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Commentary

Hypertriglyceridemia is associated with obesity, diabetes, and atherosclerosis. While lipoprotein lipase (LPL) hydrolyzes triglyceride (TG) cargo into remnant lipoproteins with atherogenic properties, how remnant lipoprotein clearance relates to atherosclerosis in people with diabetes remains unclear. In this issue of the *JCI*, Shimizu-Albergine et al. examined the effects of the basic leucine zipper transcription factor CREBH, which induces genes that activate LPL in mouse models of type I diabetes. Overexpression of a CREBH fragment reduced apolipoprotein C3 (APOC3) levels, which reduced plasma TGs. Notably, the TGs were lowered by a mechanism that was independent of LPL, and atherosclerosis was alleviated by enhanced lipoprotein remnant clearance as opposed to increased lipolysis of TG-rich lipoprotein precursors. A proinflammatory mechanism likely underlies the atherogenicity of remnant lipoproteins. These findings suggest that modifying CREBH expression in the liver may ameliorate atherosclerosis and, perhaps, other diabetes complications.

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Recruiting a transcription factor in the liver to prevent atherosclerosis

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Triglycerides and atherosclerosis

Hypertriglyceridemia is the most common of dyslipidemias and often accompanies obesity, insulin resistance, type 2 diabetes, and poorly controlled type 1 diabetes. Hypertriglyceridemia correlates with decreases in HDL cholesterol and with increased risk of atherosclerosis. For many years, it was unclear whether elevated triglycerides (TGs) or low HDL was responsible for atherosclerosis. Through Mendelian randomization studies, it became possible to answer this question by analyzing the effect of variants that independently affect serum TGs versus HDL. TGs have emerged as the more likely causal agent (1, 2). However, this determination does not necessarily mean that TGs are the direct causal agent for atherosclerosis.

TGs are transported in the bloodstream primarily on chylomicrons, which

are secreted by the intestine after a meal, or by very-low-density lipoproteins (VLDLs), which are continuously produced by the liver. In both cases, the TG cargo is hydrolyzed by lipoprotein lipase (LPL), which resides at the luminal surface of the capillary endothelium, giving rise to TG-depleted remnant lipoproteins. Chylomicron remnants are largely cleared by the liver, whereas VLDL remnants have two competing fates; they can be directly cleared by the liver like chylomicron remnants or further processed in the bloodstream to become LDL. Mutations that affect remnant clearance cause dysbetalipoproteinemia and atherosclerosis in humans (3). Remnant particles may be more atherogenic than LDL (4, 5). Remnant clearance largely depends on the binding of remnant-bound apolipoprotein E (APOE) to the LDL receptor, LDL receptor-related protein 1 (Lrp1), and syndecan-1

(6). In mice, genetic deletion of *ApoE* leads to massive remnant accumulation and severe atherosclerosis (7) and indeed, the *ApoE*^{-/-} mouse is the most widely used animal model of atherosclerosis.

APOC3 is carried on TG-rich lipoproteins. Its levels correlate with serum TG levels. Treatment of hypertriglyceridemic humans (8) or mice (9) with an antisense oligonucleotide (ASO) against APOC3 markedly lowers serum TGs. APOC3 inhibits the access of LPL to its lipoprotein-borne TG substrate and also blocks the interaction of lipoprotein remnants with their cellular receptors (10). Diabetes leads to an increase in APOC3 levels (9), accounting in part for the hypertriglyceridemia frequently seen in people with diabetes. Mutations causing a lower level of APOC3 are associated with reduced risk of atherosclerosis. For example, a large study involving 110,970 individuals showed that those carrying mutations in *APOC3* experienced an average TG reduction of 39% and had a 40% lower risk of atherosclerosis (11). Similarly, treatment of diabetic mice with an ASO against APOC3 lowers TGs and reduces atherosclerosis (9).

CREBH expression affects lipoprotein metabolism and atherosclerosis

In this issue of the *JCI*, Shimizu-Albergine et al. reported on the effects of the transcription factor CREBH on lipoprotein metabolism and atherosclerosis (12). SREBP and CREBH are synthesized as transmembrane proteins. CREBH is transported through the secretory pathway to the Golgi where it is cleaved by site-1 protease, the same enzyme that releases the active fragment of SREBP. To bypass the dependence on site-1 protease, the authors expressed the active N-terminal fragment of CREBH in vivo using adeno-associated virus in *Ldlr*-KO mice with type 1 diabetes elicited by lymphocytic choriomeningitis virus glycoprotein-induced autoimmunity.

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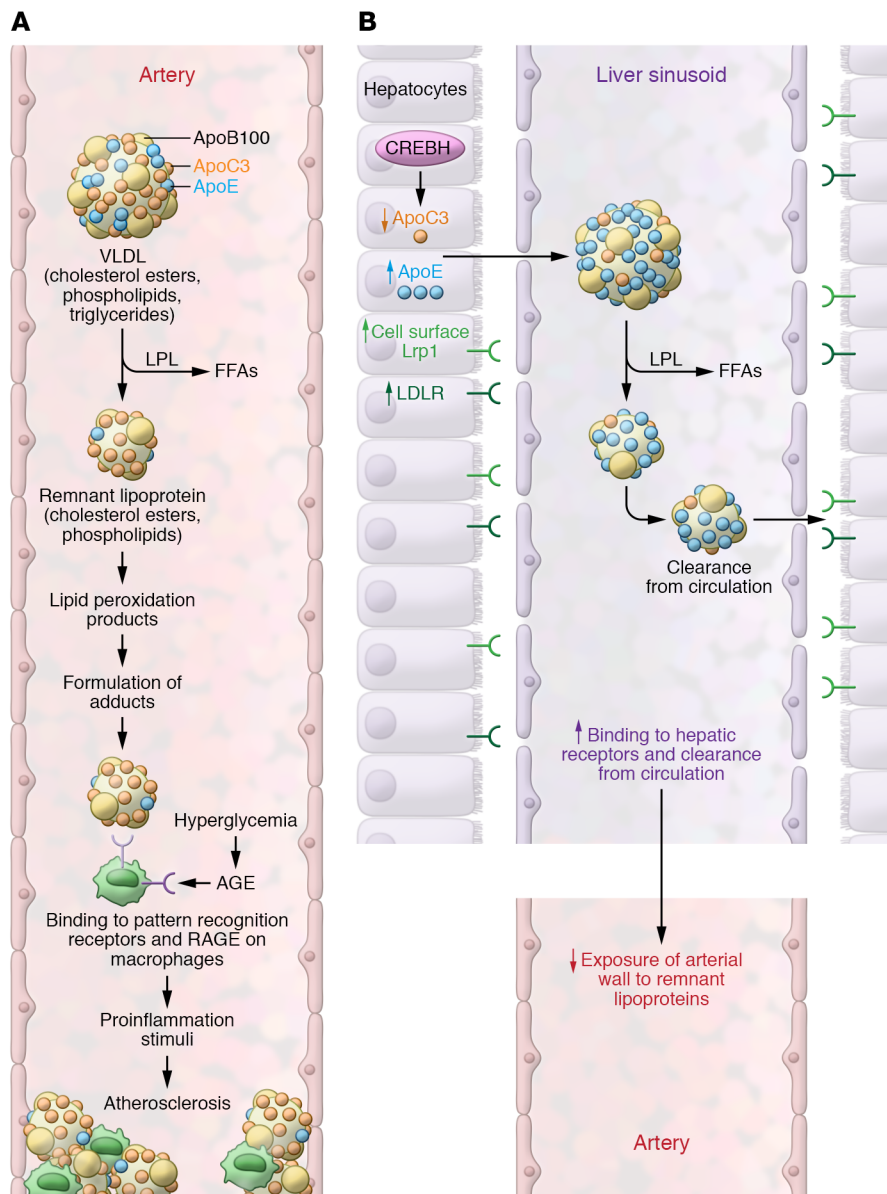


Figure 1. Enhanced lipoprotein remnant clearance ameliorates atherosclerosis in mice ectopically expressing CREBH. (A) In diabetes-related atherosclerosis, triglyceride-rich lipoproteins, VLDL, and chylomicrons (not shown) form remnant lipoproteins after lipoprotein lipase-mediated (LPL-mediated) triglyceride hydrolysis. Lipids associated with the lipoprotein particles are subject to oxidation. Oxidized lipids form complexes with proteins and bind to pattern recognition receptors on macrophages, inducing proinflammatory stimuli. Glucose can also form advanced glycosylation end products (AGEs), which bind to a distinct receptor for AGEs, termed RAGE, which can interfere with remnant clearance. CREBH also enhances the cell surface expression of the Lrp1 protein, one of several receptors that mediate remnant clearance. Clearance of remnant particles from the circulation reduces diabetes-related atherosclerosis. FFAs, free fatty acids.

APOC3 levels were markedly reduced by ectopic expression of the CREBH fragment, bringing about a substantial reduction in plasma TGs and to a lesser extent, plasma cholesterol. Contrary to published work, CREBH did not affect LPL levels in muscle or white adipose tissue, suggesting an LPL-independent mechanism for

TG lowering. Using *Lrp1*-knockdown and *ApoE*-KO mice, and examining plasma cholesterol versus TG, the authors surmised that the effect of CREBH on plasma TG was from APOE loading and remnant clearance via LDLR-family members, primarily *Lrp1*, rather than an activation of LPL. Shimizu-Albergine and colleagues concluded

that type 1 diabetes-induced insulin deficiency increased APOC3 levels, decreased cell surface hepatic *Lrp1* (insulin normally increases plasma membrane *Lrp1*), and caused hypertriglyceridemia. The studies are likely translatable to humans, as the authors went on to demonstrate that variants in the human homolog, *CREB3L3*, were associated with increased concentrations of remnant lipoproteins (12).

A major conclusion of Shimizu-Albergine et al. is that enhanced lipoprotein remnant clearance, rather than increased lipolysis of TG-rich lipoprotein precursors, ameliorated atherosclerosis in mice ectopically expressing CREBH (ref. 12 and Figure 1). This concept was supported by the much less severe atherosclerosis resulting from mutations that led to increased TG-rich lipoproteins rather than increased remnant particles (13). The results lend strong support to the concept, proposed nearly 50 years ago, that lipoprotein remnant particles are especially atherogenic (14).

Conclusions and implications

The precise mechanism underlying the atherogenicity of remnant lipoproteins is likely related to their proinflammatory effects. Oxidized lipids form oxidation-specific epitopes that stimulate the innate immune response (15). In addition, some of the fatty acids are substrates for proinflammatory prostanoids, such as PGE2 (16). Perhaps a detailed lipidomic analysis of the remnant lipoprotein particles in CREBH-knock-down mice would help to identify additional proinflammatory lipids.

Glucose, like some oxidized lipids, is an aldehyde and also forms complexes with proteins, which bind to the receptor for advanced glycosylation end products (RAGE). RAGE plays a role in diabetes complications (17, 18) and inflammatory processes in pancreatic islets (19). Perhaps there is synergism between the glucose-induced and oxidized lipid-induced adducts that leads to diabetes complications. Indeed, glucose- and lipid-modified epitopes can be detected in kidneys from patients with diabetic nephropathy (20, 21). This raises the exciting possibility that induction of CREBH may ameliorate diabetes complications like nephropathy.

Shimizu-Albergine et al. clearly demonstrate that effectively clearing lipoprotein remnants minimized their time in

the circulation, their interaction with the vascular wall, and perhaps also their interaction with tissue macrophages and circulating monocytes (12). The specificity of CREBH in clearing lipoprotein remnants in the liver suggests that CREBH does not augment other processes (e.g., gluconeogenesis) that would discourage the development of drugs or gene-based therapies. These studies (12) provide a plausible path toward preventing or treating atherosclerosis and perhaps other diabetes complications through CREBH expression in the liver.

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