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ApoC-III: a potent modulator of hypertriglyceridemia and cardiovascular disease

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Abstract

Purpose of review—To summarize the recent epidemiological, basic science, and pharmaceutical research linking apoC-III with the development and treatment of cardiovascular disease.

Recent findings—ApoC-III is an important emerging target linking hypertriglyceridemia with cardiovascular disease (CVD). ApoC-III is a potent modulator of many established CVD risk factors, and is found on chylomicrons, VLDL, LDL, and HDL particles. Recent studies show that in humans, apoC-III levels are an independent risk factor for CVD, and its presence on lipoproteins may promote their atherogenicity. This year, two large-scale epidemiological studies have linked mutations in apoC-III with increased incidence of cardiovascular disease and hypertriglyceridemia. ApoC-III raises plasma triglycerides through inhibition of LPL, stimulation of VLDL secretion, and is a novel factor in modulating intestinal triglyceride trafficking. ApoC-III also stimulates inflammatory processes in the vasculature and in the pancreas. The combination of raising plasma triglycerides and independently stimulating inflammatory processes makes apoC-III a valuable target for reducing the residual CVD risk in patients already on statin therapy, or for whom triglycerides are poorly controlled. Clinical trials on apoC-III antisense oligonucleotides are in progress.

Summary—ApoC-III is a potent direct modulator of established cardiovascular disease risk factors: plasma triglycerides and inflammation. Recent findings show that changes in apoC-III levels are directly associated with changes in cardiovascular risk, and with the atherogenicity of the lipoproteins on which apoC-III resides. Emerging roles of apoC-III include a role in directing the atherogenicity of HDL, intestinal dietary triglyceride trafficking, and modulating pancreatic β -cell survival. The combination of these roles makes apoC-III an important therapeutic target for the management and prevention of cardiovascular disease.

Keywords

apolipoprotein C-III; intestinal lipid transport; diabetes; obesity; cardiovascular disease; vascular inflammation

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Conflicts of interest

The author has no conflicts of interest.

Introduction

In the past 30+ years, there has been a significant focus on defining the factors that lead to cardiovascular disease (CVD), and the lifestyle and pharmacological approaches to its prevention and treatment. Major clinical indicators of CVD risk include high plasma cholesterol and triglycerides. Due in part to the definitive link between raised concentrations of low-density lipoprotein (LDL) cholesterol and CVD, for many years research has focused on approaches to lower plasma LDL cholesterol^{1–3}. This has resulted in the highly effective use of statins in CVD treatment and prevention⁴. Treatment of LDL cholesterol alone, however, does not completely reduce CVD risk^{5,6}. Therefore, another approach has been to focus on raising HDL levels, which are inversely related to CVD risk in many people⁷. However, after a variety of clinical trials and large-scale epidemiological studies, we are now certain that simply raising plasma HDL levels is not sufficient to decrease CVD risk^{8–14}. With the loss of increased HDL levels as a potential drug target, there is now renewed interest in understanding how raised plasma triglycerides can be targeted for further elimination of CVD risk.

Plasma triglycerides are found in the triglyceride-rich lipoproteins (TRLs): in the fasted state they are found in very-low density lipoproteins (VLDL) and in the postprandial state in chylomicrons. Chylomicrons and VLDL are converted to remnants (containing a fraction of their original triglyceride) by the action of lipoprotein lipase (LPL) on capillary endothelial cells and extrahepatic tissues. Ultimately, the liver takes up these remnants through receptor mediated mechanisms. In 1979, Zilversmit first postulated that postprandial TRL-remnants are integral to the development of atherosclerosis¹⁵. Since that time, numerous studies have shown that nonfasting plasma triglyceride levels are a strong marker for CVD risk^{16,17}. Nonfasting plasma triglycerides are strongly predictive of ischemic events in both sexes, even taking into account differences in plasma HDL¹⁷. As nonfasting triglyceride levels increase, so does the risk of all-cause mortality¹⁶. In addition, in patients with already low LDL cholesterol, elevations in plasma triglycerides increase CVD risk, and the greatest reduction in CVD risk occurs when both LDL and triglycerides are reduced¹⁸.

This relationship between plasma triglycerides and CVD is in part because increased plasma triglycerides are associated with the generation of a greater proportion of smaller, dense LDL, which are highly susceptible to oxidation, resistant to removal from the blood, and these LDL particles easily bind to the arterial wall^{18–20}. In addition to promoting the formation of these atherogenic LDL particles, plasma triglycerides are themselves atherogenic since their hydrolysis by LPL results in the release of free fatty acids and other inflammatory mediators at the arterial wall^{21–23}.

Approximately 30% of the US population have moderate to high plasma triglyceride levels (greater than 150 mg/dl)^{24,25}. Therefore, understanding the factors that modify plasma triglyceride levels (including the absorption of triglyceride from the diet, secretion of VLDL, and the metabolism and clearance of TRLs and their remnants from the plasma) is important for the treatment and prevention of CVD. Apolipoprotein C-III (apoC-III) is one important factor in the maintenance of plasma triglyceride levels. ApoC-III is an exchangeable apolipoprotein located in the apoA-I/C-III/A-IV gene cluster on chromosome

11q23^{26,27}. Genome-wide association studies (GWAS) have repeatedly linked genes in the apoA-I/C-III/A-IV gene cluster with maintenance of plasma triglycerides and in determining CVD risk^{27–30}. In humans, plasma apoC-III levels are elevated during both hyperlipidemia and diabetes^{31–33}. ApoC-III levels are themselves an independent predictor of cardiovascular disease risk^{34,35}. ApoC-III is produced by both the intestine and liver, and is found in both the postprandial and fasting state on chylomicrons, VLDL, and HDL³⁶. ApoC-III was first described as an inhibitor of LPL, but it also interferes with clearance of TRL-remnants by the LDL-receptor in the liver^{37–40}. These actions of apoC-III increase the plasma resident time of TRLs and their remnants. ApoC-III also promotes the synthesis and secretion of larger, more triglyceride-rich VLDL from the liver, and modulates intestinal lipid absorption^{41,42*}. The combination of these actions makes apoC-III a critical factor in the development of hypertriglyceridemia.

ApoC-III: activity and expression is strongly linked with CVD risk

There are several important mutations in the APOC3 gene in human populations, and these were first described in isolated populations. The Lancaster Amish, for example, is a population enriched in the nonsense mutation R19X in the apoC-III gene, which leads to a 40–50% decrease in apoC-III levels and a reduction in both fasting and postprandial triglycerides⁴³. This decrease in apoC-III levels is associated with a significant decrease in coronary artery calcification. This cardio-protective variant of apoC-III was recently shown to exist at a low level within the general population, and is associated with a 50% reduction of plasma triglyceride levels^{44*}. The protective effect of the R19X variant extends to other null mutations, including the –641C allele, located within the promoter region of apoC-III⁴⁵. This mutation is found at high levels in a small cohort of exceptionally long-lived Ashkenazi Jews, and results in reductions in plasma apoC-III levels and plasma triglycerides, and lower risk of hypertension and insulin insensitivity. In a prospective study of the children of this cohort, the –641C allele conferred a significant survival advantage⁴⁵.

These and other studies indicate that apoC-III levels are independently associated with CVD risk, even after adjusting for other plasma lipid levels^{35,46}. In June 2014 it was shown for the first time that this protective effect extends to a large-scale human cohort. In two parallel large-scale epidemiological studies, it was revealed that mutations in apoC-III result in a striking decrease in ischemic cardiovascular disease and coronary heart disease risk in humans^{47**,48**}. In the first of two parallel studies, four mutations in the APOC3 gene were identified as associated with plasma triglyceride levels in participants in the Exome Sequencing Project^{47**}. These mutations included three loss-of-function mutations: a nonsense mutation (R19X) and two splice-site mutations (IVS2+1G→A and IVS3+1G→T), and a missense mutation (A43T). These mutations resulted in a 46% decrease in circulating apoC-III levels. This reduction in apoC-III expression was coupled with a 39% decrease in triglyceride levels as compared with non-carriers. This apoC-III deficiency conferred a 40% reduction in CVD risk. The second study (using a 75,725 person general population cohort), found that carriers of loss-of-function mutations in the apoC-III gene had a 44% reduction in nonfasting triglycerides, which corresponded to a 41% reduction in risk for ischemic vascular disease and a 36% decrease in ischemic heart disease^{48**}. These epidemiological studies are the first to show in a large human cohort that mutations in apoC-III that were

previously associated with just a few individuals, exist in the general population to a rare degree, but are beneficially associated with significantly reduced plasma triglyceride levels and importantly, with a reduced risk of CVD.

ApoC-III is a potent factor in the maintenance of plasma triglycerides

These epidemiological studies define apoC-III as a potent target for treating the cardiovascular disease risk factor of increased plasma triglycerides^{49,50}. ApoC-III increases plasma triglycerides in part because it is an inhibitor of LPL. This action of apoC-III on LPL was thought to be due to competition with apoC-II, however it appears that the mechanism may involve the ability of apoC-III to displace LPL from TRLs, leading to its conversion from the active dimer to inactive monomer by angiopoietin-like protein 4 (angptl4)^{51,52**}. It has recently been shown in human studies, however, that the effect of apoC-III on LPL may be secondary to its effects on hepatic remnant uptake. In recent human kinetic studies, TRLs containing apoC-III are cleared more slowly from plasma than TRLs lacking apoC-III, and this effect does not involve a change in LPL activity but rather a modulation of hepatic remnant uptake⁵³.

ApoC-III is also a stimulator of VLDL assembly and secretion. The overexpression of apoC-III in McA-RH7777 cells increases the assembly and secretion of VLDL under lipid-rich conditions, and the naturally occurring variant of apoC-III (the Ala23-to-Thr mutation, found in hypolipidemic Mayan Indian subjects) abolished this effect^{41,54}. These *in vitro* studies link the expression of apoC-III with the synthesis and secretion of larger VLDL from the liver, by stimulating the recruitment of additional triglyceride to the nascent apoB-100 scaffold, prior to mature VLDL secretion. In human kinetic studies, apoC-III enhances the assembly and secretion of VLDL particles from the liver under lipid-rich conditions⁵⁵.

These effects of apoC-III both in the plasma (on LPL and TRL-remnants clearance) and in the liver (on VLDL synthesis and secretion) act to raise plasma triglycerides. The role of apoC-III in the intestine is just beginning to be defined. Recently, a new role for apoC-III has been delineated showing that overexpression of human apoC-III in transgenic mice decreases transport of dietary triglyceride into lymph, which is associated with accumulation of lipids in the intestinal lumen^{42**}. The mechanisms underlying the reduced lymphatic lipid transport involve both a delayed lipid uptake into enterocytes, as well as impaired reesterification of triglyceride in the enterocyte. Therefore, in addition to its other roles in the maintenance of plasma lipid levels, apoC-III is also a modulator of dietary lipid absorption.

The atherogenic effects of apoC-III go beyond raising plasma triglycerides: it is a potent marker of the atherogenicity of lipoprotein particles

It is becoming increasingly clear that apoC-III has atherogenic effects beyond its ability to raise plasma triglycerides. In a recent prospective human study, it was reported that the risk for both fatal and nonfatal myocardial infarction is significantly increased in patients with an increased fraction of VLDL or LDL containing apoC-III³⁵. In two prospective studies within the Nurses' Health and the Health Professionals Follow-Up Studies, HDL subgroups

(containing apoC-III and without apoC-III) were differentially associated with CVD, with increases in HDL enriched in apoC-III associating with a higher CVD risk^{56*}. In another study, HDL was isolated from healthy patients, and those undergoing treatment for coronary artery disease. The HDL taken from patients with CAD had significantly more apoC-III, and displayed altered activation of endothelial apoptosis than HDL from healthy patients. Pre-incubating the HDL with an antibody against apoC-III blocked these effects^{57**}. The presence of apoC-III on LDL is highly associated with the development of CVD, and the CVD risk associated with LDL may indeed be due to the subpopulation containing apoC-III^{58,59}. Finally, patients who are obese have a higher proportion of HDL containing apoC-III than healthy patients^{60*}. These data suggest that the presence or absence of apoC-III on lipoprotein particles may turn HDL, a normally protective into an atherogenic lipoprotein, and the dose of apoC-III on LDL and VLDL may determine the degree of atherogenicity. ApoC-III status may explain why drugs targeting HDL levels have been unsuccessful at lowering CVD risk, because there are actually a heterogeneous pool of lipoproteins containing apoC-III (and other factors) that are responsible for moderating CVD risk, and the presence or absence (or dose) of apoC-III determines the risk.

ApoC-III is an inflammatory factor and a modulator of apoptosis

Cardiovascular disease is not only a disease of disordered plasma lipids, but also a disease of inflammation⁶¹. ApoC-III is a critical regulator of a variety of pro-inflammatory pathways. Plasma levels of apoC-III are elevated in patients who are obese, who have cardiovascular disease⁶². The presence of apoC-III (alone, or on VLDL or LDL) stimulates monocyte adhesion through vascular adhesion molecule-1, and also activates PKC β and NF- κ B in vascular endothelial cells^{63–65}. ApoC-III-enriched HDL are not protective against monocyte adhesion, suggesting again that apoC-III enrichment may be a way of defining the different classes of HDL particles as anti-atherogenic (apoC-III rich) and protective (apoC-III poor)⁶⁴. It was recently reported that apoC-III levels are associated with an increase in the activity of the coagulation Factor II, though this association is not changed in the face of active cardiovascular disease^{66*}. Therefore, apoC-III stimulates endothelial dysfunction and adhesiveness, and promotes potentially unfavorable clotting properties in the blood. Interestingly, although statin treatment only modestly decreases plasma apoC-III levels, statins are able to inhibit the ability of apoC-III to stimulate vascular adhesion through VCAM-1⁶⁷.

Plasma levels of apoC-III are also elevated in type 1 diabetics^{68,69}. In a rodent model of type 1 diabetes, apoC-III levels increase with disease onset, and promote β -cell death⁷⁰. This effect is abolished when apoC-III levels are reduced with an antisense oligonucleotide. There is still confusion, however, about the precise role of apoC-III in pancreatic β -cell failure and disease progression. It has been reported in a variety of rodent cell lines and primary β -cells, that apoC-III promotes apoptosis^{71,72}. However, in a recent study, apoC-III was shown to reduce rat islet apoptosis in the presence of pro-inflammatory cytokines⁷³. Interestingly, HDL enriched in apoC-III from patients with incipient coronary artery disease is also pro-apoptotic in the vascular endothelium. This role of apoC-III in promoting apoptosis needs further study.

Modulating apoC-III levels may be crucial for lowering CVD risk

Although there are a variety of pharmaceuticals that reduce triglyceride levels (including statins, niacin, fibrates, and ω -3 fatty acids), apoC-III is an attractive drug target because of its additional relationship to inflammation, HDL function, pancreatic β -cell function, and reduction in cardiovascular risk. ISIS pharmaceuticals is moving forward with its human apoC-III antisense drug, based in part on the outcome of a recent phase 2 clinical study^{74**}. In this study, in both animals and humans, apoC-III reduction was dose-dependently related to a decrease in plasma triglycerides, and was not associated with other metabolic abnormalities.

Modulating apoC-III through dietary approaches may be another opportunity to beneficially change circulating apoC-III levels, though this may prove complex. In the liver, apoC-III expression is inhibited by insulin and stimulated by glucose via transcriptional regulatory elements in the promoter region^{75,76}. ApoC-III expression is also sensitive to fibrates, which reduce levels of apoC-III in addition to lowering plasma triglycerides^{77,78}. It is important to note that very little is known about how intestinal apoC-III is regulated, and this may be quite different than hepatic apoC-III regulation, since hepatic and intestinal lipoprotein synthesis pathways are unique and regulated at different steps^{79–81}. There may be a valuable difference in the regulation and function of apoC-III in these tissues, and it is likely that dietary nutrients moderate this effect. This is a critical gap in understanding how this nutrient-rich food impacts a key cardio/metabolic risk factor.

Conclusion

New insights into the role of apoC-III in modifying CVD risk make it an especially attractive clinical target, and may explain why residual CVD risk exists in the face of successful statin treatment. Understanding the complete role of apoC-III in modulating CVD risk factors, through research in animal and human models, as well as determining the regulation of apoC-III by diet and pharmaceuticals, is becoming a crucial factor in ameliorating CVD risk. The role of apoC-III in other inflammatory diseases, including diabetes, will also be an important direction for future research.

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subjects, suggesting that apoC-III plays another direct role in mediating vascular effects outside of its ability to raise plasma triglycerides.

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Key points

- Plasma triglyceride levels are associated with cardiovascular disease, and in human studies nonfasting triglyceride levels are related to risk of ischemic events and all-cause mortality
- Apolipoprotein C-III is a potent modulator of plasma triglyceride levels through effects in the intestine (on dietary triglyceride trafficking), plasma (inhibition of LPL hydrolysis of triglyceride-rich lipoproteins), and liver (through inhibition of remnant uptake and stimulation of VLDL secretion).
- There is renewed interest in apoC-III since 2 large-scale genetic studies in June 2014 showed a clear relationship between apoC-III expression and CVD.
- ApoC-III may also contribute to CVD beyond its ability to raise plasma triglycerides; it is also a stimulator of vascular inflammation, and may potentiate the atherogenicity of HDL, VLDL, and LDL.
- Lipoproteins containing apoC-III are more atherogenic, and associated with greater risk of CVD than lipoproteins lacking apoC-III.
- New pharmaceuticals are targeting apoC-III in the hopes of further reducing plasma triglycerides, and inhibiting an atherogenic factor.