

SIX WEEK FOLLOW-UP OF METABOLIC EFFECTS INDUCED BY A HIGH-FAT DIET AND STREPTOZOTOCIN IN A RODENT MODEL OF TYPE 2 DIABETES MELLITUS

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Abstract

This study was initiated to refine and characterize a nongenetic experimental model of type 2 diabetes mellitus and to follow up various metabolic parameters up to six weeks after diabetes induction. Male Wistar rats were divided into 4 groups: CON group – consumed standard rat chow and served as control; HFD group – consumed high-fat diet (45% calories as fat); STZ group was injected once intraperitoneally with streptozotocin (35 mg/kg) on day 14, and DM-2 group – consumed high-fat diet and was injected with streptozotocin. The metabolic parameters were measured one week after streptozotocin injection (week 3) and at the end of the study (week 9).

Our results confirm that HFD-group developed dyslipidaemia, obesity and insulin resistance. All metabolic parameters remained largely unaltered in STZ-group during the study. Only the combination of high-fat diet and streptozotocin (DM-2 group) induced type 2 diabetes that was characterized with moderate hyperglycaemia, insulin resistance, hypertriglyceridaemia, elevated free fatty acids, hypercholesterolaemia and increased plasma glucagon levels at the time of diabetes onset (week 3). The observed changes of the metabolic parameters after six additional weeks demonstrated an aggravated diabetic state, as confirmed from significantly increased fasting plasma glucose values, insufficient insulin secretion, severe hyperlipidaemia, increased glucagon levels, decreased serum adiponectin concentrations and significantly elevated urinary protein excretion. These results indicate that apart from its utility as a model of diabetes aetiology, this model could also be used for elucidating the role of the hormones adiponectin and glucagon in the progression of type 2 diabetes, as well as for investigating the diabetic complications.

Key words: streptozotocin, high-fat diet, type 2 diabetes, animal model.

Introduction

Type 2 diabetes mellitus is the most common endocrine disorder, affecting approximately 5% of the population worldwide. According to the report of the International Diabetes

Federation, the number of diabetic patients will reach 300 million in 2025 and more than 97% of these patients will have type 2 diabetes [1]. The two metabolic defects characterizing type 2 diabetes are derangement of insulin secretion

that is delayed or is insufficient relative to glucose load and inability of peripheral tissues to respond to insulin – called insulin resistance. Studies of the natural history of type 2 diabetes have shown that the prediabetic state is characterized by resistance to insulin-mediated glucose disposal and compensatory hyperinsulinaemia. The transition from pre-diabetes to type 2 diabetes occurs when the secretory capacity of the pancreatic beta cell is no longer able to compensate for the insulin resistance [2, 3].

With the increasing incidence of type 2 diabetes, the development of effective experimental models for evaluation of new potential therapeutic agents is very important. The ideal model should have no genetic alteration (such as db/db mice, Zucker diabetic fatty rat, Otsuka long Evans Tokushima fatty (OLETF) rat, Goto-Kakizaki rat etc), should simulate the metabolic characteristics of patients with type 2 diabetes and be cost effective [4]. We have taken into consideration the experience from previous studies showing that rodents fed a high-fat diet develop insulin resistance and that on the other hand, streptozotocin is widely used to induce damage of the pancreatic beta-cells through alkylation of the DNA. Therefore, investigators have started to develop a diabetic rodent model by feeding the animals with a high-fat diet following streptozotocin injection that would closely mimic the natural history of the disease events (from insulin resistance to beta cell dysfunction). Although the appearance of type 2 diabetes was achieved, the percentage and composition of fat in the diet, as well as the administered dose(s) of STZ, were not consistent among the studies. A higher percentage of calories as fat in the diet causes pancreatic beta-cell damage, a permanent insulin resistance and influences food intake, whereas a low percentage of fat requires a longer period of time for the development of insulin resistance [5]. Similarly, higher doses of STZ (> 50 mg/kg bw) severely impair insulin secretion mimicking type 1 diabetes, whereas lower doses (< 30 mg/kg bw) have been shown to cause only a mild impairment of insulin secretion and fail to induce diabetes [6]. Additionally, most of the investigators have evaluated this diabetic rodent model shortly (i.e. several days) after diabetes induction, so the information regarding

the long-term behaviour of the model is limited [7–11].

Therefore, the aim of this study is to refine and characterize a model of type 2 diabetes mellitus by combining a high-fat diet (45% calories as fat) and a single low dose of streptozotocin (35 mg/kg) in male Wistar rats and to follow-up various metabolic parameters up to six weeks after diabetes induction.

Material and methods

Animals and Diets

Male Wistar rats (200 ± 20 g) were kept at the experimental stable of the Institute of Preclinical and Clinical Pharmacology and Toxicology. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2°C) and humidity with a 12/12-hour light-dark cycle. Animal maintenance and research were conducted in accordance with the principles for care and use of laboratory animals [12] using experimental protocols that had prior approval from the Ethical Committee for Use of Laboratory Animals of the Ss Cyril and Methodius University Medical Faculty.

Rats had ad libitum access to water and rodent chow – either standard rat chow consisting of 10% fat, 20% protein and 70% carbohydrate or a high-fat diet consisting of 45% fat, 20% protein and 35% carbohydrate (D12451, Research Diets; New Brunswick, USA).

Induction of a diabetic model

The rats were divided into 4 groups:

- **CON** (n = 10): represents a control group that consumed standard rat chow for a period of 9 weeks and was injected intraperitoneally with the vehicle two weeks after the beginning of the experiment (day 14).
- **STZ** (n = 10): consumed standard rat chow for a period of 9 weeks and was injected intraperitoneally with streptozotocin (35 mg/kg) two weeks after the beginning of the experiment.
- **HFD** (n = 10): consumed a high-fat diet for a period of 9 weeks and was injected intraperitoneally with the vehicle two weeks after the beginning of the experiment.
- **DM-2; model of type 2 diabetes** (n = 10): consumed high-fat diet for a period of 9 weeks

eks and was injected intraperitoneally with streptozotocin (35 mg/kg) two weeks after the beginning of the experiment.

Streptozotocin (Sigma, USA) was prepared by dissolving in 0.1 mol/L sodium citrate buffer (pH 4.4–4.5) and injected (immediately to avoid degradation) once intraperitoneally at a dose of 35 mg/kg bw. Sodium citrate buffer served as a vehicle in a dose of 1 ml/kg bw.

Blood and urine samples were collected one week after streptozotocin or vehicle injection (week 3) and after 6 additional weeks of the respective diets (week 9). At the same time points, the rats were subjected to an oral glucose tolerance test (OGTT). Body weight was measured at the beginning of the study and then weekly till the end of the study. Food consumption was calculated every day by subtracting residual food from the amount given. An average weekly food intake was determined for each experimental group.

At the end of the study, the animals were sacrificed by an anaesthetic overdose of ketamine hydrochloride (Ketalar, Pfizer).

Oral glucose tolerance test (OGTT)

OGTT was carried out to observe the glycaemic response to exogenously administered glucose in unanaesthetized animals. Twelve hours before the beginning of the test, food was removed from the cages. After a 12-hour fast, each rat was administered 2 g glucose/kg bw (as a 30% solution) by intragastric tube. Glucose levels were measured by a portable glucometer (Accu-check; Roche Diagnostic, Germany) using a drop of blood from the tail vein of each rat. The determined glucose values in the specified time points: 0 (just before), 30, 60 and 120 minutes after glucose administration were used for calculation of AUC₀₋₁₂₀ (Area Under Curve) of glucose.

Blood collection and assays

After overnight fasting, blood samples were withdrawn by venipuncture from the retro bulbar veins (under mild ether anaesthesia) into plastic tubes with appropriate anticoagulant (ethylene diaminetetraacetic acid- EDTA) for plasma samples or without anticoagulant for serum samples. Plasma was collected after blood was centrifuged immediately at 3.500 rpm for 10 minutes. Serum samples were allo-

wed to clot for 30 minutes, and serum was then separated by centrifugation at 10.000 rpm for 10 minutes. An appropriate number of aliquots were separated and stored at -70°C.

Rat-specific ELISA kits were used for determination of plasma insulin (Mercodia, Uppsala, Sweden) and plasma glucagon (Wako Chemicals GmbH, Germany) concentrations. Serum adiponectin levels were measured using mouse/rat adiponectin ELISA kit (B-Bridge International Inc, USA). Serum triglycerides (Tg) and cholesterol (Chol) concentrations were measured by standard enzymatic colorimetric methods on Integra 400+ (Roche Diagnostics GmbH, Germany). For determination of serum-free fatty acids (FFA) an enzymatic colorimetric kit was applied (DiaSys Diagnostic Systems GmbH, Germany).

Insulin sensitivity of the experimental animals was evaluated using the Homeostasis Model Assessment (HOMA) – index [13]. This index incorporates the fasting glucose and insulin values and was calculated by the equation: $HOMA = \text{glucose} * \text{insulin} / 22.5$.

Determination of urinary proteins

Rats were placed in metabolic cages and urine was collected for 24 hours. Urine samples were collected in tubes with 100 µl of 10% NaN₃ added as a preservative, stored at 4°C, centrifuged and immediately processed. Total protein excretion was determined with Meulemans classical method using sulphosalicylic acid [14]. Urinary proteins were separated by horizontal ultrathin pore gradient sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), according to Görg et al. [15]. Urinary samples were used neither diluted nor concentrated; 0.9 ml urine with 0.1 ml sample buffer (1.5 m Tris/HCl, 10% SDS, pH 8.8) was incubated for 3 minutes in boiling water. SDS polyacrylamide gradient gels (4–22.5%) were prepared by standard procedure in dimensions 195 × 250 × 0.5 mm. SDS-PAGE worked at 5°C within 3 hours in a Multipor II Unit (LKB, Sweden). Pharmacia LKB low-mass calibration proteins were used for identification of protein fractions. Gels were stained with Coomassie Blue R 250 and prepared in stable preparations.

Statistics and data analysis

Data are presented as mean ± standard deviation (SD). Student's "t test" for dependant

samples was employed for analysing the data between two time points within one group, whereas Student's "t test" for independent samples was used for analyzing the data between two groups at the same time point. Comparison of values between more than two groups was performed by the analysis of variance followed by Bonferroni and Tukey's post hoc tests, through appropriate software (SPSS Statistics, version 17). Values for $p < 0.05$ were considered as statistically significant.

Calculation of AUC (Area Under Curve) from the measured glucose concentrations while the OGTT was performed with the KINETICA™ 4.4 program (Innaphase corporation, USA).

Results

The metabolic parameters of different experimental groups during the study are summarized in Table 1 and Table 2.

Table 1

Changes in markers of glucose metabolism in control (CON), streptozotocin injected (STZ), high-fat diet fed (HFD) and type-2 diabetes (DM-2) group of rats

week	CON		STZ		HFD		DM-2	
	3	9	3	9	3	9	3	9
FPG (mmol/L)	4.76±0.64	4.96±0.69	5.10±0.88	5.53±0.82	5.30±0.59	5.94±0.63 *	10.36±2.22 ‡	18.56±3.76 ‡***
Insulin (pmol/L)	97.97±9.58	104.34±11.30	95.08±14.50	103.10±11.25	198.75±24.19 ‡	287.10±31.87 ‡ ***	146.29±12.46 ‡	95.67±12.08 ***
HOMA index	2.99±0.51	3.30±0.51	3.11±0.74	3.62±0.44	6.74±1.08 ‡	10.95±1.97 ‡***	9.64±1.92 ‡	11.24±1.98 ‡
AUC (mmol/L *min)	696.00±78.89	712.40±78.78	726.40±98.37	768.30±130.19	1063.8±105.11 ‡	1185.1±124.34 ‡* *	1604.4±190.76 ‡	2441.0±739.47 ‡***

Data are shown as mean ± SD. FPG = Fasting Plasma Glucose; HOMA = Homeostasis Model Assessment Index; AUC = Area Under Curve of glucose calculated from the Oral Glucose Tolerance Test * $p < 0.05$, *** $p < 0.001$ compared with the value measured at week 3 within the same group ‡: $p < 0.001$ compared with the CON group at the same time point

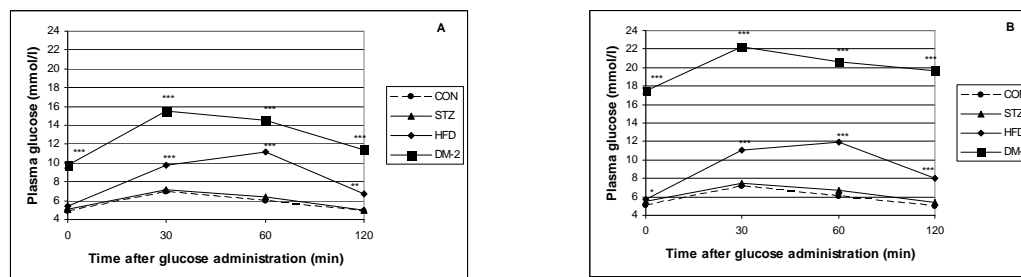
Feeding on a high-fat diet for 3 weeks induced only a moderate increase in fasting plasma glucose, but a significant increase in insulin concentrations. The HFD group showed significant impairment in glucose tolerance to exogenously administered glucose, as evidenced from elevated glycaemic values at 30, 60 and 120 minutes post-glucose challenge, compared with the CON group (Figure 1). This resulted in higher glucose AUC calculated from the OGTT compared to the CON group and was accompanied with increased HOMA-index, indicating a state of reduced insulin sensitivity in this experimental group. The induced insulin resistance progressively worsened, which is evident from the increase in all of the above-mentioned parameters at week 9. By the end of the study period the HFD group had impaired fasting plasma glucose values, but did not develop hyperglycaemia.

Additionally, serum Tg, Chol and FFA were increased at week 3, as well as at the end of the study (Table 2). This was accompanied by a significant increase in the body weight

starting only one week after the beginning of the high-fat diet and the animals in this group were heavier than the CON group throughout the study. The average weekly food intake of the HFD group (23.63 g) was similar to the CON-group (24.74 g; $p > 0.05$) (Figure 2).

On the other hand, high-fat diet alone did not produce any changes in glucagon and adiponectin levels, nor in the amount of secreted urinary proteins compared to the CON group.

The **STZ-group** manifested only a small, statistically insignificant decrease of the body weight in the third week of the study (i.e. one week after a single 35 mg/kg intraperitoneal streptozotocin injection), but the animals recovered quickly and started to gain weight in a similar manner like the CON group from week 4 until the end of the study. This dose of STZ led to a small increase of the fasting plasma glucose, insulin and glucose AUC values, but they did not reach statistical significance compared to the CON group. All other metabolic parameters remained largely unaltered in the STZ group of rats.



CON: control group; STZ: streptozotocin injected group; HFD: high-fat diet group; DM-2: type 2 diabetes mellitus group (streptozotocin+high-fat diet); ** $p < 0.01$; *** $p < 0.001$: significantly different from the control group at the same time point

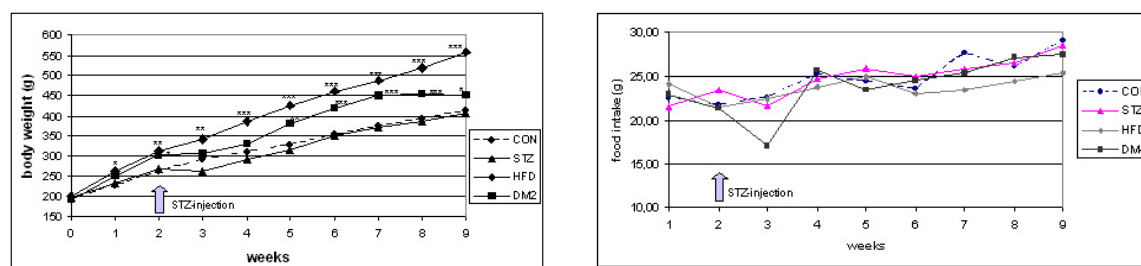
Figure 1 – Plasma glucose profiles during OGTT (Oral Glucose Tolerance Test) in different experimental groups at week 3 (A) and week 9 (B) of the study

Table 2

Changes in serum lipid metabolic parameters and urinary protein excretion in control (CON), streptozotocin injected (STZ), high-fat diet fed (HFD) and type-2 diabetes (DM-2) group of rats

week	CON		STZ		HFD		DM-2	
	3	9	3	9	3	9	3	9
Tg (mmol/L)	0.56 ± 0.07	0.60 ± 0.12	0.60 ± 0.14	0.62 ± 0.14	1.09 ± 0.22 ‡	2.49 ± 0.51 ‡ ***	1.39 ± 0.30 ‡	3.30 ± 0.67 ‡ ***
Chol (mmol/L)	1.81 ± 0.28	1.92 ± 0.26	1.87 ± 0.27	1.89 ± 0.20	3.06 ± 0.58 ‡	3.50 ± 0.64 ‡	3.13 ± 0.57 ‡	3.64 ± 0.51 ‡
FFA (mM)	0.68 ± 0.10	0.67 ± 0.09	0.73 ± 0.12	0.75 ± 0.13	0.93 ± 0.25 †	1.24 ± 0.21 ‡ *	1.05 ± 0.21 ‡	1.99 ± 0.41 ‡ ***
Urinary proteins (mg/24h)	6.03 ± 2.26	6.72 ± 1.70	5.98 ± 1.91	7.77 ± 2.30	7.07 ± 3.06	7.68 ± 2.58	7.46 ± 2.51	19.12 ± 5.07 ‡ ***

Data are shown as mean ± SD. Tg = Tryglicerides; Chol = Cholesterol; FFA = Free Fatty Acids; * $p < 0.05$, *** $p < 0.001$ compared with the value measured at week 3 within the same group †: $p < 0.05$; ‡: $p < 0.001$ compared with the control animals at the same time point



CON: control group; STZ: streptozotocin injected group; HFD: high-fat diet group; DM-2: type 2 diabetes mellitus group (streptozotocin+high-fat diet); STZ- streptozotocin injection; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: significantly different from the control group at the same time point

Figure 2 – Body weight and food intake at weekly intervals in different experimental groups

The combination of HFD and a single injection of STZ (35 mg/kg) induced a significant increase in FPG values (10.36 ± 2.22 mmol/l) that were within a diabetic range compared to CON (4.76 ± 0.64 mmol/l; $p < 0.001$) and HFD (5.10 ± 0.88 mmol/l; $p < 0.001$) groups at week 3 (Table 1). This was accompanied with hyperinsulinaemia, higher HOMA-index and glucose AUC0-120 calculated from

the OGTT in comparison to the control group ($p < 0.001$ for all parameters). The curve from the performed OGTT at week 3 shows that DM rats had increased fasting plasma glucose already at 0 min, continued rising at 30 and 60 min and sustained high at 120 min (Figure 1).

The glycaemia in this experimental group was not stable, but increased to 18.56 ± 3.76 mmol/l at week 9 ($p < 0.001$, compared to the

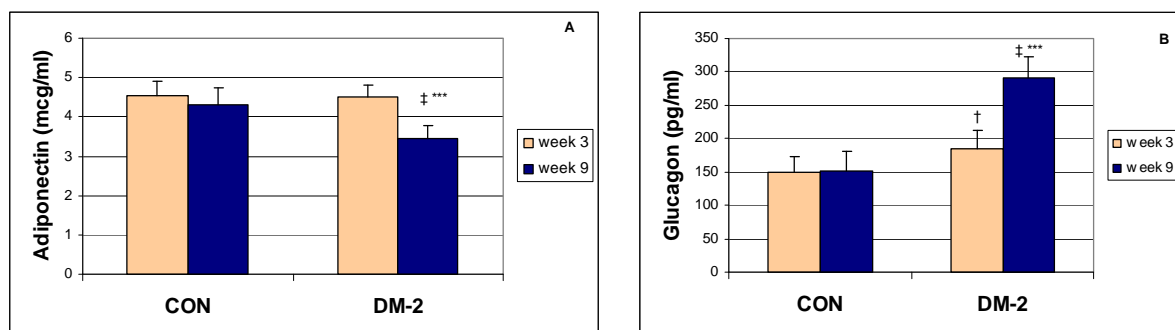
value at week 3). On the other hand, there was a marked and progressive decline in fasting insulin concentrations over the next 6 weeks (95.67 ± 12.08 pmol/l; $p < 0.001$ compared to value at week 3). At the end of the study, despite the marked hyperglycaemia, the insulinaemia in the DM-group of rats was comparable with the insulin concentrations of the CON group (104.34 ± 11.30 pmol/l; $p > 0.05$) and significantly lower than the HFD-group ($p < 0.001$). The pronounced deterioration of glucose homeostasis as diabetes progresses was also evident from the large increase in the glucose AUC ($p < 0.001$).

At the time of diabetes onset (week 3) the body weight of the DM-group (306.20 ± 10.78 g) was lower than the HFD-group (342.50 ± 35.45 g; $p < 0.05$) and comparable to values of the CON-group (290.60 ± 19.89 g; $p > 0.05$), due to the effect of the administered streptozotocin injection. In the following weeks the diabetic animals gradually increased their body weight and were heavier than the CON group from week 5 until the end of week 7. However, the progressive impairment in glucose utilization resulted in lower weight gain and even loss of body weight during the last two weeks of the study. Despite the decreased food intake within several days after STZ-injection, the average

weekly food intake of the DM-2 group (23.86 g) was not significantly different from the other experimental groups (Figure 2).

The parameters of the lipid metabolism (Tg, Chol and FFA) were significantly increased at diabetes onset (week 3) compared to the CON-group ($p < 0.001$ for all parameters). Serum Tg progressively increased from 1.39 ± 0.30 mmol/l at week 3 to 3.30 ± 0.67 mmol/l at week 9 ($p < 0.001$) and they were even higher compared to the HFD group (2.49 ± 0.51 mmol/l; $p < 0.01$), suggesting that these changes were secondary to poorer metabolic control. The increase of the Chol concentrations did not reach a statistical significance at the end of the study (3.64 ± 0.51 mmol/l; $p > 0.05$). Serum FFA increased from 1.05 ± 0.21 mM at week 3 to 1.99 ± 0.41 mM at week 9 ($p < 0.001$) which is likely to be due to decreased inhibition of lipolysis by insulin (Table 2).

Circulating adiponectin concentrations measured at diabetes onset were not statistically significant from the CON group ($p > 0.05$), but decreased over the next 6 weeks to 3.44 ± 0.33 mcg/ml ($p < 0.001$). Plasma glucagon levels were significantly elevated already at week 3 (184.78 ± 27.74 pg/ml) and the values of this hormone continued to rise until the end of the study (291.47 ± 30.24 pg/ml; $p < 0.001$) (Figure 3).



Data are shown as mean \pm SD. CON: control group; DM-2: type 2 diabetes group (streptozotocin+high-fat diet); *** $p < 0.001$ compared with the value measured at week 3 within the same group; †: $p < 0.05$; ‡: $p < 0.001$ compared with the control animals at the same time point

Figure 3 – Changes in Adiponectin (A) and Glucagon (B) levels in control and diabetic group of rats

Additionally, at the beginning of diabetes development the amount of proteins in the urine was not statistically significant (CON- 6.03 ± 2.26 mg, DM2- 7.46 ± 2.51 mg; $p > 0.05$). After six weeks, the urinary proteins increased more than two-fold in the DM2-group (19.12 ± 5.07 mg; $p < 0.001$) and these animals

had significant proteinuria compared to the CON group. The SDS-PAG electrophoretic separation revealed only a weak albumin fraction and several diffuse fractions of tubular proteins without a presence of any glomerular proteins in the urine samples of the control rats. On the other hand, the electrophoretogram of urine

from diabetic rats was characterized with intensive albumin fraction and well differentiated transferrin fraction in all samples. In the tubular area numerous, well separated and intensive fractions of urinary proteins could be noticed: alfa1-microglobulin (31 kD), monomer and dimer light chains (25 and 50 kD), retino-bin-

ding protein-RBP (22 kD) and beta2-microglobulin (14 kD). This finding confirms a presence of mixed proteinuria with both non-selective glomerular proteins and complete tubular proteins (in a range of 14–67 kD) in the urine of the diabetic rats (Figure 4).

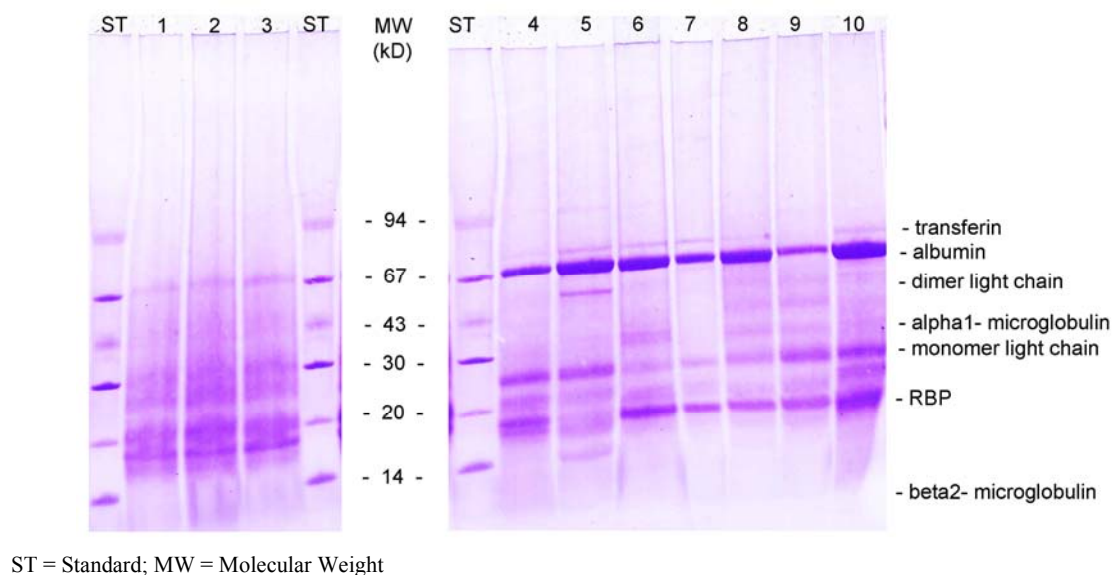


Figure 4 – SDS-PAGE of urinary proteins from control rats (lane 1–3) and diabetic rats (lane 4–10) at the end of the study (week 9)

Discussion

Type 2 diabetes mellitus is a chronic metabolic disorder that results from defects in both insulin secretion and insulin action. Both insulin resistance and beta-cell failure are genetically determined to some extent; however, environmental factors contribute to exacerbate both abnormalities [16].

The present study confirmed that high-fat diet alone induced an increase in body weight, hypertriglyceridaemia, hypercholesterolaemia and high FFA concentrations. Even after nine weeks, the rats in this group only had impaired fasting plasma glucose values according to the American Diabetes Association diagnostic criteria [17], but did not develop hyperglycaemia, because glucose homeostasis was maintained due to compensatory hyperinsulinaemia. These results are in agreement with previous reports that diets containing high fat cause glucose intolerance and insulin resistance in peripheral tissues due to lipotoxicity [18–20]. The fat content in our experiment was sufficient to induce

insulin resistance over a relatively short period of time, without significant changes in average food intake during the course of the study, which occurs in some studies where a higher percentage of fat was used [21]. In this way, equal quantities of protein, vitamin and minerals were provided to all the experimental animals and they could not have influenced the measured parameters.

In the present study we injected streptozotocin at a low dose (35 mg/kg) to induce only a light damage of the pancreatic beta cells which was not enough to affect the insulin secretion in rats fed a standard rat chow (STZ group). Therefore, the metabolic parameters in the STZ-group remained largely unaltered during the study, which is similar to the findings of Zhang et al. [10]. Other investigators reported that a single streptozotocin injection in a higher dose (50 mg/kg) induced significantly higher glucose concentrations and lower insulin concentrations in Wistar rats [22] and spontaneously hypertensive rats [23], confirming

that the severity of beta-cell destruction is dose dependant. Additional benefit of the lower streptozotocin dosage is that it minimizes the damage to other organs (especially the kidneys) and enables the research to be focused on changes resulting only from diabetes development and progression.

In the case of the DM-2 group of rats, the conversion from the pre-diabetic insulin resistant state induced by the fat diet to manifest type 2 diabetes mellitus was achieved by the single streptozotocin injection. The same dosage of streptozotocin that caused only marginal changes in chow-fed rats, led to a decline in the secretory capacity of pancreatic beta cells such that the insulin-resistant rats were unable to maintain normal glucose levels and developed hyperglycaemia. In type 2 diabetes, both insulin resistance and beta cell dysfunction are etiological contributors, but diabetes develops only when beta cell function fails to meet the increased demand.

At diabetes onset (week 3), the DM-2 rats had hyperinsulinaemia and moderately elevated blood glucose. Other investigators reported a study using a rat model similar to ours, but no data are available on glycaemia or insulinaemia at later time points [7–9]. Our results show a progressive loss of the ability to maintain glucose homeostasis and insulin production in this experimental model. Fasting plasma glucose concentrations were not stable, but increased approximately 80% by the end of the study. Plasma insulin concentrations were elevated at the beginning of the disease and declined as hyperglycaemia became more pronounced, but were not depleted even at the end of the study. Inadequate insulin secretion to compensate for insulin resistance followed by eventual beta-cell decompensation is a key component of the pathogenesis of type 2 diabetes in the human population [24]. At the end of the experiment the diabetic state was significantly worsened, which is supported by higher hyperglycaemia, reduced plasma concentrations of insulin and the animals also started to lose weight. The body weight decrease at the end of the experiment could be viewed as a pathophysiological aggravation of the disease.

Furthermore, the DM-2 rats exhibited hypertriglyceridaemia, hypercholesterolaemia and

elevated FFA levels at diabetes onset, which are also characteristic abnormalities of lipid metabolism seen in human diabetics. During the next 6 weeks, the rats manifested a moderate increase in serum cholesterol levels and a significant increase in Tg and FFA levels. This finding is in agreement with other studies [25–27], and is supported by the lipotoxicity hypothesis that lipid dysregulation contributes to insulin resistance and beta cell failure in the pathogenesis of type 2 diabetes [28]. The increased serum FFA concentrations are an important inductor for the development of the insulin resistance, because they lead to lipid accumulation in the non-adipose tissues (liver, the skeletal muscle), where the intracellular lipid metabolites interfere with the insulin signal paths, glucose transport, glycogen synthesis and/or gluconeogenesis [29]. They increase the oxidative stress, which leads to dysregulation of the adipocytokines synthesis [30]. Additionally, in the early stages of development of diabetes mellitus type 2, the increased serum FFA concentration induces a dysfunction, and later an apoptosis of the beta cells in the pancreatic islets [31].

Plasma glucagon levels were significantly elevated already at week 3 in the DM-2 rats and the values of this hormone continued to rise until the end of the experiment. Sloop et al. have revealed that glucagon-induced hepatic glucose production makes an important contribution to the hyperglycaemia observed in ob/ob mice [32]. Hyperglucagonaemia and/or an elevated plasma glucagon-to-insulin ratio have been reported in diabetic patients and point to the role of alpha-cell dysregulation in the pathogenesis of the disease [33]. On the other hand, circulating adiponectin concentrations measured at diabetes onset in DM-2 rats were similar to the control group. Adiponectin levels significantly decreased only after 6 weeks, thus potentially contributing to the disease progression. The obtained results are in agreement with other studies, performed with genetically modified animal models, such as the db/db mice [34] and the Zucker Diabetic Fat rat [35], that report lower adiponectin levels during diabetes development. Adiponectin plays an important role in the control of the insulin sensitivity of the peripheral organs, as well as in

the maintenance of glucose homeostasis [36–38]. Therefore, this nongenetic experimental model could be used to analyse the involvement of the hormones glucagon and adiponectin in the maintenance and/or progression of type 2 diabetes.

Additionally, at the beginning of diabetes development the amount of proteins in the urine was not statistically significant compared to the other groups. After six weeks, the urinary proteins increased more than two-fold in the DM2-group and these animals had significant proteinuria. The SDS-PAGE electrophoretic separation confirmed a presence of mixed proteinuria with both non-selective glomerular proteins and complete tubular proteins (in range of 14–67 kD) in the urine of the diabetic rats. This finding is similar to that of Sugano et al. (2006), where they had developed nephropathy after a high-fat diet and streptozotocin injection in heminephrectomized rats [39]. Hence, this model could also be useful for studying the diabetic nephropathy as one of the diabetic complications.

Conclusion

A combination of a high-fat diet (45% calories as fat) and a single low dose of streptozotocin (35 mg/kg) successfully induced type 2 diabetes in male Wistar rats that was characterized with moderate hyperglycaemia, insulin resistance, dyslipidaemia and elevated plasma glucagon levels at the time of diabetes onset. The observed changes of the metabolic parameters after six weeks mimicked the natural progress of diabetes development, as confirmed from increased fasting plasma glucose values, insufficient insulin secretion, aggravated dyslipidaemia, increased glucagon levels, decreased serum adiponectin concentrations and significantly elevated urinary protein excretion. Apart from its utility as a model of diabetes aetiology, this model may also provide insight into the role of adiponectin and glucagon in the progression of type 2 diabetes. Additionally, this experimental model could be used for evaluating therapeutic options for diabetic complications, such as diabetic nephropathy and lipid dysregulation.

In conclusion, this nongenetic rodent model simulates the development and progression

of type 2 diabetes mellitus and could help in developing new prevention and treatment strategies.

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Резиме

**СЛЕДЕЊЕ НА МЕТАБОЛИЧКИТЕ
ЕФЕКТИ ПРЕДИЗВИКАНИ
ОД ВИСОКОМАСНА ХРАНА
И СТРЕПТОЗОТОЦИН
КАЈ ЖИВОТИНСКИ МОДЕЛ
НА ДИЈАБЕТЕС МЕЛИТУС – ТИП 2
ВО ПЕРИОД ОД ШЕСТ НЕДЕЛИ**

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Оваа студија е спроведена со цел да се унапреди и елаборира негенетски експериментален модел на дијабетес мелитус – тип 2 и да се следат различни метаболички параметри во период од шест недели после индукција на болеста. Wistar-стаорци од машки пол беа поделени во 4 групи: КОН- контролна група која примаше стандардна храна, ХФД – група која конзумираше високомасна храна (45% масти од вкупното калориско внесување); СТЗ која група

беше инјектирана еднаш интраперитонеално со стрептозотцин (35 мг/кг) и ДМ-2 група која конзумираше високомасна храна и беше инјектирана со стрептозотцин. Метаболичките параметри беа определени една недела по инјекцијата со стрептозотцин (3. недела) и на крај од студијата (9. недела).

Нашите резултати потврдија дека ХФД-групата разви дислипидемија, зголемена телесна маса и инсулинска резистенција. Сите метаболички параметри останаа, главно, непроменети кај СТЗ-групата. Единствено комбинацијата од високомасна храна и стрептозотцин (ДМ-2 група) индуцираше дијабетес тип 2 што се карактеризираше со умерена хипергликемија, инсулинска резистенција, хипертриглицеридемија, зголемени слободни масни киселини, хиперхолестеролемија и зголемени вредности на глукагон на почетокот од болеста (3. недела). Опсервираните метаболички промени по 6 недели покажаа влошување на болеста, што се потврдува преку сигнификантно зголемување на глукозата во плазма на гладно, инсуфициентна инсулинска секреција, изразита хиперлипидемија, зголемени вредности на глукагон, намалени серумски концентрации на адипонектин и сигнификантно зголемување на екскрецијата на уринарните протеини. Овие резултати покажуваат дека, покрај корисноста на овој модел за истражување на етологијата на дијабетесот, моделот би можел да се користи за расветлување на улогата на хормоните адипонектин и глукагон во прогресијата на дијабетесот, како и за истражување на дијабетичните компликации.

Клучни зборови: стрептозотцин, високо-масна храна, дијабетес-тип 2, животински модел.