

Hepatic or intestinal ABCG5 and ABCG8 are sufficient to block the development of sitosterolemia¹

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Forty years ago, Bhattacharyya and Connor (1) were the first to describe sitosterolemia, a rare autosomal recessive lipid disorder characterized by increased absorption of dietary sterols and accumulation of sitosterol and other plant sterols (phytosterols) in the body. Patients with sitosterolemia also present with reduced biliary cholesterol secretion, decreased cholesterol biosynthesis, xanthomatosis, and premature atherosclerotic cardiovascular disease (2). In 2000, Helen Hobbs and her associates (3) discovered that sitosterolemia was caused by mutations that disrupt the expression or function of the ATP binding cassette transporters ABCG5 or ABCG8. Found on chromosome 2p21 in humans, the genes for ABCG5 and ABCG8 are in a head-to-head orientation and are only separated by 374 base pairs. The expression of ABCG5 and ABCG8 is regulated at the transcriptional level by liver X receptors (LXRs), which can be activated by synthetic LXR ligands or oxysterols derived from cholesterol (4). Localized to the apical membrane of hepatocytes in liver and enterocytes in small intestine, ABCG5 and ABCG8 form an obligate heterodimer (G5G8) that pumps sterols into the bile and intestinal lumen (5). Like humans with sitosterolemia, mice with whole-body deficiency of *Abcg5*, *Abcg8*, or both transporters have increased dietary sterol absorption, tissue and blood accumulation of phytosterols, and dramatically reduced biliary cholesterol secretion (6–9). However, mice lacking G5G8 do not display increased atherosclerosis susceptibility even on a LDL receptor-null background (10).

In this issue of the *Journal of Lipid Research*, Wang and colleagues describe the relative roles of hepatic and intestinal G5G8 in sterol homeostasis. For this work, mice were created with *LoxP* sites that 3' flanked exon 2 of *Abcg5* and exon 1 of *Abcg8*. G5G8 expression was disrupted in whole-body, liver, or intestine by crossing the floxed mice with mice carrying the Cre transgene under the control of the CMV-IE enhancer/chicken β -actin/rabbit β -globin, albumin, or villin promoter.

The levels of plasma and hepatic phytosterols in mice with G5G8 deleted in liver (*L-G5G8*^{-/-}) or intestine (*I-G5G8*^{-/-}) was elevated compared with WT mice but significantly decreased compared with mice with whole-body deletion of G5G8 (*G5G8*^{-/-}). Unlike the reductions observed in *G5G8*^{-/-} mice, cholesterol concentrations in plasma and liver were similar in WT, *L-G5G8*^{-/-}, and *I-G5G8*^{-/-} mice. These results showed that G5G8 in either liver or intestine was capable of limiting phytosterol accumulation, which may have allowed hepatic cholesterol biosynthesis to be maintained at a normal level. The findings from the *I-G5G8*^{-/-} mice were also consistent with the normalization of serum phytosterol concentrations in a sitosterolemic patient that received a liver with functional G5G8 (11).


Due to the presence of G5G8 in the liver, the level of biliary cholesterol was similar in WT and *I-G5G8*^{-/-} mice. However, because phytosterol absorption was increased in the absence of intestinal G5G8, the bile from the *I-G5G8*^{-/-} mice had high concentrations of phytosterols. As expected, the concentration of biliary cholesterol in *L-G5G8*^{-/-} was significantly decreased compared with that of WT mice. However, *L-G5G8*^{-/-} mice had a higher level of biliary cholesterol than *G5G8*^{-/-} mice, which the authors ascribed to a potentially higher cholesterol synthesis rate in the *L-G5G8*^{-/-} mice.

Even though *G5G8*^{-/-} and *I-G5G8*^{-/-} mice had similar levels of phytosterols in isolated enterocytes, the cells from the *I-G5G8*^{-/-} mice did not have a decreased cholesterol concentration. The difference in cholesterol content between the *G5G8*^{-/-} and *I-G5G8*^{-/-} enterocytes could have been due to the normal level of biliary cholesterol secretion in the *I-G5G8*^{-/-} mice. On a standard rodent diet, *G5G8*^{-/-} enterocytes were mainly exposed to dietary phytosterol. In contrast, not only dietary phytosterol but also biliary cholesterol could have been internalized by the *I-G5G8*^{-/-} enterocytes resulting in normal cellular cholesterol levels.

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In order to gauge the importance of hepatic and intestinal G5G8 in reverse cholesterol transport (RCT), mice were injected with ^3H -cholesterol, and the level of ^3H -cholesterol in bile and feces was measured. The percentage of ^3H -cholesterol in the bile of the mice was qualitatively similar to that observed for biliary cholesterol mass (WT = $I\text{-}G5G8^{-/-}$ > $L\text{-}G5G8^{-/-}$ > $G5G8^{-/-}$). Reduced biliary cholesterol secretion undoubtedly contributed to the decrease in fecal ^3H -cholesterol excretion for the $L\text{-}G5G8^{-/-}$ and $G5G8^{-/-}$ versus WT mice. However, the relative decrease in biliary ^3H -cholesterol did not closely correspond to the reduction in fecal ^3H -cholesterol. $I\text{-}G5G8^{-/-}$ compared with WT mice also had decreased fecal ^3H -cholesterol excretion, which could have resulted from increased cholesterol absorption (9). However, fractional cholesterol absorption was similar for $I\text{-}G5G8^{-/-}$ and WT mice. Thus, the levels of ^3H -cholesterol excretion in the $G5G8^{-/-}$, $L\text{-}G5G8^{-/-}$, and $I\text{-}G5G8^{-/-}$ mice could have been influenced by nonbiliary RCT. Like other mouse models with compromised biliary cholesterol secretion (12), the $G5G8^{-/-}$ and $L\text{-}G5G8^{-/-}$ mice may have maintained fecal cholesterol excretion via transintestinal cholesterol efflux (TICE), a pathway that enables enterocytes to internalize plasma cholesterol for secretion into the intestinal lumen. Although whole-body $G8^{-/-}$ mice have been reported not to have reduced TICE (13), it is possible that intestinal-specific deletion of G5G8 compromised the ability of enterocytes to efficiently secrete cholesterol into the intestinal lumen.

The highlighted work of Wang and colleagues has provided important information on the tissue-specific functions of G5G8. Future studies should focus on determining whether cholesterol homeostasis can be maintained when $L\text{-}G5G8^{-/-}$ and $I\text{-}G5G8^{-/-}$ mice are challenged with diets enriched in phytosterols or cholesterol. It will also be important to directly measure whether TICE is modulated in the absence of either intestinal or hepatic G5G8. 

- Bhattacharyya, A. K., and W. E. Connor. 1974. Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. *J. Clin. Invest.* **53**: 1033–1043.
- Salen, G., S. Shefer, L. Nguyen, G. C. Ness, G. S. Tint, and V. Shore. 1992. Sitosterolemia. *J. Lipid Res.* **33**: 945–955.
- Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science*. **290**: 1771–1775.
- Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Richardson, H. Hobbs, and D. J. Mangelsdorf. 2002. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. *J. Biol. Chem.* **277**: 18793–18800.
- Patel, S. B. 2014. Recent advances in understanding the STSL locus and ABCG5/ABCG8 biology. *Curr. Opin. Lipidol.* **25**: 169–175.
- Plösch, T., V. W. Bloks, Y. Terasawa, S. Berdy, K. Siegler, F. Van Der Sluijs, I. P. Kema, A. K. Groen, B. Shan, F. Kuipers, et al. 2004. Sitosterolemia in ABC-transporter G5-deficient mice is aggravated on activation of the liver-X receptor. *Gastroenterology*. **126**: 290–300.
- Klett, E. L., K. Lu, A. Kusters, E. Vink, M. H. Lee, M. Altenburg, et al. 2004. A mouse model of sitosterolemia: absence of Abcg8/sterolin-2 results in failure to secrete biliary cholesterol. *BMC Med.* **2**: 5.
- Yu, L., R. E. Hammer, J. Li-Hawkins, K. Von Bergmann, D. Lutjohann, J. C. Cohen, and H. H. Hobbs. 2002. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc. Natl. Acad. Sci. USA*. **99**: 16237–16242.
- Wang, H. H., S. B. Patel, M. C. Carey, and D. Q. Wang. 2007. Quantifying anomalous intestinal sterol uptake, lymphatic transport, and biliary secretion in Abcg8(−/−) mice. *Hepatology*. **45**: 998–1006.
- Wilund, K. R., L. Yu, F. Xu, G. L. Vega, S. M. Grundy, J. C. Cohen, and H. H. Hobbs. 2004. No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler. Thromb. Vasc. Biol.* **24**: 2326–2332.
- Miettinen, T. A., E. L. Klett, H. Gylling, H. Isoniemi, and S. B. Patel. 2006. Liver transplantation in a patient with sitosterolemia and cirrhosis. *Gastroenterology*. **130**: 542–547.
- Temel, R. E., and J. M. Brown. 2012. Biliary and nonbiliary contributions to reverse cholesterol transport. *Curr. Opin. Lipidol.* **23**: 85–90.
- van der Velde, A. E., C. L. Vratsis, K. van den Oever, C. Kunne, R. P. Oude Elferink, F. Kuipers, and A. K. Groen. 2007. Direct intestinal cholesterol secretion contributes significantly to total fecal neutral sterol excretion in mice. *Gastroenterology*. **133**: 967–975.