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EDITORIAL COMMENT

HDL Dysfunction

Is the Answer in the Sphinx's Riddle?*

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Sphingosine-1-phosphate (S1P) was named in 1884 (1) after the Greek mythological creature the Sphinx because of its enigmatic nature, emulating the Sphinx's riddle. S1P is a component of high-density lipoprotein (HDL) (2), but its role has not received widespread attention. In this issue of the *Journal*, Sattler et al. (3) shed light on this mysterious molecule by demonstrating S1P to be a mechanistic cause and a therapeutic target for HDL dysfunction.

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For the past 60 years, HDL has been widely considered to reduce the risk of coronary artery disease (CAD); in fact, the cholesterol carried by HDL (HDL-C) has earned the moniker of "good cholesterol" (2). This is of crucial importance because CAD is the number 1 cause of death in the Western world (4). HDL is known to possess, in addition to its well established cholesterol efflux capacity, additional salutary effects including anti-inflammatory, vasorelaxant, endothelium-protective, and antiapoptotic properties (2). These "pleiotropic" actions contribute to the benefits conveyed by HDL (2).

Recent studies cast a shadow on the benefits of HDL on CAD (2); for instance, HDL-C-increasing therapies such as niacin increase HDL-C levels without reducing CAD events (5). We previously formulated a comprehensive hypothesis that explains this paradox (2). Only 5% of the total HDL-C is derived from macrophage cholesterol efflux, and HDL-C does not represent many important antiatherogenic HDL properties (e.g., anti-inflammatory or vasorelaxant); thus, HDL-C may be an insensitive method to quantify the antiatherosclerotic properties of HDL. Therefore, we should focus on validated HDL functions that truly reflect and are responsible for the actual beneficial effects of HDL (2). The recent report that cholesterol efflux predicts CAD events, whereas HDL-C did not (6), confirms our hypothesis that HDL function (quality) is more important than HDL-C levels (quantity).

However, HDL loses its beneficial properties in certain pathological situations such as CAD, which has been termed "dysfunctional HDL" (2). The oxidation of the HDL particle is the main cause of HDL dysfunction, thus resulting in a dysfunctional HDL, which is both proinflammatory and with a reduced ability to promote cholesterol efflux or vasodilation (7). Changes in the HDL proteome and lipidome (of which S1P is an essential component) also contribute to dysfunctional HDL (2). Currently, there are no effective therapies for HDL dysfunction.

S1P is a bioactive lysophospholipid that is derived from the ubiquitous membrane lipid sphingomyelin. Sphingosine kinase is responsible for S1P synthesis, whereas S1P-lyase accounts for S1P irreversible degradation. Erythrocytes are the main source of plasma S1P because they lack S1P degrading enzymes. Only S1P-bound HDL (60% of total plasma S1P) seems to be active, whereas albumin-bound S1P (40% of plasma S1P) acts as a reservoir. Apolipoprotein (apo) M has been identified as a S1P-binding protein in HDL (8).

S1P has recently received attention for its beneficial properties, specifically for its antiatherosclerotic effects. In vivo data demonstrate that the inhibition of sphingosine kinase results in lower S1P levels and increased atherosclerosis (9), whereas, conversely, deficiency of S1P-lyase resulted in higher S1P levels and reduced atherosclerosis (10). In addition, the S1P receptor agonist fingolimod reduced atherosclerosis in murine models (11,12). CAD patients also had lower HDL-bound S1P levels than healthy volunteers (13),

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and HDL-bound S1P concentrations were inversely correlated with CAD severity (13). In vitro results explain the mechanism of S1P atheroprotective effects. S1P exerts vasodilator effects of HDL because S1P activates endothelial nitric oxide synthase, stimulates endothelial nitric oxide release, and induces vasodilation (12,14). S1P also exhibits endothelium-protective activities because HDL-bound S1P enhances survival and migration in endothelial cells (15). S1P polarizes macrophage from an M1 (classic, proinflammatory) phenotype into an M2 (alternative, anti-inflammatory) phenotype (11), reduces inflammatory cytokines in plasma, and modulates the activity and distribution of T lymphocytes, thus overall displaying anti-inflammatory properties.

Therefore, it is tempting to hypothesize that reduced S1P content in CAD-HDL could be responsible for HDL dysfunction. The authors investigated this hypothesis and the corollary of raising the S1P cargo as potential therapeutic strategy to restore CAD-HDL function (3).

Several important observations are presented in this paper (3). The authors first report that CAD-HDL contains 5-fold less S1P concentration than HDL from healthy individuals, thus confirming their previous findings (13). They also corroborate that CAD-HDL functionality was less efficient than healthy HDL, both at activating endothelial molecular pathways and at inducing vasodilation. Second, HDL-induced endothelial signaling is mediated by the S1P-load because it was completely abrogated in the presence of both S1P receptor antagonists and S1P-neutralizing antibodies. Third, the authors demonstrate that the oxidative modifications present in CAD-HDL truly decreased the S1P content in HDL. The authors subsequently designed an ingenious model of S1P-loading of HDL (i.e., incubation of HDL with erythrocytes, which have increased their S1P levels by pre-incubation with sphingosine). Finally, and most important, the authors demonstrated that S1P-loading improved CAD-HDL functionality, as demonstrated by improved HDL-mediated signaling in vitro and enhanced HDL vasodilatory capacity.

This paper's importance stems from the convincing demonstration of the mechanistic role of reduced S1P as a cause of HDL dysfunction. Moreover, HDL dysfunction seems to be exclusively due to the lower S1P content because healthy HDL and CAD-HDL with the same S1P content were equally efficient. In the most translational part of the paper, the authors develop an innovative strategy to S1P-load the HDL in vivo (transfusion of S1P-loaded erythrocytes), thus hinting at its potential applicability in humans. It is crucial to note that this S1P-loading completely corrects the functional impairment of CAD-HDL. Of note, this procedure loads CAD-HDL with S1P as efficiently as healthy HDL and transfers 100% of the erythrocyte S1P content to HDL in 5 min, thereby increasing S1P content in HDL by 4-fold. Finally, the discovery that HDL does not require apo M to effectively carry S1P is of extreme importance. S1P was absent in HDL from apo M knockout mice (8), which led to the belief that S1P only could bind HDL via apo M. The authors show that CAD-HDL, despite exhibiting very low levels of apo M, was able to be efficiently loaded with S1P. This fact prompts the investigation of the specific molecule responsible for binding S1P, and targeting that structure appears an attractive therapeutic strategy.

Some questions remain unanswered and warrant further investigation. Although the authors explore in depth the endothelium-protective and vasodilatory effects of HDL, HDL also exerts cholesterol-efflux inducing, anti-inflammatory, antioxidant, and anti-apoptotic effects. Whether S1P-loading the HDL will also enhance these additional beneficial effects of HDL remains unexplored. Furthermore, the identity of S1P receptors responsible for the atheroprotective effects of S1P has not been unequivocally established so far. Also, the authors have selected a mild CAD population; whether S1P-loading the HDL in patients with more severe CAD is possible and whether it improves mores severe HDL dysfunction still need to be proved.

S1P was named in reference to the Sphinx due its enigmatic nature. More than a century later, we are just beginning to unravel the riddle of S1P. Sattler et al. (3) found that S1P improves HDL dysfunction. Perhaps the key to understanding HDL dysfunction is in the Sphinx's riddle, specifically in S1P.

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REFERENCES

1. Thudichum JLW. A Treatise on the Chemical Constitution of Brain. London: Bailliere, Tindall and Cox, 1884.

2. Santos-Gallego CG, Badimon JJ, Rosenson RS. Beginning to understand high-density lipoproteins. Endocrinol Metab Clin North Am 2014;43:913–47.

3. Sattler K, Gräler M, Keul P, et al. Defects of high-density lipoproteins in coronary artery disease caused by low sphingosine-1-phosphate

content: correction by sphingosine-1-phosphate–loading. J Am Coll Cardiol 2015;66:1470–85.

4. Santos-Gallego CG, Picatoste B, Badimon JJ. Pathophysiology of acute coronary syndrome. Curr Atheroscler Rep 2014;16:401.

5. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 2011;365:2255–67.

6. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 2014;371:2383–93.

7. Huang Y, Didonato JA, Levison BS, et al. An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med 2014;20:193–203.

8. Christoffersen C, Obinata H, Kumaraswamy SB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci U S A 2011;108:9613–8.

9. Poti F, Ceglarek U, Burkhardt R, Simoni M, Nofer JR. SKI-II–a sphingosine kinase 1 inhibitor-exacerbates atherosclerosis in low-density lipoprotein receptor-deficient (LDL-R-/-) mice on high cholesterol diet. Atherosclerosis 2015;240:212–5.

10. Bot M, Van Veldhoven PP, de Jager SC, et al. Hematopoietic sphingosine 1-phosphate lyase deficiency decreases atherosclerotic lesion development in LDL-receptor deficient mice. PLoS One 2013;8:e63360.

11. Nofer JR, Bot M, Brodde M, et al. FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation 2007;115:501–8.

12. Keul P, Tolle M, Lucke S, et al. The sphingosine-1-phosphate analogue FTY720 reduces atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2007;27:607–13.

13. Sattler KJ, Elbasan S, Keul P, et al. Sphingosine 1-phosphate levels in plasma and HDL are altered in coronary artery disease. Basic Res Cardiol 2010;105:821–32.

14. Nofer JR, van der Giet M, Tolle M, et al. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. J Clin Invest 2004;113:569–81.

15. Kimura T, Sato K, Malchinkhuu E, et al. High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. Arterioscler Thromb Vasc Biol 2003;23:1283–8.

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