Hepatic Nuclear Sterol Regulatory Binding Element Protein 2 Abundance Is Decreased and That of ABCG5 Increased in Male Hamsters Fed Plant Sterols 1,2

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Abstract

The effect of dietary plant sterols on cholesterol homeostasis has been well characterized in the intestine, but how plant sterols affect lipid metabolism in other lipid-rich tissues is not known. Changes in hepatic cholesterol homeostasis in response to high dietary intakes of plant sterols were determined in male golden Syrian hamsters fed hypercholesterolemia-inducing diets with and without 2% plant sterols (wt:wt; Reducol, Forbes Meditech) for 28 d. Plasma and hepatic cholesterol concentrations, cholesterol biosynthesis and absorption, and changes in the expression of sterol response element binding protein 2 (SREBP2) and liver X receptor-β (LXRβ) and their target genes were measured. Plant sterol feeding reduced plasma total cholesterol, non-HDL cholesterol, and HDL cholesterol concentrations 43% (P < 0.0001), 60% (P < 0.0001), and 21% (P = 0.001), respectively, compared with controls. Furthermore, there was a 93% reduction (P < 0.0001) in hepatic total cholesterol and >6-fold (P = 0.029) and >2-fold (P < 0.0001) increases in hepatic β-sitosterol and campesterol concentrations, respectively, in plant sterol-fed hamsters compared with controls. Plant sterol feeding also increased fractional cholesterol synthesis >2-fold (P < 0.03) and decreased cholesterol absorption 83% (P < 0.0001) compared with controls. Plant sterol feeding increased hepatic protein expression of cytosolic (inactive) SREBP2, decreased nuclear (active) SREBP2, and tended to increase LXRβ (P = 0.06) and ATP binding cassette transporter G5, indicating a differential modulation of the expression of proteins central to cholesterol metabolism. In conclusion, high-dose plant sterol feeding of hamsters changes hepatic protein abundance in favor of cholesterol excretion despite lower hepatic cholesterol concentrations and higher cholesterol fractional synthesis. J. Nutr. 140: 1249–1254, 2010.

Introduction

Disruption of cholesterol homeostasis by dietary plant sterols is mainly reported to be linked to modulation of intestinal cholesterol metabolism and absorption. Studies that bypass intestinal plant sterol delivery, using intraperitoneal and subcutaneous injections of plant sterols, have shown them to have hypcholesterolemia effects in humans and also modulate gene expression involved enterohepatic cholesterol regulation in rodent and avian models (1–3). Also, Xu et al. (4) demonstrated that transcriptional regulation of hepatic cholesterol responsive genes responds to dietary plant sterol supplementation in apolipoprotein E-deficient mice. However, it is not known whether the plant sterol effect on hepatic gene expression is direct or secondary to a decrease in hepatocyte cholesterol concentrations resulting from a decrease in cholesterol absorption. Recent clinical work from our laboratory and others has shown that plant sterols can mediate cholesterol lowering even when they are not consumed as part of a meal (5–7). Based on the combined available clinical and animal data, it is our hypothesis that plant sterols themselves target sterol responsive proteins in the liver, which modulate whole-body cholesterol metabolism.

Under normal omnivorous dietary conditions, humans consume relatively equal amounts of cholesterol and plant sterols. However, most mammals have evolved to absorb >55% of the dietary cholesterol, whereas <1% of plant sterols are absorbed (8), illustrating that despite the structural similarity between these sterols, mammalian cellular plant sterol concentrations are kept very low. Higher basal dietary consumption of plant sterols is known to reduce cholesterol absorption and numerous preclinical and clinical studies have demonstrated that supplemental plant sterols lower circulating cholesterol levels by ~10% (8). However, the effects of dietary plant sterols on gene and protein expression have not been well characterized in a hepatic...
context. There may be specific effects on protein expression in the liver that are elicited by the small but increased percentage of plant sterols that are absorbed and reach the liver.

Under normal physiological conditions when hepatic cholesterol levels decrease, there is a constitutive response to increase cholesterol uptake from the periphery while increasing cholesterol synthesis and decreasing cholesterol losses. These changes in cholesterol homeostasis are achieved mainly through changes in the processing of sterol response element binding protein 2 (SREBP2) from the cytosolic to active nuclear form. From a protein expression perspective, this will appear as an increase in the relative amount of processed SREBP2 found in the nuclear fraction compared with the unprocessed SREBP2 in the cytosolic fraction. The reverse is true in situations of high cellular free cholesterol, where lower levels of nuclear SREBP2 are present due to the inhibited processing of the cytosolic form.

Liver X receptor (LXR) agonist ligand binding, which includes various sterols and oxysterols, directly increases the expression of the sterol transporters ATP binding cassette transporter G5/G8 (ABCG5/G8) (9). In the intestine, plant sterols act as ligands to LXR, thereby increasing the excretion of all neutral sterols, including cholesterol, from the enterocyte via ABCG5/G8, which are unidirectional and luminaly expressed. However, ABCG5/G8 are also expressed in hepatocytes, primarily at the canalicular membrane, facilitating free cholesterol incorporation into biliary secretions. Yu et al. (10) demonstrated in an experiment using both wild-type and ABCG5/G8 knockout mice that LXR agonists achieve increased cholesterol excretion directly via functional ABCG5/G8 transporters. Furthermore, Batta et al. (11) reported stigmastanol alone was capable of reducing plasma cholesterol levels in a normally plant sterol nonresponsive rodent model. If the plant sterols reaching the liver stimulate ABCG5/G8 expression in these hepatocytes in a similar fashion to that observed in enterocytes, the net effect on hepatic cholesterol could contribute considerably to cholesterol excretion. Therefore, the present study examines the hepatic-specific changes in cholesterol trafficking in male golden Syrian hamsters in response to feeding plant sterols from a tall oil source.

Methods and Materials

**Study design and hamsters.** Sixteen male golden Syrian hamsters (*Mesocricetus auratus*; *n* = 8; Charles Rivers Laboratories) were randomized to receive either control hypercholesterolemia-inducing diet alone, prepared in our laboratory (Table 1), or the same diet with added plant sterol mixture (Reducol, Forbes Meditech). Hamsters have been previously identified as a useful model for human cholesterol metabolism (12) because of their similar lipoprotein distribution and response to diet-induced hypercholesterolemia. All hamsters consumed a hypercholesterolemic diet (13,14) ad libitum for 28 d with body weight and food consumption measured every 3 d. The total fat concentration in this diet was 5% (wt/wt), from a mixture of lard and sunflower oil (50%/50%), with the final diet PUFA:SFA ratio of 0.4. Free cholesterol was premixed with the oils before being mixed with other diet ingredients. All diets were stored at 4°C until fed to hamsters. On d 26, 5 mg of [3,4,4-13C2]cholesterol (99% enriched; CDN Isotopes) to assess fractional cholesterol synthesis (15). Hamsters were anesthetized with inhaled isoflurane and blood was sampled by cardiac puncture 2 h after 1H2O injections. Hamsters were killed with an overdose of sodium pentobarbital; body composition was determined immediately by dual emission X-ray absorptiometry. The study protocol was approved by the University of Manitoba Animal Care Committee in accordance to the Canadian Council on Animal Care Guidelines.

**Blood chemistry.** Blood was collected in heparinized tubes and separated into plasma and RBC by centrifugation at 1500 × g. Plasma glucose, triglycerides, total cholesterol, HDL cholesterol, total protein, and albumin were measured using an autoanalyzer (Vitros MicroSlide chemistry, Vitros 350, Ortho-Clinical Diagnostics). Non-HDL cholesterol is the difference between the measured total cholesterol and HDL cholesterol and includes the sum of VLDL, intermediate-density lipoproteins, and LDL cholesterol in the blood. Plasma insulin was measured by ELISA (Millipore).

| TABLE 1 Compositions of control and plant sterol-containing hypercholesterolemia-inducing diets |
|-------------------------------------------------|------------------|------------------|
| Ingredients                                      | Control          | Plant sterol     |
| Casein                                          | 200.0            | 200.0            |
| Corn starch                                     | 260.0            | 240.0            |
| Plant sterols†                                   | 0.0              | 20.0             |
| Sucrose                                         | 330.3            | 330.3            |
| Lard-sunflower mix (50:50)                      | 50.0             | 50.0             |
| Cholesterol                                     | 2.5              | 2.5              |
| Cellulose                                       | 100.0            | 100.0            |
| α-Methionine                                    | 5.0              | 5.0              |
| Mineral mixture†                                 | 40.0             | 40.0             |
| Vitamin mixture†                                 | 10.0             | 10.0             |
| Choline bitartrate                               | 2.0              | 2.0              |
| BHT‡                                           | 0.2              | 0.2              |

1 Plant sterol mixture from Forbes Meditech: total sterols, 98%; δ-sitosterol, 71%; δ-sitostanol, 16%; campestanol, 7%; stigmasterol, <1%.
2 Vitamin mix AIN-76A (CA40077; Harlan Teklad, Madison, WI) (33).
3 Mineral mix AIN-93M (TD94047; modified for hamsters; Harlan Teklad, Madison, WI (34).
4 BHT, butylated hydroxytoluene.

99.9% enriched; CDN Isotopes) to assess fractional cholesterol synthesis (15). Hamsters were anesthetized with inhaled isoflurane and blood was sampled by cardiac puncture 2 h after 1H2O injections. Hamsters were killed with an overdose of sodium pentobarbital; body composition was determined immediately by dual emission X-ray absorptiometry. The study protocol was approved by the University of Manitoba Animal Care Committee in accordance to the Canadian Council on Animal Care Guidelines.

**Liver and fecal cholesterol concentrations.** Approximately 0.5 g of liver and dried feces was saponified with freshly prepared KOH-methanol at 100°C for 1 h. The nonsaponifiable sterol fraction was extracted with petroleum ether and dried under nitrogen gas. Prior to analysis, internal standard α-cholestanol was added to each sample. The sterol fractions of liver and feces were analyzed for cholesterol concentrations using an Agilent 6890N GC fitted with a flame ionization detector (16). A SAP-5 capillary column (30m x 0.25 mm x 0.25 μm, Supelco) was used for all of the sterol analyses.

**Cholesterol absorption and fractional cholesterol synthesis.** Approximately 0.5 g RBC was saponified with freshly prepared KOH-methanol at 100°C for 1 h and the sterol fraction was extracted with petroleum ether (15). As an indicator of cholesterol absorption, GC-combustion-isotope ratio MS (Delta V Plus, Thermo Scientific) was used to determine the δ 13C enrichment (δ 13C/12C ratio) of free cholesterol in RBC compared with the nonenriched hamster RBC cholesterol δ 13C enrichment over 48 h (17,18). To measure cholesterol synthesis, GC-thermal conversion-isotope ratio MS (Delta V Plus, Thermo Scientific) was used to determine the δ H2O/H ratio compared with VSMOW, as previously described (19). The cholesterol precursor pool is taken as the mean plasma water deuterium enrichment, which was determined by thermal conversion elemental analyzer-isotope ratio MS from plasma prepared by membrane filtration and centrifugation removing proteins >
5 kDa. Cholesterol fractional synthesis rates were calculated using the following equation:

\[
\text{fractional synthesis rate (\% per day)} = \frac{(\delta \text{cholesterol}/\delta \text{plasma H2O} \times 0.478)}{(\text{deuterium incorporation period}) \times 24\times100}
\]

where \(\delta\) is deuterium enrichment of cholesterol or plasma water above baseline and time refers to the 2-h deuterium incorporation period. The factor 0.478 is the fraction of hydrogen atoms per cholesterol molecule possibly labeled by a deuterium (20).

**Immunoblotting.** Immunoblots were prepared as previously described (21). Nuclear and cytoplasmic extracts for immunoblot analyses of SREBP2 (SC-5603, Santa Cruz Biotechnology) were separated using a CellLytic NuCLEAR extraction kit (Sigma). Microsomal extracts for the immunoblot analyses of 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase (SC-33827, Santa Cruz Biotechnology) and \(\beta\)-actin (Ab8229, Abcam) were prepared based on the previously published procedures (22). Hepatic crude membranes for immunoblot analyses of ABCG5 and LDL receptor (LDLr) were prepared as previously described (21). \(\beta\)-Actin was used as the housekeeping protein for all target proteins (Ab8229, Abcam).

**Statistical analysis.** All outcomes were assessed by 1-way ANOVA using SPSS statistical software (version 11) with differences of \(P < 0.05\) considered significant. The variance was not homogenous for plasma total cholesterol and non-HDL cholesterol concentrations, so these data were log-transformed for statistical testing but reported as arithmetic means in this manuscript. All data are reported as mean ± SEM.

**Results**

**Feed intake, body weight, and composition.** Feed intake and body weight gain did not differ between groups over the 28 d experiment. The percent body fat and absolute lean mass and fat mass did not differ between groups after 28 d (Table 2).

**Plasma lipids, glucose, insulin, and proteins.** Plasma total cholesterol, non-HDL cholesterol, and HDL cholesterol concentrations were 43% (\(P < 0.0001\)), 60% (\(P < 0.0001\)), and 21% (\(P = 0.001\)) lower in the plant sterol-fed hamsters compared with controls, respectively (Table 2). Plasma glucose did not differ, but plasma insulin was 1-fold higher (\(P < 0.001\)) in the plant sterol-fed hamsters. The plasma total protein concentration tended to be 6% lower (\(P = 0.052\)) in plant sterol-fed hamsters, whereas albumin did not differ compared with controls.

**Hepatic sterol concentrations.** Hepatic free cholesterol concentrations did not differ between groups (Table 3). Hepatic total cholesterol was reduced by 93% (\(P < 0.0001\)) in the plant sterol-fed group compared with the control group. However, hepatic \(\beta\)-sitosterol and campesterol concentrations were >6-fold higher (\(P = 0.029\)) and >2-fold higher (\(P < 0.0001\)) in the plant sterol-fed group than in the controls, respectively.

**Fecal sterols.** Daily fecal sterol output was 23% higher (\(P = 0.006\)) in the plant sterol-fed group, but fecal cholesterol content did not differ between groups (Table 4). Total fecal neutral sterols and stanols were 5-fold higher (\(P < 0.0001\)) in the plant sterol-fed group compared with controls. There was no difference in fecal \(^{13}\text{C}\)-enriched cholesterol following the orally gavaged \(^{13}\text{C}\) cholesterol tracer to measure cholesterol absorption.

**Table 2** Feed intake, body weight, and plasma biochemistry of male golden Syrian hamsters fed diets with and without plant sterols for 28 d \(^1\)

<table>
<thead>
<tr>
<th>Feed intake, g/d</th>
<th>Control</th>
<th>Plant sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometry</td>
<td></td>
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<tr>
<td>Initial body weight, g</td>
<td>115.2 ± 3.4</td>
<td>115.0 ± 2.4</td>
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<tr>
<td>Final body weight, g</td>
<td>121.1 ± 4.8</td>
<td>118.6 ± 2.6</td>
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<tr>
<td>Change, %</td>
<td>5.3 ± 3.5</td>
<td>3.2 ± 1.8</td>
</tr>
<tr>
<td>Lean mass, g</td>
<td>55.4 ± 2.9</td>
<td>55.9 ± 1.4</td>
</tr>
<tr>
<td>Fat mass, g</td>
<td>59.5 ± 3.6</td>
<td>56.3 ± 1.4</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>51.6 ± 2.2</td>
<td>50.1 ± 0.6</td>
</tr>
</tbody>
</table>

**Plasma biochemistry**

<table>
<thead>
<tr>
<th>Total cholesterol, mmol/L</th>
<th>Control</th>
<th>Plant sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>8.3 ± 0.5</td>
<td>4.7 ± 0.2*</td>
</tr>
<tr>
<td>Non HDL cholesterol, mmol/L</td>
<td>3.9 ± 0.1</td>
<td>3.1 ± 0.2*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>4.0 ± 0.2</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>8.1 ± 1.5</td>
<td>6.4 ± 0.6</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>121 ± 17.2</td>
<td>258 ± 34.4*</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>65.6 ± 1.3</td>
<td>61.3 ± 1.6</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>32.5 ± 0.7</td>
<td>31.3 ± 1.0</td>
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</table>

1 Values are mean ± SEM, \(n = 8\). *Different from control, \(P < 0.05\).

**Cholesterol synthesis and absorption.** Cholesterol synthesis was >2-fold higher (\(P < 0.03\)), whereas cholesterol absorption was reduced by 83% (\(P < 0.0001\)) in the plant sterol-fed hamsters compared with controls (Fig. 1).

**Hepatic protein expression.** The abundance of the liver cytosolic or inactive form of SREBP2 was >3-fold (\(P < 0.0001\)) higher, whereas the abundance of the nuclear or active form of SREBP2 was reduced by >50% (\(P = 0.003\)) compared with controls (Fig. 2). The expression of the SREBP2 target gene HMG-CoA reductase was 43% (\(P = 0.005\)) higher compared with controls, but the expression of LDLr did not differ between treatments (Fig. 3). The expression of LXR\(\beta\) tended to be higher (\(P = 0.06\)) in the plant sterol-fed group and that of the LXR target gene ABCG5 was higher (\(P = 0.03\)) in the plant sterol-fed group compared with controls (Fig. 4).

**Discussion**

Our key findings in this study were that dietary plant sterols substantially lowered hepatic cholesterol ester but not free cholesterol content and differentially affected the abundance of proteins central to cholesterol metabolism. There was specifically a differential effect on hepatic SREBP2, where cytosolic expression of SREBP2 was >3-fold higher, whereas the nuclear
active form was 50% lower in the face of a 93% lower hepatic cholesterol ester concentration and no change in free cholesterol levels. We speculate that increases in specific individual plant sterols may be responsible for this observation. Supporting this speculation, Yang et al. (23) demonstrated differential expression of precursor and mature SREBP2 in response to individual plant sterols in a cell culture model.

While the effects of plant sterol supplementation on circulating cholesterol concentrations has been well characterized, recent work has focused on tissue-specific changes in cholesterol metabolism by plant sterols (24). Others have shown that dietary supplementation of plant sterol indeed increases the absolute content of the individual plant sterols in most, if not all, lipid-rich tissues (24). However, the metabolic effects of increased plant sterol concentrations in these tissues is not known. Therefore, in this study, we have focused on the liver and the protein expression patterns of several key transcription factors and enzymes central to cholesterol metabolism.

While hepatic total sterol (cholesterol and plant sterol) content was similar, there was a >6-fold increase in β-sitosterol and >2-fold increase in campesterol in plant sterol-fed hamsters. The binding affinity of the sterol binding sites of SREBP cleavage-activating protein (SCAP) and insulin-induced gene (Insig) 1/2 are different. In the case of SCAP, the sterol sensing site preferentially binds cholesterol or nonpolar sterols, whereas the Insig-1/2 sterol binding site has a greater affinity for oxysterols. Intracellular concentrations of oxysterols are higher during periods of increased cholesterol synthesis when intracellular free cholesterol concentrations are low. The binding of sterols to the SCAP sterol sensing domain result in a stable Insig-SCAP-SREBP2 complex and no translocation for active SREBP2 to the nucleus. However, the binding of oxysterols to the Insig-1/2 sterol sensing domain results in a conformational change within the Insig-SCAP-SREBP2 complex, facilitating the cleavage and translocation of active SREBP2 to the nucleus. In the present study, we observed reduced nuclear active with concurrent high cytosolic expression in plant sterol-fed hamsters that would appear contradictory in the face of low hepatic cholesterol concentrations. While speculative, the considerable structural similarity between free cholesterol and free plant sterols, differing only in the side chain bonding at the C24 position, may result in binding of the plant sterols to the SCAP sterol sensing domain. This could explain the expression pattern of nuclear and cytosolic SREBP2 we observed in this study.

As expected from previous studies by our group using the hamster model, circulating cholesterol concentrations were substantially decreased following 28 d of plant sterol feeding (25–27). This decrease in circulating and hepatic cholesterol content was accompanied by a >2-fold increase in fractional cholesterol synthesis and 43% higher abundance of hepatic HMG-CoA reductase in these hamsters. Although the increase in HMG-CoA reductase appears incongruent with a decrease in nuclear active SREBP2, it should be noted that it is one of the most highly post-translationally regulated hepatic enzymes and we did not measure these regulators or mRNA levels of HMG-CoA reductase. Furthermore, protein expression of LDLr, also transcriptionally regulated by SREBP2, did not differ in response to dietary supplementation with plant sterols.

**TABLE 4** Fecal output, fecal sterol excretion, and fecal [3, 4]-13C cholesterol enrichment in male golden Syrian hamsters fed diets with and without plant sterols for 28 d

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Plant sterols</th>
</tr>
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<tbody>
<tr>
<td>Total fecal output, g/d</td>
<td>1.05 ± 0.07</td>
<td>1.30 ± 0.04*</td>
</tr>
<tr>
<td>Fecal sterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/(g d)</td>
<td>2.6 ± 0.6</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Neutral sterols+stanols, mg/(g d)</td>
<td>4.7 ± 0.5</td>
<td>28 ± 1.4*</td>
</tr>
<tr>
<td>Tracer output, 613C-cholesterol-d</td>
<td>160 ± 21</td>
<td>174 ± 17*</td>
</tr>
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</table>

1 Values are mean ± SEM, n = 7–8. *Different from control, P < 0.05.
2 Total neutral sterols are the sum of cholesterol, coprostanol, β-sitosterol, and campesterol.

**FIGURE 1** Fractional cholesterol synthesis and absorption rates in male hamsters fed control or plant sterol-containing diets for 28 d. Values are mean ± SEM, n = 8. *Different from control, P < 0.05.

**FIGURE 2** Hepatic cytosolic and nuclear SREBP2 protein abundance in male hamsters fed control or plant sterol-containing diets for 28 d. (A) Expression of hepatic cytosolic and nuclear SREBP2 protein relative to control hamsters. Values are mean ± SEM, n = 6–8. *Different from control, P < 0.05. (B) Representative immunoblots of cytosolic and nuclear SREBP2 and corresponding β-actin.
to plant sterol feeding. Lower circulating cholesterol in response to plant sterol feeding has been shown by others not to be in response to increased LDLr expression but rather an increased affinity and efficiency of the LDLr to remove lipoproteins from circulation (28,29).

Interestingly, total fecal output in our plant sterol-fed hamsters was 25% higher over the final 48 h of the study. Whereas free cholesterol and [3, 4]^{13}C_{2}-cholesterol enrichment in the fecal samples did not differ between groups, total neutral sterols and stanols were markedly higher in the plant sterol-fed hamsters. This increase in total sterol excretion corresponds to the higher consumption of dietary plant sterols, but it is possible that the increased fecal output of plant sterol-fed hamsters was also a contributing factor to the increased sterol excretion.

Our data also show a role for hepatic LXRβ and its transcriptional targets (i.e. ABCG5/G8) in plant sterol-mediated cholesterol excretion. The expression of ABCG5/G8 in the polarized hepatocytes of the canalicular membrane increases the excretion of free cholesterol from the liver to bile and the gall bladder (30). Yang et al. (23) demonstrated that plant sterols have a direct stimulatory effect on sterol responsive genes in an ABCG5/G8 knockout mice, a model that develops superphysiological blood concentrations of plant sterols. But here we show a moderate increase in the protein expression of both hepatic LXRβ and its target gene, ABCG5, in a hamster model with no genetic manipulation and challenged only with dietary plant sterols.

In summary, ABCG5/G8 expression in intestinal cells is the dominant mechanism for the cholesterol-lowering effect of plant sterols (31). Briefly, supplemental plant sterols maximally stimulate the expression of sterol transporters (i.e. ABCG5/G8) located on the luminal surface of the enterocytes, which unidirectionally export sterols, primarily plant sterols, back into the digesta for fecal excretion. Under these conditions, excretion of both dietary cholesterol and sterols from pancreatic and biliary excretions is also increased. Furthermore, the overexpression of hepatic ABCG5/G8 in genetically manipulated animal models has demonstrated increased sterol efflux via the hepatobiliary route (32), specifically via the increased cholesterol efflux into the hepatic canaliculus. Here, we show that high-dose plant sterol feeding and the resulting increase in hepatic plant sterol levels downregulate the processing of SREBP2 to the active nuclear form, an effect usually observed when intracellular free cholesterol levels are high, and increase the expression of the excretory sterol half transporter ABCG5 in the liver, possibly increasing the amount of free cholesterol in biliary secretions.

**Acknowledgments**

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Literature Cited


42. Plat J, de Jong A, Volger OL, Princken HM, Mensink RP. Preferential campesterol incorporation into various tissues in apolipoprotein e3-4leiden mice consuming plant sterols or stanols. Metabolism. 2008;57:1241–7.