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Role of Niemann-Pick C1–Like 1 (NPC1L1) in Intestinal Sterol Absorption

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Abstract. Absorption of cholesterol by the proximal small intestine represents a major pathway for entry of cholesterol into the body pools. This cholesterol is derived primarily from the bile and diet. In adult humans, typically several hundred milligrams of cholesterol reach the liver from the intestine daily, with the potential to impact the plasma low-density lipoprotein cholesterol (LDL-C) concentration. There are three main phases involved in cholesterol absorption. The first occurs intraluminally and culminates in micellar solubilization of unesterified cholesterol, which facilitates its movement up to the brush border membrane (BBM) of the enterocyte. The second phase involves transport of cholesterol across the BBM by Niemann-Pick C1 Like-1 (NPC1L1), whereas the third phase entails a series of steps within the enterocyte involving esterification of cholesterol and its incorporation, along with other lipids and apolipoprotein B-48, into nascent chylomicrons. Discovery of the role of NPC1L1 in intestinal sterol transport occurred directly as a consequence of efforts to identify the molecular target of ezetimibe, a novel, potent, and specific inhibitor of sterol absorption that is now widely used in combination therapy with statins for management of hypercholesterolemia in the general population. Some aspects of the role of NPC1L1 in cholesterol absorption nevertheless remain controversial and are the subject of ongoing research. For example, one report suggests that NPC1L1 is located not in the plasma membrane, but intracellularly, where it is believed to be involved in cytosolic trafficking of cholesterol, whereas another concludes that a protein other than NPC1L1 is responsible for the high-affinity binding of cholesterol on intestinal BBM. However, other new studies that show that in vivo responsiveness of different species to ezetimibe correlates with NPC1L1 binding affinity further support the widely held belief that NPC1L1 does facilitate sterol uptake by the enterocyte and is the target of ezetimibe. Added to this is the unequivocal finding that deletion of the gene for NPC1L1 in mice results in near complete prevention of cholesterol absorption and an accelerated rate of fecal neutral sterol excretion. In summary, the development of ezetimibe and the identification of NPC1L1 as a key player in sterol absorption have taken research on the molecular control of this pathway to an exciting new level. From this, it is hoped that we will now be able to determine more precisely what effect, if any, other classes of lipid-lowering agents, particularly the statins, might exert on the amount of intestinal cholesterol reaching the liver.

The body of a 70-kg nonobese individual contains about 140 g sterol, essentially all of which is cholesterol.[1] Each day, normally about 1 to 1.5 g of new cholesterol enters the body from synthesis by the tissues and from dietary intake. The average daily cholesterol intake for individuals consuming a typical Western diet is assumed to be around 400 mg, but clearly this value varies over a wide range. The rate at which the body synthesizes cholesterol averages about 10

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mg/day/kg body weight, but this can vary with changes in cholesterol intake.[2,3] During the course of the day, an amount of cholesterol equal to what enters the body is eliminated in various forms, predominantly as bile acids and various metabolites of cholesterol in the stools. This balance between input and output ensures that whole body cholesterol content remains essentially constant over long periods of time. The liver plays a central role in the maintenance of whole body cholesterol balance because, not only is it the organ that receives most of the cholesterol absorbed from the small intestine, but it is also the site for degradation and excretion of cholesterol through the bile.[4] The liver also synthesizes cholesterol, but the rate at which it does so varies widely, depending on multiple factors, in particular, the amount of cholesterol being delivered to it from the small intestine.[4] It is well documented that manipulation of the enterohepatic flux of cholesterol or of bile acids can markedly impact the intrahepatic handling of cholesterol in a way that leads to clinically significant shifts in the circulating low-density lipoprotein cholesterol (LDL-C) concentration.[5–9]

Transport of cholesterol from the small bowel to the liver is very substantial because it reflects movement of not only dietary cholesterol but also of reabsorbed biliary cholesterol. In an adult human consuming a typical Western diet, the total amount of dietary and biliary cholesterol entering the lumen of the small intestine likely exceeds 1000 mg/day in most individuals.[10–12] Fractional cholesterol absorption values (the proportion of the luminal pool that gets absorbed) in humans vary widely, but average about 50%.[13,14] From these values, it therefore becomes apparent that a large quantity of intestinal cholesterol reaches the liver throughout the day. Such data thus explain why the cholesterol absorption pathway has long been a key target in the management of dyslipidemia. As reviewed elsewhere, attempts to develop effective and tolerable inhibitors of cholesterol absorption span almost half a century.[15] Although several different classes of inhibitors have been developed, often these had to be taken in gram quantities and usually only modest reductions in the plasma LDL-C level were achieved. In marked contrast, a daily dose of just 10 mg ezetimibe inhibits cholesterol absorption by about 50% and lowers plasma LDL-C concentration by ~18 to 20%.[16] Although ezetimibe is now widely used in combination with statins for treating hypercholesterolemia, it has also proved to be of immense value as a tool for advancing our knowledge of how cholesterol absorption is regulated at a molecular level. In particular, studies on the mechanism of action of ezetimibe led to the discovery of a major role for Niemann-Pick C 1-like-1 (NPC1L1) in intestinal sterol transport. The primary objective of this article is to review our current understanding of the role of NPC1L1 and other proteins in facilitating the uptake and intracellular handling of cholesterol by the enterocyte. It also discusses how our new knowledge in this aspect of intestinal sterol metabolism will potentially be of value in determining, at a mechanistic level, whether other classes of lipid-lowering drugs, particularly statins, might directly or indirectly have an impact on the amount of chylomicron cholesterol (CM-C) reaching the liver.

Atherogenic potential of intestinally derived cholesterol

As depicted in the schematic shown in Fig 1, the delivery of intestinal cholesterol into the circulation can potentially impact events at the vessel wall in at least two ways. One (1) is through an effect on the plasma LDL-C concentration. The liver is the primary regulator of circulating LDL-C levels, not only because it is the site of formation of very low–density lipoproteins (VLDL), which can be converted to LDL, but also because the bulk of receptor-mediated clearance of LDL normally takes place in the liver.[17] The sustained delivery of excess intestinal cholesterol to the liver can potentially increase hepatic cholesterol content. This, in turn, can lead to an accelerated rate of VLDL cholesterol secretion into the plasma, as well as a down-regulation of hepatic LDL receptor (LDL-R) activity.[17] The extent to which these events take place is dictated not only by the amount of CM-C reaching the liver, but also by the quantity and type of fatty acid that accompany the cholesterol.[18]

A second (2) way in which intestinal cholesterol can potentially contribute to atherosclerotic plaque formation is through chylomicron remnant (CMr) particles, which are normally rapidly cleared from the circulation by the liver, primarily through the LDL receptor and LDLR–related protein (LRP) pathways.[19] In diabetes and various other disorders, the clearance of these CMrs can be markedly delayed.[19,20] These particles, which carry a marker protein, apolipoprotein B-48 (ApoB-48), are small enough to penetrate the vessel wall. ApoB-48 has been found in human atherosclerotic plaque.[21] The relative contribution of LDL and CMrs (and other lipoproteins, such as VLDL remnants) to cholesterol contained in atherosclerotic plaque is unknown. Nevertheless, the underlying objective in using inhibitors of cholesterol absorption is to diminish the amount of CM-C entering the circulation that can potentially contribute to atherogenesis through one mechanism or another.

Intestinal cholesterol absorption

In a recent classic review of this subject,[22] the author defines *intestinal cholesterol* absorption as "the transfer of intraluminal cholesterol into intestinal or thoracic duct lymph," and *intestinal uptake* of cholesterol as "its entry from the lumen into intestinal absorptive cells." This point is emphasized here because of a common misconception that "uptake" constitutes "absorption." As will be discussed in a subsequent section, the uptake step in the absorption pathway has been intensely studied as a consequence of