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Inhibitory effects of ursolic acid on hepatic polyol pathway and glucose production in streptozotocin-induced diabetic mice

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Abstract

The effects of ursolic acid on the polyol pathway and glucose homeostasis–related metabolism were examined in the livers of streptozotocin (STZ)-induced diabetic mice fed a high-fat (37% calories from fat) diet for 4 weeks. Male mice were divided into nondiabetic, diabetic control, and diabetic–ursolic acid (0.05% wt/wt) groups. Diabetes was induced by the injection of STZ (200 mg/kg body weight, intraperitoneally). Although an ursolic acid supplement lowered the blood glucose level, it did not affect the plasma leptin and adiponectin levels. The present study shows that the blood glucose levels have a positive correlation with the hepatic sorbitol dehydrogenase activities ($r = 0.39$, $P < .05$). Ursolic acid significantly inhibited sorbitol dehydrogenase activity as well as aldose reductase activity in the liver. The supplementation of ursolic acid significantly increased glucokinase activity, while decreasing glucose-6-phosphatase activity in the livers of STZ-induced diabetic mice. Ursolic acid significantly elevated the hepatic glycogen content compared with the diabetic control group. Supplementation with ursolic acid significantly lowered the plasma total cholesterol, free fatty acid, and triglyceride concentrations compared with the diabetic control group, whereas it normalized hepatic triglyceride concentration. A negative correlation was found between the hepatic triglyceride concentration and blood glucose levels ($r = -0.50$, $P < .01$) in regard to insulin-dependent diabetic mice. The hepatic fatty acid synthase activity was significantly lower in the ursolic acid group than in the diabetic control group, whereas hepatic fatty acid β-oxidation and carnitine palmitoyltransferase activities were significantly higher. These results indicate that ursolic acid may be beneficial in preventing diabetic complications by improving the polyol pathway as well as the lipid metabolism and that it can function as a potential modulator of hepatic glucose production, which is partly mediated by up-regulating glucose utilization and glycogen storage and down-regulating glyconeogenesis in the liver.

1. Introduction

The polyol pathway is an alternative glucose metabolism route. Hyperglycemia induces the polyol pathway in tissues that do not require insulin for glucose uptake purposes [1]. Recent studies indicate that the polyol pathway may be related to hyperglycemia-induced oxidative stress and that there may be a metabolic connection between the polyol pathway and oxidative stress [2,3].

The polyol pathway consists of a 2-step metabolic pathway in which glucose is reduced to sorbitol and then is converted to fructose. Aldose reductase (AR) is the first rate-limiting enzyme of the polyol pathway [4]. It has been suggested that the high level of AR activity may cause functional and structural abnormalities in diabetes and may contribute to the development of late complications [1]. A number of AR inhibitors, such as tolrestat, epalrestat, zenarestat, zopolrestat, and sorbinil, have been found to improve several diabetic complications in animal experiments and have been developed to the point of clinical evaluation. Nonetheless, clinical trials of AR inhibitors in neuropathy and retinopathy have achieved limited success; and some synthetic AR inhibitors have been associated with deleterious side effects and poor penetration of target tissues, such as the nerve and retina [5]. Therefore, in recent years,

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there has been increased interest in identifying natural sources of AR inhibitors that can be tested for their therapeutic value against diabetic complications [6,7].

Ursolic acid (UA) (3β-hydroxy-urs-12-en-28-oic acid, Fig. 1) is a common phytochemical that is naturally found in various plants and many medicinal herbs, such as rosemary, thyme, oregano, and lavender [8]. Ursolic acid is known to possess a wide range of biological functions, including anti-inflammatory [9], hepatoprotective [10], gastroprotective [11], and hypolipidemic activity [12], immunoregulatory effects [13], and anticancer functions [14-16].

The incidence of a new form of diabetes increases in children and adolescents, with the characteristics of a mixture of the type 1 and type 2 diabetes mellitus effects [17]. Overnutrition in childhood may contribute to the increasing incidence rate of childhood-onset type 1 diabetes mellitus that has been reported in many countries [18]. In a previous study, we demonstrated the antidiabetic effects of UA by means of a reduction in the blood glucose levels and increasing insulin levels with the preservation of pancreatic β-cells in streptozotocin (STZ)-induced diabetic mice fed a high-fat diet [19]. Therefore, this study investigated the role of UA in ameliorating the effects of an impaired polyol pathway and the glucoregulatory mechanism in STZ-induced diabetic mice fed high-fat diet.

2. Materials and methods

2.1. Animals and diets

Eight-week-old male ICR mice were purchased from Biogenomics (Seoul, South Korea). The animals were all individually housed in polycarbonate cages in a room at 22°C ± 2°C on a 12-hour light-dark cycle. They were fed a pelletized commercial chow diet for 1 week after arrival, and then the mice were randomly divided into nondiabetic (n = 10) and diabetic (n = 25) groups. Diabetes was induced by a single injection of STZ (200 mg/kg body weight in 0.1 mol/L citrate buffer, pH 4.2) (Sigma, St Louis, MO) into the intraperitoneum. The nondiabetic mice were only injected with the citrate buffer. After 5 days, only mice with plasma glucose levels exceeding 300 mg/dL were identified as diabetic. Diabetic mice were randomly subdivided into a diabetic control group (n = 9) and a diabetic-UA group (n = 9). Thereafter, the mice were fed a high-fat diet (37% calories from fat) (AIN-76 supplemented with beef tallow) [20,21] with or without UA (0.5 g/kg diet; TCI, Tokyo, Japan) for 4 weeks. The mice had free access to food and water ad libitum.

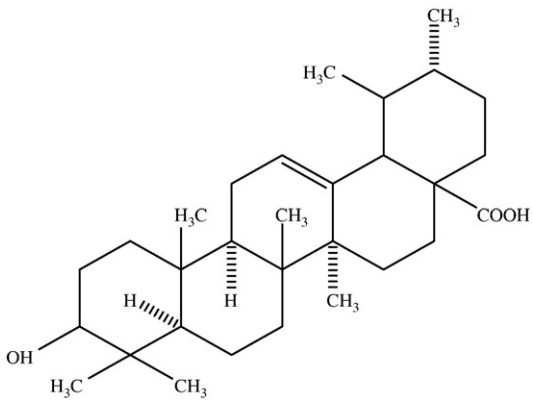

Fig. 1. The chemical structure of UA.

At the end of the experimental period, the mice were anesthetized with ether after the withholding of food for 12 hours; and blood samples were taken from the inferior vena cava to determine the plasma biomarkers. The liver had been removed after collecting the blood, was rinsed with a physiologic saline solution, and was immediately stored at −70°C. The mice were all handled in strict accordance with the Sunchon National University guidelines for the care and use of laboratory animals.

2.2. Blood glucose, plasma leptin, and plasma adiponectin levels

The blood glucose concentration was measured with whole blood obtained from the tail veins after withholding food for 6 hours, using a glucose analyzer, which was based on the glucose oxidase method (GlucoDr SuperSensor; Allmedicus, Anyang, Korea).

Blood was collected in a heparin-coated tube and centrifuged at 600*g* for 15 minutes at 4°C. The plasma leptin (ELISA kit; R&D Systems, Minneapolis, MN) and adiponectin (ELISA kit, R&D Systems) levels were then determined by using a quantitative sandwich enzyme immunoassay kit.

2.3. Hepatic tissue processing

The hepatic enzyme source fraction in the liver was prepared according to the method developed by Hulcher and Oleson [22] with slight modifications. A 20% (wt/vol) homogenate was prepared in a buffer containing 0.1 mol/L of triethanolamine, 0.02 mol/L of EDTA, and 2 mmol/L of dithiothreitol (pH 7.0), and then centrifuged at 600*g* for 10 minutes to discard any cell debris. The supernatant was centrifuged at 10 000*g*, followed by 12 000*g* for 20 minutes at 4°C to remove mitochondrial pellets. Thereafter, the supernatant was ultracentrifuged twice at 100 000*g* for 60 minutes at 4°C to obtain the cytosolic supernatant. The mitochondrial and microsomal pellets were then redissolved in 800 μL of a homogenization buffer, and the protein content was determined by the Bradford method [23] using bovine serum albumin as the standard.

2.4. Polyol pathway enzyme activities

The AR activity was determined by using a spectrophotometric continuous assay as described by Nishimura et al [24] with a slight modification. Enzyme reactions were conducted in 0.1 mol/L of sodium phosphate buffer (pH 6.8)

containing 0.4 mmol/L of ammonium sulfate, 0.1 mmol/L of EDTA, 25 μmol/L of DL-glyceraldehyde, 25 μmol/L of nicotinamide adenine dinucleotide phosphate reduced form (NADPH), and an enzyme. The activity was estimated by measuring differences in the NADPH absorbance values at 340 nm for 5 minutes with DL-glyceraldehyde as a substrate at 25°C. The sorbitol dehydrogenase (SDH) activity was measured by using the Ulrich method [25] with a slight modification. The reaction mixture contained 0.2 mol/L of triethanolamine buffer (pH 7.4), 12 mmol/L of NADH, and an enzyme. The reaction was initiated after 30 minutes by means of treatment with 4 mol/L fructose at 25°C. The activity was estimated by oxidation of the NADH at 340 nm for 5 minutes.

2.5. Hepatic glucose-regulating enzyme activities and glycogen content

The glucokinase (GK) activity was determined by using a spectrophotometric continuous assay as described by Davidson and Arion [26] and Newgard et al [27] with slight modifications, in which the formation of glucose-6-phosphate (G6P) was coupled to its oxidation by G6P dehydrogenase and NAD^+ at 37°C. The glucose-6-phosphatase (G6Pase) activity was determined by using the method of Alegre et al [28] with slight modifications; the reaction mixture contained 40 mmol/L of sodium HEPES (pH 6.5), 14 mmol/L of G6P, 18 mmol/L of EDTA, 2 mmol/L of $NADP^+$, 0.6 IU/mL of mutarotase, and 0.6 IU/mL of glucose dehydrogenase. The phosphoenolpyruvate carboxykinase (PEPCK) activity was monitored in the direction of oxaloacetate synthesis using the spectrophotometric assay developed by Bentle and Lardy [29] with slight modifications. The final volume of the purified enzyme was pipetted with a reaction mixture (pH 7.0) containing 77 mmol/L of sodium HEPES, 1 mmol/L of inosine-5′-diphosphate, 1 mmol/L of $MnCl_2$, 1 mmol/L of dithiothreitol, 0.25 mmol/L of NADH, 2 mmol/L of phosphoenolpyruvate, 50 mmol/L of $NaHCO_3$, and 7.2 U of malic dehydrogenase. The enzyme activity was then measured for 2 minutes at 25°C based on a decrease in the absorbance at 340 nm.

The hepatic glycogen concentration was determined as described previously by Seifter et al [30] with modifications. The liver tissue was homogenized in 5 vol of a 30% (wt/vol) KOH solution and dissolved at 100°C for 30 minutes. The glycogen concentration was determined by treatment with an anthrone reagent and measurement of the absorbance at 620 nm.

2.6. Plasma and hepatic lipid concentrations

The plasma total cholesterol (TC) and triglyceride concentrations were determined using an enzymatic method (Asan Diagnostics, Seoul, Korea). The plasma free fatty acid concentration was determined using an enzymatic colorimetric method (Wako Chemicals, Richmond, VA). The hepatic lipids were extracted using the procedure developed by Folch et al [31], whereas the hepatic cholesterol and triglyceride concentrations were analyzed with the same enzymatic kit as that used in the plasma analysis process.

2.7. Hepatic lipid-regulating enzyme activities

The fatty acid synthase (FAS) activity was determined as described by Nepokroeff et al [32] with slight modifications. The cytosolic enzyme (100 μL) was mixed with 125 mmol/L of potassium phosphate buffer (pH 7.0), 165 μmol/L of acetyl–coenzyme A (CoA), 50 μmol/L of malonyl-CoA, 50 μmol/L of NADPH, 1 mmol/L of β-mercaptoethanol, and 1 mmol/L of EDTA. Absorbance was then measured for 2 minutes at 340 nm (30°C) on a spectrophotometer. The β-oxidation activity was determined as described by Lazarow [33] with slight modifications, in which the reaction was initiated by adding 47 mmol/L of Tris-HCl (pH 8.0), 0.2 mmol/L of NAD, 990 μmol/L of dithiothreitol, 5 μL of albumin (1.5%), 5 μL of Triton X-100 (2%), 0.1 mmol/L of CoA, 0.01 mmol/L of flavin adenine dinucleotide (FAD), 1 mmol/L of potassium cyanide, and 5 μL of the mitochondrial fraction, followed by the addition of 10 μmol/L of palmitoyl-CoA. The formation of NADH was measured for 5 minutes at 340 nm (37°C) on a spectrophotometer. The carnitine palmitoyltransferase (CPT) was assayed spectrophotometrically by following the release of CoA-SH from palmitoyl-CoA using the general thiol reagent 5,5′-dithiobis (2-nitrobenzoate) as described by Bieber et al [34] with slight modifications. The reaction mixture contained 0.1-mL aliquot of a premix containing 232 mmol/L of Tris-HCl (pH 8.0), 1.1 mmol/L of EDTA, 220 μmol/L of L-carnitine, 24 μmol/L of 5,5′-dithiobis (2-nitrobenzoate), 7 μmol/L of palmitoyl-CoA, and 0.09% Triton X-100. The reaction was initiated by the addition of enzymes at 25°C. Absorbance was measured for 2 minutes at 412 nm on a spectrophotometer.

2.8. Statistical analysis

All data are presented as means ± standard error. The data were evaluated by means of 1-way analysis of variance using SPSS software (SPSS, Chicago, IL). The differences between the means were assessed using Duncan multiple-range test, and statistical significance was considered to be valid at $P < .05$. The Pearson correlation coefficients were calculated to examine the association of blood glucose levels with the hepatic SDH activities and hepatic triglycerides, respectively.

3. Results

3.1. Blood glucose, plasma leptin, and plasma adiponectin levels

The blood glucose concentration had been significantly elevated in the diabetic mice, with recorded values being 3 times greater than that in regard to the nondiabetic mice; however, UA supplementation had effectively lowered the blood glucose level by 12.3% (Table 1).

Table 1
The effect of UA on the levels of blood glucose, plasma leptin, and plasma adiponectin in STZ-induced diabetic mice fed a high-fat diet

	Nondiabetic (n = 10)	Diabetic (n = 9)	Diabetic-UA (n = 9)
Blood glucose (mg/dL)	165.40 ± 3.82[a]	503.30 ± 10.91[c]	441.37 ± 14.34[b]
Plasma leptin (μg/mL)	414.75 ± 20.15[a]	132.00 ± 1.61[b]	133.33 ± 7.85[b]
Plasma adiponectin (μg/mL)	1.56 ± 0.08[a]	0.68 ± 0.06[b]	0.73 ± 0.05[b]

Mean ± SE. Means in the same row not sharing a common letter ([abc]) are significantly different between groups ($P < .05$).

Both the plasma leptin and adiponectin levels were significantly lower in the diabetic group than in the nondiabetic group. Ursolic acid did not affect the plasma leptin and adiponectin levels in diabetic mice (Table 1).

3.2. Hepatic polyol pathway enzyme activities

The hepatic AR and SDH activities were significantly higher in the diabetic groups when compared with the nondiabetic group (1.3 and 2.0 times, respectively). However, the supplementation of UA significantly lowered both the AR and SDH activities when compared with the diabetic control group (Fig. 2A, B). The ratio of AR to SDH of diabetic mice was also significantly improved by UA supplementation (Fig. 2C). Significantly, the SDH activity exhibited a positive correlation with blood glucose levels ($r = 0.39$, $P < .05$) (Fig. 2D). Thus, UA significantly inhibits the hepatic polyol pathway.

3.3. Hepatic glucose-regulating enzyme activities and glycogen level

The hepatic GK activity in the diabetic group was 15% that of the nondiabetic group value; however, the UA supplement significantly increased the GK activity when compared with the diabetic control group (Table 2). The G6Pase activity was significantly higher in the diabetic group than in the nondiabetic group; however, UA normalized the G6Pase activity at the same level as that of the nondiabetic mice. In addition, UA supplementation had significantly improved the G6Pase/GK ratio compared with the diabetic control group by 52.8% (Table 2). The PEPCK activity was not significantly different between the groups (Table 2).

The hepatic glycogen concentration in the diabetic group was significantly higher than that in the nondiabetic group. Ursolic acid had elevated the glycogen level in the liver when compared with the diabetic control group (Table 2).

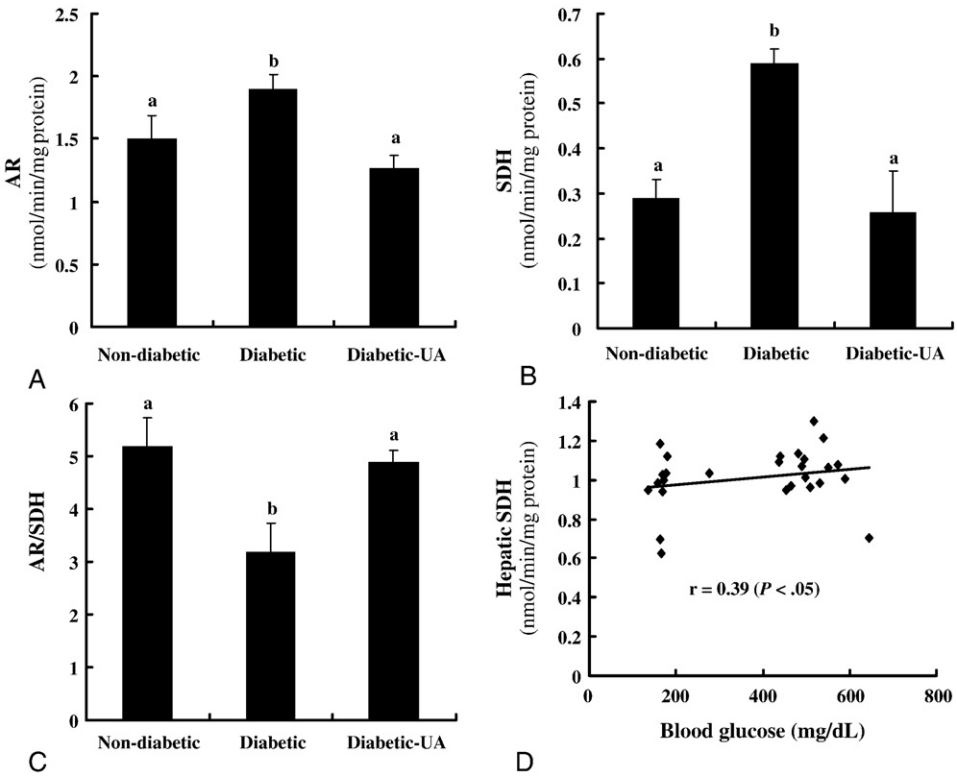

Fig. 2. The effect of UA supplementation on the activities of hepatic AR (A) and SDH (B), AR/SDH ratio (C), and correlation between SDH activities and blood glucose levels (D) in STZ-induced diabetic mice fed a high-fat diet. Values are expressed as means ± SE (nondiabetic, n = 10; diabetic, n = 9; diabetic-UA, n = 9). The means not sharing a common letter ([ab]) are significantly different between groups ($P < .05$).

Table 2
The effect of UA on hepatic glucose metabolism and glycogen content in STZ-induced diabetic mice fed a high-fat diet

	Nondiabetic (n = 10)	Diabetic (n = 9)	Diabetic-UA (n = 9)
GK (nmol/[min mg protein])	22.23 ± 1.33[a]	3.40 ± 0.27[c]	6.17 ± 0.34[b]
G6Pase (nmol/[min mg protein])	82.41 ± 7.28[a]	101.19 ± 4.60[b]	87.91 ± 1.70[a]
G6Pase/GK	3.71 ± 0.31[a]	30.16 ± 6.78[c]	14.25 ± 2.90[b]
PEPCK (nmol/[min mg protein])	30.87 ± 1.30	30.21 ± 1.21	27.21 ± 1.29
Glycogen (mg/g)	40.72 ± 5.17[a]	60.70 ± 4.00[b]	81.65 ± 8.98[c]

Mean ± SE. Means in the same row not sharing a common letter ([abc]) are significantly different between groups ($P < .05$).

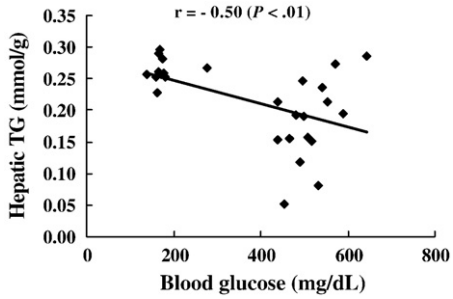

Fig. 3. The correlation between hepatic triglyceride concentration and blood glucose levels in STZ-induced diabetic mice fed a high-fat diet.

3.4. Plasma and hepatic lipids

Supplementation with UA significantly lowered the plasma TC, free fatty acid, and triglyceride concentrations when compared with the diabetic control group (Table 3). The supplementation of UA lowered the hepatic cholesterol concentration, while normalizing the hepatic triglyceride concentration (Table 3). The hepatic triglyceride concentration especially showed a negative correlation with the blood glucose levels ($r = -0.50$, $P < .01$) (Fig. 3).

3.5. Hepatic lipid-regulating enzyme activities

The hepatic lipid-metabolizing enzyme activities are presented in Table 4. The FAS activity was significantly lower in the UA group than in the diabetic control group by 41.3%, whereas the hepatic fatty acid β-oxidation activity and CPT activity were significantly higher in the UA-supplemented group by 31.3% and 21.7%, respectively.

4. Discussion

In our previous study, we demonstrated that UA increased plasma and pancreatic insulin levels with the preservation of pancreatic β-cells in STZ-induced mice fed a high-fat diet [19]. Therefore, we conducted the present study to investigate whether a UA supplement improves the hepatic glucose metabolism, including the polyol pathway, in the STZ-induced diabetic animal model fed a high-fat diet. Some reports suggested that insulin could be an important stimulator of leptin synthesis in rodents as well as in humans [35,36]. Kiess et al [37] suggested that diminished serum leptin levels in insulin-dependent diabetes mellitus patients could be caused by an insulin deficiency and/or increased lipolysis. We found that the plasma insulin concentration had been dramatically lower with declining leptin levels in diabetic control mice by 68% when compared with the nondiabetic mice. The present study showed that UA did not affect the plasma leptin levels in STZ-induced diabetic mice. Furthermore, the body weight and food intake did not differ between the diabetic groups (data not shown). However, our previous study had shown that UA supplements significantly elevated the plasma insulin level (1.18 ± 0.07 vs 0.80 ± 0.08 ng/mL) when compared with the diabetic group [19]. Recently, it has been reported that UA exerted an insulin-sensitizing effect as an insulin receptor activator in vitro [38].

The liver is the principal organ responsible for the maintenance of systemic glucose homeostasis in mammals. The present study showed that the GK activity was dramatically reduced in the diabetic mice when compared with the nondiabetic mice, whereas the G6Pase activity was higher in the liver. Glucokinase catalyzes a key regulatory step in the liver to control the formation of glycogen [39] and also acts as a sensor of glucose-stimulated insulin release in

Table 3
The effect of UA on lipid contents in STZ-induced diabetic mice fed a high-fat diet

	Nondiabetic (n = 10)	Diabetic (n = 9)	Diabetic-UA (n = 9)
Plasma			
Triglyceride (mmol/L)	1.70 ± 0.07[a]	2.06 ± 0.11[b]	1.76 ± 0.11[a]
Free fatty acid (mmol/L)	0.69 ± 0.04[a]	0.83 ± 0.02[b]	0.70 ± 0.03[a]
TC (mmol/L)	5.21 ± 0.25[a]	6.30 ± 0.46[b]	5.32 ± 0.44[a]
Liver			
Triglyceride (mmol/g)	0.26 ± 0.01[a]	0.17 ± 0.01[b]	0.23 ± 0.01[a]
Cholesterol (mmol/g)	0.08 ± 0.003[a]	0.15 ± 0.008[c]	0.12 ± 0.008[b]

Mean ± SE. Means in the same row not sharing a common letter ([ab]) are significantly different between groups ($P < .05$).

Table 4
The effect of UA on hepatic lipid metabolism in STZ-induced diabetic mice fed a high-fat diet

	Nondiabetic (n = 10)	Diabetic (n = 9)	Diabetic-UA (n = 9)
FAS (nmol/[min mg protein])	0.59 ± 0.07[a]	0.46 ± 0.03[a]	0.27 ± 0.05[b]
β-oxidation (nmol/[min mg protein])	15.46 ± 0.89[a]	17.36 ± 1.66[a]	22.80 ± 0.81[b]
CPT (nmol/[min mg protein])	2.51 ± 0.14[a]	2.76 ± 0.21[a]	3.36 ± 0.21[b]

Mean ± SE. Means in the same row not sharing a common letter ([ab]) are significantly different between groups ($P < .05$).

β-cells [40]. A previous study had reported that STZ-induced diabetic rats do not have detectable levels of hepatic GK because of their lack of insulin [41]. However, UA significantly elevated the hepatic GK activity and glycogen content when compared with the diabetic control group. Thus, UA may induce glucose utilization both by the conversion of glucose into glycogen and by the increasing glycolysis. Although the hepatic GK activity of the diabetic control group was 15% that of the nondiabetic value, the glycogen content of diabetic mice was significantly higher in comparison with the nondiabetic mice. This result indicated that early-onset hyperglycemia might adapt the metabolism in diabetic mice. However, UA continuously accelerated the conversion of glucose to glycogen in the hyperglycemic condition, although the exact mechanism was not elucidated. Ursolic acid had significantly inhibited the hepatic G6Pase activity, which catalyzes the dephosphorylation of G6P and represents the ultimate step in glucose production in the liver and kidney. The levels of the key metabolite G6P have been restored in UA-supplemented mice, probably through the combined effects regarding the increase of GK activity and the inhibition of G6Pase. In this study, the ratio of G6Pase/GK had been significantly lowered in the UA-supplemented group in comparison with the diabetic group, suggesting that UA effectively inhibits hepatic glucose production by means of the stimulation of glucose utilization and the suppression of gluconeogenesis.

The present results indicate that UA may be a potential hepatic polyol pathway inhibitor, as evidenced by the down-regulation of hepatic AR and SDH activities when compared with the diabetic control group by 36.8% and 55.9%, respectively. In hyperglycemic conditions, the accelerated flux through the polyol pathway has been suggested as contributing to diabetic complications in the peripheral nerve and other tissues that contain this pathway [42]. Maekawa et al [43] had determined the levels of AR and SDH gene expression in various rat tissues. They had concluded that different tissues contain varying amounts of AR messenger RNA (mRNA) and SDH mRNA; the polyol pathway activity in regard to each tissue differs from each other. Although the liver showed the lowest levels of AR mRNA, it had the most abundant SDH mRNA level as compared with that of the other tissues. Other researchers [44,45] had also reported high levels of SDH mRNA detected in the livers of the rats and mice. Interestingly, we found a positive correlation between blood glucose levels and SDH activities ($r = 0.39$, $P < .05$).

Aldose reductase, the first rate-limiting enzyme, catalyzes the reduction of glucose to sorbitol with the concomitant conversion of NADPH to $NADP^+$ in the polyol pathway. Aldose reductase inhibition has been considered as an ideal target regarding the reduction of any deleterious effects associated with polyol pathway activation [46]. The oxidation of sorbitol to fructose by SDH causes oxidative stress because its cofactor NAD^+ is converted to NADH in the process, and NADH is the substrate for the NADH oxidase to generate reactive oxygen species [47]. It has been reported that the activity and protein level of SDH were highest in the liver [48]. The SDH activity has been reported to be elevated in diabetic rats, therefore leading to increased fructose availability [49]. Because of the fact that fructose and its metabolites, fructose-3-phosphate and 3-deoxyglucosone, are more potent nonenzymatic glycation agents than glucose, the flux of glucose through the polyol pathway may increase the formation of advanced glycation end products, which are known to cause oxidative stress [47]. In this study, an approximately 2-fold increase in the hepatic SDH activity was observed in diabetic mice when compared with nondiabetic mice. Ursolic acid significantly lowered the blood glucose level and hepatic SDH activity when compared with diabetic mice. Latha and Pari [49] also reported that the hepatic SDH activity had increased in STZ-induced rats. This elevated SDH activity in diabetic mice may have been due to the increased availability of sorbitol, as evidenced by the increased AR activity in diabetic mice compared with the control group. Our results showed that UA was able to significantly reduce the AR/SDH ratio in the liver. Thus, this current study illustrated the fact that UA might be expected to create a nontoxic inhibitor of diabetic complications.

Although type 1 diabetes mellitus and type 2 diabetes mellitus originate from different pathogenetic causes, there is a significant association between hyperglycemia and diabetic microvascular complications in both type 1 and type 2 diabetes mellitus [46]. A number of experimental studies have attempted to elucidate the mechanism of hypertriglyceridemia associated with diabetes by using STZ-induced diabetic rats as animal models [50,51]. It is well known that the hypertriglyceridemia in STZ-induced diabetic rats is due solely to a triglyceride catabolism defect because hepatic triglyceride production is suppressed in an insulin-deficient state [52]. Most triglyceride kinetic studies in STZ-induced diabetic rats have revealed that the plasma triglyceride level was elevated, but the triglyceride secretion rate was not increased [53]. We also observed that the plasma triglyceride and TC levels were significantly higher in the diabetic mice in comparison with those of the nondiabetic mice, whereas the hepatic triglyceride had been significantly lowered. Sparks et al [54] reported that apolipoprotein B, an essential apolipoprotein for very low-density lipoprotein assembly, has been substantially degraded in the hepatocytes of STZ-induced rats, thereby inhibiting the assembly of very low-density lipoprotein. Interestingly, we confirmed fact that there is a negative correlation between the blood glucose level and the hepatic triglyceride concentration. Ursolic acid effectively improved the plasma lipid concentration and normalized the hepatic triglyceride content when compared with the diabetic control group. Sharma et al [55] suggested that hypoglycemic and hypolipidemic effects could prevent or be helpful in reducing the complications of lipid profiles, as seen in diabetic patients having hyperglycemia and hypercholesterolemia.

Recently, Jayaprakasam et al [56] reported that the consumption of UA might regulate the lipid metabolism by affecting the hepatic lipid oxidation and lipogenesis in high-fat–fed mice. Accordingly, we had determined the activities of lipid-regulating enzymes in the liver. In this study, the UA supplement markedly reduced the FAS activity in comparison with the control group by approximately 41.3%, with a simultaneous increase of the fatty acid β-oxidation and CPT activity by approximately 31.3% and 21.7%, respectively, in the livers of high-fat–fed diabetic mice. Streptozotocin-induced diabetes resulted in decreasing FAS activity [57], whereas hepatic FAS, in this study, showed an insignificant tendency to decrease. Fatty acid synthase is mainly regulated by the long-term effects of insulin and glucose on enzyme synthesis [58]. When considering that the liver is the major site regarding the anabolism and catabolism of fatty acids, the suppression of hepatic FAS and the stimulation of fatty acid β-oxidation, such as those induced by supplementation with UA, play an important role in diet-induced hyperglycemia in diabetic mice.

In conclusion, these results demonstrate that UA may play a significant role in diabetes mellitus by means of attenuating hyperglycemia, hepatic glucose production, hyperlipidemia, and the influx of glucose through the polyol pathway, although further detailed elucidation is required.

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