

Cholesterol Metabolism in Diabetes Mellitus

The Role of Diet

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SUMMARY

There is extensive evidence that cholesterol metabolism is abnormal in diabetes. Total plasma cholesterol is elevated, and more subtle indices of sterol homeostasis are also deranged.

Insulinization of the poorly controlled diabetic reduces fecal bile acid excretion. Fecal neutral sterol excretion increases compensatorily, at least when caloric intake is maintained during insulinization. Thus, net sterol balance is not changed by insulin treatment, again assuming the diet remains constant. Other workers have found decreased sterol synthesis after insulinization if calories are reduced. Using isotopically labeled squalene turnover as a reflection of cholesterol synthesis, insulin treatment increases the flux of squalene.

Pursuing diabetes' effect on cholesterol synthesis at a more basic level, we found that HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, is increased in small intestine, and decreased in liver by diabetes in rats eating ad libitum.

These changes in reductase activity are due to hyperphagia (increased caloric and/or cholesterol intake), not to insulin deficiency alone, since diabetic rats restricted to a normal food intake have normal reductase activity in liver and small intestine. It appears that increased input of dietary and newly synthesized cholesterol by the small intestine raises plasma cholesterol level and represses reductase activity in liver of chronically diabetic rats.

Experiments measuring the incorporation of tritiated water into cholesterol find that diabetes has a net suppressive effect on cholesterol synthesis, consistent with insulin's increasing squalene turnover, and consistent with the suppressed liver synthesis being quantitatively more significant than increased intestinal synthesis. When dietary cholesterol intake is included in the calculation, however, hyperphagia

may tip the balance in favor of increased net cholesterol input (synthesis plus diet).

We hypothesize that diet plays a central role in the diabetic's cholesterol homeostasis. The hyperphagia of experimental diabetes increases net cholesterol intake and increased HMG-CoA reductase in a hypertrophic small intestine. *DIABETES 30 (Suppl. 2): 76-81, 1981.*

The study of cholesterol metabolism in diabetes mellitus has been neglected, perhaps because the more common and obvious abnormality of plasma lipids in diabetic patients is hypertriglyceridemia. A few introductory points, however, will serve to highlight the importance of abnormal cholesterol homeostasis in diabetes.

Plasma cholesterol concentration is variably elevated in diabetic populations. Table 1 summarizes 18 studies,¹⁻¹⁸ widely varying in design, subject population, and conclusions. To be sure, the number of variables makes comparisons difficult: diabetes comes in many forms. And it is also evident from Table 1 that not all studies have found plasma cholesterol increased. But the larger, better-controlled studies do suggest that diabetes raises total plasma cholesterol. Nonhuman primates¹⁹ and rats²⁰ made diabetic also have hypercholesterolemia.

All major studies point to total plasma cholesterol as a potent risk factor for cardiovascular disease. Hypertriglyceridemia is far less significant²¹ and then probably only through its inverse correlation with HDL cholesterol. Not only are elevated total plasma cholesterol and LDL cholesterol strong risk factors, but they are predictors of atherosclerotic disease even throughout the "normal" range of cholesterol for western populations. One suspects, in fact, that we live and die with three to four times the plasma cholesterol we, as a species, maintained in earlier evolutionary development. Baboons, for example, have a basal plasma cholesterol of 60-83 mg/dl.²²

There are data suggesting that diabetics with elevated lipids do, in fact, have increased susceptibility for athero-

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TABLE 1
Total plasma cholesterol in diabetes: 18 studies

1st author	Ref.	Year	No. of diabetic subjects	Type of diabetes	PCh (mg/dl)		Comments
					Diabetes	Controls	
Rabinowitch	(1)	1929	46	II	243	176	↑ PCh above "normal" in 43% of diabetics Correlation of oral glucose tolerance with PCh in males only 200 diabetics with vascular disease had the higher PCh ↑ PCh in 23% of diabetics vs. literature control ↑ PCh > 280 mg/dl in 40% Strong correlation between PCh and diabetes Age matched, no significant difference between diabetics and controls Literature controls both sexes
Chaikoff	(2)	1936	26	I	181	170	
Adlersberg	(3)	1959	94	II	276	248	
Lee	(4)	1962	28M 35F	II	216	204	
New	(5)	1963	35	I	231	226	
Chance	(6)	1969	160	II	181	150	
Kaufmann	(7)	1969	135 220	I IGT	235	213	
Ahuja	(8)	1969	100	I	235	200	
Wilson	(9)	1970	98	II	306		
Sharma	(10)	1970	25	I	242	182	
Hayes	(11)	1972	102	II			
Medalie	(12)	1974	10,059	I & II			
O'Neal	(13)	1974	62	I & II			
Kaufmann	(14)	1975	129M 141F	I I	202	178	
Nikkilä	(15)	1978	170	I	193 M259 F278	178 259 262	
Kannel	(16)	1979	2,506	I & II	M230 F260	233 250	
Sosenko	(17)	1980	105	I	151 158 163	148	
Simpson	(18)	1980	14 13	I II			

Abbreviations: I and II: current terminology for types of diabetes, roughly equivalent to juvenile-onset or maturity-onset categories used in the studies presented; IGT: impaired glucose tolerance; PCh: plasma cholesterol; M: male; F: female; HbA_{1c} = hemoglobin A_{1c}.

sclerosis,²³ and that diabetes and high plasma cholesterol act as independent risk factors.¹⁶ Furthermore, diabetic non-human primates develop more atherosclerosis in response to an atherogenic diet than do nondiabetics.²⁴

It may well be that even though plasma cholesterol concentration in individual diabetic patients is often defined as "normal," the distribution curve of plasma cholesterol is shifted toward higher concentrations in diabetic populations. The Framingham study suggests that increases in plasma cholesterol even in this "normal" range would have significant effects on cardiovascular morbidity.²⁵ With over 50% of the American people dying of cardiovascular disease,²⁶ a relatively subtle shift of diabetics into higher risk groups by virtue of mildly increased cholesterol concentrations might contribute to the even more devastating mortality from cardiovascular disease seen in diabetes.

Finally, plasma cholesterol determination provides a very limited view of cholesterol metabolism as a whole. Only some 5% of the normal adult's whole body cholesterol content is found in the plasma, and the most significant body pools—quantitatively and pathologically—are in large vessel atherosclerotic plaques. This deposited cholesterol equilibrates very slowly with plasma cholesterol. So while plasma cholesterol concentration provides a readily accessible look at obvious abnormalities, there is reason to doubt that this keyhole alone provides an adequate field of vision.

We chose to ask what role insulin—its lack and its clinical replacement—plays in the regulation of cholesterol production. We started, optimistically, with studies of the intact human, only later addressing specific enzyme assays of rat tissue.

Initial studies used sterol balance techniques before and during intensive insulin therapy of five nonobese, type II (non-insulin-dependent) diabetics.²⁷ Subjects were adults (mean age 64 yr), chosen for their poor diabetic control. Three were inadequately treated with insulin initially, and two were on diet treatment alone. Patients were on a metabolic ward for the entire study, with a repeating diet containing 0.5 g cholesterol. Fecal neutral steroids (FNS) and fecal bile acids (FBA) were analyzed during a 3–8-wk baseline (poor control) period. Insulin was then initiated to achieve improved glycemic control abruptly, with patients remaining on constant diet and with fecal sterol collections continuing for 6 wk of an improved control period.

Figure 1 illustrates the study design and the results for one subject. In the group as a whole, daytime glycemia was improved by 53%. There was a modest, sudden weight gain upon insulinization, consistent with salt and water retention.²⁸ In all subjects, FBA excretion dropped (Table 2), with a predominant decrease in cholic acid. We found, however, a compensatory increase in FNS, so that total sterol balance was not changed (Table 2).

Bennion and Grundy earlier published another assessment of sterol balance during diabetic control.²⁹ Their study design was similar but not identical with ours. Rather than remaining hospitalized with constant diet and continual fecal collections, subjects were discharged for several weeks after the baseline, poor control period, and reequilibrated at a new caloric intake after achieving improved control. Subjects ingested 42% fewer calories daily during the good control period than during the poor control period.

Bennion and Grundy found, as we did, a significant de-

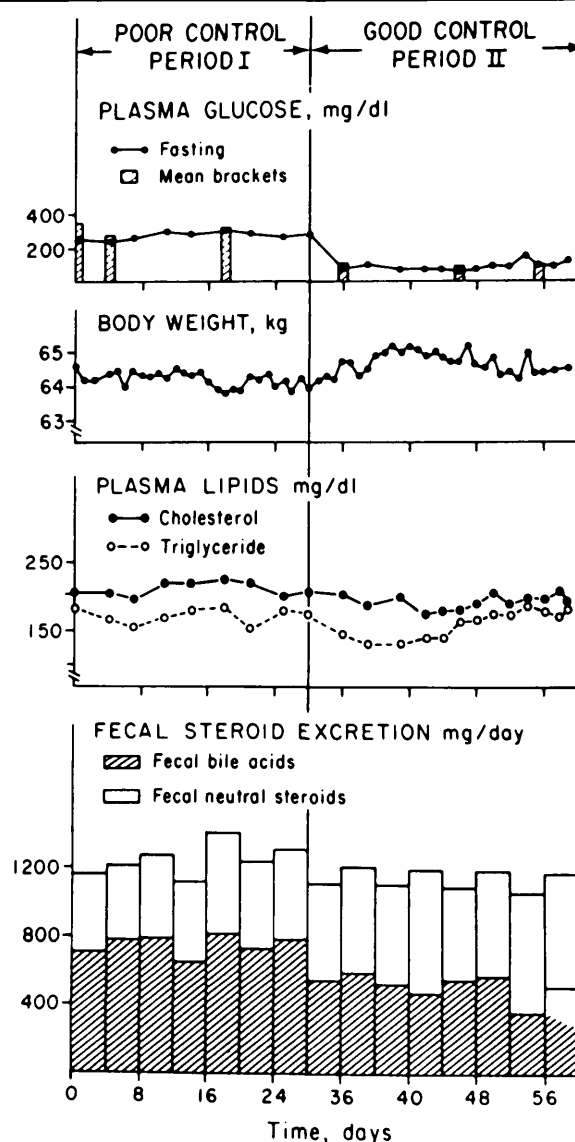


FIGURE 1. One subject (data from ref. 27), studied with sterol balance techniques before (period I) and after (period II) insulinization. Fecal bile acid excretion was significantly decreased on insulin, while fecal neutral sterol excretion was increased. Net sterol balance was unchanged.

crease in FBA upon insulinization. However, they did not note an increase in FNS. Therefore, net sterol balance was changed: insulin treatment decreased endogenous cholesterol production.

Data discussed below suggested that the apparently minor difference in study design—whether to decrease or maintain caloric intake when tight control is achieved—might explain the discrepancy between our study²⁷ and that of Bennion and Grundy.²⁹ On the one hand, reducing calories in the good control period, as they did, might of itself reduce sterol excretion, independent of an insulin effect. On the other hand, by continuing the same caloric intake in both periods, our subjects may have been mildly hypercaloric during the improved control period, as they utilized calories more efficiently. This problem is not readily resolved using sterol balance methodology.

A second problem with the application of balance methods is that a "metabolically steady state" is assumed,

TABLE 2
Change in fecal bile acids (FBA), fecal neutral steroids (FNS), and net balance upon insulinization (data from ref. 27)

Subject	Δ FBA (mg/day)	Δ FNS (mg/day)	Balance (mg/day)
1	↓ 56*	↑ 328†	↑ 274†
2	↓ 37	↓ 16	↓ 53
3	↓ 32	↑ 72†	↓ 40
4	↓ 243†	↑ 128†	↓ 115
5	↓ 32	↓ 107*	↓ 138

* Significantly changed, $P < 0.05$.

† Significantly changed, $P < 0.01$.

in which there is no net transfer of cholesterol into or out of the body's slow-turnover pools of cholesterol. This condition is considered met if subjects are stabilized on constant diet, with weight and plasma cholesterol remaining constant. But it is debatable whether a metabolically steady state exists in diabetes, either during insulinization or for some indeterminate time thereafter.

Another approach to the measurement of cholesterol synthesis is the analysis of isotopically labeled squalene kinetics. Mevalonic acid (MVA) is formed from the reduction of HMG-CoA by HMG-CoA reductase, generally considered to be the rate-limiting step in cholesterol biosynthesis in most organs. All MVA, however, does not enter into cholesterol synthesis; there is a branch point at which isopentenylpyrophosphates may either enter sterol synthesis or be diverted into a shunt pathway that produces free fatty acids, CO_2 , and other metabolites. By infusing ^{14}C -MVA, then, a portion of the label passes through squalene to cholesterol, while the rest is metabolized to other products, including $^{14}\text{CO}_2$.

Using MVA labeled with ^{14}C in the 5 position, and with ^3H -cholesterol as a simultaneous marker to correct for MVA not metabolized to sterol, Liu et al. described the kinetics of isotopically labeled squalene as a measure of cholesterol synthesis.³⁰ Because of squalene's rapid turnover in plasma, the technique requires only a 7-h study, an obvious advantage over balance methodology.

We applied the squalene kinetic method to measure cholesterol synthesis of five non-insulin-dependent, nonobese adult diabetics, mean age 60 yr, with a mean duration of diabetes of 12 yr.³¹ Four of the five were not taking insulin despite mean daytime plasma glucose values of 349 ± 20 (SE) mg/dl, measured with six blood sugar determinations spaced throughout the day. After the first squalene turnover study, they were discharged and improved diabetic control was established with insulin on an outpatient basis. At the time of the second study, daytime plasma glucose was 175 ± 15 mg/dl, and the mean insulin dose was 57 ± 21 U daily. On a diabetic diet, subjects were free to consume calories ad libitum.

Upon insulinization, cholesterol synthesis estimated by squalene kinetics was increased from 964 ± 75 to 1206 ± 111 mg/day ($P < 0.025$) (Figure 2). Two factors enter into the calculation of squalene synthesis: the administered ^{14}C -MVA converted into ^{14}C -squalene and the area under the squalene die-away curve in plasma. Of these, an increased fractional conversion of MVA to squalene accounted for the calculated increase in cholesterol synthesis.

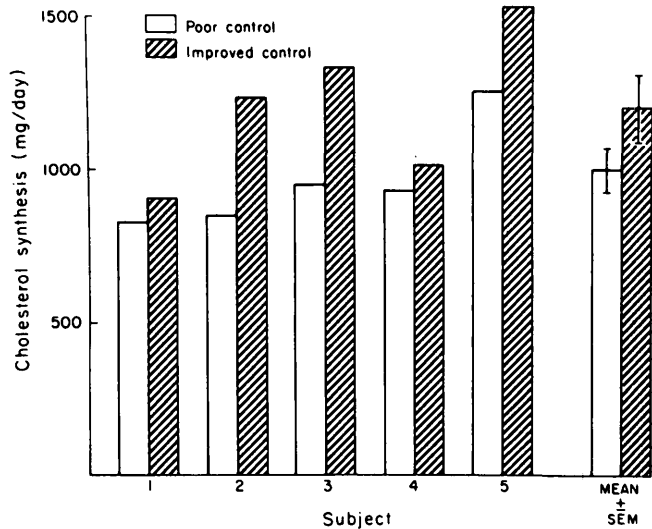


FIGURE 2. Cholesterol synthesis as measured by the squalene kinetic technique (data from ref. 31), before and after insulinization. Improved diabetic control (with ad libitum caloric intake) was associated with increased cholesterol synthesis.

Summarizing the results of human studies of insulinization, then, we and Bennion and Grundy each found that insulin causes a shift of fecal sterol excretion from FBA to FNS. When dietary intake was held constant, net sterol production did not change with insulin treatment;²⁷ when dietary intake was decreased, net sterol synthesis decreased upon insulinization.²⁹ By analysis of squalene kinetics, insulinization increased the calculated cholesterol synthetic rate.

Faced with these somewhat discrepant and hard to interpret human studies, we decided to use the streptozotocin-diabetic rat model to pursue the effect of diabetes on tissue HMG-CoA reductase. It has been known for some time that diabetes suppresses reductase activity in rat liver,^{32,33} an observation difficult to reconcile with hypercholesterolemia and accelerated atherosclerosis. As important as hepatic cholesterol synthesis is, however, there are at least three other potentially crucial aspects of cholesterol homeostasis: peripheral disposition of cholesterol, hepatic excretion of cholesterol, and intestinal cholesterol production. Clarenburg and Chaikoff found that cholesterol is increased in chyle from diabetic rats.³⁴ Nakayama and Nakagawa showed higher HMG-CoA reductase activity in mucosal cell scrapings of small intestine from experimentally diabetic rats.³⁵ We decided to pursue these observations by studying HMG-CoA reductase in small intestine, by following the sequence of events after diabetes is induced, and by considering the importance of food intake.

Measuring reductase activity in homogenate of whole small intestine offers two advantages over the isolated mucosal cell assay. First, since the whole organ is homogenized, the recovery of enzyme activity is maximized. Second, processing time is minimized, since the organ can be removed rapidly and homogenized on ice rather than requiring cell separation. Rapid handling is essential to reduce spontaneous, nonphysiologic activation of the enzyme.

In our procedure, the whole small intestine homogenate is assayed for reductase activity. Recovery of enzyme from ho-

mogenate is greater than from any subcellular fraction. This contrasts with liver, where recovery of activity is greater in microsomes. The small intestine assay was validated by showing requirement for NADPH, purity of product, kinetics for time, protein, and substrate concentration, as well as control of the reductase inhibition activity that was initially present in intestinal homogenate. Activity per gram tissue in homogenate was multiplied by organ weight to give whole organ activity.

Diabetes markedly increased total reductase activity in small intestine. In rats with diabetes for 1–3 wk, this increase coexisted with elevated plasma cholesterol and reduced reductase in liver. A pancreatectomized rat showed the same series of changes in plasma, liver, and small intestine, indicating that they were not secondary to streptozotocin administration, but to diabetes. Insulin treatment of rats largely reversed the abnormalities.

We next pursued time-course studies to help determine cause and effect. Animals were killed periodically from 4 h to 22 days after injection of streptozotocin. We found that after 12 h, reductase activity in liver was already reduced, together with decreased food intake, low insulin concentration, hyperglycemia, and significantly reduced plasma cholesterol. Reductase activity in small intestine was unchanged at this early time after streptozotocin, increasing above control only 3 days later.

It was apparent, then, that the initial suppression of reductase in liver could not be due to increased intestinal cholesterol production or hypercholesterolemia, since ingested dietary cholesterol was low, reductase activity in small intestine was unchanged, and plasma cholesterol was reduced. The possible importance of intestinal cholesterol production was highlighted, however, by the observation that plasma cholesterol concentration closely followed small intestine reductase activity, being low initially and rising above control at 3 days.

We also noted, as others had before, that the small intestine itself is significantly hypertrophied in diabetic rats. This hypertrophy occurred coincident with the increased total reductase activity of small intestine, but did not totally explain it. Both organ weight and increased reductase specific activity (per mg protein) contributed to the elevated total organ reductase.

A final "clinical" observation was that rats diabetic for more than 3 days were markedly hyperphagic. To clarify the relationships between hyperphagia, intestinal hypertrophy, increased small intestine reductase, and hypercholesterolemia, we conducted food restriction experiments. These were accomplished by delivering rat chow pellets periodically throughout the normal feeding period, but allowing diabetic rats only the same amount of food as controls.

Food restriction eliminated both hypertrophy and increased reductase activity in small intestine, and reduced the hypercholesterolemia. It also eliminated the suppression of reductase activity in liver, although plasma insulin remained low and plasma glucose high.

Each of these effects of food restriction was specific to the diabetic rat except the effect on small intestine HMG-CoA reductase. Hypocaloric feeding of nondiabetic rats did not alter plasma glucose or cholesterol, small intestine, or liver weight as a percent of whole body weight, or reductase activity in liver. Throughout a broad range of food intake, how-

TABLE 3
Effects of streptozotocin

	Time after streptozotocin	
	Immediate	Late
Plasma glucose	↕	↕
Plasma insulin	↓	↑
Food intake	↓	↑
Intestinal size	→	↑
Plasma cholesterol	↓	↑
HMG-CoA reductase		
Liver	↓	↓
Intestine	→	↑

ever, in diabetic and nondiabetic rats, HMG-CoA reductase total activity in small intestine correlated extremely closely with food intake.

The consequences of streptozotocin diabetes, then, may be divided into immediate and late effects (Table 3). Initially, food intake declines, reductase activity in liver is reduced, and plasma cholesterol concentration is reduced. Later, hyperphagia occurs coincident with intestinal hypertrophy, increased reductase activity in small intestine, and hypercholesterolemia.

With these opposite effects of diabetes on hepatic and intestinal reductase, we wanted to know the net effect of diabetes on whole body cholesterol synthesis. We approached the problem by measuring the incorporation of ³H from ³H₂O into cholesterol. Diabetic rats eating ad libitum incorporated less ³H into cholesterol than did controls. The suppression of endogenous synthesis in liver and perhaps other organs thus appears more significant quantitatively than increased new cholesterol synthesis in gut. But total cholesterol input includes that ingested, and, as noted, the rats were markedly hyperphagic. We have calculated cholesterol input (synthesis plus dietary) assuming equal fractional absorption of dietary cholesterol in diabetic and nondiabetic rats. According to this calculation, cholesterol input in diabetics is not less than in controls. Since absorption is greater in diabetes,³⁶ total cholesterol input may in fact be increased in diabetic rats.

A hypothesis may be advanced to explain our findings in the rat and to link them with the human studies noted earlier. The hypothesis (Figure 3) is that hyperphagia of whatever cause (insulin lack or insulin excess) leads to intestinal hypertrophy and elevated intestinal reductase activity. Increased cholesterol production from the intestine in diabetes is the consequence of increased cholesterol ingested as well as enhanced new synthesis of cholesterol from the hypertrophied gut. This cholesterol production more than compensates for the reduced reductase activity in liver, and contributes to hypercholesterolemia in diabetes.

The hypothesis emphasizes the importance of hyperphagia. It also takes into account the major types of human diabetes, and the effect of both under- and overinsulinization. The seriously underinsulinized diabetic is hyperphagic; hyperphagia is a classic symptom of diabetes. The overinsulinized diabetic may also overeat. While an appetite stimulating effect of vigorous insulin treatment is difficult to quantitate, it remains a common complaint of patients. Finally, even when type II diabetics are not treated with insu-

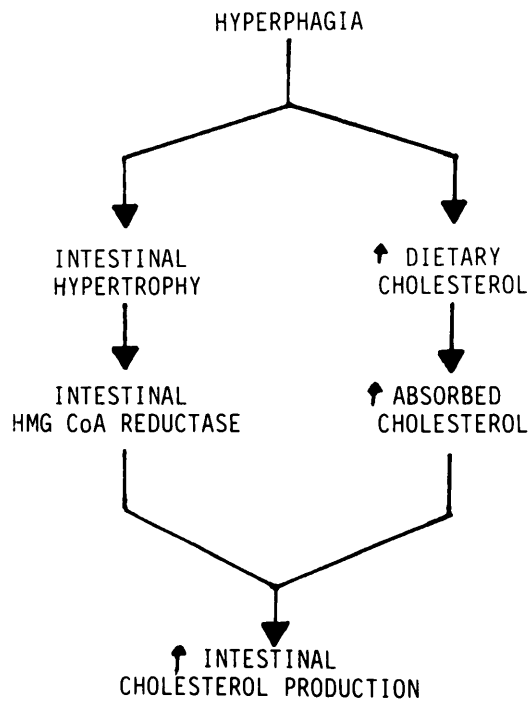


FIGURE 3. Hypothesis to explain the influence of hyperphagia on increased intestinal cholesterol production.

lin, they are usually obese, hyperinsulinemic, and hyperphagic.

The data support our contention that diet plays a significant role in the hypercholesterolemia of diabetes, which itself may be important in atherogenesis. We have considered only total food intake, but the various dietary components—cholesterol content, fat content and saturation, fiber content, etc.—must be studied. From the evidence derived thus far, it may be concluded that diet is more important in the diabetic's cholesterol homeostasis than was previously recognized.

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