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Original research paper

Comparative effects of pravastatin and rosuvastatin on carbohydrate metabolism in an experimental diabetic rat model

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ABSTRACT

Statin treatment may increase the risk of diabetes; there is insufficient data on how statins affect glucose regulation and glycemic control and the effects of statins on liver enzymes related to carbohydrate metabolism have not been fully studied. Therefore, we aimed to compare the effects of the statin derivatives, pravastatin, and rosuvastatin, on carbohydrate metabolism in an experimental diabetic rat model. Female Wistar albino rats were used and diabetes was induced by intraperitoneal injection of streptozotocin. Thereafter, 10 and 20 mg kg^{-1} day^{-1} doses of both pravastatin and rosuvastatin were administered by oral gavage to the diabetic rats for 8 weeks. At the end of the experiment, body masses, the levels of fasting blood glucose, serum insulin, insulin resistance (HOMA-IR), liver glycogen, and liver enzymes related to carbohydrate metabolism were measured. Both doses of pravastatin significantly increased the body mass in diabetic rats, however, rosuvastatin, especially at the dose of 20 mg kg^{-1} day^{-1} reduced the body mass significantly. Pravastatin, especially at a dose of 20 mg kg^{-1} day^{-1}, caused significant increases in liver glycogen synthase and glucose 6-phosphate dehydrogenase levels but significant decreases in the levels of glycogen phosphorylase, lactate dehydrogenase, and glucose-6--phosphatase. Hence, pravastatin partially ameliorated the adverse changes in liver enzymes caused by diabetes and, especially at the dose of 20 mg kg^{-1} day^{-1}, reduced the fasting blood glucose level and increased the liver glycogen content. However, rosuvastatin, especially at the dose of 20 mg kg^{-1} day^{-1}, significantly reduced the liver glycogen synthase and pyruvate kinase levels, but increased the glycogen phosphorylase level in diabetic rats. Rosuvastatin, 20 mg kg^{-1} day^{-1} dose, caused significant decreases in the body mass and the liver glycogen content of diabetic rats. It can be concluded that pravastatin, especially at the dose of 20 mg kg^{-1} day^{-1} is more effective in ameliorating the negative effects of diabetes by modulating carbohydrate metabolism.

Keywords: diabetes mellitus, pravastatin, rosuvastatin, rat, streptozotocin

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Diabetes mellitus (DM) is a serious disease characterized by abnormalities in protein, lipid, and carbohydrate metabolism resulting from defects in insulin secretion, insulin action, or both (1).

The main symptom of diabetes is chronic hyperglycemia which causes many metabolic complications. The liver plays a central role in maintaining glucose homeostasis. Uncontrolled hepatic glycogenolysis, gluconeogenesis, and decreased glucose utilization are the main factors causing hyperglycemia in diabetics (2). Enzymes involved in the regulation of hepatic glucose production are potential targets for the regulation of glucose homeostasis in diabetes.

In addition to hyperglycemia, dyslipidemia is a common feature of diabetes and plays a role in the emergence of cardiovascular complications, which are the most important causes of death in diabetes. Therefore, lipid-lowering drugs are recommended to prevent cardiovascular events in diabetic patients.

Statins are inhibitors of cholesterol synthesis and are the most widely prescribed lipid-lowering drugs for the treatment of dyslipidemia since the 1980s. Although statins have beneficial effects on blood lipid profiles and vascular events, it has been mentioned that there may be a relationship between statin usage and the development of DM in recent years (3–5). There are different types of statins according to their chemical structures. Hydrophilic statins such as pravastatin and rosuvastatin may cause fewer side effects than lipophilic ones such as simvastatin and atorvastatin (6, 7). Some clinical studies have shown that rosuvastatin may cause the onset of diabetes (8–11) while pravastatin reduces the risk of DM (12). The effect of statins on glycemic status can also differ according to the dose of the drugs (13, 14).

Although there are some clinical studies regarding the positive and negative effects of statins on the development of DM, there are not enough experimental studies on this subject. Moreover, there is insufficient data on how these statins affect glucose regulation and glycemic control. The effects of statins on liver enzymes related to carbohydrate metabolism have also not been fully studied. Therefore, the aim of this study was to investigate the effects of 10 and 20 mg kg^{-1} day^{-1} doses of pravastatin and rosuvastatin on carbohydrate metabolism in a streptozotocin-induced diabetic rat model.

EXPERIMENTAL

Chemicals

Streptozotocin (STZ), phosphate-buffered saline (PBS), bovine serum albumin (BSA), and $CuSO_4 \times 5\ H_2O$ were obtained from Merck KGaA (Germany). Citric acid monohydrate, sodium citrate dihydrate, diethyl ether, NaOH, Na_2CO_3, sodium potassium-tartrate × 4 H_2O, and Folin-Ciocalteu phenol reagent were obtained from Merck (Germany). Pravastatin tablets (10 mg and 20 mg, Pravachol) were purchased from Deva Company (Turkey), and rosuvastatin tablets (10 mg and 20 mg, Livercol) were purchased from Ilko Pharmaceuticals (Turkey).

Animals and experimental design

All experimental protocols were performed according to the guidelines for the care and the use of laboratory animals. Ethical approval for the research was obtained from the

committee for animal experiments at the Dicle University Medical Research Center, Diyarbakır, Turkey.

Forty-two Wistar albino female rats, 8–12 weeks old, with a body mass of 260–300 g, were included in this study. Animals were housed individually in stainless steel cages under standardized lighting conditions (12 hours daylight/12 hours dark) at a constant temperature (25 ± 2 °C) with a standard pellet diet and water available *ad libitum*.

Rats were divided into six groups, one healthy control, and five experimental (diabetic) groups. Each group was comprised of seven rats. All rats in the control and experimental groups were alive until the end of the experiment.

Diabetes was induced by a single injection of STZ at a dose of 40 mg kg^{-1}. Streptozotocin solution was prepared in 0.01 mmol L^{-1} citrate buffer (pH 4.5) and injected into the peritoneal cavity of each rat. The healthy control group of animals (C) was given citrate buffer in the same way as a placebo. After 48 hours of STZ injection, fasting blood glucose was measured by a glucometer (Tyson Bioresearch, Inc., Taiwan) in the blood samples of the tail veins, and rats with blood glucose levels ≥ 13.9 mmol L^{-1} were considered diabetic and included in the experimental (diabetic) groups.

After 72 hours of the induction of diabetes, diabetic rats were randomly divided into five groups: untreated diabetic rats (D), diabetic rats treated with pravastatin at a dose of 10 mg kg^{-1} day^{-1} (PRV10), diabetic rats treated with pravastatin at a dose of 20 mg kg^{-1} day^{-1} (PRV20), diabetic rats treated with rosuvastatin at a dose of 10 mg kg^{-1} day^{-1} (RSV10) and diabetic rats treated with rosuvastatin at a dose of 20 mg kg^{-1} day^{-1} (RSV20). Pravastatin and rosuvastatin were dissolved in fresh drinking water at concentrations of 1 mg mL^{-1} and 2 mg mL^{-1}. Drugs were administered by oral gavage every evening for 8 weeks according to their dosing of 10 or 20 mg kg^{-1} day^{-1}. The doses of pravastatin and rosuvastatin were chosen according to the previous studies in diabetic rats (15–18). C and D groups of animals received a placebo (1 mL of tap water per day) by oral gavage every evening for eight weeks.

Samples collection and storage

All animals were weighed at the beginning of the study (baseline) and at the end of the study (final). Food intake and water consumption of all groups were also monitored on a daily basis at a fixed time during the experimental period.

At the end of the study, following a 12-hour fasting, blood samples were collected from the tail vein under ether anesthesia for the immediate analyses of blood glucose levels. All animals were then sacrificed by cardiac puncture, and blood samples were immediately centrifuged at 3700 rpm for 15 minutes at 4 °C. Serum supernatants were separated and stored at –80 °C until the analyses of fasting insulin levels. Liver tissues were rapidly removed, washed in ice-cold saline, and then divided into two halves. One half was minced and homogenized at a 1:10 ratio (*m*/*V*) in ice-cold 0.1 mol L^{-1} PBS (pH 7.4). The homogenates were centrifuged at 3700 rpm for 15 minutes at 4 °C. The supernatants were stored at –80 °C for further analyses of glycogen content and carbohydrate metabolizing enzymes including glycogen synthase (GS), glycogen phosphorylase (GP), lactate dehydrogenase (LDH), glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBP1), hexokinase (HK), pyruvate kinase (PK), and glucose 6-phosphate dehydrogenase (G6PD). The other half of the liver was stored at –80 °C for total protein determination.

Biochemical analysis

Fasting blood glucose levels of all groups of animals were measured by using a glucometer. Fasting serum insulin levels were measured by the electrochemiluminescence's immunoassay using an automated analyzer (Cobas e601 module; Roche Diagnostics, Germany). Insulin resistance was calculated from the formula for homeostasis model assessment of insulin resistance (HOMA-IR) (19):

$$\text{HOMA-IR} = \text{fasting insulin (mU L}^{-1}) \times \text{fasting glucose (mmol L}^{-1})/22.5$$

Liver glycogen, GS, GP, LDH, G6Pase, FBP1, HK, PK, and G6PD levels were measured by ELISA kits. All procedures were performed in accordance with the manufacturer's instructions. Total protein levels in liver samples were measured by the Lowry method using bovine serum albumin and Foline-phenol reagent (20). The liver glycogen content, GS, GP, LDH, G6Pase, FBP1, HK, PK, and G6PD levels were calculated according to the ratio of ELISA measurements of each parameter to total protein levels.

Statistical analysis

Statistical analyses were carried out using SPSS software (SPSS Version 24.0, IBM Corp., USA). Measurement data were tested for normal distribution and homogeneity of variance. Kruskal-Wallis non-parametric test was performed to compare all groups. The Mann-Whitney U test with Bonferroni correction was applied for pairwise *post hoc* comparisons. A value of $p \leq 0.003$ was considered to be statistically significant. Continuous variables were expressed as mean ± SD. Categorical variables were expressed by percentages (%).

RESULTS AND DISCUSSION

Body mass changes, daily food intake, and daily water consumption

The baseline body masses of the C group and all experimental groups (D, PRV10, PRV20, RSV10, RSV20) are shown in Table I. The final body mass of the C group was increased by 20.7 % compared to its baseline value. However, the final body mass of D, PRV10, PRV20, RSV10, and RSV20 groups decreased, resp., by 18.6, 15.6, 15.3, 18.1, and 20.4 % compared to their own baseline values. The final body mass of both PRV10 and PRV20 groups was significantly higher (both, $p \leq 0.003$), but the final body mass of the RSV20 group was significantly lower ($p \leq 0.003$) than that of the D group. As shown in Table I, daily food intake and water consumption in all experimental groups were significantly higher than in the C group (all, $p \leq 0.003$). However, there were no significant differences in daily food intake and water consumption between D and any of the groups treated with statins (PRV10, PRV20, RSV10, RSV20, resp.) ($p = 0.143$, $p = 0.157$, $p = 0.265$, $p = 0.357$, and $p = 0.337$, $p = 0.197$, $p = 0.056$, $p = 0.135$, resp.).

Body mass loss may occur in diabetic patients, especially those not under medical control. It results from the catabolism of fats and proteins, as glucose cannot be used as an energy source in muscle and other tissues (21). In the present study, the body mass of all diabetic rats decreased significantly. Both doses of pravastatin significantly increased the

body mass in our diabetic rats. However, we noticed that rosuvastatin treatment, especially at the dose of 20 mg kg^{-1} day^{-1}, reduced the body mass even more than that in D rats. Similar to our results, a previous study had shown that body mass had been decreased in diabetic rats treated with rosuvastatin (22).

Blood glucose, serum insulin and HOMA-IR index

Table II illustrates the levels of fasting blood glucose, fasting serum insulin, and HOMA-IR indices in C and in all experimental groups at the end of the study. There were significant increases in fasting blood glucose levels in all experimental groups compared to the C group (all, $p \leq 0.003$). Fasting blood glucose levels were significantly lower in the PRV20 group than in both D and RSV20 groups (both, $p \leq 0.003$). Moreover, fasting blood glucose level was lower in the PRV10 group than in the RSV10 group ($p \leq 0.003$). Fasting serum insulin levels in all experimental groups decreased approximately up to 50 % (all, $p \leq 0.003$), while the HOMA-IR indices in those increased by approximately three-fold (all, $p \leq 0.003$) compared to the C group. Fasting serum insulin levels did not show significant differences between any statin-treated group (PRV10, PRV20, RSV10, and RSV20, resp.) and the D group ($p = 0.522$, $p = 0.249$, $p = 0.798$, and $p = 0.853$, resp.). There were also no significant differences in HOMA-IR indices between any statin-treated group (PRV10, PRV20, RSV10, and RSV20, resp.) and D group ($p = 0.848$, $p = 0.655$, $p = 0.482$, and $p = 0.585$, resp.).

STZ-induced diabetic rats in our study had higher fasting glucose levels and insulin resistance, but lower insulin levels compared to healthy control rats. Previous studies also indicated that STZ injection in rats induced diabetes by increasing the levels of fasting glucose and insulin resistance, but decreasing insulin levels (23, 24). Both of the statins used in this study were hydrophilic and 20 mg kg^{-1} day^{-1} dose of pravastatin treatment reduced the fasting blood glucose levels significantly compared to the untreated diabetic rats and diabetic rats treated with 20 mg kg^{-1} day^{-1} rosuvastatin. Both doses of rosuvastatin, on the other hand, caused a slight, but not significant, increase in fasting glucose levels in diabetic rats. Similar to our results, some studies have shown that pravastatin treatment has beneficial effects on glycemic control and was associated with a reduction in the incidence of diabetes (12, 25–28). In contrast to this, the study by Keech *et al*. (29) indicated no effect of pravastatin on diabetes. In another study, an even higher incidence of DM was observed in pravastatin users (30). In that study, patients were in old ages, between 70 and 82 years old, and treated with 40 mg kg^{-1} day^{-1} of pravastatin. Moreover, there are also contradictory results about the effect of rosuvastatin on diabetes. Some studies reported that rosuvastatin treatment had no effect on fasting blood glucose levels (7, 31, 32); our results are similar to these studies. However, there are some studies showing that rosuvastatin treatment increased fasting glucose levels (8, 11, 33). The dosage and duration of statin use might affect the fasting glucose level and development of DM in patients (5, 34, 35).

In our study, neither of the statins' doses seemed to cause any significant changes in insulin levels and insulin resistance in diabetic rats. It was also reported that pravastatin treatment had no effect on insulin levels (36) and insulin sensitivity/resistance in hypercholesterolemic and diabetic patients (37). Similarly, *in vitro* studies indicated that insulin secretion was not affected in β cells treated with pravastatin (4). A study by Thongtang *et al*. (38) showed that serum insulin levels significantly increased in hyperlipidemic patients

Table I. Body mass changes, daily food intake, and daily water consumption in control and experimental animals

Parameters	Group					
	C[a]	D[a]	PRV10[a]	PRV20[a]	RSV10[a]	RSV20[a]
Baseline body mass (g)	271.6 ± 15.1	277.3 ± 12.7	275.5 ± 14.9	278.8 ± 14.4	273.2 ± 11.3	278.7 ± 12.6
Daily water consumption (mL)	74.17 ± 6.4	158.2 ± 10.8[b]	151.5 ± 11.1[b]	149.3 ± 10.9[b]	156.5 ± 12.2[b]	161.1 ± 9.6[b]
Daily food intake (g)	25.4 ± 2.1	47.3 ± 3.9[b]	45.9 ± 5.8[b]	43.3 ± 4.3[b]	45.6 ± 3.7[b]	47.5 ± 5.1[b]
Final body mass (g)	327.9 ± 13.3	225.8 ± 7.9[b]	232.4 ± 10.7[b,c]	236.1 ± 9.9[b,c]	223.7 ± 11.5[b]	221.8 ± 10.7[b,c]
Body mass change (%)	+20.7	–18.6	–15.6	–15.3	–18.1	–20.4

C – healthy control, D – untreated diabetic, PRV10 – diabetic treated with 10 mg kg^{-1} day^{-1} pravastatin, PRV20 – diabetic treated with 20 mg kg^{-1} day^{-1} pravastatin, RSV10 – diabetic treated with 10 mg kg^{-1} day^{-1} rosuvastatin, RSV20 – diabetic treated with 20 mg kg^{-1} day^{-1} rosuvastatin

[a] Mean ± SD ($n = 7$).

Significant difference (Bonferroni correction *post hoc* test): [b] $p \leq 0.003$ compared with the C group; [c] $p \leq 0.003$ compared with the D group.

Table II. Blood glucose, serum insulin levels, and HOMA-IR indices in control and experimental animals

Parameter	Group					
	C[a]	D[a]	PRV10[a]	PRV20[a]	RSV10[a]	RSV20[a]
Fasting blood glucose (mmol L^{-1})	5.54 ± 0.16	25.83 ± 0.83[b]	24.78 ± 0.81[b,e]	23.78 ± 0.79[b,c,e]	26.22 ± 0.77[b]	26.43 ± 0.99[b]
Fasting insulin (mU L^{-1})	14.67 ± 1.76	7.81 ± 0.61[b]	8.10 ± 0.70[b]	8.40 ± 0.93[b]	7.67 ± 0.85[b]	7.9 ± 0.87[b]
HOMA-IR	3.61 ± 0.04	8.97 ± 0.12 [b]	8.92 ± 0.18[b]	8.88 ± 0.25[b]	8.94 ± 0.19[b]	9.28 ± 0.21[b]

C – healthy control, D – untreated diabetic, PRV10 – diabetic treated with 10 mg kg^{-1} day^{-1} pravastatin, PRV20 – diabetic treated with 20 mg kg^{-1} day^{-1} pravastatin, RSV10 – diabetic treated with 10 mg kg^{-1} day^{-1} rosuvastatin, RSV20 – diabetic treated with 20 mg kg^{-1} day^{-1} rosuvastatin

[a] Mean ± SD ($n = 7$).

Significant difference (Bonferroni correction *post hoc* test): [b] $p \leq 0.003$ compared with the C group; [c] $p \leq 0.003$ compared with the D group; [d] $p \leq 0.003$ compared with the RSV10 group; [e] $p \leq 0.003$ compared with the RSV20 group.

treated with 40 mg day^{-1} rosuvastatin, but in another study, it was shown that rosuvastatin treatment caused an increase in insulin resistance (34). The effect of statins on insulin level and insulin resistance might vary according to factors such as characteristics of the subjects used, the type and the dose of the drug, and the duration of the use. Furthermore, it was also shown that rosuvastatin treatment is associated with a significant dose-dependent increase in insulin resistance. Similarly, intensive-dose statin therapy resulted in an increased risk of developing diabetes compared to moderate-dose statin therapy (14, 35).

Glycogen metabolism

Table III shows the liver glycogen content and the enzymes related to glycogen metabolism in C and all experimental groups. Significant decreases in liver glycogen contents and GS levels were observed while there were significant increases in GP levels in all experimental groups compared to the C group (all, $p \leq 0.003$). Glycogen content was higher in the PRV20 group ($p \leq 0.003$) but lower in the RSV20 group ($p \leq 0.003$) than in the D group. GS levels increased significantly in both PRV10 and PRV20 groups (both, $p \leq 0.003$) while decreased significantly in both RSV10 and RSV20 groups (both, $p \leq 0.003$) compared to the D group. However, GP levels significantly increased in RSV10 and RSV20 groups (both, $p \leq 0.003$) but decreased in PRV10 and PRV20 compared to the D group (both, $p \leq 0.003$).

Glycogen content in various tissues such as skeletal muscles and the liver indicates insulin activity since insulin regulates glycogen storage by stimulating glycogen synthase and inhibiting glycogen phosphorylase enzymes (23, 39). It has been shown that glycogen content and GS activity are decreased and GP activity is increased in STZ-induced diabetic rats (2, 23, 39). Similar to these previous studies, our study also showed that there were significant decreases in GS levels but significant increases in GP levels in all diabetic groups compared to the C group. As a result of these changes, we also observed significant decreases in liver glycogen levels in all diabetic groups compared to the C group. Both doses of pravastatin caused a significant increase in GS levels but a decrease in GP levels in our diabetic rats. Consequently, an increase in glycogen level was observed, especially in the diabetic group treated with 20 mg kg^{-1} day^{-1} pravastatin. However, both doses of rosuvastatin decreased the GS and increased the GP levels and consequently, a decrease in glycogen level was observed, especially in the diabetic group treated with 20 mg kg^{-1} day^{-1} rosuvastatin. In the literature review, we could not find any study examining the effects of statin therapy on enzymes related to liver glycogen metabolism. Based on our results, 20 mg kg^{-1} day^{-1} dose of pravastatin might have positive effects on glycogen metabolism while rosuvastatin might have negative effects.

Glucose metabolism

Table IV shows the gluconeogenic and glycolytic enzymes related to glucose metabolism in the control and all the experimental groups. To the best of our knowledge, this is the first study to measure the effects of pravastatin and rosuvastatin on enzymes related to glucose metabolism.

Gluconeogenic enzymes. – The levels of LDH, G6Pase, and FBP1 were significantly increased in all experimental groups compared to the C group (all, $p \leq 0.003$). LDH levels were lower in both PRV10 and PRV20 groups than in the D group (both, $p \leq 0.003$). G6Pase level

Table III. Glycogen metabolism in control and experimental animals

Parameter	Group					
	C[a]	D[a]	PRV10[a]	PRV20[a]	RSV10[a]	RSV20[a]
Glycogen (mg g^{-1} tissue)	41.76 ± 2.13	19.22 ± 2.10[b]	24.1 ± 2.27[b]	25.87 ± 1.97[b,c]	17.38 ± 2.32[b]	15.44 ± 1.52[b,c]
GS (ng g^{-1} protein)	758.64 ± 11.82	437.69 ± 11.83[b]	509.9 ± 17.61[b,c]	519.64 ± 14.91[b,c]	383.72 ± 20.06[b,c]	350.33 ± 10.03[b,c]
GP (ng g^{-1} protein)	607.97 ± 21.33	791.03 ± 22.74[b]	729.43 ± 15.95[b,c]	704.5 ± 22.80[b,c]	839.04 ± 13.97[b,c]	895.56 ± 14.27[b,c]

C – healthy control, D – untreated diabetic, PRV10 – diabetic treated with 10 mg kg^{-1} day^{-1} pravastatin, PRV20 – diabetic treated with 20 mg kg^{-1} day^{-1} pravastatin, RSV10 – diabetic treated with 10 mg kg^{-1} day^{-1} rosuvastatin, RSV20 – diabetic treated with 20 mg kg^{-1} day^{-1} rosuvastatin

[a] Mean ± SD (n = 7).

Significant difference (Bonferroni correction *post hoc* test): [b] $p \leq 0.003$ compared with the C group; [c] $p \leq 0.003$ compared with the D group.

Table IV. Enzymes related to glucose metabolism in control and experimental animals

Parameter	Group					
	C[a]	D[a]	PRV10[a]	PRV20[a]	RSV10[a]	RSV20[a]
Gluconeogenic enzymes						
LDH (ng g^{-1} protein)	254.07 ± 7.74	353.58 ± 10.24[b]	301.61 ± 17.36[b,c]	287.44 ± 17.03[b,c]	361.34 ± 14.47[b]	374.89 ± 15.89[b]
FBP1 (ng g^{-1} protein)	461.64 ± 25.51	825.67 ± 21.85[b]	786.86 ± 19.78[b]	777.62 ± 19.75[b]	857.24 ± 23.13[b]	868.35 ± 27.15[b]
G6Pase (ng g^{-1} protein)	971.25 ± 37.18	1878.65 ± 62.18[b]	1784.64 ± 41.96[b]	1653.96 ± 95.33[b,c]	1934.73 ± 76.61[b]	2011.76 ± 103.72[b]
Glycolytic enzymes						
HK (μg mg^{-1} protein)	252.61 ± 5.85	131.35 ± 3.53[b]	137.48 ± 4.97[b]	140.85 ± 3.84[b]	127.66 ± 4.46[b]	124.56 ± 3.47[b]
PK (μg mg^{-1} protein)	206.94 ± 5.02	101.53 ± 3.98[b]	107.09 ± 4.93[b]	111.8 ± 4.48[b]	96.98 ± 2.55[b]	92.28 ± 3.49[b,c]
G6PD (ng g^{-1} protein)	512.19 ± 26.84	250.79 ± 10.83[b]	291.94 ± 19.01[bc]	303.25 ± 14.49[b,c]	240.04 ± 11.54[b]	228.09 ± 17.10[b]

C – healthy control, D – untreated diabetic, PRV10 – diabetic treated with 10 mg kg^{-1} day^{-1} pravastatin, PRV20 – diabetic treated with 20 mg kg^{-1} day^{-1} pravastatin, RSV10 – diabetic treated with 10 mg kg^{-1} day^{-1} rosuvastatin, RSV20 – diabetic treated with 20 mg kg^{-1} day^{-1} rosuvastatin

[a] Mean ± SD (n = 7).

Significant difference (Bonferroni correction *post hoc* test): [b] $p \leq 0.003$ compared with the C group; [c] $p \leq 0.003$ compared with the D group.

was significantly lower in the PRV20 group than in the D group ($p \leq 0.003$). LDH levels were not significantly different in both RSV10 and RSV20 groups compared to the D group ($p = 0.565$ and $p = 0.025$, resp.). G6Pase levels did not show any significant differences in both RSV10 and RSV20 groups compared to the D group ($p = 0.225$ and $p = 0.018$, resp.). FBP1 levels did not show significant differences between any statin-treated group (PRV10, PRV20, RSV10, and RSV20, resp.) and the D group ($p = 0.006$, $p = 0.004$, $p = 0.025$, and $p = 0.018$, resp.). However, there was a trend for FBP1 levels to decrease in PRV10 ($p = 0.006$) and PRV20 ($p = 0.004$).

LDH is an enzyme that plays an important role in obtaining energy under anaerobic conditions. It has been shown that LDH activity increases in diabetes (40). Increased LDH level affects normal glucose metabolism and insulin secretion in pancreatic β cells and may be directly responsible for insulin secretion defects in diabetes. Also, high levels of LDH in hepatic tissue indicate increased cell damage (23). In our study, hepatic LDH level was found to be significantly higher in all diabetic rats compared to the C group (all, $p \leq 0.003$). Both 10 and 20 mg kg^{-1} day^{-1} doses of pravastatin significantly decreased the LDH levels in diabetic rats (both, $p \leq 0.003$). This might be one of the causes of the significant decrease in fasting blood glucose levels in the PRV20 group.

G6Pase plays a key role in the gluconeogenesis and glycogenolysis pathway and catalyzes the hydrolysis of G6P to glucose, resulting in an increase in blood glucose level; glucose production can be slowed down by suppression of G6Pase activity (41). Increases in fasting blood glucose levels are accompanied by increases in G6Pase levels (23, 42). Our study also showed that G6Pase levels were significantly increased in all diabetic rats compared to the C group (all, $p \leq 0.003$) but 20 mg kg^{-1} day^{-1} of pravastatin decreased the G6Pase levels in diabetic rats. This might be the reason for decreased fasting blood glucose levels and increased glycogen content in the PRV20 group.

FBP1 catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate. This step is necessary for the reversal of glycolysis. The activity of this enzyme in diabetic animals increases due to insulin deficiency leading to hyperglycemia (43). FBP1 levels were increased in all experimental groups of diabetic rats. Both pravastatin doses of 10 and 20 mg kg^{-1} day^{-1} did not cause any significant effect on FBP1 levels in our diabetic rats ($p = 0.006$ and $p = 0.004$, resp.). Similarly, rosuvastatin doses of 10 and 20 mg kg^{-1} day^{-1} also did not have any significant effect on FBP1 levels in the diabetic rats ($p = 0.025$ and $p = 0.018$, resp.). However, there was a trend for a decrease in FBP1 concentrations in the diabetic rats treated with 10 and 20 mg kg^{-1} day^{-1} doses of pravastatin ($p = 0.006$ and $p = 0.004$, resp.). With pravastatin treatment resulting in a trend for a decrease in FBP1 concentration at both doses, pravastatin might be more efficacious than rosuvastatin in reducing FBP1 concentrations in diabetic rats.

According to the above results, it seems that pravastatin might reduce diabetes by decreasing gluconeogenic enzyme levels, especially LDH and G6Pase.

Glycolytic enzymes. – The levels of HK, PK, and G6PD significantly decreased in all experimental groups compared to the C group (all, $p \leq 0.003$). HK levels did not show any significant differences between PRV10, PRV20, RSV10, and RSV20, resp.) and D group ($p = 0.035$, $p = 0.004$, $p = 0.277$, and $p = 0.013$, resp.). Nevertheless, a trend for the HK level to increase in the PRV20 group ($p = 0.004$) is evident. PK level was lower in the RSV20 group than in the D group ($p \leq 0.003$). G6PD levels were higher in both PRV10 and PRV20 groups than in the D group (both, $p \leq 0.003$).

HK is the first regulatory enzyme of the glycolytic pathway involved in glucose oxidation. Since HK is an insulin-dependent enzyme, hepatic HK activity is significantly inhibited in diabetic rats. This inhibition leads to a significant decrease in the rate of glucose utilization and, consequently, results in hyperglycemia (42). We also determined that HK level was significantly reduced in all diabetic groups compared to the C group (all, $p \leq 0.003$). There was a trend for an increase in HK level in the diabetic rats treated with the 20 mg kg^{-1} day^{-1} dose of pravastatin ($p = 0.004$). 20 mg kg^{-1} day^{-1} dose of pravastatin might be the most efficacious among the examined doses of statin treatments to increase HK levels in diabetic rats.

PK is a ubiquitously expressed key glycolytic enzyme. Changes in PK expression affect glucose metabolism and energy production. We found that PK levels were significantly lower in all diabetic groups compared to the C group. Similarly, in a previous study, the liver PK levels had been significantly decreased in streptozotocin-induced diabetic rats (23). The decrease in liver PK level might indicate that glucose utilization is decreased and, consequently, energy balance is changed in diabetic rats. PK level was also lower in the RSV20 group than in the D group. The significant decrease in PK level observed especially in the RSV20 group suggests that diabetic complications may be more severe in this group.

G6PD contributes to the synthesis of fats from carbohydrates and ultimately lowers plasma glucose levels. An increase in liver G6PD levels increases glucose entry into the pentose monophosphate shunt, resulting in a decrease in fasting blood glucose levels (44). It was noticed that hepatic G6PD activity was lowered in diabetic rats (45). Similarly, we also have found that G6PD levels to be significantly decreased in all diabetic groups compared to the C group. Interestingly, significant improvement in G6PD levels was observed in our diabetic rats treated with both 10 and 20 mg kg^{-1} day^{-1} doses of pravastatin. According to our results, this improvement in G6PD level is parallel to the decrease in fasting glucose level in our PRV20 group. This might show an improvement in glucose utilization in diabetic rats treated with 20 mg kg^{-1} day^{-1} dose of pravastatin.

CONCLUSIONS

To the best of our knowledge, this is the first study to measure the effects of statin derivatives, pravastatin, and rosuvastatin, on liver enzymes related to carbohydrate metabolism in diabetic rats. We found that pravastatin was more effective in balancing the carbohydrate metabolism than rosuvastatin. Both doses of pravastatin (10 mg kg^{-1} day^{-1} and 20 mg kg^{-1} day^{-1}) increased the body mass in diabetic rats. The dose of 20 mg kg^{-1} day^{-1} pravastatin decreased the blood glucose level, but increased the liver glycogen content. Moreover, pravastatin, especially at the dose of 20 mg kg^{-1} day^{-1}, increased the liver glycogen synthase and glucose 6-phosphate dehydrogenase levels, but decreased the levels of glycogen phosphorylase, lactate dehydrogenase, and glucose-6-phosphatase. Although both doses of pravastatin partially ameliorated the adverse changes in liver enzymes caused by diabetes, 20 mg kg^{-1} day^{-1} dose of pravastatin was better in improving fasting blood glucose level and liver glycogen content. On the other hand, rosuvastatin, especially at the dose of 20 mg kg^{-1} day^{-1}, reduced the liver glycogen synthase and pyruvate kinase levels, but increased the glycogen phosphorylase level. Rosuvastatin, especially at the dose of 20 mg kg^{-1} day^{-1}, also reduced the body mass and liver glycogen content in diabetic rats. Therefore, it can be concluded that pravastatin has a more positive effect on

improving carbohydrate metabolism than rosuvastatin. These findings suggest that pravastatin might be considered a good choice for patients with DM and hypercholesterolemia. However, there are some limitations of this study like that only two statins with low and moderate doses were used. Different types of statins, different doses, and different time points are foreseen to be studied in future investigations. Further studies are also needed to elucidate the mechanisms by which these liver enzymes are affected by statins.

Abbreviations, acronyms, codes. – BSA – bovine serum albumin, DM – diabetes mellitus, FBP1 – fructose-1,6-bisphosphatase, G6Pase – glucose-6-phosphatase, G6PD – glucose 6-phosphate dehydrogenase, GP – glycogen phosphorylase, GS – glycogen synthase, HK – hexokinase, HOMA-IR – homeostasis model assessment of insulin resistance, LDH – lactate dehydrogenase, PK – pyruvate kinase, PBS – phosphate-buffered saline, PRV – pravastatin, RSV – rosuvastatin, STZ – streptozotocin.

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REFERENCES

1. J. C. Pickup and G. Williams, *Textbook of Diabetes*, 3rd ed., Blackwell Science, Oxford 2003, pp. 103–114.
2. T. Tella, B. Masola and S. Mukaratirwa, The effect of *Psidium guajava* aqueous leaf extract on liver glycogen enzymes, hormone sensitive lipase and serum lipid profile in diabetic rats, *Biomed. Pharmacother.* **109** (2019) 2441–2446; https://doi.org/10.1016/j.biopha.2018.11.137
3. J. D. Colbert and J. A. Stone, Statin use and the risk of incident diabetes mellitus: a review of the literature, *Can. J. Cardiol*. **28**(5) (2012) 581–589; https://doi.org/10.1016/j.cjca.2012.03.021
4. T. Yada, M. Nakata, T. Shiraishi and M. Kakei, Inhibition by simvastatin, but not pravastatin, of glucose-induced cytosolic Ca^{2+} signalling and insulin secretion due to blockade of L-type Ca^{2+} channels in rat islet beta-cells, *Br. J. Pharmacol*. **126**(5) (1999) 1205–1213; https://doi.org/10.1038/sj.bjp.0702397
5. M. J. Ko, A. J. Jo, Y. J. Kim, S. H. Kang, S. Cho, S. Jo, C. Park, S. Yun, W. J. Lee and D. Park, Time- and dose-dependent association of statin use with risk of clinically relevant new-onset diabetes mellitus in primary prevention: A nationwide observational cohort study, *J. Am. Heart Assoc.* **8**(8) (2019) e011320; https://doi.org/10.1161/JAHA.118.011320
6. Z. Zhou, A. J. Curtis, M. E. Ernst, J. Ryan, S. Zoungas, R. Wolfe, J. J. McNeil, A. M. Murray, C. M. Reid, E. K. Chowdhury, R. L. Woods, A. M. Tonkin and M. R. Nelson, Comparison of statins for primary prevention of cardiovascular disease and persistent physical disability in older adults, *Eur. J. Clin. Pharmacol*. **78**(3) (2022) 467–476; https://doi.org/10.1007/s00228-021-03239-1
7. K. K. Koh , P. C. Oh, I. Sakuma, Y. Lee, S. H. Han and E. K. Shin, Rosuvastatin dose-dependently improves flow-mediated dilation, but reduces adiponectin levels and insulin sensitivity in hypercholesterolemic patients, *Int. J. Cardiol*. **15**(223) (2016) 488–493; https://doi.org/10.1016/j.ijcard.2016.08.051

8. P. M. Ridker, E. Danielson, F. A. H. Fonseca, J. Genest, A. M. Gotto, Jr., J. J. Kastelein, W. Koenig, P. Libby, A. J. Lorenzatti, J. G. MacFadyen, B. G. Nordestgaard, J. Shepherd, J. T. Willerson and R. J. Glynn (for JUPITER Study Group), Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein, *N. Engl. J. Med.* **359**(21) (2008) 2195–2207; https://doi.org/10.1056/NEJMoa0807646
9. G. Danaei, L. A. Garcia Rodriguez, O. Fernandez Cantero and M. A Hernan, Statins and risk of diabetes: an analysis of electronic medical records to evaluate possible bias due to differential survival, *Diabetes Care* **36**(5) (2013) 1236–1240; https://doi.org/10.2337/dc12-1756
10. K. L. Wang, C.-J. Liu, T.-F. Chao, C.-M. Huang, C.-H. Wu, S.-J. Chen, T.-J. Chen, S.-J. Lin and C.-E. Chiang, Statins, risk of diabetes, and implications on outcomes in the general population, *J. Am. Coll. Cardiol.* **60**(14) (2012) 1231–1238; https://doi.org/10.1016/j.jacc.2012.05.019
11. N. Sattar, D. Preiss, H. M. Murray, P. Welsh, B. M. Buckley, A. J. M. de Craen, S. R. K. Seshasai, J. J. McMurray, D. J. Freeman, J. W. Jukema, P. W. Macfarlane, C. J. Packard, D. J. Stott, R. G. Westendorp, J. Shepherd, B. R. Davis, S. L. Pressel, R. Marchioli, R. M. Marfisi, A. P. Maggioni, L. Tavazzi, G. Tognoni, J. Kjekshus, T. R. Pedersen, T. J. Cook, A. M. Gotto, M. B. Clearfield, J. R. Downs, H. Nakamura, Y. Ohashi, K. Mizuno, K. K. Ray and I. Ford, Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials, *Lancet* **375**(9716) (2010) 735–742; https://doi.org/10.1016/S0140-6736(09)61965-6
12. D. J. Freeman, J. Norrie, N. Sattar, R. D. G. Neely, S. M. Cobbe, I. Ford, C. Isles, A. R. Lorimer, P. W. Macfarlane, J. H. McKillop, C. J. Packard, J. Shepherd and A. Gaw, Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland coronary prevention study, *Circulation* **103**(3) (2001) 357–362; https://doi.org/10.1161/01.cir.103.3.357
13. S. Lim, I. Sakuma, M. J. Quon and K. K. Koh, Potentially important considerations in choosing specific statin treatments to reduce overall morbidity and mortality, *Int. J. Cardiol.* **167** (2013) 1696–1702; https://doi.org/10.1016/j.ijcard.2012.10.037
14. S. Parida, T. R. Swain, S. N. Routray and R. Maiti, Effect of atorvastatin on glycaemic parameters in normoglycaemic and prediabetic subjects: A prospective, panel study, *J. Clin. Diagn. Res.* **11**(2) (2017) FC04-FC09; https://doi.org/10.7860/JCDR/2017/23741.9427
15. M. J. Crespo and J. Quidgley, Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats, *World J. Diabetes* **6**(10) (2015) 1168–1178; https://doi.org/10.4239/wjd.v6.i10.1168
16. J. J. Min, B. S. Shin, J. H. Lee, Y. Jeon, D. K. Ryu, S. Kim and Y. H. Shin, Effects of pravastatin on type 1 diabetic rat heart with or without blood glycemic control, *J. Diabetes Res.* **28** (2018) Article ID 1067853 (9 pages); https://doi.org/10.1155/2018/1067853
17. N. Ozturk, N. Yaras, A. Ozmen and S. Ozdemir, Long-term administration of rosuvastatin prevents contractile and electrical remodelling of diabetic rat heart, *J. Bioenerg. Biomembr.* **45**(4) (2013) 343–352; https://doi.org/10.1007/s10863-013-9514-z
18. K. Tarhzaoui, P. Valensi, G. Leger, F. Cohen-Boulakia, R. Lestrade and A. Behar, Rosuvastatin positively changes nerve electrophysiology in diabetic rats, *Diabetes Metab. Res. Rev.* **25**(3) (2009) 272–278; https://doi.org/10.1002/dmrr.920
19. D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher and R. C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia* **28**(7) (1985) 412–419; https://doi.org/10.1007/BF00280883
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* **193**(1) (1951) 265–275.
21. P. Subash Babu, S. Prabuseenivasan and S. Ignacimuthu, Cinnamaldehyde-a potential antidiabetic agent, *Phytomedicine* **14**(1) (2007) 15–22; https://doi.org/10.1016/j.phymed.2006.11.005

22. D. Mondol, M. N. Islam, S. Biswas, P. Jodder, S. Sana, M. A. Saleh and M. R. Islam, Investigation of the synergistic effect of glimepiride and rosuvastatin on alloxan-induced diabetic rat, *J. Diabetes Metab. Disord.* **19**(2) (2020) 1415–1422; https://doi.org/10.1007/s40200-020-00662-6

23. K. Gothandam, V. S. Ganesan, T. Ayyasamy and S. Ramalingam, Antioxidant potential of the aflavin ameliorates the activities of key enzymes of glucose metabolism in high fat diet and streptozotocin-induced diabetic rats, *Redox Rep.* **24**(1) (2019) 41–50; https://doi.org/10.1080/13510002.2019.1624085

24. I. Dhananjayan, S. Kathiroli, S. Subramani and V. Veerasamy, Ameliorating effect of betanin, a natural chromoalkaloid by modulating hepatic carbohydrate metabolic enzyme activities and glycogen content in streptozotocin-nicotinamide induced experimental rats, *Biomed. Pharmacother.* **88** (2017) 1069–1079; https://doi.org/10.1016/j.biopha.2017.01.146

25. N. Abboud and R. Makhous, The effect of some statins on glucose blood levels in experimental animals, *Res. J. Pharm. Technol.* **15**(6) (2022) 2661–2666; https://doi.org/10.52711/0974-360X.2022.00445

26. Y. Yu, K. Ohmori, Y. Chen, C. Sato, H. Kiyomoto, K. Shinomiya, H. Takeuchi, K. Mizushige and M. Kohno, Effects of pravastatin on progression of glucose intolerance and cardiovascular remodeling in a type II diabetes model, *J. Am. Coll. Cardiol.* **44**(4) (2004) 904–913; https://doi.org/10.1016/j.jacc.2004.04.050

27. T. Takagi, M. Matsuda, M. Abe, H. Kobayashi, A. Fukuhara, R. Komuro, S. Kihara, M. J. Caslake, A. McMahon, J. Shepherd, T. Funahashi and I. Shimomura, Effect of pravastatin on the development of diabetes and adiponectin production, *Atherosclerosis* **196**(1) (2008) 114–121; https://doi.org/10.1016/j.atherosclerosis.2007.02.013

28. S. Sugiyama, H. Fukushima, K. Kugiyama, H. Maruyoshi, S. Kojima, T. Funahashi, T. Sakamoto, Y. Horibata, K. Watanabe, H. Koga, K. Sugamura, F. Otsuka, I. Shimomura and H. Ogawa, Pravastatin improved glucose metabolism associated with increasing plasma adiponectin in patients with impaired glucose tolerance and coronary artery disease, *Atherosclerosis* **194**(2) (2007) E43-E51; https://doi.org/10.1016/j.atherosclerosis.2006.08.023

29. A. Keech, D. Colquhoun, J. Best, A. Kirby, R. J. Simes, D. Hunt, W. Hague, E. Beller, M. Arulchelvam, J. Baker and A. Tonkin (for LIPID study group), Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose: results from the LIPID trial, *Diabetes Care* **26**(10) (2003) 2713–2721; https://doi.org/10.2337/diacare.26.10.2713

30. J. Shepherd, G. J. Blauw, M. B. Murphy, E. L. Bollen, B. M. Buckley, S. M. Cobbe, I. Ford, A. Gaw, M. Hyland, J. W. Jukema, A. M. Kamper, P. W. Macfarlane, A. E. Meinders, J. Norrie, C. J. Packard, I. J. Perry, D. J. Stott, B. J. Sweeney, C. Twomey and R. G. Westendorp (for PROSPER study group), PROspective study of pravastatin in the elderly at risk. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial, *Lancet* **360**(9346) (2002) 1623–1630; https://doi.org/10.1016/s0140-6736(02)11600-x.9

31. S. Simsek, C. G. Schalkwijk and B. H. Wolffenbuttel, Effects of rosuvastatin and atorvastatin on glycaemic control in type 2 diabetes – the CORALL study, *Diabet Med.* **29**(5) (2012) 628–631; https://doi.org/10.1111/j.1464-5491.2011.03553.x

32. T. Katabami, M. Murakami, S. Kobayashi, T. Matsui, M. Ujihara, S. Takagi, M. Higa, T. Ichijo, A. Ohta and Y. Tanaka, Efficacy of low-dose rosuvastatin in patients with type 2 diabetes and hypo high-density lipoprotein cholesterolaemia, *J. Int. Med. Res.* **42**(2) (2014) 457–467; https://doi.org/10.1177/0300060513507648

33. D. Xilifu, Z. Tuerxun, B. Nuermaimaiti, A. Aili, N. Rehemu, H. Sun and X. Zhang, Effects of rosuvastatin on serum glucose and insulin in hyperuricemic rats, *BMC Pharmacol. Toxicol.* **23**(1) (2022) Article ID 66 (10 pages); https://doi.org/10.1186/s40360-022-00595-1

34. M. S. Kostapanos, H. J. Milionis, A. D. Agouridis, C. V. Rizos and M. S. Elisaf, Rosuvastatin treatment is associated with an increase in insulin resistance in hyperlipidaemic patients with impaired fasting glucose, *Int. J. Clin. Pract.* **63** (2009) 1308–1313; https://doi.org/10.1111/j.1742-1241.2009.02101.x

35. D. Preiss, S. R. Seshasai, P. Welsh, S. A. Murphy, J. E. Ho, D. D. Waters, D. A. DeMicco, P. Barter, C. P. Cannon, M. S. Sabatine, E. Braunwald, J. J. Kastelein, J. A. de Lemos, M. A. Blazing, T. R. Pedersen, M. J. Tikkanen, N. Sattar and K. K. Ray, Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis, *JAMA* **305**(24) (2011) 2556–2564; https://doi.org/10.1001/jama.2011.860

36. K. K. Koh, M. J. Quon, S. H. Han, Y. Lee, S. J. Kim, J. B. Park and E. K. Shin, Differential metabolic effects of pravastatin and simvastatin in hypercholesterolemic patients, *Atherosclerosis* **204**(2) (2009) 483–490; https://doi.org/10.1016/j.atherosclerosis.2008.09.021

37. J. H. Kim, M. Lee, J. Shin, S. Lee, J. Lee, S. J. You, K. H. Yoon and S. A. Chang, Effects of pravastatin on serum adiponectin levels in female patients with type 2 diabetes mellitus, *Atherosclerosis* **227**(2) (2013) 355–359; https://doi.org/10.1016/j.atherosclerosis.2013.01.045

38. N. Thongtang, M. Ai, S. Otokozawa, T. V. Himbergen, B. F. Asztalos, K. Nakajima, E. Stein, P. H. Jones and E. J. Schaefer, Effects of maximal atorvastatin and rosuvastatin treatment on markers of glucose homeostasis and inflammation, *Am. J. Cardiol.* **107**(3) (2011) 387–392; https://doi.org/10.1016/j.amjcard.2010.09.031

39. K. S. Shali, N. P. P. Soumya, S. Mondal and S. Mini, Hepatoprotective effect of morin via regulating the oxidative stress and carbohydrate metabolism in STZ induced diabetic rats, *Bioact. Compd. Health Dis.* **5**(3) (2022) 53–66; https://doi.org/10.31989/bchd.v5i2.893

40. E. K. Ainscow, C. Zhao and G. A. Rutter, Acute overexpression of lactate dehydrogenase-A perturbs beta-cell mitochondrial metabolism and insulin secretion, *Diabetes* **49**(7) (2000) 1149–1155; https://doi.org/10.2337/diabetes.49.7.1149

41. R. Chen, M. Meseck, R. C. McEvoy and S. L. Woo, Glucose-stimulated and self-limiting insulin production by glucose 6-phosphatase promoter driven insulin expression in hepatoma cells, *Gene Ther.* **7**(21) (2000) 1802–1809; https://doi.org/10.1038/sj.gt.3301306

42. D. Gupta, J. Raju, J. Prakash R. and N. Z. Baquer, Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: effect of insulin and vanadate, *Diabetes Res. Clin. Pract.* **46**(1) (1999) 1–7; https://doi.org/10.1016/s0168-8227(99)00067-4

43. K. Aoki, T. Saito, S. Satoh, K. Mukasa, M. Kaneshiro, S. Kawasaki, A. Okamura and H. Sekihara, Dehydroepiandrosterone suppresses the elevated hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in C57BL/Ksj-db/db mice: comparison with troglitazone, *Diabetes* **48**(8) (1999) 1579–1585; https://doi.org/10.2337/diabetes.48.8.1579

44. Y. Xu, B. W. Osborne and R. C. Stanton, Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex, *Am. J. Physiol. Renal Physiol.* **289**(5) (2005) F1040-F1047; https://doi.org/10.1152/ajprenal.00076.2005

45. B. M. McDermott, P. R. Flatt and J. J. Strain, Effects of copper deficiency and experimental diabetes on tissue antioxidant enzyme levels in rats, *Ann. Nutr. Metab.* **38**(5) (1994) 263–269; https://doi.org/10.1159/000177820