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Oxysterols increase in diabetic rats

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Abstract
To address whether diabetes enhances lipid peroxidation and attenuates nitric oxide (NO) generation resulting in tissue complications, we measured oxysterols and NO metabolites (NOx) in the tissues of diabetic Wistar rats. After 4 weeks of streptozotocin injection (STZ, 80 mg/kg, i.p.), we measured 7α- and 7β-hydroperoxycholest-5-en-3β-ol (7α-OOH and 7β-OOH), 7α- and 7β-hydroxycholesterol (7α-OH and 7β-OH) and 7-ketocholesterol (7-keto) by HPLC in the kidneys, heart, and liver. All the oxysterols were much higher in the diabetic than in sham rats, while the extent of the increase was higher in the order of the kidney, heart, and liver. Together with high blood urea nitrogen, the data indicate that the kidney is the predominant target of early diabetic complications. Plasma NOx were decreased by 20% in the STZ rats. The enhanced oxidative stress in diabetes would increase oxysterols by peroxidation, while superoxide is known to reduce NO by reaction to form another potent oxidant peroxynitrite.

Keywords: *Oxysterol, cholesterol hydroperoxide, nitric oxide, lipid peroxide, reactive oxygen species (ROS)*

Introduction

In diabetes, reactive oxygen species (ROS) are produced by NAD(P)H oxidase,[1] microsomal/mitochondrial CYP2E1,[2] mitochondrial respiratory chain,[3] inducible nitric oxide synthase,[4,5] and glycation reaction.[5,6] The ROSs promote lipid peroxidation.

Endothelial NOS (eNOS) generates NO which maintains vasodilatation and tissue circulation. NO scavenges active radicals such as lipid and lipid peroxyl radicals, thereby prevents lipid peroxidation (LPO). However, reduced availability of NO due to excessive ROS causes micro-vascular disturbance, characteristics of diabetes. eNOS also generates ROS by uncoupling this enzyme in diabetes and other diseases.[3,7]

Peroxynitrite ($ONOO^-$) is formed when NO reacts with superoxide at near equimolar ratio.[4,7–9] This peroxynitrite uncouples eNOS to generate superoxide.[7] Peroxynitrite also promotes LPO, thereby inactivates enzymes and inhibits mitochondrial respiration via protein oxidation and nitrotyrosine (NT) modification.[9] Additionally, a proteomics in the heart of diabetic mouse revealed NT modification of the mitochondrial proteins serving for energy production, antioxidant defense, and apoptosis.[10] Moreover, peroxynitrite damages DNA, activates DNA repair enzymes, and triggers cell death.[9] As such, LPOs and peroxynitrite induce diabetic complications in the kidney, retina, heart, liver, etc.[1,4,8,11–19] Therefore, LPOs and NO metabolites (NOx) have been widely measured in the blood or tissues to evaluate the pathological states in diabetes.[1,5,9,12,14,15,17,20,21] 4-Hydroxynonenal (HNE), malondialdehyde (MDA), F_2-isoprostane,

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thiobarbituric acid reacting substances (TBARS) etc. have been used to evaluate oxidative states in the diabetes.[14,15,19,22]

In this study, we measured oxysterols of which metabolism is characterized more precisely than these LPOs. Cholesterol is peroxidized by mitochondrial/microsomal CYP,[23] or oxidized to cholesterol hydroperoxides 7α- and 7β-hydroperoxycholest-5-en-3β-ol (7α-OOH and 7β-OOH) by AMVN and AAPH. 7α- and 7β-OOHs are either reduced to 7α- and 7β-hydroxycholesterol (7α-OH and 7β-OH) or further oxidized to 7-ketocholesterol (7-keto). 7α-OH is also formed by cholesterol 7α-hydroxylase, while 7-keto is formed from 7α-OH by an $NADP^+$-dependent dehydrogenase in the liver microsome.[24]

Oxysterols include variety of cholesterol derivatives such as 7α- and 7β-OOHs, cholesterol α- and β-epoxides, cholesterol-3β-5α-6β-triol, 7-keto, 4α- and 4β-hydroxycholesterols (OHs), 6β-OH, 7α- and 7β-OH, 24-OH, 25-OH, 27-OH.[23] We previously found high levels of oxysterols such as 7α- and 7β-OOH, 7α- and 7β-OH, and 7-keto in various tissues in paraquat intoxication,[25] chronic alcohol intake,[26–28] carbon monoxide poisoning,[29] and alcoholic liver injury,[30] etc. In the present study, we measured the same oxysterols in the kidney, heart, and liver as wells as blood NOx in the STZ diabetic rats.

Materials and methods

Materials

3,5-Di-tert-butyl-4-hydroxytoluene (BHT), luminol (3-aminophthaloylhydrazine) and cytochrome *c* (from horse, type IV) were purchased from Wako Pure Chemical Company (Osaka, Japan) β-Sitosterol (as IS), 7-keto, 7α- and 7β-OH were purchased from Stelaroids (Wilton, NH). 5α-Hydroperoxycholest-6-en-3β-ol (5α-OOH), 7α- and 7β-OOH, and β-sitosterol 5α-hydroperoxide (as IS) were prepared as described previously.[31]

Animals

Under the guidelines for animal experiments of the Kobe University Graduate School of Medicine, male Wistar rats (220–230 g) were fed a standard rat chow diet and had access to water *ad libitum*. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 80 mg/kg body weight) to animals. Four weeks later, under anesthesia with pentobarbital sodium (Abbott Lab, Abbott Park, IL, USA), the kidneys, heart, and liver were harvested on ice, and then kept in 10% formalin at room temperature until assay. Plasma was frozen for assay at −85°C for blood urea nitrogen (BUN) determined by urease-indophenol method, aspartate aminotransferase (AST), creatine kinase (CK-MB), following routine laboratory techniques. Insulin was determined by radioimmunoassay (Rat insulin RIA kit, Linco Research Incoporation, MO). Blood glucose was measured by a glucose analyzer (GLUTEST ACE, Sanwa Chemicals, Nagoya, Japan).

Nitrite and nitrate measurements

A 100-μl plasma sample was deproteinized by mixing with 200-μl of methanol then being centrifuged at 5200*g* for 20 min at 4°C. A 10-μl portion of the supernatant was injected to an HPLC (ENO-10, Eicom, Kyoto, Japan) with a stainless-steel column (NO-PAC. 50 × 4.6 mm I.D.) packed with polystyrene polymer using 10% methanol containing 0.15 mol/l NaCl/NH_4Cl and 0.5 g/l EDTA 4Na as a mobile phase at a flow rate of 0.33 ml/min. Then the nitrate was reduced to nitrite through a reduction column packed with copperized cadmium (NO-RED, 10 × 5 mm I.D., Eicom). The elute was mixed with Griess reagent in a reaction coil, at a flow rate of 0.1 ml/min, and the absorbance was monitored at 540 nm by use of a flow-through spectrophotometer (NOD-10, Eicom).

Extraction

Total lipids were extracted[31] and the cholesterol fraction was isolated by solid phase extraction using a silica column (Sep-Pak-NH_2), as previously described.[31]

HPLC-CL analysis

Cholesterol hydroperoxides were quantified by HPLC with post-column chemiluminescence (HPLC-CL) as previously described.[31] A TSK gel Octyl-80Ts column (Tosoh, Japan) and methanol/water/acetonitrile (89:9:2) as the mobile phase were used.

HPLC-UV analysis of oxysterols

7α-OH, 7β-OH, and 7-keto were determined by HPLC with a UV detector set at 210 and 245 nm as previously described.[27] An Inertsil ODS-2 column (GL Sciences, 5 μm, 150 × 4.6 mm internal diameter) and acetonitrile/methanol/water (46:45:9) as the mobile phase were used.

Statistical analysis

All data are expressed as means ± SD. Differences between the groups were assessed by the Student's *t*-test.

Results

Table I shows characters of the rats. The STZ-treated rat lost 12.1 ± 2.5 g of body weight by 2 days

Table I. Changes of body weight, blood glucose, and serum insulin levels in rats from control and STZ-treated rats.

	Change of body weight (g)		Blood glucose (mg/dl)		Insulin (ng/ml)
	2d	4w	2d	4w	4w
Control					
Mean	+13.2	+142.2	88.6	89.2	4.4
± SD	2.0	7.6	4.8	8.1	1.2
STZ					
Mean	−12.1	+38.0	418	508	0.3
± SD	2.5	17.8	35	75	0.1

Rats were given 80 mg/kg i.p. STZ once.

Table II. 7-Hydroperoxycholesterol concentrations in kidney, heart, and liver of rats from control and STZ groups.

Tissue	*n*	7α-OOH (nmol/g)	7β-OOH (nmol/g)
Kidney			
Control	6	10.6 ± 3.6	27.2 ± 10.5
STZ	7	51.4 ± 12.1	127.4 ± 28.4
X-increase		× 4.8	× 4.7
P		0.00006	0.00002
Heart			
Control	6	3.0 ± 1.5	6.2 ± 4.2
STZ	7	9.5 ± 4.1	21.8 ± 9.6
X-increase		× 3.2	× 3.5
P		0.005	0.004
Liver			
Control	5	22.6 ± 9.0	58.4 ± 23.9
STZ	7	41.5 ± 8.6	92.6 ± 18.7
X-increase		× 1.8	× 1.6
P		0.004	0.019

Values are mean ± SD. 7α-OOH, 7 α-hydroperoxycholest-5-en-3β-ol and 7β-OOH, 7 β-hydroperoxycholest-5-en-3β-ol. Rats were given 80 mg/kg i.p. STZ once.

post-injection. After 28 days, the STZ-rat gained weight only by 38.0 g, as compared with 142.2 g of the control rat. Blood glucose was 4.7 and 5.7-fold higher in the STZ rat than the control rat after 2 days and 4 weeks, respectively. Additionally, insulin level was 6.8% of the control after 4 weeks.

Table II shows that the levels of cholesterol hydroperoxides 7α- and 7β-OOH in the control rats. The 7α- and 7β-OOHs were higher in the order of the liver, kidney, and heart, and that 7β-OOH level was more than twice higher than 7α-OOH level in each tissue. In the diabetic rats, the extents of increases in 7α- and 7β-OOHs were higher in the order of the kidney, heart, and liver (Table II).

Table III shows that the levels of hydroxycholesterols 7α-OH and 7β-OH, 7-keto, and cholesterol. The levels of oxysterols in the control rats were higher in the order of the kidney, liver, and heart. In the diabetic rats, 7α- and 7β-OHs, and 7-keto levels were higher in these tissues than in the control rats, but the extents of the increases were higher in the order of the heart, kidney, and liver. The STZ-treatment did not affect total level of cholesterol in three tissues (Table III). Except for 7β in the liver, 7α- and 7β-OH levels were higher than 7α- and 7β-OOH levels in each tissue (Tables II and III).

Figure 1 shows the percentage of cholesterol hydroperoxide (7α- and 7β-OOHs), hydroxycholesterol (7α- and 7β-OHs), and 7-keto to the sum of these oxysterols. In the kidney of the control rat, 7α- and 7β-OHs are about half of the sum of these oxysterols. 7α- and 7β-OOHs were increased in the STZ-rat, whereas 7α- and 7β-OHs were decreased. In the heart, 7α- and 7β-OOHs were decreased, while 7α- and 7β-OHs were increased. In the liver, 7α- and 7β-OOHs were decreased, whereas 7-keto was increased. Since it is known that 7α- and 7β-OOHs are supposed to be the most injurious to tissues among oxysterols, since the chemical reactivity is the highest because of the successive alignment of hetero-atoms in –OOH. Together with the high plasma level of BUN but not CK and AST (Table IV), the percentage of increase in 7α- and 7β-OOHs to the sum of oxysterols in the kidney but not in the liver and heart explains why kidney rather than the liver or heart is the target organ in the early diabetes. These metabolites were undetectable in the blood (data not shown).

Table III. Changes of oxysterols in kidney, heart, and liver of rats from control and STZ groups.

	n	7α-OH (nmol/g)	7β-OH (nmol/g)	7-keto (nmol/g)	Cholesterol (nmol/g)
Kidney					
Control	6	64.5 ± 36.9	52.3 ± 48.8	74.0 ± 56.7	7451 ± 1857
STZ	7	215.7 ± 90.9	141.6 ± 65.6	258.7 ± 96.3	6306 ± 227
P		0.003	0.019	0.002	0.194
Heart					
Control	5	7.7 ± 3.3	9.3 ± 3.5	21.8 ± 10.3	1744 ± 134
STZ	6	45.5 ± 14.7	39.3 ± 19.8	87.4 ± 37.6	1641 ± 123
P		0.0009	0.015	0.006	0.216
Liver					
Control	6	52.2 ± 15.0	20.8 ± 5.6	43.6 ± 6.9	2334 ± 486
STZ	7	95.0 ± 32.7	63.9 ± 20.8	126.1 ± 32.5	2469 ± 304
P		0.014	0.001	0.0003	0.552

Values are mean ± SD. 7-keto, 3β-hydroxycholest-5-en-7-one; 7β-OH, cholest-5-ene-3β, 7β-diol; and 7α-OH, cholest-5-ene-3β, 7α-diol.

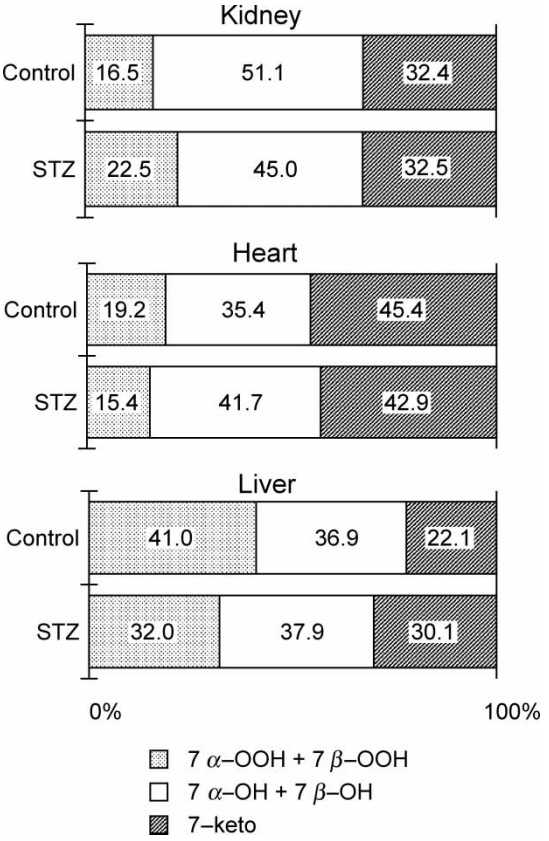

Figure 1. The percentage of cholesterol hydroxide (7α- and 7β-OOHs), hydroxycholesterol (7α- and 7β-OHs), and 7-keto to the sum of the oxysterols.

Histological examination did not show significant changes in the tissues of the STZ rats (data not shown).

In the plasma, NOx were decreased by 20% in the STZ-rats, as compared with the control rats (Table IV), in contrast with the increase in the oxysterols.

Discussion

The major findings of this study were the prominent increase of oxysterols (7α-OOH, 7β-OOH, 7α-OH, 7β-OH, and 7-keto) in diabetic rat tissues using HPLC (Tables II and III), and 20% decrease in plasma NO metabolites (NOx). The increase in the oxysterols after STZ-treatment was the highest in the kidney, in consistent with the increase in BUN, as compared with the liver and heart (Table IV). These results are in line with earlier appearance of diabetic complications in the kidney than the heart and liver. This is the first report on the simultaneous measurements of multiple oxysterols in the three tissues and NOx in the plasma, although it was reported that 7β-OH and 7-keto are increased in the myocardium.[32]

Tissue lipid peroxides (oxysterols) were increased by more than 4-folds in the diabetic rat in this study. The oxysterols were increased much greater than MDA, HNE, etc., as reported previously in diabetic animals and patients.[33–36] MDA is not increased,[33,34] or increased by 1.2-fold[35] in similar tissues after 6–10 weeks of STZ injection. HNE is slightly increased in the aorta after 28 weeks of STZ treatment.[36]

Except for 7β-OH and OOH in the liver, 7α- and 7β-OHs levels were higher than 7α- and 7β-OOH in each diabetic tissue (Tables II and III). The latter is explained by the findings that superoxide dismutase (SOD), and glutathione peroxidase are activated in the tissues of diabetic animal,[35,37] which reduce OOHs to OHs. On the other hand, basal levels of oxysterols were higher in the liver than in the kidney and heart (Tables II and III). The latter may be explained by the high hepatic CYP activity, which is involved in the metabolism of cholesterol and detoxification in general.

The extent of increase in oxysterols was much higher in the diabetic rat (this study) than in various tissues after paraquat administration,[25] chronic alcohol intake,[26–28] or CO exposure,[29] as reported. The increase was at most 2.5-fold for 7β-OOH in the kidneys after paraquat administration,[25] as compared with more than 4-folds increase of oxysterols in diabetes.

Oxysterols are physiological intermediates or products in metabolism and transport of cholesterol.[38] They also enhance transcriptional activation of genes that encode enzymes and ligands of the liver nuclear receptors LXR-α, and -β.[38,39] The latter promotes metabolism of cholesterol, bile acids, and sphingolipids, and suppresses atherogenesis. In contrast, oxysterols constitute oxidized LDL, which promotes smooth muscle cell apoptosis[40] and is a constituent of atheroma or its plaque.[22] Moreover, oxysterols promote cell death, inflammation, immuno-suppression, cell proliferation, cytokine production, and platelet activation.[25,38] Thus, oxysterols are useful markers for lipid peroxidation that reflect various pathological activities.

Table IV. Total NO metabolite level and BUN in rats from control and STZ-treated rats.

		$NO_2^- + NO_3^-$ (μM)	BUN (mg/dl)	CK-MB (IU/l)	AST (IU/l)
Control ($n = 6$)	Mean ± SD	9.18 ± 0.78	23.8 ± 1.0	597 ± 58	89 ± 17
STZ ($n = 7$)	Mean ± SD	7.34 ± 0.83	29.8 ± 2.4	604 ± 84	84 ± 21
P		0.003	0.00005	NS	NS

Rats were given 80 mg/kg i.p. STZ once.

NOx levels in the plasma were decreased by 20% in the STZ-rat, as compared with the control (Table IV). Diabetic state promotes ROS production.[1–5] Although excessive NO neutralizes and eliminates ROS, relative shortage of NO enables ROS to promote lipid peroxidation, while NO also enhances peroxynitrite generation through reaction with ROS.[7,8] Peroxynitrite inactivates various enzymes and proteins, and also promotes lipid peroxidation, DNA damage, and cell death.[8] Thus, our data on mild NOx reduction in contrast with a prominent increase in lipid peroxides suggest that this NOx reduction is a result of the enhanced generation of ROS in diabetic states.

In conclusion, tissue oxysterols prominently increase in association with mild decrease in plasma NO metabolites in the rat 1 month after STZ injection. The kidney may be the most vulnerable to STZ, judging from the highest oxysterol levels and elevation in BUN.

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References

[1] Onozato ML, Tojo A, Goto A, Fujita T, Wilcox CS. Oxidative stress and nitric oxide synthase in rat diabetic nephropathy, effects of ACEI and ARB. Kidney Int 2002;61:186–194.

[2] Raza H, Prabu SK, Robin MA, Avadhani NG. Elevated mitochondrial cytochrome P450 2E1 and glutathione *S*-transferase A4-4 in streptozotocin-induced diabetic rats, tissue-specific variations and roles in oxidative stress. Diabetes 2004;53:185–194.

[3] Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Investig 2001;108:1341–1348.

[4] Schaefer S, Kajimura M, Tsuyama S, Uchida K, Sato E, Inoue M, Suematsu M, Watanabe K. Aberrant utilization of nitric oxide and regulation of soluble guanylate cyclase in rat diabetic retinopathy. Antioxid Redox Signal 2003;5:457–465.

[5] Ihm SH, Yoo HJ, Park SW, Ihm J. Effect of aminoguanidine on lipid peroxidation in streptozotocin-induced diabetic rats. Metabolism 1999;48:1141–1145.

[6] Chappey O, Dosquet C, Wautier MP, Wautier JL. Advanced glycation end products, oxidant stress and vascular lesions," Eur J Clin Investig 1997;27:97–108.

[7] Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Investig 2002;109:817–826.

[8] Cowell RM, Russell JW. Nitrosative injury and antioxidant therapy in the management of diabetic neuropathy. J Investig Med 2004;52:33–44.

[9] Virag L, Szabo E, Gergely P, Szabo C. Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. Toxicol Lett 2003;140-141:113–124.

[10] Turko IV, Li L, Aulak KS, Stuehr DJ, Chang JY, Murad F. Protein tyrosine nitration in the mitochondria from diabetic mouse heart. Implications to dysfunctional mitochondria in diabetes. J Biol Chem 2003;278:33972–33977.

[11] Yavuz D, Kucukkaya B, Haklar G, Ersoz O, Akoglu E, Akalin S. Effects of captopril and losartan on lipid peroxidation, protein oxidation and nitric oxide release in diabetic rat kidneys. Prostaglandins Leukot Essent Fatty Acids 2003;69:223–227.

[12] Muruganandan S, Gupta S, Kataria M, Lal J, Gupta PK. Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. Toxicology 2002;176:165–173.

[13] Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA,3-oxoacid CoA-transferase. Am J Physiol Heart Circ Physiol 2001;281:H2289–H2294.

[14] Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersole, de Strihou C, Monnier VM, Witztum JL, Kurokawa K. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic neuphropathy. J Clin Investig 1997;100:2995–3004.

[15] Zhang W, Khanna P, Chan LL, Campbell G, Ansari NH. Diabetes-induced apoptosis in rat kidneys. Biochem Mol Med 1997;61:58–62.

[16] Lubec B, Aufricht C, Amann G, Kitzmuller E, Hoger H. Arginine reduces kidneys collagen accumulation, cross-linking, lipid peroxidation, glycoxidation, kidneys weight and albuminuria in the diabetic kk mouse. Nephron 1997;75:213–218.

[17] Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. The effect of verapamil on the antioxidant defence system in diabetic kidneys. Clin Chim Acta 2002;322:105–112.

[18] Pekiner B, Ulusu NN, Das-Evcimen N, Sahilli M, Aktan F, Stefek M, Stolc S, Karasu C. *In vivo* treatment with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca^{2+}-ATPase activity in heart and liver of streptozotocin-diabetic rats, comparison with vitamin E. Biochim Biophys Acta 2002;1588:71–78.

[19] Haber CA, Lam TK, Yu Z, Gupta N, Goh T, Bogdanovic E, Giacca A, Fantus IG. *N*-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance *in vivo*, possible role of oxidative stress. Am J Physiol Endocrinol Metab 2003;285:E744–E753.

[20] Davi G, Chiarelli F, Santilli F, Pomilio M, Vigneri S, Falco A, Basili S, Ciabattoni G, Patrono C. Enhanced lipid peroxidation and platelet activation in the early phase of type 1 diabetes mellitus, role of interleukin-6 and disease duration. Circulation 2003;107:3199–3203.

[21] Pekiner BD, Das EN, Nebioglu S. Determination of Ca^{2+}-atpase activity in streptozotocin-induced diabetic rat liver. Diabetes Nutr Metab 2002;15:116–120.

[22] Bagi Z, Koller A, Kaley G. PPARgamma activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with Type 2 diabetes. Am J Physiol Heart Circ Physiol 2004;286:H742–H748.

[23] Brown AJ, Jessup W. Oxysterols and atherosclerosis. Atherosclerosis 1999;142:1–28.

[24] Song W, Pierce WM Jr, Saeki Y, Redinger RN, Prough RA. Endogenous 7-oxocholesterol is an enzymatic product: characterization of 7 alpha-hydroxycholesterol dehydrogenase

activity of hamster liver microsomes. Arch Biochem Biophys 1996;328:272–282.
[25] Adachi J, Tomita M, Yamakawa S, Asano M, Naito T, Ueno Y. 7-Hydroperoxycholesterol as a marker of oxidative stress in rat kidneys induced by paraquat. Free Radic Res 2000; 33:321–327.
[26] Fujita T, Adachi J, Ueno Y, Peters TJ, Preedy VR. Chronic ethanol feeding increases 7-hydroperoxycholesterol and oxysterols in rat skeletal muscle. Metabolism 2002; 51:737–742.
[27] Adachi J, Kudo R, Ueno Y, Hunter R, Rajendram R, Want E, Preedy VR. Heart 7-hydroperoxycholesterol and oxysterols are elevated in chronically ethanol-fed rats. J Nutr 2001;131:2916–2920.
[28] Ariyoshi K, Adachi J, Asano M, Ueno Y, Rajendram R, Preedy VR. Effect of chronic ethanol feeding on oxysterols in rat liver. Free Radic Res 2002;36:661–666.
[29] Kudo R, Adachi J, Uemura K, Maekawa T, Ueno Y, Yoshida K. Lipid peroxidation in the rat brain after CO inhalation is temperature dependent. Free Radic Biol Med 2001;31:1417–1423.
[30] Asano M, Adachi J, Ueno Y. Cholesterol-derived hydroperoxides in alcoholic liver disease. Lipids 1999;34:557–561.
[31] Adachi J, Asano M, Naito T, Ueno Y, Tatsuno Y. Chemiluminescent determination of cholesterol hydroperoxides in human erythrocyte membrane. Lipids 1998; 33:1235–1240.
[32] Matsui H, Okumura K, Mukawa H, Hibino M, Toki Y, Ito T. Increased oxysterol contents in diabetic rat hearts, their involvement in diabetic cardiomyopathy. Can J Cardiol 1997;13:373–379.
[33] Dogru, Pekiner B, Das, Evcimen N, Ulusu NN, Bali M, Karasu C. Effects of vitamin E on microsomal Ca^{2+}-ATPase activity and calcium levels in streptozotocin-induced diabetic rat kidneys. Cell Biochem Funct 2003;21:177–182.
[34] Yue KK, Chung WS, Leung AW, Cheng CH. Redox changes precede the occurrence of oxidative stress in eyes and aorta, but not in kidneys of diabetic rats. Life Sci 2003;73:2557–2570.
[35] Aksoy N, Vural H, Sabuncu T, Aksoy S. Effects of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. Cell Biochem. Funct 2003;21:121–125.
[36] Meng J, Sakata N, Takebayashi S, Asano T, Futata T, Nagai R, Ikeda K, Horiuchi S, Myint T, Taniguchi N. Glycoxidation in aortic collagen from STZ-induced diabetic rats and its relevance to vascular damage. Atherosclerosis 1998; 136:355–365.
[37] Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, Isimer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus, effects of glycemic control. Clin Biochem 2001;34:65–70.
[38] Bjorkhem I, Diczfalusy U. Oxysterols, friends, foes, or just fellow passengers? Arterioscler Thromb Vasc Biol 2002;22:734–742.
[39] Millatt LJ, Bocher V, Fruchart JC, Staels B. Liver X receptors and the control of cholesterol homeostasis, potential therapeutic targets for the treatment of atherosclerosis. Biochim Biophys Acta 2003;1631:107–118.
[40] Panini SR, Sinensky MS. Mechanisms of oxysterol-induced apoptosis. Curr Opin Lipidol 2001;12:529–533.