Lead Article

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Plasma Concentrations of Plant Sterols: Physiology and Relationship with Coronary Heart Disease

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Recently, it has been questioned whether elevated levels of circulating plant sterols increase the risk of coronary heart disease (CHD). To date, no definitive conclusions regarding such a relationship have been reached, nor have there been any studies summarizing the factors that contribute to the observed elevations in plant sterol concentrations in plasma. Thus, the purpose of this review is to systematically compare the plant sterol levels of subjects from the general population and to describe factors that contribute to the variations observed. The question of whether elevated plasma concentrations of plant sterols are associated with an increased risk of CHD was also assessed. Results indicate that the key factors accounting for variations in circulating plant sterol concentrations include: apolipoprotein E phenotypes, ATP-binding cassette transporter polymorphisms, use of statin drugs, presence of metabolic syndrome, dietary intake of plant sterols, gender, and analytical techniques used in the measurement of plant sterols in the plasma. An analysis of the studies examining the relationship between circulating levels of plant sterols and CHD risk in non-sitosterolemic populations revealed no clear associations. Furthermore, it was shown that the above-mentioned factors play an important role in determining the levels of plant sterols in plasma. Since these factors may act as potential confounders, they must be controlled for before more solid conclusions can be reached.

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INTRODUCTION

Plant sterols are naturally occurring constituents of plants that are structurally similar to cholesterol yet differ in side chain configuration. These compounds are not synthesized in mammals, and therefore are derived solely from the diet. It is well established that dietary plant sterols lower plasma cholesterol concentrations by inhibiting intestinal cholesterol absorption. Compared with cholesterol, plant sterol concentrations in the plasma are low due to their limited capacity to be absorbed. Although several epidemiological studies have investigated circulating levels of plant sterols in populations consuming habitual diets, this has yet to be systematically compared across populations. In addition, the underlying factors responsible for these differences have not been well established. Furthermore, since some investigators have recently suggested that circulating levels of plant sterols may be linked to an increased risk of coronary heart disease (CHD),[1-5] a comprehensive analysis of the literature in this area is warranted. Thus, in order to address these queries and concerns, the aim of the present review is to systematically compare plant sterol levels of subjects from the general population and to describe the factors that may contribute to the variations observed. An additional objective of this review is to assess whether elevated plasma concentrations of plant sterols are associated with an increased risk of CHD.

PLANT STEROL LEVELS IN THE GENERAL POPULATION

To determine plant sterol concentrations in the general population, studies conducted from 1986 to 2005 were analyzed.[6-50] Both population-based studies and clinical trials were included to generate the data pool. For clinical trials, baseline plant sterol concentrations were

utilized. When baseline values were not available, plant sterol concentrations measured post-control intervention were used instead. In addition, since some of the studies only reported plant sterol data as the plant sterol-to-cholesterol ratio, the absolute value for the plant sterol concentration was attained by multiplying the given ratio by the cholesterol level reported.

Table 1 displays the mean values of campesterol and sitosterol concentrations, as well as the campesterol-to-cholesterol and sitosterol-to-cholesterol ratios, for studies published between 1986 and 2005. From the 45 studies examined, campesterol concentrations were shown to range from 6.9 to 27.9 μmol/L, whereas sitosterol levels were shown to range from 2.8 to 16.0 μmol/L. Mean values for campesterol and sitosterol were calculated to be 14.2 ± 5.0 μmol/L and 7.9 ± 2.7 μmol/L, respectively. There was a large variability across standard deviations from each of the studies for both campesterol and sitosterol. For example, the highest standard deviation for campesterol between studies, which had a mean value of 19.8 μmol/L, was shown to be 11.6.[16] In comparison, in another study[36] in which a similar mean value was reported (19.2 μmol/L), the standard deviation was shown to be 4.7.

With respect to the frequency distribution of plant sterol mean values, campesterol and sitosterol were shown to vary widely (Table 1). Thus, based on the available data, no clear picture can be drawn with regard to the distribution patterns between populations (i.e., whether they are normal or skewed) of these two individual plant sterols. In addition to the absolute values of plant sterol concentrations, circulating plant sterol levels have been expressed as plant sterol-to-cholesterol ratios. Since both cholesterol and plant sterols are carried by lipoproteins in the plasma, more lipoprotein particles are present in the plasma of hypercholesterolemic subjects. Therefore, it can be assumed that hypercholesterolemic subjects might have a higher absolute plasma concentration of plant sterols, even with the same dietary background or with a similar plant sterol absorption rate. To improve the estimation of cholesterol or total sterol absorption, circulating plant sterol levels are often standardized by reporting the plant sterol-to-cholesterol-ratio. In comparing the absolute concentrations of campesterol with the campesterol-to-cholesterol ratios and the absolute concentrations of sitosterol with the sitosterol-to-cholesterol ratios, the distribution shows a similar tendency (Table 1). These data indicate that plant sterol absolute values, as well as plant sterol-to-cholesterol ratios, provide similar information regarding variations in circulating plant sterol concentrations. In addition, these data reflect large variability in circulating plant sterol levels within and across populations. For example, in the Finnish population, campesterol concentrations have been shown to range from 9.3 μmol/L[49] to 20.5 μmol/L.[43] Although variability in plasma concentrations of plant sterols is large across and within different population groups, there is very little known about the underlying factors that may contribute to such differences. To date, the following factors have been recognized to play a role in affecting plant sterol concentrations, and thus may explain some of the reported variability.

Factors Responsible for Variations in Circulating Plant Sterol Concentrations

Dietary Intake of Plant Sterols

Habitual diet composition, without plant sterol supplementation, may be a factor in determining baseline plasma levels of plant sterols in the general population. Although commercially available foods enriched with plant sterols, such as margarine, milk, yogurt, orange juice, and cereal bars, contain the largest amount of plant sterols per quantity ingested,[51] certain naturally occurring foods also contain high amounts. Plant oils, for example, have been identified as an excellent source of plant sterols.[52] In addition, nuts and seeds have been shown to contain moderate levels, while fruits and vegetables generally contain the lowest concentrations of plant sterols.[52] In a study by Miettinen et al.,[49] the plasma levels of plant sterols were correlated with the type of dietary constituent consumed in a randomly selected male population. Results revealed that plasma levels of plant sterols, specifically campesterol and sitosterol, exhibited positive correlations with the polyunsaturated/saturated fatty acid ratio of dietary fat, the linoleic acid contents of plasma and dietary lipids, and the amount of dietary plant sterols ingested.[49]

In examining consumption patterns throughout the world, it has been shown that different populations vary widely in the amount of plant foods consumed.[52-56] In Japan, where plant food consumption has been noted to be particularly high, typical plant sterol intakes are approximately 373 mg/d.[53] In contrast, Western populations typically consume a much lower level of plant-derived foods.[54,55] For example, Morton et al.[54] showed that the British consumed only about 167 mg/d of plant sterols, and the primary source was vegetable oil. However, plant sterol consumption varies greatly within population groups. Individuals in Western society choosing to follow a vegetarian diet consume, on average, about 385 mg/d of plant sterols, corresponding to 325 mg/d of β-sitosterol and 60 mg/d of campesterol.[56] In a recent cross-sectional analysis of the European Prospective Investigation into Cancer (EPIC),[57] daily plant sterol intakes of the 22,256 participants were separated into

quintiles. Results revealed that the within-population variability for plant sterol consumption was quite large: the participants in the highest quintile consumed 463 mg/d of plant sterols, while those in the lowest quintile consumed only 178 mg/d.[57]

The effect of consuming a diet high in plant-based foods, and thus high in plant sterols, on circulating plant sterol levels was analyzed recently in a randomized, controlled trial by Muti et al.[13] After an 18-week intervention period, it was shown that subjects who participated in the plant-based diet intervention experienced a 20% increase in β-sitosterol levels compared with controls. Similarly, in a randomized, controlled trial by Tammi et al.,[58] it was shown that 13-month-old children consuming high levels of plant sterols (132 mg/d) experienced 75% and 44% increases in circulating campesterol and sitosterol concentrations, respectively, compared with those on a low-plant-sterol diet (65 mg/d). Thus, depending on the type and amount of plant foods ingested, plant sterol consumption, and therefore plant sterol levels appearing in the blood, may vary greatly within and between populations. However, in general, the circulating levels of plant sterols seem to be related to the amount of plant sterols consumed in habitual diets of the general population in such a manner that they reflect dietary intake. Which factors are responsible for the large variation in responses will be addressed in the following sections.

Although the main focus of this paper is to describe circulating plant sterol levels in the general population, the effect of plant sterol and stanol supplementation on sterol levels in the plasma is also important. Recent findings indicate that when plant sterols are administered in supplement form at a dose of 1.8 to 2.0 g/d for 4 to 8 weeks, there is a 52% to 99% increase in campesterol levels and a 23% to 96% increase in sitosterol levels.[7,8,21,22] In contrast, when plant stanols are supplemented at a dose of 1.5 to 3.0 g/d for a 4-week period, decreases of 28% to 113% in campesterol levels and 24% to 50% in sitosterol levels have been observed.[21,23] These results suggest that higher plant sterol intake in the form of supplements increases circulating levels of plant sterols, while plant stanol supplementation decreases these levels.

Apolipoprotein E Phenotypes

Six phenotypes of human apolipoprotein E (ApoE) stem from three common alleles: E2, E3, and E4. Evidence suggests that ApoE polymorphisms may affect the efficiency of cholesterol absorption. Although the precise mechanism of cholesterol absorption is not known, it is believed that the absorption route for plant sterols is similar to that for cholesterol. Thus, plasma concentrations of plant sterols may be considered to be an indicator of fractional cholesterol absorption,[50] and therefore different ApoE phenotypes may modify plasma concentrations of plant sterols, reflecting shifts in cholesterol absorption. Recently, Uusitupa et al.[42] demonstrated that hypercholesterolemic subjects with the ApoE4 allele had 60% and 40% higher campesterol-to-cholesterol and sitosterol-to-cholesterol ratios, respectively, compared with those with the ApoE3 allele. Similar findings have been reported in other studies.[47,48,59,60] However, the opposite results have also been observed. Ketomaki et al.[15] reported that sitosterol-to-cholesterol ratios were lower in children with the ApoE4 phenotype than in those with ApoE2 and ApoE3 phenotypes. Moreover, phenotype distribution of ApoE has been shown to vary across different ethnic groups, which may contribute to the observed variations in plant sterol concentrations between populations. In a study by Ehnholm et al.,[61] it was shown that the prevalence of the E4 allele was higher in Finnish than in other Caucasian subjects. That same study also reported that the frequency of the E2 allele was greater in American subjects.[61] To date, there is limited evidence to support the mechanistic relationship between ApoE phenotypes and their influence on plasma concentrations of plant sterols. In a study by Welty et al.,[62] it was shown that subjects with the E4 allele had a decreased rate of LDL catabolism compared with those with the E3 allele.[62] This observation led the authors to conclude that this decreased catabolic rate may contribute to the accumulation of plant sterols in plasma.[62] To summarize, although no solid conclusions can be made, it is likely that the ApoE phenotype plays a role in explaining why variations in plant sterol concentrations exist within and across different population groups.

ABCG5/ABCG8 Phenotypes

Two recently discovered ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, have been shown to play an important role in regulating intestinal plant sterol absorption by excreting plant sterols that have already been taken up from the enterocyte back into the intestinal lumen.[63,64] ABCG5 and ABCG8 are half-transporters that form a functional heterodimer. Mutations in only one of the half-transporters have been shown to cause the rare inheritable autosomal recessive disease sitosterolemia.[65,66] Sitosterolemic patients are characterized by severely elevated plant sterol concentrations in the plasma, approximately 50 to 100 times higher than that in healthy subjects. Accordingly, ABCG5 and ABCG8 have been identified as plant sterol transporters, present not only in the intestinal lumen but also in the liver, where they are responsible for excreting plant sterols from plasma into the bile. Therefore, muta-

Table 1. Mean (±SD) Values of Circulating Plant Sterol Concentrations and Plant Sterol-to-Cholesterol Ratios in Studies Published Between 1986 and 2005

Author	Age	Sex	*N*	Area	Method	Cholesterol	Campesterol	Sitosterol	Campesterol/ Cholesterol	Sitosterol/ Cholesterol	Campesterol/ Sitosterol
						mmol/L	*μmol/L*			*μmol/mmol*	
Smahelova 2005[6]			105	Czech	GC-MS	5.26 ± 1.17	7.94 ± 4.80	6.86 ± 4.28	1.62 ± 1.37	1.32 ± 0.84	1.16
Varady et al., 2004[7]	54.4	M = 20 F = 54	74	Canada	GC	5.83 ± 1.21†	8.41 ± 4.91	3.78 ± 2.01	1.44	0.64	2.22
Clifton et al., 2004[8]	54	M = 23 F = 35	58	Australia	GC	6.43 ± 0.71*†	9.28 ± 4.01	8.53 ± 4.43	1.44	1.32	1.09
Clifton et al., 2004[9]	55.3	M = 12 F = 23	35	Australia	GC	6.59 ± 1.01†	7.83 ± 3.81	8.00 ± 3.54	1.19 ± 0.47	1.20 ± 0.48	0.98
Gylling et al., 2004[10]	52.6	M = 144 F = 119	263	Finland	GC	5.75 ± 1.05†	14.08	7.53	2.45 ± 0.84	1.31 ± 0.40	1.87
Pihlajamaki et al., 2004[11]	54	M = 72	72	Finland	GC	5.99 ± 1.12†	13.71	7.66	2.29 ± 0.19	1.28 ± 0.09	1.79
Quilez et al., 2003[12]	30.9	M = 25 F = 32	57	Spain	GC-MS	4.25 ± 0.62†	7.31	6.33	1.72 ± 0.89	1.49 ± 0.64	1.15
Muti et al., 2003[13]		F = 99	99	Italy	GC		14.46 ± 8.19	8.75 ± 4.16			1.65
Ketomaki et al., 2003[14]	6.8	M = 10 F = 13	23	Finland	GC	6.3 ± 0.95	27.9	12.28	4.43 ± 1.36	1.95 ± 0.51	2.27
Ketomaki et al., 2003[15]	11.16	M = 24 F = 23	47	Finland	GC	5.45 ± 0.83	18.37 ± 7.02	8.29 ± 3.02	3.71 ± 1.18	1.76 ± 0.57	2.22
Jones et al., 2003[16]	22–68	M = 9 F = 6	15	Canada	GC	6.11 ± 1.04†	19.8 ± 11.61	8.03 ± 4.37	3.24	1.31	2.47
Homma et al., 2003[17]	47.3		105‡ 35	Japan	GC	6.07 ± 0.43†	12.5 ± 6.71	16.07 ± 8.12	2.05	2.64	0.78
Naumann et al., 2003[18]	33.7	M = 15 F = 29	44	The Netherlands	GC	4.47 ± 1.06*†	15.15	5.27	3.39 (1.42–7.51)§	1.18 (0.18–2.57)§	2.87
Miettinen et al., 2003[19]		M = 140 F = 128	268	Finland	GC	5.93 ± 0.57	11.14	7.70	1.88 ± 0.80	1.30 ± 0.46	1.45
Chan et al., 2003[20]	54.5	M = 44	44	Australia	GC	5.51 ± 0.47†	15.2		2.76 ± 0.21		

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						mmol/L	*μmol/L*			*μmol/mmol*	
Vanstone et al., 2002[21]	47.8	M = 9 F = 6	15	Canada	GC	6.18 ± 1.11†	15.2 ± 7.54	8.35 ± 3.87	2.51 ± 1.17	1.36 ± 0.64	1.82
De Graaf et al., 2002[22]	57	M = 30 F = 32	62	The Netherlands	GC	6.7†	12.30	8.46	1.85	1.27	1.45
Mensink et al., 2002[23]	36	M = 16 F = 44	60	The Netherlands	GC	4.87 ± 0.81*†	18.60	8.08	3.82 ± 1.42	1.66 ± 0.64	2.30
Mussner et al., 2002[24]	42	M = 24 F = 38	62	Germany	GC-MS	6.08 ± 0.8*†	8.9 ± 3.66	4.43 ± 2.00	1.33 ± 0.50	0.66 ± 0.26	2.01
Gylling et al., 2002[25]	55.04		50	Finland	GC	5.76 ± 1.08†	17.62	8.35	3.06 ± 1.24	1.45 ± 0.54	2.11
Tammi et al., 2002[26]	6	M = 44 F = 35	79	Finland	GC	4.29 ± 0.57†	17.54	8.53	4.09 ± 1.22	1.99 ± 0.63	2.06
Berge et al., 2002[27]	55		148	USA	GC	5.29 ± 0.98	8.23 ± 3.62	6.02 ± 2.41	1.60 ± 0.61	1.15 ± 0.43	1.37
	25		48	USA	GC	4.58 ± 1.04	10.80 ± 3.11	7.23 ± 2.04	2.33 ± 0.50	1.58 ± 0.32	1.47
Nestel et al., 2001[28]	60	M = 18 F = 4	22	Australia	GC	7.03 ± 1.26*†	13.14		1.87 ± 0.93		
Christiansen et al., 2001[29]	50.7		52	Finland	GC-MS	6.66 ± 0.82†	6.9 ± 2.99	2.82 ± 1.04	1.03	0.42	2.45
Neil et al., 2001[30]	50.0		29	UK	GC	7.2 ± 1.04*†	8.28	5.4	1.15 (0.12–4.16)	0.75 (0.30–2.10)	1.53
Plat et al., 2001[31]	33	M = 41 F = 71	112	The Netherlands	GC	4.93†	14.93	5.66	3.03 ± 0.97	1.15 ± 0.43	2.64
Davidson et al., 2001[32]	45.9	M = 46 F = 38	84	USA	HPLC	5.3 ± 0.97†	14.02 (6.1–25.1)§	5.34 (2.4–10.5)§	2.89	1.13	2.63
Maki et al., 2001[33]	58.5	M = 101 F = 123	224‡	USA	HPLC	6.14 ± 0.63†	19.64§	6.00§	3.19	0.97	3.27
Tammi et al., 2001[34]	13 months	M = 22 F = 14	36	Finland	GC	3.87 ± 0.59†	9.14 ± 3.27	5.82 ± 1.83	2.45 ± 0.8	1.56 ± 0.44	1.57
Jones et al., 2000[35]	37–61	M = 15	15	Canada	GC	6.41 ± 0.74†	13.2 ± 5.93	9.03 ± 3.09			1.46

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						mmol/L	*μmol/L*			*μmol/mmol*	
Hallikainen et al., 2000[36]	48.8		34	Finland	GC	6.10 ± 0.69*†	19.21 ± 4.79	8.19 ± 2.12	3.74 ± 0.87	1.60 ± 0.40	2.35
Hallikainen et al., 2000[37]	50.5	M = 8 F = 14	22	Finland	GC	6.51 ± 1.03*†	18.6 ± 7.31	8.48 ± 3.43	3.42 ± 1.25	1.55 ± 0.56	2.19
Simonen et al., 2000[38]	52.3	M = 13 F = 3	16	Finland	GC	6.00 ± 0.70†	10.74	5.70	1.79 ± 0.56	0.95 ± 0.26	1.88
Jones et al., 1999[39]	25–60	M = 32	32	Canada	GC	6.77 ± 1.22†	21.65 ± 8.85	6.45 ± 2.6	3.21 ± 1.24	0.98 ± 0.35	3.36
Gylling et al., 1999[40]			153	Finland	GC	5.97 ± 0.72	16.10 ± 7.30	8.28 ± 3.35	2.69	1.38	1.94
Sutherland et al., 1998[41]	56	M = 21 F = 23	44	New Zealand	GC	6.84 ± 1.07†	11.53 ± 9.76	8.21 ± 8.48	1.69 ± 1.38	1.20 ± 1.20	1.40
Uusitupa et al., 1997[42]	50	M = 20 F = 16	36	Finland	GC	7.13 ± 0.82†	19.53	11.55	2.74 ± 1.25	1.62 ± 0.63	1.69
Gylling et al., 1997[43]	53	M = 31	31	Finland	GC	6.31 ± 0.70†	20.50	9.46	3.25 ± 0.93	1.50 ± 0.42	2.17
Gylling et al., 1995[44]	9.1	M = 7 F = 7	14	Finland	GC	7.68 ± 1.34*†	27.11 ± 8.6	13.67 ± 4.11	3.53 ± 1.12	1.78 ± 0.56	1.98
Chisholm et al., 1994[45]	51	M = 8 F = 11	19	New Zealand	GC	5.92 ± 0.84*†	15.50 ± 6.03	12.39 ± 5.16	2.63 ± 0.95	2.10 ± 0.83	1.25
Sutherland et al., 1991[46]	39	M = 24	24	New Zealand	GC	5.6 ± 0.81†	8.90	10.69	1.59 ± 0.71	1.91 ± 0.67	0.83
Kempen et al., 1991[47]	46.8	M = 158 F = 160	318	The Netherlands	GC	5.73 ± 1.07†	12.49 ± 5.41	6.87 ± 2.98	2.17	1.19	1.82
	16.7	M = 161 F = 159	320	The Netherlands	GC	4.25 ± 0.75†	12.21 ± 5.64	6.73 ± 3.01	2.87	1.58	1.81
Gylling et al., 1989[48]	51.3	M = 12 F = 28	38	Finland	GC		20.21 ± 10.63	13.63 ± 6.44	1.89 ± 0.84	1.28 ± 0.52	1.48

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						mmol/L	*µmol/L*			*µmol/mmol*	
Miettinen et al., 1990[49]	50	M = 63	63	Finland	GC	6.47 ± 1.2†	9.31 ± 3.69	6.19 ± 2.22	1.4	0.95	1.50
Tilvis et al., 1986[50]	41.5	M = 11 F = 6	17	Finland	GC	5.5 ± 1.23	8.3 ± 2.96	4.9 ± 1.92	1.5	0.89	1.69
Mean ± SD						5.88 ± 0.87	14.25 ± 5.07	7.93 ± 2.71	2.46 ± 0.89	1.36 ± 0.42	1.87 ± 0.58
Maximum							27.9	16.07	4.43	2.64	
Minimum							6.9	2.82	1.03	0.42	
Maximum − Minimum (Total variation considered as 100%)							21.00	13.25	3.40	2.22	

GC, Capillary gas-chromatography; MS, mass spectrometry; HPLC, high-performance liquid chromatography
*Control diet.
†Cholesterol was measured by the enzymatic method.
‡Plant sterols measured in ⅓ of subjects.
§Data reported as median value.

tions in ABCG5 or ABCG8, as in sitosterolemia, have a double effect on circulating plant sterol levels in that intestinal uptake is increased while hepatic secretion is concomitantly decreased. For this reason, dietary plant sterols are contraindicated in homozygous sitosterolemic patients because elevated plant sterol intake would result in severe accumulation of plant sterols in the circulation.

Interestingly, obligate heterozygotes for sitosterolemia not only showed similar or only marginally elevated cross-sectional plant sterol concentrations,[67-69] but the observed elevation in serum sitosterol and campesterol concentrations during 4 to 12 weeks of consumption of plant sterol-enriched margarines (at a dose level of 2.2–3.0 g/d) was somewhat higher but still in line with those observed in healthy controls.[68,69] In general, the intake of 2 to 3 g/d of plant sterols elevates serum sitosterol and campesterol concentrations by 30% and 70%, respectively, in healthy subjects,[36,70] while these figures were around 50% and 125%, respectively, in obligate heterozygous sitosterolemic patients.[68,69] These data suggest that having only one functional ABCG5 or ABCG8 allele is sufficient for nearly normal function.

Aside from the rare mutation of a homozygous form resulting in sitosterolemia, several common sequence variations have been described.[71] Different studies have shown independently that the cross-sectional plant sterol-to-cholesterol ratio is associated with different ABCG5 and ABCG8 polymorphisms.[10,27,72] Out of the five polymorphisms analyzed in relation to concentrations of plant sterols, D19H, Y54C, T400K, A632V, and Q604E, the polymorphisms D19H in exon 1 and T400K in exon 8 of ABCG8 show the most pronounced association. Carriers of the H allele of the D19H polymorphism in ABCG8 were found to have a lower plasma campesterol-to-cholesterol ratio[10,27] and sitosterol-to-cholesterol ratio,[10] suggesting a higher activity of this variant. A similar finding was observed for the K allele of the T400K polymorphism in exon 8 of ABCG8.[27,72] Interestingly, no consistent cross-sectional associations between plasma cholesterol concentrations and any of these ABCG5 or ABCG8 polymorphisms have been described so far.[10,27,72,73]

In addition to these cross-sectional associations, genetic variations in these ABC transporters may also predict which subjects are prone to develop elevated plant sterol concentrations. In this respect, carriers of the T allele of the T400K polymorphism showed a larger reduction in both the campesterol-to-cholesterol and the sitosterol-to-cholesterol ratios during interventions known to lower plasma plant sterols.[72] Although not evaluated, it can be speculated that these subjects would also show larger elevations in circulating plant sterol levels as a result of consuming plant sterol-enriched functional foods[18,70] or undergoing treatment with statins[74]

(see below). In conclusion, the plasma campesterol-to-cholesterol and sitosterol-to-cholesterol ratios, as well as changes in situations known to modulate plasma concentrations of plant sterols, are related to variations in the ABCG5 and ABCG8 genes. No consistent relationship with plasma lipid and lipoprotein concentrations have been observed, which suggests that changes in the functionality of the ABCG5/G8 heterodimer mainly affect plasma concentrations of plant sterols, not those of cholesterol.

Influence of Metabolic Syndrome and Diabetes

It has been recently shown that individuals with type 1 or type 2 diabetes or insulin resistance or those who are obese have decreased cholesterol absorption.[6,11,43,76] Simonen et al.[38] demonstrated that low cholesterol absorption may play a role in the metabolic syndrome. This conclusion was made after observing dramatic increases in plant sterol levels in plasma following significant weight loss in obese people with type 2 diabetes.[38] Individuals with metabolic syndrome tend to have lower plasma concentrations of plant sterols, which was also confirmed in another study.[20] In 2003, Chan et al.[20] indicated that metabolic syndrome patients had significantly lower campesterol-to-cholesterol ratios compared with controls. Recently, it was shown that the gene expression of the hepatic ABC transporter (MDR2) increased in streptozotocin-induced diabetic rats, which in turn enhanced the biliary excretion of sterols.[77] It was also demonstrated that insulin administration could reverse the increase in the expression of this gene. Thus, these findings may in part explain why subjects with metabolic syndrome or diabetes have lower plasma concentrations of plant sterols.

Gender

The precise effect of gender on circulating plant sterol levels has yet to be fully clarified. In a study by Tilvis et al.,[50] it was shown that women had higher absolute plant sterol concentrations than did men. These results were confirmed by Sutherland et al.[41] In contrast, in a population-based study of 160 Dutch families, although gender differences were not observed in the raw data, a multivariate analysis showed that gender did significantly affect absolute levels of campesterol and sitosterol in the plasma.[47] In this same study,[47] it was reported that men had higher plant sterol concentrations than did women.

Statin Treatment and Plant Sterol Concentrations in Plasma

Cholesterol is derived from internal biosynthesis and from intestinal absorption of dietary and biliary cholesterol. Statins are commonly used to lower plasma cholesterol concentrations via inhibiting internal cholesterol synthesis. A putative hypothesis is that cholesterol absorption from the gut increases to partly compensate for the decrease in cholesterol synthesis. Thus, as mentioned previously, since plant sterols have been used as markers for cholesterol absorption,[49] statin treatment may increase plant sterol concentrations in the plasma by means of increasing intestinal sterol absorption or lowering the free cholesterol pool in the liver, which may result in a decreased secretion of cholesterol and plant sterols into bile.

As described previously, plant sterol-to-cholesterol ratios in the plasma are commonly used to indicate changes in plant sterol concentrations. In non-statin-users, plant sterol-to-cholesterol ratios and absolute plant sterol concentrations provide similar information. However, the parameter of plant sterol-to-cholesterol ratios is not suitable to indicate changes in plant sterol concentrations in statin users. This is because statins could greatly increase the value of plant sterol-to-cholesterol ratios via decreasing the plasma cholesterol concentration, while plant sterol concentrations would not be changed. Therefore, in this review, the absolute values of plant sterol concentrations have been used to evaluate the effects of statins.

Effect of Statins on Plant Sterol Concentrations in Hypercholesterolemic Subjects. There are no consistent conclusions concerning the effect of statins on plasma concentrations of plant sterols in randomly selected hypercholesterolemic subjects. Among the studies examined, four showed that statin treatment increased plant sterol levels in the plasma,[78-81] while the other seven showed that statins either had no effect[82] or reduced these levels.[83-88] Similarly, no consistent effect of individual statins on plasma concentrations of plant sterols has been shown. In general, the reported studies indicate that lovastatin, pravastatin, and simvastatin lowered plasma concentrations of campesterol and sitosterol, while atorvastatin slightly increased the concentrations of these two individual plant sterols. At present, there are no plausible mechanisms that may explain the potential differences between atorvastatin and other statins with regard to the absorption of plant sterols. When the data from all of the reported statin-plant sterol studies were pooled, plasma concentrations of campesterol were shown to be 16.8 and 16.6 μmol/L and sitosterol concentrations were 9.9 and 9.6 μmol/L at baseline and after statin treatment, respectively. These data indicate that, in general, statins do not alter plant sterol concentrations in plasma. In addition, no clear relationships have been observed between dose, period of statin treatment, and change in plasma concentrations of plant sterols.

Effect of Statins on Plant Sterol Concentrations in High- and Low-Sterol-Absorbers. As mentioned above, plant sterols are considered to be markers for cholesterol absorption.[49] Thus, subjects with high baseline plant sterol concentrations in plasma can be defined as high-sterol-absorbers. In high-sterol-absorbers, in whom baseline campesterol and sitosterol concentrations were 15.4 and 10.4 μmol/L, respectively, simvastatin increased the plasma campesterol concentration up to 32.1 μmol/L within 240 weeks.[74] This simvastatin-induced increase of campesterol was sustained in a time-dependent manner, and was independent of the decrease in plasma cholesterol, because simvastatin reached its maximal cholesterol-lowering effect within 6 weeks. In contrast, the sitosterol concentration was not considerably altered by simvastatin in these subjects. In low-sterol-absorbers, in whom baseline campesterol and sitosterol concentrations were 7.9 and 5.7 μmol/L, respectively, simvastatin treatment for 240 weeks only mildly increased plasma campesterol concentrations up to 10.1 μmol/L, while sitosterol concentrations decreased to 4.9 μmol/L.[74] However, a more recent study showed a less considerable increase in campesterol by 12 weeks of simvastatin treatment in high-sterol-absorbers compared with low-sterol-absorbers.[89] It is unclear why simvastatin only selectively increased campesterol, but not sitosterol, in both high- and low-sterol-absorbers.

Effect of Statins on Plant Sterol Concentrations in Good and Poor Responders to Statin Treatment. Some hypercholesterolemic subjects respond well to low doses of statins and are thus termed "good-responders," while others need a larger dose of statins for a similar cholesterol-lowering effect and are thus termed "poor-responders." This phenomenon is explained by the hypothesis that poor-responders may have a higher capacity to absorb cholesterol and plant sterols from the gut than good-responders. In other words, in poor-responders, the cholesterol-lowering effect of statins would be counterbalanced partly by an increase in intestinal sterol absorption. Therefore, a larger dose of statins would be needed to lower plasma cholesterol in poor-responders. If this hypothesis were true, statins would increase plant sterol levels more pronouncedly in the plasma of poor-responders. Data from a 52-week study with simvastatin, however, do not support this hypothesis. This study showed that simvastatin did not increase plant sterol levels in the plasma of good-responders or in poor-responders.[19] These findings further support the conclusion that statins do not markedly alter plant sterol concentrations in plasma, as was observed in the randomly selected hypercholesterolemic subjects described above.[74,89,90] Although statin treatment does not increase absolute plasma concentrations of plant sterols, it may generate LDL particles relatively rich in plant sterols, as indicated by an increased plant sterol-to-cholesterol ratio. However, the biological implications of the plant sterol (also stanol)-rich LDL particles have not yet been investigated.

Analytical Methods Used to Assess Plant Sterol Concentrations in Plasma and Their Effect on Observed Values

Another factor that may contribute to the variability seen in plant sterol concentrations is the different methods applied to quantify these compounds. The assessment of plasma levels of plant sterols is usually performed with capillary gas-chromatography coupled with either flame ionization detection or mass spectrometry.[91,92] Such methods are limited in that they usually require large sample volumes and involve laborious sample pretreatment procedures, including hydrolysis, extraction, and derivatization. In addition, issues regarding peak separation and poor resolution seen with gas chromatography-flame ionization detection methods are also of concern. Quantification of the low concentrations of plant sterols in plasma has also been problematic. Different injection techniques, such as on-column injection versus the more common split/splitless injection, may have also contributed to differences in reported values in the data pool (Table 1). Thus, since standardized procedures were not applied to all of the studies examined in this review, it is probable that the differing analytical techniques may confound plant sterol value results, and that this may be partly responsible for the differences noted in reported plant sterol concentrations between individual studies.

Estimated Impact of Different Factors on Baseline Circulating Plant Sterol Concentrations

In examining the campesterol and sitosterol concentrations, as well as the campesterol-to-cholesterol and sitosterol-to-cholesterol ratios reported in Table 1, it is evident that large variations in the baseline plant sterol concentration exist within the general population. The factors outlined above play a crucial role in determining circulating plant sterol concentrations. However, the degree to which each factor contributes to the variations observed has yet to be determined. Therefore, in order to estimate the degree to which each factor effects variations in plant sterol levels, data from Table 1 were pooled and an overall range for absolute values of campesterol and sitosterol concentrations calculated. The impact of each factor on circulating absolute values of plant sterol concentrations was then determined using the following method.

Using the factor of gender as an example, an average concentration for the absolute campesterol and sitosterol concentrations for both males and females was first calculated from the studies reporting differences based

on gender.[41,50] Next, the difference between male and female values for each campesterol and sitosterol concentration was assessed, and this value was divided by the overall range calculated in Table 1. Averaging the percentages of campesterol and sitosterol yielded absolute concentrations that allowed for the estimation of the overall impact of each factor. Since there were limited data from which to estimate the impact of diet on plasma concentrations of plant sterols, a regression equation estimating the impact of plant sterol intake on circulating plant sterol levels was developed using the results of supplementation studies. In addition, it should be noted that since the overall range generated from the data pool in Table 1 is based on healthy subjects not taking statins or consuming plant sterol-enriched food products or dietary supplements, these factors were not included to account for the source of the variations in plant sterol concentrations in the plasma. Additionally, the impact of each factor on the campesterol-to-cholesterol and sitosterol-to-cholesterol ratios was calculated in the same way as were absolute values of plant sterol concentration mentioned above.

Figure 1 portrays the estimated degree to which each factor affects circulating absolute plant sterol concentrations, whereas Figure 2 shows the degree to which each factor affects plant sterol-to-cholesterol ratios. In comparing Figure 1 with Figure 2, it can be seen that each factor affects absolute plant sterol concentrations and plant sterol-to-cholesterol ratios similarly. Analytical methods used in the assessment of plant sterol levels appear to contribute the greatest degree of variation. Gender, diabetes/metabolic syndrome, and genetic factors such as ApoE and ABCG5/8 phenotypes all have a similar yet moderate degree of influence on absolute concentrations and ratios. Interestingly, dietary intake of plant sterols without plant sterol supplementation played only a minor role in the variations observed. Additionally, since the contributions of these individual factors do not add up to 100%, it can be assumed that other factors may account for the residual 15% to 20% variation in plant sterol concentrations and plant sterol-to-cholesterol ratios.

Interactions between factors may also alter circulating plant sterol concentrations and their ratios to cholesterol. For example, it has been shown that the 604E allele of the ABCG5 gene is associated with higher BMI values and decreased insulin sensitivity,[10] two factors implicated in metabolic syndrome. However, due to the limited data available, the percentage of the impact of these interactive effects cannot be calculated at present. Thus, as demonstrated in Figures 1 and 2, analytical methods, gender, diabetes/metabolic syndrome, and genetic factors seem to account for the majority of the variations in

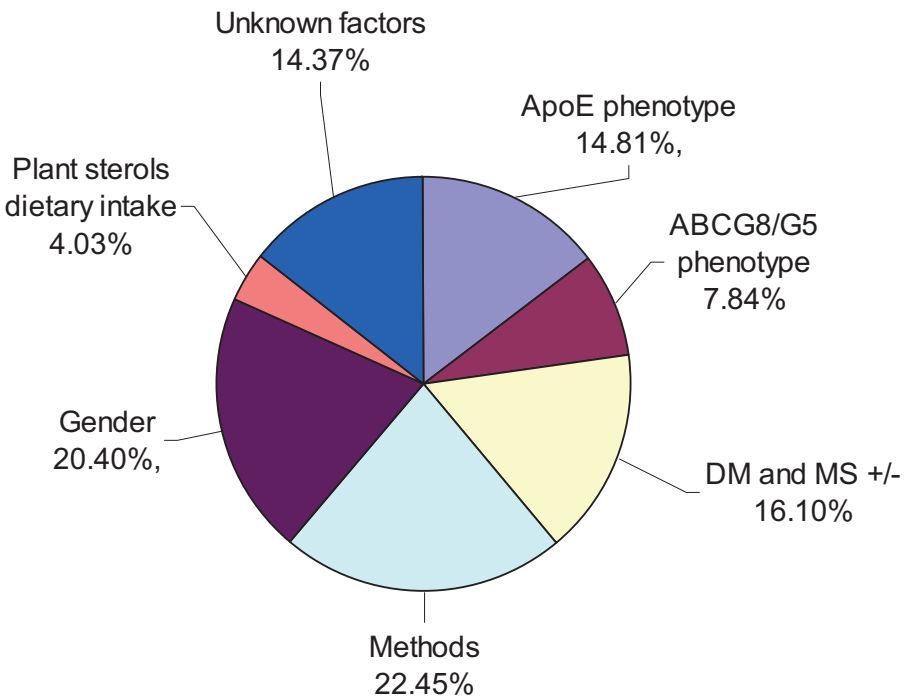

Figure 1. Estimated impact of different factors on absolute values of circulating plant sterol concentrations. Percent variations were calculated using data from studies reported in Table 1. ApoE, apolipoprotein E polymorphism; ABCG5/8, ATP-binding cassette transporter G5/G8 polymorphism; DM +/–, presence or absence of diabetes mellitus; MS +/–, presence or absence of metabolic syndrome.

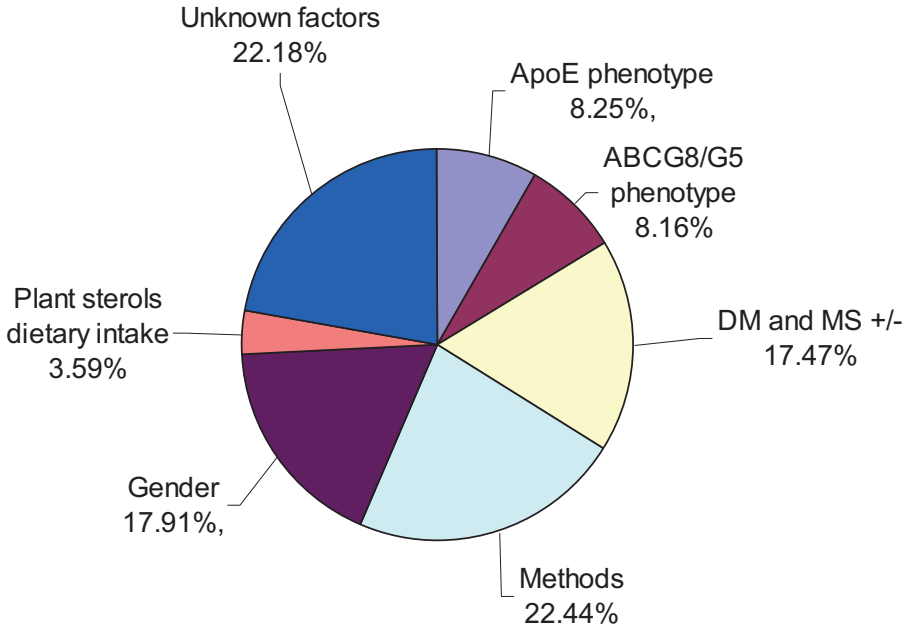

Figure 2. Estimated impact of different factors on circulating plant sterol-to-cholesterol ratios. Percent variations were calculated using data from studies reported in Table 1. ApoE, apolipoprotein E polymorphism; ABCG5/8, ATP-binding cassette transporter G5/G8 polymorphism; DM +/–, presence or absence of diabetes mellitus; MS +/–, presence or absence of metabolic syndrome.

plant sterol concentrations observed, while habitual diet seems to play only a minor role.

PLASMA PLANT STEROLS AND CORONARY HEART DISEASE RISK

As mentioned previously, sitosterolemia is an autosomal-recessive disorder that results from mutations in either of the ABC transporters, ABCG5 or ABCG8. The disease is characterized by a 50- to 100-fold elevation in the plasma levels of plant sterols, normal to moderately elevated plasma cholesterol concentrations, and an increased susceptibility to premature CHD development.[93] Therefore, it is reasonable to question whether increased plant sterol levels promote the development of CHD, even in subjects without sitosterolemia.

Studies Indicating That Plant Sterol Levels May Be Associated with Increased Risk of Coronary Heart Disease

Human Studies

Glueck et al.[1] concluded that elevated plant sterol levels may be correlated with increased risk of premature CHD. These investigators examined the relationship between plant sterol and cholesterol levels in plasma and the association between plant sterol levels and a personal or family history of CHD by evaluating 595 hypercholesterolemic patients 55 years of age and under. Results indicated that plasma campesterol ($r = 0.15$; $P \leq 0.001$), sitosterol ($r = 0.34$; $P \leq 0.0001$), stigmasterol ($r = 0.10$; $P \leq 0.02$), and total plant sterol ($r = 0.29$; $P \leq 0.0001$) levels were associated, although weakly, with cholesterol levels. In addition, the authors concluded that the incidence of a personal or family history of CHD was correlated with absolute campesterol ($P = 0.097$) and stigmasterol ($P = 0.096$) concentrations. These analyses were performed by comparing the mid-range campesterol values of the premature CHD subjects with those of the healthy subjects, and by comparing the percentage of subjects with premature CHD in the top stigmasterol decile with those in the bottom decile. Thus, the analytical procedures implemented to derive these results were neither standardized nor comparable.

Additionally, the authors reported that there were no significant differences in plasma sitosterol, stigmasterol, and cholesterol levels between subjects with premature CHD and those without. However, it was found that the prevalence of family CHD history was elevated more than two-fold ($P = 0.013$) in the 21 hyperphytosterolemic patients, those having one of the sterol concentrations above the 95th percentile and a second above the 75th percentile. Nevertheless, since plant sterol plasma levels of hyperphytosterolemic subjects were positively correlated with LDL cholesterol and ApoB levels in their first-degree relatives, the authors concluded that absolute plant sterol concentrations predicted certain parameters for atherogenesis in these families. However, the actual percentage of CHD in the first-degree relatives was not

reported, and therefore it is not possible to conclude from this endpoint that circulating plant sterols are familial markers of CHD.

Rajaratnam et al.[2] investigated whether a relationship existed between cholesterol metabolism and coronary artery disease (CAD) in postmenopausal women. These investigators established that the risk of CAD was greater in women who possessed elevated plasma campesterol and sitosterol concentrations and campesterol-to-cholesterol and sitosterol-cholesterol ratios. In this study, non-cholesterol sterol levels were assessed in 48 postmenopausal women with CAD and then compared with those of 61 postmenopausal controls to investigate whether plant sterol concentrations were indicators of CAD risk. The CAD subjects exhibited comparable plasma cholesterol levels but elevated campesterol- and sitosterol-to-cholesterol ratios relative to the control group. Based on these findings, the authors concluded that women with a higher CAD risk have a higher prevalence of elevated plasma campesterol- and sitosterol-to-cholesterol ratios.

More recently, Sudhop et al.[3] compared two groups consisting of patients undergoing elective artery coronary bypass graft with either no family history of CHD or with proven CHD and/or a family history of CHD. There was a lack of a true control group in this study. The objective of this study was to determine whether the latter group had a higher prevalence of elevated plant sterol levels. The study consisted of 42 men and 11 women. The 26 patients who were categorized as having a positive family history of CHD had 30% (0.012 μmol/L) and 29% (0.009 μmol/L) higher absolute concentrations of plasma campesterol and sitosterol, respectively. From these data, it was concluded that plant sterols might be an additional risk factor for CHD.

Assmann et al.[4] performed a nested case-control study comparing 318 controls with 159 subjects who had experienced a myocardial infarction or sudden coronary death within the past decade. When the two groups were compared, the authors concluded that the occurrence of elevated absolute sitosterol levels was greater in the group who had had a coronary event. However, the concentrations of total cholesterol, LDL cholesterol, and triglycerides, as well as systolic blood pressure, were significantly higher in the cardiac patients than in the controls. Thus, the impact of elevated sitosterol levels on the development of CHD can only be determined if factors such as LDL cholesterol levels, which are known to play a role in the development of heart disease, are matched between cases and controls. Furthermore, the conclusion was drawn based only on sitosterol values, since the campesterol data did not show any significant differences between cases and controls. Moreover, while the absolute sitosterol concentration was significantly higher in the cardiac patients than in the controls, the sitosterol-to-cholesterol ratio was not. Therefore, it cannot be ruled out that differences in cholesterol contributed to the association between high sitosterol and CHD risk. In addition, the study population only included men; since gender has been shown to affect plant sterol levels in the plasma, it can be questioned whether the inclusion of females in the analysis would have yielded similar conclusions.

Studies Indicating That Plant Sterol Levels Are Not Associated with Increased Risk of Coronary Heart Disease

Human Studies

In contrast to the four observational studies described above, one comprehensive and well-controlled study has demonstrated that plant sterol levels are not associated with atherosclerosis.[5] The association between plant sterol levels and CHD risk was studied in 2542 middle-aged subjects. Patients were required to give their familial history of myocardial infarction and to undergo electron beam computer tomography to measure coronary calcium levels. Results indicated that neither a family history of CHD nor coronary calcium level was associated with an elevated plant sterol-to-cholesterol ratio in the plasma. In contrast, plasma cholesterol concentrations were significantly higher in subjects with a family history of CHD and in those with high calcium levels. Thus, the results of this study suggest that the development of coronary atherosclerotic lesions is not augmented in those individuals with elevated plant sterol-to-cholesterol ratios. However, the age of this study population was much lower than that in previous studies,[2-4] so the percentage of patients with CHD, as determined by the level of coronary calcium, was probably lower compared with previous studies.

It is possible that this confounder may have been responsible for the lack of a positive relationship between elevated plant sterol-to-cholesterol ratios and coronary calcium levels in plasma. However, since electron beam computer tomography was not conducted in any other study, a comparison of the results of these studies based on this indicator cannot be made. Nevertheless, another marker related to plant sterol levels in plasma and plant sterol-to-cholesterol ratios that cannot be affected by age is the presence or absence of family history of CHD. Family history of CHD has been used as an outcome variable in previous studies, so the results presented for this variable are comparable.[2-4] Moreover, due to the large sample size implemented in this study, as well as the wide age range and ethnic diversity, it is probable that the results of this study may be generalized to the greater population.

Emerging preliminary results from human studies have shown that elevated plant sterol concentrations in plasma were not positively associated with CHD risk,[104] and indicated a protection from CHD.[105] The results were from case-control studies.[104,105] Higher plant sterol concentrations in plasma or their ratios to cholesterol were observed in controls as compared with cases.[104,105] However, more information will be needed for further discussion on the above-mentioned preliminary data, which are currently available only in abstract form.

Animal Studies

To date, only animal trials have been conducted to investigate the effect of dietary plant sterols on atherogenesis. Human intervention trials are lacking. Pollak et al.[95] summarized the early studies evaluating the impact of plant sterol feeding on experimental atherosclerosis models in rabbits and chickens. The inhibition of atherosclerotic development was observed in most of the studies. In the more recent studies, different species of animals were used, including ApoE-deficient mice, ApoE*3-Leiden transgenic mice, rabbits, obese Zucker rats, and hamsters.[96-103] Most animal studies have concluded that plant sterols exert protective effects on atherosclerotic lesion development, plaque formation, foam cell formation, and vascular endothelium damage. Furthermore, it has been reported that the attenuation of foam cell formation by the consumption of plant sterols occurs in a dose-dependent manner.[101]

In the most recent published animal work,[5] different types of transgenic mice were used. For example, ABCG5/8-knockout mice, which have a 30- to 100-fold increase in plant sterol levels in the plasma compared with normal mice, and hypercholesterolemic mice, which lack both the ABCG5/G8 ($G5G8^{-/-}$) and the LDL receptor (LDLr)($LDLr^{-/-}$), were used as models for human sitosterolemia. These mice were fed either a standard chow with 0.4% fat and 0.02% cholesterol or a Western-type diet with 21% fat and 0.2% cholesterol. Plant sterols made up about 30% of the circulating sterols in the chow-fed $G5G8^{-/-}$ mice and about 12% in those fed the Western-type diet. Even in $G5G8^{-/-}$ mice with very high plant sterol levels in their plasma after 7 months of feeding, no or only a few atherosclerotic lesions were found. Moreover, even though the severely hypercholesterolemic mice (with only $LDLr^{-/-}$) had significant atherosclerosis, $LDLr^{-/-}$ with $G5G8^{-/-}$ mice did not show larger aortic lesions compared with the $LDLr^{-/-}$ mice. These data suggest that the increase in plant sterol levels in plasma did not lead to additional aortic lesion development. Thus, plant sterols appear to be no more atherogenic than cholesterol.

Plasma Levels of Plant Sterols and the Risk of Coronary Heart Disease: Discussion and Summary of Findings

The absolute concentrations of plasma campesterol, sitosterol, and their ratios to cholesterol in the five studies[1-5] in humans (Table 2) fall into the normal range compared with the data pool generated by the analysis of the 45 previous studies (Table 1). As mentioned previously, several established factors play a role in influencing plasma concentrations of plant sterols. However, these factors, which may act as confounders, were not taken into consideration in the design of the studies that found associations between higher plant sterol concentrations and increased CHD risk. In the study by Glueck et al.,[1] subject exclusion/inclusion criteria were not reported, and therefore the information concerning each individual's statin intake was not available. Therefore, the use of these lipid-altering pharmaceuticals may further confound the relationship between plant sterol levels and CHD risk. Moreover, since detailed subject characteristic data were not available in the studies by Glueck et al.[1] and Sudhop et al.,[3] it is possible that the authors may have included subjects with metabolic syndrome. Therefore, if the number of the subjects with metabolic syndrome in the case and control groups was not even, this confounder may hide the real relationship between plant sterol concentrations and family history of heart disease.

ApoE phenotypes and dietary intake were only reported in the study by Rajaratnam et al.,[2] and the polymorphisms of the ABCG5/ABCG8 phenotypes were not tested in any of the studies. Both of these factors cause alterations in plasma concentrations of plant sterols, but these variables may be further linked to other real pathological factors that play a role in atherogenesis. It is possible that the elevated plasma levels of plant sterols observed in the epidemiological studies were just a marker, not a risk factor, for a higher risk of CHD. Therefore, it is possible that each of these confounding factors may have contributed to a false-positive relationship between circulating levels of plant sterols and increased risk of CHD. Thus, the effect of these confounders must be eliminated or controlled for by study design, or a proper model must be established to investigate the role of circulating plant sterols in CHD pathology before the role of circulating plant sterol levels in heart disease development can be validly determined.

Direct evidence is still lacking to show that plant sterols themselves are atherogenic in the concentrations typically found in non-sitosterolemic subjects. Furthermore, most of the animal studies demonstrated that plant sterol consumption inhibited atherosclerotic development. It is well known that after the consumption of plant

Table 2. Plasma Plant Sterols and Coronary Heart Disease Risk: Summary of Findings from Human Trials*

Author	Subjects	Age	Sex	Design	Results	Comments
Glueck et al., 1991[1]	$n = 595$, HC	57.8	M = 231 F = 364	Observational study	Cholesterol levels weakly correlated with plasma campesterol ($r = 0.15$; $P \leq 0.001$), stigmasterol ($r = 0.10$; $P \leq 0.02$), sitosterol ($r = 0.34$; $P \leq 0.0001$), and total plant sterol levels ($r = 0.29$; $P \leq 0.0001$); high campesterol ($P = 0.097$) and stigmasterol ($P = 0.096$) concentrations associated with family history of CHD	Exclusion criteria not reported; statin intake, presence/absence of MS, ApoE phenotype, $ABCG_5$/G8 phenotype, and dietary intake of plant sterols not reported; % CHD in first-degree relatives not reported so cannot conclude that plant sterols are familial markers of CHD
Rajaratnam et al., 2000[2]	$n = 48$, CAD $n = 61$, control	53.1 53.6	F = 109	Observational study comparing CAD subjects to control	CAD subjects had similar plasma cholesterol levels but elevated campesterol- and sitosterol-to-cholesterol ratios relative to control group ($P \leq 0.05$); plasma campesterol- and sitosterol-to-cholesterol ratios significantly associated with CAD	Statin intake, presence/absence of MS, and $ABCG_5$/G8 phenotype not reported; included women only
Sudhop et al., 2002[3]	$n = 26$, FH $n = 27$, no FH	60.9 64.0	M = 42 F = 11	Observational study comparing patients with or without FH	FH patients had 30% (0.012 μmol/L) and 29% (0.009 μmol/L) ($P \leq 0.05$) higher absolute concentrations of plasma campesterol and sitosterol, respectively	Lack of a true control group; statin intake, presence/absence of MS, ApoE phenotype, $ABCG_5$/G8 phenotype, and dietary intake of plant sterols not reported
Assmann et al., 2005[4]	$n = 159$, CVD $n = 318$, control		M = 477	Case-control study comparing CVD patients to control	CVD group had elevated absolute sitosterol levels ($P \leq 0.05$); concentrations of total cholesterol, LDL cholesterol, and triglycerides, and systolic blood pressure were also higher ($P \leq 0.05$) in the cases compared with the controls	Study included men only; LDL cholesterol levels, which play a role in CVD development, not matched between cases and controls; conclusions drawn based only on sitosterol values
Wilund et al., 2004[5]	$n = 1032$, FH $n = 1792$, no FH	42.3	M = 1424 F = 1828	Observational study comparing patients with or without FH	FH not associated with elevated plasma plant sterol-to-cholesterol ratios; plasma cholesterol concentrations higher ($P \leq 0.05$) in subjects with FH than in controls	Age of subjects lower than in other studies; large sample size

*Circulating plant sterol concentrations and sterol-to-cholesterol ratios for these studies are within the normal range when compared with the data pool generated by the analysis of the 45 other studies (Table 1).

CAD, Coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; FH, family history of cardiovascular disease; HC, hypercholesterolemic; MS, metabolic syndrome.

sterol-enriched food products, plant sterol concentrations are modestly increased in plasma, while cholesterol levels are substantially decreased. This reduction in circulating cholesterol concentrations is associated with a decrease in CHD risk. In view of these findings, there is no overall clear body of evidence at present to conclude that elevated levels of plant sterols in plasma play a role in the development of CHD. In contrast, based on evidence from plant sterol feeding trials in both animal models and humans, elevated plant sterol concentrations in the plasma as a result of plant sterol supplementation are associated with a decreased risk of CHD.

CONCLUSIONS

Based on the evidence available to date, a series of factors have been identified that contribute to the variability in circulating plant sterols in the general population. Since these factors may have a considerable effect on the levels of plant sterols within the blood, it is essential that they are controlled for in studies seeking to determine the relationship between circulating plant sterol levels and CHD risk. In addition, the conclusion that elevated plant sterols in plasma are associated with CHD should be drawn based upon direct evidence of cause-effect relationships between plasma levels of plant sterols and CHD. However, to date, such evidence is lacking and the pathological mechanisms of plasma concentrations of plant sterols in CHD development have yet to be established. Thus, to conclude that elevated plasma concentrations of plant sterols play a role in the development of CHD is not appropriate at the present time. Further research is needed that controls for the confounding factors described in this review before any solid conclusions can be reached. However, until such strong evidence is produced, the benefits of consuming plant sterols to lower LDL cholesterol levels appear to outweigh the risks. As a natural alternative to pharmaceuticals, plant sterol use should be considered as a valid and useful strategy in reducing risk for CHD, a recommendation that is consistent with the position taken by several leading authorities in lipid management.

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