Preventing hyperphagia normalizes 3-hydroxy-3-methylglutaryl-CoA reductase activity in small intestine and liver of diabetic rats

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Abstract Rats with streptozotocin-induced diabetes stop growing, develop high cholesterol and triacylglycerol levels in plasma, and have decreased activity of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.34), in liver and increased activity in small intestine. They also eat more than normal. To determine the contribution of hyperphagia to these changes in lipid metabolism, we restricted intake of chow to the amount eaten ad lib by normal rats. Rats were meal-fed for 8 or 22 days from the time diabetes was induced. This regimen normalized reductase activity in both liver and intestine at mid-dark and mid-light, and all but eliminated high plasma cholesterol and triacylglycerol levels, although plasma insulin remained low and glucose remained high. Activation of hepatic reductase by endogenous phosphatase in vitro was reduced in hyperphagic diabetic rats but was normal in diabetic rats eating a normal amount of food. We conclude that hyperphagia, rather than direct effects of insulin deficiency as is usually assumed, is responsible for perturbations of lipid metabolism in chronically diabetic rats. These results support the proposal that hyperphagia increases the input of dietary and newly synthesized cholesterol from the small intestine, and that this increased input raises plasma cholesterol level and inhibits reductase activity in liver.—**Young, N. L., C. D. Saudek, L. Walters, J. Lapeyrolerie, and V. Chang.** Preventing hyperphagia normalizes 3-hydroxy-3-methylglutaryl-CoA reductase activity in small intestine and liver of diabetic rats. *J. Lipid Res.* 1982. **23:** 831–838.

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When rats are made diabetic and permitted to eat ad lib, activity of the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase, decreases in liver during the first day when food intake is low, and begins to increase in small intestine at about 3 days when the rats start eating more than normal (1). By 3 weeks total reductase activity in small intestine is 2.5 times normal, while activity in liver remains low. Plasma cholesterol is lower than normal during the early anorexic period, and higher than normal after the onset of hyperphagia. These data suggest that hyperphagia increases reductase activity in small intestine. The data also support the suggestion by Nakayama and Nakagawa (2) that the influx of more dietary cholesterol and newly synthesized cholesterol from the small intestine that occurs with hyperphagia raises plasma cholesterol level and contributes to the inhibition of cholesterol synthesis in the liver.

In the present study, we examined the possibility that these perturbations of cholesterol metabolism in chronically diabetic rats eating ad lib are due to their hyperphagia by reducing food intake in diabetic and normal rats and then measuring plasma constituents and HMG-CoA reductase activity in liver and small intestine.

MATERIALS AND METHODS

Treatment of animals

Male Wistar rats initially weighing 200–250 g (Charles River Laboratories, Wilmington, MA) were equilibrated for 2 weeks on fixed lighting schedules (as given in Tables 1 and 5) and fed Purina Formulab chow 5008 during the dark period. Diabetes was induced by the intravenous injection of streptozotocin at a dose of 65 mg/kg at mid-dark as previously described (1). Thereafter, rats were caged individually, with both the daily food ration and the timing of meals rigidly controlled as follows.

A weighed daily ration was given in 3 to 9 roughly equal meals delivered automatically by a mechanical feeder. The feeder was a modified fraction collector placed above the racks of cages. At fixed intervals, the feeder dropped pellets through chutes to each cage, delivering meals to as many as 24 individual rats simultaneously. To encourage prompt and complete con-

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; TG, triacylglycerol; HM, homogenizing medium; *r*, correlation coefficient; *P*, probability; ns, not significant; D, diabetic; C, control; S, stunted.

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sumption of meals, the daily ration was at least 1 g less than would have been consumed ad lib. Delivering meals at fixed intervals assured control of the time of eating as well as the amount consumed. It prevented, for example, underfed, hungry rats from gorging their ration in a single meal.

The amount of food given each day, the number and timing of meals, the number of days of feeding, and the time that rats were killed in each experiment are shown in Tables 1 and 5. Each of the five experiments in Tables 1–4 and the last one in Tables 5–7 were done at different times, and each experiment included its own group of control rats studied at the same time and under the same conditions as the experimental group.

Plasma constituents

Plasma was prepared from EDTA-treated blood obtained by heart puncture from rats anesthetized with ether. Insulin was measured by radioimmunoassay. Glucose, TG, and cholesterol were measured by enzymatic assays as previously described (1).

Assay of HMG-CoA reductase activity

The whole liver and the small intestine from the pylorus to the ileocecal junction were removed from rats anesthetized with ether. The organs were rinsed with cold saline, weighed, added to homogenizing medium (HM), cut up with scissors, and homogenized with a Tissumizer (Tekmar Co., Cincinnati, OH) as previously described (3). A 20% (w/v) homogenate was thus prepared and then immediately diluted with HM containing NaF and with HM without NaF to give 5% homogenates. Subsequently, endogenous phosphatase activated HMG-CoA reductase in the medium without NaF, but was inhibited from doing so in the medium with NaF. Thus, both activated and nonactivated forms of reductase were prepared from each organ. Liver microsomes were prepared by differential centrifugation (3). After freeze-thawing and preincubation, reductase activity was measured in intestinal homogenate and in liver microsomes. Samples for assay were derived from 2.5 mg of small intestine or 10 mg of liver and contained 0.36 or 0.23 mg of protein, respectively, in 50 μl. Activity was assayed by the method of Shapiro et al. (4) modified as described previously (3). In this method, [^{14}C]HMG-CoA, an NADPH-generating system and [^{3}H]mevalonic acid were incubated with the enzyme sample, and the [^{14}C]mevalonic acid formed was subsequently isolated by thin-layer chromatography and measured by scintillation counting. The [^{3}H]mevalonic acid was used to correct

TABLE 1. Effect of restriction of food intake and diabetes on body and organ weights of rats killed at mid-dark

Experiment	Group	Food Intake	n	Body Weight		Intestine Weight		Liver Weight	
				Initial	Final				
		g/day		*g*	*g*	*g*	*% of body*	*g*	*% of body*
1. Food ad lib 8 days	D	36 ± 3 $P < 0.001$	8	299 ± 2	297 ± 11 $P < 0.001$	13.2 ± 0.5 $P < 0.001$	4.43 ± 0.13 $P < 0.001$	12.2 ± 0.7 ns	4.1 ± 0.1 ns
	C	23 ± 1	9	295 ± 10	345 ± 9	9.7 ± 0.4	2.80 ± 0.07	13.4 ± 0.6	3.9 ± 0.1
2. Food restricted 8 days	D	21	11	304 ± 5	270 ± 6 $P < 0.001$	7.6 ± 0.2 ns	2.82 ± 0.04 $P < 0.001$	9.6 ± 0.2 $P < 0.001$	3.5 ± 0.1 ns
	C	21	10	311 ± 8	328 ± 6	7.8 ± 0.2	2.41 ± 0.07	11.0 ± 0.2	3.4 ± 0.1
3. Food ad lib 22 days	D	42 ± 1 $P < 0.001$	11	312 ± 9	293 ± 9 $P < 0.001$	14.6 ± 0.3 $P < 0.001$	5.01 ± 0.18 $P < 0.001$	13.4 ± 0.8 ns	4.6 ± 0.2 $P < 0.001$
	C	24 ± 3	10	307 ± 9	380 ± 10	8.9 ± 0.3	2.35 ± 0.07	13.0 ± 0.7	3.4 ± 0.1
4. Food restricted 22 days	D	21	8	324 ± 6	262 ± 8 $P < 0.001$	7.1 ± 0.1 ns	2.75 ± 0.06 $P < 0.001$	9.1 ± 0.3 $P < 0.001$	3.5 ± 0.1 ns
	C	21	5	322 ± 10	370 ± 3	7.3 ± 0.1	1.98 ± 0.06	12.4 ± 0.4	3.4 ± 0.1
5. Food restricted 12 days	S	12	7	306 ± 7	292 ± 6 $P < 0.001$	5.8 ± 0.1 $P < 0.01$	2.00 ± 0.05 ns	9.3 ± 0.3 $P < 0.005$	3.2 ± 0.1 ns
	C	25	5	298 ± 8	343 ± 12	6.6 ± 0.1	1.94 ± 0.05	12.1 ± 0.6	3.5 ± 0.2

Rats in Group D were diabetic, in group C were control, and in S were stunted by underfeeding. Food intake is shown for the day prior to killing in Experiments 1, 3, and 5, for the previous 8 days in Experiment 2, and for the previous 22 days in Experiment 4. Food was delivered in five meals/day at 100-min intervals from 8:40 AM to 3:20 PM in Experiments 2 and 4, and in three meals/day at 3-hr intervals from 9 AM to 3 PM in Experiment 5. The amount of food in Experiment 5 was adjusted to maintain the body weight of rats in Group S at 95% of initial weight and to maintain normal growth in Group C. The dark period was 6 AM to 6 PM, and rats were killed at 11:30 AM to 12:30 PM in Experiments 1–5.

for losses during and after the incubation. An assay control consisting of all components except $NADP^+$ was used to correct for NADPH-independent, non-mevalonate ^{14}C-labeled products isolated with [^{14}C]mevalonate. Activity expressed per g of tissue was multiplied by organ weight to give total activity per organ. Activity per mg of protein can be calculated by dividing activity per g of tissue by protein recovery, which was 143 ± 12 mg protein/g of tissue in small intestine homogenate and 23 ± 1 mg/g in liver microsomes. Protein was measured with a fluorescamine-based assay (5), and recovery was not affected by diabetes (1).

Statistics

Data are presented as means ± standard error of the mean. The unpaired Student's t test was used to determine the significance of difference between means of experimental and control groups. A $P > 0.05$ was considered not significant.

RESULTS

In a previous study (1) of diabetic rats eating ad lib for 8 or 22 days after streptozotocin injection, their food intake, intestine weight, plasma TG and cholesterol levels, and HMG-CoA reductase activity in small intestine were all elevated, while reductase activity in liver was low (Experiments 1 and 3, Tables 1–4). The activation of hepatic reductase in vitro by endogenous phosphatase was decreased in ad lib fed rats with diabetes for 1 to 22 days ($P < 0.005$), whereas activation of intestinal reductase was unaffected by diabetes at any time (1).

In the present study, we tested the possibility that these changes in diabetic rats eating ad lib were due to hyperphagia by restricting their food intake to the amount eaten ad lib by normal rats, i.e., approximately half the amount eaten ad lib by diabetic rats (Experiments 2 and 4, Tables 1–4). Diabetic rats on this regimen for 8 or 22 days lost more weight than their hyperphagic counterparts (**Table 1**), but they all survived. Their intestine weight was normalized, and their liver weight decreased in proportion to body weight. Plasma insulin remained low and plasma glucose remained high (**Table 2**), so diabetes per se evidently was not affected. Plasma TG level was reduced to at least normal, and plasma cholesterol level was reduced to nearly normal. HMG-CoA reductase activity in liver and small intestine were normalized except for activated total activity in liver at 8 days, which was low (**Tables 3 and 4**).

We next examined the responses of normal rats to restricting food intake to about half their ad lib amount (Experiment 5, Tables 1–4). Body weight was reduced to that of hyperphagic diabetics (Table 1). Liver and small intestine weights decreased in proportion to body weight and hence to food intake. Plasma insulin in both

TABLE 2. Effect of restriction of food intake and diabetes on levels of insulin, glucose, TG, and cholesterol in plasma of rats killed at mid-dark

Experiment	Group	Insulin	Glucose	TG	Cholesterol
		μU/ml	*mg/dl*	*mg/dl*	*mg/dl*
1. Food ad lib 8 days	D	49 ± 10 $P < 0.05$	518 ± 28 $P < 0.001$	991 ± 213 $P < 0.005$	94 ± 10 $P < 0.001$
	C	85 ± 12	167 ± 4	114 ± 8	50 ± 1
2. Food restricted 8 days	D	33 ± 8 $P < 0.001$	573 ± 26 $P < 0.001$	155 ± 21 ns	54 ± 2 ns
	C	96 ± 14	158 ± 12	122 ± 8	52 ± 2
3. Food ad lib 22 days	D	11 ± 2 $P < 0.001$	581 ± 40 $P < 0.001$	1504 ± 421 $P < 0.005$	130 ± 18 $P < 0.001$
	C	88 ± 11	162 ± 10	102 ± 11	51 ± 2
4. Food restricted 22 days	D	36 ± 6 $P < 0.05$	611 ± 39 $P < 0.001$	97 ± 5 $P < 0.001$	69 ± 3 $P < 0.01$
	C	83 ± 21	164 ± 3	142 ± 6	55 ± 2
5. Food restricted 12 days	S	162 ± 21 $P < 0.005$	142 ± 5 ns	88 ± 6 ns	48 ± 3 ns
	C	284 ± 17	154 ± 4	115 ± 23	45 ± 2

Experimental details are given in Table 1.

TABLE 3. Effect of restriction of food intake and diabetes on activated HMG-CoA reductase activity in small intestine and liver of rats killed at mid-dark

Experiment	Group	Activated HMG-CoA Reductase Activity					
		Specific		Total		Total/Body Weight	
		Intestine	Liver	Intestine	Liver	Intestine	Liver
		nmol/(min·g tissue)		*nmol/(min·organ)*		*pmol/(min·g body weight)*	
1. Food ad lib 8 days	D	6.6 ± 0.5 $P < 0.005$	1.6 ± 0.3 $P < 0.005$	86 ± 5 $P < 0.001$	18 ± 3 $P < 0.005$	296 ± 26 $P < 0.001$	62 ± 13 $P < 0.005$
	C	4.2 ± 0.3	4.7 ± 0.7	42 ± 4	63 ± 10	119 ± 10	180 ± 28
2. Food restricted 8 days	D	5.5 ± 0.4 ns	6.0 ± 1.8 ns	42 ± 4 ns	59 ± 18 $P < 0.025$	154 ± 11 ns	211 ± 62 ns
	C	5.1 ± 0.5	10.7 ± 1.4	40 ± 4	119 ± 16	124 ± 15	362 ± 48
3. Food ad lib 22 days	D	4.9 ± 0.4 $P < 0.05$	1.3 ± 0.3 $P < 0.001$	71 ± 7 $P < 0.001$	18 ± 4 $P < 0.001$	242 ± 21 $P < 0.001$	60 ± 14 $P < 0.005$
	C	3.7 ± 0.4	6.0 ± 1.2	33 ± 4	79 ± 15	87 ± 11	205 ± 35
4. Food restricted 22 days	D	5.2 ± 0.5 ns	6.0 ± 1.2 ns	37 ± 4 ns	56 ± 13 ns	142 ± 12 ns	211 ± 42 ns
	C	5.4 ± 0.3	4.2 ± 0.6	40 ± 2	52 ± 7	108 ± 8	140 ± 17
5. Food restricted 12 days	S	4.1 ± 0.4 ns	6.4 ± 1.0 ns	24 ± 3 $P < 0.05$	59 ± 11 ns	83 ± 10 ns	205 ± 39 ns
	C	7.0 ± 1.5	3.4 ± 1.0	46 ± 10	40 ± 9	133 ± 25	117 ± 28

Samples for reductase assay were prepared in the absence of NaF to permit activation by endogenous phosphatase as described in Materials and Methods. Other experimental details are given in Table 1.

the stunted rats and their controls was higher than seen previously (Table 2). These rats were fed three meals per day, whereas other groups were fed five meals or ate ad lib; all were killed during the middle of their feeding period. Thus, the recent consumption of a relatively large meal may account for the high plasma insulin levels in Experiment 5. In any case, insulin levels in rats in the underfed group were lower than in their controls. Neither glucose nor lipid levels were significantly affected. Except for a decrease in total reductase activity in intestine (Table 3) and an increase in activation of reductase by endogenous phosphatase in liver (Table 4), reductase activities were unaffected.

The data summarized in Tables 1–4 were obtained from rats killed at mid-dark, the middle of their feeding period. Handling of these rats (transferring to portable cages and then to the lab, and weighing) started about 3 hr before they were killed and disturbed the feeding behavior of some rats. Although meals were provided during this period as scheduled, they were not entirely consumed by those rats receiving close to the amount they would eat ad lib. The rats on reduced food intake were hungrier and did consume their meals entirely. Therefore, it was possible that variable food intake just before killing influenced some responses, in particular that of reductase activity in liver. To eliminate this variable in the next experiment, we permitted rats to completely consume their day's ration before they were handled. Thus we killed rats at mid-light, from 7.5 to 9 hr after consuming their last meal. Diabetic and non-diabetic rats were fed either the amount each would have eaten ad lib or about half that amount for 22 days.

The responses to reduced food intake measured at mid-light (**Tables 5–7**) were qualitatively the same as those measured at mid-dark (Tables 1–4), with one exception. Total reductase activity in liver of non-diabetic rats at mid-light was decreased by food reduction (Table 7), whereas that at mid-dark was not (Experiment 5, Table 3). Possibly the lack of effect at mid-dark was due to poor control of recent food consumption.

The weights of the whole body, liver, and small intestine all decreased with decreasing food intake in both normal and diabetic rats (**Fig. 1**). The weights of whole body and to a lesser extent, liver also decreased with diabetes, but small intestine weight was unaffected by diabetes.

Total HMG-CoA reductase activity in the small intestine, like its weight, decreased linearly with decreasing

TABLE 4. Effect of restriction of food intake and diabetes on nonactivated HMG-CoA reductase activity and on its activation in vitro by endogenous phosphatase from small intestine and liver of rats killed at mid-dark

Experiment	Group	HMG-CoA Reductase			
		Nonactivated Specific Activity		Activation in vitro	
		Intestine	Liver	Intestine	Liver
		nmol/(min · g tissue)		*fold*	
1. Food ad lib 8 days	D	1.7 ± 0.1 $P < 0.005$	0.4 ± 0.1 $P < 0.005$	3.8 ± 0.6 ns	4.1 ± 0.5 $P < 0.05$
	C	1.2 ± 0.1	0.8 ± 0.1	3.8 ± 0.2	5.9 ± 0.5
2. Food restricted 8 days	D	2.0 ± 0.1 ns	2.3 ± 0.6 ns	2.9 ± 0.3 ns	2.7 ± 0.6 ns
	C	2.0 ±0.2	3.8 ± 0.5	2.7 0.3	4.1 ± 1.0
3. Food ad lib 22 days	D	1.4 ± 0.1 ns	0.2 ± 0.1 $P < 0.005$	3.6 ± 0.2 ns	7.1 ± 0.8 ns
	C	1.0 ± 0.1	0.6 ± 0.1	4.0 ± 0.3	9.4 ± 0.9
4. Food restricted 22 days	D	1.1 ± 0.1 ns	0.7 ± 0.1 ns	4.7 ± 0.5 ns	8.2 ± 0.4 ns
	C	1.1 ± 0.2	0.7 ± 0.1	5.9 ± 1.2	7.2 ± 0.7
5. Food restricted 12 days	S	1.0 ±0.1 ns	1.1 ± 0.2 ns	4.4 ± 0.4 ns	6.1 ± 0.2 $P < 0.05$
	C	1.2 ± 0.2	0.8 ± 0.2	6.0 ± 1.1	4.4 ± 0.8

Activation of HMG-CoA reductase by endogenous phosphatase in vitro was inhibited with NaF in the homogenizing medium. Activity measured in the absence of NaF (Table 3) divided by activity measured in the presence of NaF (this table) is the 'fold' activation. Other experimental details are given in Table 1.

food intake, and was unaffected by diabetes per se (**Fig. 2A**). As total reductase activity in intestine divided by body weight decreased, plasma cholesterol level decreased (Fig. 2B). As plasma cholesterol decreased, total reductase activity in liver increased (Fig. 2C). These relationships suggest that decreasing reductase activity in intes-

TABLE 5. Effect of diabetes and restriction of food intake for 22 days on body and organ weights of rats killed at mid-light

Group	Food Intake	Body Weight		Intestine Weight		Liver Weight	
		Initial	Final				
	g/day	*g*	*g*	*g*	*% of body*	*g*	*% of body*
D1	37	303 ± 1	284 ± 5 $P < 0.001$	12.8 ± 0.3 $P < 0.001$	4.49 ± 0.08 $P < 0.001$	12.3 ± 0.5 ns	4.32 ± 0.18 $P < 0.01$
D2	22	303 ± 1	231 ± 9 $p < 0.001$	8.2 ± 0.4 ns	3.60 ± 0.29 $P < 0.005$	8.4 ± 0.3 $P < 0.001$	3.64 ± 0.06 ns
S	13	301 ± 3	273 ± 6 $P < 0.001$	6.0 ± 0.4 $P < 0.005$	2.20 ± 0.11 ns	8.6 ± 0.4 $P < 0.001$	3.10 ± 0.09 $P < 0.005$
C	22	304 ± 5	365 ± 8	7.9 ± 0.3	2.20 ± 0.05	12.9 ± 0.5	3.60 ± 0.09

For 22 days after injection of streptozotocin (D1, D2) or buffer (S, C), chow was delivered in equally spaced meals from 7:10 PM to 1:50 AM. Rats in D1 received nine meals; and others received five meals per day. Food delivered to rats in D1 was gradually increased from 22 g/day on the day of injection to 40 g/day on day 6 and then held at that level until day 22. The amount of food delivered to rats in Group S was adjusted to maintain body weight equal to that of rats in Group D1. Values for food intake in the table show the amount of food actually consumed by rats in Groups D2 and C for the previous 22 days, and by rats in Groups D1 and S for the previous 2 days. For the last 3 days, food not consumed by 2 AM was removed from the cages. There were four rats in Group D1, and five rats in each of the other groups. The dark period was 4:30 PM to 4:30 AM, and rats were killed at 10–11 AM.

TABLE 6. Effect of diabetes and restriction of food intake for 22 days on levels of glucose, TG, and cholesterol in plasma of rats killed at mid-light

Group	Glucose	TG	Cholesterol
	mg/dl	*mg/dl*	*mg/dl*
D1	498 ± 23 $P < 0.001$	510 ± 113 $P < 0.005$	114 ± 10 $P < 0.001$
D2	510 ± 39 $P < 0.001$	91 ± 12 $P < 0.02$	74 ± 7 $P < 0.02$
S	136 ± 9 ns	34 ± 4 $P < 0.02$	61 ± 2 $P < 0.05$
C	137 ± 6	49 ± 3	52 ± 3

Experimental details are described in Table 5.

tine, decreasing plasma cholesterol, and increasing reductase activity in liver constitute a chain of events initiated by reducing food intake.

The only parameter that varied diurnally was reductase activity in liver (Fig. 2C). The 2- to 3-fold amplitude of this variation ($P < 0.01$) was not abolished by diabetes or by hyperphagia in these rats who were fed only during the dark period.

DISCUSSION

The present study demonstrates that HMG-CoA reductase activity in liver and small intestine is unaffected by chronic diabetes if food intake is held to a normal level. Thus, the decreased activity and the decreased activation in vitro of reductase from liver and the increased activity in small intestine in chronically diabetic rats eating ad lib result from hyperphagia and not from direct effects of insulin deficiency. Our results also show that hyperphagia is necessary to obtain the very high levels of TG and cholesterol in plasma of diabetic rats eating chow.

Our findings with chronically diabetic rats (that reductase activity in small intestine is either normal or increased but never decreased, and that reductase activity in liver is decreased only when hyperphagia is permitted but is otherwise normal) are in distinct contrast to other studies showing that insulin stimulates and glucagon inhibits reductase in both liver and small intestine (see (6) for review). However, we do see a decrease in hepatic reductase activity in acutely diabetic rats (1) that is associated with anorexia and low plasma insulin level, either of which could be the effective agent. Nevertheless, this low activity does not persist; by 8 days, if food intake is normalized, hepatic reductase specific activity is also normal. These findings are consistent with the possibilities that hormonal effects are short-lived and eventually are overridden by cholesterol feedback regulation, or that insulin sensitivity increases after prolonged insulin deficiency. In any event, it is clear from our results that reductase activity can be normal, and can even increase in the intestine, in the face of insulin deficiency.

Nepokroeff et al. (7) showed that the diurnal rhythm of hepatic HMG-CoA reductase is essentially abolished in diabetic rats eating ad lib. In contrast, we find that the rhythm persists even in hyperphagic diabetic rats (Fig. 2C). However, the rhythm in our rats may have been enforced by feeding meals only during the dark period. Nevertheless, the present data show that a normal rhythm in hepatic reductase activity does not require normal plasma insulin concentrations.

Others have recently shown that the fraction of reductase in the active, dephosphorylated form in freshly isolated hepatocytes is rapidly increased by insulin and decreased by glucagon in the medium (8, 9). From these

TABLE 7. Effect of diabetes and food restriction for 22 days on activated HMG-CoA reductase activity in small intestine and liver of rats killed at mid-light

	Activated HMG-CoA Reductase Activity					
	Specific		Total		Total/Body Weight	
Group	Intestine	Liver	Intestine	Liver	Intestine	Liver
	nmol/(min · g tissue)		*nmol/(min · organ)*		*pmol/(min · g body)*	
D1	5.0 ± 0.6 ns	0.5 ± 0.1 $P < 0.001$	63 ± 7 $P < 0.001$	6 ± 1 $P < 0.001$	224 ± 26 $P < 0.001$	22 ± 4 $P < 0.001$
D2	4.5 ± 1.2 ns	2.3 ± 0.3 ns	38 ± 10 ns	19 ± 2 ns	163 ± 46 ns	84 ± 1 ns
S	3.9 ± 0.2 ns	1.7 ± 0.3 ns	23 ± 2 $P < 0.005$	14 ± 3 $P < 0.02$	85 ± 5 ns	54 ± 1 ns
C	4.7 ± 0.4	1.9 ± 0.2	37 ± 3	25 ± 3	103 ± 8	69 ± 7

Reductase activity was assayed after activation by endogenous phosphatase in the absence of NaF. Other experimental details are given in Table 5.

results, we would expect the fraction of active enzyme to be decreased, or its reciprocal, activation in vitro, to be increased by diabetes. We find, on the contrary, that activation of hepatic reductase from ad lib fed diabetic rats is lower than normal (1). The pattern of response of activation and that of nonactivated reductase activity are similar in that both decrease in acutely diabetic rats that are eating less and have a low plasma insulin level, and both remain low in chronically diabetic rats only when they overeat (Table 4) (1). Activation of intestinal reductase is unaffected by diabetes or by altering food intake.

The apparent discrepancy between our results with activation of reductase from diabetic rats and results with hepatocytes may be due to one or more of the following

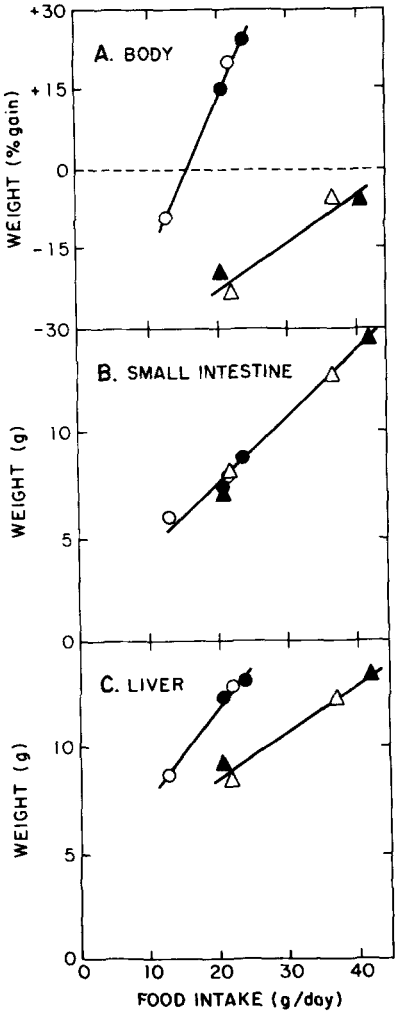

Fig. 1. Effect of food intake on body weights and weight of small intestine and liver at 22 days after injection. Mean body (panel A), small intestine (panel B), and liver (panel C) weights for groups of diabetic rats at mid-dark (▲) and at mid-light (△), and for non-diabetic rats at mid-dark (●) and at mid-light (○) at 22 days after injection are from Experiments 3 and 4 (Table 1) and the last experiment (Table 5).

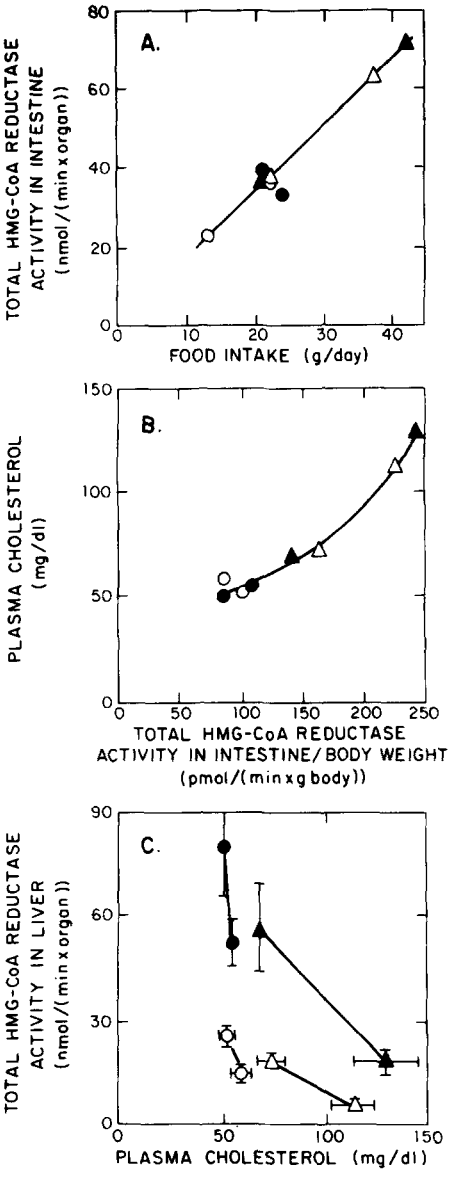

Fig. 2. Relationships between food intake, total HMG-CoA reductase activity in small intestine, plasma cholesterol, and total HMG-CoA reductase activity in liver 22 days after injection. Data are means for groups of rats from Experiments 3 and 4 (Tables 2 and 3) and the last experiment (Tables 6 and 7). The symbols are defined in Fig. 1.

factors. First, since we relied on endogenous phosphatase to activate endogenous reductase, the decrease in activation may be due to a decrease in phosphatase activity rather than to an increase in the active fraction of reductase in diabetic rat liver. Second, effects of insulin and glucagon on hepatocytes occur within 10 min (9). If these effects are short-lived we would have missed them since the earliest time diabetic rats were examined was 4 hr (1). Third, handling the rat prior to homogenization of the liver may affect phosphorylation. The recent finding that phenylephrine, a catecholamine and alpha-adrenergic stimulator, decreases the active fraction

of reductase in hepatocytes (9) supports this possibility. Perhaps catecholamines released before organs are removed lead to phosphorylation of reductase and thus mask any prior effects of altered hormonal status on the fraction of active enzyme.

We suggest that diabetes alters reductase activities and plasma cholesterol level as follows. With low plasma insulin level, transport of glucose into cells is impaired, consequently glucose and ketone bodies are excreted in urine. This inefficient use of ingested calories impairs growth (Fig. 1A) and leads to a compensatory increase in food intake. Eating more food increases dietary cholesterol intake, and also causes hypertrophy (Fig. 1B) (10), increased total HMG-CoA reductase activity (Fig. 2A) and increased cholesterol synthesis (2) of the small intestine. Since intestinal absorption of cholesterol (11) and bile acid (12) also increase, while their excretion remains normal (13), the net effect is an increase in secretion of cholesterol in chylomicrons in thoracic lymph (11). The increased influx of intestinal lipoproteins saturates mechanisms for clearance from blood so that cholesterol accumulates (Fig. 2B). This accumulation is enhanced by failure to grow and hence to utilize cholesterol for new tissue formation. Cholesterol in chylomicron remnants is eventually taken up by the liver where it inhibits HMG-CoA reductase activity (Fig. 2C) (14).

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