Journal of Clinical Lipidology (2015) 9, 14–25

Journal of Clinical Lipidology

Review Article

Fractional cholesterol absorption measurements in humans: Determinants of the blood-based dual stable isotope tracer technique

Frans Stellaard, PhD*, Dieter Lütjohann, PhD

Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands (Dr Stellaard); and Institute for Clinical Chemistry and Clinical Pharmacology, University Clinics, Bonn, Germany (Dr Lütjohann)

KEYWORDS:
Cholesterol absorption;
^{13}C;
^{2}H;
^{18}O;
Isotope;
Mass spectrometry;
Stable isotope

BACKGROUND: The flux of absorbed cholesterol is a controlling element in the regulation of cholesterol biosynthesis and catabolism. A review of 5 published methods to measure cholesterol absorption is presented, including 2 dual stable isotope approaches. The continuous dual isotope feeding procedure is accurate, but only suitable for small-scale studies. The blood-based dual stable isotope technique is the least invasive and complex procedure, but leads to large variations in individual (<10%–>90%) and mean population values (24%–70%) for healthy subjects. The results may be partly determined by the experimental and analytical procedures.

SOURCES OF MATERIAL: Fifteen blood-based dual stable isotope studies published between 1993 and 2013 have been analyzed. The results were related to the methodologies applied and evidence was sought for accordance to the test principles.

FINDINGS: Seven different isotopic tracers, 3 cholesterol subcompartments in blood, and 6 mass spectrometry techniques were applied. The oral and intravenous test formulations were presented in only 1 study. Time points for blood sampling and methodologies for blood sample preparation and analysis were highly variable. No definite proofs were supplied for the fates of the oral and intravenous cholesterol tracers. Isotope enrichment measurements in free and total cholesterol in plasma and erythrocytes were never compared. Fractional cholesterol absorption rate values depend strongly on the mass spectrometry methodology. Dual-inlet isotope ratio mass spectrometry appears to be the method of choice.

CONCLUSIONS: Dual stable isotope approaches require validation and standardization of administration and analysis procedures. A control group must always be included to correct for methodological differences.

Intestinal cholesterol absorption is an important multistep process in cholesterol homeostasis. Cholesterol entering the intestine is derived from 3 sources (ie, dietary cholesterol, biliary cholesterol, and blood cholesterol excreted directly into the intestine [trans-intestinal cholesterol excretion]).[1] The latter option is not proven in humans, however. The flux of absorbed cholesterol is a

Conflict of interest: none.

* Corresponding author. University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail address: f.stellaard01@umcg.nl

Submitted February 24, 2014. Accepted for publication November 5, 2014.

1933-2874/$ - see front matter © 2015 National Lipid Association. All rights reserved.
http://dx.doi.org/10.1016/j.jacl.2014.11.002

controlling element in the endogenous biosynthesis and catabolism of cholesterol, finally regulating blood cholesterol levels. Patients with high fractional cholesterol absorption (FCA) may be on increased risk for hypercholesterolemia and cardiovascular disease,[2] in particular when cholesterol biosynthesis and catabolism are not properly regulated. According to recent guidelines,[3] hypercholesterolemic patients are treated with statins to reduce endogenous cholesterol synthesis, bile acid–binding resins to stimulate cholesterol catabolism, or ezetimibe to reduce cholesterol absorption. It has been shown in animal models that the Niemann-Pick C1-like 1 sterol transporter is responsible for intestinal cholesterol uptake, whereas the combined action of the adenosine triphosphate–binding cassette transporters Abcg5 and Abcg8 remove excess cholesterol from the enterocyte into the intestinal lumen.[4,5] Intensive research in experimental animals focuses on the mechanisms of cholesterol absorption to establish other targets for drug treatment. The drug ezetimibe has already been developed and applied successfully to patients to decrease cholesterol uptake[6] by a competitive interaction with the Niemann-Pick C1-like 1. The ezetimibe-induced reduction of cholesterol absorption in humans could be documented by using isotope labeling.[7] A combination therapy including statins and ezetimibe is most effective[8] because enhanced cholesterol synthesis is induced when absorption is down-regulated. Also, plant sterols/stanols are added to the diet to competitively reduce cholesterol uptake.[9] Pathologically altered cholesterol absorption has been indicated in certain ethnic groups such as the Tarahumara Indians,[10,11] small intestinal diseases such as celiac disease,[12] and improvement occurred with a gluten-free diet. Decreased cholesterol absorption has also been described in inflammatory bowel disease,[13] in particular active Crohn's disease,[14] and after partial ileal bypass operation.[15,16] To document altered cholesterol absorption or beneficial effects after drug treatment, accurate analytical techniques must be available. The clinical and pharmaceutical studies mentioned previously used various different techniques measuring the fractional cholesterol absorption. Besides differences observed between pathological states and health or treated patients vs untreated patients, a large variation in individual FCA values are observed for healthy subjects and even between mean values of healthy populations of different studies. Are these inter-individual and group differences solely determined by the individuals or also by the applied methodologies?

In the following section, we present an overview of the potentials and drawbacks of available methods. One method, indicated as the blood-based dual stable isotope techniques is by far the simplest in performance and requires collection and analysis of only 2 blood samples. Its simplicity makes it the method of choice. This article analyzes the certainties and uncertainties of the method of choice.

Overview of available methods

A gold standard method producing absolute values for cholesterol absorption does not exist. Methods available to measure cholesterol absorption in humans are mostly relative methods resulting in a fractional cholesterol absorption rate expressed as a percentage of an administered dose. The available methods all have their advantages and disadvantages. They have different degrees of invasiveness and complexity in performance and/or analysis. Some excellent reviews have already dealt with the characteristics of the methods.[17–19] In principle, all methods apply isotope labeled sterols. In 1969 Grundy et al labeled the endogenous cholesterol pool[20] applying an intravenous (IV) dose of [^{14}C] or [^{3}H]cholesterol. The fecal recovery of endogenous cholesterol as neutral sterols (cholesterol and its bacterial degradation products coprostanol and coprostanone) and acidic sterols (bile acids) was measured as the endogenous cholesterol turnover rate. In the steady-state situation, daily bile acid synthesis equals fecal bile acid excretion. The sum of daily cholesterol intake + synthesis = daily fecal excretion of cholesterol-derived sterols + bile acids. Through the isotopic part of the study, the endogenous cholesterol turnover rate was measured. Subtraction of this part from the total cholesterol output leads to a value for the diet-derived cholesterol output. The fraction dietary input–diet-derived output/dietary input reflects the fractional cholesterol absorption. Cholesterol intake is known to be underestimated in the situation where patients have to register food intake. Controlled feeding during the study period is cumbersome. Measurement of fecal neutral and acid sterol output requires 3 days of collecting feces during the end of the study week and particular analytical skills. This approach has been used to obtain general information on cholesterol metabolism and is overdone when only cholesterol absorption is the target of analysis.

Strict isotopic methods for specific measurement of cholesterol absorption employ the oral administration of isotope labeled cholesterol and measurement of the absorbed fraction in blood or the nonabsorbed fraction in feces. The applied isotopes were radioactive isotopes (^{3}H, ^{14}C) in the past, and are stable, nonradioactive isotopes (^{2}H, ^{13}C, ^{18}O) today. One isotopic method originally described by Grundy et al in 1977 may be considered the only direct method. It is based on administration of radiolabeled [4-^{14}C]cholesterol or [1,2-^{3}H]cholesterol via a triple lumen intestinal perfusion tube and the measurement of the amount of labeled cholesterol that is recovered unabsorbed after traveling a predefined distance through the proximal small intestine.[21] To correct for possible transformations during the intestinal passage, unlabeled β-sitosterol was introduced as a minor absorbable marker. Fractional absorption rates between 19% and 77% were obtained for healthy subjects within a single study. No follow-up studies were performed to compare values for groups of healthy