulin and glucose levels. Considering that the higher the score, the lower the sensitivity [33], Fig. 7D shows a score 4 times higher in the STZ group in accordance with the data obtained from biological assays.

This clear picture of insulin resistance in isolated adipocytes has been seen in this model during the peripubertal period regardless of the presence of high glucose levels, and recent studies have shown that it might postpone the beginning of puberty in these animals (submitted manuscript), but the insulin resistance seems to worsen by the duration of diabetes. Besides, this reduced insulin sensitivity could be related to the diminished fat pad found in these animals (data not shown), represented here by significant reduction in cell size (Table 1). Other authors demonstrated the same pattern of reduced fat pad using STZ as the diabetogenic drug [26,34,35]. Besides, there are reports of insulin treatment in diabetic animals being able to increase body fat parallel to normalization of blood glucose levels, glucose tolerance, and insulin sensitivity [35,36,26], indicating a possible role of adipose tissue on the diabetes development in this model. Recent studies have shown that adipose tissue is no longer just a reservoir for energy store, but has endocrine functions, expressing and secreting a variety of bioactive peptides (adipokines). Thus, adipose tissue excess or deficiency has adverse metabolic consequences, associated with features of metabolic syndrome [37]. The results presented here seem to corroborate with these facts.

Taking these results together, we may conclude that the neonatally STZ-induced DM promotes insulin resistance of considerable intensity that sustains for long periods, showing that this model resemble, in certain aspects, the DM development seen in humans. Besides, adipose tissue might play a role in this "permanent" picture of disruption in the CO of carbohydrate metabolism, leading to aggravation of the diabetes status.

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