Effect of Hypocholesterolemia on Cholesterol Synthesis in Small Intestine of Diabetic Rats

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SUMMARY

Studies by our and other laboratories have demonstrated that cholesterol synthesis is increased in the small intestine of insulinopenic diabetic animals. In normal animals, many factors have been shown to regulate cholesterol synthesis in the small intestine, including changes in plasma cholesterol levels. The purpose of this study was to determine the effect of lowering plasma cholesterol levels on small intestine cholesterol synthesis in streptozocin-induced diabetic rats. In diabetic rats, 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP)-induced hypocholesterolemia (plasma cholesterol levels <20 mg/dl) resulted in a 2.5-fold increase in small intestine cholesterol synthesis, which was most marked in the distal small intestine, decreasing proximally. In the distal small intestine the incorporation of ^3H_2O into cholesterol was 0.28 ± 0.04 μmol · h^{-1} · g^{-1} in diabetic rats versus 1.60 ± 0.38 in diabetic rats administered 4-APP ($P < .01$). This stimulation of cholesterol synthesis occurred in the upper villus, middle villus, and crypt cells isolated from the middle intestine of the 4-APP–treated diabetic animals. In agreement with these observations, "functional hypocholesterolemia" due to Triton WR-1339 administration also stimulated cholesterol synthesis 2.5-fold in the small intestine of normal and diabetic animals. In the distal small intestine, cholesterol synthesis was 0.43 ± 0.10 μmol · h^{-1} · g^{-1} in the diabetic rats versus 1.08 ± 0.21 in diabetic rats treated with Triton WR-1339 ($P < .05$). In both the 4-APP and Triton WR-1339 experiments, the response of the diabetic rats was similar to that observed in normal rats. Moreover, cholesterol administered orally could prevent the Triton WR-1339–induced increase in small intestine cholesterol synthesis, indicating that providing cholesterol via an alternate route (i.e., intraluminal) can block the increase in synthesis. The results reported in this article, together with our earlier observations demonstrating that luminal factors regulate cholesterol synthesis, indicate that small intestine cholesterol synthesis in diabetic animals is regulated by both luminal and circulating factors in the expected fashion. *Diabetes* 36:1223–29, 1987

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Previous studies by this and other laboratories have demonstrated that cholesterol synthesis is increased two- to threefold in the small intestine of insulinopenic diabetic rats (1–4). One week of insulin therapy that normalized blood glucose levels markedly decreased intestine cholesterol synthesis in the diabetic rats to a level similar to that observed in controls (1). With the exception of the large intestine, de novo cholesterol synthesis in other organs, specifically the liver, skin, and remaining carcass, is not altered in the diabetic rat (1). However, other laboratories have observed a decrease in hepatic hydroxymethylglutaryl coenzyme A reductase activity in diabetic rats (3,4).

Studies of spontaneously diabetic Chinese hamsters and BioBreeding/Worcester rats, other animal models of insulinopenic diabetes, have also shown that cholesterol synthesis in the small intestine is increased in diabetic animals compared with controls (5,6), suggesting that such increases may be a general phenomenon. In contrast, studies of obese insulin-resistant diabetic mice have shown that the chief alteration in cholesterol synthesis is an increase in hepatic cholesterogenesis (5).

Studies from this laboratory with control and insulinopenic diabetic rats with thoracic duct cannulas have demonstrated that the newly synthesized cholesterol transported from the intestine to the circulation is increased fourfold in the diabetic animals (7). In both control and diabetic animals, most of the labeled cholesterol transported from the small intestine

to the bloodstream is localized in the chylomicron lipoprotein fraction (7). Young et al. (8), employing entirely different experimental methods, have also shown that cholesterol synthesized in the small intestine is transported to the circulation to a greater extent in diabetic animals than in controls. These findings suggest that the increase in small intestine cholesterol synthesis observed in diabetic animals may contribute to the total-body cholesterol pool and to the elevated plasma cholesterol levels that are observed in diabetes mellitus (9,10).

In normal animals, among the many factors that have been shown to regulate cholesterol synthesis in the small intestine are plasma cholesterol concentrations (11–13). A reduction in plasma cholesterol levels leads to an increase in small intestinal cholesterol synthesis in normal animals. In these studies, plasma cholesterol levels were altered by treating animals with either 4-aminopyrazolo[3,4-*d*]pyrimidine (4-APP) or Triton WR-1339 (11–13). 4-APP causes a profound and prompt decrease in lipoprotein secretion by the liver, leading to a marked lowering of plasma cholesterol levels (14–16). This decrease in plasma cholesterol concentrations results from the reduction in the cholesterol carried by both the low-density and high-density lipoprotein fractions (12). Triton WR-1339, a nonionic detergent, is thought to trap triglycerides and cholesterol in the plasma. This trapping of cholesterol in the vascular compartment induces a state of "functional hypocholesterolemia," because circulating cholesterol is unavailable for utilization by the tissues (17–21). The purpose of this study was to determine in diabetic animals the effect of lowering plasma cholesterol levels with these two compounds on small intestine cholesterol synthesis.

MATERIALS AND METHODS

Materials. Tritiated water (1 Ci/g) was purchased from ICN Biochemical (Irvine, CA). 26-[^{14}C]cholesterol (0.5 mCi/0.33 mg) was purchased from New England Nuclear (Boston, MA). Streptozocin and 4-APP were purchased from Sigma (St. Louis, MO). The thin-layer chromatography polygram sil G plates were purchased from Brinkmann (Westbury, NY). Ultrafluor scintillation fluid was purchased from National Diagnostics (Somerville, NJ). Ketodiastix were obtained from Ames (Elkhart, IN). Triton WR-1339 was purchased from Ruger (Irvington, NJ).

Animal procedures. Female Sprague-Dawley rats were purchased from Simonsen Animal Laboratories (Gilroy, CA). The animals were maintained on a reverse 12-h light cycle (0300–1500 h dark, 1500–0300 h light) and were fed Simonsen rat and mouse diet and water ad libitum. Diabetes was induced by injecting the animals intraperitoneally after an overnight fast with 40 mg/kg streptozocin in a 1 M sodium citrate buffer (pH 4.5). The urine of the animals was periodically analyzed with Ketodiastix, and animals were eliminated from the diabetic group if they did not have at least 1% glycosuria at all times. Ketotic animals were not utilized in these studies.

Hypocholesterolemia was induced by injecting 4-APP (20 mg/kg i.p.) in a 0.9% sodium chloride/25 mM phosphate buffer (pH 4). Control animals received buffer alone. Animals were injected either once 24 h before the study or daily for 3 days before the study. In other experiments, functional hypocholesterolemia was induced by injecting 100 mg/100 g Triton WR-1339 i.p. in 0.9% sodium chloride 24, 16, and 1 h before the study. Control animals received saline injections at identical times. After initiating treatment with either 4-APP or Triton WR-1339, both the treated and control animals were fasted. Where indicated, 50 mg cholesterol dissolved in 2.5 ml corn oil or corn oil alone was administered by an orally passed gastric tube 16 h before initiating the experiments.

Experimental protocol. Between 0800 and 1000 h the diabetic (10–20 days of diabetes) and control rats were injected with $^{3}H_2O$ (50 mCi i.p.). One hour later (3 h later in the crypt villus experiments) the animals were killed and weighed, and a blood specimen was obtained. The small intestine was removed, divided into five equal-length segments, gently rinsed free of luminal material with iced saline, and blotted dry; the segments were weighed individually, and the lipids were saponified by refluxing overnight in a solution of 45% KOH, water, and 70% ethyl alcohol (2:1:5).

Epithelial cell fractions along the villus crypt axis were isolated by a modification of the Weiser (22) technique. In control animals, the small intestine segments were inverted onto a steel rod, placed in a test tube containing 1.5 mM KCl, 96 mM sodium chloride, 27 mM sodium citrate, 8 mM KH_2PO_4, and 5.6 mM Na_2HPO_4 (pH 7.4; buffer A), and incubated at 37°C in a gyratory water bath for 10 min. The bath solution was discarded, and the small intestine segments were then incubated in 2.7 mM KCl, 137 mM NaCl, 1.5 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 1.5 mM EDTA, and 1 mM dithiothreitol (pH 7.4; buffer B) at 37°C in a gyratory water bath for 10 min. Cells dislodged from the small intestine into the buffer were then isolated by centrifugation at 900 × *g* for 10 min. This process was repeated four times with incubation periods in buffer B of 10, 20, 20, and finally 40 min. In the diabetic animals a similar procedure was employed to isolate epithelial cells, except the incubation periods in buffer B were 20, 20, 20, 40, and 20 min. Prior studies had demonstrated that longer incubation times were required to isolate the cells in diabetic animals. Alkaline phosphatase activity was measured on an aliquot of cells from each fraction by freeze-thawing and then incubating for 15 min in a buffer consisting of 0.5 M Trizma (Sigma), 0.3 mM zinc chloride, 10 mM magnesium chloride, and 0.4 mg *p*-nitrophenol phosphate (pH 9.4) (22). The absorption was determined at 420 nM on a Beckman spectrophotometer (Fullerton, CA). Protein was measured in each fraction after freeze-thawing by the Bradford method with a Bio-Rad kit (Richmond, CA). Based on the alkaline phosphatase activity per milligram protein, the five fractions were combined into three fractions representing the upper villus, middle villus, and crypts. These fractions were then saponified in a KOH-ethanol solution.

Lipid analysis. After saponification the flasks were cooled and an internal standard of [^{14}C]cholesterol was added before extraction of the nonsaponifiable material three times with 25 ml petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and then applied to thin-layer chromatography plates. The plates were developed in ethyl acetate:benzene (1:5) for 50 min, and the band, corresponding to a standard of cholesterol, was cut from the plate

TABLE 1
Effect of 4-APP–induced hypocholesterolemia on small intestine cholesterol synthesis in controls

	Total body weight (g)	Total small intestine weight (g)	Plasma cholesterol (mg/dl)	Cholesterol synthesis (μmol ^3H_2O incorporated · h^{-1} · g^{-1}) A	B	C	D	E	Total small intestine
Control (n = 4)	206 ± 4.5	4.00 ± 0.28	54 ± 5.4	0.51 ± 0.07	0.33 ± 0.04	0.30 ± 0.04	0.30 ± 0.02	0.57 ± 0.09	0.42 ± 0.05
Control + 4-APP (n = 4)	213 ± 4.1	4.23 ± 0.20	1 ± 0.20	1.14 ± 0.12	1.20 ± 0.15	0.83 ± 0.02	0.92 ± 0.08	1.72 ± 0.15	1.14 ± 0.08
P	NS	NS	<.001	<.01	<.001	<.001	<.001	<.001	<.001

Animals were administered 20 mg/kg 4-APP or saline for 3 days. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and small intestine was divided into 5 equal-length segments (A, most proximal; E, most distal) and saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE.

and counted. The window settings at the scintillation counter were adjusted so that <0.2% of the tritium counts were recorded in the ^{14}C window and ~10% of the ^{14}C counts were recorded in the tritium window. Calculations were corrected for the spillover of tritium and ^{14}C, for background, and for recovery of the internal standard. The specific activity of the ^3H_2O was determined individually for each animal by measuring the disintegrations per minute per milliliter of plasma at the end of the experiment and dividing by millimoles of water per milliliter of plasma (52 mmol/ml plasma, assuming that plasma is 93% water). The validity of our methodology for measuring cholesterol synthesis has been demonstrated in earlier publications (1,23).

Serum cholesterol concentrations were determined with Sigma diagnostic kit #351. Statistical significance was determined with either a single or two-tailed Student's t test. The single-tailed t test was used only when the direction of change expected was already known from other studies before carrying out the experiment. Because we have observed considerable variability in cholesterol synthesis between groups of animals studied at different times in this and prior studies, we only compared results from animals studied simultaneously. We believe that comparing results from animals studied at different times can be misleading and could result in erroneous conclusions.

RESULTS

Effect of fasting on cholesterol synthesis. Both 4-APP and Triton WR-1339 administration markedly decrease food intake. To prevent differences in food intake between saline- and drug-treated animals, all animals were fasted during these studies. After a 24-h fast, cholesterol synthesis in the small intestine was increased 57% in the diabetic animals compared with controls [control (n = 5) 4.14 ± 0.42 vs. diabetic (n = 6) 6.6 ± 0.52 μmol incorporated/h per entire small intestine, P < .01]. Similarly, after a 72-h fast, small intestine cholesterol synthesis was increased 131% in the diabetic animals [control (n = 4) 1.63 ± 0.12 vs. diabetic (n = 5) 3.76 ± 0.46 μmol incorporated/h per entire small intestine, P < .01)]. These observations are similar to results reported previously in animals fed ad libitum (1) and demonstrate that fasting does not obliterate the increase in small intestine cholesterol synthesis observed in diabetic animals.

Effect of 4-APP on small intestine cholesterol synthesis in control and diabetic animals. The effect of 4-APP–induced hypocholesterolemia on small intestine cholesterol synthesis in control animals is shown in Table 1. As expected, 3 days of 4-APP administration markedly decreased plasma cholesterol levels. Cholesterol synthesis in the small intestine was increased 2.7-fold in the 4-APP–treated animals (con-

TABLE 2
Effect of 4-APP–induced hypocholesterolemia on small intestine cholesterol synthesis in diabetic rats

	Total body weight (g)	Total small intestine weight (g)	Plasma cholesterol (mg/dl)	Cholesterol synthesis (μmol ^3H_2O incorporated · h^{-1} · g^{-1}) A	B	C	D	E	Total small intestine
1 Day									
Diabetic (n = 5)	155 ± 6.2	7.70 ± 0.22	25.8 ± 5.0	0.82 ± 0.06	0.65 ± 0.06	0.65 ± 0.13	0.53 ± 0.10	0.34 ± 0.06	0.57 ± 0.07
Diabetic + 4-APP (n = 5)	148 ± 7.8	6.59 ± 0.36	2 ± 0.12	0.86 ± 0.12	0.93 ± 0.13	1.14 ± 0.15	1.07 ± 0.20	0.64 ± 0.08	0.90 ± 0.12
P	NS	<.05	<.01	NS	<.05	<.05	<.05	<.02	<.05
3 Day									
Diabetic (n = 5)	178 ± 3.5	6.74 ± 0.40	55 ± 4.7	1.13 ± 0.18	0.84 ± 0.14	0.55 ± 0.07	0.36 ± 0.04	0.28 ± 0.04	0.57 ± 0.08
Diabetic + 4-APP (n = 5)	177 ± 5.0	5.94 ± 0.18	19.4 ± 2.8	1.19 ± 0.21	1.53 ± 0.30	1.67 ± 0.49	1.34 ± 0.25	1.60 ± 0.38	1.42 ± 0.27
P	NS	NS	<.001	NS	<.05	<.05	<.01	<.01	<.02

Diabetes was induced 10 days before study in 1-day experiment and 12 days before study in 3-day experiment by injecting 40 mg/kg streptozocin i.p. Before study, animals were administered 20 mg/kg 4-APP or saline for either 1 or 3 days. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and small intestine was divided into 5 equal-length segments (A, most proximal; E, most distal) and saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE.

TABLE 3
Effect of 4-AAP–induced hypocholesterolemia on cholesterol synthesis along villus-crypt axis

	Alkaline phosphatase (mg *p*-nitrophenol formed · mg^{-1} protein · 15 min^{-1})			Cholesterol synthesis (μmol ^3H_2O incorporated · mg^{-1} protein · 3 h^{-1})		
	Upper villus	Middle villus	Crypts	Upper villus	Middle villus	Crypts
Control (n = 4)	77 ± 9	37 ± 7	12 ± 3	0.046 ± 0.005	0.052 ± 0.003	0.077 ± 0.017
Control + 4-AAP (n = 4)	104 ± 20	38 ± 6	6 ± 1	0.111 ± 0.010	0.197 ± 0.062	0.229 ± 0.039
P	NS	NS	NS	<.01	<.05	<.01
Diabetic (n = 4)	130 ± 54	47 ± 19	8 ± 3	0.025 ± 0.006	0.047 ± 0.011	0.089 ± 0.020
Diabetic + 4-AAP (n = 4)	117 ± 16	50 ± 11	12 ± 2	0.148 ± 0.043	0.188 ± 0.033	0.284 ± 0.037
P	NS	NS	NS	<.05	<.01	<.01

Control and diabetic animals (10 days of diabetes) were administered 20 mg/kg 4-APP or saline for 3 days. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 3 h, animals were killed, and a 6-cm segment of mid intestine was removed. Epithelial cell fractions were isolated as described in MATERIALS AND METHODS and saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE.

trol 0.42 ± 0.05 vs. 4-APP 1.14 ± 0.08 μmol ^3H_2O incorporated · h^{-1} · g^{-1}, $P < .001$). Moreover, this increase in small intestine cholesterol synthesis is generalized, occurring in the proximal, middle, and distal segments of the small intestine.

In diabetic animals, 4-APP administration for 3 days also decreased plasma cholesterol levels (Table 2). As observed in control animals, cholesterol synthesis in the small intestine of diabetic animals is also stimulated by 4-APP administration (diabetic 0.57 ± 0.08 vs. diabetic 4-APP 1.42 ± 0.27 μmol ^3H_2O incorporated · h^{-1} · g^{-1}, 2.5-fold increase, $P <$ .02). This increase in cholesterol synthesis in 4-APP–treated diabetic animals was most marked in the distal small intestine (5.7-fold) and decreased proximally (3.7-, 3.0-, 1.8-, 1.1-fold). In the most proximal segment, no significant increase in small intestine cholesterol synthesis was observed. The administration of 4-APP to diabetic animals for a shorter period (1 day) resulted in similar findings (Table 2). In the small intestine as a whole, cholesterol synthesis was increased 1.6-fold in the 1-day 4-APP–treated animals; cholesterol synthesis in the distal segments of the diabetic small intestine was stimulated to a greater extent than in the proximal portions.

Table 3 shows the effect of 4-APP administration on cholesterol synthesis along the villus crypt axis in the mid intestine of control and diabetic animals. In both the control and diabetic animals, alkaline phosphatase activity was highest in the upper villus cells and lowest in the crypt cells, indicating the fractions isolated are representative of upper villus, middle villus, and crypt cells (22). In control animals, 4-APP–induced hypocholesterolemia stimulated cholesterol synthesis 2.4-fold in the upper villus fraction, 3.8-fold in the middle villus fraction, and 3-fold in the crypt cells. In the diabetic animals, 4-APP–induced hypocholesterolemia increased cholesterol synthesis 5.9-fold in the upper villus fraction, 4-fold in the middle villus fraction, and 3.2-fold in the crypt cells. Thus, in both control and diabetic animals, 4-APP administration led to hypocholesterolemia, and this decrease in the plasma cholesterol concentration was accompanied by an increase in small intestine cholesterol synthesis. In the control animals, this increase in small intestine cholesterol synthesis was generalized, occurring in the proximal, middle, and distal small intestine and in all epithelial cell fractions. In diabetic animals the increase in small intestine cholesterol synthesis occurred in the mid and distal portions of the small intestine but not in the most proximal segment. As observed in control animals, cholesterol synthesis in all epithelial cell fractions isolated from the mid

TABLE 4
Effect of Triton WR-1339–induced functional hypocholesterolemia on small intestinal cholesterol synthesis in controls

	Total body weight (g)	Total small intestinal weight (g)	Plasma cholesterol (mg/dl)	Cholesterol synthesis (μmol ^3H_2O incorporated · h^{-1} · g^{-1})					
				A	B	C	D	E	Total small intestine
Control (n = 4)	201 ± 4.4	5.20 ± 0.56	71 ± 8.0	0.82 ± 0.09	0.50 ± 0.04	0.42 ± 0.01	0.54 ± 0.07	0.76 ± 0.10	0.62 ± 0.01
Control + Triton WR-1339 (n = 4)	213 ± 6.0	4.70 ± 0.29	369 ± 12.8	1.14 ± 0.08	1.19 ± 0.16	1.27 ± 0.16	1.41 ± 0.14	1.83 ± 0.20	1.37 ± 0.12
P	NS	NS	<.001	<.05	<.01	<.01	<.01	<.01	<.001

Animals were administered 100 mg/100 g Triton WR-1339 or saline 24, 16, and 1 h before study. After initiating treatment, both control and treated animals were fasted. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and small intestine was divided into 5 equal-length segments (A, most proximal; E, most distal) and saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE.

TABLE 5
Effect of Triton WR-1339–induced functional hypocholesterolemia on small intestinal cholesterol synthesis in diabetic rats

	Total body weight (g)	Total small intestinal weight (g)	Plasma cholesterol (mg/dl)	Cholesterol synthesis (μmol ^3H_2O incorporated · h^{-1} · g^{-1}) A	B	C	D	E	Total small intestine
Diabetic (n = 5)	176 ± 9.0	7.2 ± 0.54	37 ± 6.3	0.55 ± 0.10	0.50 ± 0.12	0.62 ± 0.15	0.51 ± 0.10	0.43 ± 0.10	0.52 ± 0.11
Diabetic + Triton WR-1339 (n = 5)	193 ± 10.0	7.8 ± 0.69	268 ± 20.8	1.60 ± 0.26	1.15 ± 0.17	1.46 ± 0.19	1.37 ± 0.15	1.08 ± 0.21	1.31 ± 0.18
P	NS	NS	<.001	<.01	<.02	<.01	<.01	<.05	<.01

Diabetes was induced 14 days before study by injecting 40 mg/kg streptozocin i.p. Animals were administered 100 mg/100 g Triton WR-1339 or saline 24, 16, and 1 h before study. After initiating treatment, both control and treated animals were fasted. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and small intestine was divided into 5 equal-length segments (A, most proximal; E, most distal) and saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE; n, number of animals. NS, not significant.

intestine was increased in the diabetic animals treated with 4-APP.

Effect of Triton WR-1339 on small intestine cholesterol synthesis. The effect of Triton WR-1339 on small intestine cholesterol synthesis in control animals is shown in Table 4. As expected, plasma cholesterol levels increased in animals treated with Triton WR-1339. The functional hypocholesterolemia caused by the trapping of cholesterol in the vascular compartment induced by Triton WR-1339 administration resulted in a 2.2-fold increase in small intestine cholesterol synthesis. This increase in small intestine cholesterol synthesis in control animals occurred in the proximal, mid, and distal segments of the small intestine.

In diabetic animals, Triton WR-1339 administration also resulted in an increase in plasma cholesterol levels (Table 5). Small intestine cholesterol synthesis increased 2.5-fold in these diabetic animals and to a similar extent in all segments of the small intestine: 2.9-fold in the proximal small intestine, ~2.5-fold in the mid intestine (2.3-, 2.4-, 2.7-fold), and 2.5-fold in the distal small intestine. These observations demonstrate that functional hypocholesterolemia induced by Triton WR-1339 treatment results in a generalized stimulation of small intestine cholesterol synthesis in both control and diabetic animals.

To determine whether the increase in small intestine cholesterol synthesis that occurs in the Triton WR-1339–treated animals results from the decreased delivery of cholesterol to cells secondary to the trapping of cholesterol in the circulation, we quantitated proximal small intestine cholesterol synthesis in animals administered a large oral dose of cholesterol. In control animals, cholesterol administration 16 h before the study resulted in inhibition of the Triton WR-1339–induced increase in cholesterol synthesis in the proximal small intestine (control 1.36 ± 0.14 vs. Triton WR-1339 treated 1.71 ± 0.09 μmol ^3H_2O incorporated · h^{-1} · g^{-1}, $P < .05$; control 1.36 ± 0.14 vs. Triton WR-1339 plus cholesterol treated 1.29 ± 0.10, NS; Triton WR-1339 vs. Triton WR-1339 plus cholesterol, $P < .02$) (Fig. 1). In diabetic animals, cholesterol feeding also blocked the increase in proximal small intestine cholesterol synthesis that occurs in Triton WR-1339–treated animals (diabetic 1.09 ± 0.11 vs. diabetic treated with Triton WR-1339 1.56 ± 0.21 μmol tritiated water incorporated · h^{-1} · g^{-1}, $P < .05$; diabetic 1.09 ± 0.11 vs. diabetic treated with Triton WR-1339 plus cholesterol 0.99 ± 0.08, NS; diabetic treated with Triton WR-1339 vs. diabetic treated with Triton WR-1339 plus cholesterol, $P < .05$) (Fig. 2). These observations indicate that providing cholesterol to the small intestine cells via an alternate route can prevent the increase in cholesterol synthesis induced by Triton WR-1339 administration in both control and diabetic animals.

DISCUSSION

Previous studies from this and other laboratories have demonstrated that insulinopenic diabetes results in a two- to threefold increase in cholesterol synthesis in the small intestine (1–6). In animals with diabetes of relatively short duration (<1 wk), small intestine cholesterol synthesis is increased on both a total-organ basis and on a per-gram basis in diabetic animals (24). In animals with diabetes of extended duration (>1 wk), the small intestine is markedly hypertrophied, and small intestine cholesterol synthesis is increased on a total-organ basis; but on a per-gram basis, cholesterol synthesis can increase, remain the same, or even decrease compared with control animals (24). In this study, small intestine cholesterol synthesis on a per-mass basis was similar

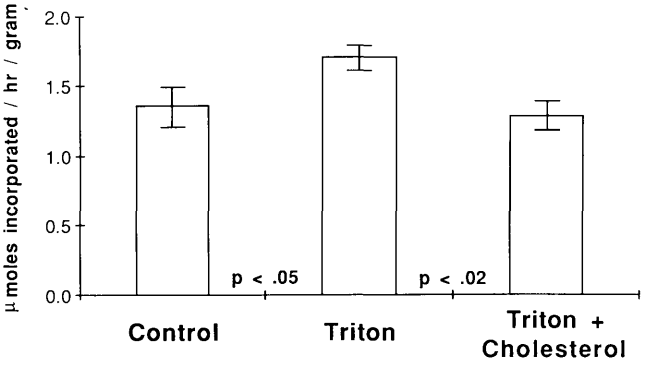

FIG. 1. Control animals were administered 100 mg/100 g Triton WR-1339 or saline 24, 16, and 1 h before study. Sixteen hours before study, 50 mg cholesterol dissolved in 2.5 ml corn oil or corn oil alone was administered by an orally passed gastric tube. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and proximal third of small intestine was saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE; n = 5 for each group. $P < .05$, control vs. Triton WR-1339. $P < .02$, Triton WR-1339 vs. Triton WR-1339 + cholesterol.

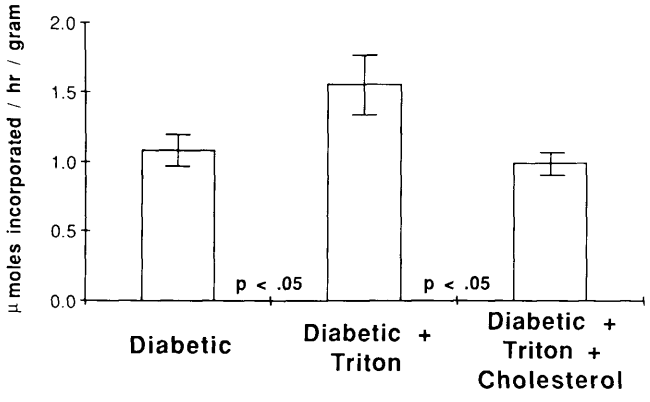

FIG. 2. Diabetes was induced 14 days before study by injecting 40 mg/kg streptozocin i.p. Diabetic animals were administered 100 mg/100 g Triton WR-1339 or saline 24, 16, and 1 h before study. Sixteen hours before study, 50 mg cholesterol dissolved in 2.5 ml corn oil or corn oil alone was administered by an orally passed gastric tube. On day of study, animals were injected with 50 mCi ^3H_2O i.p. After 1 h, animals were killed, and proximal third of small intestine was saponified in KOH-ethanol solution. [^{3}H]cholesterol was assayed after extraction with petroleum ether and thin-layer chromatography. Values are means ± SE; n = 5 for each group. $P < .05$, diabetic vs. diabetic + Triton WR-1339. $P < .05$, diabetic + Triton WR-1339 vs. diabetic + Triton WR-1339 + cholesterol.

in control and diabetic animals, but note that the intestinal weight was greater in the diabetic animals, and therefore total small intestine cholesterol synthesis increased in the diabetic animals. Thus, soon after the onset of diabetes, cholesterol synthesis on a per-gram basis is increased in the small intestine, and this increased rate per unit mass is responsible for the increase in total small intestine cholesterol synthesis. With a longer period of diabetes, the small intestine hypertrophies to such a degree that at this stage the increase in total small intestine cholesterol synthesis in diabetic animals is primarily due to an increase in intestinal mass and not to an increased rate of cholesterol synthesis per unit weight of tissue.

Previous studies in this laboratory have demonstrated that cholesterol synthesis in the small intestine of diabetic animals is affected by luminal factors (24,25). Cholesterol feeding greatly inhibits small intestine cholesterol synthesis. In fact, the two- to threefold stimulation of small intestine cholesterol synthesis observed in diabetic animals is completely obliterated by cholesterol feeding. In contrast, colestipol feeding and biliary drainage, procedures that reduce bile acid pool size, stimulate small intestine cholesterol synthesis in diabetic animals. These observations indicate that cholesterol synthesis in the small intestine of diabetic animals can be altered by experimental manipulations that affect the luminal contents.

In normal animals, studies by other investigators have demonstrated that cholesterol synthesis in the small intestine is regulated by plasma cholesterol levels (11–13). When plasma cholesterol levels are decreased by either 4-APP or Triton WR-1339 administration, small intestine cholesterol synthesis is increased. The purpose of this study was to determine if circulating cholesterol concentrations also regulate small intestine cholesterol synthesis in diabetic animals.

This study demonstrates that in diabetic animals, 4-APP–induced hypocholesterolemia results in an increase in small intestine cholesterol synthesis. In control animals, this increase in cholesterol synthesis occurs in the proximal, mid, and distal small intestine, whereas in the diabetic animals, this effect is most marked in the distal small intestine, decreasing proximally. In the most proximal small intestine segment, no effect on cholesterol synthesis is observed in the 4-APP–treated diabetic animals. In both control and diabetic animals, 4-APP–induced hypocholesterolemia stimulated cholesterol synthesis in the upper villus, middle villus, and crypt cells of the mid intestine.

In agreement with our observations in 4-APP–treated animals, functional hypocholesterolemia due to Triton WR-1339 treatment also stimulated small intestine cholesterol synthesis in control and diabetic animals. This increase in cholesterol synthesis in the small intestine of control and diabetic animals was generalized, occurring in the proximal, mid, and distal small intestine. Moreover, cholesterol administered orally could prevent the Triton WR-1339–induced stimulation of small intestine cholesterol synthesis. In both control and diabetic animals, small intestine cholesterol synthesis was the same in saline- and Triton WR-1339–treated animals administered cholesterol. In the animals treated with Triton WR-1339 alone, proximal small intestine cholesterol synthesis increased in comparison with either the animals treated with saline or the animals treated with Triton WR-1339 plus cholesterol. These observations indicate that providing cholesterol via an alternate, i.e., intraluminal, route can block the increase in cholesterol synthesis induced by the functional hypocholesterolemia caused by Triton WR-1339 administration.

The results reported herein, together with our earlier observations demonstrating that luminal factors regulate cholesterol synthesis, indicate that small intestine cholesterol synthesis in diabetic animals is regulated by both luminal and circulating factors in the expected fashion.

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