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## Interindividual variability in the cholesterol-lowering effect of supplementation with plant sterols or stanols

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► **To cite this version:**

Frédéric Fumeron, Jean-Marie Bard, Jean-Michel Lecerf. Interindividual variability in the cholesterol-lowering effect of supplementation with plant sterols or stanols. *Nutrition Reviews*, 2017, 75 (2), pp.134-145. 10.1093/nutrit/nuw059 . inserm-02452775

**HAL Id: inserm-02452775**

**<https://inserm.hal.science/inserm-02452775v1>**

Submitted on 23 Jan 2020

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1 *Special Article*

2 **Plant sterols and stanols: Physiology, Metabolism and Inter-individual**  
3 **variability in the cholesterol-lowering effect of supplementation**  
4 **with plant sterols/stanols**

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22 **Abbreviations:** ABCA1, ATP-binding cassette subfamily A member 1; ABCB4, ATP-  
23 binding cassette subfamily B member 4; ABCG5/G8, ATP-binding cassette subfamily G  
24 member 5/8; ACAT2, Acyl coenzyme A: cholesterol acyltransferase 2; APOE,  
25 Apolipoprotein E; CETP, Cholesterol ester transfer protein; CYP7A1, Cholesterol 7  $\alpha$ -  
26 hydroxylase; FCH, familial combined hyperlipidemia; FH, familial hypercholesterolemia;  
27 FSR, fractional cholesterol synthesis rate; HDL(-C), high-density lipoprotein (cholesterol);  
28 LDL(-C), low-density lipoprotein (cholesterol); MDR2, multidrug resistance 2; MetS,  
29 Metabolic syndrome; NPC1L1, Niemann-Pick C1 Like 1 protein; SCARB1, scavenger  
30 receptor BI; SOAT2, sterol O-acyltransferase 2; STRIP, Special Turku Coronary Risk Factor  
31 Intervention Project  
32

**33 Abstract**

34           Low-density lipoprotein cholesterol (LDL-C) plays a causal role in atherosclerosis.  
35   One way to reduce LDL-C levels is to inhibit cholesterol absorption. Plant sterols and stanols  
36   compete with cholesterol for absorption in the intestine and induce an average decrease in  
37   LDL-C by 5 to 15% in a dose-dependent manner. However, a decrease does not occur in all  
38   individuals. This review focuses on the inter-individual variability in response to  
39   supplementing the diet with plant sterols and stanols. Dietary plant sterols and stanols have no  
40   significant effect on substantial numbers of subjects. Higher responses, in absolute value and  
41   percentage of LDL-C, are observed in subjects with higher cholesterol absorption and a lower  
42   rate of cholesterol synthesis. Some data evidenced the influence of genetics on the response to  
43   plant sterols and stanols. Further studies on large populations are required to extend these  
44   conclusions about genetic influences.

45

46   **Keywords:** plant sterols, cholesterol transporters, intestinal absorption, LDL-cholesterol,  
47   individual response, genetics

48

## 49 **Introduction**

50 Plant sterols are natural compounds found in plants, especially seeds and oilseeds.  
51 They have a similar structure to cholesterol, but differ in the side chain at carbon 24 and in the  
52 position and configuration of the double bond structure.<sup>1</sup> (**Figure 1**). Stanols are produced by  
53 the hydrogenation of plant sterols, with no double bond at carbon 5. Plant sterols and plant  
54 stanols have a cholesterol lowering effect that was first identified in 1951,<sup>2</sup> and sitosterol was  
55 introduced as a lipid-lowering therapeutic agent by Eli Lilly in 1957.<sup>1</sup> Its low aqueous  
56 solubility and low bioavailability have made it a forgotten and abandoned agent. The  
57 esterification of plant sterols and stanols has not only improved their solubility, but given a  
58 renewed interest in these molecules used in functional foods. These compounds compete with  
59 cholesterol for absorption in the intestine<sup>3</sup> and induce an average decrease in low-density  
60 lipoprotein cholesterol (LDL-C) of 5 to 15% in a dose-dependent manner.<sup>4</sup>

61 There is inter-individual variability in the response to diverse interventions, and the  
62 metabolic response to plant sterols and stanols in the diet is no exception. In this review, we  
63 consider the range of this variability, and examine the metabolic and genetic factors that have  
64 been studied in an attempt to clarify this variability.

65

## 66 **Nature and sources of plant sterols and stanols**

67 Plant sterols are the botanical analogs of cholesterol, and are found in the parts of  
68 plants rich in fibers and fats. The main sources of plant sterols are nuts, oilseeds, cocoa butter,  
69 legumes, and grains. Vegetables and fruits contain also small amounts of plant sterols (**Table**  
70 **1**).<sup>3, 5-7</sup> Plant stanols are naturally found in some cereals (rice, maize, wheat) and plant  
71 products, for example the wood of conifers such as pine and spruce.

72 More than 200 types of plant sterols and stanols have been identified<sup>1,8</sup> but the most  
73 abundant in the diet are sitosterol (about 60%), and campesterol (about 20%), then  
74 brassicasterol, stigmasterol, sitostanol and campestanol.<sup>9,10</sup>

75 In nature, plant sterols may be free, or esterified with fatty acids or with ferulic acid,  
76 or glycosylated with a hexose (usually glucose) or a 6-fatty-acyl hexose, on carbon 3 (**Figure**  
77 **1**). These glycosylated classes are frequently not measured, because their measurement needs  
78 an additional acid hydrolysis cleaving the glycosyl link or direct analysis of intact glycosides.  
79 The exclusion of glycosylated derivatives underestimates the total amount of plant sterols by  
80 up to 37%.<sup>5</sup>

81 In addition, the analysis of the main plant sterols excludes minor sterols.<sup>5</sup> It is unclear  
82 to what extent the glycosylated plant sterols are bioactive in humans because they are not  
83 hydrolyzed by pancreatic enzymes *in vitro*.<sup>11</sup>

84

### 85 **Role of plant sterols and stanols in cholesterol metabolism**

86 Cholesterol is involved in many biological functions as a cell membrane component  
87 and as a precursor of steroid hormones, bile acids and vitamin D, but can play a deleterious  
88 role in diseases like atherosclerosis. Cholesterol homeostasis depends on a complex balance  
89 between absorption and synthesis. This reflects the limited role of dietary cholesterol on  
90 plasma cholesterol and its very controversial role in cardiovascular risk.<sup>12</sup> The absorption of  
91 cholesterol is carried out by many systems and receptors, allowing a redundancy of  
92 mechanisms. Like plant sterols, plasma cholestanol, the 5 $\alpha$ -dihydro derivative of cholesterol,  
93 is a marker of cholesterol absorption under normal conditions.<sup>13,14</sup>

94 Plant sterols and stanols come into competition with free cholesterol in the micelles of  
95 lecithin and bile salts, which causes a reduction in cholesterol solubilization. When the  
96 concentration of plant sterols/stanols is high, intestinal cholesterol loses almost all

97 solubility.<sup>15-17</sup> The affinity of plant sterols/stanols for micelles exceeds that of cholesterol.  
98 Accordingly, the addition of plant stanols in a test meal reduces the absorbable cholesterol of  
99 micelles (measured by duodenal aspiration in humans) which leads to decreased intestinal  
100 absorption.<sup>16</sup>

101         The mechanism of action of plant sterols and stanols is not limited to the intestinal  
102 lumen. They are picked up with cholesterol by the intestinal mucosa via the membrane  
103 transporter Nieman Pick Disease C1 Like1 (NPC1L1) and can be absorbed into the systemic  
104 circulation. However, the majority of plant sterols and stanols are expelled from the  
105 enterocytes into the intestinal lumen by the ATP-binding cassette subfamily G members 5 and  
106 8 (ABCG5 and ABCG8) heterodimeric pumps. Plant sterols and stanols ejected from the  
107 enterocyte are excreted in the feces with unabsorbed and non-micellar cholesterol, catabolized  
108 as coprostanol by the intestinal microbiota. Alteration of the micellar solubilization of  
109 intestinal cholesterol seems to be the only mechanism of action of plant sterols and stanols  
110 that is unequivocally established. Nevertheless, other mechanisms involving modifications in  
111 cholesterol esterification, cholesterol efflux or bile acid synthesis, composition and excretion,  
112 have been discussed.<sup>11,17-21</sup>

113         Acyl coenzyme A: cholesterol acyltransferase 2 (ACAT2), also known as sterol O-  
114 acyltransferase 2 (SOAT2), is a key step in the metabolism of cholesterol, allowing its  
115 enterocyte esterification, followed by its incorporation into chylomicrons and export.<sup>22</sup>  
116 ACAT2 esterifies cholesterol much more efficiently than sitosterol (between 7.2 and 60 times,  
117 as estimated from different studies).<sup>18,23</sup> Because of this strong preference, there is no  
118 evidence that plant sterols are esterified by ACAT2. Consequently, plant sterols are not  
119 incorporated into chylomicrons and most of them are ejected into the intestinal lumen. It is  
120 also possible that a certain fraction of plant sterols may be incorporated into high-density  
121 lipoprotein (HDL) particles after basolateral efflux through ATP-binding cassette A1

122 (ABCA1) transporter.<sup>24</sup> In animals, it has been shown that plant sterols and stanols reduced  
123 the enzymatic activity of ACAT2, causing a reduction in the net absorption of dietary  
124 cholesterol.<sup>17</sup> The reduced activity of ACAT2 could be explained by a diminished trafficking  
125 of cholesterol from the plasma membrane to the endoplasmic reticulum.<sup>17</sup>

126 Plant sterols and stanols also induce the expression of ABCA1, a cholesterol  
127 transporter, *in vitro*.<sup>19,20</sup> ABCA1 regulates the efflux of cholesterol and phospholipids from  
128 the cell to the extracellular space. However, ABCA1 carries out its functions in the basolateral  
129 membrane of enterocytes, and not in the apical membrane, where it could excrete cholesterol  
130 into the lumen.

131 A diet rich in plant sterols alters the expression of more than 130 hepatic genes and  
132 their transcripts involved in the regulation of sterol metabolism in mice.<sup>21</sup> Nevertheless, it is  
133 possible that such changes are due to the inhibition of cholesterol absorption or reductions in  
134 plasma cholesterol, and are not a direct effect of plant sterols.

135 The addition of sitosterol (1 g) to a test meal containing cholesterol (500 mg) reduced  
136 cholesterol absorption by an average of 42%.<sup>25</sup> In another experimental protocol where both a  
137 liquid diet and sitosterol or sitostanol were infused in the intestinal lumen, sitosterol and  
138 sitostanol reduced cholesterol absorption by 50% and 85% respectively.<sup>26</sup> This mimics the  
139 effect of a deficit in hepatic cholesterol, and causes an increase in the expression of LDL  
140 receptors. This higher expression of LDL receptors induces an increase in the hepatic uptake  
141 of cholesterol and a decrease in plasma LDL-C. A compensatory increase in the synthesis of  
142 cholesterol takes place: the synthesis of whole body cholesterol (as measured through the  
143 incorporation in cholesterol of deuterium from water marked with deuterium) increases from  
144 38 to 53%.<sup>27</sup> Even at high doses (from 1.6 up to 8.6 g/d), plant sterols and stanols cause a  
145 decrease in the synthesis and excretion of bile acids,<sup>28</sup> which could explain the reduction in  
146 the cholesterol-lowering effect after a month. The decrease in bile acid synthesis is caused by



147 increased net absorption of plant sterols from the diet leading to higher plasma levels.<sup>28</sup>  
148 Nevertheless, a short term experimental study in an ileostomy model showed that a modest  
149 increase in plant sterols (584 mg/d vs. 258 mg/d), increased synthesis and excretion of bile  
150 acids.<sup>29</sup> Stanols do not lead to changes in the composition of bile acids, or the percentage of  
151 biliary cholesterol.<sup>28,30</sup> This might be due to the fact that stanol absorption and plasma levels  
152 are much lower than those of plant sterols. (See *infra*).

### 153 **Absorption characteristics and plasma levels of plant sterols and stanols**

154 The absorption rate of plant sterols and stanols depends on their chemical structure.  
155 The side chain group at carbon 24 (methyl for campesterol and campestanol, ethyl for  
156 sitosterol and sitostanol) and the presence of a double bond at carbon 5 have independent  
157 effects on absorption efficiency.<sup>31</sup> (**Figure 1**). Sterols with longer side chains and no double  
158 bond are less absorbed because of increased hydrophobicity and decreased micellar solubility.  
159 This explains why sterols are better absorbed than stanols, and why campesterol is better  
160 absorbed than sitosterol. Consequently, plasma levels of campesterol are higher than plasma  
161 sitosterol, although higher amounts of sitosterol are generally consumed. The net systemic  
162 absorption of plant sterols and stanols after a few days goes from 0.04% for sitostanol to 1.9%  
163 for campesterol.<sup>31</sup> In the general population, 3 to 16  $\mu\text{mol/L}$  (0.12 to 0.66 mg/dL) of sitosterol  
164 and 7 to 28  $\mu\text{mol/L}$  (0.28 to 1.12 mg/dL) of campesterol are found in plasma.<sup>32</sup>  
165 Concentrations of plant sterols are 15 to 30 times higher than those of stanols, but 200 times  
166 lower than those of cholesterol. The plasma concentrations of plant sterols are modulated by  
167 plant sterol intake. The percentage absorption of plant sterols is lower when intakes are  
168 higher, despite higher mass absorption.<sup>8</sup> With supplementation of 1.8 to 2.0 g/d plant sterols  
169 for 4 to 8 weeks in adults,<sup>32</sup> plasma campesterol increased from 52 to 99%, and sitosterol  
170 from 23 to 96%. When doubling the usual intake in children (132 mg/d vs. 65 mg/d), plasma

171 campesterol and sitosterol increased by 75% and 44% respectively.<sup>33</sup> Conversely, stanols (1.5  
172 to 3.0 g/d for 4 weeks) reduced campesterol from 28 to 113% and sitosterol from 24 to 50%.<sup>32</sup>

173 Sitosterolemia is a rare recessive disease characterized by excessively high plasma  
174 plant sterol levels (30 to 100 times higher than normal values)<sup>34,35</sup> due to mutations in the  
175 *ABCG5* or *ABCG8* transporter genes.<sup>36-38</sup> These mutations cause abnormal retention of plant  
176 sterols and stanols. Heterozygous subjects are generally healthy, but have higher plasma plant  
177 sterol levels (+30 to +50%) than the general population.<sup>34</sup> Some genetic polymorphisms of  
178 *ABCG5* and *ABCG8* have been associated with variations in plasma plant sterol levels (see  
179 *infra*).

180 Apolipoprotein E (APOE) is a major component of all lipoprotein classes except LDL.  
181 It is involved in the removal of chylomicron and very-low-density lipoprotein remnants from  
182 the circulation. Three common isoforms of APOE, E2, E3 and E4 are encoded by the alleles  
183  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$ , respectively. The most frequent is  $\epsilon 3$ . Some very rare isoforms have also been  
184 described. The  $\epsilon 4$  allele (or E4 isoform) is generally associated with higher total cholesterol  
185 and LDL-C levels in the general population, and higher cholesterol absorption, with the  $\epsilon 2$   
186 allele (E2 isoform) having the opposite effects.<sup>39</sup> Elevated plasma plant sterol levels are  
187 observed in subjects with the *APOE* E4 isoform.<sup>40</sup>

188 Statins decrease endogenous cholesterol synthesis, but induce a compensatory increase  
189 in sterol absorption, including plant sterols, with higher plasma levels in statin users.<sup>41,42</sup>

190 Conversely, familial combined hyperlipidemia (FCH), obesity, metabolic syndrome  
191 (MetS), and type 2 diabetes with insulin resistance are associated with a decreased absorption  
192 of plant sterols.<sup>40,43-45</sup> This has been linked to increased expression of the *ABCB4* (*MDR2*)  
193 transporter gene involved in the biliary excretion of sterols in cases of insulinopenia or insulin  
194 resistance.<sup>32</sup>

195

## 196 **The physiological intake of plant sterols and stanols**

197           The dietary intake of plant sterols and stanols is low but not absent, roughly equivalent  
198 to that of cholesterol. However, cholesterol has essential physiological functions that cause  
199 the body to keep it stable by adjusting the absorption and synthesis. Conversely, plant sterols  
200 and stanols are at an extremely low plasma concentration and have no known role, which can  
201 account for the very powerful processes to eliminate them. The response to plant sterol and  
202 stanol treatment is lower in subjects with high basal cholesterol synthesis (generally low  
203 absorption) than in subjects with low basal synthesis (high absorption).<sup>46</sup> (See *infra*)

204           Their presence in the gut has an effect on the intestinal absorption of cholesterol and  
205 its plasma level even at very low doses.<sup>47</sup> The spontaneous level of intake of plant sterols and  
206 stanols is inversely correlated with LDL-C levels and intestinal cholesterol absorption.<sup>48</sup>  
207 Several studies are in favor of a non-negligible role of plant sterols and stanols in the  
208 cholesterol-lowering effect of some oils (e.g. corn) rich in plant sterols and stanols, especially  
209 when unrefined, beyond their fatty acid composition.<sup>11,49,50</sup>

## 210 **General variability in the cholesterol lowering effect of plant sterols/stanols**

211           A recent meta-analysis of randomized controlled studies (including 124 studies)  
212 clearly showed a dose-response effect of plant sterols and stanols.<sup>4</sup> For intakes of 1 to 4 g/d,  
213 LDL-C concentrations reduced on average by 6 to 12%. When plant sterols and stanols were  
214 analyzed separately, similar dose-response relationships were observed.

215           Nevertheless, there is inter-individual variability in the response to supplementing the  
216 diet with plant sterols and stanols. Some studies report that dietary plant sterols and stanols  
217 have no significant effects on substantial numbers of subjects.<sup>46,51-54</sup> In one study with a  
218 spread providing 0.8 g/d plant sterols, there was no lowering of LDL-C in 37% of the subjects  
219 after 3 weeks.<sup>51</sup> Similarly, 33% of subjects were non-responders after 4 weeks of consuming a

220 spread providing 2.0 g/d plant sterols,<sup>52</sup> and no change was observed in 20% of the subjects  
221 consuming 1.3 to 1.6 g/d plant sterols in enriched milk.<sup>53</sup> A 4-week cross-over study in  
222 hypercholesterolemic subjects assessing the effects of plant sterol doses of between 1.6 and  
223 2.0 g/d (meta-analysis of three trials) showed an average lowering of LDL-C by  $-7.3\pm 1.2\%$ ;  
224 however, 47 subjects (42%) were non-responders (LDL-C:  $+3.7\pm 1.0\%$ ), and 66 were  
225 responders ( $-15.2\pm 1.0\%$ ).<sup>46</sup> In this study, the responders were those with a decrease in LDL-C  
226 of 5% or more. Among hypercholesterolemic male subjects (n=82), 31 (38%) were non-  
227 responders to 2.0 g/d plant sterols (spread). Plasma total cholesterol increased by  $4.4\pm 1.2\%$   
228 and LDL-C by  $9.4\pm 5.9\%$  in non-responders, whereas in responders, the total cholesterol  
229 decrease was  $12.7\pm 1.2\%$  and that of LDL-C was  $18.3\pm 2.0\%$ .<sup>54</sup>

230 Other than the factors linked to the characteristics of the trials, and the origin and the  
231 doses of the plant sterols and stanols, or the food matrix used, main sources of variation in  
232 these studies are the subjects themselves.

### 233 **Role of the initial plasma cholesterol concentration**

234 The first source of variation linked to the subject is the initial plasma cholesterol  
235 concentration. In a meta-analysis of four randomized trials with plant stanol esters, there was  
236 a highly significant effect ( $P<0.001$ ) of baseline plasma LDL-C on the final response.<sup>55</sup> The  
237 lowering obtained with 2.0 g/d stanols was between  $-0.24$  mmol/L and  $-0.17$  mmol/L ( $-8.0$  to -  
238  $5.7\%$ ) for an initial LDL-C value of 3 mmol/L, between  $-0.32$  mmol/L and  $-0.22$  mmol/L (-  
239  $8.0$  to  $-5.5\%$ ) for an initial LDL-C value of 4 mmol/L, and between  $-0.41$  mmol/L to  $-0.27$   
240 mmol/L ( $-8.2$  to  $-5.4\%$ ) for an initial LDL-C of 5 mmol/L. Therefore, the effects of plant  
241 stanols as a percentage of the baseline LDL-C value were constant, such that the change  
242 increased in absolute value with the value at baseline. The authors calculated that with 3.55

243 mmol/L initial LDL-C, the variation would be -5.8%, -7.5% and -10.2% for stanol doses of  
244 1.7 g/d, 2.2 g/d and 3.0 g/d, respectively.

#### 245 **Relationship with cholesterol synthesis and/or absorption**

246 Other individual characteristics that modulate the LDL-C response to plant sterols and  
247 stanols in the diet are those which influence the absorption and endogenous synthesis of  
248 cholesterol. Generally subjects with high basal plasma plant sterol levels, an indirect measure  
249 of absorption efficiency, display large reductions in LDL-C in response to plant sterol  
250 therapy.<sup>56</sup> In the study by Rideout et al.,<sup>46</sup> the fractional cholesterol synthesis rate (FSR,  
251 assessed directly by stable isotope analysis) was 23% higher in non-responders than in  
252 responders. The lowering of the LDL-C was only 3.2% in the highest synthesis quartile  
253 whereas it was 12.3% in the lowest quartile. When selecting subjects with high vs. low  
254 cholesterol synthesis (assessed by the cholesterol precursor lathosterol to cholesterol ratio),  
255 MacKay et al.<sup>57</sup> confirmed that cholesterol synthesis rate predicted the responsiveness to 2.0  
256 g/d plant sterols. In subjects defined as high synthesizers, the LDL-C lowering was 0.05  
257 mmol/L vs. 0.29 mmol/L in low synthesizers. Zhao et al.<sup>54</sup> reported that responders had a  
258 lower synthesis rate than non-responders. In subjects with low basal plasma plant sterol  
259 levels, absorption rate was low and the FSR was high, and the opposite was observed in  
260 subjects with high plasma plant sterol levels. There was a trend for subjects with high basal  
261 levels to be responders, and for a higher frequency of non-responders among those with low  
262 levels ( $P=0.09$ ). Post-experiment plasma plant sterol concentrations were highly positively  
263 correlated with absorption rates of campesterol and cholesterol, and negatively correlated with  
264 cholesterol FSR.<sup>58</sup>

265 Metabolic syndrome (MetS) is generally associated with high cholesterol synthesis  
266 and low cholesterol absorption.<sup>43,45,59</sup> In the study by MacKay et al.,<sup>57</sup> subjects with high

267 cholesterol synthesis and low response to plant sterols had higher body mass index and lower  
268 HDL-C, both characteristics associated with MetS. In a randomized parallel trial, 24 moderate  
269 hypercholesterolemic patients with MetS were compared to 24 moderate  
270 hypercholesterolemic patients without MetS. Enrichment of a “healthy” diet with plant sterols  
271 (2.0 g/d) for 3 months lowered LDL-C by 10.5% in patients without MetS but had no effect in  
272 patients with MetS.<sup>60</sup> The authors suggest that this difference is due to poor absorption  
273 efficiency, as demonstrated by lower campesterol and sitosterol concentrations (before and  
274 after diet) in patients with MetS than those without MetS. In particular, the plasma  
275 concentration of plant sterols following their consumption increased only by 20 to 30% in  
276 patients with MetS, but by 50 to 60% in controls. Nevertheless, these results contrast with  
277 those of another study<sup>61</sup>: plant sterol supplementation (4.0 g/d) of the habitual diet for 2  
278 months lowered total cholesterol and LDL-C by 15.9% and 20.3%, respectively, in patients  
279 with MetS. The discrepancy between these two studies might be explained by the type of diet  
280 during the intervention period (“healthy” in one case, “westernized” in the other) and by the  
281 dose of plant sterols (2.0 g/d and 4.0 g/d). The second study also included 5 times more  
282 patients and therefore had greater statistical power. As noted in <sup>62</sup>, the discrepancies in trials  
283 involving patients with MetS might also be due to the broad range in criteria that define MetS.

284 In type 2 diabetic patients, a meta-analysis of 5 randomized, placebo controlled trials,  
285 showed that plant sterols and stanols (1.6 to 3.0 g/d) significantly reduced LDL-C (combined  
286 effect: -12.2%).<sup>63</sup> Although stanols seemed more effective than sterols, no significant  
287 heterogeneity between studies was found in the meta-analysis. Nevertheless, few studies were  
288 examined. Type 2 diabetes (insulin resistance) has been associated with high synthesis and  
289 low absorption of cholesterol.<sup>40,59</sup> Type 2 diabetic patients should therefore have a low LDL-  
290 C response to plant sterol supplementation. Since it does not seem to be the case, Plat et al.<sup>62</sup>  
291 raised the question of the value of surrogate markers (plasma plant sterols and cholesterol

292 precursors) in the affected population. Plant stanols were effective for LDL-C lowering in  
293 patients with type 1 diabetes<sup>64</sup>; of note, type 1 diabetes is characterized by low synthesis and  
294 high absorption of cholesterol.<sup>65</sup>

## 295 **Genetic factors**

### 296 Monogenic diseases

297 Healthy subjects heterozygous for the mutations of *ABCG5* or *ABCG8*, responsible for  
298 sitosterolemia in homozygotes, might have a particular response to plant sterol therapy. This  
299 possibility has been tested, although sample sizes are small due to the rarity of the mutations.  
300 In a study of both (obligate) heterozygote parents of a patient with sitosterolemia, LDL-C  
301 decreased by 11% after 4 weeks on a diet containing 3.3 g/d plant sterols (margarine), and  
302 plasma concentrations of plant sterols increased by 83 to 139%.<sup>66</sup> These subjects were also  
303 obese and hypercholesterolemic. In 12 obligate heterozygotes from 2 families (with 2  
304 different mutations), a low fat diet, poor in saturated fatty acids and cholesterol, led to a  
305 decrease in LDL-C of 11.2% after 6 weeks and 16% after 12 weeks. The addition of 2.2 g/d  
306 plant sterol esters to this diet led to an additional 5.9% decrease.<sup>67</sup> The LDL-C response is  
307 similar to that of subjects who do not carry the mutations. Seven heterozygotes and 10  
308 controls received portions of margarine containing 2.0 g of plant sterols, 2.0 g of stanols or a  
309 control margarine daily for 6 weeks each.<sup>68</sup> Similar decreases in total and LDL-C were  
310 observed in both groups. The changes in plasma concentrations of plant sterol expressed as  
311 percentages did not differ between the two groups. With sterols in the diet, plasma plant  
312 sterols increased by 14.5  $\mu\text{mol/L}$  (0.59 mg/dL, +23%) in heterozygotes, and by 4.9  $\mu\text{mol/L}$   
313 (0.20 mg/dL, +20.5%) in controls. With stanols, plasma plant sterols decreased by 34.2  
314  $\mu\text{mol/L}$  (1.40 mg/dL, -54.2%) in heterozygotes and by 12.2  $\mu\text{mol/L}$  (0.50 mg/dL, -50.6%) in  
315 controls.<sup>68</sup> Similar changes in LDL-C were observed in 10 individuals heterozygous for the

316 *ABCG8* S107X mutation (-10.7%) and 15 controls (-9%) following the change from placebo  
317 during 4 weeks to 1.6 g/d plant sterols. Cholesterol absorption was decreased and synthesis  
318 increased similarly in both groups (stable isotope measurements).<sup>69</sup>

319 In conclusion, inclusion of plant sterol and stanols in the diet leads to a relative  
320 decrease in total cholesterol or LDL-C that is similar both in individuals heterozygous for  
321 sitosterolemia mutations and in control subjects (normal healthy subjects from other studies).  
322 With plant sterols in the diet, the relative increase in plasma plant sterols (as a percentage of  
323 baseline levels) was similar in heterozygotes and control subjects (although the absolute value  
324 is much larger). With dietary stanols, the percentage decrease in plasma plant sterols was also  
325 similar in the two groups, but again the change in absolute values was different.

326 **(Supplementary table S1 online only)**

327 The effects of these products have been studied in cases of monogenic familial  
328 hypercholesterolemia (FH), an inherited autosomal dominant disorder of the lipoprotein  
329 metabolism caused by mutations on the LDL-receptor gene. A meta-analysis of 4 studies of  
330 patients heterozygous for a LDL-receptor gene mutation was performed in 2006.<sup>70</sup> Three of  
331 these studies were in children.<sup>71-73</sup> The meta-analysis demonstrated that the decrease in total  
332 cholesterol and LDL-C was similar in heterozygous FH patients and in other  
333 hypercholesterolemic subjects after 2.3±0.5 g/d plant sterols or stanols. The effects of 1.6 to  
334 2.0 g/d (depending on body weight) plant sterols for 12 weeks were compared between  
335 different groups of hypercholesterolemic children. The groups studied included FH  
336 heterozygotes, cases of FCH and cases with unknown cause of hypercholesterolemia.<sup>74</sup> The  
337 decrease in LDL-C was slightly smaller in FH heterozygotes (-10.7%) than in groups with  
338 FCH (-14.2%) or undetermined hypercholesterolemia (-16.0%). However, the number of  
339 subjects included was small, and consequently the difference observed is probably not  
340 significant. **(Supplementary table S1 online only)**



341 *APOE polymorphism*

342 As already mentioned, the E4 isoform (or  $\epsilon 4$  allele) has been associated with higher  
343 total cholesterol and LDL-C in the general population, and higher cholesterol absorption.  
344 Consequently, a number of studies have investigated whether the effects of plant sterols and  
345 stanols are greater in carriers of E4 than other subpopulations.<sup>75-83</sup> (**Figure 2, supplementary**  
346 **table S2 online only**).

347 One study<sup>75</sup> with 67 moderate hypercholesterolemic subjects yielded results in the  
348 expected direction: total cholesterol and LDL-C concentration in the plasma decreased by  
349 7.5% and 10%, respectively in the intervention group. Subjects with phenotypes E4E4 or  
350 E3E4 were better responders to plant stanols (LDL-C:-11.8%) than E3E3 subjects (LDL-C:-  
351 6%). Plasma sitosterol and campesterol concentrations were lower and cholesterol precursors  
352 higher in E4E4 or E3E4 than E3E3 subjects, indicating a greater decrease in cholesterol  
353 absorption with a compensatory increase in synthesis. The same team<sup>76</sup> studied the effects of a  
354 small dose (700 mg/d) of plant sterols or plant stanols in 31 moderate hypercholesterolemic  
355 subjects. Four diets were tested: rapeseed mayonnaise, rapeseed + sitosterol, rapeseed +  
356 sitostanol and rapeseed + sitostanol esters. Serum total cholesterol and LDL-C reductions  
357 were small in the plant sterol-fed groups, and tended to be highest in the sitostanol ester group  
358 (-7%). The reduction in LDL-C (5%) was significant when sterol/stanol groups were  
359 combined. The decrease in LDL-C in E4 carriers (n=8) was 0.28 mmol/L ( $P<0.05$ ) and in  
360 E3E3 subjects (n=15) 0.06 mmol/L (NS). The LDL-C decrease correlated with a decrease in  
361 absorption and an increase in endogenous synthesis, which was more pronounced in E4  
362 carriers.

363 However, other studies do not find any greater effect of plant sterols/stanols in  
364 subjects carrying E4. In a study of 105 healthy subjects receiving either a placebo, or 2.0 g/d

365 or 3.0 g/d stanols for 4 weeks, there was no difference in plasma total cholesterol and LDL-C  
366 responses between subjects with E3 and those with E4.<sup>77</sup> A crossover trial was performed in  
367 normocholesterolemic children selected from the STRIP (Special Turku Coronary Risk Factor  
368 Intervention Project) cohort.<sup>78</sup> In the group carrying the E4 isoform, there was a decrease in  
369 LDL-C of 8.4% after consumption of a margarine containing plant stanols; the corresponding  
370 value for non-carriers was 7.6%. Nevertheless, cholesterol synthesis (assessed by assaying  
371 plasma sterol precursors) was increased only in E4 carriers and absorption was similarly  
372 lowered in both groups. In healthy adult subjects (crossover trial with a margarine providing  
373 3.2 g/d plant sterols, for 3 weeks), the decrease in LDL-C was 12.2% in subjects not carrying  
374 E4 and 9.8% in E4 carriers (not significant).<sup>79</sup> In another crossover trial with 1.7 g/d plant  
375 sterols,<sup>81</sup> there was no statistically significant difference in total cholesterol and LDL-C  
376 response according to genotype. The response was significant only in E3E3 subjects, but the  
377 number of E3E4 subjects was small. A similar result was obtained with hypercholesterolemic  
378 patients subjected to a diet during 5 weeks (control margarine/+1.1 g/d plant sterols/+2.2 g/d  
379 plant sterols). There was a significant response in E3E3 subjects but not in E4 carriers.<sup>82</sup> Once  
380 again, this result might have been due to a lack of statistical power because of the small  
381 number of E4 subjects. Nevertheless, the greatest response in the study was observed in E2  
382 carriers. In another study of 75 moderate hypercholesterolemic adult subjects, no difference in  
383 response was observed according to APOE isoforms after a period of 3 months on either a  
384 standard NCEP/ATIII diet, or the same diet + 2.0 g/d plant sterols and stanols in low-fat  
385 milk.<sup>83</sup> Again, the sample sizes were small (13 E4 subjects, 24 E3E3 subjects).

386 In the most recent study, a crossover trial was performed in 63 mildly  
387 hypercholesterolemic subjects (2 periods of 4 weeks with a margarine providing either 2.0 g/d  
388 plant sterols or no plant sterols).<sup>84</sup> The LDL-C decrease in *APOE* E3 carriers (-0.13 mmol/L,  
389  $P<0.05$ ,  $n=40$ ) was smaller than in *APOE* E4 carriers (-0.31 mmol/L,  $P<0.001$ ,  $n=23$ ). In this

390 study, a higher response was found in subjects with low endogenous cholesterol synthesis  
391 (assessed by the lathosterol/cholesterol ratio), but the cholesterol synthesis phenotype was not  
392 associated with *APOE* polymorphism. Nevertheless, the authors of this study suggested as a  
393 potential mechanism that the enhanced LDL-C response to plant sterols/stanols may be due to  
394 the accelerated clearance of chylomicrons with E4 isoform, leading to a faster hepatic  
395 delivery of cholesterol.<sup>84</sup> Overall, these studies have not fully demonstrated the expected  
396 better response of E4 isoform carriers due to high basal LDL-C and high cholesterol  
397 absorption. The association of E4 isoform with cholesterol absorption has not always been  
398 found, and may depend on the amount of dietary cholesterol consumption.<sup>85</sup> Another  
399 explanation is that most of these studies lack statistical power due to small samples.

#### 400 *Polymorphisms of the genes encoding the sterol transporters ABCG5/G8 and NPC1L1*

401 ABC transporters, and in particular ABCG5/G8, play a major role in intestinal sterol  
402 absorption. Mutations in the genes coding for these two proteins explain the high plasma plant  
403 sterol and cholesterol concentrations associated with sitosterolemia.<sup>36-38</sup> The associations  
404 between genetic polymorphisms of these transporters and plasma sterols have been studied. In  
405 100 Japanese hypercholesterolemic subjects, the *ABCG8* 1285A/G (M429V) polymorphism  
406 was associated with higher plasma sitosterol levels and sitosterol/cholesterol ratio, and with  
407 lower lathosterol (cholesterol precursor).<sup>86</sup> The rare H variant of *ABCG8* D19H  
408 polymorphism has been consistently found to be associated with a higher risk of gallstone  
409 disease, and with lower cholesterol absorption (lower levels of plant sterols in plasma) and  
410 higher cholesterol synthesis (higher levels of sterol precursors).<sup>87,88</sup> Nevertheless, a meta-  
411 analysis of the associations of *ABCG5/G8* polymorphisms (859T/C (C287R) and 1810C/G  
412 (Q604E) in *ABCG5*, 1285A/G (M429V), 161G/A (C54Y), 1199C/A (T400K) and 1895C/T

413 (A632V) in *ABCG8*) found little evidence of any effect on plasma LDL-C concentrations.<sup>88</sup>

414 **(Supplementary table S3 online only).**

415 An intervention study on the effects of plant stanols according to 3 of these  
416 polymorphisms<sup>89</sup> found that, after a stabilization period of 4 weeks and before the  
417 experimental sequence, plasma LDL-C levels were higher in subjects with the QQ genotype  
418 than in E carriers for the *ABCG5* Q640E polymorphism ( $3.04 \pm 0.75$  vs.  $2.70 \pm 0.81$  mmol/L,  
419  $P=0.039$ ). Subjects with the TT genotype of *ABCG8* T400K polymorphism had higher levels  
420 of plasma campesterol and sitosterol than allele K carriers ( $16.9$  and  $6.6$   $\mu\text{mol/L}$  vs.  $13.5$  and  
421  $4.8$   $\mu\text{mol/L}$  respectively) ( $0.68$  and  $0.27$  mg/dL vs.  $0.54$  and  $0.20$  mg/dL), suggesting higher  
422 absorption efficiency in TT subjects. Nevertheless, at the end of the experiment, LDL-C had  
423 decreased in all genotypes and no difference was found according to *ABCG5/G8* transporter  
424 polymorphisms. In a study where the average effects of plant stanols and plant sterols were -  
425  $4.2\%$  and  $-4.4\%$  respectively relative to control values, *ABCG5/G8* polymorphisms were not  
426 found to have any effect.<sup>90</sup> The interaction between *ABCG5/G8* transporter gene  
427 polymorphisms and plant sterol absorption level has also been examined.<sup>54</sup>  
428 Hypercholesterolemic subjects were ranked according to plant sterol absorption, as assessed  
429 by a stable isotope method, and compared according to their basal concentrations of plasma  
430 sterols. Subjects with high basal plant sterol concentrations showed high sterol absorption and  
431 low cholesterol FSR. The response to plant sterols varied according to 1199C/A (T400K)  
432 polymorphism and the basal plant sterol levels. Among subjects carrying the A allele  
433 (400TK+KK), the LDL-C decline was 4 times larger in the group with high than low basal  
434 plant sterol concentrations ( $-16.6 \pm 6.3\%$  vs.  $-3.4 \pm 5.7\%$ ,  $P < 0.05$ ). No such interactions were  
435 found for the other polymorphisms studied (D19H and Q604E). This study also investigated  
436 another gene important for sterol absorption, *NPC1L1*. A haplotype involving the two  
437 polymorphisms 872 C/G (L272L) and 3929 G/A (Y1291Y) was associated with a 2.4 greater

438 change in LDL-C:  $-13.4 \pm 3.0\%$  for minor allele carriers vs.  $-3.9 \pm 2.9\%$  ( $P < 0.05$ ) for common  
439 allele carriers. In the study by MacKay et al.,<sup>84</sup> where significant results for *APOE* were  
440 found, no significant association was found between *ABCG8* T400K polymorphisms and the  
441 response to plant sterols.

#### 442 Other polymorphisms

443 Other factors, such as the scavenger receptor B1 (SR-B1 coded by the gene *SCARB1*)  
444 and apolipoprotein AIV (coded by *APOA4*), may influence the effects of dietary plant  
445 sterols/stanols. SR-B1 is a receptor for high density lipoproteins and might be involved in  
446 cholesterol absorption by enterocytes. Apolipoprotein AIV plays a role in chylomicron  
447 formation, allowing the sterols present in intestinal cells to be exported into lymphatic vessels.  
448 The consequences of genetic polymorphisms of these proteins (Gln<sup>360</sup> → His, Thr<sup>347</sup> → Ser for  
449 *APOA4*, *SCARB1* HaeIII) have been evaluated together with those of other proteins involved  
450 in lipoprotein intravascular remodeling (*CETP* TaqIB) and transport (*APOE* E2/E3/E4), and  
451 endogenous cholesterol synthesis (HMGCoA Reductase VNTR).<sup>80</sup> Three groups of  
452 normocholesterolemic subjects were included in a parallel trial for 8 weeks: control rapeseed  
453 margarine (n=42), the same margarine with 3.8 g/d plant stanols from vegetable oil (n=34),  
454 and the same margarine with 4.0 g/d plant stanols from wood (n=36). Before the intervention,  
455 plasma high density lipoprotein cholesterol and cholesterol synthesis (lathosterol/cholesterol  
456 ratio) were lower in *SCARB1* allele 2 carriers, and LDL-C was lower in *APOE* E2 carriers.  
457 Nevertheless, none of the polymorphisms studied were associated with the LDL-C response to  
458 plant stanols.<sup>80</sup>

459 In another study, two *CETP* polymorphisms were studied: TaqIB and I405V.<sup>81</sup> This  
460 crossover trial included 60 moderate hypercholesterolemic subjects (average 7 mmol/L) and  
461 involved 2 periods of 4 weeks, one with a margarine providing 2.8 g/d plant sterol esters, the

462 other with control margarine. The change in total cholesterol was -7.2%, -4.2% and -0.4% in  
463 subjects with genotypes II (n=15), IV (n=27) and VV (n=9), respectively; the changes in  
464 LDL-C were -9.5%, -6.3% and +4.8, respectively. Consequently in this experiment, 18% of  
465 the subjects were non-responders (VV homozygotes). There was no difference in response  
466 according to *CETP* TaqIB polymorphism.

467 Cholesterol 7 $\alpha$ -hydroxylase (*CYP7A1*) is the rate limiting enzyme in the bile acid  
468 synthesis pathway, the major way to remove cholesterol from the body. The -204A>C  
469 (rs3808607) polymorphism of the *CYP7A1* gene promoter has been studied.<sup>91</sup> The C allele is  
470 functional, enhancing promoter activity and reducing the binding of transcription inhibitors of  
471 the *CYP7A1* gene. Two trials (4 and 8 weeks duration, 3.2 and 2.0 g/d plant sterols) were  
472 pooled, including 67 subjects (males and females, mean age 42 years). In subjects carrying the  
473 C allele (n=36), the change in total cholesterol was -0.43 mmol/L vs. 0.14 mmol/L in AA  
474 subjects. The lathosterol/cholesterol ratio (cholesterol synthesis marker) increased more in C  
475 carriers than in AA subjects (0.75 vs. 0.10).<sup>91</sup> The very recent study by MacKay et al.  
476 confirmed this effect of the *CYP7A1* gene polymorphism.<sup>84</sup> In this crossover trial with 2.0 g/d  
477 plant sterols in mildly hypercholesterolemic subjects, the C allele was associated with a  
478 statistically significant LDL-C response in a dose-dependent fashion (-0.05, -0.22, and -0.46  
479 mmol/L in AA, CA, and CC genotypes respectively). Interestingly, the C allele was more  
480 frequent in the group of subjects with low cholesterol synthesis on the basis of  
481 lathosterol/cholesterol ratio. Nevertheless the genotype was not significantly associated with  
482 FSR measured by an isotopic technique. There was a trend for an interaction between  
483 *CYP7A1* and *APOE* genotypes, with the lowest response observed in subjects carrying the  
484 combination E3-AA. These interesting results need to be confirmed with a larger sample. An  
485 alternative approach to large samples of unselected subjects may be to select individuals on

486 the basis of the most relevant genotypes for *APOE* and *CYP7A1*. The same study did not show  
487 a significant effect of *CETP* I405V polymorphisms.

#### 488 **Conclusion**

489 There is some evidence for substantial diversity in the individual response to dietary  
490 supplementation with plant sterols or stanols, and in particular the absence of response in 20-  
491 42% of subjects. One of the factors responsible for the variability is the initial LDL-C  
492 concentration. The decrease in LDL-C is apparently constant as a percentage, and is therefore  
493 higher in absolute value in subjects with high basal plasma LDL-C. Also, the effect is  
494 positively correlated with the rate of intestinal absorption of sterols and negatively correlated  
495 with the rate of endogenous cholesterol synthesis. Trials with *APOE* genotyping are not  
496 concordant. The first studies indicated a higher efficiency in E4 isoform carriers, but  
497 subsequent investigations suggest that there were no such differences, or even that there was a  
498 high efficiency in E2 and no apparent effect in E4 carriers. Polymorphisms of *ABCG5/G8* do  
499 not seem to play an important role. Only one study showed a greater change in subjects  
500 carrying the A allele (K) of *ABCG8* T400K with high sterol absorption levels. Few studies  
501 have been performed with polymorphisms of other lipid metabolism genes. Nevertheless,  
502 polymorphisms of *NPC1L1*, *CETP* (I405V) and *CYP7A1* have been reported to have  
503 significant effects, but only the results with *CYP7A1* have been replicated once. These results  
504 need to be replicated. Indeed, more generally, all of the relevant studies lack statistical power;  
505 therefore, new studies with larger populations are needed to assess the relationship between  
506 genetic polymorphisms and dietary supplementation with plant sterols and stanols. Moreover,  
507 inter-individual variability in responsiveness to any nutrient or food component cannot be  
508 explained using just one polymorphism, rather a combinatorial approach should be used. To  
509 this end, a score combining polymorphisms of genes involved in cholesterol metabolism  
510 might be calculated.<sup>92</sup> By analogy, the intake of sugar-sweetened beverages was associated

511 with adiposity in interaction with a genetic predisposition score calculated on the basis of 32  
512 BMI-associated loci.<sup>93</sup> Future research towards this direction would enhance our  
513 understanding on the role of genetics in cholesterol lowering responsiveness to plant  
514 sterols/stanols.

515

## 516 **Acknowledgments**

517 *Declaration of interest.* The authors have no relevant interests to declare.

## 518 **SUPPORTING INFORMATION**

519 The following Supporting Information is available through the online version of this article at  
520 the publisher's website:

521 *Table S1* Response to dietary plant sterols/stanols in subjects with mutations for  
522 sitosterolemia and familial hypercholesterolemia

523 *Table S2* Response to dietary plant sterols/stanols in subjects according to *APOE*  
524 polymorphism

525 *Table S3* Experimental protocols of studies on plant sterols/stanols according to sterol  
526 transporter polymorphisms

527

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795

796 **Table 1:** Plant sterol content (mg/100 g) in a selection of foods (from <sup>3,5</sup>)

797

<b>Food</b>	<b>Plant sterol content</b>
<b><i>Fruits and vegetables</i></b> <sup>3</sup>	
Broccoli (frozen)	44
Green peas (frozen)	25
Orange	24
Apple	13
Cucumber	6
Tomato	5
<b><i>Cereals</i></b> <sup>3</sup>	
Wheat bran	200
Swedish knackebrot	89
Wholemeal bread	53
Rolled oats	39
Wheat bread	29
<b><i>Fats and oils</i></b> <sup>3</sup> (except *)	
Corn oil	912
Rapeseed (canola) oil	668
Liquid margarine	522
Sunflower oil	213
Spreadable butter	153
Olive oil	154
Peanut butter* <sup>5</sup>	146
<b><i>Nuts and seeds</i></b> <sup>5</sup>	
Almond	199
Cashew	150
Hazelnut	121
Macadamia nut	187
Peanut	135
Pecan	157
Pistachio	279

Sesame seed	400
Sunflower seed kernel	270
Walnut	113

798

799 Plant sterol content:

800 for reference <sup>3</sup> = sum of sitosterol, campesterol, stigmasterol, sitostanol and campestanol801 for reference <sup>5</sup> = sum of sitosterol, campesterol, stigmasterol,  $\Delta$ 5-avenasterol, sitostanol,

802 campestanol, other sterols

803 **Figure legends**

804 **Figure 1: Structure of sterols and stanols**

805

806 **Figure 2: LDL-C change (%) in response to dietary plant sterols/stanols according to**

807 ***APOE* polymorphism**

808 Differences significant only for reference <sup>75,76</sup> and <sup>84</sup>. Changes in % were found in the articles

809 except for references <sup>80,82,83,84</sup> (calculated by the authors of the present review). More details

810 are given in Supplementary table S2 (online only).

811