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WEANING STZ-INDUCED HYPERGLYCEMIC RATS FED GLUCOSE-FRUCTOSE SYRUP AS A NOVEL MODEL FOR THE NATURAL HISTORY OF TYPE 2 DIABETES

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Abstract

Weaning is a crucial period for the development of the full glucose-stimulated insulin secretion (GSIS) response, since β-cell is challenged by switching from high-milk fat diet to high-carbohydrate chow, promoting high proliferation rates. Therefore, it is hypothesized that a streptozotocin (STZ) injection in this period could enhance β-cell proliferation, accelerating β-cell exhaustion and preserving an impaired GSIS response phenotype like that observed in type 2 diabetes (T2D). In the current study, it is proposed a new animal model which mimics the main features of T2D (insulin resistance and pancreatic β-cell dysfunction) by administering a syrup solution (8.8% glucose and 5.2% fructose concentrations) for 6 weeks to STZ hyperglycemic rats induced with two different doses (45 or 65 mg/kg b. w.) at weaning. The results showed that two stages of T2D were obtained: an early phase, where hyperinsulinemia and high β-cell performance were the principal attributes, and an advanced phase, where severe hyperglycemia and poor β-cell function were perceived. Both stages' parameters improved after the administration of hypoglycemic agents; thus, this animal model can be used for the evaluation of potential hypoglycemic agents, although it can also be useful to study other aspects of T2D, such as β-cell regeneration for therapeutic purposes.

Keywords: *type 2 diabetes model, dietary animal model, glucose-fructose-STZ animal model, insulin resistance model, weaning, added sugars, sugar-sweetened beverages*

Introduction

Type 2 diabetes (T2D) is a multifactorial hyperglycemic chronic condition characterized by relative insulin deficiency and inadequate peripheral insulin response (1). Because of the heterogeneity and complexity of T2D, assorted animal models have been developed to study specific characteristics of the disease. For instance, insulin resistance (IR) has been induced by giving specific diets, such as high fat or rich in carbohydrates, to generate intracellular fat accumulation, oxidative stress, and activation of the inflammatory pathways (2). On the other hand, β-cell dysfunction has been mimicked by injecting β-cell toxic agents, such as streptozotocin (STZ), to promote the selective destruction of this cell type, causing a variable insulin deficiency (3). Therefore, to make a complete T2D model, that has the two main characteristics of the disease, the combination of diet intake plus STZ injection can be used (4,5).

Despite the wide use of these models, several concerns could be highlighted: poor standardization due to a high variability of diet composition, high costs because of the complexity of the diets and the maintenance of possible long induction periods, and STZ response variability owing to the induction age of animals (6–10). Regarding the latter, the neonatal induction of hyperglycemia could be a promising alternative since it causes a progressive loss of β-cell mass in adulthood owing to an early acute dysfunction caused by a single STZ injection during neonatal stages. After 4 to 8 weeks of age, frank hyperglycemia, glucose intolerance, depletion of pancreatic insulin stores, lack of insulin response to glucose, and reduced basal insulin levels are developed (11).

It is reported that after a 60% loss of existing β-cells and a moderate hyperglycemia in neonatal STZ-induced rats, glucose levels returned to control values after 20 days, namely after weaning (12). Recent evidence suggests that this period is critical for the pancreas development, as the high-fat milk diet is replaced by one that is high in carbohydrates, stimulating β-cell proliferation and an enhanced glucose-stimulated oxidative phosphorylation, and thus promoting the maturation of the full glucose-stimulated insulin secretion (GSIS) response (13,14). This period of high rates of proliferation could be the reason for the transient "improvement" exhibited by the neonatal STZ-induced rats before weaning. Moreover, it is documented that exogenous stimuli, such as STZ administration, can promote β-cell proliferation in young rodents due to the induction of spontaneous β-cell regeneration derived from the damage (15).

Considering the aforementioned, a STZ insult in this period could exacerbate replication rates, which could damage the normal cell development, preventing reaching functional maturity (16) and preserving an impaired GSIS response phenotype similar to that observed in T2D (17–19). Therefore, we hypothesized that a STZ injection at weaning may cause a critical β-cell dysfunction that accelerates the obtention of persistent hyperglycemia without the presence of the transient enhancement period of several weeks observed in neonatal STZ-induced rats. On the other hand, the IR-component can be generated by the intake of added sugars, which are the main constituent of sugar-sweetened beverages (SSB) (20,21), and are easy to implement in drinking water.

Over the years, the high intake of beverages with added sugars has been associated with the growing epidemic of obesity, T2D, and cardiovascular disease (22,23). According to the U.S. Food and Drug Administration, added sugars are those supplemented during the food processing and packaging, such as monosaccharides, disaccharides, and sugars from syrups, honey, concentrated fruit, or vegetable juices (24).

Both glucose and fructose are the main constituents of syrups used to prepare SSB. They can be added mainly as sucrose (50% bound fructose) or high-fructose corn syrup (HFCS, 42% to 55% free fructose) (25). Several studies have specifically linked fructose with metabolic syndrome and T2D (26). The physiological effects of the chronic consumption of fructose include dyslipidemia, fatty liver, hypertension, and decreased insulin sensitivity, which are consequences of its efficient hepatic metabolism (27,28).

Despite the extensive literature on the altered metabolic effects derived from pure fructose intake, there are evidence that glucose, as an independent

component of SSB, have a major role in the apparition of metabolic disorders (29), such as increased postprandial glucose and insulin responses, and higher 24-h circulating free fatty acids, which are different to those observed with fructose. Remarkably, other studies proposed a detrimental potentiating effect when consuming both monosaccharides (30).

Therefore, we aimed to develop an alternative model that mimics the β-cell dysfunction and the IR of T2D in a short period of time, that is easy to implement and responds to pharmacological treatment with oral hypoglycemic agents, considering the STZ-induced hyperglycemia at weaning as a critical period to accelerate β-cell dysfunction, and the proposed harmful synergistic effects of the coadministration of both fructose and glucose as important components of SSB.

First, we proposed two STZ doses used for stable hyperglycemia induction in order to select the most suitable one to produce β-cell dysfunction: 45 and 65 mg/kg b.w. (an intermediate dose and a high dose, respectively) (31,32). Second, we selected the animals that developed significant hyperglycemia and thereafter administrated a solution of a commercial glucose-fructose syrup (GFS) orally for 6 weeks for the purpose of producing IR. Finally, we assessed the effectiveness of the model by evaluating the amelioration of metabolic parameters after the chronic administration of hypoglycemic agents.

Methods

Animals

All procedures and animal handling were carried out according to the guidelines of the Committee for the Update of the Guide for the Care and Use of Laboratory Animals (33) and approved by the Academic Ethics and Scientific Responsibility Commission (CEARC) of the School of Sciences, UNAM (PI_2020_02_002).

Twenty-one-day-old Wistar rats were obtained from the research animal facility of the School of Sciences. They were weaned and subsequently acclimatized during two weeks with free access to food and water under standard conditions (25°C, 55% humidity and 12:12 h light: dark periods).

Induction of hyperglycemia

Hyperglycemia was induced in the newly weaned rats by fasting them for 12 h and then injecting them intravenously with 45 or 65 mg/kg of STZ (Sigma-Aldrich) dissolved in acetate buffer 0.1 M, pH 4.5. Animals with fasting blood glucose (FBG) greater than 150 mg/dl two weeks later were selected for the administration of the GFS solution.

Preparation of GFS solution and sugar composition of syrup

Fresh GFS solution was daily prepared by diluting 10% natural Madrileña® syrup in tap water (final glucose and fructose concentrations: 8.8% and 5.2%). Sugar composition of a 0.5 mg/ml syrup solution determined by HPLC was 0.34 ± 0.058 mg/ml of glucose and 0.2 ± 0.021 mg/ml of fructose. Based on this data, the glucose and fructose concentrations in the pure syrup were 875.8 mg/ml (87.58%) and 515.2 mg/ml (51.52%), respectively (syrup density: 1 288 mg/ml).

Experimental design

Two weeks after the STZ injection, the animals were assigned into seven experimental groups (*n*=6 per group; Figure 1). Tap water or GFS solution were placed for drinking at free access for 6 weeks. Vehicle (saline) or metformin-glibenclamide (MG, Aurax®, 500 mg/kg – 5 mg/kg) were daily administered by gavage using an esophageal cannula.

Measured parameters

FBG was measured weekly using an Accu-Chek® Active glucometer. Both triglycerides and total cholesterol were measured every two weeks using an Accutrend® Plus glucometer (Figure 2). All blood samples were obtained from the tail vein.

Fasting serum insulin (FSI) was quantified using a specific immunoassay kit (Rat/Mouse Insulin ELISA kit EZRMI-13K EMD Millipore/Merck) at the beginning and the end of the experimental period. In brief, serum was obtained by centrifuging blood samples at 10 000 g for 10 min. Then, they were frozen at –40°C until further analysis.

For comparison purposes (34), IR and β-cell function were assessed through the calculation of the homeostatic model assessment indices (HOMA-

IR and HOMA-%B), while insulin sensitivity was evaluated by calculating the quantitative insulin sensitivity check index (QUICKI), according to the next formulas:

HOMA-IR = [FSI (mU/l) × FBG (mmol/l)]/22.5

HOMA-%B = [20 × FSI (mU/l)] / [FBG (mmol/l) – 3.5]

QUICKI = 1 / [log FSI (mU/l) + log FBG (mg/dl)]

Conversion factors: FBG: 1 mmol/l = 18 mg/dl; FSI: 1 mU/l = 6 pmol/l (35).

Calculation of calorie consumption

Daily calorie intake per animal was calculated from recording both the drinking water (ml) and food (g) consumption. All experimental groups received Rodent Laboratory Chow 5001 (Purina®), which contains 48.7% carbohydrate, 24.1% protein, 5% fat (ether extract) and 5.7% fat (acid hydrolysis) (total 3.36 Kcal/g). On the other hand, the GFS solution contributed 0.33 Kcal/ml (total Madrileña® syrup 3.33 Kcal/ml) to the total calorie intake. In addition, the rats' body weights were measured each week.

Oral glucose tolerance tests

At the end of the experimental period, oral glucose tolerance tests (OGTT) were performed on the 12-h fasting animals by measuring the basal blood glucose before administering a glucose load (2 g/kg) dissolved in 1.5 ml of vehicle. Blood glucose was monitored every 30 min for two hours.

Statistical analysis

Normal distribution was assessed, and non-parametrical tests were carried out as necessary if normality was not obtained, even after log-transformation. For the analysis of initial parameters, ordinary one-way ANOVA followed by Tukey's test were applied to compare means among groups, while unpaired t-tests were used to compare each mean parameter value versus its control at the end of the experimental period. Repeated measures ANOVA followed by Dunnett's tests were used to compare means versus its baseline. Areas under curve (AUC) were calculated to assess the total effect. *p*-values less than 0.05 were considered significant.

Results

Modification of insulin-related indices by weaning STZ-induced hyperglycemia, consumption of GFS solution, and administration of therapeutic treatment

Two weeks after the STZ injection at weaning, animals from H45 and H65 groups developed markedly high glucose levels compared with those in control. As shown in Table 1, STZ induction also promoted higher insulin values at both doses; however, a differential behavior was observed between both hyperglycemic groups, which could be related to the degree of damage caused by the different doses of STZ. The H45 group presented the highest insulin levels, although it had lower glucose levels than the H65 group. Moreover, its β-cell function was almost 1.7 times superior to that observed in control, and its HOMA-IR value was 7.6 times higher. On the other hand, the H65 group showed slightly superior values of both insulin and HOMA-IR relative to the control group; nevertheless, its β-cell function diminished 2.7 times. Regarding QUICKI, it is observed a significant reduction of this value in H45 group compared with that in control.

Table 1. Initial parameters measured two weeks after the STZ injection (mean ± SEM).

Parameter	H45 (*n*=18)	C (*n*=6)	H65 (*n*=18)
FBG (mg/dl)	165 ± 3 ^{a}	119 ± 10 ^{b}	226 ± 21 ^{a}
FSI (pmol/l)	436.7 ± 125.5 ^{a}	81.3 ± 16.2 ^{b}	120.9 ± 23.3 ^{b}
HOMA-IR	29.7 ± 8.7 ^{a}	3.9 ± 0.9 ^{b}	10.9 ± 2.1 ^{b}
HOMA-%B	259.2 ± 72.8 ^{a}	150.2 ± 81.7 ^{a,b}	56.6 ± 13.6 ^{b}
QUICKI	0.264 ± 0.007 ^{b}	0.317 ± 0.008 ^{a}	0.293 ± 0.011 ^{a,b}

Different letters in the same row indicate statistical difference at $p<0.05$ (a > b). C: non-hyperglycemic control; H45: hyperglycemic group injected with 45 mg/kg STZ; H65: hyperglycemic group injected with 65 mg/kg STZ; FBG: fasting blood glucose; FSI: fasting serum insulin.

After the 6-week experimental period, both H45 and H65 hyperglycemic controls worsened in proportion to the amount of STZ injected (Table 2),

which was significantly reflected in higher glucose and worse β-cell function over time, as previously reported (36). In contrast, daily administration of the GFS solution produced greater insulin levels (S45: 273%; S65: 137%), higher HOMA-IR values, and superior β-cell performance in reference to their hyperglycemic controls. Similarly, both syrup groups had a diminished insulin sensitivity index compared with those in their hyperglycemic controls, although only the value of S45 group reached significant difference.

On the other hand, coadministration of the oral hypoglycemic agents (MG) ameliorated insulin functionality by inducing lower insulin levels and significantly enhancing the insulin sensitivity index in both MG45 and MG65 groups compared with their syrup controls. However, only the MG65 group managed to achieve normal glucose levels, and its β-cell functionality and insulin sensitivity index showed a significantly greater improvement than that observed in the MG45 group.

Effect of weaning STZ-induced hyperglycemia, consumption of GFS solution, and therapeutic treatment on intake of food, drinking water and calories, and on body weight change

As shown in Figure 3, the major significant changes on food and drinking water intake, during the 6-week experimental period, occurred in the latter at the high STZ dose. First, the STZ injection resulted in a significant proportional polydipsia increase between H45 and H65 groups, although polyphagia was significantly increased only at the 65 mg/kg STZ dose. At the same time, GFS solution administration tended to diminish food consumption of both S45 and S65 groups even though it remarkably augmented drinking water intake compared with their hyperglycemic controls. On the other hand, MG treatment significantly reduced both polyphagia and polydipsia.

Regarding calorie consumption, only 65 mg/kg STZ dose produced the biggest changes, since H65 group consumed a greater number of calories due to an increased polyphagia compared with the non-hyperglycemic control. In addition, even though S65 group consumed the major quantity of calories, both groups fed GFS solution incremented their calorie consumption because of an evident syrup intake. Nevertheless, MG treatment was able to diminish total calorie intake mainly due a lower consumption of calories contributed by the syrup solution. Overall, the higher GFS solution intake was the main source of the total calorie increase. In reference to body weight change, only the high dose of STZ promoted a significant less change compared with the low dose of STZ (Figure 4).

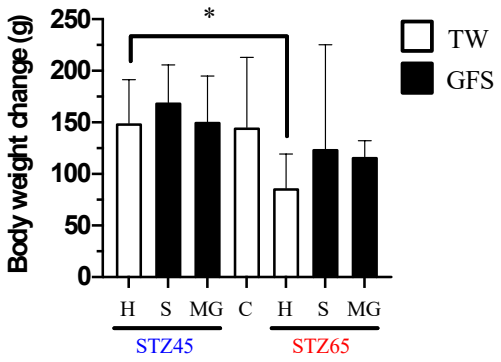

Figure 4. Final body weight change (mean ± SD, *n*=6). Asterisk indicates statistical difference ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg; TW: tap water; GFS: glucose-fructose syrup solution.

Changes in fasting blood glucose and triglyceride follow-up due to weaning STZ-induced hyperglycemia, administration of GFS solution, and therapeutic treatment

As shown in Tables 3 and 4, blood glucose and triglyceride levels of the H45 group showed no significant changes over the experimental period, while the hyperglycemia of the H65 group worsened over time, increasing to 72% more than its baseline value. Moreover, the triglycerides of this group doubled at the end of the period. On the other hand, the GFS solution significantly accelerated the appearance of high triglyceride levels in both S45 and S65 groups from week 2 by 59% and 175%, respectively, without an evident modification of glucose levels compared with their hyperglycemic controls.

Even though the antihyperglycemic and antihyperlipidemic effects of MG treatment were

observed at both STZ doses, the MG65 group exhibited better outcomes. Daily administration of these therapeutic agents prevented an increase in glucose and triglycerides from week 2, diminishing them subsequently until reaching levels similar to those observed in non-hyperglycemic control.

Overall, the most significant changes in both glucose and triglycerides occurred using the 65 mg/kg dose of STZ (Figure 5). The AUC analysis showed that even though severe hyperglycemia and hypertriglyceridemia developed in the H65 and S65 groups, the therapeutic treatment was able to significantly reduce these metabolic parameters to the normal state. In contrast, when using a 45 mg/kg STZ dose, only a change in blood glucose was detected, and neither syrup nor hypoglycemic agents were able to modify these parameters. Total cholesterol is not reported because the values were below the detection threshold.

Effect of weaning STZ-induced hyperglycemia, consumption of GFS solution, and therapeutic treatment on glucose intolerance

After the 6-week experimental period, administering a glucose load significantly increased the blood glucose of the H45 and H65 groups compared with the non-hyperglycemic control (Figure 6); however, the values in the entire curve of the H65 group were doubled compared to the H45 group, indicating worse impaired fasting glucose and peripheral glucose clearance due to greater damage to insulin secretion. On the other hand, chronic administration of GFS solution induced no significant modifications of the glucose curve behavior although the levels in the S45 and S65 groups were slightly lower than those in H45 and H65 groups, respectively.

The MG65 group presented the most relevant change since the administration of MG returned the impaired fasting glucose to normal values, and its curve was similar to those obtained with the 45 mg/kg STZ dose; nevertheless, this improvement was not significant in the AUC analysis owing to the high dispersion of data in the latter. In contrast, although the MG45 group's glucose intolerance did not appear to have any improvement, the impaired fasting glucose was significantly lower than that observed in the S45 group (133 ± 10 vs 166 ± 10 mg/dl, respectively).

Discussion

Weaning is a critical period for β-cells since it is matured the full GSIS response. This period is characterized by the high expression of genes involved in β-cell replication and metabolic function, which is associated to the transition from high-fat milk diet to high-carbohydrate chow (13). According to previous reports, β-cell proliferative capacity can be induced in young rats by increased metabolic demands or the β-cell deficiency resulting from tissue injury (15); therefore, a STZ insult at weaning could exacerbate proliferation rates, damaging the development of the full GSIS response.

In the current work, after two weeks of the hyperglycemia induction at weaning, it was observed that β-cell function was increased at the intermediate STZ dose of 45 mg/kg, while it was depleted at the elevated one of 65 mg/kg. This differential behavior could be generated since the intermediate dose sufficiently stimulated the β-cell proliferation to promote a greater release of insulin in response to the metabolic challenge of a carbohydrate-rich diet, while the elevated dose could lead to increased β-cell death, resulting in lower insulin levels capable to face this new metabolic demand. These results are outstanding since time to obtain frank hyperglycemia was accelerated compared with the 8 weeks observed after one STZ injection (100 mg/kg) on day of birth (no-STZ rat model); furthermore, in weaning STZ-induced hyperglycemic rats at 45 mg/kg, insulin levels were 444% higher than in the control, in contrast to a 50% decrease in pancreatic insulin in no-STZ rats (11).

After 6 weeks (11-week-old), hyperglycemic rats' overall metabolic status at both STZ doses worsened as β-cell function was declining, causing more severe hyperglycemia. Nevertheless, the continuous stimulus of the GFS solution could generate a greater metabolic demand that promoted the overstimulation of β-cell function and high levels of insulin to compensate for hyperglycemia. In this context, the main goal of this study was to select the most appropriate STZ dose so that, in combination with the administration of a

GFS solution, suitable metabolic parameters were obtained to generate a rapid T2D model with the classic characteristics of β-cell dysfunction and IR. The results showed that both STZ doses, together with the GFS solution, could produce T2D phenotypes to be used as experimental models; however, their usefulness will depend on what stage of the disease is being assessed.

The main feature of T2D is hyperglycemia, which is mainly caused by the incapacity of the cells to respond adequately to insulin (IR) (37). Nevertheless, during the natural history of the disease, several stages can be recognized, whose characteristics will be influenced by the β-cell deterioration from the uncontrolled IR (38). Hence, the possibility of using a specific model for a particular stage could be necessary.

In the early stage of T2D, namely a prediabetic condition, IR is a predominant feature and therefore high insulin levels are present to compensate for the inadequate performance of this hormone. At this point, there is high β-cell function and moderate hyperglycemia (39,40). Over 6 weeks, the administration of GFS solution to weaning STZ-induced hyperglycemic rats with a 45 mg/kg dose was able to reproduce this stage since moderate hyperglycemia, and significantly elevated insulin levels, HOMA-IR, and HOMA-%B were obtained compared to those observed in its hyperglycemic control. In contrast, it has been reported that a single i.v. STZ injection of 45 mg/kg on adult rats produces a type 1 diabetes phenotype, namely they exhibit significant hyperglycemia (>300 mg/dl) and hypoinsulinemia (41), while a single i.p. STZ injection of 40 or 45 mg/kg on adult rats pretreated with fructose generates a more severe insulin-resistant-T2D phenotype with high glucose levels (>300 mg/dl), but low insulin levels and β-cell function (42,43). Moreover, the pure supplementation of fructose to adult rats needs long times to induce a significant hyperinsulinemic state (44).

In this model, the advanced stage of T2D was obtained with the administration of the GFS solution to weaning STZ-induced hyperglycemic rats with a 65 mg/kg dose, generating significantly high glucose levels (>300 mg/dl), reduced insulin values and a lower β-cell function index in comparison to those exhibited in the 45 mg/kg STZ group supplemented with the GFS solution. However, the insulin levels and β-cell function obtained in the present study were not as lower as in the works mentioned above (42,43), indicating a not so severe T2D phenotype.

In addition to hyperglycemia and hyperinsulinemia, an accelerated hypertriglyceridemia was observed. Overall, the induction of these characteristics is attributed to the combination of fructose and glucose consumption. For instance, it is reported that chronic administration of fructose is associated with reduced insulin function, hyperinsulinemia, and elevated rates of *de novo* lipogenesis (28,45,46). Moreover, it prevents triglyceride clearance from the bloodstream of STZ hyperglycemic rats (47). On the other hand, it has been recently suggested that the glucose component from diets with a high carbohydrate content may have a more relevant role in the adverse metabolic effects (29). HFCS causes a greater increase in postprandial LDL-cholesterol and apolipoprotein B levels than fructose in subjects who participated in a study designed to evaluate the metabolic effects of consuming beverages sweetened with aspartame, glucose, fructose, HFCS, and sucrose (48). The authors proposed a synergistic effect between fructose and glucose, which could increase the lipoprotein risk factors of cardiovascular disease due to HFCS consumption.

Although metabolic parameters close to those observed in T2D were obtained by administering the GFS solution over 6 weeks to weaning STZ hyperglycemic rats, glucose intolerance was not obviously modified in the OGTTs. After the glucose challenge, both hyperglycemic and hyperglycemic plus syrup groups had the same behavior and the latter even tended to have lower blood glucose levels than the groups without syrup. A possible explanation is that it has been reported a β-cell adaptative response to hyperglycemia promoted by the chronic exposure to carbohydrates, such as fructose and glucose, leading to glucose hypersensitivity along with hyperinsulinemia after a glucose load (49,50).

Even though glucose intolerance was not meaningfully improved in the OGTTs after chronic administration of hypoglycemic oral agents, metformin and glibenclamide, there was an

enhancement in overall insulin functionality (51,52), namely lower insulin levels were needed to maintain glycemia, which was reflected in increased QUICKI indices, lower HOMA-IR values, and a reestablishment of β-cell performance. Nevertheless, there was a differential STZ dose impact in this amelioration. First, at the 45 mg/kg STZ dose, their insulin levels and HOMA-IR were significantly diminished, resulting in better β-cell functioning and a significantly lower HOMA-%B value. However, this treatment did not have much of an impact on their blood glucose levels. On the other hand, at the 65 mg/kg STZ dose, insulin levels were not meaningfully reduced, yet their blood glucose levels, and HOMA-IR values were radically lowered, which translates into better insulin functioning.

In summary, chronic administration of a syrup solution containing 8.8% glucose and 5.2% fructose over 6 weeks to weaning STZ-induced hyperglycemic rats produced a rapid, easy-to-implement, and drug-responsive T2D model that can mimic the classical features seen in different stages of the disease: β-cell dysfunction and IR. In addition, it is proposed that this model be used to evaluate potential hypoglycemic agents and study some aspects of T2D, such as β-cell regeneration for therapeutic purposes.

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Table 2. Final parameters measured after the 6-week experimental period (mean ± SEM, *n*=6).

Parameter	STZ45			C	STZ65		
	H	S	MG		H	S	MG
FBG (mg/dl)	180 ± 13 [a]	163 ± 12	151 ± 4	107 ± 8	441 ± 37 [a,b]	388 ± 79 [c]	115 ± 3 [d,e]
FSI (pmol/l)	159.5 ± 60.3 [c]	594.6 ± 168.7	173.5 ± 33.2 [c]	217.1 ± 57.8	140.0 ± 45.5	331.8 ± 129.4	270.0 ± 108.8
HOMA-IR	11.9 ± 4.4 [c]	43.7 ± 15.9	10.8 ± 2.1 [c]	9.6 ± 2.5	27.3 ± 9.8	34.4 ± 7.6	12.7 ± 5.1 [e]
HOMA-%B	84.4 ± 32.2 [a,c]	329.6 ± 62.6	120.0 ± 24.5 [c]	337.9 ± 106.2	21.2 ± 6.5 [a,b]	172.2 ± 238.0 [c]	324.0 ± 133.2
QUICKI	0.288 ± 0.016 [c]	0.245 ± 0.011	0.277 ± 0.006 [c]	0.292 ± 0.015	0.268 ± 0.019	0.245 ± 0.007	0.287 ± 0.015 [e]

[a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [d] versus MG45; [e] versus S65 ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg; FBG: fasting blood glucose; FSI: fasting serum insulin.

Table 3. Follow-up of fasting blood glucose expressed in mg/dl (mean ± SEM, *n*=6) and percentage change.

Group		Time (weeks)						
		0	1	2	3	4	5	6
STZ45	H	169 ± 5 [a] **100%**	167 ± 8 [a] **99%**	166 ± 3 [a] **98%**	179 ± 8 [a] **106%**	182 ± 22 [a] **108%**	170 ± 11 [a] **101%**	180 ± 13 [a] **107%**
	S	161 ± 5 **100%**	160 ± 4 **99%**	167 ± 5 **104%**	174 ± 12 **108%**	168 ± 7 **104%**	192 ± 39 **119%**	163 ± 12 **101%**
	MG	164 ± 3 **100%**	162 ± 7 **99%**	164 ± 6 **100%**	154 ± 13 **94%**	151 ± 6 **92%**	144 ± 6 **88%**	151 ± 4 * **92%**
C		119 ± 10 **100%**	120 ± 7 **101%**	120 ± 6 **101%**	117 ± 6 **98%**	121 ± 5 **102%**	122 ± 8 **102%**	107 ± 8 **90%**
STZ65	H	257 ± 45 [a] **100%**	349 ± 64 [a,b] **136%**	411 ± 45 [a,b]* **160%**	436 ± 31 [a,b]* **170%**	441 ± 25 [a,b] **172%**	412 ± 15 [a,b] **160%**	441 ± 37 [a,b] **172%**
	S	196 ± 27 **100%**	211 ± 42 **108%**	330 ± 69 [c] **168%**	317 ± 83 **162%**	362 ± 74 [c] **185%**	331 ± 74 **169%**	388 ± 79 [c]* **198%**
	MG	225 ± 36 **100%**	200 ± 38 **89%**	163 ± 17 [e] **72%**	140 ± 10 [e] **62%**	126 ± 4 [d,e]* **56%**	141 ± 8 [e] **63%**	115 ± 3 [d,e]* **51%**

In the same column: [a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [d] versus MG45; [e] versus S65 ($p<0.05$). **In the same row:** * versus its initial time ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg.

Table 4. Follow-up of triglycerides expressed in mg/dl (mean ± SEM, *n*=6) and percentage change.

Group		Time (weeks)			
		0	2	4	6
STZ45	H	103 ± 14 **100%**	105 ± 15 **102%**	116 ± 22 **113%**	123 ± 18 **119%**
	S	81 ± 5 **100%**	129 ± 15 **159%**	133 ± 13 * **164%**	127 ± 13 **157%**
	MG	118 ± 19 **100%**	144 ± 14 **122%**	125 ± 15 **106%**	106 ± 10 **90%**
C		113 ± 9 **100%**	111 ± 7 **98%**	107 ± 7 **95%**	97 ± 10 **86%**
STZ65	H	105 ± 11 **100%**	158 ± 27 **150%**	211 ± 21 [a,b]* **201%**	229 ± 31 [a,b]* **218%**
	S	89 ± 6 **100%**	245 ± 33 [c] **275%**	247 ± 26 [c]* **278%**	301 ± 47 [c]* **338%**
	MG	107 ± 5 [d] **100%**	193 ± 19 [d]* **180%**	143 ± 14 [e] **134%**	119 ± 4 [e] **111%**

In the same column: [a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [d] versus MG45; [e] versus S65 ($p<0.05$). **In the same raw:** * versus its initial time ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg.

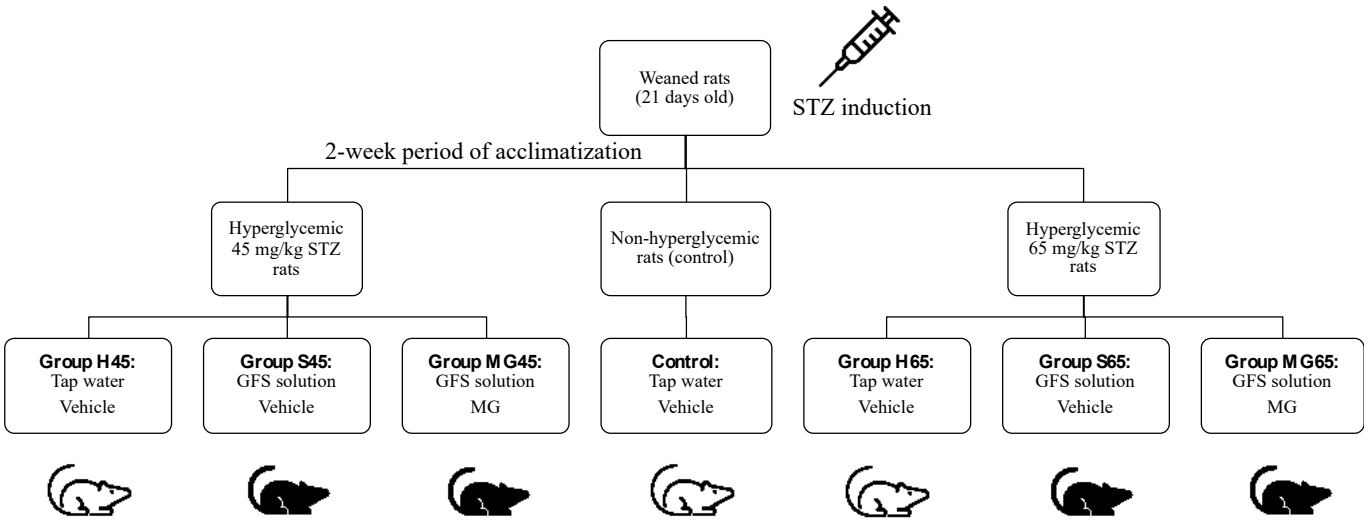

Figure 1. Assignment of experimental groups by treatment. STZ: streptozotocin; GFS: glucose-fructose syrup; MG: metformin-glibenclamide.

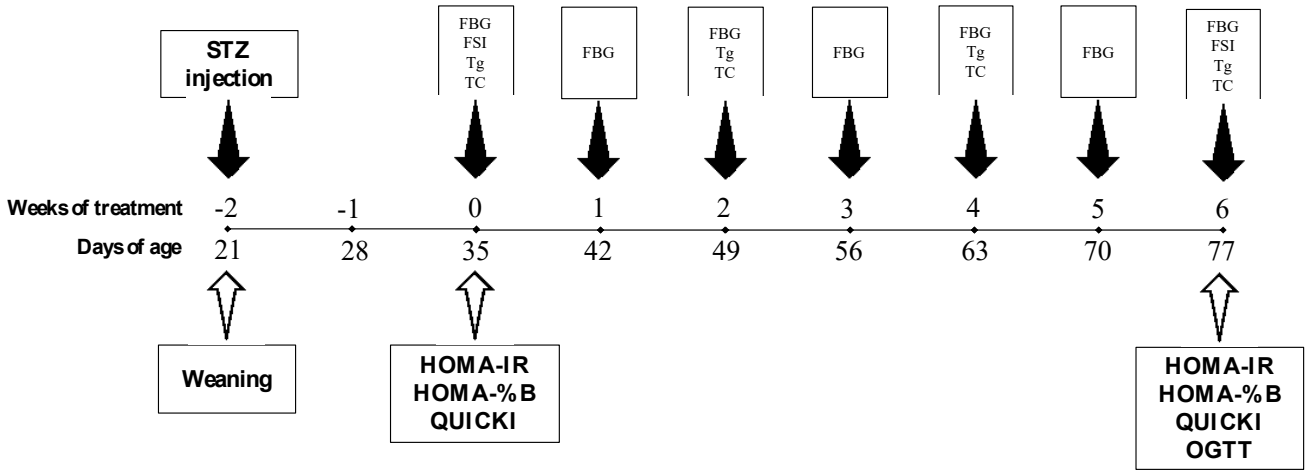

Figure 2. Experimental design. STZ: streptozotocin; FBG: fasting blood glucose; FSI: fasting serum insulin; Tg: triglycerides; TC: total cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; HOMA-%B: homeostatic model assessment of β-cell function; QUICKI: quantitative insulin sensitivity check index; OGTT: oral glucose tolerance test.

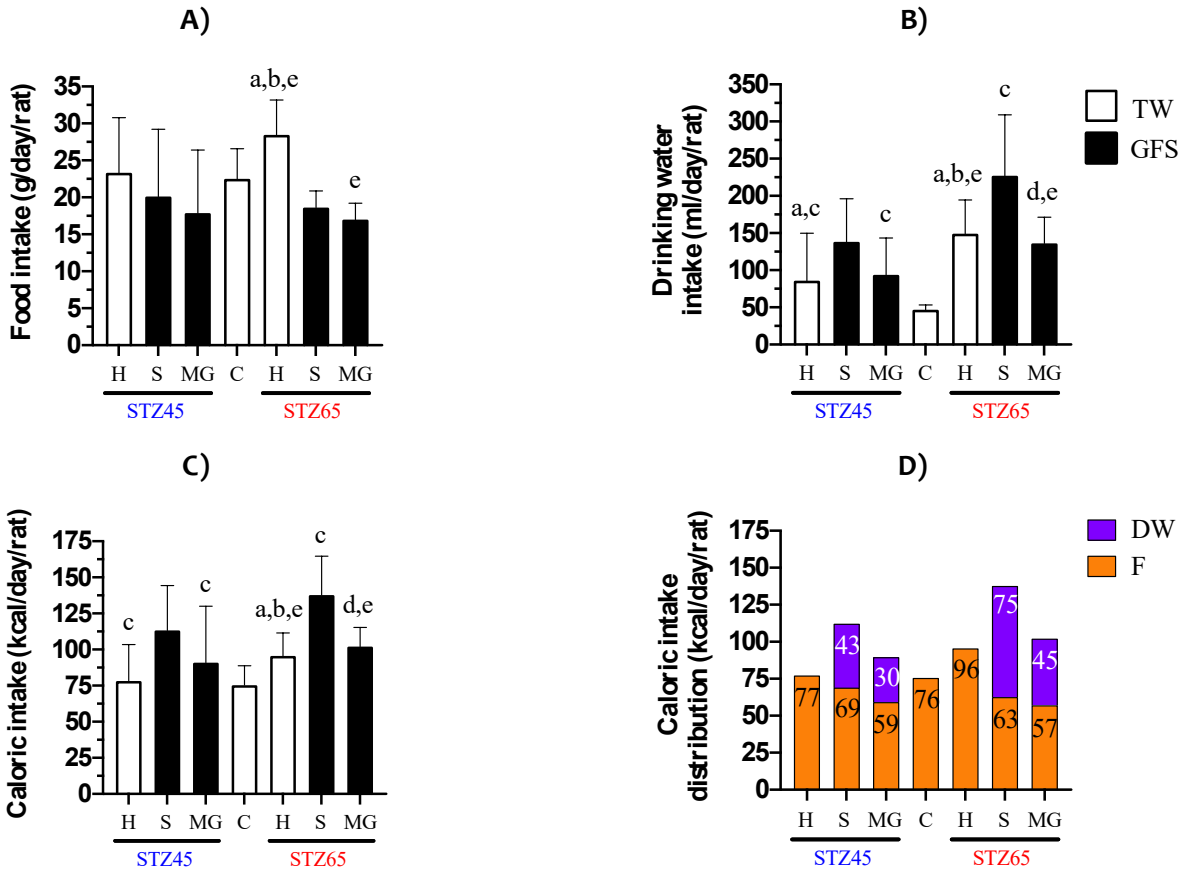

Figure 3. Consumption of food, drinking water and calories (mean ± SD, *n*=6). **A)** Food intake; **B)** Drinking water intake; **C)** Total calorie intake; **D)** Distribution of calorie sources. [a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [d] versus MG45; [e] versus S65 ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg; TW: tap water; GFS: glucose-fructose syrup solution; DW: drinking water; F: food.

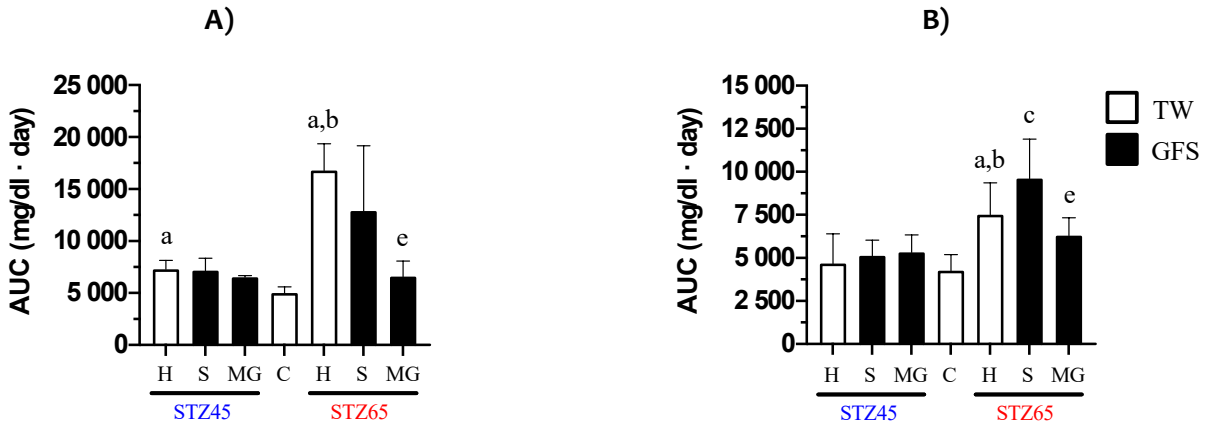

Figure 5. AUC of blood parameters (mean ± SEM, *n*=6). **A)** Fasting blood glucose; **B)** Fasting triglycerides. [a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [d] versus MG45; [e] versus S65 ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg; TW: tap water; GFS: glucose-fructose syrup solution.

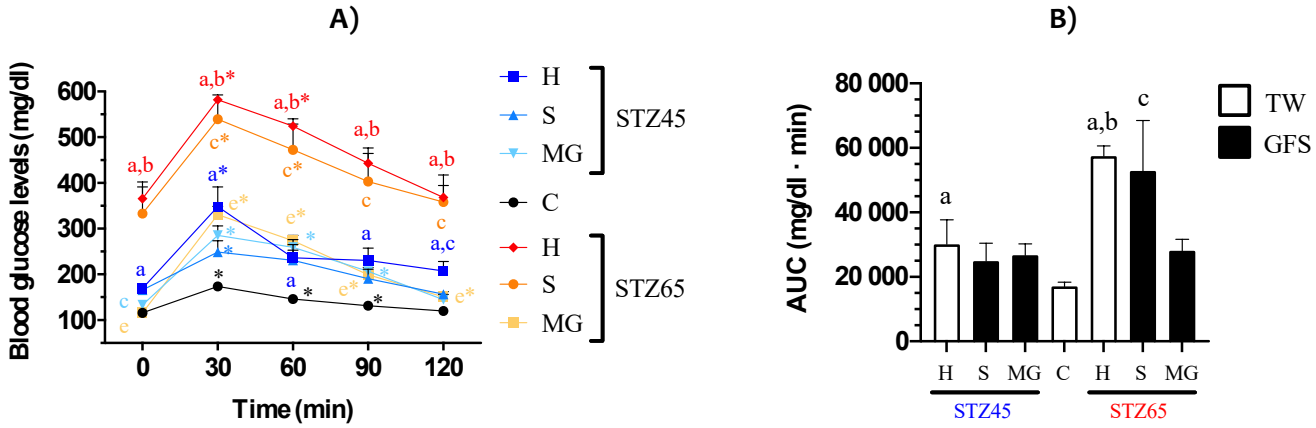

Figure 6. Oral glucose tolerance tests (mean ± SEM, *n*=6). **A)** Blood glucose 2-h curves; **B)** AUC of blood glucose 2-h curves. [a] indicates statistical difference versus C; [b] versus H45; [c] versus S45; [e] versus S65; * versus its initial time in 2-h curves ($p<0.05$). C: non-hyperglycemic control; H: hyperglycemic controls; S: hyperglycemic groups plus syrup solution; MG: hyperglycemic groups plus syrup solution and metformin-glibenclamide; STZ45: streptozotocin dose of 45 mg/kg; STZ65: streptozotocin dose of 65 mg/kg; TW: tap water; GFS: glucose-fructose syrup solution.