Sucrose administration to streptozotocin-nicotinamide induced diabetic rats as a model of type 2 diabetes

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Abstract

Type 2 diabetes is a public health problem that is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. Many authors agree that there is no single animal model that mimics human type 2 diabetes; therefore, the development of new, cost effective models that are easily inducible is necessary. This study sought to develop an animal model with the two major characteristics of type 2 diabetes mellitus: failure to secrete insulin and insulin resistance. To reach this goal, we combined a chemically induced model, injection with 150 mg/kg nicotinamide fifteen minutes before the administration of 65 mg/kg streptozotocin (STZNA), with a diet-induced model, a sucrose-rich diet (20%) administered in water for sixteen weeks. Four groups with five rats each were formed: Control, Sucrose, STZNA and NSZT + Sucrose (STZNAS). The weight and glucose levels were measured every four weeks, and the glycated hemoglobin, triglyceride and cholesterol levels were measured at 0, 8 and 16 weeks. At the end of the sixteen-week period, the HOMA-IR index was calculated and an oral glucose tolerance test was performed (OGTT). The STZNA and the STZNAS groups presented higher levels of glucose, triglycerides and glycated hemoglobin than the control group. During the OGTT, we observed an increase in the glucose levels in the STZNA and STZNAS groups, and the insulin levels were only slightly increased in the Sucrose and STZNA groups, whereas the STZNAS group showed a considerable increase in the insulin levels. The results of the HOMA-IR were 1.52 for the CON group, 2.45 for the SUC group, 6.64 for the STZNA group and 7.31 for the NSZTS. In conclusion, we postulate that the combination of a chemically induced model, STZ-NA, with a diet-induced model, sucrose feeding for 16 weeks, produces animals with the two main components of type 2 diabetes: failure to secrete insulin, due to the nicotinamide-streptozotocin injection, and insulin resistance, due to the sucrose feeding.

KEYWORDS: TYPE 2 DIABETES, HYPOGLYCEMIC AGENT, MEDICINAL PLANT, RHIZOPHORA MANGLE

ABBREVIATIONS: RM, RHIZOPHORA MANGLE; STZ-NA, NICOTINAMIDE-STREPTOZOTOCIN; EWE, ETHANOLIC EXTRACT; WE, WATER EXTRACT; T2D, TYPE 2 DIABETES; DER, DRUG EXTRACTION RATIO.
Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of the DM, the factors that contribute to hyperglycemia could include reduced insulin secretion, decreased glucose utilization and increased glucose production. [1]

Type 2 diabetes (T2D), is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. An individual may present primarily with insulin resistance or beta cell deficiencies, and these abnormalities can range from mild to severe [2].

T2D is a public health problem. According to the World Health Organization [3], more than 347 million people worldwide are affected by T2D. In 2010, the WHO acknowledged that this disease is a major cause of mortality in Mexico, and the 2008 Mexican health services report found that diabetes is the second-highest cause of mortality [4].

An animal model is a living, non-human animal that is used for the research and investigation of human disease to better understand the disease without the added risk of harming a human during the process. The results obtained from animal models can be extrapolated to human medicine.

In a review of animal models of diabetes mellitus [5], the authors highlight that animal models of T2D are likely to be as complex and heterogeneous as the human condition. Insulin resistance predominates in some animals, while α-cell failure is preeminent in other animals. Dyslipidemia and hypertension may also provide valuable insights into human Type 2 diabetes in models where glucose intolerance is part of a wider obesity phenotype. The authors list the following models: monogenetic models of obesity, Ob/Ob mouse and db/db mice that are leptin deficient, Zucker (fa/fa) rats (leptin resistant), Goto Kakizaki rats, KK mice, NSY mice, OLETF rats, Israeli sand rats, Fat-fed streptozotocin-treated rats, CBA/Ca mice, Diabetic Torri rats and New Zealand obese mice. Some strains do not develop hyperglycemia, such as ob/ob mice and fa/fa rats, but these strains develop hyperinsulinemia, which matches the insulin resistance. Other models such as Israeli sand rats and db/db mice rapidly develop hyperglycemia and have α-cells that are unable to maintain the high levels of insulin secretion that are required throughout life. There is little doubt that some animal models of diabetes have provided invaluable insight into the pathogenesis of the human disease; however, there have also been ‘blind-alleys’ in the research, such as the lack of reproducible paradigms of human diabetic complications.

In another review [6], the authors categorized the models in spontaneous or genetically derived, diet nutrition induced, chemically induced, surgical and transgenic/knock-out diabetic animals. In each category, the animals are further subdivided into models with or without obesity. The authors conclude that no single model is exactly equivalent to human diabetes, but each model acts as an essential tool for investigating the genetic, endocrine, metabolic, and morphological changes and the underlying etiopathogenic mechanisms that contribute to the evolution of type 2 diabetes in humans.

The combination of diabetes, obesity and hypertension is known as metabolic syndrome and is common in humans. Recently, some authors [7], [8] reviewed animal models that can reproduce some aspects of metabolic syndrome.

In general, the authors agree that no a single animal model can mimic human type 2 diabetes; therefore, the development of new, cost effective models that can be easily induced is necessary.

In 1998, Masiello [9] and coworkers developed an animal model of diabetes that exploits the partial protection of nicotinamide (NA) against the β-cell cytotoxic effects of the streptozotocin (STZ). These animals had moderate and stable non-fasting hyperglycemia and respond to insulin secretagogues such as sulfonylureas. It was postulated that the β-cells of the rats protected by NA remain well
differentiated and at least partially responsive to physiological or pharmacological stimuli; however, the researchers could not show insulin resistance in the model. In 2008 [10], Tahara compared the action of hypoglycemic agents in STZ and STZ-NA diabetic rats. STZ-NA induced mild diabetes in rats, characterized by a mild decline in glucose tolerance associated with the loss of early phase insulin secretion and reduced pancreatic insulin stores; however, insulin resistance was barely detected in this model. Based on these studies, we conclude that the STZ-NA rats present a relative deficiency in insulin secretion, a shared characteristic observed in type 2 diabetic patients.

High-carbohydrate intake has been associated with metabolic syndrome and insulin resistance [8]. In 1983, Wright et al. [11] made the isocaloric substitution of sucrose for starch in the diet of rats, which resulted in hyperinsulinemia and the deterioration of glucose tolerance caused by a loss of insulin sensitivity. In 2001, Oliart et al. [12] successfully used sucrose water to produce metabolic alterations. Central obesity, insulin resistance and higher blood pressure were observed after 8 weeks of adding 30% sucrose to the drinking water of young male adult Wistar rats. Other authors [13] also observed significantly increased visceral fat accumulation, serum lipid concentrations, glucose levels and insulin levels in Wistar rats after a 20-week administration of 30% sucrose through the drinking water. It has been proposed that the insulin resistance resulting from chronic fructose feeding (60%) is caused by the diminished ability of insulin to suppress hepatic glucose output [14].

Considering the mentioned works, we concluded that the rats that were given sucrose presented insulin resistance, the second shared characteristic observed in type 2 diabetic patients. Logically, we propose that a combination of STZ-NA and sucrose could produce an animal model with deficiency in insulin secretion and insulin resistance.

The aim of this study was to develop an animal model with the two major characteristics of type 2 diabetes mellitus: failure to secrete insulin and insulin resistance.

Methods

Animals

Eight-week-old Wistar rats weighing 200-220 g were obtained from the Bioterium of the Science School, UNAM, and were acclimatized with free access to food and water for at least one week in an air conditioned room (25°C with 55% humidity) with a 12 h light-dark cycle prior to performing the experiments. The animals were handled according to the National Institute of Health Guide for the Care and Use of Laboratory Animals [15].

Induction of experimental diabetes sucrose diet

Experimental diabetes was induced as described by Masiello [9]. The rats fasted overnight and were injected intraperitoneally with 150 mg/kg nicotinamide (NA) (Sigma, N3376) 15 min before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130) to produce model STZ-NA. A sucrose-rich diet (20%) was prepared by dissolving 40 g sucrose in 200 ml of water.

Experimental Groups

The animals were placed into 4 groups (1-4) of five rats each. Group 1 was the control group (CG). Group 2, the sucrose group, received 20% sucrose in water ad libitum (SUC). Group 3 was injected with nicotinamide streptozotocin (STZNA). Group 4 was injected with nicotinamide streptozotocin and received 20% sucrose in water ad libitum (STZNAS).

Chronic Experiment: After the induction of hyperglycemia for groups 3 and 4, all of the animals were separated into individual cages. Groups 1 and 3 received water, and groups 2 and 4 received sucrose water as described above. All groups were fed with Purina Rodent Laboratory Chow 5001. The experiments were conducted over a sixteen-week period.

Collection of blood and measured parameters

Glucose and weight were monitored each month. Plasma glucose concentrations were measured with
a Reflotron instrument and were confirmed with an Accutrend GC (Roche). In total, 32 µl of blood was collected for each assay.

Glycated hemoglobin levels (HbA1c), high density lipoprotein (HDL) and triglycerides (TG) were monitored at 0, 8, and 16 weeks.

HbA1C was measured with a Siemens DCA Vantage® Analyzer, and CHOL, HDL and TG were measured with a CardioChek® analyzer.

**Glucose Tolerance Test**

At the end of the chronic experiment, an Oral Glucose Tolerance Test (OGTT) was performed for each animal. After 12 hours of fasting, the rats orally received anhydrous glucose (2 g/Kg) diluted in 1.5 ml of saline (NaCl 0.9%) by a gastroesophageal gavage to ensure that the liquid reached the digestive tract of the rat. Blood samples for glucose and insulin measurements were taken at 0, 30, 60, 90 and 120 min. Insulin was measured by ELISA with a Millipore (EZRMI-13K) Kit using Awareness Stat Fax ® equipment.

**Data analysis**

The data were analyzed by one-way ANOVA followed by Tukey's test. The plasma glucose, cholesterol, triglyceride, Hba1C and insulin levels were expressed as the mean ± S.E.M. For the OGTT test, the Homeostasis Model of Assessment - Insulin Resistance (HOMAR-IR) was calculated as follows: (fasting Glucose (mmol/L) x fasting Insulin (mU/L)) / 22.5. For this calculation, the glucose values, measured in mg/dl, were converted to mmol/L, and the insulin values, measured in ng/ml, were converted to mU/l. Values above 3 for the HOMA-IR indicated a 90% probability of insulin resistance [16].

**Results**

**Chronic Experiment**

The STZNA and STZNAS groups gained more weight after four weeks compared with the control group; however, this trend reversed at 8 weeks until the end of the experiment. The SUC group did not show any weight differences from the control group. Table 1.

The glucose levels of the STZNA and STZNAS groups significantly increased after four weeks, and this increase was sustained over the 16-week period, with significantly higher glucose levels than the control group. The SUC group did not show an increase in the glucose values compared with the control group; however, a slight increase was observed over the 16-week period when compared with their own time 0, as shown in Table 2.

The triglyceride levels in the CON and SUC groups were stable over the 16-week period, and the SUC group did not show any difference in triglyceride levels compared with the CON group or time 0. The STZNA and STZNAS groups showed a statistically significant difference in triglyceride levels compared to the control group and to time 0 of both groups from 8 weeks to 16 weeks. Table 3. The cholesterol and HDL levels remained stable throughout the experiment for all of the groups, data not shown.

The Hb1Ac levels of the CON and SUC groups did not change throughout the experiment, and the Hb1Ac levels of the SUC group were not different from those in the CON group. The STZNA and the STZNAS group shown an increase at eight and sixteen weeks over the control group and time 0 for both groups.

**Oral Glucose Tolerance Test and HOMA-IR**

An OGTT was performed as described above at the end of the 16-week period. The plasmatic glucose increased 30 min after the glucose challenge in the CON group and significantly increased 90 min after the glucose challenge. The levels of the SUC group were higher at the beginning of the experiment than the control group after the glucose load, and the levels in the SUC group increased at 30 and 60 min, but the values were not significantly different from the control group. The STZNA and the STZNAS groups presented significantly higher levels of glucose compared with the control group. After the glucose challenge, the values of the STZNA and STZNAS groups increased. At 120 minu-
tes, the values for the STZNA group returned to the basal level while the values for the STZNAS group remain high. Table 4.

The insulin levels of the CON group did not show significant differences compared to the initial value at time 0. The SUC group showed slight increases in insulin at 30 min and 120 min when compared to the control group. The STZNA group also exhibited a slight increase from 60 min to 120 min. The NSZTS group showed an increase in the insulin levels between 30 min and 120 min, Table 5.

The results of the HOMA-IR were 1.52 for the CON group, 2.45 for the SUC group, 6.64 for the STZNA group and 7.31 for the NSZTS, Figure 1.

Discussion

As previously mentioned, type 2 diabetes mellitus is a metabolic disease with worldwide importance. This disease produces serious disabilities among the working population of many countries and is an important economic burden for many health services. Reverting the diabetic condition in a patient is not possible, and controlling the blood glucose levels to avoid further complications is the best course of treatment. Therefore, developing animal models to explore new therapeutic options is important. In this work, we combined a chemically induced animal model with a diet-induced animal model to reproduce the two main aspects of type 2 diabetes: deficient insulin secretion and insulin resistance. In this model (STZ-NA injection and 20% Sucrose Fed), we did not observe sustained variations in the weight of the animals; therefore, it can be assumed that this dose of sucrose does not produce obesity, which is observed when animals are treated with 30% sucrose \cite{12} and \cite{13}. The glucose values of the STZNA and STZNAS groups were higher at four weeks compared with the control group and the initial values at time 0 for both groups.

The NSZTS group presented higher glucose values than the STZNA after 4 weeks. These results suggest that STZ-NA injection elevates the glucose levels and adding sucrose to the diet produces higher glucose levels. The triglyceride levels were higher in the STZNA and STZNAS groups compared with the control group and the initial values at time 0, which suggests that the STZ-NA injection could elevate the triglycerides. The HbA1c levels were higher in the STZ-NA injected rats. The STZNAS group presented higher levels of HbA1c compared with the STZNA group, suggesting that the combination of the chemical induction with STZ-NA and the diet induction with 20% sucrose produced higher levels of HbA1c.

The glucose values of the OGTT showed that the CON and SUC groups behaved similarly; the glucose levels are elevated 30 min after the glucose challenge and returned to the basal levels by 60 min. The STZ-NA injected rats present higher levels of glucose 30 min after the glucose challenge, and the glucose levels remained elevated until 120 min, when the glucose returned to basal levels. The STZNAS group showed a similar increase in the glucose level over time, but the glucose levels did not return to the basal values at 120 min. The insulin levels of the CON group were stable during the 120 min period, and the SUC group presented higher levels of insulin than the CON group. The insulin levels of the STZNA group were higher at 60 and 90 min than the CON group, and the NSZTS group presented higher insulin levels at all times. These results suggest that 16 weeks of treatment with 20% sucrose increases the insulin levels in animals.

After the calculation of the HOMA-IR values, we observed that the STZ-NA injected rats develop insulin resistance after a period of 16 weeks, and this insulin resistance was more evident when sucrose (20%) is added to the diet.

Although other studies are necessary to better understand this model, we conclude that combining a chemically induced model (STZ-NA, 150 mg/kg-65 mg/kg) with a diet-induced model (feeding 20% sucrose for 16 weeks) produces animals with the two main components of type 2 diabetes: failed insulin secretion due to the nicotinamide-streptozotocin injection and insulin resistance due
to the sucrose feeding.

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References

13. Chen C.C., Huang C.Y., Chang M.Y., Chen C.H., Chen S.W., Ching JH., Pei-Min C. Two unhealthy dietary habits featuring a high fat content and a sucrose-containing beverage intake, alone or in combination, on inducing metabolic syndrome in Wistar rats and C57BL/6J mice 2011; Metabolism 60; 155–164.
### Table 1. Weight, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in weeks

<table>
<thead>
<tr>
<th>Weight Groups</th>
<th>(g) T0</th>
<th>(g) T4</th>
<th>(g) T8</th>
<th>(g) T12</th>
<th>(g) T16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CON</td>
<td>189 ± 8</td>
<td>216 ± 11</td>
<td>279 ± 18</td>
<td>306 ± 25</td>
<td>333 ± 33</td>
</tr>
<tr>
<td>2 SUC</td>
<td>192 ± 5</td>
<td>200 ± 11</td>
<td>268 ± 18</td>
<td>293 ± 30a</td>
<td>317 ± 37a</td>
</tr>
<tr>
<td>3 STZNA</td>
<td>197 ± 9</td>
<td>266 ± 31a</td>
<td>299 ± 41a</td>
<td>295 ± 48a</td>
<td>290 ± 42a</td>
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<tr>
<td>4 STZNAS</td>
<td>195 ± 7</td>
<td>302 ± 13a</td>
<td>194 ± 38a</td>
<td>213 ± 37a</td>
<td>214 ± 37a</td>
</tr>
</tbody>
</table>

### Table 2. Glucose, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group, superscript numbers in the same row indicate statistical differences as compared with time 0; at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in weeks

<table>
<thead>
<tr>
<th>Glucose Groups</th>
<th>(mg/dl) T0</th>
<th>(mg/dl) T4</th>
<th>(mg/dl) T8</th>
<th>(mg/dl) T12</th>
<th>(mg/dl) T16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CON</td>
<td>95 ± 5</td>
<td>128 ± 4a</td>
<td>101 ± 5</td>
<td>84 ± 1</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>2 SUC</td>
<td>84 ± 5</td>
<td>124 ± 4a</td>
<td>109 ± 5a</td>
<td>98 ± 2a</td>
<td>91 ± 4a</td>
</tr>
<tr>
<td>3 STZNA</td>
<td>91 ± 4</td>
<td>257 ± 43a</td>
<td>394 ± 48a</td>
<td>294 ± 21a</td>
<td>372 ± 33a</td>
</tr>
<tr>
<td>4 STZNAS</td>
<td>91 ± 3</td>
<td>409 ± 39a</td>
<td>399 ± 66a</td>
<td>363 ± 42a</td>
<td>467 ± 50a</td>
</tr>
</tbody>
</table>

### Table 3. Triglycerides, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group, superscript numbers in the same row indicate statistical differences as compared with time 0; at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in weeks

<table>
<thead>
<tr>
<th>TG Groups</th>
<th>(%) T0</th>
<th>(%) T8</th>
<th>(%) T16</th>
</tr>
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<tbody>
<tr>
<td>1 CON</td>
<td>70 ± 3</td>
<td>71 ± 6</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>2 SUC</td>
<td>62 ± 3</td>
<td>74 ± 6</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>3 STZNA</td>
<td>64 ± 6</td>
<td>159 ± 8a</td>
<td>119 ± 3a</td>
</tr>
<tr>
<td>4 STZNAS</td>
<td>67 ± 3</td>
<td>271 ± 8a</td>
<td>179 ± 7a</td>
</tr>
</tbody>
</table>

### Table 4. Glycated hemoglobin, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in weeks

<table>
<thead>
<tr>
<th>HbA1c Groups</th>
<th>(%) T0</th>
<th>(%) T8</th>
<th>(%) T16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CON</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2 SUC</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3 STZNA</td>
<td>3</td>
<td>8a</td>
<td>9a</td>
</tr>
<tr>
<td>4 STZNAS</td>
<td>3-3</td>
<td>12a</td>
<td>11.6a</td>
</tr>
</tbody>
</table>
Table 5. Oral Glucose Tolerance Test; Glucose values, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group, superscript numbers in the same row indicate statistical differences as compared with time 0; at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in minutes

Table 6. Oral Glucose Tolerance Test; Insulin values, the values represent the mean ± SEM. Superscripted letters in the same column indicate statistically significant differences compared with the control group, superscript numbers in the same row indicate statistical differences as compared with time 0; at (p < 0.05). CON, control group; SUC, Sucrose group; STZNA, Streptozotocin-Nicotinamide Group; STZNAS, Streptozotocin-Nicotinamide + Sucrose group, T; time in minutes

Graphic 1. Homeostasis Model of Assessment - Insulin Resistance. Con; Control Group, SUC; Sucrose fed group (20%), STZNA; NA-STZ injected group, STZNAS; NA-STZ injected + Sucrose fed group (20%). Values higher than 3 indicates insulin resistance