- Sterol Biomarker Testing -
  What the Practitioner
  Needs to Know

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Disclosures (Last 12 months)

- Consultant
- AstraZeneca
- Health Diagnostic Labs
- Lecture Bureau
  - AstraZeneca
  - Merck

Sterol Aliphatic Side Chains

Cholesterol
Campesterol
Sitosterol
Stergmasterol
Brassicasterol
Avenasterol

Klaus von Bergmann et al. Am J Cardiol 2003;94 (suppl):169-178
Sterol and Stanol Structures

Cholesterol
Sterols have a double bond at the Δ5 position

Sitosterol

Campesterol

Saturation of the Δ5 double bond of sterols by enzymes in the liver results in 5 α-stanols

Cholesterol Molecules

Cycloalkenes

Absorption of Dietary Sterols & Stanols

- Serum concentrations of plant sterols are from 500 (campesterol) to 30,000 times (stigmastanol) less than that of cholesterol
  - Phytoestrogens are not synthesized in the human body and are exclusively derived from the diet in different amounts
  - They are absorbed to a much lesser extent than cholesterol
  - They are not metabolized to bile acids
  - They are excreted much later from the liver into bile compared with cholesterol

- These large differences in serum concentrations are due to several differences in the metabolism of plant sterols/stanols when compared with cholesterol

<table>
<thead>
<tr>
<th>Sterol/Stanol</th>
<th>Serum concentrations (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>296.5</td>
</tr>
<tr>
<td>Campesterol</td>
<td>0.474</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>0.326</td>
</tr>
<tr>
<td>Stigmastanol</td>
<td>0.047</td>
</tr>
<tr>
<td>Pinolastanol</td>
<td>0.012</td>
</tr>
<tr>
<td>Pinolastanol</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Patients had mild hypercholesterolemia

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

Cholesterol Synthesis and Absorption Markers
Cholesterol Synthesis and Absorption Markers

Cholesterol Synthesis

► Most cholesterol is synthesized and utilized in the extrahepatic organs
► 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
► 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
► Most of the cholesterol carried in LDL is taken up into the liver (indirect reverse cholesterol transport)

Kandutsch-Russell

Lanosterol
Desmosterol
7-dehydrocholesterol

Acetate
HMG-CoA
Mevolinate
HMG-CoA reductase

It is thought that these pathways may be independently regulated but that they share common control mechanisms. If these enzymes are inhibited, these pathways will seriously disrupt cholesterol synthesis.

Dietzsch, JM, Turcsey SD & Spady DK. J Lip Res 1993;34:1657-1659

Donald J. McNamara et al, JCI 1987;79:1729-39
Visceral Obesity

Cholesterol Synthesis Markers in Hypertension

Elevated TG

Cofan M et al. Nutr Metab & CV Diseases 2011;21: 651e65 7

Desmosterol is not a good surrogate for cholesterol in lipid rafts, in lipid rafts,

DHCR24) DHCR24) and its substrate desmosterol play a pivotal role in cholesterol homeostasis, and we are only just beginning to appreciate the importance of these in a variety of cellular processes and disease settings

Alzheimer’s Disease

HCV and other viral diseases

Prostate cancer

Arterial macrophage-dependent inflammation

Desmosterol is not a good surrogate for cholesterol in lipid rafts, which provide platforms for signaling molecules to interact

Desmosterol accumulation, like cholesterol, signals sterol overload in the cell

ZerenliTurk EJ et al. Prog Lipid Res 2013 in press

Identification of a new plasma biomarker of Alzheimer’s disease using metabolomics technology*

Yoshikawa Satō,* Hayato Sasaki,* Tatsuyuki Nakamura,* Francois Berneix,* Ken Asahina,* and Yodera Oda,*

Desmosterol plasma level and the desmosterol to cholesterol ratio in the same patients was significantly decreased in those with AD and mild cognitive impairment

**Intestinal Cholesterol Absorption**

- Unlike triglycerides and phospholipids which are hydrolyzed principally by colipase-dependent pancreatic lipase, cholesterol esters are hydrolyzed by the bile salt-stimulated lipase. The resultant free cholesterol can be absorbed.

**Cellular Sterol Transporters**

**Membrane Topologies & Domains**

- **NPC1L1** is critical for intestinal cholesterol absorption.

- ABCG5/ABCG8 are expressed in the same locations and are responsible for preventing accumulation of a host of dietary noncholesterol sterols (veseal sphingolipids).

**Hepatocyte & Enteroocyte Sterol Homeostasis**

- Genetic Expression of NPC1L1 and ABCG5, ABCG8 help regulate cholesterol homeostasis.
Cholesterol Synthesis and Absorption Markers

**Cholesterol Absorption**

While intestinal absorption of bile acids is essentially complete under normal conditions, cholesterol absorption in healthy adult volunteers is variable, with 29–81% (mean 56%) absorbed in the small intestine. Bosner MS et al. J Lipid Res 1998;39:2415-22.

The majority absorb about 55% of dietary sterols.


Dietary Cholesterol

There is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C.

Framingham Offspring Study (FOS)

Cholesterol Absorption Markers are Associated with Prevalent CVD

Cholesterol Synthesis and Absorption Markers

Inactivating Mutations in NPC1L1 and Plasma Lipids

- The exons of NPC1L1 in 7364 patients with coronary heart disease and in 14,728 controls without such disease who were of European, African, or South Asian ancestry were sequenced.
- Carriers of inactivating mutations (nonsense, splice-site, or frameshift mutations) were identified.
- In addition, a specific inactivating mutation (p.Arg406X) was genotyped in 22,590 patients with coronary heart disease and in 68,412 controls and tested the association between the presence of an inactivating mutation and both plasma lipid levels and the risk of coronary heart disease.


Inactivating Mutations in NPC1L1 and Plasma Lipids

<table>
<thead>
<tr>
<th>Mean difference</th>
<th>Low density lipoprotein</th>
<th>-1.12</th>
<th>0.04</th>
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<tbody>
<tr>
<td>Naturally occurring mutations that disrupt NPC1L1 function were not only found to be associated with reduced plasma LDL-C levels but also a reduced risk of coronary heart disease</td>
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<table>
<thead>
<tr>
<th>Mean difference</th>
<th>Triglycerides</th>
<th>-1.12</th>
<th>0.11</th>
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<tbody>
<tr>
<td>Carrier status was associated with a relative reduction of 53% in the risk of coronary heart disease (odds ratio for carriers, 0.47; 95% CI, 0.25 to 0.87; P = 0.009)</td>
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Drugs and Evidence-Based Medicine in The Elderly (DEBATE) Study

- Even with frequent metabolic syndrome and diabetes, a low cholesterol absorption was associated with fewer recurrent cardiovascular events, and especially with better survival in elderly cardiovascular patients.
- This may be due to selective survival in these elderly individuals or because cholesterol absorption is partly genetically determined, the mechanism may be a lower cholesterol burden during lifetime.
- Cholesterol absorption may thus modulate the risk associated with T2DMs and Metabolic Syndrome.

Cholesterol Synthesis and Absorption Markers

On subgroup analysis, patients with diabetes had a greater benefit with ezetimibe/simvastatin (HR = 0.86, p for interaction = 0.023)
Cholesterol Synthesis and Absorption Markers

Scandinavian Simvastatin Survival Study (4S)
Simvastatin Efficacy: Relationship to Cholesterol Absorption

The higher the cholesterol absorption, the less efficacious is simvastatin


Ezetimibe & Statin
Cholesterol Absorption Markers

Ezetimibe monotherapy (10 mg daily) significantly lowered plasma concentrations of both sitosterol and campesterol from baseline compared with placebo (~53.8% and ~58.2%, respectively; p for both < 0.001).

With atorvastatin monotherapy, there was a modest numerical increase in sitosterol and campesterol (18.1% and 10.1%, respectively).

Ezetimibe 10 mg plus atorvastatin (pooled across doses) produced decreases in phytosterols from baseline of a similar magnitude: ~49.4% for sitosterol and ~59.3% for campesterol (both p < 0.001 vs. placebo and atorvastatin monotherapy).

Overall, the decreases in phytosterol concentrations observed with ezetimibe co-administered with statins were of similar magnitude to those observed with ezetimibe monotherapy.


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

Hazard ratios for development of coronary events according to sitosterol concentration (mmol/L) among men in different categories of 10-year global coronary risk (hazard ratio 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
Interpreting Serum Phytosterols

► In interpreting the associations between circulating plant sterols and cardiovascular disease, it should again be considered that plant sterols reflect intestinal cholesterol absorption.

► The repeatedly observed positive correlation between circulating plant sterols and cardiovascular disease is accounted for by the atherogenic effects of high cholesterol absorption.

► This view is supported by the finding that circulating cholestanol was increased in people with cardiovascular disease and also predictive of future cardiovascular events.

► Thus, a raised phytosterol concentration may be a marker of disturbed cholesterol metabolism and not itself causally related to atherosclerosis.

Enigma: Are Phytosterols “Good or Evil”

Consider -------

► Why did humans evolve the ABCG5 and ABCG8 nonesterified sterol (phytosterol) efflux transporters?

► Why are these sterol efflux transporters expressed at the two critical locations: gut lumen/enterocyte interface and hepatobiliary interface?

► Why is cholesterol the preferred substrate and why are phytosterols poor substrates for esterification by ACAT and LCAT?

Fenofibrate Decreases Sterol Absorption

Specific activation of PPARα by fenofibrate decreases cholesterol absorption via an inhibitory effect on NPC1L1 expression in the proximal small intestine.

Cholesterol lowering and inhibition of sterol absorption by Lactobacillus reuteri NCIMB 30242

Individual changes in plasma deconjugated bile acids (DBAs) and associated changes in serum LDL-C over the intervention period. A significant association was observed in subjects taking L. reuteri NCIMB 30242 (r=0.369, P=0.003), whereas no association was observed in subjects taking placebo.