

Progress and perspectives in plant sterol and plant stanol research

Peer-reviewed author version

Jones, Peter J. H.; Shamloo, Maryam; MacKay, Dylan S.; Rideout, Todd C.; Myrie, Semone B.; Plat, Jogchum; Rouillet, Jean-Baptiste; Baer, David J.; Calkins, Kara L.; Davis, Harry R.; Duell, P. Barton; Ginsberg, Henry; Gylling, Helena; Jenkins, David; Luetjohann, Dieter; Moghadasian, Mohammad; Moreau, Robert A.; Mymin, David; Ostlund, Richard E., Jr.; Ras, Rouyanne T.; Reparaz, Javier Ochoa; Trautwein, Elke A.; Turley, Stephen; VANMIERLO, Tim & Weingaetner, Oliver (2018) Progress and perspectives in plant sterol and plant stanol research. In: NUTRITION REVIEWS, 76(10), p. 725-746.

DOI: 10.1093/nutrit/nuy032

Handle: <http://hdl.handle.net/1942/28640>

**Progress and Prospective of Plant Sterol and Plant Stanol Research: Report of the 3rd
International Plant Sterols/Stanol, Health and Disease Meeting, Winnipeg 2016**

Authors: Peter JH Jones^{1,3*}, Maryam Shamloo^{1,3}, Dylan S MacKay⁴, Todd C Rideout², Semone B Myrie^{1,3}, Jogchum Plat⁵, Jean-Baptiste Rouillet⁶, David Bear⁷, Kara Calkins⁸, Harry Davis⁹, P. Barton Duell¹⁰, Henry Ginsberg¹¹, Helena Gylling¹², David Jenkins¹³, Dieter Lütjohann¹⁴, Mohammad Moghadasian³, Robert Moreau¹⁵, David Mymn¹⁶, Richard Ostlund Jr¹⁷, Rouyanne T Ras¹⁸, Javier Ochoa Reparaz¹⁹, Elke A Trautwein¹⁸, Stephen Turley²⁰, Tim Vanmierlo²¹, Oliver Weingärtner²²

¹*Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*

²Department of Exercise and Nutrition Sciences, University of Buffalo, Farber Hall G10, Buffalo, NY 14214, USA

³Department of Food and Human Nutritional Sciences Food Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

⁴George and Fay Yee Centre for Healthcare Innovation, University of Manitoba, Winnipeg, MB, Canada

⁵Maastricht University, Department of Human Biology, 6200 MD, Maastricht, The Netherlands

⁶Division of Metabolism, CDRC-P, Department of Pediatrics, Oregon Health & Science University, 707 S.W. Gaines St., Portland, OR 97239-2998, USA

⁷Beltsville Human Nutrition Research Center, USDA, USA

⁸University of California, Los Angeles, USA

⁹CVPath Institute Inc, Gaithersburg, Maryland, USA

¹⁰OHSU, USA

¹¹Institute of Human Nutrition, New York, USA

¹²University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

¹³Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada; Clinical Nutrition & Risk Factor Modification Centre, St. Michael's Hospital, Toronto, ON, Canada

¹⁴Institute for Clinical Chemistry and Clinical Pharmacology, Bonn University, Bonn, Germany

¹⁵USDA ARS ERRC, Wyndmoor, Pennsylvania, USA

¹⁶Health Sciences Centre, Winnipeg, Canada

¹⁷Washington University, St Louis, USA

¹⁸Unilever Research and Development, Vlaardingen, The Netherlands

¹⁹Eastern Washington University Cheney, WA United States

²⁰UT Southwestern Medical Center, Dallas, USA

²¹Hasselt University, Hasselt, Belgium

²²Abteilung für Kardiologie, Klinikum Oldenburg, European-Medical School Oldenburg-
Groningen Carl von Ossietzky Universität, Oldenburg, Germany

***Corresponding author:** Peter JH Jones, *Richardson Centre for Functional Foods and
Nutraceuticals*, University of Manitoba, Winnipeg, MB R3T 6C5, Canada

Tel: +1(204) 474 8883, Email: Peter.Jones@umanitoba.ca

Keywords: Plant sterols, Plant stanols, Cholesterol, Nutrition, Sitosterolemia

1. Introduction

A meeting of experts in the field of plant sterols and stanols was convened September 30 - October 2, 2016, in Winnipeg, Manitoba, to enable discussion of developments and controversies in this active area of functional food science. The first day's sessions were oriented to understanding contemporary topics surrounding metabolic aspects of dietary plant sterol and stanol (plant sterols/stanols) supplementation, while the second day focused on clinical aspects, including disorders pertaining to plant sterols/stanols absorption and physiology. Case reports of families with sitosterolemia were also discussed on the second day. Overall, most of the experts considered that an important role continues to exist for plant sterols/stanols provided as functional foods and supplements as effective cholesterol-lowering agents. It was also apparent from the data presented that an improved understanding exists in the mechanisms through which cholesterol-lowering actions of plant sterols/stanols occurs, compared with the state of the art in 2011¹. The purpose of the present report is to identify the high-level points arising from the presentations and ensuing discussions that capture recent developments in the field.

2. Low Density Lipoprotein-Cholesterol (LDL-C) Efficacy of Plant Sterols/Stanol

2.1 Factors that Influence the Cholesterol-lowering Efficacy of Plant Sterols/Stanol

Hundreds of studies have investigated a variety of aspects of the clinical efficacy of plant sterols and stanols for lowering LDL-C. Firstly, comparing plant sterols with plant stanols, consistent evidence demonstrates that plant sterols/stanols lower LDL-C levels by 7.5 to 12% with intakes of 1.5 to 3 g/d ². At intakes of up to 3 g/d, which is the current recommended range of intake in most countries, equal LDL-C lowering effects occur between plant sterols and plant stanols. A systematic review of 14 studies showed a non-significant weighted mean difference in LDL-C lowering ³ between plant sterols and plant stanols. Moreover, compiling data from 124

studies revealed a clear dose-dependent reduction in LDL-C at plant sterol and stanol intakes up to 4 g/d. In this meta-analysis, at an average plant sterol/stanol intake of 2.1 g/d, an 8.4% reduction of LDL-C was observed, while with an average intake of 3.3 g/d a 12.4% reduction was found ². It appeared that at 2.1 g/day intake, there was about a 2% difference in LDL-C, with plant stanols achieving a more pronounced LDL-C lowering whereas at higher average intakes of 2.6 and 3.3 g/d comparable lowering of LDL-C was found ¹. These findings persisted in the results of several additional analyses ⁴. The consistency of the food format, either solid/edible or liquid/drinkable, is critical to compare plant sterols/stanols. As described by Ras et al. ², in the dose category ≥ 2.0 dose < 2.5 g/d, average 2.1 g/d, fifteen of forty plant sterol studies used liquid food formats, whereas only four of eighteen plant stanol studies used this type of food format. Irrespective of the type of plant sterols/stanols used, liquid foods lowered LDL-C concentrations by, on average, 6.5%, whereas solid foods lowered LDL-C concentrations by, on average, 9.2% ². So, the limited sample size of studies that used the liquid food formats as plant stanol carrier warrants caution in drawing sweeping conclusions. Additional research with head to head plant sterol vs. stanol comparisons is needed.

A second factor influencing the cholesterol-lowering efficacy of plant sterols/stanols is food matrix. Liquid versus solid food matrix, the fat content and fat type of the food, supplement form (capsules or tablet), use of free or esterified plant sterols/stanols and the fatty acid used for esterification, all exist as matrix effects. In addition, frequency of administration, e.g. single vs. multiple daily intakes, intake with or without a meal, as well as the time of administration during the day, e.g. morning vs. later during the day, are factors contributing to the degree of plant sterol or stanol efficacy. A systematic review of dietary plant sterols/stanols coming from food or tablets showed a similar mean difference in LDL-C lowering ⁵. However, in most tablet studies,

particle size and dissolution activity data were missing. Tablet characteristics represent a critical aspect for future reported research using tablets.

Plant sterols/stanols have been examined across multiple food formats and there is no apparent difference in efficacy between fat-based and low or non-fat based foods ^{6,7}. In terms of type of the carrier fat, a recent study found no difference in the relative reduction in LDL-C levels ⁸. Higher efficacy of solid (e.g. spreads and margarines) vs liquid food formats (milk and juices) was seen in two meta-analyses ^{2,7}. No differences exist between the efficacy of free vs esterified plant sterols ^{7,9,10}, however, the particle size of plant sterols should be taken into account. Nor does the fatty acid used for esterification have an impact on the cholesterol-lowering efficacy of plant sterols/stanols ¹¹⁻¹³. However, data from meta-analyses show that intake frequency matters and that once a day seems sub-optimal ^{2,7}. Larger LDL-C lowering effects of 9.4% were found when a yogurt drink was consumed together with a lunch meal compared to a 6.0% lowering when consumed before breakfast ¹⁴. Another study with plant stanol-enriched biscuits also found that biscuits consumed with a meal resulted in a greater cholesterol-lowering effect compared to biscuits consumed between meals ¹⁵. In 2000, Law found that in the plant sterol and stanol intervention studies published, the absolute decrease in LDL-C increased with age ¹⁶, however, relative changes were comparable across age ranges.

The design of clinical studies is also of interest. In the earliest published research with plant sterols, 9 males consumed 5-6 g/d of beta-sitosterol showing mean serum total cholesterol decreases of as great as 15 to 20% over 6 weeks ¹⁷. Another early research, in which 15 males all with previous myocardial infarctions consumed 12 to 18 g/d of beta-sitosterol, also showed large declines in serum total cholesterol ¹⁸. Neither of these studies, however, were randomized trials and the results focused on changes in total cholesterol. Since these initial publications, important

advances in trial design and analytical methods have occurred. Miettinen et al. conducted a landmark, one year-long study of 153 subjects in a double-blind, randomized control trial and observed a 14.1% decrease in circulating LDL-C with 2.6 g/d of plant stanols compared to the placebo, without a decrease in high density lipoprotein-cholesterol (HDL-C) ¹⁹.

Overall, summarizing data from meta-analyses from 2000 through 2016, most studies report an LDL-C reduction between 0.3 and 0.4 mmol/L ^{2,5,6,8,16,20}. As LDL-C is recognized as an important causal risk factor for coronary heart disease ²¹ such a reduction in LDL-C would correspond to a 25% reduction in the risk of heart disease. However, to date, direct evidence on cardiovascular disease (CVD) is not available as studies exploring hard endpoints including CVD events and mortality have not been conducted as they are expensive and challenging like all dietary intervention studies to perform in light of long-term compliance.

2.2 Diversity of Natural Plant Sterols/Stanol

Experts agree that a minimum of 1 g of plant sterols/stanol consumed per day is necessary to significantly lower circulating LDL-C levels ²¹. However, plant sterols in fruits and vegetables naturally range from about 38 to 439 mg/kg fresh weight and 329 to 1780 mg/kg in grains, so to consume 1 g of plant sterols, one would need to eat about 2 kg fruits/vegetables or about 1 kg of grains per day ²². Plant oils contain higher levels of plant sterols/stanol but one would need to eat about 100 g of oil per day to reach a daily intake of 1g. Therefore, fruits/vegetables, grains and plant oils are not practical sources of dietary plant sterols/stanol, so one needs to look at other approaches. Tall oil and vegetable oil deodorizer distillates continue to be major feedstocks for plant sterols/stanol destined for functional foods, but other sources are under investigation. For example, corn fiber oil and rice bran oil contain 10-15% and 2% total plant sterols, respectively, but have not been used as a commercial feedstock for plant sterols/stanol ²². In

plants, most sterols/stanols occur either in the free un-esterified form or esterified to fatty acids. However, plant sterols/stanols also occur as steryl glucosides (SG) and as acylated steryl glucosides (ASG) with the SG esterified to a fatty acid. Unlike sterol esters, SG can inhibit cholesterol absorption in their intact form, without being hydrolyzed by digestive enzymes such as pancreatin^{23,24}. A future option therefore could be cloning the gene to produce SG, which may be useful if future clinical studies indicate additional benefits of dietary SG, when compared to common forms of free and esterified plant sterols²⁵. Inclusion of lecithin as a food ingredient, as another strategy, may contribute significant amounts of plant sterols/stanols to the diet. Lecithin also has been reported to be a valuable organogelator. An organogel is defined as an organic liquid entrapped within a thermo-reversible, three-dimensional gel. Some of the other main organogelators include sitosterol plus oryzanol and plant waxes^{26,27}. Hence, further research on organogels is warranted.

3. Effects of Plant Sterols/Stanoles Beyond Cholesterol-Lowering

3.1 Plant Sterols/Stanoles and Immune Function

Nutrition, whether considered as whole diets, specific nutrients, or bioactive phytochemicals, is a powerful modulator of the immune system, regulating defense against pathogens and the chronic inflammatory response that underlies many disease states²⁸. Previous *in vitro*²⁹, animal²⁹, and human³⁰ studies suggest that plant sterols/stanols affect immune response. Calpe-Berdiel et al. reported that, independent of cholesterol-lowering effects, 2% dietary plant sterol supplementation in apolipoprotein E (apoE) deficient mice increased secretion of the type 1 T helper cells (Th1), interleukin (IL-2) and interferon gamma (IFN) from cultured spleen lymphocytes treated with turpentine²⁹. An effective biological response to an immune challenge involves the balance of specific patterns of pro- and anti-inflammatory

cytokines by Th1 and Th2 helper T cells, respectively³¹. Nashed et al. demonstrated that in addition to cholesterol lowering, 2% dietary plant sterol supplementation in apoE deficient mice for 14 weeks decreased plasma IL-12 concentrations³². Brull et al. previously reported evidence that physiological concentrations of both sitosterol and sitostanol induce a Th1 shift in human peripheral blood mononuclear cells³³. More recently, the same group addressed whether these *in vitro* plant sterol/stanol-induced changes could be applied clinically to enhance immune function in asthma patients³⁴. In a randomized, double-blind clinical trial, asthma patients receiving plant stanol enriched soy-based yogurts (4.0 g/d plant stanols) vs control demonstrated higher antibody titers against hepatitis A virus vaccination and reductions in plasma total immunoglobulin E, interleukin (IL)-1 β , and tumor necrosis factor- α concentrations. Changes in plant stanol concentrations correlated positively with changes in antibody titers and the Th1/Th2 cytokine index and negatively with changes in IL13 concentrations. Although these results are promising, further studies designed to explore clinical benefits in immune compromised populations are required.

3.2 Plant Sterols/Stanol and Triglyceride-Lowering

The rising global obesity epidemic is associated with a characteristic dyslipidemic phenotype that includes elevated serum/plasma cholesterol and triglyceride (TG) concentrations. Previous work suggests that approximately 80% of overweight and obese subjects have serum TG concentrations >150 mg/dL (1.7 mmol/L). Although plant sterols/stanols have a rich history as effective cholesterol-lowering compounds, their benefit in reducing hypertriglyceridemia is a relatively recent discovery. Results of previous randomized controlled studies conducted in normo-triglyceridemic subjects suggest that daily supplementation of plant sterols/stanols (1.6-9 g/d) for 1-2 months resulted in a TG-lowering response of 0.8-7%.³⁵⁻³⁸. However, in subjects

with elevated serum TG concentrations (>1.7 mmol/L), randomized control trials results suggest that plant sterol/stanol supplementation (1.8-4 g/d) may lower circulating TG concentrations in the range of 11-28% ³⁹⁻⁴⁵.

Previous animal studies indicate that the TG-lowering effects of plant sterols may be related to altered intestinal fat metabolism including increased fecal fatty acid excretion in plant sterol supplemented mice ⁴⁶ and reduced postprandial lymphatic transport of TG (5-7 hours following a meal) in thoracic duct-cannulated Sprague-Dawley rats ⁴⁷. However, clinical studies investigating postprandial fat handling in normo-triglyceridemic subjects failed to support animal data suggesting that plant sterols can interfere with intestinal fat digestion/absorption ^{48,49}. Studies investigating potential alterations in TG absorption or postprandial handling in response to plant sterol/stanol supplementation in subjects with hypertriglyceridemia are needed.

Additionally, previous work implies that plant sterol supplementation may reduce hepatic *de novo* lipogenesis in Golden Syrian hamsters ⁵⁰, however, species differences have been noted ⁴⁶. In support of a TG-lowering mechanism of hepatic origin, Plat et al., reported a reduction in large and medium plasma very low density lipoprotein (VLDL) particles in dyslipidemic metabolic syndrome subjects consuming 2 g/d of plant stanols provided in a yogurt ⁴². This was also confirmed in an animal study looking at hepatic VLDL production ⁵¹.

Future research priorities with respect to plant sterols/stanols and TG metabolism include human intervention studies specifically powered to detect TG responses in hypertriglyceridemic subjects, a direct examination of fatty acid absorption, as well as whole body lipogenesis in response to plant sterol/stanol supplementation. Additionally, identification of both metabolic and genetic factors that determine the magnitude of plant sterol/stanol-induced TG reductions, needs more attention.

3.3 Plant Sterols/Stanol and the Central Nervous System

Consumption of plant sterol-enriched foods increases circulating plant sterol levels and may enhance accumulation of plant sterols in tissues such as aortic valves, liver, but also in the central nervous system (CNS) ⁵²⁻⁵⁵. In a study by Simonen et al consumption of plant sterols/stanols did not enhance accumulation of plant sterols/stanols in stenotic aortic valves ⁵⁶. The mean duration of this intervention was 2.6 ± 0.2 months (range 0.6-5.0 months) ⁵⁶.

Although sterols are poorly transported across the blood brain barrier (BBB), sterols with a lower molecular side-chain complexity such as cholesterol and campesterol cross the BBB more easily compared to other plant sterols possessing a more complex side chain (e.g. sitosterol and stigmasterol) ⁵⁷⁻⁵⁹. The exact mechanism by which plant sterols are delivered to the endothelial monolayer of the BBB remains speculative. As ATP-binding cassette sub-family G member 5 and member 8 (*ABCG5/G8*) transporter proteins are not expressed within the brain, or at the BBB ⁶⁰, this transporter complex would not be expected to modulate plant sterol transport at the level of the BBB. An HDL-mediated plant sterol transport pathway across the BBB has been suggested given that plant sterols are predominantly transported via HDL in wild type and *ABCG5*^{-/-} mice, and scavenger receptor class B member 1 (SR-BI), the major HDL receptor, is highly expressed on the apical membrane of endothelial cells of the BBB ⁶¹. Regardless of the uptake mechanism, animal plant sterol feeding and depletion studies suggest that accumulation of plant sterol in the CNS is virtually irreversible ⁵⁸. Although the conversion of cholesterol to 24(*S*)-hydroxycholesterol in neurons accounts for over 60% of cholesterol efflux from the CNS ⁶²⁻⁶⁶, once plant sterols enter the CNS, they are not metabolized by the *CYP46A1* gene into 24(*S*)hydroxysterol ^{58,67}, likely due to steric hindrance with respect to the ethyl or methyl group at the C24 position.

Although quantitative data on spatio-temporal accumulation of plant sterols in the human CNS are limited, the total content of plant sterols in the CNS of non-neurologic elderly is estimated at ~75 ng/mg dry tissue, representing about 0.5% of the total amount of sterols in the CNS⁵⁴. Pyramidal cells of the cortex and Purkinje cells of the cerebellum have a cholesterol turnover rate of more than 20%/day^{63,68-70}. The high flux of sterols in these metabolically active cells allow fast incorporation of plant sterols in detergent-resistant parts of neuronal membranes, thereby actively modulating CNS cholesterol metabolism^{58,71}. A mechanistic study from Burg et al. shows that cleavages of the amyloid precursor protein were beneficially modified by incorporation of plant sterols in neuronal membranes⁷². To date, it is largely unclear whether accumulation of plant sterols in the CNS has functional implications. Long-term exposure to increased levels of plant sterols in transgenic mice did not lead to an overt cognitive phenotype with respect to memory or anxiety⁷³. Similarly, a randomized double-blind placebo-controlled dietary intervention study showed no negative influence of long-term plant sterol or stanol consumption on neurocognitive function or mood in hypercholesterolemic patients receiving statin treatment⁷⁴. On the other hand, previous studies found that plant extracts have anxiolytic-like effects after intraperitoneal administration in mice^{75,76}. Together, data suggest that plant sterols do not enhance cognition in normo-cognitive settings. However, accumulating *in vitro* and *in vivo* findings support a therapeutic potential for plant sterols in a disease-related cognitive impairment.

4. LDL- Responsiveness to Plant Sterols/Stanol

4.1 Increased Cholesterol Excretion as an Alternative Measure of Plant Sterols/Stanol Efficacy

Reduction of cholesterol absorption by plant sterols/stanols is clearly important in their LDL-C lowering action, but it may not be the only mechanism. Plant sterols/stanols also may affect reverse cholesterol transport and whole body cholesterol metabolism⁷⁷, which are emerging areas of interest in cardiovascular risk analysis studies. Plant sterols/stanols exert their principal effects most likely through disruption of the intraluminal solubilization step⁷⁸. In a controlled feeding study with 20 subjects, in which dietary nutrient and plant sterols intakes were measured and carefully controlled, fecal cholesterol excretion rose by 36% as the diet plant sterol content was increased from 59 mg/day to 459 mg/day and by a total of 74% as the plant sterol dose was further increased to 2059 mg/day⁷⁹. In contrast, LDL-C levels were reduced by 5% and 9%, respectively, with each stepwise increase in dose. Additionally, in many studies, plant sterol consumption reduces cholesterol absorption efficiency by 30-45%⁸⁰⁻⁸⁴, yet circulating levels are not affected to such a large extent. Taken together, these data emphasize that the effects of plant sterols/stanols on whole body cholesterol metabolism are broad and not limited to only LDL-C lowering, but that there should be additional pathways involved. More studies demonstrating enhanced reverse cholesterol transport and reductions in hard cardiovascular outcomes following plant sterol/stanol feeding should improve the ability to make public health recommendations. To successfully achieve this goal better biomarkers to assess plant sterol/stanol consumption precisely are needed. Better biomarkers are needed as measuring plasma plant sterols/stanols alone does not allow a precise estimation of dietary intake because of the large between-individual variation in non-cholesterol sterol handling. Validation of biomarkers of dietary plant sterol/stanol consumption on controlled diets where plant sterol intake is precisely known suggests that a better indicator is the ratio of plasma campesterol (the most avidly absorbed plant

sterols), to 5 α -cholestanol (an endogenous cholesterol metabolite). This ratio has been found to be significantly and directly associated with dietary plant sterol intake ($R^2 = 0.79$, $P < 0.0001$ ⁸⁵).

4.2 The Genetics Behind Plant Sterols/Stanol Responsiveness

Several clinical studies have investigated the genetics behind plant stanol responsiveness. Effects of small amounts of sitosterol, sitostanol and sitostanol esters (< 1 g/day of free sterols) dissolved in rapeseed oil (RSO) were studied on serum lipids and cholesterol metabolism in patients with primary hypercholesterolemia, but with different apolipoprotein E (apo E) phenotypes on a RSO diet. LDL-C reduction was -8% in subjects with apo E epsilon 4 allele and insignificant in those with apo E3/3 phenotype⁸⁶. The relationship of genetic variation in genes encoding apolipoprotein A-IV, scavenger receptor BI, HMG-CoA reductase, CETP and apo E with the response of cholesterol metabolism to plant stanol ester consumption was examined by Plat and Mensink⁸⁷. This group examined 112 non-hypercholesterolemic subjects, 70 of whom consumed 3.8-4.0 g plant stanols in the form of plant stanol esters per day for 8 weeks. No significant differences between the polymorphisms and dietary responsiveness to plant stanol consumption was found, thus indicating it is unlikely that one of the single polymorphisms analyzed in this study was a major factor in explaining the variation in serum LDL-C responses⁸⁷. However, in another study in which changes in serum plant sterol concentrations with *ABCG5/G8* polymorphisms were investigated after consumption of plant stanol esters, cholesterol-standardized serum campesterol and sitosterol concentrations were significantly associated with the *ABCG8* T400K genotype, as were changes in serum plant sterol concentrations after consumption of plant stanols. However, despite the shifts in circulating plant sterol levels, no associations with serum LDL-C levels were found⁸⁸. Gylling et al. also determined whether common polymorphisms of *ABCG5* and *ABCG8* regulate the responses of

serum cholesterol levels and vascular function during long-term inhibition of cholesterol absorption. Here, 282 subjects completed a 1-year study consuming plant stanol or sterol esters (2 g/d plant stanols or sterols) or a control spread. Neither serum cholesterol lowering, nor absorption inhibition, were found to be associated with polymorphic sites of *ABCG5* and *ABCG8*. However, regulation of baseline cholesterol metabolism and vascular function and structure, and intima media thickness (IMT) progression during 1 y seemed to share some common polymorphic sites of these genes, suggesting a gene-regulated interaction between cholesterol metabolism and vascular function and structure⁸⁹. Taken together, although provocative data exist suggesting that genetic architecture influences the response of sterol metabolism to plant sterols/stanols, such mechanisms need further study.

Clinical trials, as shown in **Figure 1**, reveal that substantial inter-individual variability in LDL-C lowering exists in response to plant sterols consumption^{40,90}, with responses ranging from better than average to non-response or even adverse-responsiveness (please include in citations: Weingärtner O, Bogeski I, Kummerow C et al. Plant sterol ester diet supplementation increases serum plant sterols and markers of cholesterol synthesis, but has no effect on total cholesterol levels. *J. Steroid Biochem Mol Biol.* 2017; 169: 219-225.)^{1,91}. Distinct inter-individual responses to plant sterol consumption have been shown to be reproducible in individuals across repeated plant sterols interventions⁹², indicating other potential determinants of responsiveness. Factors responsible for this variability have been investigated. One explanation has focused on individual differences in cholesterol synthesis rates as determined by the circulating lathosterol-to-cholesterol ratio. This was shown to be a biomarker predicting an individual's response of cholesterol biomarkers to plant sterol intervention, as reported by Mackay et al⁹³. Response of cholesterol synthesis and plasma cholesterol levels were found

subsequently to be influenced by SNP rs38038607 in CYP7A1- and APOE polymorphisms ⁹⁴. In particular, *CYP7A1*-rs3808607 and APOE isoforms were correlated with the extent of reduction in circulating LDL-C levels in response to plant sterol consumption. Thus, these could serve as potential predictive genetic markers to identify individuals who would derive maximum LDL-C lowering with plant sterol consumption ⁹⁴. Mackay's study confirmed the results of De Castro-Oros et al ⁹⁵, which assessed whether a common A to C substitution at position –204 of the promoter of *CYP7A1*-rs3808607 was related to variability in plasma sterol responses to plant sterol supplementation. They found that compared with carriers of the A allele, those bearing the –204C variant had a significantly higher adjusted mean reductions in total cholesterol and increases in lathosterol-to-cholesterol ratios ⁹⁵.

To investigate if other evidence exists in support of genetic mechanisms explaining inter-individual differences in responsiveness to dietary bioactives, Abdullah et al., reviewed the current knowledge on cholesterol-related genetic variations in association with responses of fasting circulating cholesterol levels in epidemiological and intervention studies ⁹⁶. The reviewed studies indicate that carriers of certain genotypes within cholesterol-related genes respond better to a given dietary intervention than others, and the clinical effects of this responsiveness seem to be significant for most cases reported ⁹⁶. For example, a 3.9-fold greater reduction in serum LDL-C levels was observed in hypercholesterolemic men carrying the SNP rs4148217-A, but not the other allele, in the *ABCG8* gene when intake of plant sterols was 2.0 g/d for 4 weeks ⁹⁷. These findings could represent a first step in evaluating the use of common genetic variations to predict an individual's response to plant sterol/stanol intervention, which would potentially enhance plant sterol/stanol efficacy in reducing CVD risk factors. Taken together, it has been considered that a tipping point has been reached in understanding that genomic architecture plays

a role in modulating the degree of responsiveness of biomarkers to dietary intervention. A number of cholesterol-related gene-diet interactions have been identified, suggesting that such interactions may represent a further advance for meaningful conclusions that may eventually lead to genetically targeted dietary recommendations in the era of personalized nutrition ⁹⁶.

5. Challenges in Measuring Plant Sterols/Stanoles in Biological Samples and their Use as Surrogate Markers of Cholesterol Metabolism:

5.1 Measuring Plant Sterols/Stanoles

Plant sterols/stanoles fall broadly into the category of non-cholesterol sterols (NCS), which encompasses a category of biological non-cholesterol and non-steroid hormone sterols. NCS share the steroid skeleton with cholesterol, and are comprised of precursors in the cholesterol synthesis pathway, sterols/stanoles of plant origin, and certain cholesterol derivatives ⁹⁸. Serum or plasma concentrations of the cholesterol precursors, such as lanosterol, lathosterol, and desmosterol, are widely used as surrogate markers of endogenous cholesterol synthesis ^{99,100}. Reciprocally, plant sterols, such as campesterol or sitosterol and the cholesterol metabolite 5 α -cholestanol, are used as markers of cholesterol absorption ¹⁰¹⁻¹⁰³.

These NCS are often so similar in structure to cholesterol that enzymatic methods to quantify cholesterol will actually measure the NCS species as well, artificially inflating cholesterol concentrations ¹⁰⁴. Conceptually, very little in the quantitation of NCS has changed since they were measured by Bhattacharyya and Connor in the first sitosterolemic children identified ¹⁰⁵. The various species of sterols must be separated chromatographically, often by gas or liquid chromatography and then measured, which typically either uses flame ionization detection or mass spectrometry ¹⁰⁶. Even with careful chromatographic techniques it can still be impossible to separate certain species of sterols; therefore, separation of these species must occur

during the detection using mass spectrometry with mass selective detection ¹⁰⁷. While NCS may share a similar chemical structure as cholesterol, they are found in biological fluids in concentrations which are profoundly different, ranging from mmol/L for cholesterol, umol/L for plant sterols/stanols and cholesterol precursors, down to pmol/L or lower for their oxidized sterol derivatives ¹⁰⁸. The large range of concentrations in NCS renders it difficult to capture all using a single analytical method, which have contributed to the numerous methods which have been specifically developed for measuring NCS ¹⁰⁶. These methods for NCS measurement often vary in chromatographic separation techniques and detection methods ¹⁰⁷. This variability in methodology used to measure NCS is a substantial challenge to their use as surrogate measures of cholesterol metabolism because it hinders the ability to compare NCS values reported from different laboratories. In fact, measurement methodology has been identified as the greatest contributor to variability in plant sterols concentrations reported in the scientific literature ¹⁰⁹. This variability has led to an attempt by researchers in the field to work towards harmonizing NCS measurement and to conduct ring-trials to measure the amount of variability across various laboratories ¹⁰⁶. In summary, comparing plant sterol or stanol concentrations reported from different laboratories must be done with caution, realizing that methodology may be the biggest single contributor to differences, rather than diet or other biological mechanisms.

5.2 Plant Sterols/stanols as Surrogate Markers of Cholesterol Metabolism

As mentioned above, circulating plant sterol/stanol levels are often used as surrogate measures of cholesterol absorption ¹⁰². Compared to direct and indirect methods of measuring whole body cholesterol absorption or synthesis, measuring NCS is faster, affordable and less invasive. However, occasions occur when using plant sterols or stanols as surrogate markers of cholesterol absorption is not appropriate and may not accurately represent intestinal sterol

405 absorption even in the absence of supplemental intake of plant sterols/stanols. When intakes of
406 plant sterols or stanols are changing, such as in a trial involving plant sterol/stanol
407 supplementation, use of concentrations of those compounds as surrogate measures of cholesterol
408 absorption is invalidated ¹¹⁰. When plant sterols, or other NCS, are to be used as surrogate
409 measures, they should be expressed as ratios to total cholesterol, which standardizes for
410 variations in sterol transport protein concentrations ¹⁰¹ and show even stronger correlations with
411 cholesterol absorption and synthesis. Plant sterols and other NCS, as surrogates for cholesterol
412 absorption, have been associated with CVD risk ^{111,112}. NCS have also been used to differentiate
413 between different types of dyslipidemias ^{98,113,114}; predict response to statin therapy ^{115,116}; and
414 could be used to guide lipid lowering therapy ^{117,118}. ^{190; (please include this citation)}. Beyond their use
415 individually as markers of cholesterol absorption or synthesis, the ratios of cholesterol synthesis
416 to cholesterol absorption surrogates, such as the lathosterol to campesterol ratio, are also utilized
417 to assess the overall balance of cholesterol metabolism, with higher values representing more
418 synthesis and lower absorption ¹¹⁹. However, due to the inherent nature of ratios, use of the ratio
419 of synthesis to absorption markers does not take into account the absolute values of each marker.
420 This hypothetically means that an individual with the unlikely scenario of high concentrations of
421 both synthesis and absorption surrogate markers could have the same ratio as someone with very
422 low values, which likely does not fit well with the actual impact of these different values on
423 biology. To overcome this limitation it is possible to arrange the synthesis and absorption
424 markers in a Cartesian plane and relate an outcome in a third plain as was done by Qi et al. ¹²⁰. A
425 new approach of using both absorption and synthesis markers together as a method of measuring
426 cholesterol metabolism was proposed (**Figure 2**). By taking the length of the hypotenuse of a

triangle created by graphing cholesterol absorption surrogates against synthesis surrogates, a potential overall measure of cholesterol metabolism is obtained.

Due to their ease of use, measuring plant sterol or other NCS, as surrogates of cholesterol metabolism is not likely to become less common. Improvements and standardization in the measurements of NCS and how they are used as surrogate markers of cholesterol metabolism will further improve their utility.

6. Plant Sterols/Stanol as Adjuncts with Diet and Drugs

6.1 Lipid Lowering Drugs and Plant Sterols: Ezetimibe

Ezetimibe (Zetia, Ezetrol) is a selective cholesterol absorption inhibitor that potently inhibits the uptake and absorption of biliary and dietary cholesterol and non-cholesterol sterols from the intestinal lumen without affecting the absorption of other nutrients. Clinically, ezetimibe reduced fractional cholesterol absorption and this was accompanied by an LDL-C lowering of 20.4% in 18 patients with mild hypercholesterolemia ¹²¹. Ezetimibe alone reduces plasma total cholesterol and LDL-C levels by 18% in patients with primary hypercholesterolemia, and when ezetimibe was added to on-going statin treatment, an additional 25% reduction in LDL-C levels occurred ¹²². On the other hand, ezetimibe also blocks plant sterol absorption. In clinical studies, after just two weeks of ezetimibe at 10 mg/day, plasma sitosterol and campesterol were reduced 41% to 48%, respectively. Ezetimibe also reduced serum plant sterol levels by about 50% in combination with statins (simvastatin and atorvastatin) ¹²³.

Sitosterolemia is caused by mutations in the ATP-binding cassette (ABC) co-transporters, either *ABCG5* and/or *ABCG8*, leading to an accumulation of plant sterols in plasma and tissues which, in turn, results in accelerated cardiovascular disease, anemia, platelet defects, and other disorders. Case studies have examined ezetimibe treatment for sitosterolemia, and in some

450 instances ezetimibe treatment caused xanthomas to resolve, platelet counts to increase, and
451 cardiovascular symptoms to improve ¹²⁴. Ezetimibe reduced the serum levels of the atherogenic
452 sterols campesterol and sitosterol in 37 patients with sitosterolemia ¹²⁵.

453 The intestinal transporter for cholesterol and plant sterols is Niemann Pick C1 Like 1
454 (NPC1L1) ¹²⁶. Ezetimibe works by inhibiting the NPC1L1 mediated uptake of sterols into the
455 enterocyte and it also blocks the re-uptake of sterols from the bile back into hepatocytes in
456 humans ¹²⁷. This blockage results in enhanced excretion of fecal neutral sterols and a reduction
457 of both plasma and tissue cholesterol and plant sterol levels.

458 Pre-clinically, ezetimibe treatment or the lack of NPC1L1 in mice has been shown to
459 reduce atherosclerosis ¹²⁸. The effect of NPC1L1 mutations on human atherosclerosis was not
460 known. Sekar Kathiresan et al led a study where they exon-sequenced >22,000 individuals and
461 found 15 inactivating mutations of NPC1L1. Then they screened for these inactivating NPC1L1
462 mutations in >100,000 individuals and looked at their CVD risk and found that being
463 heterozygous for an inactivating mutation of NPC1L1 was associated with an average plasma
464 LDL-C reduction of about 12 mg/dl and a fall in the risk of coronary heart disease (CHD) by
465 53% ¹²⁹. Since these are heterozygotes, this is a lifelong 50% inhibition of NPC1L1. So, whether
466 the use of ezetimibe to inhibit NPC1L1 will cause a similar large decrease in CHD in a hard
467 outcomes trial needed to be addressed.

468 The IMPROVE-IT was an acute coronary syndrome (ACS) secondary prevention
469 outcomes trial in over 18,000 patients ¹³⁰. The objective was to reduce LDL-C levels to either 70
470 mg/dl with simvastatin alone or to 55 mg/dl by adding ezetimibe, seeing if even lower than the
471 70 mg/dl LDL-C guideline recommendations is better with the combination. The baseline LDL-
472 C levels were 94 mg/dl at the start of this trial. In contrast to previous data ¹³¹, there was about a

16 or 17 mg/dl difference between the treatment groups; with simvastatin alone, LDL-C levels were 70 vs 53 mg/dl with the combination with ezetimibe. There was a significant reduction of 6.4% treatment effect on top of simvastatin with ezetimibe for the primary CVD outcome endpoints in the intention to treat population ¹²⁴. In another study, the addition of plant sterols to ezetimibe improved the effects of ezetimibe on whole-body cholesterol metabolism and plasma LDL-C as shown by Lin et al, ¹³². Recently, Gomez et al., reported that the combination of plant sterols and ezetimibe was associated with lower LDL-C levels ¹³³. In that regard, long-term use of sitostanol-ester margarine as a substitute for part of normal dietary fat had a favorable effect in subjects with mild hypercholesterolemia in lowering serum total cholesterol and LDL-C levels ¹⁹. Therefore, this indicates that LDL-C lowering with ezetimibe is probably causing the reduction in CV events. These data help emphasize the primacy of LDL-C lowering as ‘a strategy to prevent coronary heart disease’ ¹³⁴.

A question still remains whether it is just LDL-C reduction with ezetimibe that lowers the CV event rates. Ezetimibe also blocks plant sterol absorption, and possibly oxysterol absorption, which may add to the anti-atherosclerotic activity of ezetimibe, but this requires further investigation.

6.2 Guidelines for Lowering Serum Cholesterol Levels: Is There a Place for Plant Sterols/Stanol?

There has been a long-standing argument over the “statin hypothesis” - the idea that statins have a unique efficacy in atherosclerotic vascular disease not shared by other lipid-modifying agents, and that reductions in LDL-C levels are not the only basis for the beneficial effect of statins. The efficacy and safety of statin therapy treatment was explored in a prospective meta-analysis of data from over 90,000 individuals in 14 randomized trials. The study concluded that,

on average, a reduction of 1 mmol per liter (38.7 mg/dl) in LDL-C levels by statin therapy yields a consistent 23% reduction in the risk of major coronary events over 5 years¹³⁵.

In this regard, the recent development of PCSK9 inhibitors is also of note. These agents reduce LDL-receptor degradation, thereby enhancing LDL clearance from the circulation, and reducing LDL-C levels by as much as 60%¹³⁶. Definitive clinical outcomes trials with these agents are ongoing. Sabatine et al found that PCSK9 inhibition with the PCSK9 inhibitor Evolocumab on a background of statin therapy reduced LDL-C levels and the risk of CVD¹³⁷.

6.3 Plant Sterols and Other Dietary Agents

Like fiber, plant sterol intake appears to have contracted substantially in modern diets. It has been estimated from studies of early ancestral diets that one would have consumed ~1 g/d of plant sterols 4-5 million years ago when splitting genealogically from the gorillas and chimpanzees.

When this early diet was recreated and fed to healthy volunteers, major increases in fecal output (1 kg/d) and marked reductions in circulating LDL-C levels of 30-35% were observed¹³⁸. This fall in cholesterol was related to increased intakes of fiber, vegetables, vegetable proteins, nuts and plant sterols in the diet that was very low in saturated fat with zero cholesterol content. It can be reasoned that the lack of these components in the current diet, together with the consumption of significant amounts of animal products, high in saturated fat, cholesterol and animal proteins, was responsible for the current elevated LDL-C levels seen in humans consuming Western-type diets. This current intake has resulted in the need to take statin drugs instead of employing diet modification to improve cholesterol levels.

The key elements of the ancestral dishes, which were individually been approved by FDA for cholesterol reduction claims, were taken to create a new diet, which required consumption of

a very large volume of plant foods. Elements included vegetable protein (soy); nuts; viscous fibers (oats, barley and psyllium); and plant sterols, incorporated in standardized amounts into a single diet termed the “dietary portfolio”. This portfolio diet lowered LDL-C and CRP levels by 20-35% in hyperlipidemic participants on metabolic diets ¹³⁹. In an ad libitum study over 6 month on a self-selected dietary portfolio in a cross-Canada multicenter trial of 335 participants, LDL-C levels were decreased by 13-14%, and by~20% on the West Coast ¹⁴⁰! It is believed that plant sterols were a major reason for the dietary portfolio’s LDL-C reducing effect, since a 10-15% reduction can be seen with 2 g/d intake and isotopic studies have shown that both plant stanols and sterols reduce cholesterol absorption comparably. Plant sterols therefore appear to have a very useful role in maintaining healthy cholesterol levels.

7. Plant Sterols/Stanol and Cardiovascular Disease (CVD) Risk

7.1 Vascular Function Effects of Plant Sterols/Stanol

The LDL-C lowering effect of plant sterols/stanol is well established ^{2,7,84}. Nevertheless, direct evidence linking the intake of foods with added plant sterols/stanol and CVD risk is still lacking. As mentioned earlier, CVD endpoint trials with plant sterols/stanol are prohibitively expensive and challenging to perform. Depending on the length of follow-up and the annual risk level, 36,000 to 636,000 subjects would be needed to have enough power to show a LDL-C lowering benefit. A typical CVD endpoint study was deemed therefore not feasible for foods with added plant sterols/stanol due to the large sample size required, compliance aspects and costs. Therefore, surrogate endpoint markers will remain to serve as an alternative to study the direct effect of plant sterols/stanol on CVD risk. As atherosclerosis progression occurs from an early age onwards, the function and structure of the arterial wall is influenced. Endothelial

function may be impaired, arteries may become stiffer, and thickness of the arterial wall may increase and low-grade inflammation may occur.

7.2 Plant Sterols/stanols and Endothelial Function

Several types of evidence support a link between LDL-C and endothelial function, including data from patients with familial hypercholesterolemia ¹⁴¹, LDL apheresis ¹⁴² and other LDL-C lowering treatments such as statins ^{143,144} and ezetimibe ^{145,146}. Furthermore, a significant inverse association between flow-mediated dilation (FMD) and CVD risk seems to exist, so people with a higher FMD possess a lower risk of CVD ¹⁴⁷.

After consumption of plant sterols their concentrations in plasma and tissues increases. This raises the question of whether this may affect surrogate endpoint markers in a beneficial or perhaps detrimental way. The change in plasma plant sterols after an intake of plant sterol-enriched foods was investigated in a meta-analysis including 41 studies ¹⁴⁸. On an absolute scale, sitosterol and campesterol were increased modestly, on average by 2.2-5.0 $\mu\text{mol/L}$ especially compared to the average change in LDL-C (-0.33 mmol/L). However, on a relative scale, increases were considerable, on average 31-37%. Plasma plant sterol concentrations have been linked to increased CVD risk in homozygous sitosterolemic patients ¹⁴⁹ and in some, but not all, observational studies ¹⁵⁰. However, there are also controversial findings as demonstrated by the results of another study in five sitosterolemic subjects. In spite of massive hypercholesterolemia and high plant sterol/stanol levels, none of these individuals had symptoms of CVD or positive clinical markers of atherosclerosis ¹⁵¹. It should be realized that intake of foods with added plant stanols, the saturated form of plant sterols, increases plasma plant stanol concentrations despite a lower absorption rate compared to plant sterols. A randomized trial with a 4-week intake of 3 g/d of plant stanols showed increased plasma plant stanol concentrations by about 400% ¹⁵². On the

absolute scale, however, these increases were minor, being far less than those in plant sterols when their intake was increased.

The effects of plant sterols/stanols on endothelial function have been investigated in several animal and human studies. In wild-type mice fed for 4 weeks extremely high doses of plant sterol esters (2%; ~100 times higher than the 2 g/d recommended dose for lowering LDL-C in humans), intake of plant sterols increased plasma plant sterol concentrations and impaired endothelial-dependent vasodilatation, as measured by vascular relaxation of aortic rings ⁵³. Furthermore, cerebral lesion size increased after plant sterol intake. However, plasma cholesterol concentrations in these mice were not affected, questioning whether these wild-type mice were suitable for studying the effects of plant sterols. In another animal study with an atherogenic apoE^{-/-} mouse model, plant sterol and plant stanol supplementation reduced serum cholesterol and increased plant sterol and plant stanol concentrations, as expected ¹⁵³. Elevated levels of plant sterols/stanols were associated with impaired endothelial function. Atherosclerotic lesion retardation was more pronounced in response to plant stanol compared to plant sterol supplementations, however, this effect was not significant ¹⁵³. Diet supplementation with plant sterols and ezetimibe, alone and in combination reduced the atherosclerotic lesion compared to control, however the reduction was significantly greater in the ezetimibe versus the plant sterol fed group ⁵³. Contrary to the findings in mice studies, 6-week intake of sitosterol and stigmasterol in hamsters improved aortic functioning as measured by acetylcholine induced endothelium-dependent relaxation ¹⁵⁴. Taken together, animal studies reporting effects of plant sterol/stanol intake on endothelial function show conflicting results.

A few human studies have investigated the effect of plant sterol/stanol intake on FMD as summarized by Plat et al. ¹. Despite significant reductions in LDL-C in these studies, none

showed statistically significant effects on FMD. However, when the effects seen in five of these studies were combined, an indication for a modest improvement in FMD was found^{89,131,155-157}.

Recently, the large randomized trial focusing on vascular function effects of plant sterols (the INVEST study), investigated the influence of plant sterol intake on FMD as a primary outcome measure together with other vascular function markers¹⁵⁸. The study included 240 subjects who consumed margarine enriched with 3 g/d of plant sterols for 3 months. The INVEST study showed that plant sterol intake had a neutral effect on endothelial function based on a placebo-corrected change in FMD of 0.01 percentage points (95% CI: -0.73, 0.75). Also, arterial stiffness as measured by pulse wave velocity and augmentation index, was not affected. This neutral effect supports neither a worsened nor an improved vascular function with plant sterol intake. It should be realized that the LDL-C lowering effect observed in this study was only -0.26 mmol/L (95% CI: -0.46; -0.07) or -7% compared to control, which is smaller than anticipated for a plant sterol intake of 3 g/d. In general, it is estimated that 3 g/d of plant sterols would lower LDL-C by ~12%.

In the INVEST study, plasma plant sterol concentrations were significantly increased in the plant sterol group as expected, but these increases were not related to changes in FMD (**Figure 3** permission to re-use required). On the other hand, although not very strong, a larger reduction in LDL-C was significantly correlated with an increase in FMD, suggesting that lowering LDL-C could lead to improvements in endothelial function.

Also, several plasma biomarkers of endothelial dysfunction, E-selectin, soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble intercellular adhesion molecule-1 (sICAM-1), measured as well in the INVEST study, were not significantly affected by plant sterol intake compared to control¹⁵⁹.

Taken together, plant sterols/stanols have not been shown to consistently improve endothelial function, despite significant reductions in LDL-C. This could be because the plant sterols/stanols doses used were below the threshold needed to trigger measurable differences in endothelial function. Furthermore, populations used in studies so far may have been too healthy. Improvements in endothelial function may only be detectable in individuals with impaired endothelial function. Furthermore, a longer intervention period is perhaps needed to detect effects on the endothelium. Importantly, the evidence shows that plant sterol intake does not weaken endothelial function, despite increases in plasma plant sterol concentrations.

7.3 Plant Sterols/Stanols and Other Surrogate Markers of Arterial Health

Recently a few other studies with plant sterols/stanols investigated surrogate endpoint markers including arterial stiffness, intima media thickness (IMT) and inflammation. In a randomized controlled study by Gylling et al., the effects of plant stanols on arterial stiffness were investigated ¹⁶⁰. The study found that lowering LDL-C by ~10% with plant stanol esters reduced arterial stiffness in small arteries with some indications of a beneficial effect on that in large arteries only in men. It should, however, be noted that these effects were mainly driven by increases in arterial stiffness in the control group. Endothelial function, as measured by reactive hyperemia index (RHI), was overall not improved with plant stanol intervention. However, changes in LDL-C correlated significantly with changes in RHI in the plant stanol group, which is consistent with the findings of the INVEST study.

In an observational study with Old Order Amish people who are prone to be heterozygous for sitosterolemia ¹⁶¹, carriers of a specific *ABCG8* variant had higher plasma sitosterol concentrations compared to non-carriers of this variant, whereas LDL-C levels did not differ between groups. Compared to non-carriers, carriers had decreased carotid intima-media wall

thickness, suggesting less plaque formation in their vessels with increased plasma plant sterol concentrations.

Inflammation is also involved in the process of atherosclerosis. Recently, a meta-analysis was published that summarized the effects of plant sterol/stanol intake on inflammation markers, and particularly on C-reactive protein (CRP)¹⁶². A beneficial effect on this marker was not seen.

Evidence regarding effects on surrogate markers of CVD risk, such as endothelial function, is still inconclusive. Noteworthy, no worsening of endothelial function with elevated plasma plant sterols concentrations has been shown.

7.4 Personalizing and Optimizing Lipid-Lowering Therapies

Statins reduce cardiovascular morbidity and mortality in primary and secondary prevention trials¹⁶³⁻¹⁶⁵. However, statin efficacy shows individual differences which can be because of the cholesterol metabolism variations between individuals^{135,166,167,168}, with some subjects demonstrating a genetically determined rather high cholesterol synthesis and others a higher cholesterol absorption¹¹⁸. In subjects with high cholesterol synthesis, statins are potent cholesterol lowering drugs, but in those who are high absorbers, statins are less effective than cholesterol absorption inhibitors in lowering LDL-C¹⁶⁹⁻¹⁷¹. However, some studies have found controversial results. For instance, Lakoski et al reported that combination therapy using ezetimibe and simvastatin lowered LDL-C by 15% or greater in more than 95% of participants¹⁷². Moreover, inhibition of cholesterol synthesis results in increased cholesterol absorption, with increased uptake of plant sterols¹⁷³. As a consequence, in patients with high cholesterol absorption, statins have been shown to increase cardiovascular event rates¹⁷⁴. These findings suggest that individuals with low synthesis and high absorption of cholesterol should be treated with combined cholesterol lowering using a statin and a cholesterol absorption inhibitor¹⁷⁴.

Genetic studies have shown that life-long lower cholesterol levels are associated with lower CVD risk¹⁷⁵. In individuals with inactivating mutations of *NPC1L1* a minor cholesterol lowering of 12 mg/dl reduced cardiovascular risk dramatically by 53%¹²⁹. Moreover, it has been shown for the sterol transporter gene *ABCG8* that plant sterol levels are associated with cardiovascular risk in the general population^{112,149}. Other studies have demonstrated that high cholesterol absorption is associated with coronary artery disease severity¹⁷⁶, and high cholesterol absorption is associated with higher cardiovascular mortality¹⁷⁷. Interestingly, the ratio of cholesterol absorption to cholesterol synthesis has been shown to be associated with coronary artery disease severity¹⁷⁸. These results have been verified in the Framingham-offspring-study, with the ratio of cholesterol absorption to cholesterol synthesis being the best lipid parameter to predict cardiovascular risk¹⁷⁹. New studies using intravascular optical devices show the same direction. In patients with stable and unstable angina pectoris, those with high cholesterol absorption markers and low cholesterol synthesis demonstrated thinner fibrous caps and larger lipid cores¹⁸⁰. In patients with coronary heart disease, the atorvastatin treatment effect on lesion progression was assessed with intravascular ultrasound. In those patients not responding adequately to statin treatment, atherosclerotic plaque progression was most pronounced¹⁸¹. In the PRECISE-IVUS trail, statin monotherapy was compared to combined lipid-lowering with a statin and ezetimibe combination in patients with suspected coronary heart disease¹⁸². After a study period of 9-12 months, LDL-C lowering was greater with combined lipid-lowering than with statin monotherapy (63 mg/dl vs. 73 mg/dl). Moreover, intravascular ultrasound demonstrated a more pronounced atherosclerotic plaque regression with combined lipid lowering. The effect of an ezetimibe-statin combination on lesion regression was more pronounced than the effect of a combination of a statin with a PSCK9-inhibitor in the **GLAGOV** study¹⁸³.

In patients on dialysis, statins did not show any effect on cardiovascular mortality^{184,185}. A possible explanation for this is that patients on dialysis are characterized by high cholesterol absorption and low cholesterol synthesis, with high cholesterol absorption being associated with greater mortality¹⁸⁶. This may also explain why in the study of heart and renal protection (SHARP) a comparably less effective LDL-C lowering resulted in a significant reduction of cardiovascular events with combined lipid-lowering¹⁸⁷. A post-hoc analysis of the AURORA study (a study to evaluate the use of rosuvastatin in subjects on regular hemodialysis: an assessment of survival and cardiovascular events) points in the same direction. In this analysis only patients on dialysis who were known to be high cholesterol synthesizers showed a reduction in cardiovascular mortality on statins¹⁸⁸. Since the publication of the IMPROVE-IT trial additional evidence has surfaced that a combined lipid-lowering in high risk patients can reduce cardiovascular mortality¹³⁰. With these risk calculations in mind, one can speculate that a combined lipid-lowering approach – assessed on an individual basis on differences in cholesterol metabolism – can further reduce cardiovascular risk^{189,190}.

8. Sitosterolemia: Clinical Perspective, Diagnosis, Treatment, Screening Programs

8.1 Microbiota Therapeutics: Perspectives on Management of Sitosterolemia

The gut microbiome is "the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space"¹⁹¹. Many studies have shown that nutrition can affect gut microbiota^{192,193}. Some studies show associations between microbiome and serum lipid levels¹⁹⁴. The composition of the microbiome was recently evaluated during early stages of sitosterolemia. Those animals that developed severe forms of the disease had an overall different composition of the microbiome compared with those that either did not develop the disease, or only a mild form of it. Furthermore, differences in the microbial population across

groups were identified¹⁹⁵. Specifically, levels of lactobacillus were found to be down-regulated in those with severe experimental autoimmune encephalomyelitis (EAE)¹⁹⁵. Lactobacillus is a big component of all of those probiotics in the market. Could one use a probiotic to treat something so specific such as sitosterolemia? Some studies show that plant sterols can affect the microbiome. As an example, dietary supplementation with 5% plant sterol esters induced alterations in the fecal microbiota of hamsters¹⁹⁶. However, a recent study could not confirm this finding in human volunteers¹⁹⁷.

For sitosterolemia management, ezetimibe is the standard treatment. Although it has been shown to reduce plasma sitosterol levels by about 30-40%, this may not be sufficient to treat severe symptoms of the disorder. Could one modify the abundance and the function of the microbiome in order to treat sitosterolemia? How about using a genetically modified vector as a delivery system? Can one deliver a probiotic that proliferates in the gut, and which is able to carry a gene that might actually be able to be transferred into the epithelial cells of the gut? Bacterial vectors have been used in the past to induce protective peripheral immunity. For example, *Salmonella* has been successfully adapted for live-vector vaccine delivery^{198,199}. This shows that such delivery systems can be effective in carrying human genes and transferring them into cells. How about using a genetically modified probiotic that can target the *ABCG5* and *ABCG8* genes in enterocytes? Many issues require consideration including the pathogenic factors of potential vectors; however, these are provocative concepts to explore as potential adjunctive treatment options for sitosterolemia.

8.2 Clinical Perspective: When to Add Sitosterolemia to the Differential Diagnosis List

In 1974 Drs. William Connor and Ashim Bhattacharyya reported the first cases of sitosterolemia¹⁰⁵. The index patients were two young adult sisters who had onset of tendon

xanthomas at the ages of 7 and 8 years, progressing at 13-14 years, which led to medical evaluation. They otherwise had normal development, including “normal” plasma cholesterol concentrations. The total circulating cholesterol levels in both subjects were around 200 mg/dl¹⁰⁵, which at the time was considered an oddity in the context of prominent tendon xanthomas because the level is much lower than what one would expect to see due to a disorder such as familial hypercholesterolemia (FH). FH is an autosomal dominant disorder that affects about 1 in 250 individuals in the general population, is associated with severe hypercholesterolemia, and is the most common cause of tendon xanthomas. FH is caused by defects in the LDL receptor, apolipoprotein B (apo-B), proprotein convertase subtilisin/kexin type 9 (PCSK9), and homozygous defects in the LDL receptor adaptor protein. Roughly one-third of patients with a clinical diagnosis of FH do not have an identifiable mutation even when all of the known genes are sequenced, suggesting other genes involved²⁰⁰. At the time these sisters were evaluated, one would have expected a total cholesterol concentration of 350 mg/dl to 400 mg/dl or higher in a patient with FH. Furthermore, at the time, the presence of tendon xanthomas was usually consistent with a diagnosis of FH, or rarely cerebrotendinous xanthomatosis (CTX) caused by mutations in CYP27A1 that encodes sterol 27-hydroxylase, a key enzyme in the bile acid synthetic pathway²⁰¹. However, it has been suggested that some individuals with undiagnosed sitosterolemia may masquerade as pseudo-FH as a consequence of marked diet-induced hypercholesterolemia that may be seen in some patients with sitosterolemia in response to high intake of dietary cholesterol and plant sterols²⁰². The proportion of patients with a clinical diagnosis of presumed FH who actually have sitosterolemia is unknown.

Sitosterolemia is caused by mutations in sterol transporter genes *ABCG5* and/or *ABCG8*, resulting in several consequences, including intestinal hyper-absorption of all dietary sterols,

impaired hepatic excretion of sterols into bile, increased tissue content of plant sterols, and the development of extensor tendon xanthomas and atherosclerosis.

An important question in relation to clinical practice relates to when a diagnosis of sitosterolemia should be considered. It is a rare disorder, so random screening of patients is not indicated or useful, but there are several situations in which it is reasonable to consider the diagnosis of sitosterolemia. In line with the clinical presentation of the index patients described by Drs. Connor and Bhattacharyya, sitosterolemia should be considered when tendon xanthomas are present in the absence of severe hypercholesterolemia ¹⁰⁵. Another situation that may be a sign of occult sitosterolemia is the development of extreme hypercholesterolemia after consumption of high cholesterol/saturated fat diets. As a consequence of mutations in *ABCG5* or *ABCG8*, patients with sitosterolemia hyper-absorb dietary cholesterol and plant sterols/stanols, resulting in exaggerated diet-induced hypercholesterolemia. One patient was identified with sitosterolemia on the basis of an increase in the LDL-C concentration from 120 mg/dl to 295 mg/dl during consumption of a diet high in saturated fat and cholesterol. Other conditions that may be suggestive of a diagnosis of sitosterolemia include a paradoxical hypercholesterolemia in response to pharmacological treatment with plant sterols. Unlike normal individuals who may achieve an 8-10% decrease in the plasma concentration of LDL-C because of plant sterol-mediated inhibition of micelle formation resulting in inhibition of cholesterol absorption, patients with sitosterolemia will hyper-absorb the plant sterols, and may actually have a hypercholesterolemic response. Hypo-responsiveness to the LDL-C lowering efficacy of statins is another indicator that the patient may have sitosterolemia, but this finding may be confounded by noncompliance with statin treatment, gain of function mutations in PCSK9, or other factors

unrelated to sitosterolemia. Hence, the vast majority of patients who are hypo-responsive to the LDL-C lowering efficacy of statins are unlikely to have sitosterolemia.

A key step in the diagnosis of sitosterolemia is measurement of serum/plasma plant sterols using gas chromatography/ mass spectrometry. Some patient groups have false positive elevations in the concentration of plasma sitosterol equivalent to sitosterolemia, such as babies and patients with severe liver disease who are treated with soy-based parenteral nutrition high in plant sterols. In these individuals, the sitosterolemia is found to be completely reversible after cessation of parenteral administration of plant sterols. Clinical features that may facilitate with diagnosis of sitosterolemia can include extensor tendon xanthomas (rarely tuberous xanthomas), normal to elevated plasma cholesterol, thrombocytopenia, chronic hemolytic anemia and stomatocytosis, and occasionally elevated liver enzymes and acute liver failure, but the absence of these features does not exclude the diagnosis²⁰³. Management of sitosterolemia includes decreasing dietary intake of plant sterols and cholesterol, as well as treatment with ezetimibe, possibly bile acid binding resins, and treatment of hypercholesterolemia with statins as indicated.

In summary, the diagnosis of sitosterolemia should be considered in a variety of clinical settings, including hyper-responsiveness to dietary sterol intake, paradoxical responses to treatment with plant sterols, the presence of tendon xanthomas in the absence of hypercholesterolemia, hypo-responsiveness to statins, findings of platelet and red blood cell abnormalities, as well as early onset coronary artery disease without significant hypercholesterolemia.

8.3 Sterol Metabolism in Sitosterolemia

Although the clinical symptoms of sitosterolemia may vary across individuals, a consistently important diagnosis of the disorder is highly elevated circulating levels of plant

sterols. Abnormal sterol homeostasis has been observed in individuals with sitosterolemia²⁰⁴. It is characterized by increased retention of plant sterols and cholesterol, reduced removal, and expanded whole body pools which compensate for the reduced cholesterol synthesis in sitosterolemia²⁰⁴. Using *in vivo* radiolabeled isotopic techniques, Salen et al.²⁰⁴ observed that the turnover rates of plasma cholesterol and sitosterol in sitosterolemia patients were similar and significantly slower compared to a control subject. It has been shown that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and synthase, and other key enzymes involved in cholesterol synthesis, are down regulated in sitosterolemia patients²⁰⁵⁻²⁰⁷. Accumulation of plant sterols may account for the low cholesterol synthesis rates observed in sitosterolemia²⁰⁸. Strategies such as feeding either the cholesterol precursor mevalonic acid, or low sterol diets²⁰⁷ failed to stimulate *de novo* cholesterol synthesis in patients with sitosterolemia. While ezetimibe is the current standard therapy for sitosterolemia, its effect on the rates of cholesterol synthesis and sterol turnover in sitosterolemic patients are undefined and need further investigation.

9. Intravenous Plant Sterols and Pediatric Intestinal Failure Associated Liver Disease

When enteral nutrition is limited due to insufficient intestinal length and/or poor function, intestinal failure develops. In order to prevent dehydration and malnutrition, patients with intestinal failure are prescribed parenteral nutrition (PN), or intravenous nutrition. PN serves as an important source of water, electrolytes, and macro- and micronutrients. While PN is life sustaining for intestinal failure patients, it can lead to intestinal-failure associated liver disease (IFALD), a potentially fatal liver disorder. IFALD is defined by the presence of intestinal failure, or prolonged PN use, and liver dysfunction, which includes elevated serum transaminases and/or a conjugated hyperbilirubinemia. On liver biopsy, IFALD is characterized by cholestasis,

inflammation, and steatosis. After a short course of PN, liver fibrosis can develop. In some patients, IFALD culminates in cirrhosis, liver failure, and death. Once liver failure develops, a liver transplant is the only life-saving option.

IFALD and sepsis are the top two causes of mortality for children with intestinal failure²⁰⁷. For several reasons, IFALD is more common in children than adults. PN duration, gestational age, birth weight, and underlying gastrointestinal disorders are important risk factors for IFALD. 70% percent of infants who have received greater than 60 days of PN will develop IFALD²⁰⁹. Moreover, gestational age and birth weight are inversely correlated to the incidence of IFALD. Premature neonates and low birth weight neonates are at high risk for IFALD due to prolonged PN courses, immature livers, feeding intolerance, and a high incidence of necrotizing enterocolitis²⁰⁷. Last, children with gastroschisis, volvulus, distal intestinal atresias, and short bowel syndrome commonly develop IFALD²⁰⁷.

Intravenous lipids are prescribed with PN as a source of non-protein calories and essential fatty acids. In the US, the only FDA-approved intravenous lipid emulsion for children is entirely soy-based (Intralipid™(Fresenius Kabi, Uppsala, Sweden). SO-based lipid emulsions have a long-standing association with IFALD^{195,196,210,211}. Intravenous soybean oil contains a high concentration of plant sterols (>350-400 mg/L)^{195,196,210,211}. In contrast to intravenous soybean oil, a non-FDA approved fish oil-based lipid emulsion (Omegaven™, Fresenius Kabi, Bad Homburg, Germany) contains a negligible amount of plant sterols. Fish oil-based lipid emulsions are prescribed in the US under compassionate use protocols and serves as an important rescue treatment for children with advanced IFALD^{209,210,211,212}. Studies have demonstrated that intravenous fish oil is a safe, effective treatment for IFALD; IFALD resolves in approximately

838 75% of children treated with fish oil and is associated with a decrease in both the incidence of
839 liver failure and need for liver transplantation ^{210,211}.

840 While there are several differences between soybean and fish oil lipid emulsions, the plant
841 sterol concentration cannot be overlooked. In comparison to healthy controls, infants with
842 IFALD have higher circulating concentrations of various plant sterols. When IFALD infants are
843 compared to IFALD children, IFALD infants have higher plant sterol concentrations ^{213,214}.
844 Furthermore, plasma sterol concentrations correlate with hepatic sterol concentrations and
845 histological changes on liver biopsy ²¹⁵. Last, in IFALD children whose intravenous soybean oil
846 was replaced with intravenous fish oil, plasma sterol concentrations not only dramatically
847 decreased, but early changes in plasma stigmaterol predicted later changes in conjugated
848 bilirubin ²¹⁰. This suggests that stigmaterol may serve as surrogate for disease severity and
849 treatment response.

850 Animal experiments provide mechanistic evidence that stigmaterol may be one of the main
851 culprits driving IFALD. Mice infused with PN and intravenous soybean oil have decreased
852 expression of hepatic nuclear transcription factors, liver X receptor (LXR) and farnesoid X
853 receptor (FXR), and decreased mRNA expression of bilirubin, bile acid, and sterol liver
854 transporters. Also, mice exposed to PN plus intravenous soybean oil developed cholestasis and
855 elevated liver function tests, mimicking pediatric IFALD ²¹⁶. In contrast, when mice were
856 infused with PN plus intravenous fish oil, FXR, LXR, and transporter expression were similar to
857 control mice, and they were protected against IFALD ²¹⁶. However, when stigmaterol was
858 added to fish oil, FXR, LXR and transporter expression were similar to the soybean oil group
859 and the mice developed IFALD ²¹⁶.

From these studies, it can be concluded that the type of intravenous lipid emulsion and, more specifically, intravenous plant sterols are important players in IFALD pathogenesis. With the advent of new lipid formulations, careful attention should be paid to sterol content. It remains unknown if specific sterols are safer than others, and if there is a “safe” sterol content for lipid emulsions. Further research is needed to answer these questions.

10. Plant Sterols: Patients’ Perspectives

10.1 Introductory Remarks

The National Institutes of Health (NIH) has defined a rare disease as one that affects less than 200,000 people in the US population, which corresponds to 1 in 16,000 to <1 in 500,000 individuals. However, the prevalence of various rare diseases is quite variable, with some incidences being highly infrequent. Currently, 7,000 separate diseases have been identified as rare, with many of these being inherited. Multiple challenges exist with studying rare diseases, including limited recruitment of patients, unknown natural history of the disorder and considerable phenotypic variability in these diseases. This adds to the complications in investigating not only the disease itself, but also therapeutic approaches to these diseases. Very few investigators are trained specifically in rare disease research largely because of the rarity of most of these disorders. Most physicians fail to recognize diseases when they encounter them because they have never seen a case of a disease that occurs one in 100,000 incidents. So many challenges exist. The NIH well recognizes the challenges in diagnosing and treating the very large constellation of rare diseases that exists. This is demonstrated by its establishment of a rare disease clinical research network (RDCRN) which now specifically targets 22 diseases. The Sterol and Isoprenoid Research Consortium (STAIR), one of the 22 in the RDCRN network, is a consortium that is focused on sterol metabolism disorders. The consortium itself has a number of

advantages, such as including recruitment of patients. The idea behind it is that no center will encounter enough patients with a rare disease to be able to conduct a valid clinical study alone, and therefore efforts should be pooled in carrying out multi-center studies on these diseases.

10.2 Sitosterolemia, Clinical and Treatment Aspects. Observations from the Manitoba Cohort

The Manitoba Sitosterolemia Cohort is a kindred of Hutterite patients living mostly in Manitoba. They are a religious isolate based in rural communities. A specific case was a five-year-old girl who died suddenly and was found at autopsy to have extensive aortic and coronary atheroma²¹⁷. Her medical history was anemia and recurring abdominal pain²¹⁸. This led to searching for a diagnosis and eventually a determination of sitosterolemia before the specific mutation was identified^{217,218}. Subsequent cascade screening over a period of some sixteen years has built up a cohort of 21 patients all having the *ABCG8* S107X mutation. All 20 survivors have responded very favorably to ezetimibe therapy^{219,220}.

11. Summary and Conclusions

The present review provides a comprehensive overview of past and recent developments in the basic biology of plant sterols and stanols, largely in the context of their value as therapeutic agents for dyslipidemia management in the general population. It also presents guidance for the clinical management of rare disorders resulting from mutations in sterol metabolism at various levels that lead to the retention in the circulation and tissues of cholesterol, plant sterols and stanols, as well as other types of non-cholesterol sterols. Particularly novel in the area of plant sterol/stanol physiology is the recognition that even low levels of intake of plant sterols or stanols can influence cholesterol absorption efficiency and circulatory pools in both adults and infants. Also, the reciprocity between cholesterol synthesis and absorption and how

that ratio impacts the efficacy of plant sterol/stanol action in LDL-C lowering is being increasingly recognized. How polymorphisms within genes coding for enzymes active in lipid pathways affect the LDL-C lowering action are now better understood. Advantages of combining plant sterols/stanols with other dietary elements such as fiber, soy protein and nuts have been recognized. Overall importance of LDL-C lowering in CVD risk has been further established from combined drug trials such as IMPROVE-IT¹³⁰, FOURIER¹³⁷. Additionally, Ference et al., recently found a clear association between LDL and atherosclerotic cardiovascular disease, from investigating numerous and multiple clinical and genetic studies²²¹. In best approaches to clinical management of sitosterolemia, ezetimibe continues to prevail as the drug of choice. The disparity in degree of severity of this disorder across patients was emphasized, as well as the importance of proper screening using both levels of circulatory plant sterols as well as confirmation of the specific mutation as diagnostic criteria. It is considered important to rely on these tools for correct identification of patients with sitosterolemia so as not to confuse them with FH. In summary, plant sterols and stanols continue to offer an efficacious and convenient dietary approach to cholesterol management and serve as an important natural health product as well as functional food ingredient. Their clinical benefit through long-term studies addressing CVD endpoints has however not been established.

Acknowledgements

Authors would like to express their gratitude to the International Plant Sterol and Stanol Association (IPSSA) as key sponsor for their financial support, which made the organization of this meeting possible. Also, we thank Louise Grapentine and Jennifer Palichuk at the Richardson Centre for Functional Foods and Nutraceuticals for outstanding administrative support. Finally,

928 we thank all speakers and participants for their engagement in the discussion during this meeting.
929 (Appendix 1).

930 **Declaration of Interest**

931 **Oliver Weingärtner has received** speaker honoraria from AMGEN, Berlin-Chemie
932 Menarini, MERCK, Sanofi and serves on advisory boards for AMGEN, MERCK and
933 Berlin-Chemie Menarini.

934

Invited speakers and contributors	Affiliation
Elke Trautwein	Unilever R&D, Vlaardingen, The Netherlands
Robert Moreau	USDA ARS ERRC, Wyndmoor, Pennsylvania, USA
David Bear	Beltsville Human Nutrition Research Center, USDA, USA
Jogchum Plat	Maastricht University, Maastricht, The Netherlands
Tim Vanmierlo	Hasselt University, Hasselt, Belgium
Peter Jones	University of Manitoba, Winnipeg, Canada
Richard Ostlund Jr.	Washington University, St Louis, USA
Jean- Baptiste Roullet	Oregon Health & Science University, Portland, USA
Dieter Lütjohann	Institute for Clinical Chemistry and Clinical Pharmacology, Bonn University, Bonn, Germany
Dylan MacKay	University of Manitoba, Winnipeg, Canada
Mohammad Moghadasian	University of Manitoba, Winnipeg, Canada
Henry Ginsberg	Institute of Human Nutrition, New York, USA
David Jenkins	Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada; Clinical Nutrition & Risk Factor Modification Centre, St. Michael's Hospital, Toronto, ON, Canada.
Rouyanne T Ras	Unilever R&D, Vlaardingen, The Netherlands
Oliver Weingärtner	Abteilung für Kardiologie, Klinikum Oldenburg European-Medical School Oldenburg-Groningen Carl von Ossietzky Universität Oldenburg, Germany
Helena Gylling	University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland
Todd Rideout	University at Buffalo, NY, USA
Javier Ochoa Reparaz	Eastern Washington University Cheney, WA United States.
P. Barton Duell	OHSU, USA
Semone Myrie	University of Manitoba, Winnipeg, Canada
William Rizzo	University of Nebraska Medical Center, USA
David Mymn	Health Sciences Centre, Winnipeg, Canada
Kara Calkins	University of California, Los Angeles, USA
Stephen Turley	UT Southwestern Medical Center, Dallas, USA.
Ken Hofer	Barrickman & Pineland Hutterite Colonies, MB, Canada
Ingmar Wester	Raisio Group, Benecol Unit, Raisio, Finland
Harry Davis	CVPPath Institute Inc, Gaithersburg, Maryland, USA
Susanna Rosin	Raisio Group, Benecol Unit, Raisio, Finland
Dietrich Rein	BASF Plant Science Holding GmbH, Limburgerhof, Germany
Alex Eapen	Cargill's regulatory affairs group, Cargill, Minneapolis, USA
Cyndi Jones	Sitosterolemia Foundation, Huntsville, Alabama, USA

References

1. Plat J, Mackay D, Baumgartner S, Clifton PM, Gylling H, Jones PJ. Progress and prospective of plant sterol and plant stanol research: report of the Maastricht meeting. *Atherosclerosis*. Dec 2012;225(2):521-533.
2. Ras RT, Geleijnse JM, Trautwein EA. LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomised controlled studies. *The British journal of nutrition*. 2014;112(2):214-219.
3. Talati R, Sobieraj DM, Makanji SS, Phung OJ, Coleman CI. The comparative efficacy of plant sterols and stanols on serum lipids: a systematic review and meta-analysis. *Journal of the American Dietetic Association*. May 2010;110(5):719-726.
4. Musa-Veloso K, Poon TH, Elliot JA, Chung C. A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: Results of a meta-analysis of randomized, placebo-controlled trials. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2011/07/01/ 2011;85(1):9-28.
5. Amir Shaghaghi M, Abumweis SS, Jones PJ. Cholesterol-lowering efficacy of plant sterols/stanols provided in capsule and tablet formats: results of a systematic review and meta-analysis. *Journal of the Academy of Nutrition and Dietetics*. Nov 2013;113(11):1494-1503.
6. AbuMweis SS, Barake R, Jones PJH. Plant sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. *Food & Nutrition Research*. 2008;52:10.3402/fnr.v3452i3400.1811.

- 958 7. Demonty I, Ras RT, van der Knaap HC, et al. Continuous dose-response relationship of
959 the LDL-cholesterol-lowering effect of phytosterol intake. *J Nutr.* Feb 2009;139(2):271-
960 284.
- 961 8. Ferguson JJ, Stojanovski E, MacDonald-Wicks L, Garg ML. Fat type in phytosterol
962 products influence their cholesterol-lowering potential: A systematic review and meta-
963 analysis of RCTs. *Prog Lipid Res.* Oct 2016;64:16-29.
- 964 9. Nestel P, Cehun M, Pomeroy S, Abbey M, Weldon G. Cholesterol-lowering effects of
965 plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. *Eur J*
966 *Clin Nutr.* Dec 2001;55(12):1084-1090.
- 967 10. Amir Shaghaghi M, Harding SV, Jones PJH. Water dispersible plant sterol formulation
968 shows improved effect on lipid profile compared to plant sterol esters. *Journal of*
969 *Functional Foods.* 1// 2014;6:280-289.
- 970 11. Demonty I, Chan YM, Pelled D, Jones PJ. Fish-oil esters of plant sterols improve the
971 lipid profile of dyslipidemic subjects more than do fish-oil or sunflower oil esters of plant
972 sterols. *The American journal of clinical nutrition.* 2006;84.
- 973 12. Jones PJH, Demonty I, Chan Y-M, Herzog Y, Pelled D. Fish-oil esters of plant sterols
974 differ from vegetable-oil sterol esters in triglycerides lowering, carotenoid bioavailability
975 and impact on plasminogen activator inhibitor-1 (PAI-1) concentrations in
976 hypercholesterolemic subjects. *Lipids in health and disease.* 2007;6(1):28.
- 977 13. Carr TP, Krogstrand KL, Schlegel VL, Fernandez ML. Stearate-enriched plant sterol
978 esters lower serum LDL cholesterol concentration in normo- and hypercholesterolemic
979 adults. *J Nutr.* Aug 2009;139(8):1445-1450.

- 980 14. Doornbos AM, Meynen EM, Duchateau GS, van der Knaap HC, Trautwein EA. Intake
981 occasion affects the serum cholesterol lowering of a plant sterol-enriched single-dose
982 yoghurt drink in mildly hypercholesterolaemic subjects. *Eur J Clin Nutr.* Mar
983 2006;60(3):325-333.
- 984 15. Kriengsinyos W, Wangtong A, Komindr S. Serum cholesterol reduction efficacy of
985 biscuits with added plant stanol ester. *Cholesterol.* 2015;2015:9.
- 986 16. Law M. Plant sterol and stanol margarines and health. *BMJ (Clinical research ed.).* Mar
987 25 2000;320(7238):861-864.
- 988 17. Best MM, Duncan CH, Van Loon EJ, Wathen JD. Lowering of serum cholesterol by the
989 administration of a plant sterol. *Circulation.* 1954;10(2):201.
- 990 18. Farquhar JW, Smith RE, Dempsey ME. The Effect of Beta Sitosterol on the Serum
991 Lipids of Young Men with Arteriosclerotic Heart Disease. *Circulation.* 1956;14(1):77.
- 992 19. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of Serum
993 Cholesterol with Sitostanol-Ester Margarine in a Mildly Hypercholesterolemic
994 Population. *New England Journal of Medicine.* 1995;333(20):1308-1312.
- 995 20. Musa-Veloso K, Binns MA, Kocenas A, et al. Impact of low v. moderate intakes of long-
996 chain n-3 fatty acids on risk of coronary heart disease. *The British journal of nutrition.*
997 Oct 2011;106(8):1129-1141.
- 998 21. Moreau RA. Composition of Plant Sterols and Stanols in Supplemented Food Products.
999 *Journal of AOAC International.* May-Jun 2015;98(3):685-690.
- 1000 22. Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in
1001 foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in*
1002 *Lipid Research.* 11// 2002;41(6):457-500.

- 1003 23. Lin X, Ma L, Moreau RA, Ostlund RE, Jr. Glycosidic bond cleavage is not required for
1004 phytosteryl glycoside-induced reduction of cholesterol absorption in mice. *Lipids*. Aug
1005 2011;46(8):701-708.
- 1006 24. Moreau RA, Hicks KB. The in vitro hydrolysis of phytosterol conjugates in food matrices
1007 by mammalian digestive enzymes. *Lipids*. Aug 2004;39(8):769-776.
- 1008 25. Solaiman DK, Liu Y, Moreau RA, Zerkowski JA. Cloning, characterization, and
1009 heterologous expression of a novel glucosyltransferase gene from sophorolipid-producing
1010 *Candida bombicola*. *Gene*. Apr 25 2014;540(1):46-53.
- 1011 26. Sawalha H, den Adel R, Venema P, Bot A, Floter E, van der Linden E. Organogel-
1012 emulsions with mixtures of beta-sitosterol and gamma-oryzanol: influence of water
1013 activity and type of oil phase on gelling capability. *Journal of agricultural and food*
1014 *chemistry*. Apr 04 2012;60(13):3462-3470.
- 1015 27. Han L, Li L, Li B, et al. Structure and physical properties of organogels developed by
1016 sitosterol and lecithin with sunflower oil. *Journal of the American Oil Chemists' Society*.
1017 2014// 2014;91(10):1783-1792.
- 1018 28. Albers R, Bourdet-Sicard R, Braun D, et al. Monitoring immune modulation by nutrition
1019 in the general population: identifying and substantiating effects on human health. *The*
1020 *British journal of nutrition*. Aug 2013;110 Suppl 2:S1-30.
- 1021 29. Calpe-Berdiel L, Escola-Gil JC, Benitez S, et al. Dietary phytosterols modulate T-helper
1022 immune response but do not induce apparent anti-inflammatory effects in a mouse model
1023 of acute, aseptic inflammation. *Life sciences*. May 01 2007;80(21):1951-1956.

30. De Smet E, Mensink RP, Boekschoten MV, et al. An acute intake of plant stanol esters alters immune-related pathways in the jejunum of healthy volunteers. *The British journal of nutrition*. Mar 14 2015;113(5):794-802.
31. Berger A. Th1 and Th2 responses: what are they? *BMJ (Clinical research ed.)*. Aug 12 2000;321(7258):424.
32. Nashed B, Yeganeh B, HayGlass KT, Moghadasian MH. Antiatherogenic effects of dietary plant sterols are associated with inhibition of proinflammatory cytokine production in Apo E-KO mice. *J Nutr*. Oct 2005;135(10):2438-2444.
33. Brull F, Mensink RP, van den Hurk K, Duijvestijn A, Plat J. TLR2 activation is essential to induce a Th1 shift in human peripheral blood mononuclear cells by plant stanols and plant sterols. *The Journal of biological chemistry*. Jan 29 2010;285(5):2951-2958.
34. Brull F, De Smet E, Mensink RP, et al. Dietary plant stanol ester consumption improves immune function in asthma patients: results of a randomized, double-blind clinical trial. *The American journal of clinical nutrition*. Feb 2016;103(2):444-453.
35. Plana N, Nicolle C, Ferre R, et al. Plant sterol-enriched fermented milk enhances the attainment of LDL-cholesterol goal in hypercholesterolemic subjects. *Eur J Nutr*. Feb 2008;47(1):32-39.
36. Maki KC, Lawless AL, Reeves MS, et al. Lipid effects of a dietary supplement softgel capsule containing plant sterols/stanols in primary hypercholesterolemia. *Nutrition (Burbank, Los Angeles County, Calif.)*. Jan 2013;29(1):96-100.
37. Shaghaghia MA, Harding SV, Jones PJH. Water dispersible plant sterol formulation shows improved effect on lipid profile compared to plant sterol esters. *Journal of Functional Foods*. 2014;6:280–289.

- 1047 38. Davidson MH, Maki KC, Umporowicz DM, et al. Safety and tolerability of esterified
1048 phytosterols administered in reduced-fat spread and salad dressing to healthy adult men
1049 and women. *Journal of the American College of Nutrition*. Aug 2001;20(4):307-319.
- 1050 39. Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation
1051 of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters.
1052 *Journal of lipid research*. May 2000;41(5):697-705.
- 1053 40. Rideout TC, Chan YM, Harding SV, Jones PJ. Low and moderate-fat plant sterol
1054 fortified soymilk in modulation of plasma lipids and cholesterol kinetics in subjects with
1055 normal to high cholesterol concentrations: report on two randomized crossover studies.
1056 *Lipids in health and disease*. Oct 20 2009;8:45.
- 1057 41. Theuwissen E, Plat J, van der Kallen CJ, van Greevenbroek MM, Mensink RP. Plant
1058 stanol supplementation decreases serum triacylglycerols in subjects with overt
1059 hypertriglyceridemia. *Lipids*. Dec 2009;44(12):1131-1140.
- 1060 42. Plat J, Mensink RP. Plant stanol esters lower serum triacylglycerol concentrations via a
1061 reduced hepatic VLDL-1 production. *Lipids*. Dec 2009;44(12):1149-1153.
- 1062 43. Plat J, Brufau G, Dallinga-Thie GM, Dasselaaar M, Mensink RP. A plant stanol yogurt
1063 drink alone or combined with a low-dose statin lowers serum triacylglycerol and non-
1064 HDL cholesterol in metabolic syndrome patients. *J Nutr*. Jun 2009;139(6):1143-1149.
- 1065 44. Sialvera TE, Pounis GD, Koutelidakis AE, et al. Phytosterols supplementation decreases
1066 plasma small and dense LDL levels in metabolic syndrome patients on a westernized type
1067 diet. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. Oct 2012;22(10):843-
1068 848.

45. Naumann E, Plat J, Kester AD, Mensink RP. The baseline serum lipoprotein profile is related to plant stanol induced changes in serum lipoprotein cholesterol and triacylglycerol concentrations. *Journal of the American College of Nutrition*. Feb 2008;27(1):117-126.
46. Rideout TC, Harding SV, Jones PJ. Consumption of plant sterols reduces plasma and hepatic triglycerides and modulates the expression of lipid regulatory genes and de novo lipogenesis in C57BL/6J mice. *Mol Nutr Food Res*. May 2010;54 Suppl 1:S7-13.
47. Tomoyori H, Kawata Y, Higuchi T, et al. Phytosterol oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein E-deficient mice. *J Nutr*. Jul 2004;134(7):1690-1696.
48. Relas H, Gylling H, Miettinen TA. Acute effect of dietary stanyl ester dose on post-absorptive alpha-tocopherol, beta-carotene, retinol and retinyl palmitate concentrations. *The British journal of nutrition*. Feb 2001;85(2):141-147.
49. De Smet E, Mensink RP, Lutjohann D, Plat J. Acute effects of plant stanol esters on postprandial metabolism and its relation with changes in serum lipids after chronic intake. *Eur J Clin Nutr*. Jan 2015;69(1):127-133.
50. Rideout TC, Ramprasath V, Griffin JD, Browne RW, Harding SV, Jones PJ. Phytosterols protect against diet-induced hypertriglyceridemia in Syrian golden hamsters. *Lipids in health and disease*. 2014;13:5.
51. Schonewille M, Brufau G, Shiri-Sverdlov R, Groen AK, Plat J. Serum TG-lowering properties of plant sterols and stanols are associated with decreased hepatic VLDL secretion. *Journal of lipid research*. Dec 2014;55(12):2554-2561.

- 1091 52. Vanmierlo T, Popp J, Kolsch H, et al. The plant sterol brassicasterol as additional CSF
1092 biomarker in Alzheimer's disease. *Acta psychiatrica Scandinavica*. Sep 2011;124(3):184-
1093 192.
- 1094 53. Weingärtner O, Lütjohann D, Ji S, et al. Vascular effects of diet supplementation with
1095 plant sterols. *Journal of the American College of Cardiology*. Apr 22 2008;51(16):1553-
1096 1561.
- 1097 54. Shafaati M, Marutle A, Pettersson H, et al. Marked accumulation of 27-
1098 hydroxycholesterol in the brains of Alzheimer's patients with the Swedish APP 670/671
1099 mutation. *Journal of lipid research*. May 2011;52(5):1004-1010.
- 1100 55. Fransen HP, de Jong N, Wolfs M, et al. Customary use of plant sterol and plant stanol
1101 enriched margarine is associated with changes in serum plant sterol and stanol
1102 concentrations in humans. *J Nutr*. May 2007;137(5):1301-1306.
- 1103 56. Simonen P, Lommi J, Hallikainen M, et al. Dietary plant stanols or sterols neither
1104 accumulate in stenotic aortic valves nor influence their structure or inflammatory status.
1105 *Clinical nutrition (Edinburgh, Scotland)*. Dec 2015;34(6):1251-1257.
- 1106 57. Smiljanic K, Vanmierlo T, Djordjevic AM, et al. Aging induces tissue-specific changes
1107 in cholesterol metabolism in rat brain and liver. *Lipids*. Nov 2013;48(11):1069-1077.
- 1108 58. Vanmierlo T, Weingartner O, van der Pol S, et al. Dietary intake of plant sterols stably
1109 increases plant sterol levels in the murine brain. *Journal of lipid research*. Apr
1110 2012;53(4):726-735.
- 1111 59. Saeed AA, Genove G, Li T, et al. Effects of a disrupted blood-brain barrier on cholesterol
1112 homeostasis in the brain. *The Journal of biological chemistry*. Jun 27 2014.

- 1113 60. Jansen PJ, Lutjohann D, Abildayeva K, et al. Dietary plant sterols accumulate in the
1114 brain. *Biochimica et biophysica acta*. Apr 19 2006.
- 1115 61. Panzenboeck U, Balazs Z, Sovic A, et al. ABCA1 and scavenger receptor class B, type I,
1116 are modulators of reverse sterol transport at an in vitro blood-brain barrier constituted of
1117 porcine brain capillary endothelial cells. *The Journal of biological chemistry*. Nov 8
1118 2002;277(45):42781-42789.
- 1119 62. Xie C, Lund EG, Turley SD, Russell DW, Dietschy JM. Quantitation of two pathways for
1120 cholesterol excretion from the brain in normal mice and mice with neurodegeneration.
1121 *Journal of lipid research*. Sep 2003;44(9):1780-1789.
- 1122 63. Björkhem I, Lütjohann D, Diczfalusy U, Stahle L, Ahlborg G, Wahren J. Cholesterol
1123 homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a
1124 cerebral origin of most of this oxysterol in the circulation. *Journal of lipid research*. Aug
1125 1998;39(8):1594-1600.
- 1126 64. Lund EG, Xie C, Kotti T, Turley SD, Dietschy JM, Russell DW. Knockout of the
1127 cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of
1128 cholesterol turnover. *The Journal of biological chemistry*. Jun 20 2003;278(25):22980-
1129 22988.
- 1130 65. Björkhem I, Lütjohann D, Breuer O, Sakinis A, Wennmalm A. Importance of a novel
1131 oxidative mechanism for elimination of brain cholesterol. Turnover of cholesterol and
1132 24(S)-hydroxycholesterol in rat brain as measured with ¹⁸O₂ techniques in vivo and in
1133 vitro. *The Journal of biological chemistry*. Nov 28 1997;272(48):30178-30184.
- 1134 66. Lütjohann D, Breuer O, Ahlborg G, et al. Cholesterol homeostasis in human brain:
1135 evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the

circulation. *Proceedings of the National Academy of Sciences of the United States of America*. Sep 3 1996;93(18):9799-9804.

67. Mast N, Norcross R, Andersson U, et al. Broad substrate specificity of human cytochrome P450 46A1 which initiates cholesterol degradation in the brain. *Biochemistry*. Dec 9 2003;42(48):14284-14292.

68. Lütjohann D, Vanmierlo T, Mulder M. Cholesterol Trafficking in the Brain. In: Ehnholm C, ed. *Cellular Lipid Metabolism*. Heidelberg: Springer; 2008:131-156.

69. Dietschy JM, Turley SD. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *Journal of lipid research*. Aug 2004;45(8):1375-1397.

70. Li H, Turley SD, Liu B, Repa JJ, Dietschy JM. GM2/GD2 and GM3 gangliosides have no effect on cellular cholesterol pools or turnover in normal or NPC1 mice. *Journal of lipid research*. Aug 2008;49(8):1816-1828.

71. Vanmierlo T, Bogie JF, Mailleux J, et al. Plant sterols: Friend or foe in CNS disorders? *Prog Lipid Res*. Apr 2015;58:26-39.

72. Burg VK, Grimm HS, Rothhaar TL, et al. Plant sterols the better cholesterol in Alzheimer's disease? A mechanistical study. *J Neurosci*. Oct 09 2013;33(41):16072-16087.

73. Vanmierlo T, Rutten K, van Vark-van der Zee LC, et al. Cerebral accumulation of dietary derivable plant sterols does not interfere with memory and anxiety related behavior in Abcg5^{-/-} mice. *Plant Foods Hum Nutr*. Jun 2011;66(2):149-156.

74. Schiepers OJ, de Groot RH, van Boxtel MP, et al. Consuming functional foods enriched with plant sterol or stanol esters for 85 weeks does not affect neurocognitive functioning

1159 or mood in statin-treated hypercholesterolemic individuals. *J Nutr.* Jul 2009;139(7):1368-
 1160 1373.

1161 75. Aguirre-Hernandez E, Rosas-Acevedo H, Soto-Hernandez M, Martinez AL, Moreno J,
 1162 Gonzalez-Trujano ME. Bioactivity-guided isolation of beta-sitosterol and some fatty
 1163 acids as active compounds in the anxiolytic and sedative effects of *Tilia americana* var.
 1164 *mexicana*. *Planta medica*. Sep 2007;73(11):1148-1155.

1165 76. Kalariya M, Parmar S, Sheth N. Neuropharmacological activity of hydroalcoholic extract
 1166 of leaves of *Colocasia esculenta*. *Pharmaceutical biology*. Nov 2010;48(11):1207-1212.

1167 77. Racette SB, Lin X, Lefevre M, et al. Dose effects of dietary phytosterols on cholesterol
 1168 metabolism: a controlled feeding study. *The American journal of clinical nutrition*.
 1169 2010;91(1):32-38.

1170 78. Nissinen M, Gylling H, Vuoristo M, Miettinen TA. Micellar distribution of cholesterol
 1171 and phytosterols after duodenal plant stanol ester infusion. *American journal of*
 1172 *physiology. Gastrointestinal and liver physiology*. Jun 2002;282(6):G1009-1015.

1173 79. Racette SB, Lin X, Lefevre M, et al. Dose effects of dietary phytosterols on cholesterol
 1174 metabolism: a controlled feeding study. *The American journal of clinical nutrition*. Jan
 1175 2010;91(1):32-38.

1176 80. Gylling H, Simonen P. Phytosterols, Phytostanols, and Lipoprotein Metabolism.
 1177 *Nutrients*. Sep 17 2015;7(9):7965-7977.

1178 81. Ling WH, Jones PJ. Dietary phytosterols: a review of metabolism, benefits and side
 1179 effects. *Life sciences*. 1995;57(3):195-206.

82. Ostlund RE, Jr., Racette SB, Okeke A, Stenson WF. Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. *The American journal of clinical nutrition*. Jun 2002;75(6):1000-1004.
83. Ostlund RE, Jr., Lin X. Regulation of cholesterol absorption by phytosterols. *Current atherosclerosis reports*. Nov 2006;8(6):487-491.
84. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clinic proceedings*. Aug 2003;78(8):965-978.
85. Lin X, Racette SB, Ma L, Wallendorf M, Spearie CA, Ostlund RE, Jr. Plasma biomarker of dietary phytosterol intake. *PloS one*. 2015;10(2):e0116912.
86. Miettinen TA, Vanhanen H. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis*. Feb 1994;105(2):217-226.
87. Plat J, Mensink RP. Relationship of genetic variation in genes encoding apolipoprotein A-IV, scavenger receptor BI, HMG-CoA reductase, CETP and apolipoprotein E with cholesterol metabolism and the response to plant stanol ester consumption. *European journal of clinical investigation*. Apr 2002;32(4):242-250.
88. Plat J, Bragt MC, Mensink RP. Common sequence variations in ABCG8 are related to plant sterol metabolism in healthy volunteers. *Journal of lipid research*. Jan 2005;46(1):68-75.
89. Gylling H, Hallikainen M, Raitakari OT, et al. Long-term consumption of plant stanol and sterol esters, vascular function and genetic regulation. *The British journal of nutrition*. Jun 2009;101(11):1688-1695.

90. Casas-Agustench P, Serra M, Perez-Heras A, et al. Effects of plant sterol esters in skimmed milk and vegetable-fat-enriched milk on serum lipids and non-cholesterol sterols in hypercholesterolaemic subjects: a randomised, placebo-controlled, crossover study. *The British journal of nutrition*. Jun 2012;107(12):1766-1775.
91. Rideout TC. Getting personal: considering variable interindividual responsiveness to dietary lipid-lowering therapies. *Current Opinion in Lipidology*. 2011;22(1):37-42.
92. Rudkowska I, AbuMweis SS, Nicolle C, Jones PJH. Association between non-responsiveness to plant sterol intervention and polymorphisms in cholesterol metabolism genes: a case-control study. *Applied Physiology, Nutrition, and Metabolism*. 2008/08/01 2008;33(4):728-734.
93. MacKay DS, Eck PK, Gebauer SK, Baer DJ, Jones PJ. Lathosterol-to-cholesterol ratio in serum predicts cholesterol-lowering response to plant sterol consumption in a dual-center, randomized, single-blind placebo-controlled trial. *The American journal of clinical nutrition*. 2015;101:432-439.
94. MacKay DS, Eck PK, Gebauer SK, Baer DJ, Jones PJ. CYP7A1-rs3808607 and APOE isoform associate with LDL cholesterol lowering after plant sterol consumption in a randomized clinical trial. *The American journal of clinical nutrition*. 2015;102:951-957.
95. De Castro-Oros I, Pampin S, Cofan M, et al. Promoter variant -204A > C of the cholesterol 7 alpha-hydroxylase gene: Association with response to plant sterols in humans and increased transcriptional activity in transfected HepG2 cells. *Clinical Nutrition*. Apr 2011;30(2):239-246.

- 1224 96. Abdullah MM, Jones PJ, Eck PK. Nutrigenetics of cholesterol metabolism: observational
1225 and dietary intervention studies in the postgenomic era. *Nutrition reviews*. Aug
1226 2015;73(8):523-543.
- 1227 97. Zhao HL, Houweling AH, Vanstone CA, et al. Genetic variation in ABC G5/G8 and
1228 NPC1L1 impact cholesterol response to plant sterols in hypercholesterolemic men.
1229 *Lipids*. Dec 2008;43(12):1155-1164.
- 1230 98. MacKay DS, Jones PJH. Plasma noncholesterol sterols: current uses, potential and need
1231 for standardization. *Current Opinion in Lipidology*. Jun 2012;23(3):241-247.
- 1232 99. Bjorkhem I, Miettinen T, Reihner E, Ewerth S, Angelin B, Einarsson K. Correlation
1233 between serum levels of some cholesterol precursors and activity of HMG-CoA reductase
1234 in human liver. *Journal of lipid research*. Oct 1987;28(10):1137-1143.
- 1235 100. Mackay D, Jones PJ. Evaluation of methods for the determination of cholesterol
1236 absorption and synthesis in humans. *Atherosclerosis*. Oct 2011;218(2):253-262.
- 1237 101. Miettinen TA, Tilvis RS, Kesaniemi YA. Serum plant sterols and cholesterol precursors
1238 reflect cholesterol absorption and synthesis in volunteers of a randomly selected male
1239 population. *Am J Epidemiol*. Jan 1990;131(1):20-31.
- 1240 102. Tilvis RS, Miettinen TA. Serum plant sterols and their relation to cholesterol absorption.
1241 *The American journal of clinical nutrition*. Jan 1986;43(1):92-97.
- 1242 103. Miettinen TA, Tilvis RS, Kesaniemi YA. Serum cholestanol and plant sterol levels in
1243 relation to cholesterol metabolism in middle-aged men. *Metabolism*. Feb 1989;38(2):136-
1244 140.

- 1245 104. Moghadasian MH, Godin DV, McManus BM, Frohlich JJ. Lack of regression of
1246 atherosclerotic lesions in phytosterol-treated apo E-deficient mice. *Life sciences*.
1247 1999;64(12):1029-1036.
- 1248 105. Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis. A newly
1249 described lipid storage disease in two sisters. *J Clin Invest*. 1974;53(4):1033 - 1043.
- 1250 106. Mackay DS, Jones PJ, Myrie SB, Plat J, Lutjohann D. Methodological considerations for
1251 the harmonization of non-cholesterol sterol bio-analysis. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*. Apr 15 2014;957:116-122.
- 1252 107. **Lütjohann D**. Methodological Aspects of Plant Sterol and Stanol Measurement. *Journal*
1253 *of AOAC International*. May-Jun 2015;98(3):674-676.
- 1254 108. Schött H-F, Lütjohann D. Validation of an isotope dilution gas chromatography–mass
1255 spectrometry method for combined analysis of oxysterols and oxyphytosterols in serum
1256 samples. *Steroids*. 7// 2015;99, Part B:139-150.
- 1257 109. Chan YM, Varady KA, Lin Y, et al. Plasma concentrations of plant sterols: physiology
1258 and relationship with coronary heart disease. *Nutrition reviews*. Sep 2006;64(9):385-402.
- 1259 110. MacKay DS, Jones PJH. Limitations of lathosterol to plant sterol ratios and serum plant
1260 sterols as surrogate markers for cholesterol absorption during plant sterol
1261 supplementation. *Nutrition Metabolism and Cardiovascular Diseases*. Sep
1262 2012;22(9):E21-E21.
- 1263 111. Silbernagel G, Chapman MJ, Genser B, et al. High intestinal cholesterol absorption is
1264 associated with cardiovascular disease and risk alleles in ABCG8 and ABO: evidence
1265 from the LURIC and YFS cohorts and from a meta-analysis. *Journal of the American*
1266 *College of Cardiology*. Jul 23 2013;62(4):291-299.
- 1267

- 1268 112. Teupser D, Baber R, Ceglarek U, et al. Genetic regulation of serum phytosterol levels and
1269 risk of coronary artery disease. *Circulation. Cardiovascular genetics*. Aug
1270 2010;3(4):331-339.
- 1271 113. Noto D, Cefalu AB, Barraco G, et al. Plasma non-cholesterol sterols in primary
1272 hypobetalipoproteinemia. *Atherosclerosis*. Jun 2011;216(2):409-413.
- 1273 114. Noto D, Cefalu AB, Barraco G, et al. Plasma non-cholesterol sterols: a useful diagnostic
1274 tool in pediatric hypercholesterolemia. *Pediatr Res*. Feb 2010;67(2):200-204.
- 1275 115. Nissinen MJ, Miettinen TE, Gylling H, Miettinen TA. Applicability of non-cholesterol
1276 sterols in predicting response in cholesterol metabolism to simvastatin and fluvastatin
1277 treatment among hypercholesterolemic men. *Nutrition, metabolism, and cardiovascular
1278 diseases : NMCD*. Jun 2010;20(5):308-316.
- 1279 116. Miettinen TA, Strandberg TE, Gylling H. Noncholesterol sterols and cholesterol lowering
1280 by long-term simvastatin treatment in coronary patients: relation to basal serum
1281 cholestanol. *Arterioscler Thromb Vasc Biol*. May 2000;20(5):1340-1346.
- 1282 117. Wu AH. Biomarkers for cholesterol absorption and synthesis in hyperlipidemic patients:
1283 role for therapeutic selection. *Clin Lab Med*. Mar 2014;34(1):157-166, viii.
- 1284 118. Weingärtner O, Lütjohann D, Böhm M, Laufs U. Relationship between cholesterol
1285 synthesis and intestinal absorption is associated with cardiovascular risk. *Atherosclerosis*.
1286 Jun 2010;210(2):362-365.
- 1287 119. Miettinen TA, Gylling H, Nissinen MJ. The role of serum non-cholesterol sterols as
1288 surrogate markers of absolute cholesterol synthesis and absorption. *Nutrition, Metabolism
1289 and Cardiovascular Diseases*. 2011;21(10):765-769.

120. Qi Y, Liu J, Ma C, et al. Association between cholesterol synthesis/absorption markers and effects of cholesterol lowering by atorvastatin among patients with high risk of coronary heart disease. *Journal of lipid research*. 2013;54(11):3189-3197.
121. Sudhop T, Lutjohann D, Kodal A, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation*. Oct 08 2002;106(15):1943-1948.
122. Davis HR, Veltri EP. Zetia: inhibition of Niemann-Pick C1 Like 1 (NPC1L1) to reduce intestinal cholesterol absorption and treat hyperlipidemia. *Journal of atherosclerosis and thrombosis*. Jun 2007;14(3):99-108.
123. Assmann G, Kannenberg F, Ramey DR, Musliner TA, Gutkin SW, Veltri EP. Effects of ezetimibe, simvastatin, atorvastatin, and ezetimibe-statin therapies on non-cholesterol sterols in patients with primary hypercholesterolemia. *Current medical research and opinion*. Jan 2008;24(1):249-259.
124. Ajagbe BO, Othman RA, Myrie SB. Plant Sterols, Stanols, and Sitosterolemia. *Journal of AOAC International*. May-Jun 2015;98(3):716-723.
125. Salen G, von Bergmann K, Lutjohann D, et al. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation*. Mar 02 2004;109(8):966-971.
126. Davis HR, Jr., Altmann SW. Niemann-Pick C1 Like 1 (NPC1L1) an intestinal sterol transporter. *Biochimica et biophysica acta*. Jul 2009;1791(7):679-683.
127. Davis HR, Jr., Zhu LJ, Hoos LM, et al. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *The Journal of biological chemistry*. Aug 06 2004;279(32):33586-33592.

- 1312 128. Davis HR, Jr., Lowe RS, Neff DR. Effects of ezetimibe on atherosclerosis in preclinical
1313 models. *Atherosclerosis*. Apr 2011;215(2):266-278.
- 1314 129. Investigators TMIGC. Inactivating Mutations in NPC1L1 and Protection from Coronary
1315 Heart Disease. *New England Journal of Medicine*. 2014/11/27 2014;371(22):2072-2082.
- 1316 130. Cannon CP, Blazing MA, Giugliano RP, et al. Ezetimibe Added to Statin Therapy after
1317 Acute Coronary Syndromes. *New England Journal of Medicine*. 2015/06/18
1318 2015;372(25):2387-2397.
- 1319 131. Jakulj L, Trip MD, Sudhop T, von Bergmann K, Kastelein JJ, Vissers MN. Inhibition of
1320 cholesterol absorption by the combination of dietary plant sterols and ezetimibe: effects
1321 on plasma lipid levels. *Journal of lipid research*. Dec 2005;46(12):2692-2698.
- 1322 132. Lin X, Racette SB, Lefevre M, et al. Combined effects of ezetimibe and phytosterols on
1323 cholesterol metabolism: a randomized, controlled feeding study in humans. *Circulation*.
1324 Aug 02 2011;124(5):596-601.
- 1325 133. Gomes GB, Zazula AD, Shigueoka LS, et al. A Randomized Open-Label Trial to Assess
1326 the Effect of Plant Sterols Associated with Ezetimibe in Low-Density Lipoprotein Levels
1327 in Patients with Coronary Artery Disease on Statin Therapy. *Journal of medicinal food*.
1328 Jan 2017;20(1):30-36.
- 1329 134. Jarcho JA, Keaney JF. Proof That Lower Is Better — LDL Cholesterol and IMPROVE-
1330 IT. *New England Journal of Medicine*. 2015/06/18 2015;372(25):2448-2450.
- 1331 135. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering
1332 treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised
1333 trials of statins. *Lancet (London, England)*. Oct 08 2005;366(9493):1267-1278.

- 1334 136. Sabatine MS, Giugliano RP, Wiviott SD, et al. Efficacy and Safety of Evolocumab in
 1335 Reducing Lipids and Cardiovascular Events. *New England Journal of Medicine*.
 1336 2015/04/16 2015;372(16):1500-1509.
- 1337 137. Sabatine M, Robert P. Giugliano, Anthony C. Keech, et al. Evolocumab and clinical
 1338 outcomes in patients with cardiovascular disease. *The journal of the Royal College of*
 1339 *Physicians of Edinburgh*. Jun 2017;47(2):153-155.
- 1340 138. Jenkins DJ, Kendall CW, Popovich DG, et al. Effect of a very-high-fiber vegetable, fruit,
 1341 and nut diet on serum lipids and colonic function. *Metabolism*. Apr 2001;50(4):494-503.
- 1342 139. Jenkins DA, Jones PH, Lamarche B, et al. Effect of a dietary portfolio of cholesterol-
 1343 lowering foods given at 2 levels of intensity of dietary advice on serum lipids in
 1344 hyperlipidemia: A randomized controlled trial. *JAMA*. 2011;306(8):831-839.
- 1345 140. Jenkins DJ, Jones PJ, Lamarche B, et al. Effect of a dietary portfolio of cholesterol-
 1346 lowering foods given at 2 levels of intensity of dietary advice on serum lipids in
 1347 hyperlipidemia: a randomized controlled trial. *Jama*. Aug 24 2011;306(8):831-839.
- 1348 141. de Jongh S, Lilien MR, Bakker HD, Hutten BA, Kastelein JJ, Stroes ES. Family history
 1349 of cardiovascular events and endothelial dysfunction in children with familial
 1350 hypercholesterolemia. *Atherosclerosis*. Jul 2002;163(1):193-197.
- 1351 142. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL Apheresis
 1352 Improves Endothelium-Dependent Vasodilatation in Hypercholesterolemic Humans.
 1353 *Circulation*. 1997;95(1):76.
- 1354 143. Tsunekawa T, Hayashi T, Kano H, et al. Cerivastatin, a hydroxymethylglutaryl coenzyme
 1355 a reductaseinhibitor, improves endothelial function in elderly diabetic patients within 3
 1356 Days. *Circulation*. 2001;104(4):376.

144. Saluveer O, Bergh N, Grote L, Andersson O, Hrafnkelsdottir TJ, Widgren BR. Acute vascular effects of atorvastatin in hypertensive men: a pilot study. *Scandinavian cardiovascular journal : SCJ*. Oct 2013;47(5):275-280.
145. Kurobe H, Aihara K, Higashida M, et al. Ezetimibe monotherapy ameliorates vascular function in patients with hypercholesterolemia through decreasing oxidative stress. *Journal of atherosclerosis and thrombosis*. 2011;18(12):1080-1089.
146. Yunoki K, Nakamura K, Miyoshi T, et al. Ezetimibe improves postprandial hyperlipemia and its induced endothelial dysfunction. *Atherosclerosis*. Aug 2011;217(2):486-491.
147. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *International journal of cardiology*. Sep 20 2013;168(1):344-351.
148. Ras RT, Hiemstra H, Lin Y, Vermeer MA, Duchateau GS, Trautwein EA. Consumption of plant sterol-enriched foods and effects on plasma plant sterol concentrations--a meta-analysis of randomized controlled studies. *Atherosclerosis*. Oct 2013;230(2):336-346.
149. Sudhop T, von Bergmann K. Sitosterolemia--a rare disease. Are elevated plant sterols an additional risk factor? *Zeitschrift fur Kardiologie*. Dec 2004;93(12):921-928.
150. Genser B, Silbernagel G, De Backer G, et al. Plant sterols and cardiovascular disease: a systematic review and meta-analysis. *European heart journal*. Feb 2012;33(4):444-451.
151. Hansel B, Carrie A, Brun-Druc N, et al. Premature atherosclerosis is not systematic in phytosterolemic patients: severe hypercholesterolemia as a confounding factor in five subjects. *Atherosclerosis*. May 2014;234(1):162-168.

- 1378 152. Baumgartner S, Mensink RP, Husche C, Lütjohann D, Plat J. Effects of plant sterol- or
1379 stanol-enriched margarine on fasting plasma oxysterol concentrations in healthy
1380 subjects. *Atherosclerosis*. Apr 2013;227(2):414-419.
- 1381 153. Weingärtner O, Ulrich C, Lütjohann D, et al. Differential effects on inhibition of
1382 cholesterol absorption by plant stanol and plant sterol esters in apoE^{-/-} mice.
1383 *Cardiovascular research*. Jun 01 2011;90(3):484-492.
- 1384 154. Liang YT, Wong WT, Guan L, et al. Effect of phytosterols and their oxidation products
1385 on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet.
1386 *Atherosclerosis*. Nov 2011;219(1):124-133.
- 1387 155. Raitakari OT, Juonala M, Ronnema T, et al. Cohort profile: the cardiovascular risk in
1388 Young Finns Study. *International journal of epidemiology*. Dec 2008;37(6):1220-1226.
- 1389 156. Hallikainen M, Lyyra-Laitinen T, Laitinen T, et al. Endothelial function in
1390 hypercholesterolemic subjects: Effects of plant stanol and sterol esters. *Atherosclerosis*.
1391 Oct 2006;188(2):425-432.
- 1392 157. de Jongh S, Vissers MN, Rol P, Bakker HD, Kastelein JJ, Stroes ES. Plant sterols lower
1393 LDL cholesterol without improving endothelial function in prepubertal children with
1394 familial hypercholesterolaemia. *Journal of inherited metabolic disease*. 2003;26(4):343-
1395 351.
- 1396 158. Ras RT, Fuchs D, Koppenol WP, et al. The effect of a low-fat spread with added plant
1397 sterols on vascular function markers: results of the Investigating Vascular Function
1398 Effects of Plant Sterols (INVEST) study. *The American journal of clinical nutrition*.
1399 2015;101(4):733-741.

1400 159. Ras RT, Fuchs D, Koppenol WP, et al. Effect of a plant sterol-enriched spread on
1401 biomarkers of endothelial dysfunction and low-grade inflammation in
1402 hypercholesterolaemic subjects. *Journal of nutritional science*. 2016;5:e44.

1403 160. Gylling H, Halonen J, Lindholm H, et al. The effects of plant stanol ester consumption on
1404 arterial stiffness and endothelial function in adults: a randomised controlled clinical trial.
1405 *BMC Cardiovasc Disord*. Jul 10 2013;13:50.

1406 161. Horenstein RB, Mitchell BD, Post WS, et al. The ABCG8 G574R variant, serum plant
1407 sterol levels, and cardiovascular disease risk in the old order amish. *Arteriosclerosis,*
1408 *thrombosis, and vascular biology*. 12/13 2013;33(2):10.1161/ATVBAHA.1112.245480.

1409 162. Rocha DM, Caldas AP, Oliveira LL, Bressan J, Hermsdorff HH. Saturated fatty acids
1410 trigger TLR4-mediated inflammatory response. *Atherosclerosis*.244:211-215.

1411 163. Endo A. A gift from nature: the birth of the statins. *Nat Med*. 10//print 2008;14(10):1050-
1412 1052.

1413 164. Pedersen TR, Kjekshus J, Berg K, et al. Randomised trial of cholesterol lowering in 4444
1414 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S).
1415 1994. *Atherosclerosis. Supplements*. Oct 2004;5(3):81-87.

1416 165. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with
1417 pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention
1418 Study Group. *The New England journal of medicine*. Nov 16 1995;333(20):1301-1307.

1419 166. Grundy SM, Cleeman JI, Merz CNB, et al. Implications of Recent Clinical Trials for the
1420 National Cholesterol Education Program Adult Treatment Panel III Guidelines.
1421 *Circulation*. 2004;110(2):227.

- 1422 167. Group HPSC. MRC/BHF Heart Protection Study of cholesterol lowering with
1423 simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*
1424 (*London, England*). Jul 06 2002;360(9326):7-22.
- 1425 168. Boekholdt SM, Hovingh GK, Mora S, et al. Very low levels of atherogenic lipoproteins
1426 and the risk for cardiovascular events: a meta-analysis of statin trials. *Journal of the*
1427 *American College of Cardiology*. Aug 05 2014;64(5):485-494.
- 1428 169. Teoh H, Mendelsohn AA, Goodman SG, et al. Usefulness of statin-ezetimibe
1429 combination to reduce the care gap in dyslipidemia management in patients with a high
1430 risk of atherosclerotic disease. *Am J Cardiol*. Sep 15 2009;104(6):798-804.
- 1431 170. Farnier M, Aversa M, Missault L, et al. Lipid-altering efficacy of ezetimibe/simvastatin
1432 10/20 mg compared with rosuvastatin 10 mg in high-risk hypercholesterolaemic patients
1433 inadequately controlled with prior statin monotherapy - The IN-CROSS study.
1434 *International journal of clinical practice*. Apr 2009;63(4):547-559.
- 1435 171. Thuluva SC, Igel M, Giesa U, Lutjohann D, Sudhop T, von Bergmann K. Ratio of
1436 lathosterol to campesterol in serum predicts the cholesterol-lowering effect of sitostanol-
1437 supplemented margarine. *International journal of clinical pharmacology and*
1438 *therapeutics*. Jul 2005;43(7):305-310.
- 1439 172. Lakoski SG, Xu F, Vega GL, et al. Indices of cholesterol metabolism and relative
1440 responsiveness to ezetimibe and simvastatin. *The Journal of Clinical Endocrinology and*
1441 *Metabolism*. 2010;95(2):800-809.
- 1442 173. Van Himbergen TM, Matthan NR, Resteghini NA, et al. Comparison of the effects of
1443 maximal dose atorvastatin and rosuvastatin therapy on cholesterol synthesis and
1444 absorption markers. *Journal of lipid research*. Apr 2009;50(4):730-739.

- 1445 174. Miettinen TA, Gylling H, Strandberg T, Sarna S. Baseline serum cholestanol as predictor
1446 of recurrent coronary events in subgroup of Scandinavian simvastatin survival study.
1447 *BMJ (Clinical research ed.)*. 1998;316(7138):1127-1130.
- 1448 175. Ference BA, Yoo W, Alesh I et al. Effect of long term exposure to low-density
1449 lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a
1450 Mendelian randomization analysis. *J Am Coll Cardiol*. 2012; 60: 2631-2639.
- 1451 176. Silbernagel G, Fauler G, Renner W, et al. The relationships of cholesterol metabolism
1452 and plasma plant sterols with the severity of coronary artery disease. *Journal of lipid*
1453 *research*. Feb 2009;50(2):334-341.
- 1454 177. Silbernagel G, Fauler G, Hoffmann MM, et al. The associations of cholesterol
1455 metabolism and plasma plant sterols with all-cause and cardiovascular mortality. *Journal*
1456 *of lipid research*. Aug 2010;51(8):2384-2393.
- 1457 178. Weingärtner O, Weingärtner N, Scheller B, et al. Alterations in cholesterol homeostasis
1458 are associated with coronary heart disease in patients with aortic stenosis. *Coronary*
1459 *artery disease*. Sep 2009;20(6):376-382.
- 1460 179. Matthan NR, Pencina M, LaRocque JM, et al. Alterations in cholesterol
1461 absorption/synthesis markers characterize Framingham offspring study participants with
1462 CHD. *Journal of lipid research*. Sep 2009;50(9):1927-1935.
- 1463 180. Nasu K, Terashima M, Habara M, et al. Impact of cholesterol metabolism on coronary
1464 plaque vulnerability of target vessels: a combined analysis of virtual histology
1465 intravascular ultrasound and optical coherence tomography. *JACC. Cardiovascular*
1466 *interventions*. Jul 2013;6(7):746-755.

- 1467 181. Kataoka Y, St John J, Wolski K, et al. Atheroma progression in hyporesponders to statin
1468 therapy. *Arterioscler Thromb Vasc Biol.* Apr 2015;35(4):990-995.
- 1469 182. Tsujita K, Sugiyama S, Sumida H, et al. Impact of Dual Lipid-Lowering Strategy With
1470 Ezetimibe and Atorvastatin on Coronary Plaque Regression in Patients With
1471 Percutaneous Coronary Intervention: The Multicenter Randomized Controlled PRECISE-
1472 IVUS Trial. *Journal of the American College of Cardiology.* Aug 04 2015;66(5):495-507.
- 1473 183. Nicholls SJ, Puri R, Anderson T, et al. Effect of evolocumab on progression of coronary
1474 disease in statin-treated patients: The GLAGOV randomized clinical trial. *JAMA -*
1475 *Journal of the American Medical Association.* 2016;316(22):2373-2384.
- 1476 184. Wanner C, Krane V, März W, et al. Atorvastatin in Patients with Type 2 Diabetes
1477 Mellitus Undergoing Hemodialysis. *New England Journal of Medicine.* 2005;353(3):238-
1478 248.
- 1479 185. Fellström BC, Jardine AG, Schmieder RE, et al. Rosuvastatin and Cardiovascular Events
1480 in Patients Undergoing Hemodialysis. *New England Journal of Medicine.* 2009/04/02
1481 2009;360(14):1395-1407.
- 1482 186. Rogacev KS, Pinsdorf T, Weingartner O, et al. Cholesterol synthesis, cholesterol
1483 absorption, and mortality in hemodialysis patients. *Clinical journal of the American*
1484 *Society of Nephrology : CJASN.* Jun 2012;7(6):943-948.
- 1485 187. Baigent C, Landray MJ, Reith C, et al. The effects of lowering LDL cholesterol with
1486 simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and
1487 Renal Protection): a randomised placebo-controlled trial. *The Lancet.* 377(9784):2181-
1488 2192.

- 1489 188. Silbernagel G, Fauler G, Genser B, et al. Intestinal cholesterol absorption, treatment with
1490 atorvastatin, and cardiovascular risk in hemodialysis patients. *Journal of the American*
1491 *College of Cardiology*. Jun 02 2015;65(21):2291-2298.
- 1492 189. Weingärtner O, Lütjohann D, Elsässer A. Personalize and optimize lipid-lowering
1493 therapies. *J Am Coll Cardiol* 2016; 68; 325-326.
- 1494 190. Weingärtner O, Lütjohann D, Plösch T, Elsässer A. Individualized lipid-lowering therapy
1495 to further reduce residual cardiovascular risk. *The Journal of steroid biochemistry and*
1496 *molecular biology*. May 2017;169:198-201.
- 1497 191. Lederberg J, McCray AT. Ome SweetOmics--A Genealogical Treasury of Words. *The*
1498 *Scientist*. 2001;15(7):8-8.
- 1499 192. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut
1500 microbial enterotypes. *Science*. Oct 07 2011;334(6052):105-108.
- 1501 193. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the
1502 human gut microbiome. *Nature*. Jan 23 2014;505(7484):559-563.
- 1503 194. Wang Z, Koonen D, Hofker M, Fu J. Gut microbiome and lipid metabolism: from
1504 associations to mechanisms. *Curr Opin Lipidol*. Jun 2016;27(3):216-224.
- 1505 195. Ochoa-Repáraz J, Kasper LH. Gut microbiome and the risk factors in central nervous
1506 system autoimmunity. *FEBS Letters*. 2014/11/17/ 2014;588(22):4214-4222.
- 1507 196. Martinez I, Perdicaro DJ, Brown AW, et al. Diet-induced alterations of host cholesterol
1508 metabolism are likely to affect the gut microbiota composition in hamsters. *Applied and*
1509 *environmental microbiology*. Jan 2013;79(2):516-524.
- 1510 197. Baumgartner S, Mensink RP, Smet E, et al. Effects of plant stanol ester consumption on
1511 fasting plasma oxy(phyto)sterol concentrations as related to fecal microbiota

1512 characteristics. *The Journal of steroid biochemistry and molecular biology*. May
1513 2017;169:46-53.

1514 198. Yang X, Suo Z, Thornburg T, et al. Expression of Escherichia coli virulence usher
1515 protein attenuates wild-type Salmonella. *Virulence*. Jan-Feb 2012;3(1):29-42.

1516 199. Ochoa-Reparaz J, Riccardi C, Rynda A, Jun S, Callis G, Pascual DW. Regulatory T cell
1517 vaccination without autoantigen protects against experimental autoimmune
1518 encephalomyelitis. *J Immunol*. Feb 01 2007;178(3):1791-1799.

1519 200. Brautbar A, Leary E, Rasmussen K, Wilson DP, Steiner RD, Virani S. Genetics of
1520 familial hypercholesterolemia. *Current atherosclerosis reports*. Apr 2015;17(4):491.

1521 201. Russell DW. Fifty years of advances in bile acid synthesis and metabolism. *Journal of*
1522 *lipid research*. Apr 2009;50 Suppl:S120-125.

1523 202. Renner C, Connor WE, Steiner RD. Sitosterolemia Presenting as Pseudohomozygous
1524 Familial Hypercholesterolemia. *Clinical medicine & research*. Jun 2016;14(2):103-108.

1525 203. Miettinen TA, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a
1526 patient with sitosterolemia and cirrhosis. *Gastroenterology*. Feb 2006;130(2):542-547.

1527 204. Salen G, Shore V, Tint GS, et al. Increased sitosterol absorption, decreased removal, and
1528 expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with
1529 xanthomatosis. *J. Lipid Res*. September 1, 1989 1989;30(9):1319-1330.

1530 205. Nguyen LB, Salen G, Shefer S, Tint GS, Shore V, Ness GC. Decreased cholesterol
1531 biosynthesis in sitosterolemia with xanthomatosis: diminished mononuclear leukocyte 3-
1532 hydroxy-3-methylglutaryl coenzyme A reductase activity and enzyme protein associated
1533 with increased low-density lipoprotein receptor function. *Metabolism*. Apr
1534 1990;39(4):436-443.

- 1535 206. Nguyen LB, Cobb M, Shefer S, Salen G, Ness GC, Tint GS. Regulation of cholesterol
1536 biosynthesis in sitosterolemia: effects of lovastatin, cholestyramine, and dietary sterol
1537 restriction. *J. Lipid Res.* December 1, 1991 1991;32(12):1941-1948.
- 1538 207. Honda A, Salen G, Nguyen LB, Tint GS, Batta AK, Shefer S. Down-regulation of
1539 cholesterol biosynthesis in sitosterolemia: diminished activities of acetoacetyl-CoA
1540 thiolase, 3-hydroxy-3-methylglutaryl-CoA synthase, reductase, squalene synthase, and 7-
1541 dehydrocholesterol delta7-reductase in liver and mononuclear leukocytes. *Journal of lipid
1542 research.* Jan 1998;39(1):44-50.
- 1543 208. Othman RA, Myrie SB, Jones PJ. Non-cholesterol sterols and cholesterol metabolism in
1544 sitosterolemia. *Atherosclerosis.* Dec 2013;231(2):291-299.
- 1545 209. Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. Identifying patients,
1546 on the first day of life, at high-risk of developing parenteral nutrition-associated liver
1547 disease. *Journal of perinatology : official journal of the California Perinatal Association.*
1548 May 2007;27(5):284-290.
- 1549 210. Calkins KL, DeBarber A, Steiner RD, et al. Intravenous Fish Oil and Pediatric Intestinal
1550 Failure-Associated Liver Disease: Changes in Plasma Phytosterols, Cytokines, and Bile
1551 Acids and Erythrocyte Fatty Acids. *JPEN. Journal of parenteral and enteral nutrition.*
1552 May 01 2017;148607117709196.
- 1553 211. Nandivada P, Fell GL, Mitchell PD, et al. Long-Term Fish Oil Lipid Emulsion Use in
1554 Children With Intestinal Failure-Associated Liver Disease. *JPEN. Journal of parenteral
1555 and enteral nutrition.* Mar 09 2016.
- 1556 212. Calkins KL, Venick RS, Devaskar SU. Complications associated with parenteral nutrition
1557 in the neonate. *Clinics in perinatology.* Jun 2014;41(2):331-345.

- 1558 213. Clayton PT, Bowron A, Mills KA, Massoud A, Casteels M, Milla PJ. Phytosterolemia in
1559 children with parenteral nutrition-associated cholestatic liver disease. *Gastroenterology*.
1560 Dec 1993;105(6):1806-1813.
- 1561 214. Pianese P, Salvia G, Campanozzi A, et al. Sterol profiling in red blood cell membranes
1562 and plasma of newborns receiving total parenteral nutrition. *Journal of pediatric*
1563 *gastroenterology and nutrition*. Nov 2008;47(5):645-651.
- 1564 215. Mutanen A, Nissinen MJ, Lohi J, Heikkila P, Gylling H, Pakarinen MP. Serum plant
1565 sterols, cholestanol, and cholesterol precursors associate with histological liver injury in
1566 pediatric onset intestinal failure. *The American journal of clinical nutrition*. Oct
1567 2014;100(4):1085-1094.
- 1568 216. El Kasmi KC, Anderson AL, Devereaux MW, et al. Phytosterols promote liver injury and
1569 Kupffer cell activation in parenteral nutrition-associated liver disease. *Science*
1570 *translational medicine*. Oct 09 2013;5(206):206ra137.
- 1571 217. Mymin D, Wang J, Frohlich J, Hegele RA. Aortic xanthomatosis with coronary ostial
1572 occlusion in a child homozygous for a nonsense mutation in ABCG8. *Circulation*.
1573 February 11, 2003 2003;107(5):791-.
- 1574 218. Wang J, Joy T, Mymin D, Frohlich J, Hegele RA. Phenotypic heterogeneity of
1575 sitosterolemia. *J. Lipid Res*. December 1, 2004 2004;45(12):2361-2367.
- 1576 219. Othman RA, Myrie SB, Mymin D, et al. Ezetimibe reduces plant sterol accumulation and
1577 favorably increases platelet count in sitosterolemia. *J Pediatr*. Jan 2015;166(1):125-131.
- 1578 220. Lütjohann D, von Bergmann K, Sirah W, et al. Long-term efficacy and safety of
1579 ezetimibe 10 mg in patients with homozygous sitosterolemia: a 2-year, open-label
1580 extension study. *International journal of clinical practice*. Oct 2008;62(10):1499-1510.

221. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause
atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and
clinical studies. A consensus statement from the European Atherosclerosis Society
Consensus Panel. *European heart journal*. Apr 24 2017.
222. Rideout TC, Harding SV, Mackay DS. Metabolic and genetic factors modulating subject
specific LDL-C responses to plant sterol therapy. *Canadian journal of physiology and
pharmacology*. May 2012;90(5):509-514.

Figures Legends

Figure 1. Percentage change in LDL-C in individuals from baseline in response to the consumption of a low-fat plant sterol enriched soy beverage (1.95 g plant sterols /d) ²²².

Figure 2. Proposed surrogate measure of cholesterol metabolism which could overcome issues related to using ratios of surrogate synthesis to absorption markers.

Figure 3. Correlations between changes in serum LDL-C and plasma plant sterols and changes in flow-mediated dilation (copied from Ras et al.¹⁵⁸; permission to re-use required)