The prevalence of metabolic syndrome continues to increase globally and heightens the risk for cardiovascular disease (CVD). Insulin resistance is a core pathophysiologic mechanism that causes abnormal carbohydrate metabolism and atherogenic changes in circulating lipoprotein quantity and function. In particular, dysfunctional HDL is postulated to contribute to CVD risk in part via loss of HDL-associated sphingosine-1-phosphate (S1P). In this issue of the JCI, Izquierdo et al. demonstrate that HDL from humans with insulin resistance contained lower levels of S1P. Apolipoprotein M (ApoM), a protein constituent of HDL that binds S1P and controls bioavailability was decreased in insulin-resistant db/db mice. Gain- and loss-of-function mouse models implicated the forkhead box O transcription factors (FoxO1,3,4) in the regulation of both ApoM and HDL-associated S1P. These data have important implications for potential FoxO-based therapies designed to treat lipid and carbohydrate abnormalities associated with human metabolic disease and CVD.
The FoxOs are in the ApoM house

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FoxO and insulin signaling

The mammalian forkhead box O family of transcription factors (FoxO1, FoxO3, FoxO4, and FoxO6) possess an evolutionarily conserved forkhead box (also called, F-box or winged helix) DNA-binding domain (DBD) along with a nuclear localization signal, a nuclear export sequence, and a C-terminal transactivating domain (1). FoxO1,3,4 are master transcriptional regulators of the insulin/insulin growth factor signaling axis in metabolically active tissues including liver, skeletal muscle, adipose tissue, and heart (2). In response to insulin, FoxOs are phosphorylated by Akt at conserved serine/threonine residues (3). This post-translational modification promotes the translocation of FoxOs out of the nucleus into the cytoplasm, thereby inactivating them. FoxO1,3,4 control carbohydrate and lipid metabolism during physiologic adaptations to fasting; their dysregulation directly impacts pathologic gene expression caused by insulin resistance, diabetes mellitus, and metabolic syndrome (4).

There are strong pathophysiologic links between insulin resistance, which is a state proposed to result in persistent activation of FoxOs, and dyslipidemia. In this issue of the JCI, Izquierdo et al. explored the role of FoxO1,3,4 in the regulation of HDL-associated apolipoprotein M (ApoM), a protein constituent of HDL that binds S1P and controls bioavailability was decreased in insulin-resistant db/db mice. Gain- and loss-of-function mouse models implicated the forkhead box O transcription factors (FoxO1,3,4) in the regulation of both ApoM and HDL-associated S1P. These data have important implications for potential FoxO-based therapies designed to treat lipid and carbohydrate abnormalities associated with human metabolic disease and CVD.

Conflict of interest: The authors have declared that no conflict of interest exists.

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Posttranslational modifications influence transcriptional output

One conundrum related to insulin resistance is the degree to which FoxOs are activated or inactivated. Existing mod-
Figure 1. FoxO1,3,4 regulate plasma levels of the HDL-ApoM-S1P complex. Hepatic FoxO transcription factors control the expression of Apom by binding to the promoter and enhancer regions of the gene. ApoM is secreted and forms a complex with plasma S1P. The majority of plasma ApoM-S1P associates with HDL and is found to be associated with pre-β and α (HDL2 and HDL3) migrating subpopulations. ApoM may stimulate the formation of pre-β-HDL during the endothelial lipase– (EL–) and hepatic lipase–mediated (HL-mediated) conversion of α-HDL2 to HDL3 and pre-β-HDL. The HDL-ApoM-S1P complex enhances endothelial barrier integrity and vasodilation. The roles for the HDL-ApoM-S1P complex in insulin resistance, HDL CEC, and RCT have not been clearly demonstrated.

COMMENTARY

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EL – HL

PL FC

Pre-β HDL

Disosoidal HDL

LCAT

HDL3

HDL2

Endothelial barrier/vasodilation

CEC/RCT?

Insulin resistance?

The Journal of Clinical Investigation

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continuously regulated and changing. Apolipoprotein AI (ApoAI), along with a wide range of other proteins and diverse lipids, mediates HDL functions. In diseases that confer increased risks for CAD, such as diabetes (17), changes in HDL constituents and oxidative modifications can render HDL dysfunctional, increasing the atherogenic risk. Further, interventions to scavenge reactive dicarbonyls such as isolevuglandins (IsoLGs) and malondialdehyde (MDA) decrease atherosclerosis and improve HDL function (21). Low levels of HDL-SIP have also been linked to cardiovascular disease (CVD) (22). Thus, ApoM-SIP may be key contributors to HDL function.

The HDL-ApoM-SIP complex has been reported to promote endothelial barrier and vasodilation, as well as antioxidant, antiinflammatory, and cholesterol efflux capacity (CEC) (6, 23, 24). SIP signals via GPCRs, and when bound to ApoM, as opposed to albumin, more potently regulates endothelial function (7, 25). In vitro and in vivo studies suggest that HDL-ApoM-SIP protects against endothelial inflammation and promotes barrier integrity and vasodilation (7, 23). Interestingly, Izquierdo and colleagues (5) determined that the flow-mediated vasodilation in insulin-resistant individuals and controls was not associated with HDL or SIP levels. However, as the authors suggest, an association could have been masked by other factors. Indeed, total HDL-ApoM-SIP may not be a measure of endothelial protective effects. HDL from humans with type 2 diabetes versus controls has similar ApoM content, but HDL from humans with type 2 diabetes versus controls has similar ApoM content, but HDL from control mice does not modulate the flux of cholesterol from cholesterol-enriched macrophages to the liver for excretion (8, 30). Thus, roles for ApoM in modulating CEC, and RCT have yet to be clearly elucidated and require further investigation.

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1. Calissi G, et al. Therapeutic strategies targeting FOXO transcription factors. Nat Rev Drug Discov. 2021;20(1):21-38.
2. Tikhonovich I, et al. Forkhead box class O transcription factors in liver function and disease. J Gastroenterol Hepatol. 2013;28 Suppl 1:125-131.
3. Nakae J, et al. Differential regulation of gene expression by insulin and IGF-1 receptors correlates with phosphorylation of a single amino acid residue in the forkhead transcription factor FKHRL1. EMBO J. 2000;19(5):989-996.
4. Haessler RA, et al. FoxOs function synergistically to promote glucose production. J Biol Chem. 2010;285(46):35245-35248.
5. Izquierdo MC, et al. Hepatic FoxO1,3,4 mice did not change alveolar barrier function. J Clin Invest. 2011;126(11):4368-4379.
6. Christoffersen C, et al. Forkhead box class O transcription factors in liver function and disease. J Gastroenterol Hepatol. 2013;28 Suppl 1:125-131.
7. Christoffersen C, et al. Endothelial-protective sphingosine-1-phosphate-stimulated HDL formation in the arterial wall. Nature. 2006;441(7094):1833-1834.
8. Liu M, et al. Hepatic apolipoprotein M (apoM) overexpression stimulates formation of larger apoM/sphingosine-1-phosphate-enriched plasma high density lipoprotein. J Biol Chem. 2014;289(5):2801-2814.
9. Ramaswamy S, et al. A novel mechanism of gene regulation and tumor suppression by the transcription factor NFKB1. Cancer Cell.
loss of HDL endothelium protective functions. *PloS One.* 2018;13(3):e0192616.
18. Christoffersen C, et al. The apolipoprotein M/SIP axis controls triglyceride metabolism and brown fat activity. *Cell Rep.* 2018;22(1):175–188.
19. Zhou JW, et al. Apolipoprotein M gene (APOM) polymorphism modifies metabolic and disease traits in type 2 diabetes. *PloS One.* 2011;6(2):e17324.
20. Linton MF, et al. The role of lipids and lipoproteins in atherosclerosis. In: De Groot LJ, et al. eds. *Endotext.* South Dartmouth (MA); 2019.
21. Tao H, et al. Scavenging of reactive dicarbonyls with 2-hydroxybenzylamine reduces atherosclerosis in hypercholesterolemic Ldlr−/− mice. *Nat Commun.* 2020;11(1):4084.
22. Sattler K, et al. HDL-bound sphingosine 1-phosphate (SIP) predicts the severity of coronary artery atherosclerosis. *Cell Physiol Biochem.* 2014;34(1):172–184.
23. Del Gaudio I, et al. Endothelial Spns2 and ApoM regulation of vascular tone and hypertension via sphingosine-1-phosphate. *J Am Heart Assoc.* 2021;10(14):e021261.
24. Christoffersen C, et al. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. *J Biol Chem.* 2008;283(4):1839–1847.
25. Wilkerson BA, et al. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-SIP prolongs endothelial barrier enhancement as compared with albumin-SIP via effects on levels, trafficking, and signaling of S1P1. *J Biol Chem.* 2012;287(53):44645–44653.
26. Frej C, et al. A shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P complex. *Atheroscler Thromb Vasc Biol.* 2017;37(6):1194–1205.
27. Saleheen D, et al. Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. *Lancet Diabetes Endocrinol.* 2015;3(7):507–513.
28. Plomgaard P, et al. Apolipoprotein M predicts pre-beta-HDL formation: studies in type 2 diabetic and nondiabetic subjects. *J Intern Med.* 2009;266(3):258–267.
29. Wolfrum C, et al. Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. *Nat Med.* 2005;11(4):418–422.
30. Elsoe S, et al. Apolipoprotein M promotes mobilization of cellular cholesterol in vivo. *Biochim Biophys Acta.* 2013;1831(7):1287–1292.