Apolipoprotein CIII and Atherosclerosis
Beyond Effects on Lipid Metabolism
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The prevailing concept of mechanisms responsible for the development of atherosclerotic lesions largely focuses on the accumulation and retention of low-density lipoproteins in the arterial intima and their subsequent oxidative modification. This oxidation leads to activation of the endothelium, and particularly, expression of adhesion molecules that mediate leukocyte adherence and chemokines which initiate the inflammation reaction that is widely accepted as being responsible for the development and progression of atherosclerotic lesions.1,2 There is also a strong body of evidence to indicate that elevated triacylglycerides (triglycerides) are an independent risk factor for atherosclerosis.3–5

Article p 731

One mechanism that can contribute to elevated triglycerides involves apolipoprotein CIII (apoCIII). apoCIII is a small protein that resides on the surface of very-low-density lipoproteins (VLDLs), low-density lipoproteins, chylomicrons, and high-density lipoproteins (Figure). It exists as multiple species, as either a nonglycosylated isof orm (apoCIII1) or a glycosylated isof orm (apoCIII2, apoCIII3); all three isof orms have similar plasma half-lives and probably have very similar physiological functions. Increased apoCIII production is a characteristic feature of patients with hypertriglyceridemia,6 and plasma apoCIII levels have been positively correlated with plasma triacylglycerol concentrations and also have been associated with severity of hypertriglyceridemia.7 Elevated plasma apoCIII concentration and, specifically, accumulation of apoCIII in triacylglycerol-rich lipoproteins is casually related to hypertriglyceridemia in patients with metabolic syndrome and has also been associated with insulin resistance.8 apoCIII is a major regulator of lipolysis, as it noncompetitively inhibits endothelial-bound lipoprotein lipase, the enzyme that hydrolyzes triacylglycerols in triacylglycerol-rich lipoproteins, which generates smaller triacylglycerol-depleted remnant lipoproteins.9 It also inhibits the uptake of triacylglycerol-rich lipoprotein remnants by hepatic lipoprotein receptors, while high concentrations of apoCIII inhibit hepatic lipase, an enzyme with triacylglycerol lipase and phospholipase A1 activity. This latter inhibition can further reduce lipolysis. Consistent with these findings, mice deficient in apoCIII are hypotriglyceridemic and are protected from postprandial hypertriglyceridemia.10

In addition to its effects on lipolysis, apoCIII can also augment arterial inflammation. apoCIII stimulates the adhesion of peripheral monocytes to endothelial cells by activating monocytes.11 It activates protein kinase Cα and RhoA and induces cytoskeletal rearrangement, thereby activating β2-integrin. These effects of apoCIII on monocytes are dependent on signaling via G-proteins and protein kinase Cα.12 apoCIII also activates nuclear factor-κB, which is important for the inflammatory aspects of atherogenesis. Nuclear factor-κB activation in monocytes can initiate the transcription of a variety of proatherogenic genes, including those encoding chemokines and inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β.13 apoCIII also induces the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in endothelial cells, by activating protein kinase Cβ, stimulating IkB degradation and promoting the translocation of nuclear factor-κB to the nucleus.14 Thus apoCIII exerts proinflammatory effects on both monocytes and endothelial cells that are important for transendothelial migration of monocytes into the vessels’ intima and development of atherosclerosis.

In this issue of Circulation, Kawakami et al15 provide further insight as to how the actions of apoCIII on endothelial cells might promote the development of atherosclerosis. They demonstrate using cultured human endothelial cells that apoCIII inhibits insulin signaling, preventing insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation and downstream activation of phosphatidylinositol-3 kinase/Akt. Because endothelial nitric oxide synthase (eNOS) phosphorylation by insulin-activated Akt activates eNOS, the authors also investigated whether eNOS activity was affected by apoCIII. apoCIII dose-dependently attenuated insulin-stimulated eNOS activity without affecting its expression. eNOS is an important generator of nitric oxide (NO) in blood vessels. NO generated by eNOS is an endogenous vasodilator and also exerts other important vascular effects, which are atheroprotective. To confirm the in vitro effects, the authors also administered apoCIII to mice. Consistent with their in vitro observations, aortas from mice that were administered apoCIII exhibited impaired endothelium-dependent relaxation induced by NO. The endothelium is a target of insulin, and these observations indicate that insulin resistance can exist at the level of the endothelium.16 Impaired NO generation by endothelial cells is frequently used to define endothelial dysfunction. Endothelial dysfunction is regarded as a causal factor in the development of atherosclerosis and is one of the
LIPOLYSIS
- Endothelial-bound Lipoprotein Lipase

ENDOTHELIAL CELLS
- Insulin-dependent IRS-1 phosphorylation
- eNOS activity
- Endothelial Dysfunction

MONOCYTES
- beta1 integrin activity

TRL
LDL
ApoC-III
HDL

Figure. Atherogenic mechanisms of apoCIII. apoCIII on the surface of triglyceride-rich, low-density, or high-density lipoproteins can interact with endothelial-bound lipoprotein lipase to attenuate its activity. It may also more directly interact with endothelial cells to inhibit insulin-dependent IRS-1 phosphorylation and eNOS activity and thereby induce endothelial cell dysfunction. Impairment of endothelial function augments proinflammatory responses to cytokines. This interaction of apoCIII with the endothelium also elevates vascular cell adhesion molecule-1, which can augment recruitment of leukocytes to developing atherosmas. apoCIII can also increase the activity of β1-integrins on monocytes, further augmenting their adhesion to endothelium. The presence of apoCIII on high-density lipoproteins may limit its antiinflammatory properties.

earliest abnormalities that can be detected clinically in people at risk for atherosclerosis. Endothelial dysfunction is also found in subjects with insulin resistance and type 2 diabetes, 2 groups that are at high risk for developing atherosclerosis. NO limits endothelial activation by cytokines, thereby reducing the expression of the adhesion molecule vascular cell adhesion molecule-1 and decreasing monocyte adhesion to the endothelium. It also inhibits smooth muscle cell migration and proliferation, platelet aggregation, and nuclear factor-κB, all of which are important for the development of atherosclerosis. Thus, apoCIII, like hyperlipidemia and increased oxidative stress, is a significant contributor to endothelial cell dysfunction.

Endothelial dysfunction has been associated with activation of extracellular signal-regulated kinase 1/2, which induces phosphorylation of IRS-1 on Ser616, preventing Tyr phosphorylation of IRS-1 and resulting in its dysfunction as a docking protein and activator of phosphatidylinositol-3 kinase. Also, deletion of IRS-1 in mice impairs endothelium-dependent vascular relaxation. Consequently, the authors investigated whether Ser616 phosphorylation by apoCIII might be responsible for the endothelial dysfunction. apoCIII stimulates extracellular signal-regulated kinase 1/2 in endothelial cells and induced phosphorylation of IRS-1 on Ser616, which could be attenuated by inhibiting extracellular signal-regulated kinase 1/2 or protein kinase Cβ. Administration of apoCIII to mice elicited identical signaling effects in the aorta, indicating that this mechanism was also operative in vivo. Because apoCIII normally resides on the surface of VLDLs and low-density lipoproteins, the authors also examined whether apoCIII in VLDLs could elicit similar effects. apoCIII-rich VLDLs but not apoCIII-deficient VLDLs induced Ser612 phosphorylation of aortic IRS-1 in mice (equivalent to Ser616 in human IRS-1), and inhibition of protein kinase Cβ restored insulin-stimulated Tyr989 phosphorylation of IRS-1 by apoCIII-rich VLDLs and also NO release; these effects were dependent on apoCIII in VLDLs. Triglyceride-rich lipoproteins are known to prime the endothelium, enhancing inflammatory responses to cytokines such as tumor necrosis factor-α. Whether this effect can also be attributed to apoCIII remains to be determined.

A growing body of evidence indicates that apoCIII is a multifunctional protein that not only regulates the metabolism of triglyceride-rich lipoproteins; it is also an important regulator of endothelial function (Figure). Its ability to induce endothelial dysfunction links hyperlipidemia with endothelial cell dysfunction and is one mechanism by which triglyceride-rich lipoproteins may augment inflammatory responses associated with developing atherosclerotic lesions, thereby increasing the risk for atherosclerotic cardiovascular disease. Targeting apoCIII could be an important new therapeutic approach to reduce coronary heart disease risk in patients with dyslipidemia.

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