ENZYME SYSTEMS PROVIDING SUBSTRATES AND COENZYMES FOR HYPERLIPOGENESIS IN NONINSULIN-DEPENDENT DIABETES

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Disturbances of lipid metabolism, expressed as raised serum levels of antherogenic classes of lipoproteins, namely very low (VLDLP) and low (LDLP) density lipoproteins, and the development of atherosclerotic lesions of the blood vessels, are a characteristic feature of noninsulin-dependent diabetes mellitus [5]. The greater part of the VLDLP is known to be synthesized in the liver [1]; the rate of their formation depends directly on the intensity of lipogenesis [15]. Meanwhile, modern views on regulation of lipogenesis in type II diabetes are extremely limited [7], and this is undoubtedly a handicap to the production of effective hypolipidemic agents.

The aim of this investigation was to study enzyme systems providing substrates and coenzymes for lipogenesis as well as fatty acid synthetases, in the liver of C57BL/KsI (db/db) mice with diabetes, the standard model of noninsulin-dependent diabetes mellitus [7].

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/KsI (db/db) mice with diabetes weighing 26.0 ± 1.5 g and on control C57BK/KsI (db/+) and C57BL/KsI (+/+) mice weighing 14.0 ± 0.5 g. To study the intensity of lipogenesis, one group of control animals and of mice with diabetes received 1-^{14}C-acetate, and another group received 2-^{14}C-pyruvate in a dose of 11.1 kBq/g body weight (by intraperitoneal injection 1 h before decapitation), followed by determination of the con-

TABLE 1. Content and Specific Radioactivity of Free Fatty Acids and Total Lipids in Liver of Control Mice and Mice with Diabetes (M ± m, n = 8)

Parameter	Control	Diabetes
Content of:		
FFA, μmoles/g tissue	2,54±0,07	2,64±0,17
TL, mg/g tissue	62,89±3,60	118,56±9,28*
Specific radioactivity after injection of 2-^{14}C-pyruvate:		
FFA, cpm/μmole	128956±14392	347146±35249*
TL, cpm/mg	1766±468	5037±926*
Specific radioactivity after injection of 1-^{14}C-acetate:		
FFA, cpm/μmole	23859±4471	40823±4719*
TL, cpm/mg	557±57	644±80

Legend. Here and in Tables 2 and 3: *p < 0.05.

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TABLE 2. Activity of Enzymes Providing Substrates for Lipogenesis and 2-Oxyglutarate Dehydrogenase in Liver of Control Mice and Mice with Diabetes (n = 8, M ± m)

Parameter	Control	Diabetes
Pyruvate dehydrogenase complex, nmoles $K_3Fe(CN)_6$/mg protein in 20 min	215,75±3,36	222,22±4,46
ATP-citrate lyase, ng Fe hydroxamate/mg protein/min	23,2±1,24	18,6±1,15*
Acetyl-CoA synthetase, nmoles acetyl-CoA/mg protein/min	5,59±0,42	8,71±0,33*
2-oxoglutarate dehydrogenase complex, nmoles $K_3Fe(CN)_6$/mg protein in 20 min	850,05±15,11	998,12±13,94*

tent and specific radioactivity of free fatty acids (FFA) and total lipids (TL) [3] in the liver. Fatty acid synthetase activity in the cytosol was estimated by the method of Frenkel et al. [9].

The supply of substrate for lipogenesis was judged by activity of enzymes involved in acetyl-CoA formation in the mitochondria and cytosol, namely the pyruvate dehydrogenase complex [4], acetyl-CoA synthetase [2], and ATP-citrate lyase [3]; the energy supply for the process was judged by the ATP concentration, determined by an enzymic method using kits from Boehringer (West Germany) and activity of the 2-oxoglutarate-dehydrogenase complex [4]. Activity of cytoplasmic NADP-dependent dehydrogenases [3]: dehydrogenases of the pentose phosphate pathway, NADP-dependent malate dehydrogenase, isocitrate dehydrogenase, and also transketolase [4], transhydrogenase [10], and glutathione reductase [8] — were used to characterize the generation and consumption of NADPH — a coenzyme of fatty acid synthetase. Concentrations of fructose-1,6-diphosphate [6] — an allosteric effector of fatty acid synthetase [13], and of glycerol-3-phosphate [6] — an inducer of ATP-citrate lyase biosynthesis [14], were determined in chloride extracts of the liver [6]. The numerical results were subjected to statistical analysis by Student's standard t test.

EXPERIMENTAL RESULTS

The data in Table 1 demonstrate intensification of lipogenesis in the liver in noninsulin-dependent diabetes. For instance, in experiments with 1-^{14}C-acetate the specific radioactivity of FFA and TL in the liver of mice with diabetes was 1.71 and 1.15 times, respectively higher than the corresponding value in the control. The rate of incorporation of radioactive label of 2-^{14}C-pyruvate into FFA and TL of the liver of the control animals was 5.4 and 3.2 times higher than that of 1-^{14}C-acetate, and the contribution of the "pyruvate" path to lipogenesis is increased in noninsulin-dependent diabetes. This fact is confirmed by experiments with 2-^{14}C-pyruvate, in which specific radioactivity of FFA and lipids in the liver of mice with diabetes was 2.6 and 2.85 times, respectively, higher than the control values and 3.2 and 7.8 times higher, respectively, than values in animals with diabetes and receiving 1-^{14}C-acetate.

The existence of differences of this kind in the intensity of FFA and lipid biosynthesis from 1-^{14}C-acetate and 2-^{14}C-pyruvate suggests that intramitochondrial synthesis of acetyl-CoA and its transfer into the cytosol (pyruvate-dehydrogenase complex—citrate synthetase—tricarboxylate carrier—ATP-citrate lyase) is the stage responsible for hyperlipogenesis from pyruvate in noninsulin-dependent diabetes. However, the study of several enzymes providing substrates for lipogenesis (Table 2) revealed no significant changes in activity of the pyruvate-dehydrogenase complex in diabetes, whereas activity of ATP-citrate lines was actually significantly reduced (by 20.8%). The latter contradicts existing ideas on regulation of this enzyme, for despite the raised level of insulin, which is a positive modulator of ATP-citrate lyase activity [11] in the liver of mice with noninsulin-dependent diabetes (db/db) [12], and the 3.2-fold increase in the concentration of glycerol-3-phosphate, an inducer of biosynthesis of this enzyme [14], its activity was reduced. The opposite changes in ATP-citrate lyase activity and the intensity of lipogenesis in the control

TABLE 3. Activity of Enzymes Generating and Utilizing NADPH, and of Fatty Acid Synthetase in Liver of Control and Diabetic Mice (n = 8; M ± m)

Parameter	Control	Diabetes
Dehydrogenases of pentose phosphate path, nmoles/mg protein/min	10,6±0,79	8,1±1,03
NADP-dependent malate dehydrogenase, nmoles/mg protein/min	18,0±1,55	24,0±4,26
Isocitrate dehydrogenase, nmoles/mg protein/min	46,0±2,07	45,0±3,04
Transketolase, μmoles sedoheptulose-7-phosphate/g tissue/h	246,93±7,64	263,85±7,25
Transhydrogenase, nmoles/mg protein/min	20,3±1,03	19,9±1,3
Glutathione reductase, nmoles/g protein/min	13,3±0,77	8,9±1,39*
Fatty acid synthetase, nmoles/mg protein/min	50,6±3,0	70,8±5,1*

and diabetic mice may be connected not only with the fact that acetyl units are transported from the mitochondria into the cytoplasm in the mouse liver in the form of citrate, but also with the fact that ATP-citrate lyase may perhaps not play a key role in lipogenesis from pyruvate under the experimental conditions examined above. Conversely, activity of cytoplasmic acetyl-CoA synthetase, an enzyme catalyzing the initial stage of lipogenesis from acetate, is increased by 55.8% in this type of diabetes. The final conclusions regarding the velocities of the individual reactions supplying substrates for lipogenesis can be drawn, however, only after the study of the kinetic characteristics of enzymes isolated from the liver of control and diabetic mice.

As is shown in Fig. 1b,* the ATP content in the liver of diabetic mice was 34.1% greater than the corresponding value in the control animals. This may perhaps be due to intensification of the tricarboxylic acid cycle, evidence of which is given by the significant increase (by 17.4%) in 2-oxoglutarate dehydrogenase activity in the diabetic animals (Table 2). Thus more favorable conditions than in the control for energy-dependent stages of FFA and lipid biosynthesis are created in noninsulin-dependent diabetes, and this may be one of the factors maintaining hyperlipogenesis in the liver in type II diabetes.

The study of activity of the enzymes supplying reducing equivalents in the NADPH form for lipogenesis (Table 3), namely dehydrogenases of the pentose phosphate path, NADP-dependent malate dehydrogenase, isocitrate dehydrogenase, transhydrogenase, and also the key enzyme of the pentose phosphate path — transketolase — showed no significant differences between the values obtained in control and diabetic mice. Meanwhile activity of gluthathione reductase — the principal enzyme system competing for NADPH with fatty acid synthetase — was significantly depressed (by 33.1%) in the diabetic mice, thus creating more extensive prospects for the utilization of the reducing equivalents thus formed in reactions of lipogenesis. It must also be noted that the content of fructose-1,6-diphosphate, a powerful allosteric regulator of fatty acid synthetase, which reduces K_m for that enzyme with respect to NADPH [13], was increased in noninsulin-dependent diabetes by 38.6% (up to 0.097 ± 0.008 from 0.070 ± 0.005 μmole/g tissue in the control). The above facts explain the higher (by 32.4% compared with the control) activity of fatty acid synthetase — an enzyme of lipogenesis, which elongates the carbon chain of acyl-derivatives of CoA in the liver of animals with insulin-independent diabetes (Table 3).

The results of these investigations thus indicate a number of factors giving rise to hyperlipogenesis in noninsulin-dependent diabetes, foremost among which is a more intensive supply of energy for the process compared with the control, shifts in the relative levels of activity of enzymes generating and utilizing NADPH, and the more extensive potential prospects for NADPH utilization in the reaction catalyzed by fatty acid synthetase. The results are evidence that a new class of hypolipidemic agents must be created, with an action aimed

*Figure missing in Russian original — Publisher.

at correcting disturbances of the molecular mechanisms of regulation of lipid metabolism in type II diabetes.

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TIME COURSE OF LIPID PEROXIDATION AND TISSUE RESPIRATION DURING HEALING OF EXPERIMENTAL ASEPTIC AND INFECTED WOUNDS

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Much research has recently been published to show that reactions of lipid peroxidation (LPO) are intensified in various pathological processes and, in particular, in burns [6], atherosclerosis and ischemic heart disease [2, 3], and aseptic inflammation [14]. Meanwhile its role in the pathogenesis of wound healing, especially when infection is present, has been inadequately studied.

The aim of this investigation was to study LPO during healing of aseptic and infected wounds and to compare changes in LPO activity with the time course of tissue respiration.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 250 male Wistar rats weighing 180-200 g. Full-thickness aseptic and infected wounds with an area of 400 mm^2, inflicted under general hexobarbital anesthesia (150 mg/kg, intramuscularly) on the dorsal aspect of the animal's cervical region, served as the experimental model. After removal of an area of skin with the subcutaneous cellular tissue and superficial fascia, a plastic ring was inserted into the wound and covered with perforated film. To obtain an infected wound, the edges and base of the wound were traumatized by toothed forceps, and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus (strain 209), containing 1.5×10^9 microbial cells to 1 ml of physiological saline, was introduced inside the ring. The ring was removed on the 3rd day.

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