Phytosterols as a Functional Food

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Disclosures (Last 12 months)

► Employment
  ➤ True Health Diagnostics

► Lecture Bureau Sanofi Regeneron

Lipid Assessment to Evaluate CV Risk

Sterols
Were the sterols synthesized or absorbed?

Cholesterol?
Xenosterols?

Xenosterols include phytosterols and any other noncholesterol sterol
Sterol / Stanol Biochemistry

Sterols have a double bond at the \( \Delta^5 \) position.

Absorption of Sterols/Stanols

- Serum concentrations of plant sterols are from 500 (campesterol) to 20,000 times (stigmasterol) less than that of cholesterol.
- The concentrations of plant sterols are even lower than their respective stanols (e.g., campestanol is 140 times less than campesterol, and sitostanol is 28 times less than sitosterol).
- These large differences in serum concentrations are due to several differences in the metabolism of plant sterols/stanols when compared with cholesterol.
- Plant sterols 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 probably not metabolized to bile acids.
- They are excreted much faster from the liver into bile compared with cholesterol.

Dietary Phytosterols

The average American’s intake of phytosterols is around 250 milligrams per day (for stanols it is 20 mg).

Vegetarians ingest more, averaging almost twice as much, approximately 500 milligrams.

Eating around 2,000 milligrams (2 grams) of plant sterols daily can lower blood cholesterol levels about 10 percent.

References:
- Von Bergmann et al. Am J Cardiol 2005;96(suppl):10D–14D
- Dietary Phytosterols
Phytosterol Supplements

Factors Responsible for Variations in Circulating Plant Sterol Concentrations

- Dietary intake of phytosterol supplements
  - When plant sterols are administered in supplement form at a dose of 1.8 to 2.0 g/day for 4 to 8 weeks, there is a 52% to 99% increase in campesterol levels and a 23% to 96% increase in sitosterol levels
  - In contrast, when plant stanols are supplemented at a dose of 1.5 to 3.0 g/day for a 4-week period, decreases of 28% to 113% in campesterol levels and 24% to 50% in sitosterol levels have been observed

Yen-Ming Chan et al. Nutrition Reviews 2006;64:385-402

Consumption of Plant Sterol-enriched Foods and Effects on Plasma Phytosterol Concentrations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Baseline Concentration</th>
<th>Concentration after PS Use</th>
<th>Absolute change after placebo</th>
<th>Relative change vs. placebo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitosterol</td>
<td>µmol/mL</td>
<td>6.92 (5.90, 7.94)</td>
<td>12.20 (10.18, 14.22)</td>
<td>5.28 (2.73, 7.83)</td>
<td>77.3 (50.0, 94.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.22 (0.99, 1.36)</td>
<td>1.77 (1.54, 2.02)</td>
<td>0.55 (0.26, 0.84)</td>
<td>46.7 (31.0, 52.5)</td>
</tr>
<tr>
<td>Campesterol</td>
<td></td>
<td>3.00 (2.09, 4.00)</td>
<td>5.0 (3.00, 7.00)</td>
<td>2.00 (1.00, 3.00)</td>
<td>67.3 (45.0, 89.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.20 (2.60, 3.80)</td>
<td>4.00 (3.40, 4.60)</td>
<td>0.80 (0.20, 1.40)</td>
<td>70.8 (44.7, 76.9)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>µmol/L</td>
<td>1.34 (0.83, 1.85)</td>
<td>0.30 (0.20, 0.40)</td>
<td>-1.04 (-0.30, -0.70)</td>
<td>23.0 (-7.0; 36.0)</td>
</tr>
</tbody>
</table>

Yen-Ming Chan et al. Nutrition Reviews 2006;64:385-402

Weighed net effects (baseline, end-of-intervention, absolute change) of plasma sitosterol, Campesterol & LDL-C

Expressed as means (95% CI)
The role of plant sterols and stanols as cholesterol lowering agents is rarely mentioned. Several meta-analyses have shown that consumption of 2.5 g/d of plant sterols and stanols can lower serum LDL-C concentrations up to ~10%, while recent studies have shown that additional reductions can be achieved at higher intakes. Rarely mentioned is that the drop in LDL-C is often achieved at the expense of higher phytosterol levels. There is an ongoing debate whether increased serum plant sterol concentrations are associated with an increased CVD risk.

**Sterols and Stanols**

Therapeutic doses of phytosterols (e.g. sitosterol) and phytostanol esters (sitostanol) displace cholesterol from the micellar nucleus, less cholesterol is available in micelles for absorption

**Cellular Sterol Transporters**

Niemann-Pick C1 Like 1 Protein is Critical for Intestinal Cholesterol Absorption

ABC-G5/G8 (sterolin 1 and 2) are also expressed at the enterocyte-lumen efflux which prevents accumulation of a host of dietary noncholesterol sterols.
Inactivating Mutations in NPC1L1

- 15 distinct NPC1L1 inactivating mutations were identified; approximately 1 in every 650 persons was a heterozygous carrier of these mutations.
- Heterozygous carriers of NPC1L1 inactivating mutations had a mean LDL-C level that was 12 mg/dL lower than that in noncarriers (P = 0.04).
- Carrier status was associated with a relative reduction of 53% in the risk of coronary heart disease (odds ratio for carriers, 0.47; 95% confidence interval, 0.25 to 0.87; P = 0.008).


Sterolins (ABCG5, ABCG8)

- ABCG5/G8 travel as heterodimers to the apical membrane where they form full, active transporters and require ATP to function and at the cell surface, they likely promote flopping of sterols from the inner to the outer leaflets of the plasma membrane.
- The sterolin heterodimer is responsible for the exclusion of sterols by actively pumping them out of the cells (the enterocyte or the hepatocyte).
- This pump has a much higher affinity for non-cholesterol sterols compared with cholesterol - Note that this mode of action allows for the sterolins to efflux cholesterol into bile in the absence of non-cholesterol sterols in the liver.
- Sterolins do not regulate the entry of sterols into the enterocyte or the hepatocyte.

Gut lumen or bile canalicula
Sitosterol Cholesterol
Hepatocyte or enterocyte

Accumulation of Dietary Cholesterol in Sitosterolemia Caused by Mutations in Adjacent ABC Transporters

- Patients LH and RH have a mutation in ABCG5, leading to sitosterolemia.
- The distribution of sterols in the plasma lipoproteins of two sisters shows a marked increase in sitosterol levels in patients LH and RH compared to normal controls.

Bhattacharyya AK et al. Jour of Clin Invest. 1974;53:1033-1043

β-Sitosterolemia and Xanthomatosis

A NEWLY DESCRIBED LIPID STORAGE DISEASE IN TWO SISTERS

Ashim K. Bhattacharyya and William E. Connor
From the Clinical Research Center and the Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52240

The distribution of sterols in the plasma lipoproteins of two sisters.
Besides the various rare mutations in ABCG5 or ABCG8 as observed in sitosterolemic patients, more common sequence variations in both half-transporters, without the sitosterolemic phenotype, have been described.

These polymorphisms in ABCG5 and ABCG8 are related to serum plant sterol concentrations.

Caucasians seem to carry mutations in ABCG8 gene, whereas Chinese, Japanese, and Indian (20% of known cases) patients seem to have mutations in ABCG5.

The prevalence of heterozygous mutations in the population is not known.

There is a higher prevalence of polymorphisms in ABCG8, compared to ABCG5, despite its close proximity and homology with ABCG5.

A number of studies have implicated this locus in disease or physiological processes, other than sitosterolemia, ranging from gallstone formation, lipoprotein kinetics, cholesterol absorption and obesity to response to drug therapy.

Alleles of ABCG8 and ABO associated with elevated phytosterol levels displayed significant associations with increased CAD risk.

Common variants in ABCG8 and ABO are strongly associated with serum phytosterol levels and show concordant and previously unknown associations with CAD.

Alleles at ABCG8 associated with reduced phytosterol levels were associated with reduced CAD risk (rs41360247 odds ratio, 0.84; 95% CI, 0.78 to 0.91; P=1.3 x 10^-5).

Genetic Regulation of Phytosterols


Cholesterol Synthesis and Absorption Markers

Heritability of Plasma Xenosterols and Relationship to DNA Sequence Polymorphism in ABCG5 and ABCG8.

Berge K et al. J Lipid Res. 43: 486–494


ABCG5/ABCG8 Mutations


Genetic Regulation of Phytosterols


Common variants in ABCG8 and ABO are strongly associated with serum phytosterol levels and show concordant and previously unknown associations with CAD.

rs657152 odds ratio, 1.13; 95% CI, 1.07 to 1.19; P=9.4 x 10^-6.

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Ulcerative colitis is associated with increased phytosterol levels,

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Alleles at ABCG8 associated with reduced phytosterol levels were associated with reduced CAD risk (rs41360247 odds ratio, 0.84; 95% CI, 0.78 to 0.91; P=1.3 x 10^-5).
High cholesterol absorption is associated with risk alleles in ABCG8 and ABO and with CVD. Harm caused by elevated cholesterol absorption rather than by plant sterols may therefore mediate the relationships of ABCG8 and ABO variants with CVD.

On univariate analysis, a high sitosterol concentration (>2.0) was significantly associated with a CHD risk (HR = 1.81; p < 0.05) similar to that of hypertension, family CHD history, or metabolic syndrome. Of the univariate risk factors, only high LDL-C, low HDL-C and global risk > 20%, (hazard ratio = 3.56) were associated with a greater relative risk of a major coronary event than elevated sitosterol.

On multivariate analysis, the hazard ratio was 1.81 (95% CI 1.20-2.71, p = 0.006) for a high sitosterol concentration (>2.0), similar to that of hypertension, family CHD history, or metabolic syndrome.

Male Data

Sitosterol (> 5.25 µmol/L) HDL-C (< 40 mg/dL) LDL-C (> 161 mg/dL) Global Risk ≥ 20%

Hazard ratio for development of CAD

1.81** 2.06** 2.86** 3.56**

* p<0.05  **p<0.001

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Phytosterols and Family History of Coronary Heart Disease

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

Serum campesterol and sitosterol concentrations were 30% (P = .011) and 29% (P = .004) higher in patients with a positive family history compared with those without.

Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin (STELLAR)


Plant Sterols in Serum and Plaque of Carotid Endarterectomy Patients


The higher the absorption of cholesterol, the higher are the plant sterol contents in serum resulting also in their higher contents in atherosclerotic plaque. However, the role of dietary plant sterols in the development of atherosclerotic plaque is not known.

Correlation of serum ratios of campesterol (left) and lathosterol (right) to cholesterol with those of tissue campesterol.
Cholesterol Synthesis and Absorption Markers

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
- 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. It does not address the vascular effects or safety of long-term consumption of plant sterol-enriched foods

Genser B et al. European Heart Journal 2012;33:444-451

Plant sterol supplementation impairs endothelial function, aggravates ischemic brain injury, effects atherogenesis in mice, and leads to increased plasma sterol concentrations in humans. In the light of the severe premature atherosclerosis in patients with phytosterolemia and epidemiological observations suggesting an association of plant sterols with increased vascular risk, the findings of this study underline the need for prospective clinical studies with cardiovascular endpoints for functional foods supplemented with PSE that are currently advertised for patients with cardiovascular diseases

J Amer Coll Card. 2008;51:1553-61

Vascular Effects of Diet Supplementation With Plant Sterols

Oswin Wingenraus, MD; Davey Lewis, PhD; Synopsis; Markus Fuchs; Rainer Frolow; Frank Lüscher; Thomas Schaper, MD; Michael Weiss, MD; Karin Grotz, MD; Jochen Krämer, MD; Volker Völker, MD; Markus Fuchs, MD; Michael Bilski, MD; Ulrich Lüscher, MD; Wolfang Heinze, MD; Berlin; and Munich, Germany

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Nutrition in cardiovascular disease

- All cholesterol within CNS is synthesized in situ & turnover is slow
- Phytosterols trafficked on HDLs can cross the blood brain barrier (BBB) via SR-B1 and then efflux to apoE for trafficking in the CNS
- Phytosterols unlike cholesterol virtually accumulate in the CNS as they cannot be converted 24(S)hydroxycholesterol which can exit via the BBB
- Studies have shown they have neuroprotective and neurodegenerative properties
- Plant sterols must be used with caution in practice

Prog Lipid Res 2015;58:26-39

Thomas Dayspring MD, FACP, FNLA
Effects of long-term plant sterol and -stanol consumption on the retinal vasculature: A randomized controlled trial in statin users

1. The effects of long-term plant sterol and -stanol consumption on changes in retinal vessels diameter which reflex alterations in the microcirculation were compared. Three randomized groups were studied at baseline and after 85-weeks.
2. Increased serum campesterol concentration correlated positively with increased retinal venular diameter, independent from changes in serum LDL-cholesterol concentrations.
3. This may constitute an explanation for the suggested effects of plant sterols on vascular function but this novel finding needs confirmation and further study.


Sterol/Stanol Concentration Distributions

60,000
40,000
20,000
0 1 2 3 4 5 6
Sitosterol (µg/mL)

60,000
40,000
20,000
0 1 2 3 4 5 6
Campesterol (µg/mL)

60,000
40,000
20,000
0 1 2 3 4 5 6
Cholestanol (µg/mL)

60,000
40,000
20,000
0 0.5 1 1.5 2 2.5
Desmosterol (µg/mL)

Increased absorption markers were associated with apoE 4 allele & menopause.

ACC/AHA Guideline on the Assessment of Cardiovascular Risk

There were no ASCVD outcomes identified for plant sterols, sterol esters, stanols or stanol esters.

Statement removed the recommendation of phytosterol use to prevent atherosclerotic CVD.

Daily use of 2 grams lowers LDL-C by 7-10% (with some heterogeneity) with no effect on TG or HDL-C. No studies have been performed yet on the subsequent effect on CVD.

Long term surveillance is also needed to guarantee the safety of the regular use of phytosterol-enriched products. Based on LDL-C & absence of adverse signals 2 gm sterol/stanol foods may be considered in intermediate or low global risk not qualifying for pharmacotherapy or adjunct to Rx for high/very high risk not at goal or statin-intolerant.

Atherosclerosis 2016;253:281-344 specifically page 299 & 302

The US FDA classifies PS as having Generally Recognized as Safe status and the FDA has authorized a health claim stating that consuming foods that include plant sterols/stanols (at least 0.75 g/each) may reduce the risk of CHD.

The connection between high circulating phytosterol (PS) vs lower circulating PS concentrations and CHD risk has led to investigations and to date, the findings have not supported a clear link between circulating PS concentrations in the normal range and the development of CVD.

A meta-analysis included 17 studies involving 11,182 participants concluded that there is no relationship between serum concentrations of PS and CVD risk over a 3-fold difference in serum plant sterol concentrations.

Measurement of circulating PS concentrations in clinical practice is generally limited to the diagnosis of phytosterolemia.


Summary

- Sterols are the sine qua non of atherogenesis
- Sterol absorption is tightly regulated and under genetic control
- Phytosterol concentrations vary significantly among individuals and can be increased by statins
- Although phytosterol supplementation reduces LDL-C it raises lipoprotein phytosterol (eg LDL-sitosterol) levels
- There are no outcome trials evaluating using phytosterols for CV benefit

National Lipid Association Recommendations: Part 2

National Lipid Association Recommendations for Patient-Centered Management of Dyslipidemia: Part 2

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Thanks for your attention