



The good side of cholesterol: a requirement for maintenance of intestinal integrity¹

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The relationship between high plasma cholesterol levels and cardiovascular disease is well established and has led to development of cholesterol-lowering strategies that dramatically reduce the rate of cardiovascular mortality and morbidity in the general population. Although these major advances have garnered well-deserved recognition in the popular press, unfortunately, the word ‘cholesterol’ has also gained considerable negative connotations in society. It is important to note that cholesterol is essential for mammalian cell growth and survival, as evidenced from a study reported in this issue of the *Journal of Lipid Research*. Work from the laboratory of Luke Engelking and colleagues reports a remarkable phenotype in conditional, intestinal-specific SREBP-2 knockout mice (1). The authors used a conditional knockout approach to inactivate SREBP-2, the transcription factor responsible for cholesterol biosynthetic genes in the intestine, and showed that in the absence of endogenous cholesterol biosynthesis, exogenous cholesterol is required to maintain intestinal integrity and survival. In particular, they showed that the intestine of *vil-SREBP2*^{-/-} mice fed a cholesterol-free diet exhibits grossly abnormal villus morphology with cells sloughing from the villus tips. The villus:crypt ratio was also significantly reduced in the *vil-SREBP2*^{-/-} intestine. Additional studies with enteroid cultures in vitro showed that *vil-SREBP2*^{-/-} crypt units cultured in the presence (but not the absence) of cholesterol exhibit a morphology comparable to that of *vil-SREBP2*^{+/+} cells. These experiments document that cholesterol itself, but not intermediates in the SREBP-2-regulated cholesterol biosynthetic pathway, is required for normal enterocyte growth and differentiation. Although the precise mechanism underlying the cholesterol requirement for normal intestinal morphology is still not fully resolved, previous studies have shown that cholesterol is necessary for cell surface and intracellular membrane maintenance and its depletion affects the structure and function of the Golgi complex as well as trafficking of apical membrane proteins to the brush border membrane of enterocytes (2). It is possible that those structural alterations—reflecting cholesterol depletion—may also affect the migration dynamics of crypt cells, their terminal

differentiation, and/or turnover of mature villus cells, all suggestions that will require further study. The increased numbers of proliferating crypt cells and the irregular villus morphology observed in the *vil-SREBP2*^{-/-} intestine is consistent with this possibility. Finally, it is possible that enterocyte membrane cholesterol depletion may also disrupt intercellular tight junctions and consequently impair barrier function (3), thereby promoting toxin permeability and reducing survival.

The study of *vil-SREBP2*^{-/-} mice also illustrates the complexity of lipid metabolism and homeostasis in the small intestine, highlighting the differences between this tissue and the liver. In contrast to the liver, where cholesterol is derived from endogenous biosynthesis and plasma lipoproteins, intestinal cholesterol content reflects three different sources, including uptake of dietary and biliary cholesterol from the apical surface, endogenous biosynthesis, and plasma lipoprotein uptake from the basolateral surface. Additionally, intestinal cholesterol homeostasis also differs from the liver in regard to crosstalk between SREBP-2 and SREBP-1. Whereas SREBP-1 levels are reduced in SREBP-2-deficient hepatocytes (4), SREBP-1 levels in the small intestine of *vil-SREBP2*^{-/-} mice were similar to those seen in control mice. The persistence of SREBP-1 levels in the small intestine of *vil-SREBP2*^{-/-} mice likely reflects the abundance of SREBP-1a expression (5), which in turn leads to a phenotype that is less severe than that observed in intestinal-specific Scap knockout mice.

The observation that dietary cholesterol or de novo synthesized cholesterol is necessary—whereas lipoprotein-derived cholesterol is not sufficient—to maintain intestinal integrity is an important and intriguing finding with potential novel implications toward understanding the complexity of intestinal lipid transport. The *vil-SREBP2*^{-/-} mice exhibit normal SREBP2 function in the liver and plasma cholesterol levels are similar to those observed in control mice. However, neither LDL-cholesterol uptake (mediated

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by the LDL receptor in the basolateral membrane) nor HDL-cholesterol uptake can compensate for endogenously-derived cholesterol in maintaining intestinal integrity. The authors suggested that the low rate of LDL-cholesterol uptake by the intestine may account for its inability to prevent enteropathy in *vil-SREBP2*^{-/-} mice fed a cholesterol-free diet. The low LDL-cholesterol levels in mouse plasma may also limit the amount of cholesterol delivered to the intestine through this mechanism. However, although HDL-cholesterol is present abundantly in the plasma of these animals, the *vil-SREBP2*^{-/-} mice were apparently unable to utilize HDL-cholesterol as the sole cholesterol source to maintain intestinal integrity. Although the rate of HDL-cholesterol uptake may also be a factor, other possibilities need to be considered to explain these interesting observations. For example, the inability of plasma LDL and HDL to compensate for lack of endogenous cholesterol biosynthesis may be due to differences in intracellular trafficking between cholesterol endocytosed from the basolateral side of cells compared with those derived from endogenous biosynthesis or transported from the apical side of the intestinal cells. Alternatively, endocytosis may be compromised in cholesterol-depleted basolateral membrane, thereby limiting the accessibility of plasma lipoprotein-derived cholesterol to intracellular sites where it can be utilized to sustain intestinal integrity. Additional detailed kinetic studies measuring cholesterol flux (including alterations in transintestinal cholesterol excretion) as well as monitoring the fate of cholesterol from various sources in this animal model may advance our understanding regarding unique cholesterol trafficking and utilization mechanisms in the intestine.

Another curious observation in this study concerns the sources of luminal cholesterol. The authors demonstrate that although dietary cholesterol is necessary, biliary cholesterol is not sufficient to prevent enteropathy in *vil-SREBP2*^{-/-} mice. This finding is especially surprising because biliary cholesterol levels were found to be similar or perhaps even higher in *vil-SREBP2*^{-/-} mice compared with control mice. Moreover, as the majority of luminal cholesterol in the intestine is derived from the bile with only a small percentage derived from the diet (6), and both biliary and dietary cholesterol are absorbed through an identical, NPC1L1-mediated pathway (7), the preference for dietary cholesterol is difficult to explain. One potential variable that needs to be considered is the quality of the cholesterol added to the diet for the in vivo experiments and also used for in vitro enteroid culture studies. Commercial sources of cholesterol are often contaminated with oxygenated cholesterol derivatives (8), which may include the oxysterol 24S,25-epoxycholesterol that is typically generated endogenously from mevalonate and squalene (9). This oxysterol has been

reported previously to be required for viability of fibroblasts (10). Interestingly, this and other oxysterols are found to be reduced in the intestine of *vil-SREBP2*^{-/-} mice. Whether this oxysterol is also necessary to sustain intestinal integrity and whether it is supplied from the diet to rescue the enteropathy phenotype of *vil-SREBP2*^{-/-} mice may need to be considered in future studies.

In summary, this important study by Engelking and colleagues (1) reminds us about the beneficial effects of cholesterol; i.e., its obligate role in cell and tissue growth as well as overall survival. This work also clearly illustrates important differences in cholesterol metabolism and requirements between the small intestine and the liver. As with all good science, important questions remain. Additional studies that provide a better understanding of the adaptive pathways and signaling mechanisms involved with cholesterol trafficking in enterocytes along with more precise understanding of the molecular requirement of sterol species necessary to maintain small intestinal villus integrity will likely provide further advances toward our understanding of the complexity of cholesterol metabolism and homeostasis in intestine. 

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