Cholesterol Absorption and Synthesis Biomarkers

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Disclosures (Last 12 months)
- Consultant or Advisory Board
  - Abbott Labs
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  - Genetech (The Roche Group)
  - Genzyme
  - Glaxo Smith Kline (GSK)
  - Health Diagnostic Labs
  - Kowa & Eli Lilly
  - Merck
  - Omthera

Lecture Bureau
- Abbott
- GSK
- HD Lab
- Kowa/Lilly
- Merck

Steranes, Sterols, Steroids

- Sterols, also known as steroid alcohols, are steranes with a hydroxy group at the 3 position of the A-ring. They are a group of compounds which are essential components of cell membranes in both plants and animals.
- Endogenous or exogenous sterols (fungal) that are not cholesterol are referred to as noncholesterol sterols of xenosterols.
- Plant sterols (phytosterols) which are structurally similar to cholesterol, are not synthesized by the human body and are minimally absorbed from the gut.

Cholesterol Synthesis and Absorption Markers

Cholesterol

Cholesterol, from the Greek, chole (bile) and stereos (solid) followed by the chemical suffix -ol for an alcohol.

► Cholesterol occurs as a component of plant membranes and as part of the surface lipids of leaves where it is sometimes the major sterol.

► The quantity of cholesterol is generally small when expressed as a percent of total lipid. While cholesterol averages perhaps 80 mg/kg total lipid in plants, it can be as high as 5 g/kg (or more) in animals.

Cholesterol vs Phytosterols

3 hydroxy cholesterol

The majority of the differences are in the “R” tail with plant sterols having an extra methyl (campesterol) or ethyl (stigmasterol) group at the C-24 position and different levels of desaturation.

The more carbon atoms and desaturation, the less the intestinal absorption.

Sterol and Stanol Structures

Saturation of the Δ5 double bond of sterols by enzymes in the liver results in stanols.
Cholesterol Synthesis and Absorption Markers

Cholesterol Metabolism

Coprostanol is formed by the conversion of cholesterol in the gut of most higher animals by intestinal bacteria. The general scheme for its production via a ketone intermediate.

http://en.wikipedia.org/wiki/Coprostanol

Noncholesterol Sterols/Stanols

Serum concentration of cholesterol and noncholesterol sterols/stanols in patients with mild hypercholesterolemia

<table>
<thead>
<tr>
<th>Sterols</th>
<th>Serum concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>236.5</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.474</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>0.326</td>
</tr>
<tr>
<td>Brassicasterol</td>
<td>0.047</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.011</td>
</tr>
<tr>
<td>Stanols</td>
<td></td>
</tr>
<tr>
<td>Sitostanol</td>
<td>0.012</td>
</tr>
<tr>
<td>Campestanol</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Klaus von Bergmann et al. Am J Cardiol 2005;96 (suppl):10D–14D

Cholesterol Synthesis

► In the whole animal, and presumably in humans, most cholesterol is synthesized and utilized in the extrahepatic organs.
► The CNS contains as much as 25% of the total amount of unesterified cholesterol in the entire body, and that is mostly produced via local de novo synthesis.
► Under dietary conditions equivalent to those found in Western humans, the extrahepatic tissues probably account for > 80% of whole animal sterol synthesis in virtually every species that has been studied.
► Most of the cholesterol carried in LDL is taken up into the liver (indirect reverse cholesterol transport).

Dietchy JM, Turley SD & Spady DK. J Lip Res 1993;34:1637-1659
Cholesterol Synthesis and Absorption Markers

Cholesterol Synthesis

- Acetate<br>- Acetoacetyl-CoA<br>- HMG CoA<br>- Mevalonate<br>- Desmosterol<br>- Lanosterol<br>- Squalene<br>- 7-dehydrocholesterol<br>- Free or unesterified cholesterol

Dayspring T in Chap 14 Davidson, Toth, Maki Therapeutic Lipidology 2008

Esterification: joining of a sterol-alcohol with a long chain fatty acid

Free Cholesterol * (unesterified)

Esterified cholesterol or Cholesteryl Ester (CE) (Non-polar, hydrophobic or water insoluble)

Usually esterified with oleic or palmitic acid creating cholesteryl oleate or palmitate.
Cholesterol Absorption and Synthesis

- Normal human serum contains small amounts of the cholesterol precursors squalene, cholesterol, desmosterol, and lanosterol, which reflect cholesterol synthesis especially in ratios to serum cholesterol.
- Small concentrations of cholesterol, campesterol and sitosterol are also detectable in serum the ratios of which are related to cholesterol absorption.
- The two groups (synthesis and absorption markers) of xenosterols are negatively (inversely) related to each other in the general population.

Plasma Absorption / Synthesis Markers Ranges

<table>
<thead>
<tr>
<th>Substance</th>
<th>Hyper</th>
<th>Normal</th>
<th>Hypo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol (μg/mL)</td>
<td>&gt; 4.64</td>
<td>2.1 - 4.3</td>
<td>&lt; 2.10</td>
</tr>
<tr>
<td>Campesterol ratio 10^5 mmol/mol cholesterol</td>
<td>&gt; 241</td>
<td>115 - 240</td>
<td>&lt; 114</td>
</tr>
<tr>
<td>Sitosterol (μg/mL)</td>
<td>&gt; 3.10</td>
<td>1.43 - 3.17</td>
<td>&lt; 1.42</td>
</tr>
<tr>
<td>Sitosterol ratio 10^5 mmol/mol cholesterol</td>
<td>&gt; 169</td>
<td>78 - 168</td>
<td>&lt; 75</td>
</tr>
<tr>
<td>Cholesterol (μg/mL)</td>
<td>&gt; 3.40</td>
<td>2.02 - 3.47</td>
<td>&lt; 2.01</td>
</tr>
<tr>
<td>Cholesterol ratio 10^5 mmol/mol cholesterol</td>
<td>&gt; 195</td>
<td>117 - 194</td>
<td>&lt; 118</td>
</tr>
<tr>
<td>Desmosterol (μg/mL)</td>
<td>&gt; 1.28</td>
<td>0.5 - 1.27</td>
<td>&lt; 0.49</td>
</tr>
<tr>
<td>Desmosterol ratio 10^5 mmol/mol cholesterol</td>
<td>&gt; 3.65</td>
<td>31.0 - 64</td>
<td>&lt; 30</td>
</tr>
</tbody>
</table>

Note that the concentrations are reported as absolute values or as ratios of cholesterol.

Cholesterol Synthesis and Absorption Relationships

The rate of incorporation of [2,14C]acetate into sterols in freshly isolated blood monocytes in MNL (nanomoles per 10^6 cells) plotted against the mass of absorbed dietary cholesterol (milligrams per kilogram per day).

When individual data are considered rather than means of group data, the degree of suppression of MNL sterol synthesis was found to be linearly related to the mass of cholesterol actually absorbed by the volunteers (milligrams per kilogram per day).

**Note:** The data points are from individual patients and may not reflect the general population.
Using Sterol Biomarkers

- The use of surrogate markers like noncholesterol sterols is qualitative in nature and cannot replace or yield the quantitative data derived by isotopic methods.
- However, compared with the more complicated and exact isotopic measures of cholesterol synthesis and absorption, the assessment of noncholesterol sterols is affordable and can be performed on large populations.
- This has led to the wide use of noncholesterol sterols to measure and characterize cholesterol metabolism.

Cholesterol Synthesis and Absorption Markers

MacKay DS & Jones PJH. Current Opin Lipidol 2012;23:341-247

Cholesterol Absorption and Balance

Synthesized cholesterol (400 mg/day)
Secretion of bile salts of which 2 gm is cholesterol
Biliary Cholesterol Transport
Intestinal Brush Border

The intestine absorbs about 50% of cholesterol that is presented to it, but that can vary individually from 20-80%.

Cholesterol Absorption

While intestinal absorption of bile acids is essentially complete under normal conditions, cholesterol absorption in healthy adults is variable, with 25-80% (mean 55%) absorbed in the small intestine. This range of variability has been observed in many studies where cholesterol absorption ranged from 25% to 75%. J Lipid Res 1998;39:2415-22.

The majority absorb about 55% of dietary sterols.

Number of Subjects

1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th

Decile Percent Cholesterol Absorption

Hypo-absorbers
Hyper-absorbers

Cholesterol absorption measured in 100 healthy patients using dual isotope tracer technique.

Cholesterol Synthesis and Absorption Markers

Diet and Cholesterol Absorption

Neither dietary cholesterol nor dietary fat significantly altered % cholesterol absorption. Regardless of diet type, the individuals within each dietary group differed markedly in the percentage dietary cholesterol absorption.

Cholesterol Absorption and Balance

The intestine absorbs about 50% of cholesterol that is presented to it, but that can vary individually from 30-80%.

Understanding Cholesterol Consumption

- The average diet consists of 200 – 600 mg of cholesterol
- Based on numerous studies, evidence has accumulated to suggest that the amount of dietary cholesterol (consumption) has no substantial effect on CAD risk except in certain circumstances
- Canadian guidelines do not specify an upper limit for dietary cholesterol intake; there is little relationship between dietary cholesterol and CVD

Cholesterol Synthesis and Absorption Markers

Cholesterol Absorption and Balance

- Synthesized cholesterol (400 mg/day)
- Secretion of 24 gms/day of bile salts of which 2 gms is cholesterol
- Dietary Cholesterol & Plant Sterols 500 of each mg-day
- 95% of biliary secretion
- Of the absorbed cholesterol 75% is from bile and 25% from diet

Transport

Intestinal Brush Border

50% of the intestine absorbs cholesterol.

Hepatocyte & Enterocyte Sterol Absorption

Genetic Expression of NPC1L1 and ABCG5, ABCG8 help regulate cholesterol homeostasis

NPC1L1 Mediated Internalization of Cholesterol

When the extracellular cholesterol concentration is high, cholesterol is incorporated into the PM and is sensed by cell-surface localized NPC1L1. NPC1L1 and cholesterol are then internalized together through clathrin-NPC1L1 mediated endocytosis and transported along microtubules to the ERC to recycle.

The ERC is where massive amounts of cholesterol and NPC1L1 are stored. At high cholesterol levels, NPC1L1 is cleaved and transported along microtubules to the ERC to recycle. When the intracellular cholesterol levels are low, ERC-localized NPC1L1 moves back to the PM along microtubules in order to absorb cholesterol.
Phytosterolemia

- Mutations in either ABCG5 (sterolin-1) or ABCG8 (sterolin-2) cause the autosomal recessive disease of sitosterolemia
- Phytosterolemia leads to increased atherosclerosis, formation of tuberous and tendinous xanthomas, and macrothrombocytopenia, with rare cases of endocrine disruption or cirrhosis of the liver also reported


Phytosterols – Injurious?

- It is not clear whether, when ABCG5, ABCG8 function is impaired, any subsequent pathophysiological issues that arise are because of cholesterol toxicity or xenosterol toxicity (or both).
- In sitosterolemic individuals, as well as in several in vivo and in vitro studies, phytosterols can affect the activity of several metabolic pathways.

Cholesterol Synthesis and Absorption Markers

**Association of plasma markers of cholesterol homeostasis with metabolic syndrome components. A cross-sectional study**

In both dyslipidemic and healthy populations, the Metabolic Syndrome is associated with increased plasma lathosterol, a cholesterol synthesis marker, and decreased plasma sitosterol, a marker of cholesterol absorption.

Cotan M et al. Nutrition, Metabolism & Cardiovascular Diseases 2011;21:651-657

**Phytosterols and Family History of Coronary Heart Disease**

Sudhop T et al. Metabolism 2002;51:1519-1521

**Cholesterol Homeostasis Markers in Metabolic Syndrome Patients**

The plasma campesterol:cholesterol ratio was significantly lower (51%, p < 0.01), whereas the ratio of lathosterol:cholesterol and lathosterol:campesterol were 24% (p = 0.05) and 64% higher (p < 0.05), respectively, in the low-cholesterol absorption compared with the high-cholesterol absorption group. Moreover, the subjects in the low-cholesterol absorption group had significantly higher plasma VLDL- and LDL-apoB pool sizes (37% and 27%, respectively, p < 0.05) and VLDL-apoB secretion rate (30%, p 0.05) compared with the high-cholesterol group.

Chan DC et al. Obes Res 2003;11:591-596
Cholesterol Synthesis and Absorption Markers

Framingham Offspring Study (FOS)
Cholesterol Absorption/Synthesis Markers are Associated with Prevalent CVD


Case control study in 155 cases and 414 controls. Multivariable analysis adjusted for diastolic blood pressure, LDL-C, HDL-C, triglycerides, diabetes and hypertension medication, age, sex, systolic blood pressure, body mass index, and smoking.

"In conclusion, present results indicate that alterations in cholesterol homeostasis, namely high cholesterol absorption and low cholesterol synthesis, are associated with increased CVD risk in a subset of men and women with similar plasma LDL-cholesterol concentrations"

"Additionally, the cholesterol homeostasis markers appear to be better predictors of disease than traditional lipid risk factors in this study population"

PROspective CArdiovascular Munster Study (PROCAM): Elevated Phytosterols and CHD

Hazard ratios for development of coronary events according to sitosterol concentration among subjects in different categories of 10-year global coronary risk. Hazard ratios of 1 = global risk < 10%, and sitosterol < 5.25 mmol/L.

The participants in the category with low global risk (≤ 10%) were divided into groups with low (≤ 5.25 mmol/L, 39 cases, 140 controls) and high (> 5.25 mmol/L, 17 cases, 46 controls) sitosterol concentrations.

At medium level of global risk (10.0 - 19.9%), low sitosterol concentrations were observed in 29 cases and 53 controls and high sitosterol levels in 18 cases and 24 controls, while at high global risk (≥ 20%), low sitosterol levels occurred in 38 cases and 47 controls while high sitosterol levels were measured in 18 cases and 8 controls.

Assmann G et al. Nutrition, Metabolism & Cardiovascular Diseases (2006) 16, 13e21
**PROspective CArdiovascular Munster Study (PROCAM): Elevated Phytosterols and CHD**

- The PROCAM study was conducted before statins were available, and few participants were receiving other lipid-modifying medications when their blood samples were drawn.
- Two potential explanations for these data are:
  - That sitosterol is somehow involved in the disease process.
  - Sitosterol is a surrogate for some other factor or condition that is involved in atherogenesis.

*Assmann G et al. Nutrition, Metabolism & Cardiovascular Diseases (2006) 16, 13e21*

**Phytosterols and Atherogenesis**

- Hyperabsorption of phytosterols can produce premature CAD and aortic stenosis in individuals with defects in ABCG5/8 raises the possibility that increased serum levels of phytosterols can contribute to CAD in the general population.
- Variation in the plasma concentration of plant sterols is highly heritable and that genetic polymorphisms in ABCG8 contribute to this variability.

*Manoj D. Patel, Paul D. Thompson. Atherosclerosis 2006;186:12–19*

**Nutrition in cardiovascular disease**

**Plant sterols and cardiovascular disease: a systematic review and meta-analysis**

- The meta-analyses were not supportive of any relationship between serum concentrations of sitosterol and campesterol (both absolute concentrations and ratios to cholesterol) and risk of CVD.
- Our systematic review and meta-analysis did not reveal any evidence of an association between serum concentrations of plant sterols and risk of CVD.
- Our systematic review and meta-analysis have several limitations. Further research will be necessary to assess whether plant sterols are causally involved in atherogenesis — does not address the vascular effects or safety of long-term consumption of plant sterol-enriched foods.

**Cholesterol Synthesis and Absorption Markers**

**Phytosterols “Good or Evil” Enigma**

A teleology is any philosophical account that holds that final causes exist in nature, meaning that design and purpose analogous to that found in human actions are inherent also in the rest of nature. 

http://en.wikipedia.org/wiki/Teleology

- Why is cholesterol the preferred substrate and why are phytosterols poor substrates for esterification by ACAT and LCAT?
- Why did humans evolve the ABCG5 and ABCG8 nonesterified sterol efflux transporters?
- Why are these sterol efflux transporters expressed at the two critical locations (gut lumen/enterocyte interface and hepatobiliary interface)?

**Plasma noncholesterol sterols: current uses, potential and need for standardization**

The measurement and reporting of noncholesterol sterols (NCSs) is very heterogeneous and standardization would allow better comparison or meta-analyses of results across different assessment methods and improve the use of NCSs in both research and clinical settings.

- Noncholesterol sterols are still a valuable research tool for the overall assessment of cholesterol metabolism and may have clinical potential in the personalization of diet and medicine. They are an affordable means of estimating cholesterol metabolism.

- Cholesterol metabolism of different disease states can be phenotyped with NCSs and this may help in identifying appropriate hypolipidemic treatments.

**Serum levels of sitosterol in CVD cases and controls (ratios to total cholesterol).**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>SMD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajaratnam 2000</td>
<td>0.19 (-0.15, 0.53)</td>
</tr>
<tr>
<td>Rajaratnam 2001</td>
<td>0.50 (-0.04, 1.05)</td>
</tr>
<tr>
<td>Gylling 2009 D+L</td>
<td>0.54 (0.16, 0.93)</td>
</tr>
<tr>
<td>Strandberg 2006</td>
<td>0.48 (0.24, 0.73)</td>
</tr>
<tr>
<td>Genser B et al. European Heart Journal 2012;33, 444-451</td>
<td>0.28 (0.06, 0.51)</td>
</tr>
<tr>
<td>Cross Sectional</td>
<td></td>
</tr>
<tr>
<td>Wilund 2004 (M)</td>
<td>0.31 (-0.26, 0.89)</td>
</tr>
<tr>
<td>Wilund 2004 (F)</td>
<td>0.28 (0.06, 0.51)</td>
</tr>
<tr>
<td>Fassbencer 2008</td>
<td>0.28 (0.06, 0.51)</td>
</tr>
<tr>
<td>Shay 2009 (no statin)</td>
<td>0.28 (0.06, 0.51)</td>
</tr>
<tr>
<td>Silbernagel 2009</td>
<td>0.28 (0.06, 0.51)</td>
</tr>
<tr>
<td>Mathan 2009</td>
<td>-0.10 (-0.23, 0.03)</td>
</tr>
<tr>
<td>Pinedo 2007</td>
<td>-0.10 (-0.23, 0.03)</td>
</tr>
<tr>
<td>Mathan 2010</td>
<td>-0.08 (-0.22, 0.07)</td>
</tr>
</tbody>
</table>

**Studied melanoma/CVD**

- Higher Levels in Controls
- Higher Levels in Cases

**Current Opin Lipidol 2012;23:241-247**

- The measurement and reporting of noncholesterol sterols (NCSs) is very heterogeneous and standardization would allow better comparison or meta-analyses of results across different assessment methods and improve the use of NCSs in both research and clinical settings.
- Noncholesterol sterols are still a valuable research tool for the overall assessment of cholesterol metabolism and may have clinical potential in the personalization of diet and medicine. They are an affordable means of estimating cholesterol metabolism.
- Cholesterol metabolism of different disease states can be phenotyped with NCSs and this may help in identifying appropriate hypolipidemic treatments.
Cholesterol Synthesis and Absorption Markers

Principles of Sterol Testing: Review

- Markers of absorption and synthesis are reported as absolute values and as ratios adjusted for cholesterol. They have the same meaning: some believe the ratios are best used if patient is on a cholesterol lowering drug.
- Absorption markers are sitosterol and campesterol which are phytosterols and cholestanol, a metabolite of cholesterol found in some dietary products.
- The synthesis markers are desmosterol and lathosterol.
- As a very general rule typically hyperabsorbers tend to be hyposynthesizers and vice versa. Hypoabsorption is desirable and requires no treatment per se.
- The pattern associated with the most CV risk is hyperabsorption with hyposynthesis.
- The disease phytosterolemia is very rare and the sitosterol, campesterol levels will be in the 100-300 µg/mL.
- Although elevations of phytosterols are associated with CV risk, one cannot state with assurance the phytosterols are causal of atherosclerosis.

Thank You For Your Attention

See my 5 part audiovisual Lecture Series on Sterols at www.lecturePad.org