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Peroxisomal Enzyme Activities in the Liver of Overtly Diabetic Non-obese Diabetic Mouse

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Enzyme activities of hepatic peroxisomes were examined in spontaneously diabetic non-obese diabetic (NOD) mice. Peroxisomal fatty acid β-oxidation activity in the NOD diabetic mouse was the same as that of the ICR mouse. Sodium glutamate-induced obesity did not affect peroxisomal enzymes. The results indicate that peroxisomal β-oxidation does not increase in the insulin-deficient mouse. Furthermore, considering that the onset of diabetes in the NOD mouse is around 13 weeks after birth, these findings suggest that hepatic peroxisomal β-oxidation is utilized mainly in the acute state, not in the chronic state, in order to obtain adenosine triphosphate urgently for cellular demands.

Keywords——spontaneously diabetic mouse; insulin-dependent diabete; obesity; peroxisomes; β-oxidation

The non-obese diabetic (NOD) (diabetogenic subline) mouse is an inbred strain derived from outbred Jcl-ICR established at Shionogi Research Laboratory, Japan.[1] This mouse is severely diabetic due to insulitis, and can not survive without insulin treatment after the onset of overt diabetes mellitus.[1,2] Insulitis occurs spontaneously from the age of 5 weeks in the NOD mouse.[2] Ohneda *et al.* reported that insulin deficiency and hypersecretion of glucagon might contribute to the development and clinical course of diabetes mellitus in the NOD mouse.[3] Furthermore, in the diabetic condition, the blood insulin level of the NOD mouse was markedly lower than that of the C57BL/obob mouse.[4] In the female, diabetic symptoms occur mainly at 13 weeks after birth and the cumulative incidence reaches 80—90% by 30 weeks, whereas in the male it is less than 20%.[1,5] Glycosuria, persistent hyperglycemia and ketonuria, and rapid weight loss abruptly appear after the onset of overt diabetes. Thus it is considered that the NOD mouse is a useful animal model for studying human diabetes, particularly insulin-dependent diabetes.[1,2,5]

Peroxisomes are cytoplasmic organelles and the existence in them of a fatty acid β-oxidation system was discovered by Lazarow and de Duve.[6] The system is enhanced more rapidly than that of mitochondria under the condition of diabetes[7] as well as fasting[8] and high-fat diet feeding.[9] Recently, several studies on the peroxisomal β-oxidation activity of the Zucker rat, a model animal of type II diabetes, have been reported. Brady and Hoppel[10] observed that in the total liver of the obese Zucker rat, peroxisomal β-oxidation activity was about 2-fold higher than that of the lean rat.

In this study, we examined the activities of peroxisomal and mitochondrial β-oxidations in the liver of the diabetic NOD mouse and the obese NON × NOD (F1) mouse (induced by sodium glutamate[11]), in order to investigate the physiological significance of peroxisomal β-oxidation.

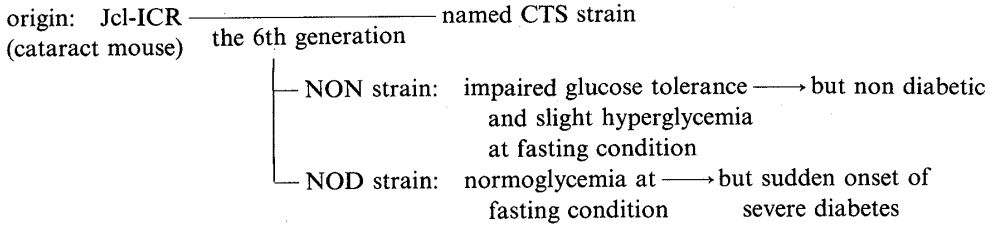

Fig. 1. The Pedigree of NOD and NON Mice

Materials and Methods

Female NOD mice at the 50th generation of inbreeding were supplied by the Shionogi Research Laboratory in Japan. The mice were used in the diabetic state at 25 weeks of age in this experiment. In the 2nd experiment, NON × NOD (F1) mice from the crossing of non obese normal (NON) and NOD strain mice were used. The pedigree of NOD and NON strain mice is shown in Fig. 1. Two sublines were separated from the CTS strain at the 6 generation. The CTS strain was characterized by cataractal eyes and was derived from the Jcl-ICR strain mouse. Mice of one subline showed slight hyperglycemia upon fasting and also glucose intolerance in old age, while those of another subline displayed normoglycemia; these sublines were named NON and NOD, respectively. For the study of obesity, monosodium glutamate (MSG) was subcutaneously injected into new born NOD-F1 mice at a dose of 4 mg/g body weight daily during the first 2 d (not 4 d[11]) of the neonatal stage. Animals were fed *ad libitum* with water and standard laboratory chow. At 2 weeks after the onset of sudden severe diabetes, NOD mice were sacrificed together with age-matched female Jcl-ICR mice as the control. Sera were obtained for measurement of glucose, cholesterol and triglyceride. The livers were quickly removed, weighed, minced and homogenized with ice-cold 0.25 M sucrose in a Potter–Elvehjem type homogenizer with a Teflon pestle. The activity of fatty acyl-coenzyme A (CoA) oxidase (FAO) was determined by measuring the palmitoyl-CoA-dependent H_2O_2 formation.[12] The activities of carnitine acetyltransferase (CAT) and carnitine palmitoyltransferase (CPT) were determined spectrophotometrically by measuring the amount of CoA-SH released.[13] Activities of other peroxisomal enzymes such as catalase, urate oxidase and D-amino acid oxidase (DAAO) were determined as described previously.[14] Serum glucose was determined by the *o*-toluidine borate method[15] and cholesterol and triglyceride contents in the liver and serum were determined as described previously.[16] Protein content was determined by the method of Lowry *et al.*[17] with bovine serum albumin as a standard.

Results

Table I shows biochemical values in the female Jcl-ICR and female NOD mice. Body weight gain of the NOD mice was not changed after the onset of diabetes and liver weight relative to body weight was almost the same as in the Jcl-ICR mice. Serum glucose was three times higher than that of the ICR mice (statistically significant). The concentration of serum cholesterol was double, though the levels of triglyceride in the serum and liver were very low. Hepatic protein content in the NOD mice was almost 1.2 times higher than that in the ICR mice. Table II shows the activities of several typical peroxisomal enzymes and enzymes related to fatty acid β-oxidation in the livers of female ICR and NOD mice. In the NOD mice, activities of catalase and urate oxidase were 67% and 137%, respectively, compared with the female ICR mice. DAAO was not detectable in either group. FAO, a rate-limiting enzyme of the peroxisomal β-oxidation system, was almost the same in both groups. Of other enzymes investigated, the activities of CAT and CPT were 1.68 and 1.74 times higher than those of the control ICR group, respectively; these differences are statistically significant. In order to clarify whether the β-oxidation enzymes participate in the obesity, biochemical values and activities of enzymes related to oxidation were determined in the liver of NON × NOD (F1) mice and the MSG-induced obese F1 mice. As described previously, NON × NOD mice are inbred strains and are genetically homogeneous. Although F1 mouse is a heterozygote, the genotype of F1 is homogeneous. We consider that F1, as a heterozygote derived from NOD, might be useful as a model of type I diabetes, while NON may be a model of type II diabetes. As shown in Table III, the body weights of male and

Table I. Biochemical Values of NOD Mice

		Jcl-ICR (5 females)	NOD[a] (4 females)	
Body weight	(g)	47.7±2.8	23.5±2.4	(49)[c]
Liver weight	(%)	5.76±0.44	5.66±0.59	(98)
Glucose	(mg/dl)	131±22	385±78	(294)[c]
Cholesterol	(mg/dl)	77±23	161±67	(209)[b]
	(mg/g)	10.5±1.3	9.4±0.7	(90)
Triglyceride	(mg/dl)	120±49	37±1	(31)[b]
	(mg/g)	12.4±5.2	4.2±1.9	(34)[b]
Protein	(mg/g)	212±5	248±6	(117)[c]

Results are expressed as mean±S.D. of 5 or 4 mice. *a*) Two weeks after the onset of over diabetes. Values in parentheses are percentages of the values of Jcl-ICR mice. Statistical evaluations were performed by using Student's *t*-test. *b*) $p<0.05$. *c*) $p<0.01$.

Table II. Activities of Peroxisomal and Mitochondrial Enzymes from the Liver of NOD Mice

	Jcl-ICR (5 females)	NOD[a] (4 females)	
Catalase	38.3±2.5	25.6±5.7	(67)[c]
Urate oxidase	2.99±0.34	4.09±0.92	(137)[b]
DAAO	N.D.	N.D.	
FAO	1748±123	1728±73	(99)
CAT	884±48	1485±234	(168)[c]
CPT	2654±265	4618±793	(174)[c]

Results are expressed as mean±S.D. of 5 or 4 mice. *a*) Two weeks after the onset of over diabetes. Values represent U/g liver. N.D. = not detectable. Values in parentheses are percentages of the values of Jcl-ICR mice. Statistical evaluations were performed by using Student's *t*-test. *b*) $p<0.05$. *c*) $p<0.01$.

Table III. Biochemical Values of NOD-F1 Obese Mice

		NON×NOD (F1) [normal] (5 males)	NON×NOD (F1) [normal] (6 females)	NON×NOD (F1) [obese][a] (5 males)	NON×NOD (F1) [obese][a] (6 females)
Body weight	(g)	47.1±1.0	36.6±3.1[c]	64.4±4.4[b]	60.0±6.4[b]
Liver weight	(%)	4.58±0.3	3.84±0.3[c]	3.68±0.5[b]	2.15±0.2[b,c]
Glucose	(mg/dl)	119±16	115±7	143±15	162±14[b]
Cholesterol	(mg/dl)	129±13	82±10[c]	204±19[b]	130±11[b,c]
	(mg/g)	8.7±0.9	10.1±0.9	10.1±0.4	10.3±0.6
Triglyceride	(mg/dl)	230±73	123±45	298±56	109±21[c]
	(mg/g)	9.8±3.8	8.9±1.8	15.5±3.4	12.7±2.1[b]
Protein	(mg/g)	218±3	220±10	223±12	223±8

Results are expressed as mean±S.D. of 5 or 6 mice. *a*) Obesity induced by sodium glutamate (4 g/kg, s.c.) at the neonatal stage. Statistical evaluations were performed by using Student's *t*-test. Normal male *vs.* obese male or normal female *vs.* obese female: *b*) $p<0.01$. Normal male *vs.* female or obese male *vs.* female: *c*) $p<0.01$.

female obese mice were higher than those of normal F1 mice. The relative liver weight of the obese mice was lower than that of normal mice. Furthermore, the liver size of female mice was smaller than that of males, and a sex difference in relative liver weight was also observed in both normal and obese mice. The serum glucose concentration in the obese female mice was

TABLE IV. Activities of Peroxisomal and Mitochondrial Enzymes from the Liver of NOD Mice

	NON × NOD (F1) [normal]		NON × NOD (F1) [obese][a]	
	(5 males)	(6 females)	(5 males)	(6 females)
Catalase	35.6±1.9	35.9±2.8	32.6±3.0	34.5±4.1
Urate oxidase	3.76±0.3	2.86±0.3	2.78±0.5[b]	2.35±0.6
DAAO	N.D.	N.D.	N.D.	N.D.
FAO	1648±117	1730±146	1532±148	1986±78[c]
CAT	775±134	1413±69[c]	1282±108[b]	1454±128
CPT	4659±544	4948±526	5137±294	5869±313[b,c]

Results are expressed as mean±S.D. of 5 or 6 mice. *a*) Obesity induced by sodium glutamate (4 g/kg, s.c.) at the neonatal stage. Values represent U/g liver. N.D. = not detectable. Statistical evaluations were performed by using Student's *t*-test. Normal male *vs.* obese male or normal female *vs.* obese female: *b*) $p<0.01$. Normal male *vs.* female or obese male *vs.* female: *c*) $p<0.01$.

1.4 times higher than that of the normal female mice (statistically significant). The level of serum cholesterol was 1.6 times higher than that of the normal group, whereas the liver cholesterol level was practically the same in both groups. Liver triglyceride contents were also higher than in the normal groups, and the difference was significant in female mice. Female mice showed a lower serum lipid level than the males, and accordingly sex difference was observed in the serum cholesterol of both normal and obese mice and in the serum triglyceride of the obese group. Table IV shows the activities of peroxisomal and mitochondrial enzymes in the liver of the normal (F1) and MSG-induced obese mice. In the male obese mice, the activity of urate oxidase was lower, whereas the activity of CAT was higher than those of the normal male mice (statistically significant). In the obese female mice, only CPT activity was 1.2-fold higher than that of the normal female mice. The obese female groups showed higher FAO activity as well as CPT activity compared with the obese male groups. However, the differences were not large.

Discussion

The NOD mice show symptoms and histological changes similar to those of human insulin-dependent diabetes mellitus.[1,2] Diabetic NOD mice had almost the same peroxisomal palmitoyl-CoA oxidation rate as Jcl-ICR mice. This observation was unexpected in view of the previous finding that the peroxisomal β-oxidation was enhanced rapidly when the utilization of fatty acids as an energy source was stimulated in rats with alloxan-induced diabetes[7] as well as in starved rats,[8] rats fed a high-fat diet[9] or neonatal rats.[18] In contrast to FAO activity, the activity of CPT, which catalyzes the import of long-chain acyl-CoA into mitochondria, was increased in diabetic NOD mice. As described previously, the onset of diabetes in NOD mice is around 13 weeks after birth, so all NOD mice used in this experiment should have passed the acute phase of the diabetic condition and have entered the chronic state, in which many metabolic systems have adapted to the diabetic conditions, and energy production has reached a new stationary state. Peroxisomal β-oxidation may be important in the response to the demand of cells for the urgent supply of energy from fatty acids. Brady and Hoppel[10] reported that obese Zucker rats, an animal model of human type II diabetes, showed about 2-fold higher peroxisomal β-oxidation activity than lean Zucker rats. Furthermore, Murphy *et al.*[19] demonstrate that peroxisomal fatty acid β-oxidation in obese (C57BL/6J-obob) mice was about three-fold higher than that of the lean mice in terms of total activity per gram of liver and over five-fold higher per total liver. However, we observed no effect of MSG-induced obesity on peroxisomal β-oxidation activity per gram of liver in either male or female NON × NOD (F1) mice, and female obese mice had lower

total liver FAO activity than the normal females. Although these differences of peroxisomal β-oxidation activities between genetically obese and MSG-induced mice may be due to the effect of MSG, which causes hypothalamic damage and disturbance of several hormon levels,[9] they may be associated with the fact that in the chronic stage, obese rats do not demand an urgent supply of energy from fatty acid catabolism. It is known that both Zucker rats and C57BL/obob mice have very high levels of insulin in the sera compared with those of the lean animals, while MSG-induced obese mice have a rather high insulin level compared with the non-treated mice. Further research on the hormonal sensitivity of animals could help to clarify the differences of peroxisomal β-oxidation between NOD obese mice and Zucker rats or C57BL/obob mice. The NOD mouse should be a useful animal model for investigating the pathology of human insulin-dependent diabetes.

It is known that in the acute phase after the onset of diabetes mellitus the activity of the peroxisomal β-oxidation system is enhanced.[9] However, our present experiment using the NOD mouse, which might be correspond to the chronic stage of diabetes mellitus, showed no enhancement of the peroxisomal β-oxidation system. These results suggest that hepatic peroxisomal β-oxidation is important mainly in the acute state, not in the chronic condition, in order to obtain adenosine triphosphate urgently for cellular demands.

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