Cholesterol Metabolism in Diabetes

I. The Effect of Diabetic Control on Sterol Balance

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SUMMARY

To evaluate the effect of diabetic control on sterol balance, five subjects (one with fasting chylomicronemia) were studied when they had little control of blood glucose (period I) and, continuously, when increased insulin dosage improved diabetic control (period II). The four subjects with no chylomicronemia showed a diminished fecal bile acid (FBA) excretion. Although fecal neutral steroid excretion increased variably, the total steroid balance was unchanged. The results support the concept that good control of diabetes shifts the fecal steroid excretion towards diminished FBA, but steroid balance methods did not reveal an effect of insulin on total steroid balance. DIABETES *27*:1059-64, November, 1978.

Although large vessel atherosclerosis is the major cause of death in diabetic patients today,[1] the effect of insulin on cholesterol metabolism is largely unknown. Several recent studies[2-5] confirmed that plasma cholesterol is elevated in diabetic populations, and there is evidence that other aspects of cholesterol metabolism are abnormal. For example, the atherogenic effect of cholesterol feeding of nonhuman primates is enhanced by experimental diabetes.[6] In rats made diabetic by streptozotocin, hepatic 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase) is reportedly suppressed,[7-9] but Nakayama and Nakagawa[10] reported recently that, in similar circumstances, intestinal HMG-CoA reductase is increased. Only one study[11] reported sterol balance in four diabetic patients.

We sought to determine whether intensive insulin treatment has an effect on cholesterol balance. Five subjects were studied for three to eight weeks while they had poor control of their diabetes and, then, continuously for three to 11 weeks when control was improved. The results support the contention[11] that fecal bile acid (FBA) excretion is diminished when strict diabetic control is maintained, but they suggest that excretion of fecal neutral steroids (FNS) is increased variably, such that insulin does not affect total sterol balance.

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METHODS

Patients. Clinical characteristics of the five patients studied are listed in table 1. All were maturity-onset diabetics with continually poor control of their disease. Three were taking suboptimal doses of insulin and two were being treated with diet alone when the study began. No patient was > 20 per cent above ideal body weight, and none had clinical evidence of renal or hepatic disease. One subject (5) had type-V hyperlipoproteinemia (increased VLDL with fasting chylomicronemia), while another (2) had slightly elevated concentration of plasma tryglyceride (type IV) (table 2).

Study Design

Patients continued their customary anti-diabetic regimen during an equilibration period and a period designated balance I (poor control). No attempt was made to worsen diabetic control; in fact the diet was more rigid in hospital than before admission. The equilibration period was defined as the time needed to establish consistent weight, plasma lipid concentrations, and fecal sterol excretion. Because stool analyses were done at a later date, the equilibration period was defined retrospectively and lasted four to 40 days (mean 17). Balance period I lasted 20 to 58 days (mean 31); it was the period of sterol balance in poor diabetic control. Balance II was the period of relatively good diabetic control; it lasted 21 to 78 days (mean 40). Short-acting and intermediate-acting insulin preparations were given two to four times daily to

TABLE 1

Clinical characteristics of subjects*

Subject	Sex	Age	Duration of diabetes, yr.	Baseline diabetic treatment	Final diabetic treatment	Diabetic complications
1	F	60	6	Ins, 15 U.	Ins, 30 U.	BR, ASHD
2	M	72	15	Diet alone	Ins, 80 U., DD	PVD, BR
3	F	64	30	Diet alone	Ins, 55 U., DD	BR
4	F	84	10	Ins, 45 U.	Ins, 140 U., DD	BR
5	M	43	7	Ins, 15 U.	Ins, 68 U., DD	ASHD

*Abbreviations: BR, background retinopathy; ASHD, arteriosclerotic heart disease; PVD, peripheral vascular disease; Ins, insulin; DD, divided doses, in intermediate-acting and short-acting preparations.

obtain optimal control of hyperglycemia. All data presented were derived from balance periods I and II, excluding the equilibration period.

Diet. A repetitive A, B, A, B, balanced diet was given throughout the study period. The diet was designed to approximate a normal American diet,[12] except no concentrated sweets were allowed. The diet consisted of 28 to 39 calories per kilogram weight and was adjusted to maintain constant body weight; 45 per cent of calories were in the form of carbohydrate, 40 per cent in fat, and 15 per cent in protein. The polyunsaturated fatty acid ratio was 0.2. Cholesterol content was analyzed in our laboratory and revealed a mean of 359 mg. dietary cholesterol per day. Dietary personnel confirmed that each meal was ingested in its entirety. As diabetic control was established in balance period II, caloric intake was reduced by more than 50 calories in only one subject (5), who ingested 121 calories less per day.

Sterol analyses. With oral chromic oxide (400 mg. in divided doses daily) used as a nonabsorbable marker of fecal flow, stools were collected every two or four days.[13] Beta sitosterol was used in the diet to correct for intestinal loss of neutral sterols.[14] Fecal sterol analyses were performed with minor modifications of the procedures of Miettinen, Grundy, and Ahrens,[15,16] as described elsewhere.[17] In brief, aliquots (1 gm.) of stool homogenate were analyzed separately for FNS and FBA. Neutral sterols were extracted after mild alkaline saponification and were separated by florisil thin layer chromatography (Floridin Co., Berkeley Springs, W. Va.) into the two classes of bacterial conversion products derived from cholesterol (3-OH and 3-keto compounds). These fractions were then exposed to trimethyl-silylating reagents (SIL-PREP and DMF-SIL-PREP, Applied Science Labs, State College, Pa.) and were measured by gas-liquid chromatography on a six-foot column using 1 per cent DC-560 Gas Chrom Q (Applied Science) at oven temperature 220° C. FBA were analyzed using 5αcholonic acid-3β-ol (Steraloids, Inc., Wilton, N. H.) as a procedural internal standard. After rigorous saponification, bile acid methyl esters were extracted and separated from fatty acid methyl esters by silica gel, thin layer chromatography (silica gel H, Applied Science). Bile acids were then prepared by trimethyl

TABLE 2

Weight, fasting plasma glucose (FBS), mean daytime plasma glucose (mean brackets), and urine glucose by qualitative assay (Clinitest). Data are means ± S.E.M. and compare period I (poor control) with period II (good control). P denotes significance of the mean change on a paired control basis.

Subject	Study period	Weight (kg.)	FBS (mg./dl.)	Mean brackets (mg./dl.)	Qualitative urine glucose (gm./dl.)
1	I	62.9 ± 0.05	160 ± 4	180 ± 3	0.55
	II	63.1 ± 0.04	106 ± 9	120 ± 1	0
2	I	58.4 ± 0.11	371 ± 18	379 ± 16	1.80
	II	60.1 ± 0.16	146 ± 21	183 ± 16	0.25
3	I	59.2 ± 0.04	249 ± 24	328 ± 1	2
	II	60.1 ± 0.08	130 ± 12	240 ± 6	0.85
4	I	64.2 ± 0.05	275 ± 6	307 ± 6	1.5
	II	64.7 ± 0.09	84 ± 9	88 ± 2	0
5	I	74.2 ± 0.18	221 ± 6	211 ± 15	2
	II	76.1 ± 0.125	128 ± 4	117 ± 12	0.3
Mean change		+1.0	−136	−131.5	−1.28
p		< 0.05	< 0.01	< 0.01	< 0.005

silylation and quantitated by gas-liquid chromatography with a six-foot column of 1 per cent hi-Eff-8 BP on Gas Chrom Q (Applied Science), run at 225° C.

Plasma cholesterol and triglyceride concentrations were measured twice weekly by AutoAnalyzer methods N-24A and N-78A (Technicon, Tarrytown, N.Y.). Fasting plasma glucose concentration was measured twice weekly, using the glucose oxidase method of the glucose analyzer (Beckman Co., Mountainside, N.J.). In addition, diabetic control was estimated by (1) weekly plasma glucose "brackets," which consisted of six glucose determinations drawn in one day and timed immediately before and one hour after each of three meals; and (2) qualitative urinary glucose measurement on each voiding with Clinitest tablets (Ames Co., Elkhart, Ind.).

Statistical analyses were performed using the Student's *t*-test with unpaired analysis of each subject's data in periods I versus II and paired analysis of means and analysis of variance when comparing data of the five subjects grouped in periods I versus II.[18]

RESULTS

Clinical parameters. Table 2 notes the changes in mean fasting blood glucose, plasma glucose brackets, urinary glucose, and body weight. There was a mean increase in body weight of 1.0 kg. (1.6 per cent). This is, in part, attributable to the sodium retention that accompanies rapid insulinization,[19] but it may also be the result of the unchanged caloric intake and more efficient utilization of ingested calories; we could not decide which of the two contributed to weight gain in our study. All three indexes of glycemia—mean fasting plasma glucose, mean glucose brackets, and mean qualitative urine glucose—indicated improved diabetic control. Mean fasting plasma glucose was decreased 53 per cent, from 255 to 119 mg. per deciliter, and mean glucose brackets, representing mean daytime glycemia, was lowered 47 per cent, from 281 to 150 mg. per deciliter. Patient 4 showed the most lowering of blood glucose (mean glucose brackets diminishing from 307 to 88 mg. per deciliter), whereas patient 3 was the least controlled (mean brackets from 328 to 240 mg. per deciliter, although FBS was reduced to 130 mg. per deciliter).

Plasma lipids. Plasma cholesterol changed inconsistently when subjects were brought into good control of their diabetes (table 3). The chylomicronemic subject (5) showed a marked decrease in plasma cholesterol, but this may be attributed to diminished chylomicron and VLDL cholesterol, since hypertriglyceridemia was greatly improved. Of the other four subjects, two had significantly increased and two had significantly decreased mean plasma cholesterol. In no patient was the mean change in plasma cholesterol concentration greater than 22 mg. per deciliter, and paired comparison of mean plasma cholesterol for all five subjects showed no significant change.

Plasma triglyceride showed a downward trend in all five patients as their diabetes became controlled (table 3), although this did not reach statistical significance. By far the greatest decrement was found in the chylomicronemic subject (5).

Fecal steroid balance. Table 4 shows the FBA, FNS, and cholesterol balance data. Sterol balance is calculated on the basis of FBA plus FNS minus dietary cholesterol. The chylomicronemic subject (5) had extremely high FBA excretion, a fact that has been observed by others in hypertriglyceridemia.[20] Insulin caused no significant change in this subject's FBA or FNS excretion. While plasma triglyceride concentration diminished, he remained chylomicronemic. Of

TABLE 3

Plasma lipids, milligrams per deciliter ± S.E.M. Using unpaired *t*-test, asterisk denotes a value in period II that is different from period I with $p < 0.05$; dagger denotes $p < 0.01$.

Subject	Study period	n	Plasma cholesterol	Plasma triglyceride
1	I	27	259 ± 3	166 ± 4
	II	20	276 ± 3†	115 ± 3†
2	I	12	236 ± 2	282 ± 19
	II	7	243 ± 2*	241 ± 10
3	I	6	279 ± 3	89 ± 3
	II	23	257 ± 2†	86 ± 2
4	I	14	211 ± 2	175 ± 3
	II	15	195 ± 3†	164 ± 5
5	I	17	296 ± 9	3,809 ± 240
	II	10	199 ± 12†	1,438 ± 261†

TABLE 4

Fecal bile acid (FBA), fecal neutral steroids (FNS), and total balance in milligrams per day ± S.E.M. Using unpaired *t*-test, significances of differences are denoted as in table 3.

Subject	Study period	n	FBA	FNS	Balance
1	I	14	329 ± 35	295 ± 28	216 ± 40
	II	11	273 ± 10*	623 ± 49†	490 ± 43†
2	I	6	331 ± 116	792 ± 27	691 ± 69
	II	5	294 ± 51	776 ± 18	638 ± 36
3	I	6	138 ± 13	535 ± 15	273 ± 35
	II	23	106 ± 8	607 ± 17†	313 ± 40
4	I	7	720 ± 31	493 ± 20	764 ± 41
	II	8	477 ± 30†	621 ± 23†	649 ± 22
5	I	7	1,083 ± 100	652 ± 41	1,353 ± 110
	II	7	1,051 ± 95	545 ± 16*	1,215 ± 103

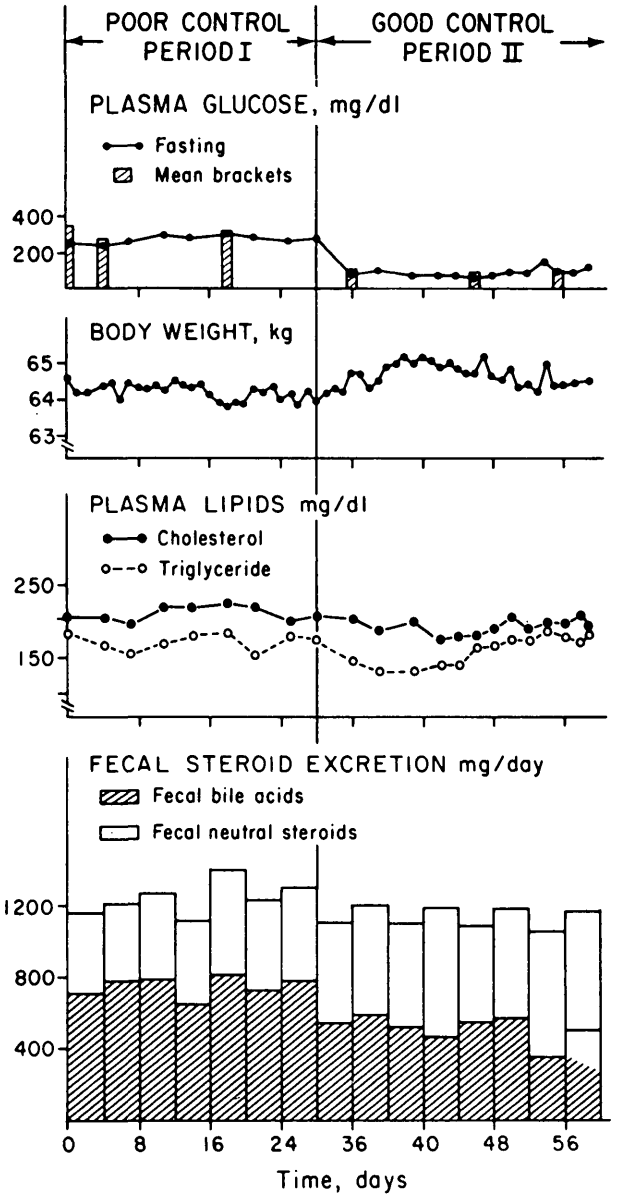

FIG. 1. Plasma glucose, body weight, plasma lipids, and fecal steroid excretion for patient 4. Time refers to days in steroid balance (see test for criteria). During period I the patient was in poor control, while period II was characterized by improved control of the diabetes.

the other four subjects, intensive insulinization (period II) caused a diminished FBA excretion that was statistically significant in two (table 4). FNS, on the other hand, increased significantly in three of the four nonchylomicronemic subjects during insulinization. These directionally opposite changes in sterol excretory products during insulin treatment (decreased acidic sterols and increased neutral sterols) resulted in total sterol excretion being unchanged in four subjects and significantly increased in one.

Figure 1 illustrates the data from patient 4, chosen because plasma glucose was most successfully controlled in this subject. Within four days of initiation of high-dose insulin administration, body weight rose abruptly and then declined for the next three weeks. The pattern suggests there was sodium and water retention rather than hypercaloric feeding. Insulinization was also associated with significantly diminished FBA excretion and increased FNS excretion. Total sterol balance was unchanged. The two subjects in whom blood glucose was least controlled (2 and 3) showed little change in FNS and FBA excretion.

Table 5 is a list of the individual major bile acids as well as the minor bile acids, as a group, excreted before and after diabetic control was enacted. Subject 5, with chylomicronemia, excreted most of his acidic sterols in the form of the primary bile acid, cholic acid; insulin did not substantially affect the pattern. By contrast, in the four nonchylomicronemic subjects, most acidic steroid was found in the form of secondary bile acids, deoxycholic and lithocholic acids. During strict diabetic control, these subjects continued to excrete largely secondary bile acids, and the mean deoxycholic acid excretion diminished 22 per cent to 39 per cent in period II. The decrease accounted for 63 per cent of the total decrease in FBA excretion in period II and is significant on a paired control basis with $p < 0.005$. Figure 2 is an illustration of the mean decrements in total and individual FBA for patients 1 through 4.

DISCUSSION

Many studies have reported abnormal lipid concentrations in the plasma of diabetic populations. In our small sample, we confirmed the generally accepted observation that chylomicronemia is the hyperlipoproteinemia most responsive to insulin treatment. We found, as others have reported, that plasma cholesterol concentration is inconsistently related to diabetic control. But plasma lipid concentration alone gives little indication of the flux into and out of the various cholesterol-containing pools, and it gives no indication of insulin's role in regulating cholesterol synthesis.

We found that insulinization of nonchylomicronemic subjects diminished bile acid excretion, specifically deoxycholic acid excretion. FNS excretion, on the other hand, increased significantly in three of four subjects, such that total steroid excretion was unchanged in three and increased in one subject. The interpretation of sterol balance data in diabetics must be approached cautiously, however.

TABLE 5

Individual major and minor bile acid excretion in milligrams per day ± S.E.M.
Using unpaired *t*-test, significances of differences are denoted as in table 3.

Subject	Study period	n	Cholic acid	Chenodeoxy-cholic acid	Litho-cholic acid	Deoxy-cholic acid	Minor bile acids
1	I	11	14	0	57	168	93
	II	11	18	0	39	115*	101
2	I	6	<5	0	54	159	118
	II	5	<5	0	43	124	127
3	I	6	12	0	41	43	42
	II	23	5	0	37	31*	33
4	I	7	5	90	135	336	154
	II	8	10*	8†	144	205†	110
5	I	7	553	104	72	75	279
	II	7	499	58	69	139*	286

The metabolic steady state is defined theoretically as the state in which there is no net flux of cholesterol into or out of the body's sterol-containing pools.[15] Excretion of cholesterol metabolites in excess of cholesterol ingested, then, would reflect quantitatively endogenous cholesterol synthesis. The steady state is operationally defined, however, as constant body weight, constant plasma cholesterol, and constant food intake; our subjects met these criteria. But diabetes is inherently an unsteady state by most metabolic parameters; it is obvious that physiologic control of blood glucose, free fatty adic, and ketone body concentrations was not achieved when our patients were in good control of their disease. It is therefore risky, at best, to assume that sterol balance accurately measures cholesterol synthesis. The net balance could still reflect sterol efflux from adipose tissue, arterial wall, or other tissue sites.

The report of Bennion and Grundy[11] is the only other published account of cholesterol balance methods applied to diabetic subjects. Our data support their contention that FBA excretion diminishes as diabetes is controlled. Additionally, we found that a significant decrease in deoxycholic acid specifically accounted for the lower total bile acid excretion. At variance with their results is our finding of increased FNS excretion in three of five patients after control was improved. The importance of this discrepancy is that we did not find support for the conclusion that control diminishes cholesterol synthesis (even assuming balance represents synthesis).

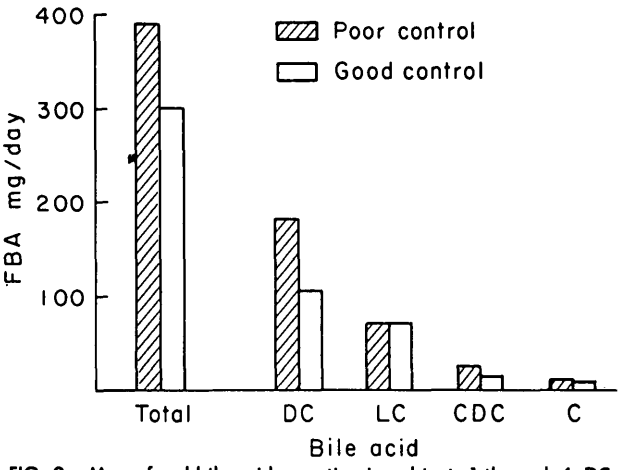

FIG. 2. Mean fecal bile acid excretion in subjects 1 through 4. DC, deoxycholic acid; LC, lithocholic acid; CDC, chenodeoxycholic acid; C, cholic acid.

A number of differences exist between the design of our study and that of Bennion and Grundy. The subjects they used were four Pima Indians, a relatively homogeneous group with an obesity-related form of diabetes[21] and with a lower incidence of coronary heart disease.[22] Our subjects were of diverse genetic background and were not obese. Their study design included a brief discharge from the hospital and then a re-equilibration on the new insulin regimen before resumption of stool collections; our design studied the period of transition continuously. Probably the most significant difference between the two studies is that, in order to maintain body weight, Bennion and Grundy reduced the caloric intake of subjects brought into good control; the mean decrease was 1498 calories per day (mean of the four subjects on whom balance data were derived was 3532 calories per day before insulin and 2034 calories per day while taking insulin). As the authors point out, a diminished calorie intake alone may reduce cholesterol synthesis.[11] Our subjects, on the other hand, may have been in slightly positive caloric balance.

The diminished bile acid excretion with unchanged or increased neutral steroid excretion would, if reflected in the bile, suggest that improved control of the disease increases the lithogenesy of bile. This is consistent with the demonstration by Bennion and

Grundy[11] of increased gallbladder bile cholesterol saturation in controlled diabetes.

Cholesterol balance studies might be expected to detect only the end result of several potentially opposing regulatory influences on cholesterol synthesis. The activity of HMG-CoA reductase appears to be increased by insulin in certain circumstances. Studies from Porter's laboratory[7-9] show depressed hepatic HMG-CoA reductase activity in diabetic rats stimulated by insulin administration. Nakayama and Nakagawa,[10] however, found increased HMG-CoA reductase in the intestinal crypt cells of diabetic rats. Their finding suggests that insulin deficiency has opposing effects on the two major cholesterol-synthesizing organs, suppressing hepatic cholesterogenesis as it stimulates intestinal synthesis. Stout et al.[23] demonstrated that insulin increases arterial wall conversion of labeled acetate into cholesterol. It is also possible that diminished delivery of free fatty acid to the liver, as is seen after treatment with insulin, will diminish the production of very low density lipoprotein and thereby reduce the production of low density lipoprotein. There are, then, a number of sites at which insulin might regulate cholesterol synthesis, and the direction of this regulation might be variable.

We found that insulin treatment of poorly controlled diabetic subjects has a definable effect on the excretion of cholesterol metabolites. Bile acid excretion is decreased, but neutral sterol excretion is variably increased, so that there is no net change in cholesterol balance. Further studies will be necessary to determine how insulin acts to regulate cholesterol synthesis, distribution, and excretion.

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