

# Cholesterol absorption efficiency declines at moderate dietary doses in normal human subjects

Richard E. Ostlund, Jr.,<sup>1,\*</sup> Matthew S. Bosner,<sup>†</sup> and William F. Stenson<sup>§</sup>

Division of Endocrinology, Diabetes, and Metabolism,<sup>\*</sup> Division of Cardiology,<sup>†</sup> and Division of Gastroenterology,<sup>§</sup> Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110

**Abstract** While unphysiologically large cholesterol doses are known to reduce percent cholesterol absorption, smaller amounts are reported to have no effect in human subjects. To determine the dose–response relation between dietary cholesterol consumed and the efficiency of intestinal cholesterol absorption, we fed 18 normal subjects two test meals containing different amounts of natural cholesterol. In each test pentadeuterated cholesterol tracer was given orally, hexadeuterated cholesterol tracer was given intravenously, and the tracer ratio was measured in plasma 4 days later by gas chromatography/negative ion mass spectrometry. Baseline cholesterol absorption in the presence of 26 mg cholesterol tracer was  $40.7 \pm 2.3\%$ . This decreased by 4.9 percentage points ( $P = 0.05$ ) when a total of 188 mg cholesterol was included in the meal and by 15.6 percentage points ( $P = 0.006$ ) when 421 mg cholesterol was given, showing that the efficiency of cholesterol absorption declines appreciably even with modest increases in cholesterol dose. Considerable variation was noted in the response of different subjects and, on the higher cholesterol dose, dietary cholesterol absorption varied 5-fold from 40 mg to 212 mg. Fasting plasma insulin was correlated with the ability to absorb higher cholesterol doses without loss of efficiency ( $r_s = 0.700$ ,  $P = 0.036$ ). Percent cholesterol absorption in a single meal is significantly influenced by the amount of cholesterol in that meal, suggesting that acute dietary factors influencing cholesterol absorption need further study.—Ostlund, R. E., Jr., M. S. Bosner, and W. F. Stenson. Cholesterol absorption efficiency declines at moderate dietary doses in normal human subjects. *J. Lipid Res.* 1999. 40: 1453–1458.

**Supplementary key words** cholesterol • absorption • diet • spectrum analysis, mass • deuterium • insulin

Dietary cholesterol absorption in human subjects is usually assessed indirectly by measuring the response of plasma cholesterol levels to dietary changes. These studies are difficult to interpret because plasma cholesterol levels are influenced by factors other than the efficiency of absorption and are confounded by biological variability. Direct measurement of cholesterol absorption has been difficult because of the large size and slow turnover of body cholesterol pools and the need to use radioactive isotopes adminis-

tered on multiple occasions. Recently we and others have developed methods to re-explore cholesterol absorption and metabolism using tracers labeled with the stable isotopes carbon-13 or deuterium in order to achieve a molecular weight 5 or 6 mass units above natural cholesterol (1, 2). Using these tracers it is possible to assess cholesterol absorption directly without radioactive exposure by measuring tracer concentrations in stool or plasma cholesterol. The large tracer dilutions in plasma cholesterol are more difficult to detect and analysis has been improved substantially by development of methods using highly sensitive negative ion mass spectrometry (3). These techniques now make possible re-evaluation and extension of our knowledge of cholesterol absorption. In contrast to the nearly complete uptake of most other nutrients, only about half of dietary cholesterol is normally absorbed with great variation existing between subjects. As a consequence, the efficiency of intestinal cholesterol absorption is an important variable in studies of the effects of diet on serum lipid levels (4, 5).

One of the most fundamental questions about the absorption of a dietary substance concerns the dose/response relation between the amount fed and the amount absorbed. Although earlier studies have addressed this question with respect to cholesterol absorption in human subjects, statistically meaningful data over physiological cholesterol doses is sparse. In order to eliminate potential adaptive changes with chronic cholesterol feeding, we have designed a study in which cholesterol absorption is measured using single meals spanning the usual range of cholesterol intake and subjects are studied twice at different cholesterol doses to make possible paired comparisons. Percent cholesterol absorption was determined by the method of Zilversmit and Hughes (6) in which one

Abbreviations: SD, standard deviation; SEM, standard error of the mean;  $r$ , Pearson correlation coefficient;  $r_s$ , Spearman rank correlation coefficient; BMI, body mass index,  $\text{kg}/\text{m}^2$ ; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol;  $m/z$ , mass to charge ratio.

<sup>1</sup> To whom correspondence should be addressed.

cholesterol tracer bolus is given orally, another intravenously, and the ratio of tracers in plasma is determined after several days. This technique has been validated for humans using radioactive and stable isotopic cholesterol tracers (1, 7). In the current work we have used pentadeuterated and hexadeuterated cholesterol as the oral and intravenous tracers, respectively, and three different oral doses of cholesterol. Our hypothesis was that the efficiency of cholesterol absorption during a single test meal would decline with increasing dose and that metabolic variables such as plasma glucose, insulin, and body mass index would be associated with the acute response to dietary cholesterol.

## METHODS

### Subjects

Eighteen healthy adults (15 women and 3 men) without active medical or surgical illnesses participated. The mean age was  $39.8 \pm 8.6$  (SD) years and body mass index was  $24.2 \pm 4.5$  (SD)  $\text{kg}/\text{m}^2$ . All subjects participated in two cholesterol absorption tests and were divided into two groups. One group of 9 subjects underwent testing with tracer alone and tracer + low-dose natural cholesterol while another group of 9 subjects received tracer alone and tracer + high-dose natural cholesterol as described below. Subject characteristics were determined as described previously (8) and are presented in the tables. Informed consent was obtained in writing and the project was approved by the Washington University Human Studies Committee.

### Study design

Each subject received two cholesterol absorption tests performed 2 weeks apart. A time interval was included between the tests to allow plasma cholesterol tracer enrichments from the first study to decline and stabilize. Each cholesterol absorption test was conducted over a 7-day period during which subjects consumed a National Cholesterol Education Program Step I diet. Fasting insulin was determined by radioimmunoassay (9). Plasma lipids and lipoproteins were measured in the Washington University Lipid Research Clinic Core Laboratory (10). On day 3 the subjects reported fasting to the Washington University General Clinical Research Center and received an intravenous infusion of 15 mg hexadeuterated cholesterol in approximately 4 ml Intralipid over 5 min as described previously (1). Immediately after this the test breakfast was consumed. The amounts of tracer cholesterol infused and eaten were quantitated by weighing. Fasting plasma samples (0.5 ml) for isotope determination were drawn on day 3 just before the test and again on day 7.

The test breakfast consisted of an orange sherbet prepared by stirring 120 ml partially frozen orange slush (44 ml orange juice, 6 ml lemon juice, 28 g sucrose, and 67 ml water) with 26 g corn oil at room temperature. After consumption the cup containing the sherbet was washed twice with water to ensure complete transfer. Coffee or tea was given optionally. The meal contained 33 g carbohydrate, 0.2 g protein, 26 g fat, and 368 calories. For the control meal 26 mg pentadeuterated cholesterol tracer was thoroughly dissolved in the corn oil by end-over-end rotation overnight. For the low-dose cholesterol test meal 26 mg tracer + 162 mg natural cholesterol (188 mg total cholesterol) was present in the oil and for the high-dose meal 26 mg tracer + 395 mg natural cholesterol (421 mg total cholesterol) was used. The order of testing was randomized and half the subjects received tracer alone first while half were given tracer + natural cholesterol first.

### Tracer enrichments

Our methods for measuring cholesterol absorption by mass spectrometry of plasma cholesterol have been reported previously (1, 3, 8). [ $2,2,4,4,6,6\text{-}^2\text{H}_5$ ]cholesterol (pentadeuterated cholesterol or cholesterol- $\text{d}_5$ ) and [ $26,26,26,27,27,27\text{-}^2\text{H}_6$ ]cholesterol (hexadeuterated cholesterol or cholesterol- $\text{d}_6$ ) were purchased from Medical Isotopes, Pelham, NH. The solid tracers were heated at  $100^\circ\text{C}$  for 30 min, taken up in ethanol, and passed through a  $0.22\ \mu\text{m}$  filter. Aliquots of the intravenous tracer solution were cultured in thioglycollate broth and tested for pyrogenicity by the limulus test (QCL-1000, BioWhittaker, Walkersville, MD) after evaporation of the ethanol. Fasting plasma obtained before and 4 days after tracer administration was saponified and neutral lipids were extracted (11), derivatized to pentafluorobenzoyl esters, and analyzed by gas chromatography/negative ion mass spectrometry on an HP-5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA) operating in negative ion mode with 0.7 torr methane reagent gas and a source temperature of  $120^\circ\text{C}$ . Cholesterol-related ion peak areas were acquired at  $m/z$  581 (M+1), 585 (M+5), and 586 (M+6) where M is the molecular anion of cholesterol pentafluorobenzoate at  $m/z$  580. Natural cholesterol is the principal contributing component at  $m/z$  581, pentadeuterated cholesterol at  $m/z$  585, and hexadeuterated cholesterol at  $m/z$  586. The enrichment of cholesterol tracers in natural cholesterol on day 7 was determined by computing the area ratios  $m/z$  585/581 and  $m/z$  586/581 and subtracting the corresponding ratios found in the baseline sample. The mole ratio of pentadeuterated oral cholesterol tracer/hexadeuterated intravenous tracer in plasma cholesterol on day 7 was read from a standard curve relating tracer mole ratios to  $m/z$  585/586 area ratios. Percent cholesterol absorption was calculated from the following mole ratios (8):

$$\frac{(\text{cholesterol-}\text{d}_5/\text{cholesterol-}\text{d}_6)_{\text{plasma day 7}}}{(\text{cholesterol-}\text{d}_5/\text{cholesterol-}\text{d}_6)_{\text{given}}} \cdot 100$$

Dietary cholesterol absorption was calculated as the percent cholesterol absorption multiplied by the known total cholesterol (including tracer) in the test meal. Results are presented as mean  $\pm$  SEM and the significance of treatment effects was calculated by Student's paired *t*-test. A general linear statistical model was used to calculate the independent significance of cholesterol dose as a variable in predicting reduction in cholesterol absorption (SAS Institute, Cary, NC).

## RESULTS

Dietary cholesterol absorption was measured in two series of 9 subjects each in which cholesterol was given as 26 mg tracer alone and 26 mg tracer + either 162 mg or 395 mg natural cholesterol. With 26 mg tracer alone percent cholesterol absorption in all 18 subjects averaged  $40.7 \pm 2.3\%$ . When a total of 188 mg cholesterol was included in the meal percent cholesterol absorption was reduced by 4.9 percentage points (Table 1,  $P = 0.05$ ). This represents a 12.7% reduction from the initial value. When a total of 421 mg cholesterol was included in the meal percent cholesterol absorption was reduced by 15.6 percentage points or 36.3% of the original value (Table 2,  $P = 0.006$ ). The change of percent cholesterol absorption was significantly different in the low and high dose groups by analysis of variance ( $P = 0.036$ ). These averages, however, do not reveal the considerable heterogeneity observed in the re-

TABLE 1. Subjects undergoing low-dose cholesterol absorption test

Subject	Sex	Age	BMI	Total Chol	Total Trig	HDL Chol	LDL Chol	Insulin	Cholesterol Absorption	
									26 mg	188 mg
		yr	kg/m <sup>2</sup>		mg/dl			μU/ml	%	
1	F	33	17.9	160	43	48	102	2.7	38.5	37.1
2	F	41	29.9	173	62	47	115	5.0	42.2	31.4
3	M	35	24.2	202	79	44	138	3.7	42.7	34.8
4	F	38	30.3	208	208	34	144	7.2	41.2	34.0
5	F	38	20.3	180	66	50	125	4.0	44.6	42.6
6	F	35	21.3	131	55	52	74	6.6	32.1	33.5
7	F	47	31.7	157	92	35	109	4.0	15.3	10.1
8	F	63	16.4	187	110	45	127	4.0	44.3	49.4
9	M	40	23.4	158	137	27	107	4.0	46.2	30.2
Mean		41.3	23.9	173	94.7	42.4	116	4.6	38.6	33.7 <sup>a</sup>
SEM		3.0	1.9	8	17.2	2.8	7	0.5	3.2	3.6

A test meal containing either 26 mg pentadeuterated cholesterol or 26 mg pentadeuterated cholesterol + 162 mg natural cholesterol was fed and percent cholesterol absorption was calculated.

<sup>a</sup>  $P = 0.05$ .

sponse of different individuals. Table 1 shows that in the low-dose test 6 of the 9 subjects had a reduction in cholesterol absorption, but all changes were modest. With the high-dose test (Table 2) 8 of the 9 subjects had reductions in cholesterol absorption. In two individuals cholesterol absorption was reduced to 9.5 and 10%, very low levels not encountered in another study of 94 normal subjects where the range of observed values was 29–80% while feeding 64 mg cholesterol (8). At the same dose of dietary cholesterol, one subject had little change in percent cholesterol absorption and two had reductions of only 4.4 and 7.5 percentage points. Thus, while increasing dietary cholesterol resulted in substantial overall reductions in the efficiency of cholesterol transport, this effect was attenuated in some individuals and accentuated in others. The order in which testing was performed (tracer only first or tracer plus natural cholesterol first) was not related to outcome variables ( $P = 0.87$  for an independent effect).

Figure 1 shows the milligram amount of dietary chole-

sterol absorbed from test meals as a function of dose. Absorption of the dietary cholesterol fed varied from  $10.6 \pm 0.8$  mg with 26 mg tracer alone to  $114.9 \pm 20.7$  mg when 421 mg cholesterol was fed. At the latter dose the standard deviation of the amount absorbed was 54% of the mean value, demonstrating large between-individual differences in dietary cholesterol absorption.

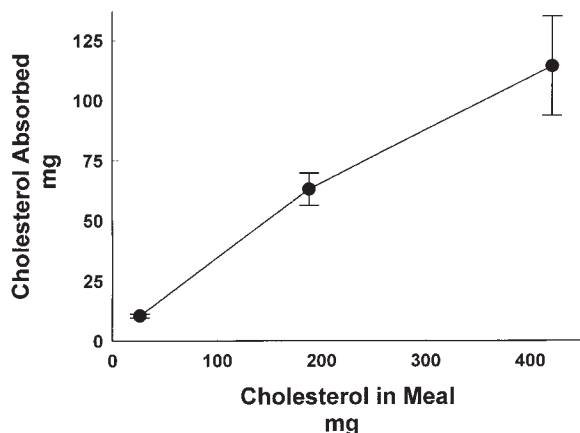
We studied the relation of measured covariates to the dietary responsiveness of percent cholesterol absorption during the high-dose test using Spearman rank correlations (Table 3). Dietary responsiveness was calculated as percent cholesterol absorption in the presence of 421 mg cholesterol minus that in the presence of 26 mg tracer alone so that increasingly negative values indicate greater dietary responsiveness. A statistically significant positive correlation was found between fasting plasma insulin concentration and dietary responsiveness ( $r_s = 0.700$ ,  $P = 0.036$ ). No significant relationships were noted between dietary cholesterol responsiveness and body mass index, age, gender, or lipoproteins.

TABLE 2. Subjects undergoing high-dose cholesterol absorption test

Subject	Sex	Age	BMI	Total Chol	Total Trig	HDL Chol	LDL Chol	Insulin	Cholesterol Absorption	
									26 mg	421 mg
		yr	kg/m <sup>2</sup>		mg/dl			μU/ml	%	
1	F	41	24.0	134	44	42	88	8.2	57.9	50.4
2	F	35	23.5	123	43	41	70	3.8	39.3	9.5
3	F	39	21.1	151	68	43	94	5.1	50.6	46.2
4	M	29	22.8	168	181	30	105	9.2	38.3	10.0
5	F	55	22.2	237	113	69	150	5.6	35.6	24.8
6	F	40	23.4	177	95	39	123	5.0	29.9	18.3
7	F	43	32.1	172	57	76	95	4.5	44.8	25.2
8	F	37	22.8	146	30	70	73	3.7	54.5	22.7
9	F	27	27.4	168	68	60	98	15.5	34.9	38.4
Mean		38.4	24.4	164	77.7	52.2	99.6	6.7	42.9	27.3 <sup>a</sup>
SEM		2.8	1.1	11	15.6	5.5	8.2	1.3	3.2	4.9

A test meal containing either 26 mg pentadeuterated cholesterol or 26 mg pentadeuterated cholesterol + 395 mg natural cholesterol was fed and percent cholesterol absorption was calculated.

<sup>a</sup>  $P = 0.006$ .



**Fig. 1.** Absorption of dietary cholesterol during different test meals. Error bars depict mean  $\pm$  SEM for 18 subjects (26 mg meal cholesterol) or for 9 subjects (remaining points).

## DISCUSSION

The principal new finding of this work is that in normal subjects intestinal efficiency for absorption of a single dose of dietary cholesterol is reduced as the amount of cholesterol is increased within a physiological range of 26–421 mg. For comparison, the cholesterol content of an egg is estimated at 213 mg (12). These acute dose/response measurements for dietary cholesterol absorption versus intake reveal that percent absorption of dietary cholesterol declines as cholesterol in the test meal is increased (Tables 1 and 2). These results are convincing evidence that acute limitation of cholesterol absorption occurs which can be measured by a dual isotope procedure.

A few previous studies have measured cholesterol absorption as a function of acute dietary cholesterol load in human subjects, but each has certain limitations. After feeding a single test meal containing radiolabeled cholesterol plus variable amounts of natural cholesterol and measuring fecal recovery of the tracer, Borgstrom (13) found that percent cholesterol absorption declined mono-

tonically over 4 dose levels from 40% when 150 mg was given to 23% when 1910 mg was used. However, this rather large difference was not statistically significant, probably because different subjects were used for each dose level and between-subject variation was quite large just as we found it to be here. It was concluded that the efficiency of cholesterol absorption was independent of the dose over wide limits. In this work we have reduced variation in baseline cholesterol absorption by using paired tests on the same individuals with a resulting increase in statistical power. Our results confirm the trends of Borgstrom's original data. Samuel et al. (7) performed cholesterol absorption studies by the Zilversmit dual isotope technique and reported that [ $^{14}\text{C}$ ]cholesterol given in a liquid formula at breakfast was absorbed equally well whether or not 750 mg cholesterol in three eggs was given at lunch the same day. However, the tracer and unlabeled cholesterol were not given at the exactly the same time and the study was limited to 5 subjects. Two reports have measured percent cholesterol absorption at single time points during chronic diet studies. McNamara and colleagues (14) found that percent absorption of oral [ $^{14}\text{C}$ ]cholesterol tracer given in standard formula was significantly reduced from 61% to 55% when chronic dietary cholesterol intake was increased from 240 mg/day to 840 mg/day. It is likely that these data reflect chronic adaptation rather than an acute response as the cholesterol tracer was apparently given in the same test meal during both dietary periods. Sehayek et al. (15) found that chronically increased dietary cholesterol did not reduce percent cholesterol absorption measured at single time points, but the oral cholesterol tracer was apparently given in the same way with milk during both diets. These studies are consistent with our earlier work (8) in showing that chronic cholesterol feeding has little or no relation to percent cholesterol absorption. However, the current data reveal that the cholesterol content of an otherwise precisely controlled test meal is a critical determinant of the percent absorption of cholesterol tracer given with that meal. Our experimental design differs from previous literature by clearly separating acute and chronic effects of cholesterol feeding. Important early work using sterol balance techniques demonstrated that percent cholesterol absorption was about the same for 11 subjects who received up to 1 g/day of dietary cholesterol (16). However, acute responses could not be measured and only 2 subjects had repeat testing on different doses of dietary cholesterol. Taken together, these data are consistent with the notion that percent cholesterol absorption at a given meal is significantly influenced by the amount of cholesterol consumed at that meal over the physiological range and that there is little or no chronic adaptation from high or low cholesterol diets.

If excess cholesterol can acutely depress the efficiency of intestinal cholesterol absorption, then it is possible that other meal-related factors might have a similar effect. For example, phytosterols found in common vegetable foods are known to reduce cholesterol absorption by acting in the intestine while they themselves are not well absorbed

**TABLE 3.** Spearman rank correlations between dietary cholesterol responsiveness and metabolic variables in the high-dose cholesterol group of nine subjects

Variable	$r_s$
Age	0.083
BMI	0.067
Waist/hip	-0.033
Gender	0.274
Fasting insulin	0.700 <sup>a</sup>
Fasting glucagon	0.283
Total cholesterol	0.234
Total triglycerides	0.343
LDL cholesterol	0.350
HDL cholesterol	0.033

Dietary responsiveness was calculated as percent cholesterol absorption in the high-dose cholesterol test minus percent cholesterol absorption in the standard test.

<sup>a</sup>  $P = 0.036$ .

(17). Use of direct measurement of cholesterol absorption in a single meal by the dual isotope technique could help to resolve differences among investigators who find different forms of phytosterols either highly effective at reducing plasma cholesterol (18) or ineffective (19). Likewise, genetic factors such as apoE polymorphism may be important in the response to increased dietary cholesterol. It has been reported that percent cholesterol absorption in Finnish men is related to apoE phenotype during a high cholesterol diet (20) but not during a low cholesterol diet (21).

Individual subjects have marked differences in response to increased dietary cholesterol. From Table 2 it can be seen that when fed 421 mg cholesterol, subject 2 absorbed only 40 mg whereas subject 1 absorbed 212 mg, a 5.3-fold difference. The reasons for this are not known, but test variability in repeated cholesterol absorption studies in the same individual is small with a reported standard deviation of less than 3 percentage points (1). Thus, some of these differences may be physiologically meaningful. Intestinal factors such as bile acid concentration and biliary cholesterol secretion could have a substantial influence on the absorption of dietary cholesterol. If biliary cholesterol is relatively reduced it is possible that increased dietary cholesterol might be more readily accommodated. In this small sample there was no statistically significant relation between plasma lipids or lipoproteins and the milligram amount of dietary cholesterol absorption observed.

The positive correlation of fasting insulin level with change in percent cholesterol absorption during high-dose cholesterol feeding ( $r_s = 0.700$ , Table 3) is a new finding and provides additional evidence that insulin is important in the regulation of whole body cholesterol metabolism. As the response to addition of extra cholesterol to the test meal was usually a reduction in percent cholesterol absorption, most changes were negative (percent absorption in the presence of extra cholesterol minus that in the absence of extra cholesterol). Individuals with high plasma insulin levels tended to have little change in percent cholesterol absorption with high-dose cholesterol feeding and absorbed more dietary cholesterol during the high-dose test meal. The plasma insulin levels were not greatly elevated in any of the subjects and varied between 3.7 and 15.5  $\mu\text{U}/\text{ml}$ . Previous work in diabetic animals and humans has emphasized a role of insulin in the intestine with respect to cholesterol metabolism. Both increased cholesterol absorption (22, 23) and increased intestinal cholesterol biosynthesis (24) have been reported in insulin-deficient diabetic rats. Reduced percent cholesterol absorption was observed in hyperinsulinemic non-diabetic subjects selected for elevated fasting plasma glucose (25) and in patients with mild type 2 diabetes (26). These results suggest that increased circulating insulin may reduce cholesterol absorption, the opposite of what we found here in non-diabetic subjects. The relation between insulin and cholesterol absorption therefore requires further work. The correlation between insulin and change in percent cholesterol absorption is not due to obesity as BMI was not related to reduction of percent cholesterol absorption (Table 3).

The use of stable isotopic cholesterol tracers has made studies involving repeated cholesterol absorption testing much easier to perform. Subjects can be recruited without need to consider possible effects of repeated doses of radioactive materials and women of reproductive age need not be excluded. Many of our current subjects are pre-menopausal women who previously have not been studied extensively. These results may help to further our understanding of cholesterol absorption and whole body cholesterol metabolism as it relates to hypercholesterolemia and coronary heart disease risk. ■■

This work was supported by NIH grant R01 HL 50420 (REO), an American Heart Association Clinical Research Grant (MSB), the Washington University Mass Spectrometry Resource (NIH grant RR-00954), and the Washington University General Clinical Research Center (RR-00036). Stephen Block, Nels Holmberg, Wei Zhang, Laura Kobayashi, and Norma Janes provided expert technical and clinical assistance.

*Manuscript received 8 December 1998 and in revised form 26 March 1999.*

## REFERENCES

1. Bosner, M. S., R. E. Ostlund, Jr., O. Osofisan, J. Grosklos, C. Fritschle, and L. G. Lange. 1993. Assessment of percent cholesterol absorption in humans with stable isotopes. *J. Lipid Res.* **34**: 1047–1053.
2. Lutjohann, D., C. O. Meese, J. R. Crouse, III, and K. von Bergmann. 1993. Evaluation of deuterated cholesterol and deuterated sitostanol for measurement of cholesterol absorption in humans. *J. Lipid Res.* **34**: 1039–1046.
3. Ostlund, R. E., Jr., F.F. Hsu, M. S. Bosner, W. F. Stenson, and D. L. Hachey. 1996. Quantification of cholesterol tracers by gas chromatography/negative ion chemical ionization mass spectrometry. *J. Mass Spectrom.* **31**: 1291–1296.
4. Grundy, S. M. 1983. Absorption and metabolism of dietary cholesterol. *Annu. Rev. Nutr.* **3**: 71–96.
5. McNamara, D. J. 1987. Effects of fat-modified diets on cholesterol and lipoprotein metabolism. *Annu. Rev. Nutr.* **7**: 273–290.
6. Zilversmit, D. B., and L. B. Hughes. 1974. Validation of a dual-isotope plasma ratio method for measurement of cholesterol absorption in rats. *J. Lipid Res.* **15**: 465–473.
7. Samuel, P., D. J. McNamara, E. H. Ahrens, Jr., J. R. Crouse, and T. Parker. 1982. Further validation of the plasma isotope ratio method for measurement of cholesterol absorption. *J. Lipid Res.* **23**: 480–489.
8. Bosner, M. S., L. G. Lange, W. F. Stenson, and R. E. Ostlund, Jr. 1999. Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J. Lipid Res.* **40**: 302–308.
9. Morgan, C. R., and A. Lazarow. 1963. Immunoassay of insulin. Two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes.* **12**: 115–126.
10. Manual of Laboratory Operations. Lipid Research Clinics Program. DHEW Publication no. NIH 75-628. 1974. United States Government Printing Office, Washington, D.C.
11. Goodman, D. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. *J. Clin. Invest.* **47**: 231–241.
12. U.S. Department of Agriculture, Agricultural Research Service. 1998. USDA Nutrient Database for Standard Reference, Release 12. Nutrient Data Laboratory Home Page, <http://www.nal.usda.gov/fnic/foodcomp>, NDB #01123.
13. Borgstrom, B. 1969. Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope-labeled meal. *J. Lipid Res.* **10**: 331–337.
14. McNamara, D. J., R. Kolb, T. S. Parker, H. Batwin, P. Samuel, C. D. Brown, and E. H. Ahrens, Jr. 1987. Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quantity. *J. Clin. Invest.* **79**: 1729–1739.

15. Sehayek, E., C. Nath, T. Heinemann, M. McGee, C. E. Seidman, P. Samuel, and J. L. Breslow. 1998. U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans. *J. Lipid Res.* **39**: 2415–2422.
16. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12**: 233–246.
17. Ling, W. H., and P. J. H. Jones. 1995. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci.* **57**: 195–206.
18. Miettinen, T. A., P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol ester margarine in a mildly hypercholesterolemic population. *N. Engl. J. Med.* **333**: 1308–1312.
19. Denke, M. A. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *Am. J. Clin. Nutr.* **61**: 392–396.
20. Kesaniemi, Y. A., C. Ehnholm, and T. A. Miettinen. 1987. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J. Clin. Invest.* **80**: 578–581.
21. Miettinen, T. A., H. Gylling, H. Vanhanen, and A. Ollus. 1992. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arteriosclerosis.* **12**: 1044–1052.
22. Young, N. L., D. R. Lopez, D. J. McNamara, and G. Benauides. 1985. 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.* **26**: 62–69.
23. Thomson, A. B. R., and R. Rajotte. 1983. Effect of dietary modification on the enhanced uptake of cholesterol in diabetic rats. *Am. J. Clin. Nutr.* **37**: 244–252.
24. Feingold, K. 1989. Importance of small intestine in diabetic hypercholesterolemia. *Diabetes.* **38**: 141–145.
25. Strandberg, T. E., V. Salomaa, H. Vanhanen, and T. A. Miettinen. 1996. Associations of fasting blood glucose with cholesterol absorption and synthesis in nondiabetic middle-aged men. *Diabetes.* **45**: 755–761.
26. Gylling, H., and T. A. Miettinen. 1997. Cholesterol absorption, synthesis, and LDL metabolism in NIDDM. *Diabetes Care.* **20**: 90–95.