Validation of a single-isotope-labeled cholesterol tracer approach for measuring human cholesterol absorption

Wang, Yanwen; Vanstone, Catherine A.; Parsons, William D.; Jones, Peter J. H.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1007/s11745-004-1206-6
Lipids, 39, January 1, pp. 87-91, 2004-01

Validation of a Single-Isotope-Labeled Cholesterol Tracer Approach for Measuring Human Cholesterol Absorption

Yanwen Wang, Catherine A. Vanstone, William D. Parsons, and Peter J.H. Jones*

School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

ABSTRACT: Cholesterol absorption is frequently determined using the plasma dual stable-isotope ratio method (PDSIRM). However, this method involves intravenous injection of stable-isotope-labeled cholesterol with simultaneous oral administration of differently labeled cholesterol, which results in high study costs and involves additional ethical considerations. The objective of the present study was to validate a simpler single-isotope method for determining cholesterol absorption against PDSIRM by using data from two previous studies. Enrichments of carbon-13 (^{13}C) and deuterium in red blood cells were analyzed by using differential isotope ratio MS. The area under the curve of ^{13}C-enrichment in the plasma free-cholesterol pool was found to be significantly correlated with cholesterol absorption measured by using PDSIRM for study 1 ($r = 0.85$, $P < 0.0001$) and study 2 ($r = 0.81$, $P < 0.0001$). Average ^{13}C-enrichment correlated with the area under the curve of ^{13}C-enrichment in the plasma free cholesterol for both study 1 ($r = 0.98$, $P < 0.0001$) and study 2 ($r = 1.00$, $P < 0.0001$). Study 1 examined the efficacy and mechanisms of unesterified plant sterols and stanols on lipid profiles in hypercholesterolemic men and women, while study 2 investigated the effects of phytosterol vs. phytostanol esters on plasma lipid levels and cholesterol kinetics in hyperlipidemic men. Experimental approaches to determine cholesterol absorption were identical between the two studies. Consequently, in both studies, correlations ($r = 0.88$, $P < 0.0001$ for study 1, and $r = 0.82$, $P < 0.0001$ for study 2) were found between the average ^{13}C-enrichment of plasma free cholesterol and cholesterol absorption measured by PDSIRM. These results suggest that a single-isotope-labeled cholesterol tracer approach can be used as a reliable noninvasive method to replace PDSIRM for examining changes in cholesterol absorption.

Paper no. L9373 in *Lipids 39*, 87–91 (January 2004).

Dietary cholesterol and the large enterohepatic recirculation of endogenous cholesterol readily mix to form a single pool of intestinal cholesterol (1–3). Cholesterol absorption from the gastrointestinal tract is a key component of whole-body cholesterol metabolism. Measurement of cholesterol absorption provides important insights into the relationships among diet and plasma cholesterol levels, cholesterol homeostasis, genetic variations, and drug effects. For this purpose, a variety of different methods have been developed for estimating cholesterol absorption in humans (4,5). Presently, three methods exist for measuring cholesterol absorption: the plasma dual-isotope ratio method (6–8), the fecal dual-isotope ratio method (9), and mass (sterol) balance (10). The simplest of these methods is the plasma dual-isotope ratio method originally introduced by Zilversmit (6).

In 1993, Lutjohann *et al.* (4) introduced the use of stable isotopes into the fecal dual-isotope ratio method for measuring cholesterol absorption. In the same year, Bosner *et al.* (11) developed a plasma dual stable-isotope-ratio method (PDSIRM) to measure cholesterol absorption, based on the plasma dual-isotope ratio method developed by Zilversmit (6). This modified method has been used extensively (12–17). Although this technique can measure cholesterol absorption with good precision and accuracy, it involves intravenous injection of labeled cholesterol. As such, the technique increases study costs dramatically, involves an invasive procedure, and requires additional ethical considerations.

In PDSIRM, the amount of labeled cholesterol absorbed is calculated by the enrichment of orally administered and intravenously injected cholesterol tracers. Isotope enrichment in the cholesterol pool following an oral dose of single-isotope-labeled cholesterol is believed to be indicative of the cholesterol absorption rate (18). However, the relationship between the isotope enrichment of plasma free cholesterol following an oral dose of single-isotope-labeled cholesterol and cholesterol absorption remains to be established as a noninvasive method to compare relative changes in cholesterol absorption. It was hypothesized that the area under the isotope enrichment curve, or the average isotope enrichment, in the plasma free-cholesterol pool following an oral dose of single-isotope-labeled cholesterol tracer correlates with the cholesterol absorption rate. The objective of the current study was to devise a method that would simplify the process of measuring cholesterol absorption in humans.

MATERIALS AND METHODS

Study protocols. Data from two published studies (14,15) were analyzed for the correlations between the area under the curve of a single-isotope enrichment and the cholesterol absorption rate, as measured by PDSIRM. Correlations between the measured cholesterol absorption rate and the average enrichment of the single isotope were also conducted. Experimental approaches to determine cholesterol absorption were identical between the two studies. Study 1 involved 15 otherwise healthy

*To whom correspondence should be addressed at School of Dietetics and Human Nutrition, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9. E-mail: jonesp@macdonald.mcgill.ca

Abbreviations: IRMS, isotope ratio MS; PDB, Pee Dee belemnite; PDSIRM, plasma dual stable-isotope ratio method; SMOW, Standard Mean Ocean Water.

hypercholesterolemic subjects (9 male, 6 female; 35–58 yr) who had plasma total cholesterol concentrations in the range of 5.2–9.0 mmol/L and TAG concentrations <3.5 mmol/L. Subjects completed each of four dietary treatments in a randomized crossover double-blind design. The four diets were comprised of solid foods typical of those consumed in North America, and contained 1.8 g/d of cornstarch (control), plant sterols, plant stanols, and a 50:50 mixture of sterols and stanols, respectively. Cholesterol concentrations of the diets were not measured but were calculated to provide approximately 300 mg cholesterol/d, i.e., within the range of typical North American diets. Each treatment phase consisted of a 21-d feeding period and was separated by a 4-wk washout. Ninety-six hours before the end of each phase, a baseline blood sample was drawn prior to subjects receiving an intravenous injection of 15 mg D_7-cholesterol and a 75-mg oral dose of ^{13}C-cholesterol (CDN Isotopes, Pointe-Claire, Quebec, Canada).

Study 2 contained 15 healthy hyperlipidemic males (37–61 yr) who had fasting plasma total cholesterol concentrations in the range of 6.0–10.0 mmol/L and TAG concentrations <3.0 mmol/L. Using a randomized crossover double-blind design, subjects were assigned to one of three typical North American solid-food diets that contained margarine alone, margarine with 8% (w/w based on free sterol content) plant sterol esters, and 8% (w/w based on free stanol content) plant stanol esters, respectively. Cholesterol concentrations of the diets were calculated to provide approximately 300 mg cholesterol/d. Each dietary phase consisted of 21 feeding days followed by a 5-wk washout. Ninety-six hours before the end of each phase, subjects were intravenously injected with 15 mg of D_7-cholesterol and simultaneously ingested 90 mg of ^{13}C-cholesterol.

Determination of cholesterol absorption. Cholesterol absorption was determined in both studies using PDSIRM following the procedure of Bosner *et al.* (11). Briefly, lipids were extracted from red blood cells in duplicate using a modified Folch extraction procedure (19). Free cholesterol was purified by TLC and transferred into combustion tubes each containing 0.5 g cupric oxide, and sealed under vacuum. After combustion at 520°C for 4 h, ^{13}C-enriched CO_2 was vacuum-distilled into sealed tubes; D-enriched water was transferred under vacuum into tubes containing 0.06 g zinc. Tubes containing water and zinc were reduced to D-enriched H_2 gas by combusting at 520°C for 30 min. The ^{13}C-enrichment in CO_2 and D-enrichment in H_2 gas were measured by differential isotope ratio MS (IRMS). Enrichments were expressed relative to the Pee Dee belemnite (PDB) limestone standard of the National Bureau of Standards) (NBS).

The ratio of ingested ^{13}C-cholesterol to injected D_7-cholesterol enrichment relative to baseline samples ($t = 0$) in the plasma free cholesterol after 48 and 72 h was taken as an indicator of the cholesterol absorption rate:

$$\text{absorption (pool/pool)} = \frac{\Delta^{13}\text{C} \times 7 \times 27 \times \text{i.v. dose of D}_7\text{-cholesterol} \times 0.0112}{\Delta\text{D} \times 2 \times 46 \times \text{i.g. dose of } ^{13}\text{C-cholesterol} \times 0.000155} \quad [1]$$

where Δ (‰) for ^{13}C and D is the difference between the enriched sample at 48 or 72 h and the baseline abundance (at $t = 0$) in parts per thousand relative to PDB and Standard Mean Ocean Water (SMOW) standards, respectively. The factors 7:46 and 2:27 reflect the ratios of labeled to unlabeled hydrogen and carbon atoms in the cholesterol tracers, respectively. The constants 0.0112 and 0.000155 represent factors converting the parts-per-thousand units to the equivalent atom percent excess for the PDB and SMOW scales, respectively.

Correlation analysis. The average ^{13}C-enrichment and area under the curve of ^{13}C-enrichment in plasma free cholesterol during a 24–96 h period following the oral dose of ^{13}C-cholesterol were calculated for each subject during each phase. Data from the four treatments in study 1 and the three treatments in study 2 were pooled, respectively. The correlations among cholesterol absorption as measured by PDSIRM, the area under the ^{13}C-enrichment curve, and the average ^{13}C-enrichment were analyzed using Pearson correlations. The same method also was used to analyze the relationship between the area under the ^{13}C-enrichment curve and the average ^{13}C-enrichment in plasma free cholesterol during a 24–96 h period after an oral dose of ^{13}C-cholesterol. Separate analyses were performed for each study.

RESULTS

Study 1. The correlation between the measured cholesterol absorption rate and the area under the curve of ^{13}C-enrichment is presented in Figure 1. The correlation between the measured cholesterol absorption rate and the average ^{13}C-enrichment in the plasma free-cholesterol fraction is presented in Figure 2. We found that the area under the curve of ^{13}C-enrichment in plasma free cholesterol over a 24–96 h period following an oral dose of ^{13}C-cholesterol was correlated ($r = 0.85$, $P < 0.0001$) with the cholesterol absorption rate as measured by PDSIRM. Similarly, the average ^{13}C-enrichment in plasma free cholesterol was also correlated ($r = 0.88$, $P < 0.0001$) with the measured cholesterol absorption rate. A correlation was observed between the area

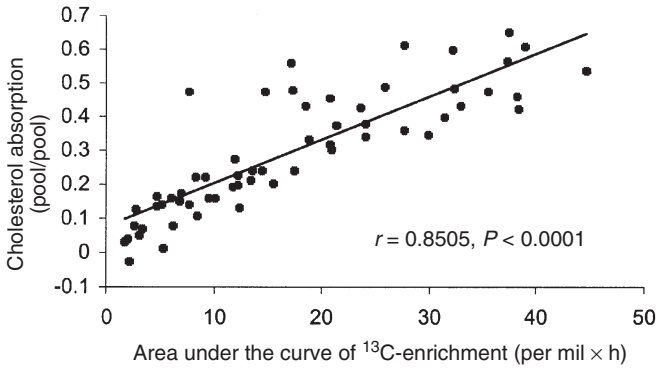

FIG. 1. Relationship between the area under the curve of ^{13}C-enrichment in the cholesterol pool [per thousand atom excess (per mil) × h] and the cholesterol absorption rate (pool/pool) measured using the plasma dual stable-isotope ratio method in study 1. Data were analyzed using a Pearson correlation.

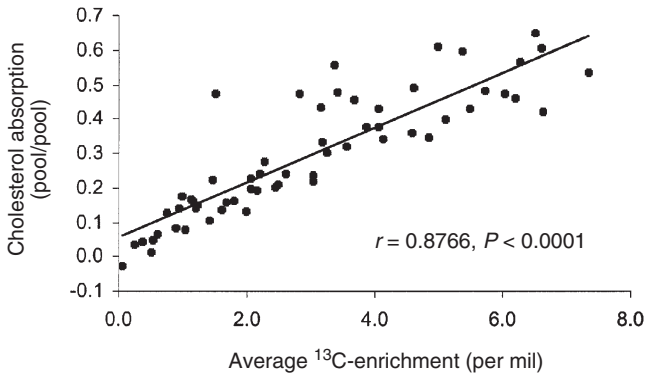

FIG. 2. Relationship between the average ^{13}C-enrichment in the cholesterol pool (per mil) and cholesterol absorption as measured using the plasma dual stable-isotope ratio method in study 1. Data were analyzed using a correlation.

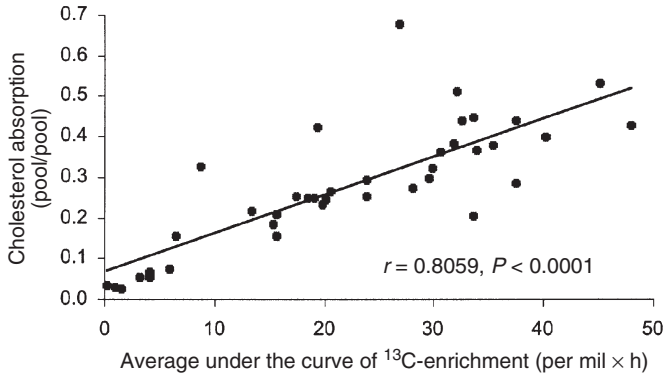

FIG. 4. Relationship between the area under the curve of ^{13}C-enrichment in the plasma cholesterol pool (per mil × h) and the cholesterol absorption rate (pool/pool) as measured using the plasma dual stable-isotope ratio method in study 2. Data were analyzed using a Pearson correlation.

under the curve of ^{13}C-enrichment and the average ^{13}C-enrichment in the plasma free-cholesterol pool after an oral dose of ^{13}C-cholesterol (Fig. 3). The correlation coefficient was 0.99 ($P < 0.0001$).

Study 2. Identical analyses were performed for the data in study 2. As shown in Figure 4, the area under the curve of ^{13}C-enrichment in plasma free cholesterol during a period of 24–96 h following an oral dose of ^{13}C-cholesterol was correlated ($r = 0.82$, $P < 0.0001$) with the cholesterol absorption rate as measured by PDSIRM. The average ^{13}C-enrichment in plasma was also correlated ($r = 0.81$, $P < 0.0001$) with the measured cholesterol absorption rate (Fig. 5). The area under the curve of ^{13}C-enrichment was significantly correlated with the average ^{13}C-enrichment in the plasma free-cholesterol pool after an oral dose of ^{13}C-cholesterol (Fig. 6), with a correlation coefficient of 1.00 ($P < 0.0001$).

DISCUSSION

PDSRIM has been used over the years to measure the cholesterol absorption rate. However, the high cost and ethical considerations of this technique limit its use in human studies. It is very important to devise a less expensive and noninvasive alternative to PDSRIM to compare the changes in cholesterol absorption. Results of the present study suggest that either the area under the ^{13}C-enrichment curve or the average ^{13}C-enrichment during a 24–96-h period following oral ^{13}C-cholesterol administration could be used as a reliable index to compare the differences or changes in cholesterol absorption.

The average ^{13}C-enrichment was found to be correlated with the area under the curve of ^{13}C-enrichment in a 4-d period following the oral dose of ^{13}C-cholesterol, so it was not surprising that the average ^{13}C-enrichment also showed a significant correlation with cholesterol absorption measured by PDSIRM, as did the area under the curve of ^{13}C-enrichment. Integration of the area under the curve of ^{13}C-enrichment is more complicated than calculating the average ^{13}C-enrichment with the use of an integrating program. For example, if statistical software is chosen to integrate the area under the enrichment curve, understanding the integration procedure and the related commands is essential to complete this task. For this reason, calculating the average ^{13}C-enrichment has advantages over calculating the area under the curve of ^{13}C-enrichment for comparing relative changes in the cholesterol absorption rate.

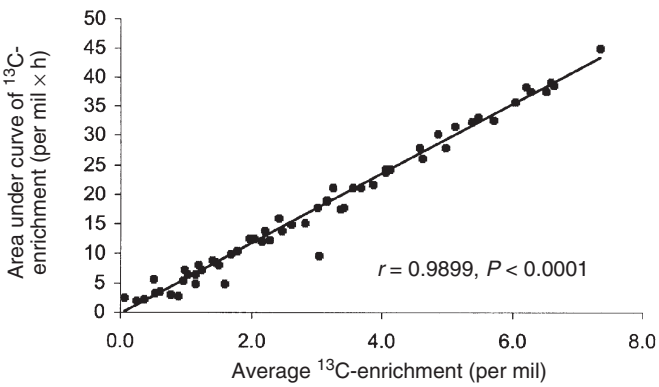

FIG. 3. Correlation between the average ^{13}C-enrichment (per mil) and the area under the curve of ^{13}C-enrichment of plasma free cholesterol (per mil × h) in study 1. Data were analyzed using a Pearson correlation.

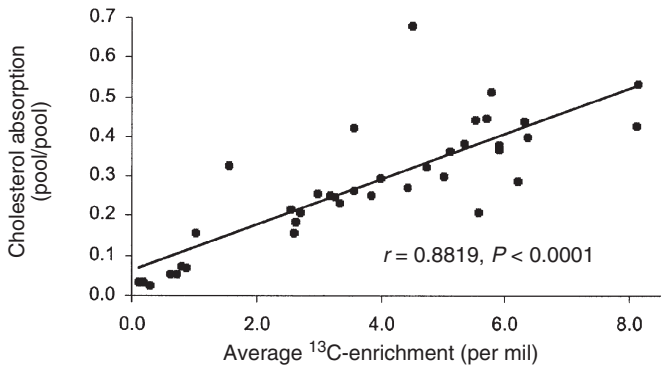

FIG. 5. Relationship between the average ^{13}C-enrichment in the cholesterol pool (per mil) and cholesterol absorption as measured using the plasma dual stable-isotope ratio method in study 2. Data were analyzed using a Pearson correlation.

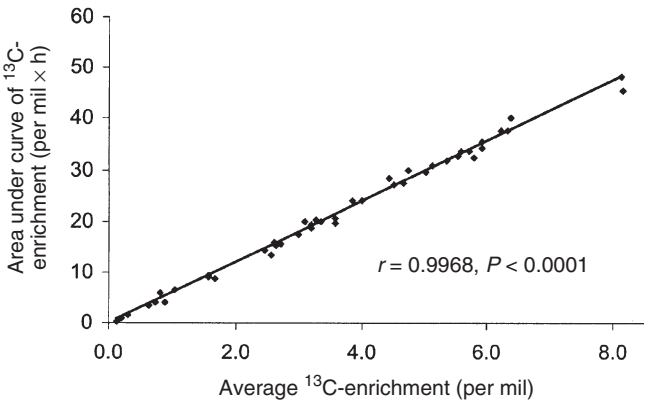

FIG. 6. Correlation between the average ^{13}C-enrichment (per mil) and the area under the curve of ^{13}C-enrichment in the plasma free-cholesterol pool (per mil × h) in study 2. Data were analyzed using a Pearson correlation.

It is well-documented that intestinal cholesterol absorption is a major determinant of whole-body cholesterol homeostasis and plasma LDL cholesterol levels (2,3,20–22), particularly in Western populations that consume significant quantities of cholesterol. Despite the extensive body of literature, important questions remain regarding the measurement of human cholesterol absorption. These shortcomings in our understanding of cholesterol metabolism are, at least in part, due to methodological limitations (11). As such, investigators have developed a wide range of isotope tracer methods to study cholesterol absorption (10,23). However, the methods that were developed initially relied on radioisotope tracers, thus limiting their application in human studies. Bosner *et al.* (11) developed a method that applied stable isotope techniques and MS, and this method was widely used in later studies that investigated the cholesterol absorption rate (14,15,24,25). Although this technique measures cholesterol absorption with good precision and accuracy, it involves both oral administration and intravenous injection of labeled cholesterol. Thus, the use of this technique is limited by the high cost and ethical considerations.

Many studies have measured the cholesterol absorption rate to compare treatment effects on the efficiency of dietary cholesterol absorption in the intestine (11,14,15,24,25). In these studies, the contribution of absorbed cholesterol from dietary sources to the body's cholesterol pool has not been calculated. In fact, most studies have compared the effects of dietary treatment(s) on cholesterol absorption instead of the exact proportion of absorbed cholesterol that contributes to the body's cholesterol pool. In these kinds of investigations, either the average enrichment or the area under the enrichment curve of a single stable isotope in a 4-d period following an oral dose of the single-isotope-labeled cholesterol tracer can be used as a reliable method to achieve the goals of these experiments.

It is evident that the single-isotope-labeled cholesterol tracer method also has limitations in its application. After absorption, the concentration of cholesterol tracer is quickly diluted as it mixes with the body's cholesterol pool. Given an amount of absorbed isotope-labeled cholesterol, the body's cholesterol pool size becomes a key factor that affects the tracer concentration, i.e., a larger cholesterol pool makes the tracer concentration lower, and vice versa. Therefore, the use of a single-stable-isotope cholesterol tracer method to compare cholesterol absorption is based on the assumption that subjects in each treatment have the same cholesterol pool size or baseline. For a crossover design with a washout period, the same subjects go through each treatment. The cholesterol pool size of each subject is assumed to have returned to the initial baseline before commencing the next treatment. Therefore, a single-stable-isotope cholesterol tracer is a reliable indictor to compare the changes caused by different treatments in a crossover design. For a parallel-arm study design, the single-isotope-labeled cholesterol tracer method may be less sensitive because subjects have different body cholesterol pool sizes, although the groups were adjusted by complete randomization of subjects to have no significant difference in their cholesterol pool sizes before receiving different treatments. For this type of study, influence of the body's cholesterol pool size on the accuracy of measurements of the single-stable-isotope cholesterol tracer can be minimized by including the initial cholesterol pool size as a covariant.

If enrichment of the single-isotope cholesterol tracer is used to calculate the percent cholesterol absorption rate, many assumptions have to be made that reduce data reliability and compatibility. These assumptions include the following: (i) that absorbed cholesterol tracers are entirely incorporated into the body's cholesterol pool; (ii) that the absorbed cholesterol tracers are completely equilibrated in the cholesterol pool; (iii) that the cholesterol pool is measured accurately; and (iv) that no significant amount of cholesterol tracers has decayed or cleared out of the cholesterol pool over a period of 24–96 h after oral administration. Therefore, the authors consider it better to use ^{13}C-enrichment to compare differences in cholesterol absorption between treatments or treatment effects relative to a control.

In summary, the area under the ^{13}C-enrichment curve or the average ^{13}C-enrichment is significantly correlated with the cholesterol absorption rate as measured by PDSIRM. It is suggested that either average ^{13}C-enrichment or the area under the curve of ^{13}C-enrichment can be used as an index of cholesterol absorption. The single-stable-isotope cholesterol tracer method has advantages over PDSIRM, as it is simpler, noninvasive, and less expensive. However, this method can be used only to compare the changes in cholesterol absorption. When measurement of the absolute cholesterol absorption rate becomes essential, PDSIRM should be used.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Fady Y. Ntanios, Mahmoud Raeini-Sarjaz, and Jian Y. Feng for their contributions to the data used in this paper.

REFERENCES

1. Grundy, S.M. (1983) Absorption and Metabolism of Dietary Cholesterol, *Annu. Rev. Nutr. 3*, 71–96.
2. Wilson, M.D., and Rudel, L.L. (1994) Review of Cholesterol Absorption with Emphasis on Dietary and Biliary Cholesterol, *J. Lipid Res. 35*, 943–955.
3. Carter, D., Howlett, H.C., Wiernsperger, N.F., and Bailey, C.J. (2003) Differential Effects of Metformin on Bile Salt Absorption from the Jejunum and Ileum, *Diabetes Obes. Metab. 5*, 120–125.
4. Lutjohann, D., Meese, C.O., Crouse, J.R., and von Bergmann, K. (1993) Evaluation of Deuterated Cholesterol and Deuterated Sitostanol for Measurement of Cholesterol Absorption in Humans, *J. Lipid Res. 34*, 1039–1046.
5. Pouteau, E., Piguet-Welsch, C., Berger, A., and Fay, L.B. (2003) Determination of Cholesterol Absorption in Humans: From Radiolabel to Stable Isotope Studies, *Isotopes Environ. Health Stud. 39,* 247–257.
6. Zilversmit, D.B. (1972) A Single Blood Sample Dual Isotope Method for the Measurement of Cholesterol Absorption in Rats, *Proc. Soc. Exp. Biol. Med. 140*, 862–865.
7. Wang, D.Q., Lammert, F., Cohen, D.E., Paigen, B., and Carey, M.C. (1999) Cholic Acid Aids Absorption, Biliary Secretion, and Phase Transitions of Cholesterol in Murine Cholelithogenesis, *Am. J. Physiol. 276*, G751–G760.
8. Wang, D.Q., Paigen, B., and Carey, M.C. (2001) Genetic Factors at the Enterocyte Level Account for Variations in Intestinal Cholesterol Absorption Efficiency Among Inbred Strains of Mice, *J. Lipid Res. 42*, 1820–1830.
9. Borgstrom, B. (1968) Quantitative Aspects of the Intestinal Absorption and Metabolism of Cholesterol and β-Sitosterol in the Rat, *J. Lipid Res. 9*, 473–481.
10. Grundy, S.M., and Ahrens, E.H., Jr. (1969) Measurements of Cholesterol Turnover, Synthesis, and Absorption in Man, Carried Out by Isotope Kinetic and Sterol Balance Methods, *J. Lipid Res. 10*, 91–107.
11. Bosner, M.S., Ostlund, R.E., Jr., Osofisan, O., Grosklos, J., Fritschle, C., and Lange, L.G. (1993) Assessment of Percent Cholesterol Absorption in Humans with Stable Isotopes, *J. Lipid Res. 34*, 1047–1053.
12. Harris, W.S., Windsor, S.L., Newton, F.A., and Gelfand, R.A. (1997) Inhibition of Cholesterol Absorption with CP-148,623 Lowers Serum Cholesterol in Humans, *Clin. Pharmacol. Ther. 61*, 385–389.
13. Ntanios, F.Y., and Jones, P.J. (1999) Dietary Sitostanol Reciprocally Influences Cholesterol Absorption and Biosynthesis in Hamsters and Rabbits, *Atherosclerosis 143*, 341–351.
14. Jones, P.J., Raeini-Sarjaz, M., Ntanios, F.Y., Vanstone, C.A., Feng, J.Y., and Parsons, W.E. (2000) Modulation of Plasma Lipid Levels and Cholesterol Kinetics by Phytosterol Versus Phytostanol Esters, *J. Lipid Res. 41*, 697–705.
15. Vanstone, C.A., Raeini-Sarjaz, M., Parsons, W.E., and Jones, P.J.(2002) Unesterified Plant Sterols and Stanols Lower LDL-Cholesterol Concentrations Equivalently in Hypercholesterolemic Persons, *Am. J. Clin. Nutr. 76*, 1272–1278.
16. Woollett, L.A., Buckley, D.D., Yao, L., Jones, P.J., Granholm, N.A., Tolley, E.A., and Heubi, J.E. (2003) Effect of Ursodeoxycholic Acid on Cholesterol Absorption and Metabolism in Humans, *J. Lipid Res. 44*, 935–942.
17. Pouteau, E.B., Monnard, I.E., Piguet-Welsch, C., Groux, M.J., Sagalowicz, L., and Berger, A. (2003) Non-esterified Plant Sterols Solubilized in Low Fat Milks Inhibit Cholesterol Absorption: A Stable Isotope Double-Blind Crossover Study, *Eur. J. Nutr. 42*, 154–164.
18. Ostlund, R.E., Jr., Spilburg, C.A., and Stenson, W.F. (1999) Sitostanol Administered in Lecithin Micelles Potently Reduces Cholesterol Absorption in Human Subjects, *Am. J. Clin. Nutr. 70*, 826–831.
19. Folch, J., Lees, M., and Sloane Stanley, G.H. (1957) A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*, 497–509.
20. Dietschy, J.M., Turley, S.D., and Spady, D.K. (1993) Role of Liver in the Maintenance of Cholesterol and Low Density Lipoprotein Homeostasis in Different Animal Species, Including Humans, *J. Lipid Res. 34*, 1637–1659.
21. Rudel, L., Deckelman, C., Wilson, M., Scobey, M., and Anderson, R. (1994) Dietary Cholesterol and Downregulation of Cholesterol 7 α-Hydroxylase and Cholesterol Absorption in African Green Monkeys, *J. Clin. Invest. 93*, 2463–2472.
22. Gylling, H., and Miettinen, T.A. (1995) The Effect of Cholesterol Absorption Inhibition on Low Density Lipoprotein Cholesterol Level, *Atherosclerosis 117*, 305–308.
23. Quarfordt, S.H. (1977) Methods for the *in vivo* Estimation of Human Cholesterol Dynamics, *Am. J. Clin. Nutr. 30*, 967–974.
24. Bosner, M.S., Lange, L.G., Stenson, W.F., and Ostlund, R.E., 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.
25. Gremaud, G., Dalan, E., Piguet, C., Baumgartner, M., Ballabeni, P., Decarli, B., Leser, M.E., Berger, A., and Fay, L.B. (2002) Effects of Non-esterified Stanols in a Liquid Emulsion on Cholesterol Absorption and Synthesis in Hypercholesterolemic Men, *Eur. J. Nutr. 41*, 54–60.

[Received August 22, 2003; accepted December 18, 2003]