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Intestinal and Hepatic Cholesterol Synthesis in the Alloxan Diabetic Rat[1] (41432)

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Abstract. The effects of alloxan diabetes and insulin treatment upon rat 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was studied. In diabetes, hepatic HMG-CoA reductase specific and whole organ activities were reduced 85 and 89%, respectively, during the diurnal high period of enzyme activity. In contrast, whole small intestinal HMG-CoA reductase activity was increased twofold in diabetic rats during this period. The diabetic rats' whole gut HMG-CoA reductase activity approximated the entire liver's. Chronic insulin therapy markedly stimulated hepatic HMG-CoA reductase activity in diabetes rats but did not increase gut enzyme activity. Thus in alloxan diabetes, the intestine may be as important a source of endogenous cholesterol as the liver.

In vivo cholesterol biosynthesis has been shown to be most active in liver and next most active in intestinal mucosa (1). In both of these tissues, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) is the rate-limiting enzyme of cholesterol synthesis (2, 3). Therefore the activity of this enzyme reflects cholesterol synthesis rate under most conditions (4, 5). Drug-induced diabetes has been shown to cause a profound depression in hepatic HMG-CoA reductase activity (6, 7). In contrast, intestinal HMG-CoA reductase has been noted to be increased in diabetes (8). However, previous studies of diabetes have measured specific activity of liver and gut HMG-CoA reductase (6–8) or activity of HMG-CoA reductase per centimeter of scraped intestine (8). HMG-CoA reductase activities of the entire liver and intestine have not been measured. Whole organs rates of sterol synthesis are of interest because the relative amount of synthesis in a given organ depends upon the relative size of the organ (9). This is especially important for diabetes where intestinal enlargement is a prominent feature (10).

To assess the relative importance of the gut as an organ of cholesterol synthesis in diabetes, we determined whole organ rates of cholesterol synthesis for liver and intestine in the alloxan diabetic rat. In addition, we examined the effect of both acute and chronic insulin administration on hepatic and intestinal HMG-CoA reductase activity.

Materials and Methods. *Animal preparation.* Male Sprague–Dawley rats (Bio Labs, St. Paul, Minn.) weighing 180–200 g were maintained *ad libitum* on water and pelleted rat chow (Ralston-Purina) and housed in individual cages. Rats were maintained in rooms that were cycled with alternating 12-hr periods of light and dark. Animals were allowed to adapt to light cycling for at least 2 weeks prior to alloxan or saline injections. Diabetes was induced with an intravenous injection of alloxan 50 mg/kg (Sigma Chemical) freshly prepared in saline. Control rats received a similar volume of intravenous saline. The mean daily food consumption of diabetic and control animals was measured for one week prior to sacrifice. After injection, rats were allocated into four experimental groups and treated as follows: *control*—normal animals fed *ad libitum; untreated*—diabetic animals

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fed *ad libitum; chronic insulin treated*—diabetic rats fed *ad libitum* and chronically receiving 6 units of NPH insulin subcutaneously 1 hr prior to onset of the dark period; and *acute insulin treated*—diabetic rats fed *ad libitum* and receiving a single dose of 20 units of regular insulin subcutaneously 3 hr prior to sacrifice. Animals in the first three groups were sacrificed both at the mid-light phase and the mid-dark phase. Animals in the fourth group were sacrificed only at mid-light. Chronic insulin-treated rats received NPH insulin for 26 days. Animals not receiving insulin received saline injections at comparable times to the insulin-treated rats. Those animals sacrificed at mid-dark phase received their usual insulin dose on the day of sacrifice. Diabetic urines were checked twice weekly and only those rats with greater than 2+ glycosuria (Diastix, Ames) were considered diabetics. Animals were sacrificed on Day 28 after alloxan or saline injections by exsanguination under light ether anesthesia.

Tissue preparation. The entire small intestines were excised and flushed with cold saline. Mucosa was scraped with a spatula and suspended in 25 vol of a cold solution of 0.1 *M* potassium phosphate buffer (pH 7.2), 0.2 *M* sucrose, and 20 m*M* EDTA (Buffer A). The mucosa was homogenized in a Ten-Broeck homogenizer with a tight-fitting glass pestle.

The livers were removed immediately after sacrifice and placed in cold 50 m*M* Tris–150 m*M* KCL buffer (pH 7.6, Buffer B) with and without NaF (50 m*M*). The livers were homogenized with a tight-fitting, motor-driven Teflon pestle using 3.0 ml of Buffer B per gram of liver. The homogenate was centrifuged at 12,000*g* for 15 min. The 12,000*g* supernatant was then centrifuged at 104,000*g* for 45 min and the sedimented microsomal pellet was then resuspended in Buffer A using a tight fitting glass pestle.

Assay for HMG-CoA reductase activity. HMG-CoA reductase was assayed in hepatic microsomes and homogenates of intestinal mucosa as previously reported (11, 12). Specific activity of HMG-CoA reductase was expressed as picomoles of mevalonate formed per milligram protein per minute. Potential whole organ enzyme activity was expressed as nanomoles mevalonate formed per whole liver per minute or nanomoles mevalonate formed per entire small intestine per minute. Hepatic microsomal recovery, utilized for determination of whole liver enzyme activity, was determined utilizing glucose-6-phosphatase assay and was approximately 50% (11).

Other methods. A portion of the liver was extracted by the Folch procedure (13), saponified, and total cholesterol was determined on the digitonin precipitate by the method of Abell (14). Serum cholesterol was also measured by the method of Abell (14) and serum glucose by the method of Zender (15). Protein was determined by the method of Lowry (16). Statistical significance of differences among means was evaluated by Student's t test.

Results. Table I presents metabolic data from the four groups of rats. Untreated diabetic rats weighed much less than controls but had 21% more intestinal mucosal protein. Serum cholesterol was significantly increased in the untreated diabetic rats but the increase in diabetics' liver cholesterol did not quite reach statistical significance ($P < 0.1$). The chronic insulin-treated diabetic rats were still markedly hyperglycemic and their growth rates did not approach that of control rats.

Table II presents data on HMG-CoA reductase activity. Specific activity of hepatic HMG-CoA reductase and whole liver enzyme activity were reduced 85 and 89%, respectively, during the diurnal peak period in untreated diabetic rats compared with controls. Control rats showed a threefold ($P < 0.001$) diurnal rhythm in hepatic HMG-CoA reductase activity but the diurnal rise was obliterated in diabetic animals. As well-known, peak enzyme activity occurred in control rats during the dark period, when food consumption is greatest.

In contrast to liver activity, intestinal HMG-CoA reductase activity was actually increased in diabetic rats during the diurnal peak period. Though specific activity of diabetic gut enzyme was not significantly different, the untreated diabetic rats' whole

TABLE I. METABOLIC DATA

	Control	Untreated diabetes	Chronic insulin-treated diabetes	Acute insulin-treated diabetes
Body weight (g)	369 ± 9.0*	231 ± 9.0†	275 ± 12†	274 ± 11†
Food consumption (g)				
Light period	9.3 ± 0.6*	17.2 ± .85†	14.6 ± 0.97†	
Dark period	17.2 ± 0.3*	22.6 ± 0.6†	20.5 ± 0.55†	
Total per 24 hr	26.5 ± 0.07*	39.8 ± 0.9†	35.1 ± 0.67†	
Total mucosal protein (mg)	461 ± 19*	557 ± 32†	599 ± 25†	586 ± 45.2†
Blood glucose (mg/100 ml)	193 ± 4.4*	773 ± 48†	460 ± 22.5‡	114 ± 17.9§
Serum cholesterol (mg/dl)	48 ± 2.01*	65.6 ± 2.95†	67.5 ± 9.5†	46.3 ± 2.6*
Liver cholesterol (mg/100 g)	210 ± 17*	244 ± 35*		

Note. Values are mean ± SE of 6–31 animals per group. Methods are discussed in text. There were not significant differences in serum values obtained by sacrifice at mid-dark or mid-light. Means within the same horizontal row not followed by a common superscript are significantly different ($P < 0.05$).

small intestinal HMG-CoA reductase activity was more than twice that of control rats. In addition, a diurnal cycle in enzyme activity (both whole organ and specific) was noted in intestine of both diabetic and control rats ($P < 0.01$).

Chronic NPH insulin treatment, as expected, markedly increased diabetics' hepatic HMG-CoA reductase activity compared with untreated animals during the period of peak insulin effect (7 hr after the dose). In contrast, chronic insulin therapy did not increase gut HMG-CoA reductase activity.

To determine if an acute dose of insulin could stimulate HMG-CoA reductase activity, 20 units of regular insulin was administered during the diurnal low period of

TABLE II. HMG-CoA REDUCTASE

	Control	Untreated diabetes	Chronic insulin-treated diabetes	Acute insulin-treated diabetes
		Specific activity		
Intestine[a]				
Light	18.7 ± 2.8*	17.8 ± 3.7*	13.9 ± 2.8*	21.8 ± 5.5*
Dark	31.0 ± 3.6†	49.3 ± 9.2†	35.6 ± 7.3†	
Liver[a] (−NaF)				
Light	106 ± 9.0*	69.4 ± 16†	68.7 ± 10†	239 ± 49‡
Dark	395 ± 37.8‡	59.4 ± 11.7†	236 ± 30§	
		Whole organ activity		
Intestine[b]				
Light	8.54 ± 1.4*	8.58 ± 1.6*	8.3 ± 1.6*	13.5 ± 3.7*
Dark	14.0 ± 3.7†	29.6 ± 6.5‡	20.6 ± 3.2†,‡	
Liver[c] (−NaF)				
Light	76 ± 9.8*	34.8 ± 9.6†	43.0 ± 6.4†	142 ± 28‡
Dark	282 ± 31.6‡	31.6 ± 6.2†	213 ± 47‡	

Note. Values are mean ± SE of 6–14 animals per group. Light: midpoint of light period. Dark: midpoint of dark period. Methods are discussed in text. Means within the same horizontal row not followed by a common superscript are significantly different ($P < 0.05$). Diurnal values not followed by a common superscript are significantly different ($P < 0.05$).

[a] pmole mevalonate formed/mg protein (microsomal for liver, whole tissue homogenate for gut)/min.

[b] nmole mevalonate formed per whole small intestine/min.

[c] nmole mevalonate formed per whole liver/min.

enzyme activity. This dose of regular insulin brought blood glucose down to slight hypoglycemic levels and caused a threefold increase in hepatic HMG-CoA reductase specific activity. Gut specific activity and whole organ activity did not significantly increase after acute insulin treatment.

Since endogenous phosphatases convert inactive (phosphorylated) HMG-CoA reductase to its active (dephosphorylated) form during liver microsomal isolation, hepatic microsomes were prepared with and without the phosphatase inhibitor, NaF (17). HMG-CoA reductase activity of NaF-treated microsomes is felt to represent the active enzyme at the time the homogenates were prepared while the activity of microsomes isolated in the absence of fluoride approximates total reductase activity (17). The activity of NaF-treated microsomes varied between 24 and 28% of total activity in all four groups. All relationships between groups held regardless of whether active or total enzyme activity was considered.

Discussion. Results of the present study confirm previous work demonstrating depressed hepatic HMG-CoA reductase specific activity in streptozotcin diabetes and stimulation of enzyme activity by insulin in this condition (6, 7). The present study also confirms Nakayama's observation that intestinal cholesterogenesis is increased in streptozotocin diabetic rats (8). Since our study also measured whole organ activity, we have demonstrated that the intestine's total cholesterol synthesis approximates the liver's in untreated diabetic rats. Thus the gut is a much more important source of endogenous cholesterol in the diabetic rat. In addition, we found that chronic insulin treatment increases hepatic HMG-CoA reductase (total and specific activity). However, we are unable to demonstrate a statistically significant increase in intestinal HMG-CoA reductase activity in response to either acute or chronic insulin administration. This is in contrast to the acute stimulation of HMG-CoA reductase by insulin that we demonstrated in the canine intestine (12).

The reason for the increased intestinal HMG-CoA reductase activity in diabetes is of interest. The likely cause of this finding is the diabetic rat's hyperphagia. Increased food intake could stimulate gut cholesterol synthesis by requiring increased cholesterol for the transport of dietary fat in intestinal lipoproteins (18). On the other hand, the etiology for the diabetic's depressed hepatic HMG-CoA reductase activity may be a decreased serum insulin level, or an increased serum cholesterol with feedback suppression of liver HMG-CoA reductase activity. In support of the former possibility is the fact that chronic insulin treatment for diabetes markedly increased hepatic HMG-CoA reductase activity without any real change in serum cholesterol.

Results of the present study indicate that the elevated serum cholesterol level in the diabetic rat is not related to increased hepatic cholesterogenesis. The possibility that the intestine is a more important source of circulating cholesterol in untreated diabetes is intriguing and needs further study.

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