Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry

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Abstract **Percent cholesterol absorption was measured in 94 normal subjects aged 17–80 years while consuming diets generally low in cholesterol (mean intake = 226 ± 126 mg/day). A new dual stable isotope method was used where a cholesterol tracer containing 6 extra mass units was given intravenously and another tracer with 5 extra mass units was given orally during a standard test meal. The ratio of tracers in plasma was determined by negative ion mass spectrometry of pentafluorobenzoyl sterol esters. Absorption values ranged widely from 29.0% to 80.1% with mean 56.2 ± 12.1 (SD) %. Cholesterol absorption was significantly increased in African-Americans (63.4 ± 11.8% vs. 55.1 ± 11.9%, $P = 0.027$) but was similar for women (53.3 ± 11.9%) and men (57.6 ± 12.1%). It was not related to plasma lipoproteins, age, apoE3/E3 or E3/E4 genotype, or chronic dietary intake of energy, fat, or cholesterol quantitated from 7-day food records. However, dietary cholesterol intake was positively related to plasma cholesterol ($P = 0.036$) and triglycerides ($P = 0.026$). The milligram amount of dietary cholesterol absorbed (but not percent absorption) was positively correlated with fasting plasma insulin ($r = 0.525$, $P < 0.0001$), C-peptide ($r = 0.367$, $P = 0.0003$) and glucagon ($r = 0.421$, $P < 0.0001$) independent of gender, body fat percent and age. The efficiency of intestinal cholesterol absorption and the milligram amount of dietary cholesterol absorbed were not related to plasma cholesterol or LDL cholesterol in individuals consuming a low-cholesterol low-fat diet. The dominant factor determining dietary cholesterol absorption was intake rather than absorption efficiency. Dietary cholesterol and fat were strongly and independently related to hormonal measures of insulin resistance.**—Bosner, M. S., L. G. Lange, W. F. Stenson, and R. E. Ostlund, Jr. **Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry.** ***J. Lipid Res.*** **1999.** 40: **302–308.**

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Despite an extensive literature, important questions remain regarding dietary cholesterol in human nutrition (1, 2). Detailed knowledge of the effects of dietary cholesterol on serum cholesterol levels and whole body cholesterol metabolism must include direct measurements of cholesterol absorption, but few such studies have been reported. One of the principal factors restricting the number and characteristics of human subjects in whom cholesterol absorption studies have been performed has been the need to administer multiple radioisotopic tracers. Because radioactive cholesterol may persist in the circulation for over a year (3), cholesterol absorption studies have often been focused on individuals with coronary heart disease or hyperlipidemia or on middle-aged men who are at risk for coronary heart disease. An alternative to radioactive isotopes is the use of cholesterol modified with the stable isotopes carbon-13 and deuterium and detection by mass spectrometry (4–6). However, the development of suitable methods has been hindered by the large amount of natural cholesterol present in plasma. Recently we described an improved method for measurement of cholesterol tracers diluted in plasma cholesterol in which substantially increased sensitivity was achieved through the use of negative ion chemical ionization mass spectrometry (7). In these experiments cholesterol and cholesterol tracers were derivatized with pentafluorobenzoyl chloride. We report here the determination of percent cholesterol absorption using negative ion mass spectrometry in a normal United States population of both sexes and a wide range of ages.

Cholesterol absorption is incomplete so that percent cholesterol absorption, the efficiency with which the intestine absorbs cholesterol, is a potentially important component of cholesterol metabolism in human nutrition. Per-

Abbreviations: SD, standard deviation; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; *r*, coefficient of correlation; apo, apolipoprotein; P/S, polyunsaturated fat divided by saturated fat; BMI, body mass index or kg weight divided by height in meters^2.

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cent cholesterol absorption in middle-aged Finnish men selected for varying LDL cholesterol levels averaged 45% and was positively correlated with plasma LDL (8). It was proposed that percent cholesterol absorption regulates plasma LDL in this population. In another group of middle-aged Finnish men, percent cholesterol absorption was also positively correlated with total plasma cholesterol and LDL cholesterol (9). In American middle-aged men, percent cholesterol absorption declined by 6 percentage points during chronic treatment with 840 mg dietary cholesterol/day compared to 240 mg/day (10). Reduction in percent cholesterol absorption was thought to contribute to metabolic compensation for the higher dietary cholesterol intake in individuals whose plasma cholesterol level did not change. In a dramatic case, cholesterol absorption in a man who ate 25 eggs per day and had normal plasma cholesterol levels was shown to be only 18% (compared to 46% in controls) (11).

Fewer studies have been performed in women. Cholesterol absorption has apparently been measured in only 6 healthy women (12), but the values were not reported separately. Cholesterol absorption averaged 51% in women with familial hypercholesterolemia (13) and 51% in anovulatory women (14). In the latter study cholesterol absorption declined by 6 percentage points after dietary cholesterol was increased to 780 mg/day from 170 mg/day. These reports suggest that percent cholesterol absorption might be related to plasma lipids and dietary cholesterol intake.

To investigate further the importance of dietary cholesterol absorption in determining plasma cholesterol levels, percent cholesterol absorption was measured during a standard test meal which contained a cholesterol tracer enriched by 5 mass units. Another cholesterol tracer enriched by 6 mass units was injected intravenously at the same time to measure the body pool within which the oral cholesterol tracer was diluted. Percent cholesterol absorption was calculated by dividing the plasma tracer ratio by the administered tracer ratio. Adult subjects of all ages and both genders were included and diet was recorded and analyzed but not changed during the study week. Metabolic variables such as lipids and lipoproteins, plasma insulin, and body fat were measured as covariates to investigate possible relationships with dietary cholesterol absorption.

MATERIALS AND METHODS

Cholesterol tracers

[2,2,4,4,6-$^{2}H_5$]cholesterol ([$^{2}H_5$]cholesterol, 93.4% total enrichment) and [26,26,26,27,27,27-$^{2}H_6$]cholesterol ([$^{2}H_6$]cholesterol, 99% total enrichment) were synthesized by Medical Isotopes, Pelham, NH. [23,24,25,26,27-$^{13}C_5$]cholesterol ([$^{13}C_5$]cholesterol, 99% total enrichment) was obtained from Mass Trace, Inc., Woburn, MA. Pentafluorobenzoyl chloride and reagent grade cholesterol were purchased from Sigma, St. Louis, MO.

In order to remove water of hydration and to destroy adventitious viruses, solid cholesterol tracers were treated with dry heat at 100°C for 0.5 h (15, 16). Tracer for oral administration ([$^{13}C_5$]cholesterol or [$^{2}H_5$]cholesterol) was then dissolved in corn oil at 15 mg/g by rotating overnight at room temperature and 2 g (30 mg cholesterol tracer) was placed on a bagel for the test breakfast. Tracer for intravenous administration ([$^{2}H_6$]cholesterol) was dissolved in USP ethanol at 20 mg/ml and filtered through a 0.2-μ solvent-resistant filter (Millex-FG, Millipore, Bedford, MA). Pyrogens were measured by the limulus colorimetric assay (Bio-Whittaker, Walkersville, MD) using 5-μl aliquots of ethanolic cholesterol solution that had been dried on microtiter plates with or without added endotoxin standard. Using reagents warmed to 37°C, the ethanolic cholesterol tracer was added dropwise over 1 min to 4 volumes of freshly opened 10% Intralipid (Pharmacia, Inc., Clayton, NC) and gently mixed. After incubating at 37°C for 5 min, the preparation was allowed to cool to room temperature for 15 min and then passed through a 1.2-micron particulate filter (IV6120, EPS, Inc., Feasterville, PA). The infusate preparation (freezing point −18°C) was stored at −12°C for up to 4 weeks in a commercial freezer modified to maintain temperature to within ±1°C with an A319 electronic temperature control unit (Johnson Controls, Inc., Milwaukee, WI). Standard refrigeration equipment was unsuitable because of excessive temperature variation and occasional freezing of the tracer with partial disruption of the Intralipid particles. The infusate was refiltered within 1 week of each use and was stored for up to 1 additional week at 4°C before administration. These procedures satisfy the draft recommendations of the American Society of Hospital Pharmacists for parenteral pharmaceuticals (17) and also permit the cholesterol tracer infusate to be transported for use at remote sites. In test samples stored 5 weeks at −15°C followed by 7 days at 4°C, the cholesterol concentration of the infusate after repassage through a 1.2-μ filter was 99.5% of the amount originally present, showing that aggregation of the cholesterol–Intralipid particles did not occur during storage. Examination of these aged and refiltered cholesterol–Intralipid particles by phase contrast microscopy at 200 power showed them to be identical to material freshly prepared.

Subjects

Ninety-four healthy adults (62 women and 32 men, 79 Caucasian, 12 African-American, and 3 Asian) without active medical or surgical illnesses participated. None took medication affecting lipid metabolism or drank alcohol during the study. Subject characteristics are listed in **Table 1**. Informed consent was obtained in writing and the project was approved by the Washington University Human Studies Committee. Fasting plasma insulin (18), C-peptide (19), glucagon (20), and leptin (21) were determined by radioimmunoassay. Plasma cholesterol, triglycerides, VLDL, LDL, and HDL cholesterol were measured after ultracentrifugation by the Washington University Lipid Research Clinic Core Laboratory (22) and HDL_2 was determined after selective precipitation of HDL_3 (23). Skinfold thicknesses of the triceps, suprailiac, and thigh regions were measured in women and of the chest, abdomen, and thigh in men using a Lange caliper. Percent body fat was calculated from these values and age using gender-specific regression equations validated by comparison to underwater weighing (24). ApoE genotype was determined by polymerase chain reaction in 80 subjects (25).

Subjects met with a registered dietitian and kept a food diary during the 7-day protocol while consuming their usual diet, and dietary intakes were calculated using the University of Minnesota Nutrient Data System. On day 3 the subjects reported fasting to the Washington University General Clinical Research Center and received an intravenous infusion of 15 mg [$^{2}H_6$]cholesterol tracer in approximately 4 ml Intralipid over 5 min as described previously (6). Immediately after this, a test breakfast was given consisting of 240 ml orange juice, 240 ml whole milk, 21 g corn flakes, and a 60-g bagel saturated with 2 g corn oil that contained

TABLE 1. Characteristics of the study subjects

Variables	Mean ± SD
Women/men	62/32
Age	40.8 ± 15.6
Body fat %	27.3 ± 9.7
BMI, kg/m^2	27.6 ± 6.0
Weight, kg	76.2 ± 18.3
Calories/day	1971 ± 650
Percent cholesterol absorption	56.2 ± 12.1
Dietary cholesterol, mg	226 ± 126
Absorbed dietary cholesterol, mg	125 ± 68
% Calories from fat	32.1 ± 7.9
% Calories from carbohydrate	53.1 ± 8.6
% Calories from protein	15.3 ± 4.8
P/S	0.60 ± 0.22
Dietary fiber, g/day	16.7 ± 5.8
Plasma cholesterol, mg/dl	186 ± 46
Plasma triglycerides, mg/dl	138 ± 194
VLDL cholesterol, mg/dl	19.5 ± 27.7
LDL cholesterol, mg/dl	116 ± 37
HDL_2, mg/dl	8.3 ± 7.6
HDL_3, mg/dl	41.4 ± 10.0
Insulin, μU/ml	8.0 ± 7.1
C-peptide, ng/ml	2.1 ± 1.2
Glucagon, pg/ml	108 ± 25.2
Leptin, ng/ml	14.2 ± 11.3
ApoE genotype 33/34	54/26

30 mg oral cholesterol tracer. The breakfast contained 510 calories, 64 mg cholesterol (including the tracer) and 1.7 g total dietary fiber. The amounts of tracer cholesterol infused and eaten were quantitated by weighing. Standard weighed mixtures of aliquots of oral tracer in oil and intravenous tracer in Intralipid were set aside for analysis. Fasting plasma samples for cholesterol enrichment were drawn on day 3 just before the test for use as a baseline and again on day 7 after isotope administration.

Mass spectrometry and calculation of percent cholesterol absorption

Our general methods for measuring cholesterol tracers diluted in plasma cholesterol have been reported previously (6, 7). In this work all samples were analyzed by negative ion mass spectrometry. Plasma samples (0.5 ml) were saponified (26) and the non-saponifiable sterols were extracted into petroleum ether and converted to pentafluorobenzoyl esters. Approximately 1 mg of sterol from 0.5 ml plasma was dried in a 16 × 100 glass test tube and 200 μl toluene was added followed by 20 μl dry pyridine and 5 μl pentafluorobenzoyl chloride. After vortexing and standing for 10 min at room temperature, 1 ml water was added, the pentafluorobenzoyl cholesterol was extracted twice with 2 ml petroleum ether, and the combined organic fractions were dried and taken up in 200 μl toluene (final concentration 5 mg/ml). Approximately 1 μl of a 1:10 dilution of the sterols was injected, split 1:10, and then separated on a 5-meter 0.53 mm i.d. 1 μ film thickness XTi-5 capillary column connected to a 5-meter 0.25 mm i.d. flow-restricting transfer line using a temperature program of 220°C for 1 min and then a 20°/min rise to 280°C. This technique allows an increased amount of cholesterol to be analyzed for many seconds without compromise of mass spectrometer vacuum. The effluent was admitted into a Hewlett-Packard 5988A mass spectrometer operating in negative ion chemical ionization mode with methane reagent gas at 1 torr. The principal ion observed under these mild analytical conditions was the molecular ion of cholesterol pentafluorobenzoate at m/z 580, but this ion was too intense to monitor routinely. Instead, ions monitored over time at m/z 581 (a measure of natural cholesterol), 585 (corresponding primarily to M + 5 tracer), and 586 (corresponding primarily to M + 6 tracer) at high sensitivity were integrated to give raw ion areas.

The m/z 585/581 and m/z 586/581 ratios observed in plasma cholesterol after isotope administration are a measure of the dilution of cholesterol tracers in natural cholesterol. These raw ratios in day 7 plasma cholesterol were corrected by subtracting the corresponding ratios found in day 3 plasma cholesterol before isotope administration to yield corrected m/z 585/581 and m/z 586/581 ratios and the m/z 585/586 ratio was computed. Then the mole ratio to which this area ratio corresponded was obtained by reading from a standard curve. **Figure 1** presents standard curves for mixtures of tracers used in patient studies, with or without dilution 1:2500 in cholesterol or plasma sterols prepared from a normal subject. The dilution of 1:2500 approximates the average expected dilution of intravenous cholesterol tracer 4 days after administration computed from published parameters for cholesterol turnover (3). The neat tracer mixtures and the mixtures diluted in natural cholesterol or sterols fell on the same line. The between-assay coefficient of variation for 13 assays was 1.0% for a mole ratio of 0.33 and 1.3% for a mole ratio of 1.0. Percent cholesterol absorption was calculated as the mole ratio of tracers in plasma on day 7 divided by the mole ratio administered. Mass absorption of dietary cholesterol was calculated as percent cholesterol absorption × cholesterol intake estimated from food records (mg/day).

[$^{13}C_5$]cholesterol was used as the oral tracer in 58 studies and [$^2H_5$]cholesterol was used in 36 studies. Values for percent cholesterol absorption with the two tracers were similar but not identical. Using [$^{13}C_5$]cholesterol, percent cholesterol absorption was found to be 58.5 ± 10.5% (range, 32.6–80.1%) and using [$^2H_5$]cholesterol, values of 52.4 ± 13.7% (range, 29.0–79.7%) were obtained (P for difference = 0.03). The reason for this small systematic difference is not known. Including the type of oral label used as a covariate in the statistical analyses did not alter the results obtained.

Pearson correlations and general linear statistical models were performed using Statistical Analysis System software (SAS Institute, Cary, NC). Plasma triglyceride concentration was log-transformed. Means ± standard deviation are presented.

RESULTS

A histogram showing the distribution of percent cholesterol absorption in the population is presented in **Fig. 2**. The values ranged from 29.0% to 80.1% with a mean of 56.2 ± 12.1%. Previous work has shown that percent cholesterol absorption is reproducible on repeat measurement in the same individual (6) so that the wide spectrum of values observed is likely to represent principally between-subject variation. Percent cholesterol absorption was the same in women (53.3 ± 11.9%) as in men (57.6 ± 12.1%, difference not significant, P = 0.10). However, as shown in **Table 2**, percent cholesterol absorption was significantly higher in African-Americans (63.4 ± 11.8%, n = 12) than in other racial groups (55.1 ± 11.9%, P = 0.027). Table 2 gives the characteristics of African-American participants, which are generally similar to other ethnic groups except that dietary fiber intake was lower and HDL cholesterol and plasma leptin levels were higher. Body fat percent also tended to be higher but this was not statistically significant. The increased percent cholesterol ab-

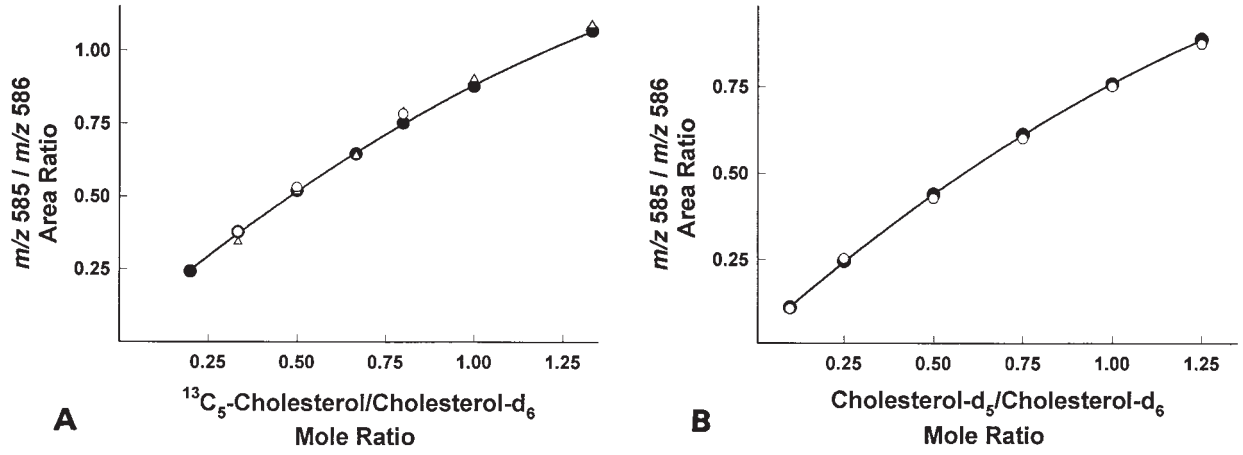

Fig. 1. Standard curves for calculating cholesterol tracer mole ratios from mass spectrometric data. Known mixtures of M + 5 and M + 6 cholesterol tracers were prepared and analyzed as described in Methods either without further addition (filled circles and regression lines) or after dilution 1:2500 in purified cholesterol (open triangles) or plasma sterols (open circles). The area ratio of selected ion chromatograms at m/z 585/586 is plotted against the known mole ratios. A: Mixtures of [$^{13}C_5$]cholesterol and [$^2H_6$]cholesterol. B: Mixtures of [$^2H_5$]cholesterol and [$^2H_6$]cholesterol. For samples diluted in natural cholesterol, the background areas found in the natural material were subtracted. Each point is the mean of duplicate determinations.

sorption observed in African-Americans did not appear to be accounted for by other variables such as plasma leptin level, body fat percent, dietary fiber, dietary cholesterol intake, or apoE genotype when tested in statistical models with each of these variables. Ethnicity remained significant or nearly significant (P = 0.031 to 0.059) while the other variables were not significant (P = 0.13 to 0.87).

Dietary cholesterol intake quantitated from 7-day food records was low and generally within the guidelines of the American Heart Association at 226 ± 126 mg/day (range 30–956 mg/day, Table 1). **Table 3** presents correlations between measured percent cholesterol absorption and plasma lipoproteins and other metabolic variables. There was a trend for percent cholesterol absorption to decrease with increasing cholesterol intake but this did not reach statistical significance (r = −0.143, P = 0.17). Percent cholesterol absorption was specifically not related to plasma total cholesterol (P = 0.94) or LDL cholesterol (P = 0.44). ApoE genotype was determined in 80 subjects and was not related to percent cholesterol absorption by analysis of variance (P = 0.33). However, percent cholesterol absorption was significantly inversely related to dietary fiber intake (r = −0.208, P = 0.046). Dietary fiber was 16.7 g/day and of this 66% ± 3% (SEM) was insolu-

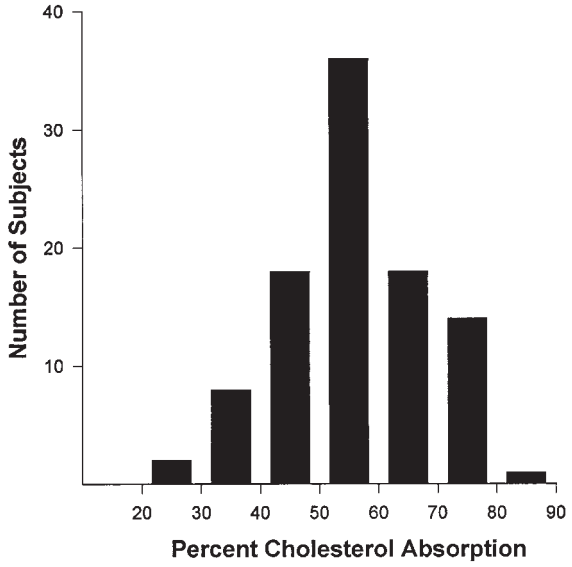

Fig. 2. Distribution of values for percent cholesterol absorption in 94 subjects by decile.

TABLE 2. Characteristics of African-American and other racial groups

Variables	African-American	Other
Women/men	11/1	51/31
ApoE genotype 33/34	7/5	47/21
Age	43.4 ± 13.4	40.4 ± 16.0
Body fat %	31.1 ± 10.5	26.7 ± 9.5
BMI, kg/m^2	28.7 ± 5.2	27.5 ± 6.2
Weight, kg	78.0 ± 15.8	75.9 ± 18.7
Calories/day	1635 ± 396	2020 ± 667
Percent cholesterol absorption	63.4 ± 11.8[a]	55.1 ± 11.9
Dietary cholesterol, mg	213 ± 79	228 ± 132
% Calories from fat	32.8 ± 6.3	31.9 ± 8.1
% Calories from carbohydrate	53.3 ± 8.2	53.1 ± 8.7
% Calories from protein	14.0 ± 2.1	15.5 ± 5.1
P/S	0.63 ± 0.14	0.60 ± 0.23
Dietary fiber, g/day	13.1 ± 4.4[a]	17.2 ± 5.8
Plasma cholesterol, mg/dl	186 ± 41	186 ± 47
Plasma triglycerides, mg/dl	114 ± 127	142 ± 202
VLDL cholesterol, mg/dl	13.9 ± 16.6	20.4 ± 29.0
LDL cholesterol, mg/dl	111 ± 27	117 ± 39
HDL cholesterol, mg/dl	60.9 ± 20.5[b]	48.4 ± 14.5
Insulin, μU/ml	8.4 ± 5.3	8.0 ± 7.3
C-peptide, ng/ml	2.0 ± 0.8	2.1 ± 1.2
Glucagon, pg/ml	108 ± 21	108 ± 26
Leptin, ng/ml	23.7 ± 13.0[b]	12.8 ± 10.4

Means ± SD are presented.
[a] $P < 0.03$.
[b] $P < 0.01$.

TABLE 3. Pearson correlations of percent cholesterol absorption, absorbed dietary cholesterol, and dietary cholesterol intake with metabolic variables

Variables	Correlation Coefficient, *r*		
	Percent Cholesterol Absorption	Absorbed Dietary Cholesterol	Dietary Cholesterol
	%	*mg*	*mg*
Age	−0.144	0.033	0.100
Body fat %	0.008	0.139	0.139
BMI, kg/m^2	−0.058	0.324[b]	0.360[b]
Weight, kg	−0.037	0.512[a]	0.548[a]
Calories/day	−0.153	0.641[c]	0.710[c]
% Fat calories	0.024	0.553[c]	0.559[c]
% Carbohydrate calories	−0.031	−0.569[c]	−0.570[c]
% Protein calories	−0.006	0.093	0.092
P/S	0.176	−0.222[a]	−0.287[b]
Dietary fiber	−0.208[a]	0.173	0.252[a]
Percent absorption	1.000	0.244[a]	−0.143
Dietary cholesterol	−0.143	0.911[c]	1.000
Plasma cholesterol	−0.075	0.174	0.227[a]
Plasma triglyceride	−0.112	0.366[b]	0.421[c]
VLDL cholesterol	−0.087	0.342[b]	0.428[c]
LDL cholesterol	−0.072	0.055	0.084
HDL_2	0.095	−0.177	−0.220[a]
HDL_3	0.107	−0.206[a]	−0.280[b]
Insulin	0.013	0.525[c]	0.466[c]
C-peptide	−0.054	0.367[b]	0.429[c]
Glucagon	0.133	0.421[c]	0.434[c]
Leptin	0.088	−0.028	−0.068

[a] $P < 0.05$.
[b] $P < 0.005$.
[c] $P < 0.0001$.

ble. However, dietary fiber was no longer related to percent cholesterol absorption after adjusting for race in a statistical model ($P = 0.13$).

The milligram amount of dietary cholesterol absorbed was estimated by multiplying percent cholesterol absorption by the dietary cholesterol intake. Average absorbed dietary cholesterol was 125 ± 67 mg day. Table 3 shows that there was no significant correlation of absorbed dietary cholesterol with either total plasma cholesterol or LDL cholesterol. However, surprisingly strong correlations were observed between absorbed dietary cholesterol and fasting plasma insulin ($r = 0.525$, $P < 0.0001$), fasting C-peptide ($r = 0.367$, $P = 0.0003$), and fasting plasma glucagon ($r = 0.421$, $P < 0.0001$). Each of these relationships remained statistically significant independent of body fat percent, age, and gender when tested in a statistical model (P values <0.0001, 0.02, and 0.0005, respectively, in an analysis of covariance containing all factors). Absorbed dietary cholesterol was highly intercorrelated with total dietary fat ($r = 0.797$), dietary saturated fat ($r = 0.787$), and energy intake ($r = 0.641$). Because of this collinearity it was not possible to determine by statistical means which was most closely related to plasma insulin and hormone levels.

Statistically significant but quantitatively lower correlations were found between absorbed dietary cholesterol and total plasma triglycerides ($r = 0.366$, $P = 0.0003$), VLDL cholesterol ($r = 0.342$, $P = 0.0008$), and HDL cholesterol ($r = -0.225$, $P = 0.03$). However, these relationships were no longer significant after considering body fat percent and gender in an analysis of covariance.

Dietary cholesterol intake was correlated with several lipoproteins. After adjusting for gender, body fat, and age, dietary cholesterol was significantly related to total plasma cholesterol ($P = 0.036$), VLDL cholesterol ($P = 0.024$), and total triglycerides ($P = 0.026$) but not to LDL cholesterol ($P = 0.53$), HDL_2 ($P = 0.49$), or HDL_3 ($P = 0.33$).

DISCUSSION

This study was designed to define the range and distribution of dietary cholesterol absorption in a diverse group of healthy individuals consuming diets generally within recommended guidelines. Women, who have not been well-studied previously, comprised 66% of the subjects. Dietary cholesterol averaged 226 mg/day and fat intake was 32.1% of calories with a polyunsaturated/saturated fatty acid ratio of 0.6. The amount of absorbed dietary cholesterol was 125 mg/day.

The mean cholesterol absorption observed was 56%. This is somewhat higher than previously reported values for normal subjects of 47% in Finnish men (9), 45% in Iowa men (27), 28% in Tarahumara Mexican men (28), and 45% in Minnesota men and women (12). It is similar to values of 54–62% found in normal middle-aged New York men (10). There were no differences between men and women in percent cholesterol absorption nor were effects of age or body fat noted (Table 3). The data for women show that none of the gender differences in lipoproteins appears to be attributable to differences in percent cholesterol absorption. However, percent cholesterol absorption was significantly higher in African-Americans than in other racial groups, the difference being 8.3 percentage points or 15% of the mean value for other subjects. The difference in percent absorption cannot be accounted for by any of the other factors measured and may represent a difference between the ethnic groups. However, this conclusion should be tempered by the fact that only 12 African-American subjects were studied.

The range of values observed for percent cholesterol absorption (29–80%) was wide, the highest being 2.8 times the lowest. However, within a given subject, percent cholesterol absorption is a highly reproducible measurement with the standard deviation of differences between repeated tests being only 3 percentage points (6). Thus, most of the variability encountered here appears to be between-subject variation due to physiology. Percent cholesterol absorption was not correlated with either plasma cholesterol or LDL cholesterol, indicating that alternative mechanisms such as biliary cholesterol secretion and cholesterol biosynthesis might adapt in order to maintain cholesterol homeostasis. On the other hand, dietary cholesterol intake was independently related to total plasma cholesterol and VLDL cholesterol even though this group consumed relatively small amounts of cholesterol. Biliary secretion of cholesterol into the intestine may be over 1000 mg/day (1) whereas the dietary intake was 226 mg/

day in this study. The plasma production rate for cholesterol is approximately 1000 mg/day (3) whereas absorbed dietary cholesterol was only 125 mg/day in this study. Thus, the impact of dietary cholesterol is limited by dilution in biliary cholesterol within the bowel, by incomplete absorption, and by a relatively high rate of endogenous synthesis. No statistically independent relation between dietary fiber and percent cholesterol absorption was found. A previous interventional study (29) and a cross-sectional study (9) failed to find an effect of dietary fiber on percent cholesterol absorption.

Our results differ from other published data in several ways. Work performed with Finnish men showed that percent cholesterol absorption was related to apoE phenotype with absorption increasing from apoE-2 through apoE-3 to apoE-4 (30). The Finnish diet is very high in cholesterol and when the amount consumed was reduced from the initial level of 573 mg/day to 210 mg/day the relationship between percent cholesterol absorption and apoE phenotype was lost (31). Our work confirms that the common apoE genotypes E3/E3 and E3/E4 are not related to percent cholesterol absorption in a population consuming a low-cholesterol diet. Individuals with E2/E2, E2/E3, and E4/E4 genotypes were not present in our group. In Finnish men, percent cholesterol absorption is also positively correlated with LDL cholesterol levels (8, 9) and inversely correlated with BMI (9). It is possible that certain apoE genotypes and body fat may increase percent cholesterol absorption and secondarily increase LDL cholesterol levels when dietary cholesterol is high but not have prominent effects when recommended levels of cholesterol are consumed. It also has been reported that cholesterol absorption is reduced in 75-year-old men compared to 50-year-old men (32). However, the older men consumed 32% fewer calories despite similar BMI, suggesting at least some degree of inactivity or disability. Our results show no general effect of age on percent cholesterol absorption throughout most of the lifespan, but sedentary elderly subjects were not specifically studied. The lack of correlation between percent cholesterol absorption and apo E genotype, BMI, age, and LDL cholesterol in our subjects may indicate, in part, the effectiveness of a reduced-cholesterol diet.

Strong and independent positive correlations were noted between absorbed dietary cholesterol and fasting plasma insulin, C-peptide, and glucagon. Elevations of these hormones are linked to insulin resistance and the metabolic obesity syndrome (33, 34). The data suggest that absorbed dietary cholesterol or factors closely linked to it such as dietary fat intake may worsen the insulin-resistant metabolic syndrome. The relationship between absorbed cholesterol and plasma insulin was independent of age, body fat percent, and gender in multivariate analysis. Percent cholesterol absorption was not significantly correlated with insulin levels, indicating that dietary cholesterol intake was the dominant associated factor. The strong correlation between dietary cholesterol and parameters related to insulin resistance is striking in that this study was performed in a very heterogeneous population of healthy individuals consuming low-cholesterol low-fat diets in which the mean plasma insulin level was only 8.0 μU/ml. This suggests that within this group dietary cholesterol, fat or other related factors might have substantial effects on insulin sensitivity.

Previous work has shown that a high fat diet can potently increase insulin resistance in rodents (35). Only a few human studies have considered a link between dietary composition and insulin resistance. In the Normative Aging Study, saturated fat intake assessed from a food frequency questionnaire was related to fasting and postprandial insulin levels in 652 men of average age 62 years independent of body mass index and other variables (36). The Stanford Coronary Risk Intervention Project, a study of 215 middle-aged men with coronary heart disease, reported a significant association between saturated fat intake or cholesterol intake and fasting insulin concentration that was independent of body mass index and other variables (37). While the study emphasized dietary saturated fat, the statistical relation between dietary cholesterol and plasma insulin was equally strong. In the Zutphen Elderly Study of 389 men over 70 years of age it was reported that area under the insulin curve during a glucose tolerance test and fasting C-peptide levels were independently correlated with saturated fat intake (38). Taken together, these clinical studies show a positive relationship between dietary fat or cholesterol and plasma insulin that is independent of body mass index in older men. The effects of fat and cholesterol intake could not be separated cleanly because of their interdependence. Our work confirms these findings and extends them to a wider age group that includes women as well as men and in which dietary cholesterol and fat intake are related to fasting plasma glucagon as well as to insulin and C-peptide. The relationship to glucagon appears not to have been reported previously. As this was an observational study, more work is needed in order to determine the exact relationship between absorbed dietary cholesterol, dietary fat intake, and plasma insulin levels.

Some limitations of the current work should be recognized. First, percent cholesterol absorption was measured which reflects the efficiency of intestinal cholesterol transport. When combined with dietary cholesterol intake it provides a measure of absorbed dietary cholesterol but it does not provide information about the total amount of cholesterol absorbed as biliary cholesterol secretion into the intestine was not estimated. Second, cholesterol absorption was measured during a standardized test meal which was low in cholesterol and may not necessarily reflect results from diets higher in cholesterol. Nevertheless, our work gives insight into basic cholesterol physiology in normal subjects consuming recommended diets and shows that stable isotopic cholesterol tracers can be used productively for the study of cholesterol metabolism. The ability to use two stable isotopic cholesterol tracers simultaneously may be useful in future studies of whole body cholesterol metabolism and reverse cholesterol transport and extends the kinds of metabolic studies that can be done conveniently by mass spectrometry.

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