

Evaluation of the contribution of dietary cholesterol to hypercholesterolemia in diabetic rats and of sitosterol as a recovery standard for cholesterol absorption

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Abstract The contribution of dietary cholesterol to hypercholesterolemia in diabetic rats fed chow ad libitum was evaluated. Diabetes was induced with streptozotocin, and the intake, absorption, and subsequent tissue distribution of dietary cholesterol were measured. Absorption was measured as the difference between [³H]cholesterol intake and fecal ³H-labeled neutral sterol excretion, using both [¹⁴C]sitosterol (added to diet) and [¹⁴C]cholesterol (added to feces) as recovery markers. [³H]Cholesterol absorption was underestimated by 1–3% using [¹⁴C]sitosterol as a recovery standard, due to the 7–8% absorption of sitosterol. After 3 weeks of diabetes, rats were hyperphagic, thereby increasing dietary cholesterol intake 2-fold. [³H]Cholesterol absorption was significantly increased from 69% in controls to 78% in diabetics, whereas [¹⁴C]sitosterol absorption was unaffected. With increased dietary cholesterol intake and decreased whole body cholesterol synthesis (*Diabetes*. 1983. 32: 811–819), influx from diet equaled for exceeded influx from synthesis. The amounts of ³H-labeled neutral sterol recovered from the small intestine, periphery, and plasma were increased 3- to 4-fold in the diabetic rats. Furthermore, the degree of hypercholesterolemia in diabetic rats was directly related to the fraction of plasma cholesterol derived from the diet. We conclude that the 2.3-fold increase in absorbed dietary cholesterol resulting from hyperphagia and, to a lesser extent, from increased fractional absorption, contributes to the hypercholesterolemia of diabetic rats fed chow ad libitum.—Young, N. L., D. R. Lopez, D. J. McNamara, and G. Benavides. Evaluation of the contribution of dietary cholesterol to hypercholesterolemia in diabetic rats and of sitosterol as a recovery standard for cholesterol absorption. *J. Lipid Res.* 1985. 26: 62–69.

Supplementary key words fecal sterols • tissue cholesterol • bile acids

This study of absorption and subsequent tissue distribution of dietary sterol was undertaken as part of a series aimed at determining the causes of hypercholesterolemia in diabetic rats (1–3). Dietary sterol assumes greater importance in cholesterol dynamics in diabetic

rats since they are hyperphagic, consuming twice the amount of chow of control animals. Hyperphagia is a critical determinant of the lipemia of diabetics: severely diabetic rats, whose food intake is restricted to normal, have nearly normal plasma cholesterol and triacylglycerol levels (2, 3). Even if insulin deficiency leads to decreased lipoprotein lipase activity, hence impaired clearance of plasma lipoproteins (4–6), it is evident that the activity of clearance mechanisms is adequate to handle a normal influx.

In addition to increased cholesterol intake with hyperphagia, the fraction of dietary cholesterol absorbed by the small intestine has been shown to increase in diabetic rats fed ad libitum (7–9). Nervi, Gonzalez, and Valdivieso (7) reported that the amount of radiolabeled cholesterol recovered in thoracic lymph after gastric intubation of a fixed volume liquid meal was doubled by diabetes. Thomson (8) and Thomson and Rajotte (9) found that the rate of flux of radiolabeled cholesterol into a section of intestinal mucosa in vitro was increased over 2-fold in diabetic rats previously fed chow or a high carbohydrate diet compared to normal rats. While it is clear from these studies that fractional cholesterol absorption is increased in hyperphagic diabetic rats, it is not possible to calculate from the data presented how much cholesterol would be absorbed from chow. Hence, the relative contributions of dietary and newly synthesized cholesterol to total cholesterol influx remains to be determined.

In the present study we estimated the in vivo absorption of [³H]cholesterol from chow consumed ad libitum by measuring the difference between intake and ³H-

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labeled neutral sterols in feces and intestinal contents. [^{14}C]Sitosterol, a poorly absorbed phytosterol, was included in the test meal as a recovery standard (10). In addition, to avoid errors introduced by [^{14}C]sitosterol absorption, we added [^{14}C]cholesterol to one-half of each sample as a recovery standard. To evaluate the contribution of dietary cholesterol to hypercholesterolemia in diabetic rats, we measured the ^3H and cholesterol mass in plasma. To completely account for all ^3H -labeled neutral sterols, we also measured those in tissues and urine. Finally, all of the data were corrected for loss of ^3H from the sterol molecule (11) and for ^3H -labeled non-sterol impurities in the [^3H]cholesterol stock.

MATERIALS AND METHODS

Animals and diet

Twenty male Wistar rats (Charles River Laboratories) weighing 200–250 g were exposed to light from 9 PM to 9 AM daily, and fed Purina Formulab 5008 ad libitum for 5 weeks prior to testing sterol absorption. The concentrations of cholesterol and sitosterol in the batch of Formulab 5008 used throughout were determined by gas-liquid chromatography of extracts (12, 13). Three weeks before the test, when rats weighed 315 ± 2 g, diabetes was induced in 13 rats by the intravenous injection of streptozotocin at 65 mg/kg (3). The 10 controls received injections of buffer only. Rats were housed individually in metabolic cages with wire-mesh bottoms through which fecal pellets fell to minimize coprophagy. Food cups were placed at the end of feeding tunnels that were suspended from the sides of the cages to minimize contamination of urine and feces with food. Chow pellets were pulverized in a blender, and the powder was fed for 7 days prior to and 2 days following a test meal containing radiolabeled sterols. During the latter 2 days food intake was measured by weighing food cups at the beginning and end of each of four consecutive feeding periods whose measured length varied from 10.5 to 14.5 hr. At the end of the 2 days, body weights were 397 ± 16 g in controls and 321 ± 7 g in diabetic rats.

Test meal

Trace amounts of [$1,2\text{-}^3\text{H}$]cholesterol (3 μCi , 47 Ci/mmol, 64 pmol) and [$4\text{-}^{14}\text{C}$]sitosterol (0.1 μCi , 58 Ci/mol, 1.7 nmol, both from Amersham) in benzene were added to 5 g of powdered chow in a glass beaker for each control rat. Twice as much of each labeled sterol was added to 10 g of powdered chow for each diabetic rat. The test meals were placed in a vacuum for 24 hr to remove the benzene.

The test meal was presented at the start of the dark period after a 12-hr fast, and was nearly all consumed in about 3 hr by the diabetic rats and in 10 hr by the controls. Since the controls would normally have consumed 10–15 g of chow in this time (3), it is evident that their feeding behavior was somewhat disturbed by the procedure. Diabetic rats have voracious appetites and their feeding is less sensitive to a change in their surroundings. The beakers were reweighed to determine the exact amount of the test meal and radiolabeled sterols consumed.

Fecal neutral sterols

Feces were collected in four consecutive pools for 50 hr after the start of the test meal. Each pool was dried in a vacuum to constant weight, pulverized, and added to 30% KOH in methanol (3 ml/g feces). Each sample was then divided exactly in half. An internal recovery standard of [$4\text{-}^{14}\text{C}$]cholesterol (59.4 Ci/mol, New England Nuclear) was added to only one half. Each half was saponified at 70°C for 3 hr, diluted 50% with water, and extracted three times with hexane. The hexane extract was dried in a vacuum and radioactivity in the residue was measured by scintillation counting in 20 ml of OCS (Fisher). ^3H dpm and ^{14}C dpm per vial were determined by correcting for counting efficiency with the external standard channel ratio method. The ^3H dpm/ ^{14}C dpm ratio in each half and the ^{14}C dpm added as recovery standard were used to calculate ^3H dpm and ^{14}C dpm in each fecal pool (see Appendix 1).

Urinary sterols

Urine was collected in four consecutive pools for 50 hr. Each pool was dried in scintillation vials in a vacuum. Radioactivity was measured after shaking with 20 ml of Scintiverse (Fisher) until the residue was dispersed.

Neutral sterols in intestinal contents

At 50 hr after the start of the test meal, a blood sample was taken by heart puncture, and then the rats were killed with ether. The cecum plus large intestine including contents and the small intestinal contents were each weighed, saponified, and extracted as described above for feces except that drying of the contents was omitted.

Neutral sterols in tissues

The liver and cleaned small intestine were separately treated as described above for intestinal contents. Remaining tissues, termed periphery, were treated similarly except that only 200 ml of the solution of tissue in methanolic KOH was extracted, and dilution of this solution with water was omitted. (Water led to a persis-

tent soapy emulsion between the top and bottom phases during hexane extraction, especially in the controls with their higher fat content.) The hexane extract was backwashed with water to remove colored material. These changes did not decrease recovery of label.

Stability of ^3H in [^3H]cholesterol

[1,2- ^3H]Cholesterol (2.21 μCi) and [4- ^{14}C]cholesterol (0.41 μCi) were added to 5 g of powdered chow and fed to each of five normal rats. Neutral sterols were subsequently extracted from each of four consecutive 12-hr fecal pools and from the body of each rat. The techniques were the same as above except that no recovery standard was used and only the $^3\text{H}/^{14}\text{C}$ ratio was measured.

The $^3\text{H}/^{14}\text{C}$ ratio in the stock solution of [^3H]- and [^{14}C]cholesterol was 5.46. After the solution was added to chow, saponified, and extracted with hexane, the ratio declined 5% to 5.20. The ratio declined 9% to 4.735 ± 0.049 in fecal extracts and did not change with time of excretion. The ratio in the corporeal extract was 4.735 ± 0.024 , thus there was no further decline after absorption. The data were corrected for this loss of ^3H by multiplying the ^3H dpm in neutral sterol extracts by $5.46/4.73$ or 1.15.

Constituents of plasma

Radioactivity in 200 μl of plasma at 50 hr after the test meal was measured after shaking for 2 hr in 10 ml of OCS plus 1 ml of NCS. Radioactivity in the total plasma volume was estimated by assuming 3.5 ml of plasma/100 g body weight (14). Glucose, cholesterol, and triacylglycerol were measured with enzymatic assays (1).

Statistics

Data are presented as means \pm standard error of the mean. The significance of difference between means was determined with the unpaired Student's *t*-test. Correlations are expressed as the Pearson correlation coefficient (*r*).

RESULTS

Food intake

The usual diurnal rhythm of food intake in controls was maintained in the diabetic rats (Fig. 1). However, diabetic rats ate more food than controls, especially during periods of peak consumption. Average daily food intake increased 2.03-fold from 22.5 ± 1.1 g in controls to 45.7 ± 1.6 g in diabetic rats.

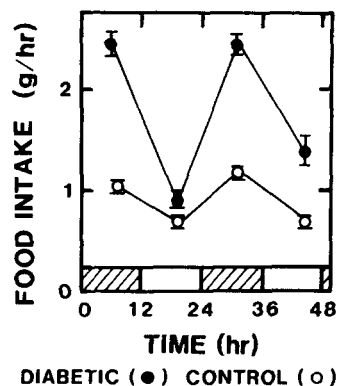


Fig. 1 Effect of diabetes on food intake. Food intake was measured as described in Materials and Methods for 2 days following the test meal during successive dark (hatched horizontal bars) and light (clear horizontal bars) periods.

Dietary sterols

The chow contained 0.24 ± 0.01 mg cholesterol and 0.35 ± 0.01 mg sitosterol per g, providing a mean daily cholesterol intake in the 2 days after the test meal of 11.0 ± 0.4 mg for diabetic rats and 5.4 ± 0.3 mg for control rats. Cholesterol intake in the test meal alone was 2.367 ± 0.009 mg for diabetic rats and 1.010 ± 0.049 mg for controls. Thus, diabetic rats consumed 2.34-fold more cholesterol in the test meal than control rats.

Fecal neutral sterols

After consuming [^3H]cholesterol and [^{14}C]sitosterol in a test meal, excretion of ^{14}C -labeled neutral sterols was maximal in the 0 to 14.5 hr pool for diabetics and in the 14.5 to 25.0 hr pool for controls (Fig. 2C). The later appearance of ^{14}C in feces of controls was most likely due to the slower consumption of the test meal by these rats. Excretion of ^3H (Fig. 2A) was delayed relative to that of ^{14}C in both groups.

The cumulative excretion of ^{14}C -labeled neutral sterol in feces leveled off at 92% in diabetics and 90% in controls by 50 hr (Fig. 2D). At 50 hr the large intestine and its contents contained only 1% and the contents of the small intestine less than 0.2% of the radioactivity consumed (Table 1). The cumulative excretion of ^3H -labeled neutral sterol in feces was much less than that of ^{14}C and was decreased by diabetes (Fig. 2B).

^3H excretion lagged behind ^{14}C excretion, so the $^3\text{H}/^{14}\text{C}$ ratio increased exponentially with time and even exceeded 1.0 in gut contents at 50 hr (Fig. 2E). The ratio of cumulative ^3H /cumulative ^{14}C increased linearly with time for the first 37 hr, and may have been leveling off in the last 13 hr (Fig. 2F).

Cholesterol absorption

Cholesterol absorption can be estimated from the

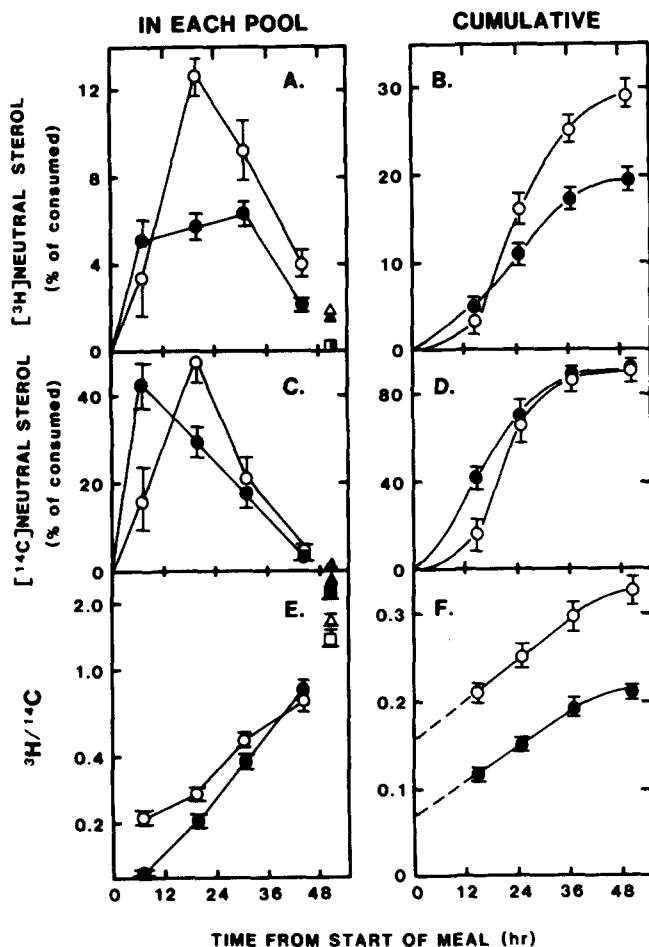


Fig. 2 Effect of diabetes on excretion of ^3H -labeled and ^{14}C -labeled neutral sterols. Data for ^3H and ^{14}C in each of four fecal pools excreted during 50 hr after the test meal containing [^3H]cholesterol and [^{14}C]sitosterol are shown on the left, panels A, C, and E. Data for cumulative radioactivity are shown on the right, panels B, D, and F. Data for $^3\text{H}/^{14}\text{C}$ were normalized for the dose of each radiolabeled sterol consumed in the test meal by each rat. Closed symbols represent data for diabetics, open symbols for controls; circles are for feces, squares for large intestine plus contents, and triangles for small intestinal contents. Data for $^3\text{H}/^{14}\text{C}$ in panel E are on a log scale; all other scales are linear.

$^3\text{H}/^{14}\text{C}$ ratio in fecal neutral sterols if absorption of [^{14}C]sitosterol is negligible. [^3H]Cholesterol absorption estimated from $^3\text{H}/^{14}\text{C}$ at time zero (from extrapolation of cumulative ^3H /cumulative ^{14}C in Fig. 2F) was 93% in diabetics and 84% in controls (Table 2). Estimates from pools accumulated for 50 hr were lower by 16–18% due to the lag in ^3H excretion. Estimates from the isotope ratio in the 50 hr pool were 1.4–2.8% lower than those from absolute ^3H radioactivity. This is the error due to neglecting sitosterol absorption in the fecal isotope ratio method (Appendix 2).

Distribution of absorbed cholesterol

The amounts of ^3H -labeled neutral sterols from the test meal in the small intestine, periphery, and plasma

were increased 3- to 4-fold, and that in the liver 1.8-fold in diabetic rats (Table 3). Since absorbed [^3H]cholesterol was 2.7-fold higher in the diabetic rats, ^3H -labeled neutral sterol remaining in the small intestine, plasma, and periphery at 50 hr was enriched and that in the liver was depleted compared to that absorbed.

Not more than 1% of either label was recovered in urine of control rats and even less in urine of diabetic rats despite a 16-fold increase in urinary volumes of diabetic rats (Table 1). The recovery of ^{14}C in urine at least equaled that of ^3H in both groups; however, these data are confounded by the possibility that the urine may have been contaminated by bits of food and feces.

Recovery of radiolabeled sterol

Total recovery of ^{14}C from feces, gut contents, tissues, plasma sample, and urine was 97–98% of the [^{14}C]sitosterol consumed (Table 1). Total recovery of ^3H was much less at 63–65%. Diabetes did not affect total recovery of either label.

Plasma constituents

In control rats and diabetic rats, respectively, plasma glucose was 156 ± 3 and 553 ± 32 mg/dl (8.7 ± 0.2 and 31 ± 2 mM), cholesterol was 55 ± 1 and 96 ± 8 mg/dl, and triacylglycerol was 178 ± 14 and 966 ± 152 mg/dl.

Cholesterol concentration was directly related to the ^3H concentration in plasma 50 hr after the test meal (Fig. 3, $r = 0.97$, $P < 0.001$). Furthermore, the degree of hypercholesterolemia in diabetic rats was directly related to the portion of total plasma cholesterol coming from the diet (Fig. 4, $r = 0.75$, $P < 0.01$).

DISCUSSION

Cholesterol absorption in control and diabetic rats

Our value of $68.6 \pm 1.6\%$ for fractional cholesterol absorption calculated from recovery of ^3H -labeled neutral sterols excreted in 50 hr by normal male Wistar rats eating chow agrees with that of $68.3 \pm 0.4\%$ reported by Mathe and Chevallier (15) from long-term feeding studies. In short-term studies, where rats received a semi-purified test meal by gastric intubation, values ranged from 41 to 70% (10, 16, 17). The differences in absorption may be due to dietary components or to the mode of delivery of the test meal.

Fractional cholesterol absorption was increased 1.14-fold ($P < 0.001$) from 69% in control rats to 78% in diabetic rats (Table 2). Thus, although diabetic rats consumed twice the normal amount of chow, they still absorbed a larger fraction of cholesterol. The total

TABLE 1. Percent of dietary sterol recovered 50 hours after start of meal

Sample	³ H]Cholesterol			¹⁴ C]Sitosterol		
	Diabetic	Control	P <	Diabetic	Control	P <
	%			%		
Feces	20 ± 1	29 ± 2	0.001	92 ± 3	90 ± 4	ns ^c
Large intestine plus contents	1.8 ± 0.2	1.8 ± 0.2	ns	0.9 ± 0.2	1.2 ± 0.2	ns
Small intestine, contents	0.20 ± 0.02	0.24 ± 0.05	ns	0.09 ± 0.01	0.20 ± 0.05	0.1
Sum excreted	22 ± 1	31 ± 2	0.001	93 ± 3	92 ± 4	ns
Small intestine	7.1 ± 0.5	4.3 ± 0.8	0.01	0.7 ± 0.1	1.9 ± 0.2	ns
Liver	9 ± 1	11 ± 2	ns	1.0 ± 0.3	1.4 ± 0.2	ns
Periphery	24 ± 1	17 ± 2	0.005	2.2 ± 0.2	2.1 ± 0.2	ns
Sum in body	40 ± 2	32 ± 3	0.1	3.9 ± 0.3	4.5 ± 0.4	0.02
Urine	0.21 ± 0.02	0.37 ± 0.02	0.001	0.25 ± 0.05	0.88 ± 0.15	0.001
Plasma sample ^a	1.6 ± 0.3	0.4 ± 0.4	na	0.21 ± 0.04	0.06 ± 0.03	na
Total recovered	63 ± 2	65 ± 4	ns	98 ± 3	97 ± 4	ns
Total plasma ^b	3.4 ± 0.4	2.1 ± 0.2	0.05	0.42 ± 0.05	0.35 ± 0.05	ns

^aThe amount of blood removed from each rat for the plasma sample was variable and happened to be greater for diabetics than for controls, hence the statistical comparison of means is not applicable (na).

^bRadioactivity in total plasma was calculated from the dpm/200 μl of plasma sample, assuming 3.5 ml plasma/100 g body weight. In actuality, plasma was distributed in tissues and in the plasma sample; thus values for total plasma radioactivity were not included in either sum in body or total recovered. To do so would result in counting some of the radioactivity twice.

^cNot significant.

effect of a 103% increase in food intake and a 14% increase in fractional cholesterol absorption was a 132% or 2.3-fold increase in absorbed dietary cholesterol.

Many of the perturbations of cholesterol dynamics including changes in plasma lipids, bile acid pools, and hydroxymethylglutaryl-CoA reductase activity in chronically diabetic rats eating chow ad libitum are due to hyperphagia rather than to direct effects of insulin deficiency per se, since these parameters are normalized when hyperphagia is prevented (2, 3). However, it is presently unknown whether this applies to increased fractional cholesterol absorption. Also unknown is whether the increase is mediated by change in bile acid composition, absorptive surface area, intestinal transit

time, or other factors. An intriguing possibility is that it is the result of stimulation of cholesterol esterase activity by increased taurocholate concentration. The cholate pool is enlarged in hyperphagic diabetic rats (18), and taurocholate stimulates activity of cholesterol esterase, an enzyme required for cholesterol absorption (19).

Measurement of cholesterol absorption

We compared two methods of correcting for efficiency of extraction of fecal ³H-labeled neutral sterols: a conventional method in which [¹⁴C]sitosterol is added to the diet with [³H]cholesterol (10), and a novel method in which [¹⁴C]cholesterol is added to half of each fecal sample already containing ³H and ¹⁴C. The former method underestimates cholesterol absorption, in this case by less than 3%, due to absorption of

TABLE 2. Estimates of dietary sterol absorption

	Time hr	Dietary Sterol Absorbed	
		Diabetic	Control
		%	
³ H]Cholesterol			
From cumulative ³ H/ ¹⁴ C	0	93.2 ^a	84.3 ^a
In feces	14.5	88.2 ± 0.8	78.8 ± 1.0
	25.0	85.0 ± 0.9	74.9 ± 1.4
	37.0	80.6 ± 0.9	70.3 ± 1.7
	50.5	78.9 ± 1.0	67.4 ± 1.6
In feces and gut	50.5	77.1 ± 1.0	65.8 ± 1.7
From cumulative ³ H			
In feces	50.5	80.5 ± 1.2	70.6 ± 1.5
In feces and gut	50.5	78.5 ± 1.2	68.6 ± 1.6
¹⁴ C]Sitosterol			
From cumulative ¹⁴ C			
In feces	50.5	7.6 ± 2.6	9.5 ± 4.2
In feces and gut	50.5	6.6 ± 2.6	8.2 ± 4.2

^a[³H]Cholesterol absorption at time zero was estimated as the intercept of the linear least squares best fit to data for the first 37 hr.

TABLE 3. Distribution of dietary [³H]cholesterol in the body

	³ H-labeled Neutral Sterol			
	Diabetic	Control	P <	D/C
	μg			
Small intestine	168 ± 12	44 ± 8	0.001	3.8
Liver	202 ± 29	112 ± 18	0.05	1.8
Periphery	565 ± 28	172 ± 19	0.001	3.3
Total plasma	80 ± 1	21 ± 1	0.001	3.8
Total tissue	973 ± 40	332 ± 35	0.001	2.9
Unrecovered	880 ± 41	357 ± 38	0.001	2.5

The microgram value of [³H]cholesterol in each tissue was calculated as the fraction of [³H]cholesterol recovered at 50 hr in the tissue (Table 1) times the micrograms of [³H]cholesterol plus unlabeled cholesterol consumed in the test meal, 2367 for diabetics and 1010 for controls. Values for small intestine, liver, and periphery were not corrected for residual plasma in the tissue. Total tissue is the sum of small intestine, liver, periphery, and plasma sample (not total plasma).

[¹⁴C]sitosterol. Theoretically, the error increases with higher sitosterol absorption and with lower cholesterol absorption (see Appendix 2). It is evident that if sitosterol absorption is unknown, the error is also unknown.

There was a 5% loss of ³H from [³H]cholesterol with saponification and extraction from chow compared to [¹⁴C]cholesterol, and an additional 9% loss after passage through the gut. The initial loss can be accounted for by ³H-labeled polar impurities in the [³H]cholesterol stock seen in thin-layer chromatograms but not extracted in hexane. The loss during passage through the gut may have been due to removal of ³H from the sterol molecule (11), or possibly to metabolic conversion of ³H-labeled nonpolar impurities to polar compounds. Correcting the data for ³H-labeled neutral sterols in feces and tissues for this loss of ³H raised ³H recoveries and lowered apparent absorption.

The ³H/¹⁴C ratio in fecal neutral sterols increased with time after the test meal due to lagging ³H excretion: 16–18% of ³H was excreted later than ¹⁴C in 50 hr. The cause of the delay, noted previously in rats (10), rabbits (20), and humans (21), is unknown. There are at least two reasonable models: in model 1, cholesterol enters mucosal cells and returns directly to the lumen; in model 2, cholesterol enters the cells, is secreted in chylomicrons in lymph and then into blood, and finally reenters the intestinal lumen either in bile after passage through the liver or by transudation from plasma into and through intestinal mucosal cells.

Zilversmit (22) predicted that model 1 would produce an increasing “instantaneous cholesterol/sitosterol ratio” in successive fecal samples collected after the first 6 hr (curve E, Fig. 4 of ref. 22) which is in agreement with our data (Fig. 2E). However, he also predicted that the cumulative cholesterol/cumulative sitosterol ratio would be constant up to 80 hr after an initial

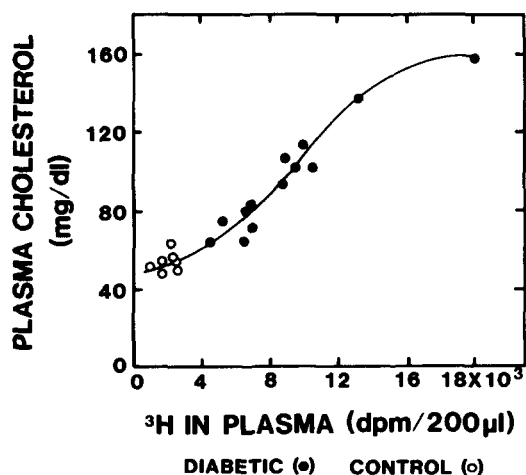
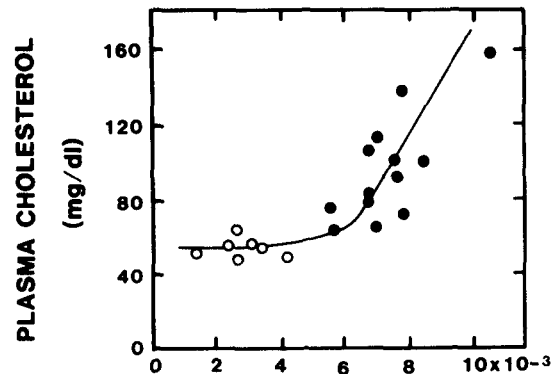


Fig. 3 Relation between ³H in plasma and plasma cholesterol level. Data are from blood samples taken 50 hr after the start of the test meal containing [³H]cholesterol.



DIETARY CHOLESTEROL IN PLASMA
TOTAL CHOLESTEROL IN PLASMA

DIABETIC (●) CONTROL (○)

Fig. 4 Relation between the fraction of total plasma cholesterol coming from the diet and plasma cholesterol level. The mass of [³H]cholesterol in plasma was calculated from the ³H dpm in plasma (Fig. 3) and the specific activity of [³H]cholesterol in the test meal, and was then divided by the total mass in plasma to give the fraction derived from the test meal.

decrease during the first 12 hr or so (curve F, Fig. 4 of ref. 22), which is in marked contrast to our data showing an ascending ratio for up to 50 hr (Fig. 2F). Consequently, it is evident that either the prediction is incorrect or that model 1 does not apply to sterol excretion in rats.

Nevertheless, there are data supporting model 1 in humans and rats. In people with abetalipoproteinemia, very little dietary fat or cholesterol reaches the blood, but excretion of dietary cholesterol relative to sitosterol is still delayed (21). In rats injected intravenously with radiolabeled cholesterol, only 3–4% of the label is recovered in feces excreted in the next 4 days (D. B. Zilversmit, personal communication). Therefore, we have provisionally assumed that model 1 is correct. If model 2 is correct, we have underestimated absorption by about 17%.

Unrecovered radioactivity—bile acids

Total recovery in the neutral sterol fraction of ¹⁴C from [¹⁴C]sitosterol was 97–98% and of ³H from [³H]cholesterol was 63–65% (Table 1). We postulate that unrecovered radioactive compounds were polar sterols² not extractable in hexane at high pH, e.g., bile acids. If so, then at most 33–34% of the absorbed [¹⁴C]sitosterol

²Both ³H cholesterol and ¹⁴C sitosterol are extensively metabolized during transit through the gut to less polar compounds. Only 15% of the ³H in a hexane extract of feces from one diabetic rat comigrated with [¹⁴C]cholesterol during high pressure liquid chromatography (unpublished data of N. L. Y. and D. R. L.).

and 47–52% of the absorbed [³H]cholesterol were converted to bile acids after being taken up by the liver. This would amount to an average daily synthesis of bile acids from dietary cholesterol of at most 4 mg by diabetic animals and 2 mg by control animals, and from sitosterol of at most 0.4 mg by diabetic animals and 0.2 mg by controls. Both diabetic and control rats excrete 10–11 mg of bile acids daily (3); thus diabetic rats synthesize at most 40% of bile acids from dietary cholesterol compared to at most 20% by controls.

Distribution of absorbed cholesterol

The amount of ³H-labeled neutral sterol recovered from all tissues was increased 2.9-fold in diabetic rats, with 3- to 4-fold increases from the small intestine, periphery, and plasma, and a 1.8-fold increase from liver (Table 3). If unrecovered ³H is assumed to be ³H-labeled bile acids formed in the liver, then total [³H]-cholesterol taken up by the liver increased 2.3-fold from 469 μg in controls to 1082 μg in diabetic animals; in both groups, about 60% of the absorbed dietary cholesterol was taken up by the liver, and 80% of this was converted to bile acids. Hepatic uptake is underestimated here due to hepatic secretion of [³H]cholesterol in lipoproteins and in bile.

It is remarkable that the increase in absorbed dietary cholesterol does not lead to an increase in the total tissue cholesterol pool; this pool remains constant for at least the first 4 weeks of diabetes (3). Evidently, increased bile acid synthesis and decreased cholesterol synthesis compensate for the increased dietary cholesterol intake (3). Using previous data for whole body cholesterol synthesis (3) we calculate that absorbed dietary cholesterol increases from about 20% of internal cholesterol influx in controls to at least 50% in diabetic animals. (Internal influx is the sum of absorbed dietary and newly synthesized cholesterol.)

Plasma cholesterol

Although the total tissue cholesterol pool is not increased, plasma cholesterol level is doubled in hyperphagic diabetic rats (1–3). Thus, the compensatory responses noted above do not maintain a normal plasma cholesterol level. Hypercholesterolemia is clearly a result of hyperphagia, since plasma cholesterol is only slightly elevated in diabetic rats restricted to a normal food intake (2). It appears that this effect of hyperphagia is mediated by increased chylomicron secretion secondary to increases in both absorbed dietary cholesterol and cholesterol synthesis by the small intestine (1–3). With these changes a larger fraction of the internal cholesterol input must pass through the blood.

Our findings, that the portion of total plasma cholesterol derived from the test meal is increased 2.6-fold (*P*

< 0.001) in hyperphagic diabetic rats and that the diet-derived portion is highest in the most hypercholesterolemic rats (Fig. 4), offer additional support for the possibility that dietary cholesterol contributes significantly to the hypercholesterolemia. Decreased clearance of plasma lipoproteins may also contribute to the increase in both ³H and cholesterol mass in plasma (Fig. 3). However, it is unlikely that decreased clearance could account for the increased proportion of dietary cholesterol in plasma unless the clearance of lipoproteins carrying dietary cholesterol was preferentially affected.

Significance

The finding that dietary cholesterol contributes significantly to hypercholesterolemia in diabetic rats may be of importance to the management of diabetes in humans. Dietary cholesterol is likely to be a greater risk factor for atherosclerosis in people with diabetes if poor diabetic control leads to hyperphagia and increased fractional cholesterol absorption in our species as it does in rats. At the very least, studies of the effect of diabetes on these parameters in humans are called for. ■

APPENDIX 1

Use of [¹⁴C]cholesterol as a recovery standard in a sample containing ³H-labeled and ¹⁴C-labeled neutral sterols

To determine ³H dpm and ¹⁴C dpm in a sample, let

$$\begin{aligned} H &= \text{³H dpm in sample} \\ C &= \text{¹⁴C dpm in sample} \\ RS &= \text{dpm [¹⁴C]cholesterol added to half sample as recovery standard} \\ R1 &= C/H \text{ measured in extract of half sample without RS} \\ R2 &= C/H \text{ measured in extract of half sample with RS} \end{aligned}$$

then

$$\begin{aligned} R2 &= (0.5 C + RS)/0.5 H \\ &= C/H + RS/0.5 H \\ &= R1 + RS/0.5 H \\ H &= 2 RS/(R2 - R1) \\ C &= H \times R1. \end{aligned}$$

In practice, amounts of isotopes added to the test meal and added to samples as RS should be chosen so that R1 and R2 are less than 0.3 to minimize spill of ¹⁴C into the ³H scintillation counting channel, and so that R2 is at least twice R1 to give a reliable difference between R1 and R2. This was achieved by, first, starting with an R1 of 0.03 in the test meal, which gave R1 in feces of about 0.15 and in tissues of about 0.004, and, second, by using RS of 0.08–0.18 × H for fecal samples and 0.002–0.2 × H for tissue samples. Also, small errors in ³H dpm and ¹⁴C dpm result in large errors in R and very large errors in (R2 - R1). Consequently, extreme care must be taken to obtain accurate quench corrections and low counting error.

APPENDIX 2

Error in estimating cholesterol absorption by the fecal isotope ratio method due to sitosterol absorption

Let

DC = [³H]cholesterol consumed in diet = 1
DS = [¹⁴C]sitosterol consumed in diet = 1
FC = ³H-labeled neutral sterol in feces
FS = ¹⁴C-labeled neutral sterol in feces
AC = absorbed [³H]cholesterol
AS = absorbed [¹⁴C]sitosterol
AC* = absorbed [³H]cholesterol estimated by the fecal isotope ratio method

then

AC = 1 - FC/DC
AS = 1 - FS/DS
AC* = 1 - FC/FS
AC = 1 - (FC/FS)(FS/DS)
AC - AC* = error in the fecal isotope ratio method
= (FC/FS) - [(FC/FS)(FS/DS)]
= (FC/FS)[1 - (FS/DS)]
= AS(FC/FS).

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