Contributions of Absorbed Dietary Cholesterol and Cholesterol Synthesized in Small Intestine to Hypercholesterolemia in Diabetic Rats

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To determine how insulin deficiency leads to hypercholesterolemia, we examined cholesterol absorption, synthesis, and utilization in streptozocin-induced diabetic rats fed a grain-based diet ad libitum. Absorbed dietary cholesterol was determined from measurement of dietary cholesterol intake and previous data for cholesterol fractional absorption. Daily rates of cholesterol synthesis in the small intestine, liver, and periphery were calculated from recovery of labeled sterols after injection of ^3H_2O at six times during 24 h. Utilization of cholesterol for growth, fecal excretion, and bile acid synthesis were also determined. Absorbed dietary cholesterol in diabetic rats was double that in control rats. The contribution of absorbed cholesterol to total cholesterol production (sum of absorbed dietary cholesterol and endogenous cholesterol synthesis) in control rats was 24% compared to 48% in diabetic rats. The increase in diabetic rats was due to overeating and, to a lesser extent, to increased fractional absorption. Overeating also induced intestinal hypertrophy and a twofold increase in cholesterol synthesis by the small intestine to 24% of whole-body production in diabetic rats. Consequently, the small intestine accounted for 72% of daily cholesterol input in diabetic rats compared to 37% in control rats, with diet accounting for two-thirds of the cholesterol input via the small intestine in both groups. In response to this increased input from the intestine, cholesterol synthesis in the periphery was 39% of whole-body production in control rats compared to 22% in diabetic rats, and synthesis in the liver was 26 and 6% of total cholesterol production in control and diabetic rats, respectively. The net effect of these changes was a decrease in whole-body synthesis equal to the increase in absorbed dietary cholesterol and no change in whole-body cholesterol production in diabetic rats. The need for cholesterol as substrate for bile acid synthesis was met largely by absorption from the diet in diabetic rats and by synthesis in the liver in control rats. The need for cholesterol in the periphery was entirely met by synthesis in situ in both groups. We conclude that *1*) an increased fraction of the net cholesterol production is derived from intestinal sources and transported through the blood in diabetic rats, and 2) cholesterol absorbed from the diet contributes more to the hypercholesterolemia of diabetic rats than the increased cholesterol synthesis in the small intestine. *Diabetes* 37:1151–56, 1988

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People with diabetes have a shortened life span due largely to atherosclerosis accelerated by high levels of plasma low-density lipoprotein (LDL) cholesterol and very-low-density lipoprotein (VLDL) triglyceride and low levels of high-density lipoprotein (HDL) cholesterol (1–18). These changes indicate that diabetes perturbs cholesterol metabolism. To determine the mechanism for this perturbation, various aspects of cholesterol metabolism were examined in rats injected with streptozocin (STZ), a β-cytotoxic drug (19–32). We report on the last of a series of such studies from our laboratories aimed at determining the causes of diabetes-induced hypercholesterolemia (28–31).

It is clear that hypercholesterolemia of diabetic rats fed a grain-based diet is due to overeating rather than to more direct effects of insulin deficiency on cholesterol metabolism. With overeating there are increases in dietary cholesterol intake, fractional cholesterol absorption (25,26,31), cholesterol synthesis by the small intestine (23,27–29), and secretion of dietary and endogenous cholesterol in intestinal lymph (20,24). The critical role of overeating in producing hypercholesterolemia is also shown by the observations that the plasma cholesterol level in diabetic rats is proportional

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TABLE 1
Effect of streptozocin on plasma glucose and cholesterol levels

	Streptozocin	Buffer
Plasma glucose (mg/dl)		
Middark	661 ± 34 (11)*	182 ± 3 (11)
Midlight	526 ± 30 (8)*	154 ± 6 (13)
Plasma cholesterol (mg/dl)		
Middark	109 ± 15 (11)†	52 ± 3 (11)
Midlight	115 ± 18 (8)‡	58 ± 3 (13)

Values are means ± SE with number of rats in each group given in parentheses. Significance of difference from rats injected with buffer: * $P < .001$, † $P < .005$, and ‡ $P < .025$.

to the fraction of cholesterol in plasma originating in the diet (31) and that hypercholesterolemia is all but eliminated when feed intake is restricted to a normal level (29).

Because absorbed dietary cholesterol and cholesterol synthesis in the small intestine both increase in hyperphagic diabetic rats (20,23,26,27,31), each may contribute to the secretion of more chylomicron cholesterol. The primary objective of this study was to compare the absolute amount of cholesterol synthesized by the whole small intestine per day with the amount absorbed from the diet to determine the relative contribution of each to cholesterol flux through the small intestine. Another objective was to compare cholesterol production in the small intestine with synthesis in liver and periphery to test the hypothesis that diabetes-induced changes in synthesis in the latter tissues are due to feedback regulation by cholesterol derived from the small intestine. Finally, we estimated rates of cholesterol utilization for growth, bile acid synthesis, and fecal excretion and summed losses through urine, skin, and steroid hormone synthesis to enable comparison with rates of cholesterol synthesis in liver and periphery. From these data the extent to which requirements for cholesterol are met by synthesis in situ as opposed to uptake from plasma can be estimated.

Rates of cholesterol synthesis are best estimated from the amount of ^3H incorporated into sterols after injection of ^3H_2O. Short labeling periods of 1–2 h are used to minimize transfer of [^3H]cholesterol between tissues and its conversion to bile acids and steroid hormones (32). In this study, we estimated daily rates of synthesis in the small intestine, liver, and periphery by averaging rates of ^3H incorporation during six 80-min periods spaced at 4-h intervals throughout 24 h. Dietary cholesterol intake and fecal steroid excretion were also measured. These data and previously published data were used to calculate sterol pool sizes and absolute rates of sterol production and utilization through various routes.

TABLE 2
Effect of diabetes on dietary cholesterol intake and fecal steroid excretion

	Diabetic (n = 19)	Control (n = 24)
Dietary cholesterol (mg/day)*	16.0 ± 0.6†	8.6 ± 0.3
Fecal steroids (mg/day)*		
Total	20.4 ± 1.5	21.3 ± 1.2
Neutral‡	10.6 ± 0.7	11.5 ± 0.8
Acidic	9.8 ± 1.1	9.8 ± 0.7

Values are means ± SE.
*Excludes phytosterols.
† $P < .001$, significantly different from control.
‡Includes unabsorbed dietary cholesterol.

TABLE 3
Effect of diabetes on daily average weight of tissues and intestinal contents

	Diabetic	Control
Body weight (g)	306 ± 6 (100)*	390 ± 6 (100)
Tissue weight (g)		
Total	257 ± 5 (84)*	366 ± 5 (94)
Periphery	219 ± 5 (72)*	334 ± 2 (86)
Liver	13.0 ± 0.3 (4.2)	14 ± 1 (3.6)
Small intestine	12.2 ± 0.4 (4.0)*	7.4 ± 0.1 (1.8)
Large intestine and cecum	4.6 ± 0.2 (1.5)*	3.1 ± 0.2 (0.8)
Blood sample	8.5 ± 0.8 (2.8)	8.3 ± 0.3 (2.1)
Contents (g)		
Small intestine	8.5 ± 0.8 (2.7)*	3.0 ± 0.3 (0.8)
Large intestine and cecum	17.7 ± 0.7 (5.8)*	8.1 ± 0.2 (2.1)
Unrecovered (g)†	23 ± 1 (7.5)*	12 ± 1 (3.1)

To calculate the daily average, the mean for rats at each time point (see n at bottom of Fig. 2) was calculated first, then the mean ± SE was calculated for all times. Values are means ± SE, with percentages in parentheses.
* $P < .001$, significantly different from control.
†Due to stomach contents, blood, and urine discarded during dissection.

MATERIALS AND METHODS

Treatment of rats. Male Wistar rats (Charles River, Wilmington, MA) were housed in metabolic cages with one group exposed to light from 0940 to 2140 and a second group from 2100 to 0900 (31). Hours of light are designated L1–L12, hours of darkness D1–D12. All rats were fed Purina Formulab 5008 pellets ad libitum from D1 to D12.

After 14–19 days of equilibration, rats weighing 330 ± 2 g were intravenously injected with a single bolus of STZ at 65 mg/kg body wt (diabetic). At the same time, control rats weighing 331 ± 3 g were injected with buffer (control). The day of injection was designated day 1 (30).

Collection of samples. Before injection or phlebotomy, rats were anesthetized with ether and weighed. One 1-ml blood sample was taken from a tail vein of each rat during L5–L8 on days 12–16 or during D5–D7 on days 13–17 for plasma glucose and cholesterol determinations. For determination of dietary and fecal steroids, daily food intake was measured and feces were collected on days 19–21 or days 20–22.

To measure rates of cholesterol synthesis, a bolus of ^3H_2O (75 mCi/ml saline) was injected into a tail vein (151 mCi/kg body wt) of each rat on day 21 or 22, ~40 min before L2, L6, L10, D2, D6, or D10. The usual conditions of light, food, and water were continued. After 80 min for sterol labeling, a 5-to 10-ml blood sample was obtained by cardiac puncture, and the liver and whole intestinal tract were removed and weighed. The intestinal tract was flushed with saline and reweighed. Tissues and contents of the small intestine were each added to 30% KOH in methanol as previously described (31).

Assays. Plasma glucose and cholesterol were measured with enzymatic assays (28). Dietary and fecal steroids were extracted and analyzed by gas-liquid chromatography

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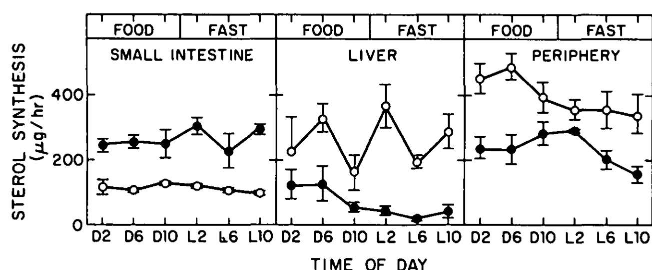

FIG. 1. Effect of diabetes on rate of C_{27}-sterol synthesis in small intestine, liver, and periphery of diabetic (●) and control (○) rats at various times of day. Times are given as number of hours into dark (D) or light (L) periods. Data are expressed as means ± SE for *n* given at *bottom* of Fig. 2.

(33,34). $[^3H]C_{27}$-sterols were extracted (31) and isolated by digitonin precipitation (35) with $[^{14}C]$cholesterol as a recovery standard and a final thin-layer chromatography step in which radioactivity in a narrow band containing the lowest $^3H/^{14}C$ ratio was measured as detailed previously (30). ^3H_2O activity in plasma was constant from 15 min to 4 h (30). Labeling of $[^3H]C_{27}$-sterols in the whole body was linear from 0 to 4 h (30). Absolute rates of C_{27}-sterol synthesis were calculated by assuming 22 H atoms of C_{27}-sterol were labeled with ^3H of the same specific activity as ^3H_2O in plasma (30).* Rates of synthesis were not corrected for the small amount of $[^3H]C_{27}$-sterols in blood removed from the body or remaining in tissues.†

Statistics. Data are presented as means ± SE. Significance of difference between means was determined by *t* test, which is applicable where the number of samples and the variance in the two groups are unequal (39).

RESULTS

Plasma constituents. STZ injection caused a 3.5-fold increase in plasma glucose and a 2.0-fold increase in plasma cholesterol as seen previously (28–31; Table 1).

Dietary and fecal steroids. The diet contained 0.348 mg cholesterol, 0.474 mg sitosterol, and 0.186 mg campesterol plus stigmasterol per gram. In diabetic rats there was a 1.9-fold increase in dietary cholesterol intake with hyperphagia but no change in fecal steroid excretion compared with control rats (Table 2), in agreement with our previous findings (30).

Weight of tissues and intestinal contents. Three weeks after injection, the body weight of control rats had increased 18%, whereas that of diabetic rats had decreased 7%. This decrease was due to weight loss in the periphery, because liver weights were unchanged and intestinal tissue and contents weights were increased in diabetic rats (Table 3).

Sterol synthesis. Synthesis of C_{27}-sterols (μg/h) in diabetic rats varied during the day from 10 to 50% of the rate in control rats in the liver and from 50 to 70% of the rate of control rats in the periphery (Fig. 1). In contrast, synthesis in the small intestine in diabetic rats was 200–300% of that in control rats. Less than 1% of the total $[^3H]C_{27}$-sterol was recovered in the contents of the small intestine (means for the 6 time points were 3.7 ± 0.2 μg/h in diabetic and 2.8 ± 0.2 μg/h in control rats). The net effect of these changes in

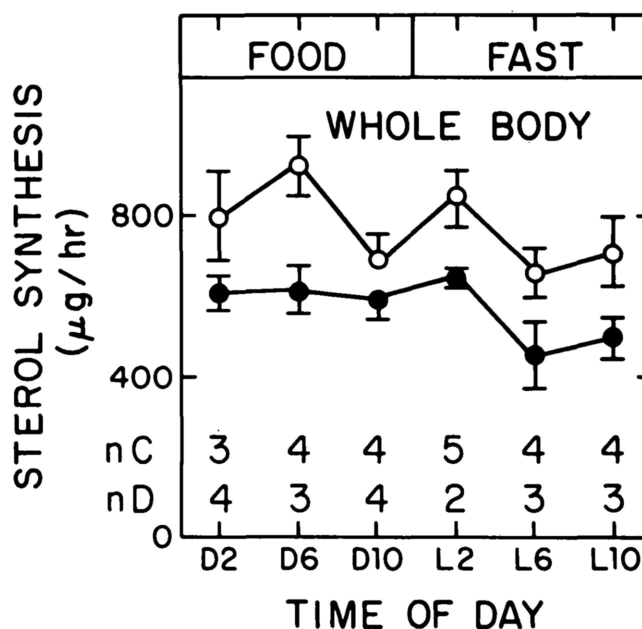

FIG. 2. Effect of diabetes on rate of C_{27}-sterol synthesis in whole body at various times of day. Whole-body synthesis was calculated from recovery of $[^3H]$sterols in liver, periphery, small intestine, and contents of small intestine. ●, Diabetic; ○, control.

TABLE 4
Effect of diabetes on the daily average rate of cholesterol synthesis

	Diabetic	Control
Total tissue [μg/(g tissue × h)]	2.2 ± 0.1	2.1 ± 0.1
Periphery [μg/(g tissue × h)]	0.98 ± 0.09	1.14 ± 0.07
Liver [μg/(g tissue × h)]	6 ± 2*	19 ± 2
Small intestine [μg/(g tissue × h)]	21.9 ± 0.8*	15.3 ± 0.6

Daily average is mean ± SE of means for 6 4-h intervals in 24 h; for *n* at each time see Fig. 2.
*$P < .001$, significantly different from control.

*We assume, as do Dietschy and Spady (32), that $[^3H]$NADPH used for cholesterol synthesis has the same specific activity (sp act) as ^3H_2O in tissues. However, $[^3H]$NADPH sp act may be lower than ^3H_2O sp act due to synthesis of unlabeled NADPH by the pentose cycle (36,37). Pullinger and Gibbons (37) argue that $[^3H]$NADPH sp act is lower when carbohydrates compared to fats are catabolized, so that ~20 H atoms per cholesterol molecule are labeled in the presence of oleate compared to 15 in its absence. Because control rats probably use more carbohydrate for energy than do diabetic rats, the $[^3H]$NADPH sp act used for cholesterol synthesis may be lower in control than in diabetic rats. If we assume 20 H/cholesterol in diabetic subjects and 15 H/cholesterol in control subjects, then the estimated rates of whole-body cholesterol synthesis would be 15.4 and 27.1 mg/day, and total sterol production would be 27.9 and 33.0 mg/day or 109 and 90 $mg^{-1} \cdot kg^{-1} \cdot$ day in diabetic and control subjects, respectively (Table 5). This estimate of sterol production in control subjects is probably unreasonably high because only 56% of this value can be accounted for by measured routes of utilization, leaving 44% to be accounted for by hormone synthesis plus excretion through urine and skin. Thus, we feel that 15 H atoms/cholesterol is too low.

†From data provided by Robbins et al. (Tables 1 and 2 in 38) and assuming a linear increase in whole-body $[^3H]$cholesterol with time (30), 16% of whole-body $[^3H]$cholesterol would be contained in the total plasma volume at 80 min after injection of ^3H_2O. After removing a blood sample, 61 and 69% of the total plasma volume remained in diabetic and control rats, respectively, assuming 3.5 ml plasma/100 g body and 50 ml plasma/dl blood. Thus, we estimate in diabetic and control rats, respectively, that ~10 and 11% of total $[^3H]$cholesterol was contained in plasma remaining in the body after removing a blood sample and that 6 and 5% of total $[^3H]$cholesterol was removed in blood and not recovered.

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TABLE 5
Effect of diabetes on cholesterol dynamics

	Diabetic	Control
Total sterol production		
mg · kg^{-1} · day^{-1}*	103 ± 3†	67 ± 2
mg/day‡	26.3 ± 0.7	24.4 ± 0.8
Sterol synthesis (mg/day)§		
Total tissue	13.8 ± 0.5‖	18.5 ± 0.8
Periphery	5.7 ± 0.3†	9.5 ± 0.5
Liver	1.7 ± 0.2†	6.3 ± 0.6
Small intestine	6.4 ± 0.3†	2.8 ± 0.1
Absorbed from diet (mg/day)¶	12.5 ± 0.5†	5.9 ± 0.2
Sterol utilization (mg/day)		
Excretion		
Neutral steroid#	7.1 ± 0.5	8.8 ± 0.6
Acidic steroid	9.8 ± 1.1	9.8 ± 0.7
Bile acid pool expansion**	3.3 ± 0.2†	0
Bile acid synthesis††	13 ± 1	9.8 ± 0.7
Growth‡‡	0†	3.4 ± 0.5
Other§§	6.1	2.5
Steroid pools (mg)		
Tissue cholesterol‖‖	515	608
Total bile acid¶¶	137	53
Fractional production rate (%/day)##		
Tissue cholesterol	5.1	4.0
Total bile acid	9.6	18.5

*The sum of C_{27}-sterols synthesized and dietary cholesterol absorbed divided by total tissue weight (Table 3).
†$P < .001$, ‖$P < .005$, significance of difference from controls.
‡Sum of sterol synthesized in total tissue and dietary cholesterol absorbed.
§Mean ± SE of the mean of mean μg/h for 6 times in 24 h (Figs. 1 and 2) times 24 h/day times mg/1000 μg.
¶Dietary cholesterol (Table 2) times fraction absorbed, 0.785 in diabetic and 0.686 in control rats (31).
#Fecal neutral steroids (Table 2) minus nonabsorbed dietary cholesterol.
**Due to increasing food intake; estimated from data in ref. 30 for rats fed ad libitum by correcting for the 21% decrease in food intake and weight of small intestinal contents due to feeding 12 h/day in this study.
††Sum of bile acid pool expansion and acidic steroid excretion.
‡‡Growth rate for the previous 6 days, 2.03 g/day, times the concentration of cholesterol plus Δ^7-cholesterol in tissues of control rats, 1.66 mg/g (30).
§§Summed losses of cholesterol through skin, urine, and steroid hormone synthesis. Total production minus utilization through excretion, bile acid pool expansion, and growth.
‖‖Total tissue weight (Table 3) times the concentration of cholesterol plus Δ^7-cholesterol in the total tissue, 2.01 mg/g in diabetic rats and 1.66 mg/g in control rats (30).
¶¶Weight of small intestine contents (Table 3) times concentration of bile acid in small intestine contents, 36.3 μmol/g in diabetic rats and 39.6 μmol/g in control rats (30), times (0.386 mg cholesterol/μmol bile acid) divided by 0.85 to give total bile acid pool (because 85% of the total pool is in small intestine contents).
##Production rate in mg/day times 100% divided by pool size in mg.

diabetic rats was a 10–30% decrease in whole-body cholesterol synthesis (Fig. 2). When synthesis is expressed as micrograms per gram/tissue times number of hours, the daily average rates in periphery and total tissue were unaffected by diabetes, and the fold increase in synthesis in the small intestine was less than when expressed as micrograms per hour (Table 4). Thus, the decrease in synthesis (μg/h) in the periphery and, to some extent, the increase in the small intestine, but not the decrease in liver, can be accounted for by the change in tissue weights (Table 3).

DISCUSSION

Cholesterol production. We have previously shown that rats with STZ-induced insulin deficiency stop growing and are hyperphagic and that overeating increases the intake and fractional absorption of dietary cholesterol and total 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase activity in the small intestine (28–31). These increases result in hypercholesterolemia and, through product feedback inhibition, decreases in HMG-CoA reductase activity in liver and in whole-body cholesterol synthesis (28–30).

Our data for daily cholesterol synthesis in small intestine, liver, and periphery and for absorbed dietary cholesterol permit the following conclusions. Cholesterol production (synthesis plus absorption) by the small intestine accounts for 72% of total-body cholesterol production in diabetic rats compared to 35% in control rats (Table 5; Fig. 3). Absorbed dietary cholesterol accounts for two-thirds of cholesterol produced in the small intestine in both groups. As cholesterol flux through the small intestine increases, cholesterol synthesis in the liver and periphery decreases so that total-body production is unchanged by diabetes.

Cholesterol utilization and steroid pools. We have previously shown that the bile acid pool in the contents of the small intestine is proportional to food intake and independent of insulin status per se, that fecal steroid excretion is not increased in spite of overeating, and that bile acid synthesis increases to support the increase in the bile acid pool size in hyperphagic diabetic rats (30). Our previous data are confirmed by the data herein. In addition, we demonstrate in this study that the bile acid pool turns over more slowly

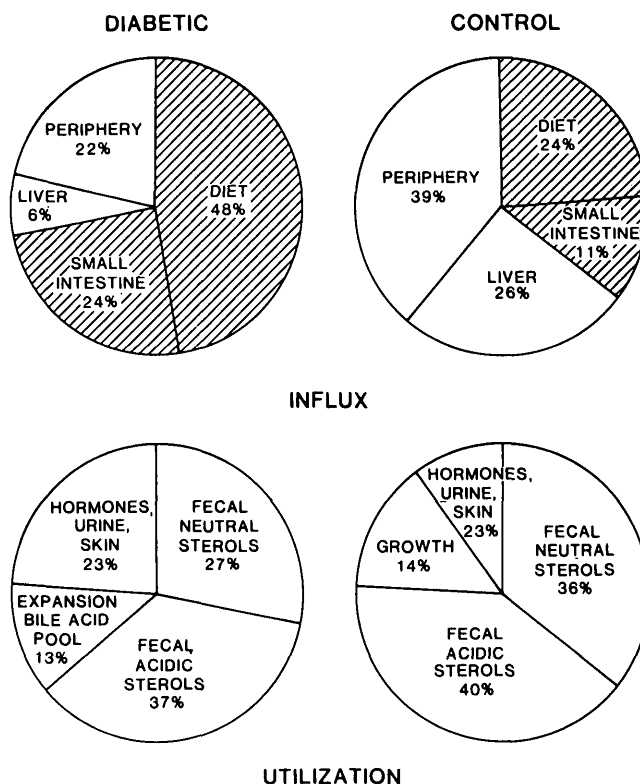

FIG. 3. Effect of diabetes on daily rates of cholesterol production and utilization through various routes. Wedges show fraction of total sterol production (mg/day) through each route calculated from data in Table 5.

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in diabetic rats because the pool is greatly enlarged, whereas excretion is not increased (Table 5).

It is interesting that hepatic cholesterol synthesis is inadequate to support bile acid synthesis in either diabetic or control rats; cholesterol synthesis in situ could provide at most 12% of the substrate for bile acid synthesis in diabetic rats and 65% in control rats. Consequently, at least 88% of the cholesterol converted to bile acids must originate outside of the liver and be transported through the bloodstream in diabetic rats compared to 35% in control rats.

There is no utilization of cholesterol for growth in diabetic rats because they are not growing, and the total tissue cholesterol pool is held constant for at least 4 wk after STZ injection (30). Because the total cholesterol pool fails to increase when cholesterol production (mg/day) is normal, the pool turns over faster in diabetic rats (Table 5). It appears that cholesterol synthesis in the periphery is at least adequate to support utilization for growth and hormone synthesis plus any losses through urine and skin in both diabetic and control rats. In control rats, synthesis in the periphery exceeds such utilization, which suggests there is a net flux of nearly synthesized cholesterol from the periphery to the liver.

Flux of cholesterol through plasma. We have not directly measured cholesterol flux through plasma. However, consideration of data for cholesterol production and utilization suggests that diabetes-induced overeating increases the fraction of cholesterol produced daily that is transported through plasma. Absorbed dietary cholesterol must be transported through the bloodstream before its utilization, e.g., for bile acid synthesis, fecal excretion, hepatic VLDL secretion, growth, and hormone synthesis, whereas cholesterol used at sites of synthesis is obviously not transported through the blood. It is clear that less cholesterol is synthesized and more is absorbed from the diet in hyperphagic diabetic rats, hence a larger fraction of the total-body cholesterol production is likely to be passing through the bloodstream. Consider, for example, cholesterol used for bile acid synthesis. As pointed out above, net transport of cholesterol to the liver to supply substrate for bile acid synthesis (bile acid synthesis minus hepatic cholesterol synthesis) must increase from 3.5 mg/day in control rats to 11.3 mg/day in diabetic rats. Thus, the fraction of total cholesterol production moving from the blood into the liver and converted to bile acids increases threefold, from 0.14 to 0.43. A major part of this increase is probably supplied by absorbed dietary cholesterol, which increases from 5.9 mg/day in control rats to 12.5 mg/day in diabetic rats. A minor part of the increased need for bile acid substrate could also be supplied by cholesterol synthesized in the small intestine, which increases from 2.8 mg/day in control rats to 6.4 mg/day in diabetic rats.

The twofold increase in absorbed dietary cholesterol in diabetic rats that results from overeating plays a major role in their hypercholesterolemia. We reach this conclusion from the previous findings that restricting food intake by diabetic rats to normal prevents hypercholesterolemia (29) and that <8% of the cholesterol absorbed from food remains in the small intestine 50 h after consumption (31) and from the finding herein that, although cholesterol synthesis in the small intestine is also doubled by overeating, this source provides only half as much cholesterol as do dietary sources in both diabetic and control rats (Table 5).

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