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Association between Disturbances in Polyunsaturated Fatty Acid Metabolism and Development of Oxidative Stress during Experimental Diabetes Mellitus

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We studied the effect of streptozotocin-induced diabetes mellitus on the level of glycemia and some other indices of lipid metabolism, including fatty acid metabolism and LPO intensity, during the development of diabetes mellitus in rats. Even at the early terms of diabetes development, hypertriglyceridemia and hypercholesterolemia were accompanied by changes in the blood content of fatty acid (at the expense of ω3 and ω6 fatty acids) that persisted throughout the observation period. Intensification of LPO against the background of suppressed activity of antioxidant enzymes and reduced level of ω3 fatty acids attested to the development of oxidative stress. These data attest to antioxidant property of ω3 fatty acids, which is seen from positive correlations between these fatty acids and activity of antioxidant enzymes.

Key Words: *diabetes mellitus; fatty acids; streptozotocin; antioxidants; ω3 fatty acids*

Diabetes mellitus is not only medical, but also social and economic problem all over the world. Life expectancy in 40-year-old patients with type 2 diabetes is reduced by on average 14 years due to the development of various complications; in more than 75% cases, these patients die of cardiovascular diseases [1,2]. Importantly, the use of ω3 polyunsaturated fatty acid (PUFA) elevates cell membrane fluidity thereby increasing the sensitivity of tissues to insulin in patients with type 1 and 2 diabetes mellitus [3,10,12].

The clinical and experimental studies showed LPO activation against the background dyslipidemia and fatty acid metabolism disturbances [5,6,13]. However, molecular mechanisms underlying association between PUFA metabolism disturbances and the development of oxidative stress as well as changes in free radical processes and peculiarities of metabolism of various PUFA families are not completely understood.

This work was designed to examine association between disturbances in PUFA metabolism and the development of oxidative stress in rats with diabetes mellitus.

MATERIALS AND METHODS

Experiments were carried out on male and virgin female Wistar rats (n=70) weighing 80-120 g and aging 8-10 months. Of them, 10 rats comprised the control group. Diabetes mellitus was modeled by single intraperitoneal injection of 2.5% streptozotocin (STZ, 60 mg/kg; Sigma) after 24-48-h fasting. The blood was drawn prior to the experiment and in 3, 7, 14, 21, 28, and 35 days after STZ injection (10 rats per term). The control rats received injection of physiological saline. The rats were sacrificed by xylazine overdose; thereupon, the blood was drawn from the heart.

All procedures with animals were carried out in strict adherence to Directive No. 267 of Ministry of Health of Russian Federation ("On Establishing the Rules of Laboratory Practice", June 19, 2003). The experimental protocols were approved by Bioethics Committee of the N. I. Pirogov Russian National Research Medical University (Protocol No. 93, November 9, 2009). The rats with STZ-induced diabetes

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were characterized with increased blood glucose (by 3-4.5 times), diminished body weight, and polyuria [11]. LPO activity in the blood plasma and erythrocyte membranes was assessed by the content of TBA-reactive products, hydroperoxides and malondialdehyde (MDA) [14]. Catalase activity in the blood was assayed as described elsewhere [4]. For fatty acid assay, the lipids were extracted [7] and hydrolysis and methylation of fatty acids were performed [9]. Liquid chromatography-mass spectrometry (LC-MS) was performed on an ITQ 900 (Thermo Scientific) gas chromatograph. The chromatograph was calibrated with standard mixtures of fatty acid methyl esters (FAME mix, Sigma-Aldrich). The peaks of chromatograms were quantified and identified using Analytica for Windows software and hardware complex based on PC Pentium IV 1800. To this end, Mainlib spectrum libraries and Xcalibur (Thermo) software were also employed.

The data were analyzed statistically using Microsoft Excel software, Student's *t* test, and Spearman correlation test at $p<0.05$. The results are summarized as $m\pm SE$.

RESULTS

On day 3 after STZ injection, glucose levels in the blood and urine increased by 3.2 and 5.7 times, respectively in comparison with the control. The maximum fasting blood sugar level of 24.5±1.8 mmol/liter was attained to postinjection day 28. In these rats, metabolic decompensation was confirmed by a decrease in plasma C-peptide by 39.6% ($p<0.05$) on day 21; water consumption increased by 2.5 times on average, and polyuria was observed. Body weight dramatically decreased by 28, 26, and 39% by days 7, 14, and 21, respectively. The concentration of glucose in the urine in the experimental group varied in the range of 1.9-3.8 mmol/liter.

In 7 days after STZ injection, plasma glucose and glycated hemoglobin significantly increased by 3.52 and 1.95 times, respectively (Table 1). In parallel, cholesterol and triglycerides increased by 30% ($p<0.05$) and 45% ($p<0.05$), respectively. In parallel with hyperglycemia, hypercholesterinemia, and hypertriglyceridemia, the diabetic rats demonstrated elevated MDA and other TBA-reactive products as well as increased plasma concentrations of glycated hemoglobin and lactate ($p<0.05$) attesting to the development of tissue hypoxia and pronounced disturbances in carbohydrate balance.

In experimental rats, the following shifts in lipid metabolism were observed: total cholesterol increased by 53% ($p<0.01$), triglycerides by 16% ($p<0.001$), LDL by 77% ($p<0.001$), and VLDL by 24% ($p<0.05$); the level of HDL decreased by 29.9% ($p<0.05$). The content of TBA-reactive substances increased by 25% due to accumulation of the primary and secondary LPO products. Activity catalase, the enzyme catalyzing decomposition of H_2O_2, decreased by 35% ($p<0.05$). Accumulation of LPO products can result in the appearance of pores in the membranes due to increased content of hydrophilic hydrocarbon tails and to increased membrane rigidity, which can decrease hormone-binding activity of insulin receptors and glucose utilization by cells [5]. Similar data were also obtained previously [15].

In addition, the revealed elevation of plasma MDA (by 52%) in parallel with down-regulated antioxidant activity can indicate activation of peroxidation processes in lipoproteins, which can be a supplementary cause of abnormal affinity of lipoproteins to their receptors that provokes the development of cholesterol plaques in the vascular walls [8,10].

Under conditions of hypoxia provoked by diabetes mellitus, suppression of aerobic oxidative processes in tissues as well as switching of energy processes from hydrocarbon to lipid metabolism impede utilization of free fatty acids (FFA) [14]. During elevation of FFA in diabetic rats, the up-regulated free radical oxidation resulted in significant increase in the contents of hydroperoxides (by more than 2.5 times) and other TBA-reactive products (Table 1). On STZ postinjection day 7, erythrocytic activities of catalase and Zn,Cu-superoxide dismutase decreased ($p<0.01$).

The diabetic mellitus is characterized with abnormal concentrations of such PUFA as α-linoleic, doco-

TABLE 1. Metabolic Changes in Rat Blood on Day 7 after STZ Injection (m±SE, n=10)

Parameter	Control	Diabetes
Glucose, mmol/liter	5.66±0.15	19.95±1.1*
Hb1Ac, %	3.42±0.07	6.69±0.25*
Cholesterol, mmol/liter	2.55±0.11	3.91±0.16*
Triglycerides, mmol/liter	0.91±0.05	2.27±0.09*
LDL, mmol/liter	3.30±0.15	5.97±0.28*
VLDL, mmol/liter	0.72±0.04	0.89±0.04
HDL, mmol/liter	1.57±0.07	1.12±0.05*
TBA-reactive products, μmol/liter	0.48±0.05	0.92±0.03*
Catalase, mcat/liter	6.04±0.19	4.48±0.21*
Hydroperoxides, μmol/liter	6.9±0.03	17.4±0.043*
Superoxide dismutase, U/ml	329±14.47	209±11.23*
Lactate, mmol/liter	2.0±0.2	5.6±0.3**
Pyruvate, mmol/liter	0.08±0.008	0.26±0.01**

Note. *$p<0.01$, **$p<0.001$.

TABLE 2. Fatty Acids in Erythrocytes of Rats with STZ-Induced Diabetes (m±SE, % Total Fatty Acids)

Fatty acids	Control	Diabetes
20:5 (eicosapentaenoic acid)	1.890±0.020	1.003±0.010*
22:6 (docosahexaenoic acid)	4.75±0.23	4.15±0.23*
18:2 (linoleic acid)	14.221±0.140	13.81±0.49*
20:3 (dihomo-γ-linolenic acid)	0.30±0.02	0.75±0.21*
20:4 (arachidonic acid)	13.746±0.479	11.09±0.091*
∑SFA	43.115±1.830	49.98±2.12
∑USFA	43.116±1.910	54.36±0.93
SFA/USFA	1.010	0.91
∑ω3	5.67±0.41	6.28±0.53*
∑ω6	28.68±1.35	25.94±0.79*
∑ω7	1.69±0.05	1.23±0.08*
∑ω9	20.983±1.330	12.19±0.45

Note. *p<0.05. SFA, saturated fatty acids; USFA, unsaturated fatty acids.

sapentaenoic, and arachidonic acids (Table 2), which are precursors of eicosanoids and other biologically active molecules involved in the control of various physiological processes, especially the antioxidant reactions [2,3]. Elevated content of saturated and monounsaturated fatty acids results in elevation of serum LDL, which are easily oxidized and turned into the atherogenic agents under inefficient performance of antioxidant system [6,12]. In diabetic rats, the relative concentration of eicosapentaenoic acid, which possesses the anticoagulant properties, is decreased by 78% in comparison with the control level (p<0.05). This decrease can up-regulate thrombosis, which is an important pathogenetic stage in atherogenesis.

On day 7 after STZ injection, we observed an increase in the levels of myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids in erythrocytes by 56, 45.8, and 24.8%, respectively, in comparison with the control levels (Table 2). It is well known that production of monounsaturated fatty acids from the saturated ones is catalyzed by 9 desaturase. Hyperexpression of this enzyme results in elevation of palmitic acid relatively to stearic one. Up-regulated activity of fatty acid synthase in erythrocytes is evidenced by the fact that in the experimental group, the level of palmitic acid decreased by 15.7% (p<0.05) in comparison with the control value, while the total content of stearic and arachic acids dropped by 38.3% (p<0.05). In contrast to other fatty acids, the level of myristic acid did not decrease in rat erythrocytes, but demonstrated a rising trend (p=0.2) probably because myristic acid is present mostly in triglycerides [6], which can provoke hypertriglyceridemia. On day 7 after STZ injection, the total level of ω3 fatty acids markedly decreased. The total levels of ω6 and ω9 fatty acids also decreased. There were positive correlations between total activity of Cu,Zn-superoxide dismutase and catalase as well as between the levels of α-linolenic, docosahexaenoic, and eicosapentaenoic acids (r=0.53; p<0.05); negative correlations were established between TBA-reactive products and ω6 fatty acids. The increase in the ratio of ω6/ω3 PUFA in the blood is accompanied by LPO intensification (p<0.05 at the expense of ω3 fatty acids). The total ω6/ω3 PUFA ratio negatively correlated with the increase in total activity of SOD+CT (r=-0.763; p<0.05; n=19).

Our experiments showed that dyslipidemia observed at the early stages of streptozotocin-induced diabetes in rats is accompanied by pronounced changes in fatty acid composition of the blood, in particular, in the content ω6 and ω3 PUFA. Intensification of LPO against the background reduced activity of antioxidant enzymes and decreased level of ω3 fatty acids indicates the development of oxidative stress. This study also revealed the antioxidant potency of ω3 fatty acids evidenced by positive correlations between the level of these acids and activity of antioxidant enzymes. The molecular mechanisms of antioxidant effects of ω3 fatty acids need further studies.

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