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HEPATIC AND INTESTINAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN GENETICALLY DIABETIC MICE

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Cholesterol synthesis rate, as determined by 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, is characterized in the major organs of genetically diabetic mice. Both C57BL/Ks db+/db+ and C57BL/6 ob+/ob+ mice are hyperinsulinemic and insulin-resistant. These animals demonstrate loss of the circadian rhythm of hepatic reductase activity and a tendency for increased intestinal activity. As a result, proportionally more endogenous cholesterol synthesis occurs in intestinal mucosa than liver in genetically diabetic animals. Thus, the alterations in activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase which are observed in animal models of diabetes are the result of diminished insulin effect rather than insulin level.

Endogenous synthesis of cholesterol is most active in liver and intestine (1,2). The relative contribution of these two organs varies between species and with alterations in diet (1,3). In both tissues, 3-hydroxy-3- methylglutaryl coenzyme A (HMG CoA) reductase is the rate determining enzyme of cholesterol synthesis (4,5). Activity of this microsomal enzyme generally reflects rate of tissue cholesterogenesis (6). Several investigators have reported a relationship between insulin, diabetes and HMG CoA reductase activity. Acute experiments have suggested that insulin stimulates HMG CoA reductase activity in both liver and intestine (7,8,9). Chronic studies of diabetes produced by islet cell toxins have shown reduced hepatic HMG CoA reductase specific and total activity, while intestinal enzyme activity increased (7,10,11,12,13).

Previous studies of these relationships have utilized insulinopenic diabetic rats produced by alloxan or streptozotocin injection (7,8,10,11,12,

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13). In contrast to these animals, several strains of genetically diabetic and hyperphagic mice with elevated insulin levels have been bred. Genetically diabetic mice (C57BL/Ks db+/db+) are markedly hyperglycemic, hyperinsulinemic and insulin resistant while demonstrating moderate hyperphagia and obesity (14,15). Genetically obese mice (C57BL/6 ob+/ob+) are markedly obese and hyperphagic while demonstrating less hyperglycemia, hyperinsulinism and insulin resistance (16,17). To compare the relative impact on cholesterogenesis of insulin level as opposed to insulin effect, we have measured hepatic and intestinal HMG CoA reductase activity in these genetic strains of mice and compared them to mice treated with streptozotocin.

MATERIALS AND METHODS

Age and sex matched 3-4 month old diabetic C57BL/Ks db+/db+ mice (db/db) and their heterozygote (db/m) and homozygote (m/m) non-diabetic controls were bred at the University of Minnesota Mouse Colony (Department of Laboratory Medicine and Pathology; Minneapolis, Minnesota) from db/m breeding pairs (Jackson Laboratries; Bar Harbor, Maine). Age and sex matched 3-4 month old obese C57BL/6 ob+/ob+ mice (ob/ob) and their non-obese littermate (ob/-) controls were also obtained from Jackson Laboratories. Healthy C57BL/6 mice obtained from the Mammalian Genetic Section of the National Cancer Institute through a VA/NCI contract were made diabetic by intraperitoneal injection of streptozotocin (180 mg/kg as a single dose) 5-6 weeks prior to study. Streptozotocin mice were compared to age and sex matched normal C57BL/6 mice. All animals were kept under standard conditions with 12 h dark-12 h light cycles and free access to Purina laboratory chow and water. Animals were allowed access to food until sacrifice by decapitation at mid-dark or mid-light time periods (as indicated). Serum glucose levels were measured by glucose oxidase kit 510 obtained from Sigma Chemical (St. Louis, Missouri). Total serum cholesterol levels were measured using Hewlett-Packard 5830A gas-liquid chromatography (Palo Alto, California) following extraction by the method of Abell (18).

Immediately following sacrifice, the small intestine was removed, stripped of mesentery, rinsed clean with iced saline, weighed, and homogenized in 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose and 40 mM ethylenediaminetetraacetic acid. A sample was taken for protein determination by the method of Lowry et al (19). Intestinal HMG CoA reductase activity was measured in whole homogenates as previously described (9,12,20). Assay tubes were preincubated for 5 min prior to addition of 31.5 μM [^{14}C] HMG CoA substrate for 15 min incubation. Liver was removed, weighed and a weighed portion of the right lobe was homogenized in iced 50 mM Tris buffer (pH 7.2) containing either 150 mM KCl or 100 mM KCl plus 50 mM NaF. Homogenates were centrifuged at 12,000 g for 15 min, the supernatant centrifuged at 104,000 g for 45 min and the sedimented microsomal pellet resuspended in phosphate-sucrose buffer. Protein was measured in whole homogenate and resuspended microsomes. Assay for HMG CoA reductase activity in liver microsomes was as described for intestine except that substrate concentration was 58.5 μM. Adrenal glands were also removed, cleaned and homogenized in phosphate-sucrose buffer. HMG CoA reductase activity measured in whole adrenal homogenate used the same conditions for hepatic microsomes. Chemicals were obtained from Sigma Chemical while radioisotopes were obtained from New England Nuclear (Boston, Massachusetts). Statistical analysis utilized unpaired, two-tailed Student's t-test.

RESULTS

Table 1 displays body weight, serum glucose and serum cholesterol data. The db/db and ob/ob mice were heavier than their respective controls and had higher levels of glucose and cholesterol. The db/db mice had higher glucose levels and weighed less than ob/ob mice ($p<0.05$). Streptozotocin (insulinopenic) mice were hyperglycemic but weighed 20% less than controls.

Table 2 shows HMG CoA reductase specific activity (per mg/protein) in hepatic microsomes and intestinal and adrenal homogenates for the diabetic mice and their controls. A striking circadian rhythm of HMG CoA reductase in rodent liver and a modest rhythm in gut have been reported (21,22). Consistant with this pattern, dark period hepatic activity was found to be higher than light period activity in control animals ($p<0.01$). The principle abnormality in both insulin-resistant and insulinopenic diabetes was decreased dark period hepatic reductase activity and loss of diurnal rhythm. A diurnal rhythm of HMG CoA reductase specific activity was not detected in either intestine or adrenal gland (data not shown). Also, specific activity of intestinal HMG CoA reductase in diabetic mice was not significantly different from control animals at the time of reported peak (dark cycle) activity. Adrenal specific activity was increased only in db/db animals compared to

Table 1: Metabolic Data

	Body Weight (g)	Liver Weight (g)	Gut Weight (g)	Serum Glucose (mg/dl)	Serum Cholesterol (mg/dl)
db/db	36.1±1.9*	1.70±0.10*	1.67±0.05*	482±28*	92±6*
db/m control	20.1±1.1	0.88±0.04	1.06±0.03	142± 7	52±4
m/m control	19.2±1.0	0.86±0.04	0.78±0.27	121± 9	47±3
ob/ob	51.1±0.6*	3.17±0.11*	1.92±0.09	322±39	105±13*
ob/- control	28.2±1.3	1.28±0.08	1.43±0.04	156± 6	59± 2
Streptozotocin	19.5±0.7*	1.12±0.04+	1.46±0.20°	455±23*	81±5°
Control	25.1±0.7	0.90±0.04	0.97±0.04	112±11	68±4

Total body weight, organ weight, serum glucose and serum cholesterol values at time of sacrifice.
Values are Mean ± SE
* $p<0.01$, + $p<0.02$, ° $p<0.05$ compared to respective controls.

Table 2: HMG CoA Reductase Specific Activity

	Hepatic Reductase (Dark)	Hepatic Reductase (Light)	Gut Reductase (dark)	Adrenal Reductase (dark)
db/db	192±65*	237±70	26±3	34.5±3.2*
db/m control	719±133	111±31	23±2	18.7±4.2
m/m control	439±162	not done	29±2	14.2±4.3
ob/ob	110±8*	85±15	26±4	5.2±1.6
ob/- control	211±35	95±12	31±6	3.8±0.2
Streptozotocin	194±41*	165±28+	25±3	8.8±0.6
Control	476±126	80±22	27±3	7.4±0.8

HMG CoA reductase activity per mg of microsomal or homogenate protein per minute. Control animals have a significant diurnal peak in hepatic activity during the dark period ($p<0.01$).

Values are Mean ± SE in pmol mevalonate formed/mg protein/min (n = 5-14)

* $p<0.02$, + $p<0.05$ compared to controls.

littermate controls. This finding correlates with the observation that db/db animals are hypercortisolemic (23).

Table 3 shows calculated total hepatic and intestinal HMG CoA reductase activity. As for specific activity data, total hepatic enzyme activity at

Table 3: Total Organ HMG CoA Reductase Activity

	Liver	Gut	Ratio (gut/liver)+
db/db, dark	2625±626*	4500±182*	1.71
db/m, dark	5735±716	2671±298	0.47
db/db, light	3220±936	3790±1673	1.18
db/m, light	1375±441	1693±44	1.23
ob/ob, dark	4222±308	6036±640	1.43
ob/-, dark	4739±779	5486±1046	1.16
Streptozotocin, dark	1852±391	3872±1039	2.09
Control, dark	3652±967	1939±301	0.53

Total gut activity is elevated and total hepatic activity is reduced in diabetic animals compared to controls. Changes reach significance only in the db/db studies.

Values are Mean ± SE in pmol mevalonate formed/organ/min (n = 4-12)

* $p<0.05$ compared to control at stated light cycle.

+ This ratio is a calculation rather than an absolute value. Total hepatic activity is underestimated due to approximately 50% microsomal recovery.

the diurnal peak time was depressed in diabetic animals. The degree of depression reached significance only in db/db animals compared to their db/m controls. Total intestinal activity was increased in diabetic animals, but the increase reached significance only in db/db animals and resulted from the increased mucosal mass in diabetic animals. Intestinal mucosa is responsible for a higher proportion of cholesterogenesis in diabetic animals.

DISCUSSION

This study characterizes HMG CoA reductase activity in insulin-resistant diabetic animals. The findings are comparable to those reported in drug-induced insulin deficiency (7,10,11,12). Both diabetic conditions increased the ratio of intestinal enzyme activity to hepatic enzyme activity by obliterating the circadian rhythm of hepatic activity while total intestinal activity increased.

Feingold et al measured incorporation of tritiated water into sterols in streptozotocin diabetic rats (24). These investigators found intestinal cholesterol synthesis to be increased, but reported no difference between control and diabetic rats in hepatic ^{3}H-cholesterol content 6 h after administration of $^{3}H_2O$. There are several possible explanations for this apparent discrepency between reductase activity and sterol synthesis. Since cholesterol is normally converted to bile acids or released from liver to lipoproteins, measurement of labeled hepatic sterol content after 6 h may have underestimated normal synthesis. Alternatively, diabetes may cause a dissociation between hepatic HMG CoA reductase activity and cholesterogenesis.

Inactive HMG CoA reductase is phosphorylated and is activated (dephosphorylated) by cellular phosphatases (6). This activation is prevented by sodium fluoride. Therefore, we measured hepatic reductase in microsomes isolated in 50 mM sodium fluoride. Activity in the presence of fluoride was a constant 14-20% fraction of total enzyme activity measured in the absence of fluoride (data not shown). Thus, the diurnal variations and the changes observed in diabetic animals are due to alterations in total reductase enzyme present rather than state of enzyme activation.

These studies indicate that the changes in activity of HMG CoA reductase in diabetes are due to reduced insulin effect rather than reduced insulin levels. Insulin resistant animals have the same patterns as reported for insulin deficient animals. The diabetic condition itself, therefore, is critical to the altered HMG CoA reductase relationships. Young et al suggest that hyperphagia in streptozotocin diabetes causes increased cholesterol absorption and synthesis by hypertrophic intestinal mucosa (13). The resulting elevated serum cholesterol is then postulated to reduce hepatic synthesis by feedback inhibition. Our findings in hyperphagic genetic diabetes are consistant with this thesis, but do not rule out a more direct insulin effect on hepatocyte cholesterogenesis.

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