The Effect of Diabetes Mellitus on Sterol Synthesis in the Intact Rat

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SUMMARY
Diabetes mellitus in both humans and animals is characterized by elevations in plasma cholesterol concentrations. The cause of this hypercholesterolemia is unknown. The present study employed tritiated water to quantify sterol synthesis in intact diabetic and control animals. Sterologenesis was 60–246% greater in the gut of diabetic animals than in controls. This enhancement of sterol synthesis occurred soon after the onset of diabetes and persisted for at least 3 wk. Moreover, insulin therapy markedly decreased gut sterol synthesis in diabetic animals to levels only slightly greater than in controls. Diabetes increased sterol synthesis primarily in the small intestine, but a small increase was also observed in the large intestine. Subfractionation of the small intestine into epithelial cell and muscularis cell layers revealed an increased sterologenesis in both layers. Quantitatively, though, the epithelial layer accounted for the majority of the enhancement of small intestine sterol synthesis observed in diabetic animals. De novo sterologenesis in tissues other than the intestines (liver, skin, stomach, or remaining carcass) was not significantly altered by diabetes. This study demonstrates that diabetes markedly stimulates sterol synthesis, an effect that is specifically localized to the intestines. DIABETES *31*:388–395, May 1982.

Atherosclerotic vascular disease is a major cause of the increased morbidity and mortality associated with diabetes mellitus.[1] Although the etiology of atherosclerosis is unknown, there is a strong correlation between plasma cholesterol concentrations and atherosclerosis in almost all population groups studied.[2] In the many comparisons of plasma cholesterol levels in diabetic patients and matched controls, in general, cholesterol concentrations are significantly elevated in the diabetics.[3–7] Moreover, in those diabetic populations that tend to have low plasma cholesterol levels, for example, the Pima Indians and Japanese, atherosclerosis is a far less common problem than in Caucasian diabetics.[8,9] Diabetic animal models have also exhibited increased plasma cholesterol concentrations.[10–15] Moreover, several recent studies have presented data suggesting that plasma cholesterol levels in diabetic subjects are directly correlated with the degree of hyperglycemia. Diabetics with "loose" metabolic control have been noted to have elevated plasma cholesterol concentrations in comparison with diabetics with "tight" control.[16,17] Similarly, the introduction of "tight" diabetic control results in a significant reduction in plasma cholesterol levels.[16,18–20]

While a strong relationship between the hypercholesterolemia of diabetes and the premature atherosclerosis that characterizes diabetes is well established, the cause of the elevated plasma cholesterol levels in diabetes mellitus remains unknown. The aim of the present study was to determine in vivo whether diabetes results in alterations in de novo sterologenesis. Because of its many advantages over the usual substrates employed for this purpose, we have used tritiated water to quantify sterol synthesis in the tissues of intact diabetic and control animals.

The major finding of the present study is that diabetes results in a marked increase in de novo sterol synthesis by the intestine, a tissue known to make a significant contribution to plasma cholesterol levels.[21]

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METHODS

Materials. Tritiated water (1 Ci/g) and [26-^{14}C]cholesterol (0.5 mCi/0.33 mg) were purchased from New England Nuclear (Boston, Massachusetts). The thin-layer polygram Sil G plates were purchased from Brinkmann Instruments (Burlingame, California). Radioactivity on the thin-layer strips was assayed in a solution containing 1.133 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) (Amersham/Searle),

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9.33 g of 2,5-diphenyloxazole (PPO), 1333 ml of scintillation grade toluene, and 666 ml of Triton X-100 (Beckman Instruments, Fullerton, California). Radioactivity in the plasma samples was determined in a solution containing 300 ml of Beckman Bio-Solv III, 1000 ml of Packard scintillation grade toluene, 100 ml of glass-distilled water, and 6.0 g of PPO (Amersham/Searle). Streptozotocin was purchased from Sigma Chemical (St. Louis, Missouri). Alzet pumps were purchased from Alza Inc. (Palo Alto, California). U500 regular insulin was purchased from Eli Lilly and Company (Indianapolis, Indiana). Ketodiastix were obtained from Ames Company (Elkhart, Indiana).

Animal procedures. Female Sprague-Dawley rats (~200 g) (Simonsen Laboratories, Gilroy, California) were maintained on a reverse 12-h light cycle (3 a.m.-3 p.m. dark, 3 p.m.-3 a.m. light). After an overnight fast animals were injected i.p. with either 40 mg/kg streptozotocin in a 1 M Na citrate buffer (pH 4.5) or buffer alone. After streptozotocin administration the animals were fed Simonsen Rat and Mouse Diet and water ad libitum. The animals were studied at various time points after the administration of streptozotocin as indicated in the text. Diabetes was treated by the subcutaneous implantation of Alzet pumps containing U500 regular insulin diluted with saline such that 4 U of regular insulin was delivered daily. The urines of the animals administered streptozotocin were periodically analyzed with Ketodiastix and animals were eliminated from study if they did not have at least 1% glycosuria at all times. Animals that were ketotic were not studied.

Experimental protocol. Between 8:00 and 9:00 a.m., the rats were injected i.p. with tritiated water (50 mCi). Six hours later the animals were killed and weighed and a blood specimen was obtained; the plasma was separated by centrifugation. The organs studied were removed, individually weighed, and saponified by refluxing overnight in a solution of 90% KOH, H_2O, and 70% ethyl alcohol (1:2:5). The epithelial and muscularis layers of the small intestine were separated by scraping with a glass slide. The flasks were cooled and an internal standard of ^{14}C-cholesterol was added before extracting the nonsaponifiable material three times with 25 ml of petroleum ether. The contents of the flasks containing the remaining carcasses (throughout this article carcass refers to all tissues and organs not specifically studied individually) were thoroughly mixed, the volume determined, and 50-cc aliquots were transferred to smaller flasks to which the internal standard was added. The nonsaponifiable material was extracted three times with 25 ml of petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and then applied to thin-layer chromatographic plates. The plates were developed in ethyl acetate:benzene (1:5) for 50 min and portions of the lane corresponding to standards of cholesterol, lanosterol, and squalene were counted.[22,23] Nonsaponifiable lipids represent the sum of these fractions. The gain and discriminator window settings of the scintillation counter were adjusted so that less than 0.2% of the tritium counts were recorded in the ^{14}C window and approximately 10% of the ^{14}C counts were recorded in the tritium window. Calculations were corrected for spillover of tritium and ^{14}C, for background, and for recovery of internal standard. The specific activity of the tritiated water was determined individually for each animal by measuring the dpm/cc of plasma at the end of the experiment and dividing by the millimoles of water/cc of plasma (52 mmol/cc of plasma, assuming that plasma is 93% H_2O). Samples of plasma were dried at room temperature overnight in a hood and only a very small percentage of counts were nonvolatile (94,607 cpm before air drying, 126 cpm or 0.13% after air drying). To determine that the specific activity would remain constant during the duration of the experiments, animals were injected i.p. with 312 μCi of ^3H_2O and at 1, 3, and 6 h blood was removed via the tail vein and plasma ^3H cpm determined. The ^{14}C cholesterol was solubilized in saline by the addition of 1 drop of tween 40 from Sigma Chemical Co.

RESULTS

The specific activity of the ^3H_2O in the plasma remained unchanged during the hours of the experiment, thereby providing a constant labeled substrate for the following studies of sterologenesis. [Control (N = 4): 1 h—96,000 cpm ± 250 (SEM), 3 h—97,000 ± 2250, 6 h—95,000 ± 3900; diabetic (N = 4):1 h—95,000 ± 3380, 3 h—100,000 ± 2,330, 6 h—94,000 ± 7,580.]

After the intravenous administration of ^{14}C-cholesterol, 31.7% of the labeled cholesterol is localized in the liver in control animals and 35.6% in the diabetic animals. In contrast, the large and small intestines contained only a small portion of the labeled cholesterol (control 4.6%, diabetic 5.9%). This result demonstrates that the distribution of labeled cholesterol after intravenous administration is similar in control and diabetic animals and that only a small percentage of the labeled cholesterol is localized in the intestines. These data indicate that redistribution of hepatic cholesterol can play a minor role in the findings of this study.

The effects of streptozotocin-induced diabetes on de novo sterologenesis are shown in Table 1. In the control animals, as previously reported by this laboratory,[24] the carcass (all tissues other than the liver and gut*) is the main site of in vivo, de novo cholesterol, and nonsaponifiable lipid synthesis. In the animals made hyperglycemic 4 days previously, the same general distribution of newly synthesized cholesterol and nonsaponifiable lipids is observed. The incorporation of tritiated water into cholesterol and nonsaponifiable lipids in the liver and carcass is similar in the diabetic and control groups. However, gut cholesterol and nonsaponifiable lipid synthesis is 65% and 71% greater, respectively, in the 4-day diabetic group. In vivo total body sterologenesis is greater in the diabetic animals, but this difference did not achieve statistical significance. These data indicate that hyperglycemia, even of very short duration, markedly stimulates de novo sterol synthesis in the gastrointestinal tract.

The effect of diabetes of a longer duration (i.e., 11 days) on sterologenesis is also shown in Table 1. As also noted in the short-term diabetic animals, diabetes has no significant effect on cholesterol or nonsaponifiable lipid synthesis by the liver and carcass. Again, incorporation of tritiated water into cholesterol and nonsaponifiable lipids by the gut is greatly stimulated, with the diabetic animals demonstrating a twofold greater incorporation of ^3H_2O into cholesterol and nonsaponifiable lipids. Gut weight is increased in the dia-

* In this paper, gut includes stomach, small intestine, and large intestine.

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TABLE 1
Sterologenesis in 4-, 11-, and 21-day diabetic animals

	Weight (g)			Plasma glucose (mg/dl)	^3H_2O incorporated into cholesterol (μmol)				^3H_2O incorporated into nonsaponifiable lipids (μmol)			
	Body	Liver	Gut		Liver	Gut	Carcass	Total	Liver	Gut	Carcass	Total
4 Day												
Control (N = 6)	197 ± 5	6.5 ± 0.2	13.5 ± 0.5	138 ± 5	123 ± 17	104 ± 12	197 ± 15	424 ± 36	132 ± 17	109 ± 12	439 ± 67	680 ± 70
Diabetic (N = 7)	176 ± 5	6.4 ± 0.3	15.0 ± 0.9	277 ± 23	114 ± 26	172 ± 14	198 ± 27	441 ± 86	124 ± 27	186 ± 12	561 ± 30	871 ± 60
	$P < 0.02$	NS	NS	$P < 0.001$	NS	$P < 0.01$	NS	NS	NS	$P < 0.001$	NS	$P < 0.1$
11 Day												
Control (N = 5)	188 ± 3	6.9 ± 0.2	14.0 ± 0.8	165 ± 24	70 ± 14	67 ± 6	202 ± 14	340 ± 25	75 ± 14	79 ± 8	562 ± 24	705 ± 48
Diabetic (N = 6)	189 ± 6	7.8 ± 0.2	19.5 ± 2.0	405 ± 47	88 ± 17	134 ± 22	176 ± 32	431 ± 43	96 ± 17	164 ± 22	592 ± 64	852 ± 80
	NS	$P < 0.02$	$P < 0.05$	$P < 0.01$	NS	$P < 0.02$	NS	NS	NS	$P < 0.01$	NS	NS
21 day*												
Control (N = 5)	216 ± 4	7.8 ± 0.1	17.1 ± 0.8	167 ± 6	70 ± 7	42 ± 4	84 ± 8*	247 ± 9	90 ± 9	60 ± 3	236 ± 13*	508 ± 86
Diabetic (N = 5)	186 ± 2	9.7 ± 0.6	24.6 ± 2.6	336 ± 30	77 ± 11	79 ± 8	118 ± 7*	312 ± 13	101 ± 13	96 ± 9	204 ± 15*	505 ± 21
	$P < 0.001$	$P < 0.02$	$P < 0.05$	$P < 0.001$	NS	$P < 0.01$	$P < 0.02$	$P < 0.01$	NS	$P < 0.001$	NS	NS

Diabetes was induced 4, 11, and 21 days before the study by injecting i.p. 40 mg/kg streptozotocin. On the day of the study the animals were injected i.p. with 50 mCi of ^3H_2O. At 6 h the animals were killed and the liver, gut, and remaining carcass (all tissues not studied individually) were separately saponified in a KOH-ethanol solution. ^{3}H-cholesterol and ^{3}H-nonsaponifiable lipids were assayed after extraction with petroleum ether and thin-layer chromatography. Data presented are the mean ± SEM.
N, number of animals; NS, nonsignificant.
* In this particular study the carcass does not include the skin.

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betic animals. However, this does not account for the stimulation of gut nonsaponifiable lipid synthesis observed because the enhancement of sterologenesis in diabetics is present even when the data are expressed on a per gram basis (control 5.6 ± 0.3 versus diabetic 8.3 ± 0.9 μM ^3H_2O incorporated into nonsaponifiable lipids/gram in 6 h, $P < 0.01$). Total body de novo cholesterol and nonsaponifiable lipid synthesis again is increased in the diabetic group, but this difference is not statistically significant. These data therefore demonstrate that the enhancement of cholesterol and nonsaponifiable lipid synthesis observed in the gut soon after onset of diabetes persists and that the major in vivo effect of diabetes on sterologenesis is to stimulate sterol synthesis in the gut.

De novo sterologenesis in animals with diabetes of 3 wk duration is similarly shown in Table 1. As noted in both prior experiments, liver cholesterol and nonsaponifiable lipid synthesis is not altered by diabetes. In our previous in vivo studies we observed that the skin is a major contributor to total body de novo sterologenesis,[24] and, therefore, in this experiment we specifically analyzed skin sterol synthesis in both the normal and diabetic animals. The skin in controls accounted for 24% and in diabetics 21%, of total body de novo nonsaponifiable lipid synthesis. Diabetes did not significantly alter the skin incorporation of tritiated water into nonsaponifiable lipids (control 122 ± 5 versus diabetic 104 ± 11 μM tritiated water incorporated in 6 h, NS). Additionally, nonsaponifiable lipid synthesis in the remaining carcass (all tissues other than the liver, gut, and skin) of the control and diabetic animals is similar. Tritiated water incorporation into cholesterol, however, is increased in the carcass of the 21-day diabetic animals. The stimulation of intestinal incorporation of tritiated water into cholesterol and nonsaponifiable lipids in the diabetics observed in the 4- and 11-day experiments is still present. Gut cholesterol synthesis is increased 86% and nonsaponifiable lipid synthesis is increased 60% in the diabetic animals. These and previous data demonstrate that diabetes specifically stimulates gut de novo sterologenesis and that this stimulation occurs early after the development of hyperglycemia and persists for an extended period of time, i.e., at least 3 wk.

Localization of increased gut sterologenesis in diabetics. The localization of sterologenesis within segments of the gut in diabetic and control animals is shown in Table 2. In the control animals the stomach accounts for approximately 8%, the small intestine 64%, and the large intestine 28% of the total gut cholesterol and nonsaponifiable lipid synthesis. In the 21-day diabetic animal, the stomach contains 4%, the small intestine 78%, and the large intestine 18% of the total gut-labeled cholesterol and nonsaponifiable lipids. Gastric incorporation of tritiated water into nonsaponifiable lipids is similar in diabetic and control animals. In the large intestine, nonsaponifiable lipid synthesis is enhanced in the diabetic animals but does not achieve statistical significance. In contrast, cholesterol synthesis is significantly increased in the large intestine of diabetic animals but, quantitatively, this increase is small. However, cholesterol and nonsaponifiable lipid synthesis in the small intestine is markedly stimulated in the diabetic group and, quantitatively, primarily accounts for the enhanced sterol synthesis observed in the gut of the diabetic animals. That this stimulation of nonsaponifiable lipid synthesis is not

TABLE 2
Gut localization of sterologenesis

	Weight (g)			Plasma glucose (mg/dl)	^3H_2O incorporated into cholesterol [total μmol/organ (total μmol/g)]			^3H_2O incorporated into nonsaponifiable lipids [total μmol/organ (total μmol/g)]		
	Stomach	Small intestine	Large intestine		Stomach	Small intestine	Large intestine	Stomach	Small intestine	Large intestine
Control (N = 5)	1.8 ± 0.1	6.0 ± 0.4	7.3 ± 0.5	140 ± 10	4.9 ± 0.4 (2.7 ± 0.2*)	36.6 ± 2.9 (5.0 ± 0.1*)	13.6 ± 1.9 (2.3 ± 0.3*)	5.4 ± 0.4 (3.1 ± 0.2*)	39.3 ± 3.1 (5.3 ± 0.1*)	19.1 ± 2.6 (3.2 ± 0.3*)
Diabetic (N = 6)	3.1 ± 0.9	10.3 ± 1.2	14.4 ± 1.4	483 ± 47	5.6 ± 0.5 (2.3 ± 0.5*)	113 ± 18 (8.0 ± 1.4*)	24.7 ± 3.8 (2.6 ± 0.6*)	6.2 ± 0.6 (2.6 ± 0.6*)	120 ± 16.5 (8.7 ± 1.4*)	28.8 ± 3.9 (3.0 ± 0.6*)
	NS	P < 0.02	P < 0.01	P < 0.001	NS (NS*)	P < 0.01 (P < 0.1*)	P < 0.05 (NS*)	NS (NS*)	P < 0.01 (P < 0.05*)	P < 1 (NS*)

* Values given represent μmol ^3H_2O incorporated into cholesterol and nonsaponifiable lipids; per gram of tissue. N, Number of animals; NS, nonsignificant. Diabetes was induced 21 days before the study by injecting i.p. 40 mg/kg streptozotocin. On the day of study the animals were injected i.p. with 50 mCi ^3H_2O. At 6 h the animals were killed and the stomach, small intestine, and large intestine were weighed and separately saponified in a KOH-ethanol solution. ^3H-Cholesterol and ^3H-nonsaponifiable lipids were assayed after extraction with petroleum ether and thin-layer chromatography. Data presented are the mean ± SEM.

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TABLE 3
Localization of sterologenesis in the small intestine

	Weight (g)		^3H_2O incorporated into cholesterol [total μmol/organ (total μmol/g)]		^3H_2O incorporated into nonsaponifiable lipids [total μmol/organ (total μmol/g)]	
	Epithelial	Muscularis	Epithelial	Muscularis	Epithelial	Muscularis
Controls (N = 2)	3.1	1.1	18.4 (6.0*)	3.6 (3.2*)	19.9 (6.5*)	3.7 (3.4*)
Diabetics (N = 2)	7.6	2.8	121 (16.1*)	18.3 (6.6*)	126.8 (16.7*)	19.1 (6.9*)

* Values given represent μmol ^3H_2O incorporated into cholesterol and nonsaponifiable lipids per gram of tissue. N, number of animals. Diabetes was induced 21 days before the study by injecting i.p. 40 mg/kg streptozotocin. On the day of study the animals were injected i.p. with 50 mCi ^3H_2O. At 6 h the animals were killed and the small intestine removed. The contents of the small intestine were removed by saline washes and then the epithelial layer was separated from the muscularis layer by scraping with a glass slide. These layers were weighed and separately saponified in a KOH-ethanol solution. ^3H-Cholesterol and ^3H-nonsaponifiable lipids were assayed after extraction with petroleum ether and thin-layer chromatography. Data presented are the mean of the two samples.

solely due to an increased mass of the small intestine in diabetes is shown by the fact that the incorporation of tritiated water into nonsaponifiable lipids in the small intestine of diabetics is increased, even when the results are expressed on a per gram basis (control 5.3 ± 0.1 versus diabetic 8.7 ± 1.4 μM tritiated water incorporated into nonsaponifiable lipids per gram of small intestine in 6 h, $P < 0.05$). These data demonstrate that the diabetes-induced stimulation of gut sterologenesis is chiefly due to an increase in cholesterol and nonsaponifiable lipid synthesis by the small intestine.

Cellular localization of sterol synthesis in the intestine. The cellular localization of de novo sterologenesis in the small intestine in control and diabetic animals is shown in Table 3. In the control animals, the epithelial layers account for 84%, while the muscularis contributes only 16% of the total small intestine newly synthesized cholesterol and nonsaponifiable lipids. In the diabetic animals a similar distribution is observed, with the epithelial layer synthesizing 87% and the muscularis 13% of the total small intestinal cholesterol and nonsaponifiable lipids. Epithelial cholesterol and nonsaponifiable lipid synthesis is markedly enhanced in the diabetic group, whether expressed as total epithelial synthesis or on a per gram basis. Muscularis cholesterol and nonsaponifiable lipid synthesis is also enhanced in the diabetic animals. Because of the limited contribution of the muscularis to the total small intestinal synthesis, this difference is not a major factor contributing to the difference between diabetic and control de novo sterol synthesis in the intestine. These data demonstrate that the epithelial cells of the small intestine are the site that primarily accounts for the enhancement of gut de novo sterol lipid synthesis observed in diabetic animals.

The effect of insulin therapy on sterologenesis. The effect of insulin therapy on de novo sterol synthesis in diabetic animals is shown in Table 4. Insulin administration by continuous Alzet pump infusion, as expected, results in the normalization of plasma glucose levels and an increase in total body weight. Gut weight is also affected by diabetic control, with the insulin-treated diabetic animals exhibiting a gut weight midway between control and untreated diabetic animals. In contrast to our earlier studies, where an enhancement of hepatic cholesterol and nonsaponifiable lipid synthesis in the diabetic animals was not observed, in this single experiment a significant increase in the incorporation of tritiated water into sterols by the liver is present. The explanation for this discrepancy is unknown. Insulin administration normalized hepatic sterol synthesis in the treated diabetic animals. In the carcass, as noted previously, no significant difference in de novo sterologenesis is observed between diabetic and control animals. Insulin treatment of the diabetic animals also had no significant effect on carcass sterol synthesis. As observed above, cholesterol and nonsaponifiable lipid synthesis is markedly stimulated in the gut of the diabetic animals. Moreover, insulin therapy resulted in a large reduction in the intestinal synthesis of cholesterol and nonsaponifiable lipids. This reduction resulted in sterol synthesis in the insulin-treated diabetic animals being only slightly greater than the control animals. These data demonstrate that the diabetic enhancement of de novo gut sterologenesis is readily reversible with insulin-induced normalization of the plasma glucose levels.

DISCUSSION

The increased morbidity and mortality of diabetes mellitus is, in large part, due to an earlier onset and an increased severity of atherosclerotic vascular disease.[1] The etiology of atherosclerosis is unknown, but elevations in plasma cholesterol concentrations are generally accepted as a strong risk factor in the development of this disease. Numerous studies have demonstrated that plasma cholesterol levels in both diabetic patients and experimentally induced diabetic animals are increased above normal.[3–7,10–15] Additionally, several recent reports have suggested that plasma cholesterol concentrations in diabetic patients may be strongly influenced by the degree of metabolic control.[16–20] Cholesterol balance studies in diabetics have further suggested that tight metabolic control decreases cholesterol synthesis,[16] but this finding has not been observed by all investigators.[25]

Cholesterol synthesis in diabetic animals has been extensively investigated; however, the results have been conflicting.[26–33] The present study employed tritiated water as the labeled precursor to quantify in vivo sterologenesis in control and diabetic animals because this substrate has several important advantages compared with conventional substrates such as acetate or octanoate.[34–36] Our study demonstrates that in vivo, de novo sterologenesis was stimulated

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TABLE 4
Effect of insulin therapy on sterologenesis in diabetic animals

	Weight (g)			Plasma glucose (mg/dl)	^3H_2O incorporated into cholesterol (total μmol/organ)				^3H_2O incorporated into nonsaponifiable lipids (total μmol/organ)			
	Body	Liver	Gut		Liver	Gut	Carcass	Total	Liver	Gut	Carcass	Total
Control (N = 5)	215 ± 4	6.9 ± 0.3	15.3 ± 1.0	121 ± 7	63 ± 8	58 ± 6 (3.8 ± 0.3*)	261 ± 7	382 ± 19	80 ± 8	73 ± 6 (4.8 ± 0.4*)	604 ± 49	756 ± 41
Diabetic (N = 5)	175 ± 5†	8.4 ± 0.3‖	25.8 ± 1.5†	389 ± 44†	134 ± 15‖	201 ± 9† (7.8 ± 0.6*)	286 ± 43	621 ± 44‖	188 ± 24‖	224 ± 21† (8.7 ± 0.6*)	523 ± 23	936 ± 77††
Diabetic + insulin (N = 6)	199 ± 4‡§	8.8 ± 0.4‖	18.9 ± 1.1§¶	135 ± 29**	66 ± 4§	81 ± 11**†† (4.2 ± 0.3*)	253 ± 16	400 ± 23§	95 ± 4§	108 ± 14§¶ (5.6 ± 0.4*)‡§	603 ± 32#	806 ± 28

* Values given represent μmol ^3H_2O incorporated into nonsaponifiable lipids and cholesterol per gram of tissue. N, number of animals. Diabetes was induced 21 days before the study by injecting i.p. 40 mg/kg streptozotocin. One week before the study Alzet pumps containing U500 regular insulin diluted with saline, such that 4 U of regular insulin would be delivered daily, were implanted subcutaneously in a group of diabetic rats. On the day of study the animals were injected i.p. with 50 mCi of ^3H_2O. At 6 h the animals were killed and the liver, gut, and remaining carcass (all tissues not studied individually) were separately saponified in a KOH-ethanol solution. ^{3}H-Cholesterol and ^{3}H-nonsaponifiable lipids were assayed after extraction with petroleum ether and thin-layer chromatography. Data presented are the mean ± SEM.

† $P < 0.001$ difference between control.
‡ $P < 0.02$ difference between control.
§ $P < 0.01$ difference between diabetic.
‖ $P < 0.01$ difference between control.
¶ $P < 0.05$ difference between control.
# $P < 0.10$ difference between diabetic.
** $P < 0.001$ difference between diabetic.
†† $P < 0.10$ difference between control.

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60–246% in the gut of diabetic animals. This enhancement of sterologenesis occurred soon after the onset of diabetes and persisted for an extended period of time, i.e., for at least 3 wk. Moreover, in the diabetic animals, insulin therapy, which normalized plasma glucose, markedly decreased the incorporation of tritiated water into sterols. Gut de novo sterol synthesis in the insulin-treated diabetic animals was only slightly greater than in the control group, whereas in the untreated diabetic animals, gut sterol synthesis was threefold greater than control.

This diabetic enhancement of gut sterologenesis was primarily due to an increased synthesis of cholesterol and nonsaponifiable lipids by the small intestine. Subfractionation of the small intestine into epithelial cell and muscularis layers revealed an increase in sterol synthesis in both layers. Quantitatively, though, because the epithelial layer accounts for the major portion of the total small intestine sterol synthesis (approximately 85%), it is the stimulation of this epithelial layer by diabetes that primarily accounts for the enhancement of sterol synthesis in the small intestine.

As has been noted by others, diabetes results in a significant increase in the weight of the gut.[37,38] It is unlikely, however, that the increase in intestinal sterol synthesis observed in the diabetic animals is solely due to this small intestinal hypertrophy. First, in the short-term, 4-day diabetic animals (Table 2) an enhancement of gut sterol synthesis is observed before small intestinal hypertrophy. Second, even when expressed on a per gram basis, the entire gut, small intestine, muscularis layer, and epithelial cell layer of the diabetic animals all exhibited a significant increase in the incorporation of tritiated water into sterols. These data therefore indicate that diabetes induces specific changes in the small intestine that lead to an increase in de novo sterologenesis and that these changes are reversible with insulin treatment.

It is also unlikely that alterations in the transport of lipids account for our observations of increased quantities of labeled sterols in the intestines of diabetic animals. We, and others, have demonstrated that in normal animals the rate of cholesterol synthesis in the intestines is independent of the rates of synthesis in the liver.[24,39] Thus, in normal animals, transport of sterols from the liver to the intestines is not quantitatively significant. In the diabetic animals it is also unlikely that there is a significant degree of transport to the intestine of sterols synthesized in the liver. Six hours after the intravenous injection of labeled cholesterol approximately one-third of the injected cholesterol is concentrated in the liver while only 5–6% is localized to the intestines in both control and diabetic animals. Assuming that the labeled cholesterol in the liver is handled in a manner similar to that of newly synthesized cholesterol, it is unlikely that the liver transports a significant quantity of cholesterol to the gut in either control or diabetic animals during the 6 h of study. An impaired release of lipoproteins formed in the intestine of diabetic animals is also not an explanation for our observations. Lindsey and Wilson have demonstrated that the transport of newly synthesized cholesterol from the intestines to the blood via the lymphatics is slow.[40] In their study, after 10 h only approximately 15% of the labeled cholesterol appeared in the lymphatics. Our study was only 6 h in duration and, moreover, when tritiated water is employed as the radiolabel, the rate of sterologenesis is linear throughout the duration of study. Therefore, an impaired release of lipoproteins from the diabetic rat could not quantitatively account for our results. Lastly, Nakayama and Nakagawa have demonstrated, using ^{14}C acetate, that cholesterol synthesis is enhanced in the intestine of diabetic animals in vitro.[41]

De novo sterol synthesis in organs other than the small intestine, with the exception of the large intestine, was not significantly altered by diabetes. Specifically, in vivo, neither liver, skin, nor stomach incorporated tritiated water into sterols to a greater extent in the diabetic animal. Sterol synthesis in the carcass (all organs not studied separately) was also not influenced by diabetes. Because de novo sterologenesis in the intestines accounts for only a small portion of the total body incorporation of tritiated water into sterols, the increased sterol synthesis in the small intestine of the diabetic group was not sufficient to result in an elevation of total body sterologenesis in diabetic animals.

Sterols synthesized by the small intestine have two major fates. First, these sterols can be utilized in situ by small intestinal cells for synthesis and maintenance of cell membranes. As noted above, diabetes results in small intestinal hypertrophy and it is therefore possible that the increase in de novo sterol synthesis demonstrated in this study serves to meet the requirements of increased cell growth and division. The second fate of the newly synthesized sterols is to be transported by the lymphatics to the bloodstream. The intestine is well known to be an important source of plasma cholesterol[21,40] and, therefore, it is possible that increased de novo sterologenesis observed in the intestine of diabetic animals contributes to the characteristic elevation of plasma cholesterol concentrations noted in diabetics. The fate of these newly synthesized sterols in the small intestine of diabetic animals is under further investigation.

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