Assessment of percent cholesterol absorption in humans with stable isotopes

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Abstract Dietary cholesterol restriction is a general recommendation for the medical community and emphasizes the importance of intestinal cholesterol absorption and metabolism in humans. However, several methods that may accurately quantify cholesterol absorption utilize radioactive isotopes that are undesirable for younger individuals, women, children, and normal subjects. To eliminate this hazard, we have developed a procedure for measurement of percent cholesterol absorption, based on that of Zilversmit (1972, Proc. Soc. Exp. Med. Biol. 140: 862-865), using stable nonradioactive isotopic tracers of cholesterol. [26,25,26,27,27,27-H3]cholesterol (30 mg) was administered orally and [23,24,25,26,27-13C]cholesterol (15 mg) was administered intravenously on day 0 and percent cholesterol absorption was calculated as the plasma ratio of oral/intravenous isotopic tracer on day 3 as determined by gas chromatography-mass spectrometry with selected ion monitoring. Tracer cholesterol given orally peaked in plasma on day 2 and then slowly declined in parallel with the intravenous tracer. Cholesterol absorption in 16 healthy subjects (on no medication and not ingesting alcohol) consuming a Step One Diet was 53.5% ± 8.5 SD%. Five subjects underwent repeat testing after 4-6 weeks with excellent replication (SD of difference between tests = 2.8%). No differences in the metabolism of [13C3]cholesterol, [3H2]cholesterol, and [13C1]cholesterol were observed. The use of stable isotopes for the study of percent cholesterol absorption is precise and safe, allowing repeated measurements in normal individuals and thus facilitating clinical investigation of this key component of human cholesterol metabolism. Bosner, M. S., R. E. Ostlund, Jr., O. Ososfian, J. Grosklos, C. Fritschle, and L. G. Lange. Assessment of percent cholesterol absorption in humans with stable isotopes. J. Lipid Res. 1993. 34: 1047-1053.

Supplementary key words intestine • carbon isotopes • deuterium • mass spectrometry • selected ion monitoring

When the relationship between cholesterol and atherosclerosis is considered, the usual focus of attention is on plasma cholesterol concentration. As this comprises less than 9% of total body cholesterol (1), insights from whole body cholesterol metabolism could complement and extend those obtained from the extensive literature on plasma lipoproteins. Cholesterol absorption from the gastrointestinal tract is a key component of whole body cholesterol metabolism, not only because of the dietary cholesterol content, but also because of the twofold or larger enterohepatic recirculation of endogenous cholesterol which readily mixes with dietary cholesterol to form a single pool of intestinal cholesterol (2).

Despite an extensive literature, important questions remain regarding human cholesterol absorption. This is partly because of the complex nature of cholesterol absorption physiology (see Discussion), but methodological limitations have also contributed. Of the latter, the need to use radioactive isotopes stands out. Because of ethical considerations related to radiation exposure, cholesterol absorption studies have often excluded women, children, and young persons, and a tendency to select middle-aged or older men and individuals with coronary heart disease or lipid disturbances has been evident. In order to investigate intestinal cholesterol metabolism in normal individuals, we have developed a method that applies stable isotope techniques and mass spectrometry to established methods for measuring cholesterol absorption that have previously used radioactive isotopes.

An important component of cholesterol absorption is the percent absorption of the intestinal cholesterol load. Percent cholesterol absorption investigated with radioactive tracers varies from 15% to 75% in humans, a broad range suggestive of metabolic or genetic regulation (2). A simple method for estimating percent cholesterol absorption at a single time point under standardized conditions was proposed by Zilversmit (3, 4), based upon cholesterol labeled with tritium (administered intravenously) and carbon-14 (given orally). Determination of the ratio of the two isotopes in plasma cholesterol after 3 days of...
equilibration in the rapidly miscible pool of body cholesterol provided an estimation of fractional absorption. During this time period, a negligible amount of cholesterol tracer was excreted from the body. Such an isotope ratio method has been validated in humans by comparison to other methods including those that involve oral administration of radiolabeled cholesterol and stool collection (5-7).

We utilized the Zilversmit methodology using cholesterol labeled with either deuterium or carbon-13 to achieve a mass difference of 5 or 6 daltons over natural cholesterol. One stable isotope was administered orally and one intravenously (in small amounts, 15-30 mg) and the ratio of these tracers was precisely quantified in plasma cholesterol on day 3 by gas chromatography-mass spectrometry (GC-MS). We measured cholesterol absorption in 16 normal adult subjects and evaluated the reproducibility of absorption measurements in 5 subjects after 4-6 weeks.

MATERIALS AND METHODS

Labeled cholesterol

[23,24,25,26,27-13C3]cholesterol with average substitution of 99% at each enriched site was synthesized by Dr. Alfred Ajami of Tracer Technology, Inc. (Fig. 1). The material as supplied contained 26% [23,24,25-13C3]-26,27-bis-norcholesterol, a phytosterol missing two terminal methyl groups. For some studies as well as preparation of standards, this was removed by HPLC using an octadecyl reversed phase column eluted with methanol. Purification was not routinely necessary because the material was not measured in the GC-MS analysis. A previously available tracer, [3,4-13C]cholesterol (8), was found to have insufficient mass difference from natural cholesterol to allow convenient GC-MS assays. [26,25,26,27,27,27-2H6] cholesterol (99% enrichment) was prepared by Dr. Eric Stohler of Biodesign, Inc. [4-14C]cholesterol (55 mCi/mmol) was purchased from Amersham.

Clinical studies

Sixteen healthy subjects (nine men and seven women, age 22-63 years old) participated in cholesterol absorption studies (Table 1). All consumed a National Cholesterol Education Program Step I diet for 2 days prior to and during the study. The subjects were not taking any medications and did not consume alcohol during the study period. To assess intraindividual variability of cholesterol absorption, five subjects (three men and two women) underwent an identical procedure after 4-6 weeks. The protocol was approved by the Institutional Review Boards of The Jewish Hospital of St. Louis and Washington University School of Medicine.

[13C]cholesterol suitable for intravenous administration was prepared in a laminar flow hood. The labeled cholesterol was dissolved at 5 mg/ml in USP ethanol in a sterile container, water was added to 10% by volume in order to increase virucidal activity, and the solution was allowed to stand at room temperature for at least 6 h. The solvents were then sterilly removed and the cholesterol residue was dissolved at 20 mg/ml in ethanol and passed through a 0.22-micron solvent-resistant filter. Aliquots were tested for sterility and dried aliquots for pyrogenicity using the Limulus assay, and the stock was kept frozen at -70°C. On the day of the experiment (day 0) both the [13C]cholesterol and a small amount of 10% Intralipid were warmed to 37°C and the ethanolic cholesterol was added dropwise to 4 volumes of Intralipid. After 5 min at 37°C and 15 min at room temperature the mixture was put through a 0.8-micron particulate filter. More than 94% of the labeled cholesterol passed through the filter. An aliquot containing approximately 15 mg [13C]cholesterol was drawn into a tared syringe and weighed. The syringe contents were injected intravenously over 5 min into a running saline infusion and the syringe was washed several times with saline. With the use of Intralipid, cholesterol is quantitatively solubilized and less than 0.4% of the tracer cholesterol is found in the syringe and intravenous lines.

[2H]cholesterol for oral administration was dissolved in corn oil at 15 mg/ml and stored at -70°C. An English muffin was dried to constant tare weight and an amount of the warmed oil solution containing approximately 30 mg tracer was added dropwise on an analytical balance. On day 0 the muffin was consumed with a breakfast consisting of 240 ml of orange juice, one slice dry toast, 50 g of corn flake cereal, and 240 ml of whole milk as a source of fat to assure contraction of the gallbladder. The mole ratio of administered tracer cholesterol was deter-

![Fig. 1. Tracer cholesterol.](image-url)
Subjects were given 65 mg [13C]cholesterol and 15 pCi [4-14C]cholesterol (9). Acetate esters of cholesterol were prepared by dissolving approximately 400 μg cholesterol in 160 μl anhydrous pyridine, adding 800 μl acetic anhydride, mixing, and allowing the solution to stand overnight at room temperature. Volatile reagents were removed and the solid cholesterol acetate was taken up in heptane at a concentration of 5 mg/ml.

Approximately 5 μg sample was injected onto a 122-cm packed column of 1% SE-30, chromatographed at 250°C, and admitted to a Finnigan 3300 mass spectrometer operating in electron ionization mode with a quadrupole mass analyzer. The amount injected was the largest compatible with a linear response from the mass spectrometer ion source. Selected ions were monitored with high gain at m/z 372, 373, and 374 (see Results). These ions represent principally a nonabundant component of natural cholesterol (372) and the principal components of [13C]cholesterol (373) and [2H]cholesterol (374), respectively. The very large principal ion of natural cholesterol at m/z 368 was not monitored. The sizes of peaks 372, 373, and 374 were similar.

Each ion peak area monitored consisted of a detector response due mainly to one type of cholesterol but also including smaller overlapping responses due to the other two cholesterols present. Therefore, the ion peak areas at masses 372, 373, and 374 were corrected to those expected for a single cholesterol species using three simultaneous equations as follows:

\[
A_{372,\text{corr}} = A_{372,\text{obs}} - a_1 \times A_{373,\text{corr}} - a_2 \times A_{374,\text{corr}}
\]

\[
A_{373,\text{corr}} = A_{373,\text{obs}} - b_1 \times A_{372,\text{corr}} - b_2 \times A_{374,\text{corr}}
\]

\[
A_{374,\text{corr}} = A_{374,\text{obs}} - c_1 \times A_{372,\text{corr}} - c_2 \times A_{373,\text{corr}}
\]

To validate the use of these stable isotopes for metabolic studies in humans, two experiments were performed. In the first, to assess whether [13C]cholesterol and [4-14C]cholesterols were metabolically equivalent, three healthy subjects were given 65 mg [13C]cholesterol and 15 μCi [4-14C]cholesterol prepared in ethanol and Intralipid intravenously. The percent of injected dose per mg plasma cholesterol was determined on days 3-5. In the second, to confirm preliminary data that demonstrated equivalent intestinal absorption of the stable isotopes in rabbits, a 49-year-old healthy female consumed a muffin containing 30 mg of [13C]cholesterol and 30 mg of [2H]cholesterol and the plasma isotopic enrichment derived from the orally administered labels was determined daily for 6 days.

### Mass spectrometry of human plasma cholesterol

Total cholesterol (about 2 mg) was partially purified from 1 ml EDTA-anticoagulated plasma by saponification of the sample in ethanolic base and extraction of the nonsaponifiable sterols into petroleum ether exactly as described previously (9). Acetate esters of cholesterol were prepared by dissolving approximately 400 μg cholesterol in 160 μl anhydrous pyridine, adding 800 μl acetic anhydride, mixing, and allowing the solution to stand overnight at room temperature. Volatile reagents were removed and the solid cholesterol acetate was taken up in heptane at a concentration of 5 mg/ml.

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\]

\[
A_{374,\text{corr}} = A_{374,\text{obs}} - c_1 \times A_{372,\text{corr}} - c_2 \times A_{373,\text{corr}}
\]

### TABLE 1.

<table>
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<tr>
<th>Subject</th>
<th>Age/Sex</th>
<th>BMI</th>
<th>TC</th>
<th>LDL</th>
<th>HDL</th>
<th>TG</th>
<th>Test 1</th>
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Cholesterol absorption in normal adult subjects. Repeat tests (subjects 1-5) were performed 4-6 weeks apart. Means ± SD for repeat studies in subjects 1-5 were 59.3 ± 8.4% for Test 1 and 57.6 ± 8.29% for Test 2. Abbreviations: BMI, body mass index (kg/m²); TC, total cholesterol; LDL, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, total triglyceride.
In these equations the corrected peak areas $A_{corr}$ are unknowns to be computed from the observed peak areas $A_{obs}$ and the constants $a$, $b$, and $c$ determined from pure labeled cholesterols or the natural cholesterol from each patient before administration of tracer. The Simulation, Analysis, and Modeling (SAAM) computer program obtained from the Resource Facility for Kinetic Analysis at the University of Washington was used for calculations.

Each day a standard curve was determined consisting of the ratio $A_{374,corr}/A_{373,corr}$ plotted against weighed mole ratios of $[^2H]/[^{13}C]$cholesterol diluted in natural cholesterol. The mole ratios of $[^2H]/[^{13}C]$cholesterol in the plasma on day 3 and in the administered materials were determined from the standard curve and the percent cholesterol absorption was calculated as the quotient of these ratios.

RESULTS

Mass spectrometry

When natural cholesterol acetate was subjected to electron ionization mass spectrometry, a prominent ion was found at mass 368, representing dehydrated cholesterol after loss of acetic acid from the original ester. In contrast, less than 1% of the signal was observed at masses 373 and 374. Therefore, we adopted the strategy of using tracer cholesterols 5 or 6 mass units greater than that of natural cholesterol to reduce the interference from endogenous cholesterol ($m/z = 368$). The importance of this step can be appreciated by considering that the tracers are diluted into a rapidly miscible body cholesterol pool of approximately 24,000 mg (1). In typical mass spectrometric assays, the ratio of tracer to natural material is determined, but here that is not practical because of the large mass of natural cholesterol present. Moreover, the only information needed is the ratio of the two tracer molecules in plasma. Therefore, we have ignored the large peak of natural cholesterol at mass 368 and have measured instead the tracer cholesterols at masses 373 ($[^{13}C]$cholesterol), and 374 ($[^2H]$cholesterol) using high instrument sensitivity. Mass 372, a nonabundant peak present in natural cholesterol, was also measured in order to correct for small (less than 5%) background contributions from natural cholesterol and possibly other related sterols at the masses of the tracer cholesterols.

During the GC-MS analysis of either natural cholesterol or labeled cholesterols, various area responses are observed at the different masses monitored (Fig. 2). Natural cholesterol gives a peak at mass 372 with much smaller but still appreciable peaks at 373 and 374. The distribution and relative amounts of each of these ions (masses 372, 373, and 374) are highly consistent in the same individual. Likewise, the spillover from $[^{13}C]$cholesterol and $[^2H]$cholesterol to other masses is consistent. By using simultaneous equations as described in Methods, it is possible to correct the measured peak areas of $[^{13}C]$cholesterol and $[^2H]$cholesterol for spillover from each other and from endogenous cholesterol. These corrected peak areas are then related to known weighed mixtures of the tracers in a standard curve (Fig. 3). The ratio of tracer cholesterols in an unknown sample can be read from the standard curve. When replicate extractions and GC-MS measurements of the same sample were performed on different days, the coefficient of variation in the computed values of tracer ratios read from standard curves was 1.9%.

Clinical studies

Fig. 4 shows plasma enrichment of cholesterol tracers over time during a standard cholesterol absorption test in

![Fig. 2. GC-MS with selected ion monitoring. In separate runs natural cholesterol, $[^{13}C]$cholesterol, and $[^2H]$cholesterol were subjected to gas chromatography and selected ion monitoring at masses 372, 373, and 374. The data for each cholesterol species are presented as a composite.](https://www.jlr.org/issue/34/1050/)

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which $^{[13]}\text{C}$cholesterol was given intravenously and $^{[2]}\text{H}$cholesterol orally. In contrast to triglyceride absorption which is maximum at 2-5 h, the cholesterol absorption rose gradually (upper panel, open circles) and peaked at 2 days while the intravenous tracer (closed circles) showed a slow decline over time. The lower panel shows that the plasma ratio of tracers became constant at the end of 3 days in this subject. To test the hypothesis that the ratio of oral and intravenous tracers is in fact constant after 3 days, cholesterol absorption was measured at both 3 and 5 days in five normal subjects. The percent cholesterol absorption was $46.3 \pm 6.3\%$ at 3 days and $48.0 \pm 8.1\%$ at 5 days ($P = 0.30$, not significantly different by paired t-test).

Results of cholesterol absorption in 16 healthy adults consuming a Step One diet are presented in Table 1. The mean cholesterol absorption $\pm$ SD was $53.5\% \pm 8.5\%$. There was no statistically significant relationship noted between age, sex, or any of the lipid classes analyzed and percent cholesterol absorption in this small sample size.

To assess the reproducibility of this method over time, identical absorption studies were performed in five healthy subjects who underwent a second cholesterol absorption study after 4-6 weeks (Table 1: subjects 1-5). The mean cholesterol absorption was $59.3 \pm 8.4\%$ for the Test 1 and $57.6 \pm 8.29\%$ for Test 2 (Mean $\pm$ SD). However, variability in the same individual was small so that over time low absorbers remained low absorbers and high absorbers remained high. The standard deviation of the difference between test 1 and 2 was 2.8%, which compares favorably with 3.7% calculated from data reported with the radioactive method (6). There was no difficulty in performing the repetitive cholesterol absorption studies even though a small amount of tracer cholesterol could be detected in plasma at the time of the second study because this background is accounted for in the analysis of a plasma sample obtained prior to the second study.

As previous studies of cholesterol absorption have been performed with $^{[14]}\text{C}$cholesterol, the metabolism of $^{[13]}\text{C}$cholesterol and $^{[14]}\text{C}$cholesterol was directly compared in three individuals who simultaneously received both isotopic cholesterols intravenously on day 0 solubilized in Intralipid (see Methods). On days 3-5 the fraction of the administered dose/mg plasma cholesterol was determined for each tracer and the ratio of these measurements ($^{[13]}\text{C}$cholesterol/$^{[14]}\text{C}$cholesterol) was found to be $1.06 \pm 0.05$ SD. This experiment suggests that there is no difference in the short-term metabolism of cholesterols labeled with these carbon isotopes.

To demonstrate the physiologic equivalence of $^{[2]}\text{H}$cholesterol, 30 mg of $^{[13]}\text{C}$cholesterol and $^{[2]}\text{H}$cholesterol were given orally to a normal subject. Fig. 5, panel A reveals that these tracers rose in parallel, reached a peak in plasma at 2 days, and then slowly declined over the next 4 days. Panel B shows that the ratio of the two tracers in plasma was constant over the 6-day experiment ($^{[2]}\text{H}$cholesterol/$^{[13]}\text{C}$cholesterol $= 1.029 \pm 0.009$), demonstrating that they were biologically equivalent both in absorption from the gastrointestinal tract and in short-term body metabolism.
Fig. 5. Intestinal absorption of labeled cholesterols. A healthy 49-year-old woman was given a meal containing 30 mg \[^{13}\text{C}\]cholesterol (open circles) and 30 mg \[^{2}\text{H}\]cholesterol (open triangles) and plasma was analyzed for 6 days. Panel A: Plasma enrichment of the tracers is expressed relative to the amount of natural cholesterol at mass 372. Panel B: the ratio of \[^{2}\text{H}\]cholesterol/\[^{13}\text{C}\]cholesterol is given.

The only side effect noted by two subjects was slight burning at the intravenous infusion site and this was readily relieved by increasing the rate of infusion of the carrier fluids.

DISCUSSION

The current data demonstrate that mass spectrometry can be a powerful and effective analytical tool in the study of cholesterol absorption. It was possible to detect tracer cholesterols constituting less than 1/2000 of the mass of circulating natural cholesterol and to discriminate between two tracers differing on average by only one mass unit. The analytical technique, gas chromatography–mass spectrometry (GC–MS), uses traditional gas chromatography to separate chemical species and uses the mass spectrometer as a mass-specific chromatographic detector that can record peaks from several selected ions during a single gas chromatographic run (Fig. 2). Because of the great difference in concentration of natural cholesterol and tracer cholesterol in plasma it was not possible to record ion tracings from natural and tracer cholesterol simultaneously without administering unacceptably large doses of the tracers. However, by using heavily labeled tracers and monitoring ions 4–6 mass units above the principal mass of natural cholesterol, tracer ions were easily measured in the presence of natural cholesterol (Fig. 2) while limiting the tracer doses to 15 mg (intravenous) and 30 mg (oral). The mass of the oral dose is not negligible but it is small when compared with a recommended intake of 300 mg cholesterol/day and it is likely that this could be reduced substantially without affecting precision of measurement. The mole ratio of tracer molecules in plasma was determined by comparison to a standard curve run at the same time (Fig. 3). This ratio (corrected for the amount of tracers given) represents the percent absorption of cholesterol.

The labeling of cholesterols with stable isotopes and the use of the resulting tracers in metabolic studies has been reported previously, but complicated clinical protocols were used (8, 10). In particular, the measurement of \[^{13}\text{C}\]cholesterol had to be carried out by isotope ratio mass spectrometry, an extraordinarily precise technique but one that is somewhat cumbersome and requires isolation of pure cholesterol and its combustion to CO₂ before analysis. Deuterium enrichments were measured separately. In contrast, the current GC–MS technique is much simpler in that conventional gas chromatography is performed and the mass spectrometer is used to measure both cholesterol tracers at the same time.

The measurements made here were precise. Repeated analyses of the same sample on different days gave values with a coefficient of variation of 1.9%, indicating that sample preparation and instrument response were not responsible for large errors. Biological variability was assessed by making duplicate measurements 4–6 weeks apart in five individuals under standardized clinical conditions with the same test meal (Table 1). No significant difference in the mean level of cholesterol absorption was observed and the standard deviation of the inter-test differences was only 2.8 percentage points. Because this is the critical value used in calculating the power of a clinical study, it is clear that the stable isotope method could be useful in repeated measurements of cholesterol absorption and such studies carry no cumulative radioactive hazard. It is expected that GC–MS would give precision equivalent to or greater than radioactive techniques because only weights and mass spectrometric ion peak areas are used in calculations, and the observed replicability supports this postulate. The mean value of 54% absorption in Table 1 is similar to values of 45–60% reported for studies that used radioactive isotopes (3–7, 11–13).

The \[^{1}\text{H}\] and \[^{2}\text{H}\]cholesterol tracers behaved identically over the time course of these studies (Fig. 5). In addition, there was no difference in the metabolism of \[^{1}\text{C}\] and \[^{1}\text{C}\]cholesterol. Thus, we conclude that the tracers used accurately reflect the metabolic pathways of natural...
Cholesterol. Previous work has shown that certain preparations of tritiated cholesterol labeled in the potentially labile 1, 2 or 24, 25 positions are lost from plasma more quickly than \( ^{[14}\text{C}]\)cholesterol and have been referred to as “radiochemically unreliable” (14, 15). The basis for this observation has not been determined, but it is possible that biological exchange of hydrogen for tritium (especially \( ^{2}\text{H} \)) and/or radiation damage to the cholesterol molecule before injection are responsible. Both of these difficulties are unlikely to affect our \([2\text{H}]\)cholesterol because the labeling is on stable terminal methyl groups (Fig. 1) and radioactive decay does not occur.

Although the results reported here are encouraging, the limitations of the Zilversmit technique (regardless of the isotopes employed) should be appreciated. The method measures only the percent of cholesterol absorbed rather than the absolute amount, and it is likely that regulation of body cholesterol metabolism will depend on the latter.

In addition, it measures cholesterol absorption at only a single time point. While this may be an advantage in that percent cholesterol absorption can be determined under standardized and well-defined conditions, it is also a potential disadvantage in that more data are needed to ascertain whether the single measurement made is representative of percent cholesterol absorption over the entire day and with different types of foods. Preliminary data suggest that the composition of the meal in which the labeled cholesterol is given is unimportant as long as gallbladder contraction occurs and that measurements made from a single oral dose of isotope are comparable to those in which isotope is given with each meal over the course of the day (5). Finally, the applicability of the Zilversmit technique in subjects with disordered bile acid metabolism or bowel disease is uncertain. However, we believe that stable isotopic techniques can play a key role in overcoming these limitations. Absolute cholesterol absorption, the product of percent cholesterol absorption and the intestinal cholesterol flux, requires additional information that might be obtained by combining the present method with a measure of biliary cholesterol secretion obtained by intubation (16, 17) or by chronic dual labeling of plasma and dietary cholesterol followed by measurement of the ratio of the two isotopic cholesterols in stool as previously reported for radioactive isotopes (18).

Current work is underway to study these complementary aspects of cholesterol absorption using mass spectrometric methods.

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*REFERENCES*


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