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The quartet of apolipoprotein Cs and the different roles they play in diabetes

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Abstract

Apolipoprotein A1 (APOA1) and APOB are the structural proteins of high-density lipoprotein and APOB-containing lipoproteins, such as low-density lipoprotein and very low-density lipoprotein, respectively. The four smaller APOCs (APOC1, APOC2, APOC3 and APOC4) are exchangeable apolipoproteins; they are readily transferred among high-density lipoproteins and APOB-containing lipoproteins. The APOCs regulate plasma triglyceride and cholesterol levels by modulating substrate availability and activities of enzymes interacting with lipoproteins and by interfering with APOB-containing lipoprotein uptake through hepatic receptors. Of the four APOCs, APOC3 has been best studied in relation to diabetes. Elevated serum APOC3 levels predict incident cardiovascular disease and progression of kidney disease in people with type 1 diabetes. Insulin suppresses APOC3 levels, and accordingly, elevated APOC3 levels associate with insulin deficiency and insulin resistance. Mechanistic studies in a mouse model of type 1 diabetes have demonstrated that APOC3 acts in the causal pathway of diabetes-accelerated atherosclerosis. The mechanism is likely due to the ability of APOC3 to slow the clearance of triglyceride-rich lipoproteins and their remnants, thereby causing an increased accumulation of atherogenic lipoprotein remnants in lesions of atherosclerosis. Less is known about the roles of APOC1, APOC2 and APOC4 in diabetes.

Graphical Abstrct

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Disclosures

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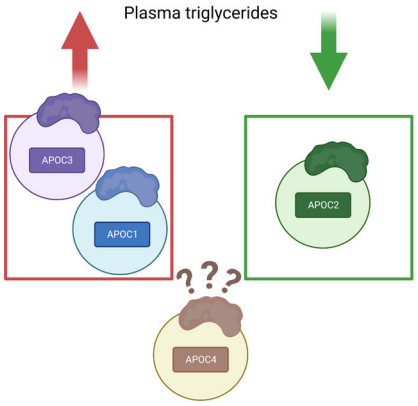

Introduction to the APOCs and diabetes

Diabetes has been known to be associated with dyslipidemia since ancient times when bloodletting was used as one of the treatment practices for the disease. A milky appearance of the blood was so common in patients with severe diabetes that it was used diagnostically.[1] Much later, in 1935, Mann and Peters[2] showed a lack of correlation between plasma cholesterol levels and levels of fatty acids and phospholipids in subjects with diabetes; differences we now know are due to elevated levels of triglyceride-rich lipoproteins (TRLs) in poorly controlled diabetes. With the knowledge that diabetes is associated with dyslipidemia and the realization that diabetic dyslipidemia might contribute to the increased risk of cardiovascular disease (CVD) that became apparent with the discovery and rapid initiation of insulin therapy, scientists started to investigate changes in specific proteins that bind to lipid to form lipoproteins – the apolipoproteins.

The history of apolipoprotein research is described in an excellent review article by Siri-Tarino and Krauss.[3] A and B apolipoproteins were first identified, but other apolipoproteins were soon discovered in chylomicron preparations (chylomicrons are large TRLs secreted from the intestine during and immediately following a meal). These proteins were termed C apolipoproteins.[4] It is now known that there are four apolipoprotein Cs, encoded by separate genes – APOC1, APOC2, APOC3 and APOC4 (Figure 1). The *APOC1*, *APOC2* and *APOC4* genes are part of the *APOE/APOC1/APOC4/APOC2* gene cluster located on chromosome 19 in humans and on chromosome 7 in mice, whereas the *APOC3* gene is part of the *APOA1/APOC3/APOA4/APOA5* gene cluster on chromosome 11 in humans and chromosome 9 in mice.

The four APOCs are small proteins secreted primarily by hepatocytes, which also secrete very low-density lipoprotein (VLDL). VLDLs are TRL particles secreted from the liver between meals. APOCs are added to the maturing VLDL particle in hepatocytes prior to secretion. The APOCs are also produced at lower levels by the intestine, which secretes chylomicrons into the lymph. Lymph chylomicrons are believed to acquire a significant amount of their APOC1, APOC2 and APOC3 cargo by transfer from lipoproteins filtered from plasma into lymph.[5, 6] APOCs are also expressed by enterocytes in the intestine. Upregulation of *Apoc2* gene expression in the jejunum following oil gavage[7] as well as the

localization of APOC3 and APOB in the villus tip regions[8] suggest that APOC2 and APOC3 are involved in chylomicron generation.

Conversely, the APOCs can be transferred from TRLs (chylomicrons and VLDL) to HDL in circulation. As the TRL particle becomes smaller when its triglyceride cargo is lipolyzed by lipoprotein lipase (LPL), the “excess” phospholipid surface material is believed to be transferred to HDL, likely carrying APOCs and other exchangeable apolipoproteins with it.[6] This mechanism is consistent with the very low levels of HDL in people with LPL deficiency. Richard Havel proposed that small amounts of APOCs are secreted from the liver in VLDL and then recycled repeatedly between VLDL and HDL before they are catabolized.[6] Thus, the APOCs are exchangeable apolipoproteins, as opposed to APOA1 (the structural apolipoprotein of HDL) and APOB (the structural apolipoprotein of TRLs, TRL remnants, and LDL).

TRL levels are often increased in individuals with sub-optimally controlled diabetes and/or insulin resistance.[9] Because of the increased APOC levels associated with elevated TRLs, and because insulin acutely suppresses large VLDL secretion,[10] many studies have investigated the association between diabetes and the APOCs. For example, people with diabetes who also had increased levels of VLDL were found to exhibit an increase in the relative amount of APOC3 in plasma.[11] While all four APOCs appear to play important roles in triglyceride metabolism, the role and regulation of APOC3 in the setting of diabetes is most well studied. Contrarily, almost nothing is known about APOC4.

Little is also known about the potential role of APOCs secreted by the intestine in the setting of diabetes. Early studies in rats suggested that the main APOCs produced by the intestine are APOC2 and APOC3.[12] Studies on mice transgenic for APOC3 have suggested that APOC3 blunts basolateral uptake of lipids into enterocytes, perhaps by interfering with lipoprotein uptake through LDLR, thereby reducing chylomicron secretion into the lymph.[13] However, the human relevance of these findings is uncertain because humans with *APOC3* loss-of-function mutations show no differences in production rates of APOB48-containing chylomicrons.[14]

This review focuses on the roles and regulation of the APOCs in diabetes, and the emerging evidence that APOCs, in particular APOC3 and perhaps APOC2, might serve as drug targets for prevention of some of the vascular complications associated with type 1 diabetes (T1D) and type 2 diabetes (T2D).

Current methodological issues hamper studies of the role of APOCs and the lipoprotein particle populations they modulate

Reviewing the literature on APOCs and the lipoprotein particles they modulate, it is important to consider several methodological issues. First, because the APOCs (and many other apolipoproteins) are exchangeable, mouse overexpression models can “overload” the lipoprotein particle, thereby causing unintended biological consequences by the overexpressed APOC, displacing endogenous apolipoproteins[15] or perhaps, blocking the access of enzymes to substrates or ligands to receptors,[16] thus altering the composition and

biological function of the particle. Issues of non-physiological alterations in composition and function of lipoproteins might also confound data interpretation on knockout models. Second, the method used for isolation of lipoprotein particles can alter the apolipoprotein composition ex vivo. For example, apolipoproteins can “fall off” the particle during ultracentrifugation. Alternatively, affinity chromatography e.g., using anti-APOC3 antibodies has been used to isolate APOC3-containing lipoproteins from human plasma.[17] Such methods are informative in that different lipoprotein populations carrying different sets of apolipoproteins can be quantified, but this approach requires the availability of suitable and specific antibodies. Third, APOC1, APOC3 and APOC4 are believed to slow the clearance of TRLs and their remnants in part by interfering with APOE binding to the hepatic receptors, LDLR (low-density lipoprotein [LDL] receptor) and LRP1 (LDLR related receptor 1).[18–20] The most widely used mouse models of atherosclerosis, *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice, therefore have deficiencies in this mechanism. Moreover, mice differ from humans in several critical aspects related to lipoprotein metabolism. For example, mice are naturally deficient in CETP (cholesteryl ester transfer protein), which transfers triglycerides from TRLs and exchanges them for cholesteryl esters from HDL and vice versa in humans. These issues must be considered when interpreting apolipoprotein and lipoprotein studies based on mouse models.

Moreover, there are not yet standardized methods available to accurately quantify concentrations and sizes of TRLs and their remnants in large clinical trials.[21, 22] Despite these methodological issues, many informative studies have revealed new information on APOCs and their relationships to diabetes.

APOC1

APOC1 is the smallest of the APOCs (Figure 1). In normolipidemic individuals the majority of APOC1 is present in HDL. However, with increased triglyceride levels, such as those often observed in people with T2D and people with suboptimally controlled T1D, the total plasma concentration and the proportion of APOC1 in TRLs increase.[23] The altered partitioning of APOC1 is thought to be important as HDL-associated APOC1 and TRL-associated APOC1 appear to have different functions. For example, HDL-associated APOC1 serves to inhibit CETP activity[24] (Figure 2) through a mechanism that may be due to APOC1’s ability to change the electrostatic charge of the HDL particle, resulting in a weaker HDL-CETP interaction.[25] Thus, APOC1-mediated inhibition of CETP would result in triglyceride enrichment of TRLs and cholesterol-enriched HDL.

Transgenic mice overexpressing APOC1 and in vitro studies have revealed that APOC1 also appears to have other functions, including inhibition of the activity of several lipases[26, 27] and at least in vitro, activation of lecithin-cholesterol acyl transferase (LCAT),[28] an enzyme involved in converting small HDL particles into larger particles. In vitro, APOC1 bound to TRLs prevents LPL from hydrolyzing triglycerides due to the displacement of LPL from the particles.[29] The inhibition of lipolysis is likely partly an in vitro phenomenon because mice overexpressing APOC1 do not exhibit impaired VLDL lipolysis; rather, the increase in plasma triglyceride levels is due to impaired hepatic uptake of VLDL and TRL remnants, likely by interfering with APOE-binding to LRP1, leading to increased

levels of cholesterol-enriched VLDL, TRL remnants and LDL.[19, 30] Mouse studies suggest that in situations where APOC1 increases levels of APOB lipoproteins, APOC1 plays a pro-atherogenic role,[31, 32] but its overexpression has also been shown to protect against obesity and insulin resistance,[30, 33] perhaps because of impaired fatty acid uptake in adipose tissue and skeletal muscle.[33] Whether APOC1 plays a role in obesity or insulin resistance in humans is unclear.[34]

Studies in mice deficient in endogenous APOC1 have confirmed that APOC1 increases VLDL, TRL remnants and LDL triglyceride and cholesterol levels, without altering intestinal triglyceride absorption.[35] Because these studies were performed in *Apoe*$^{-/-}$ mice, which have a defect in hepatic uptake of TRLs, endogenous APOC1 in this model was shown to act by increasing hepatic VLDL production and by reducing lipolysis via LPL inhibition. Thus, the triglyceride-elevating mechanism of APOC1 is multifactorial (Figure 2).

Plasma levels of APOC1 are elevated in people with T1D and T2D who also have elevated plasma triglycerides.[34, 36] Similarly, an *Ldlr*$^{-/-}$ mouse model of T1D exhibits increased plasma levels of APOC1, concomitant with increased plasma triglyceride levels.[37] However, T2D appears to increase APOC1 plasma levels in part independently of plasma triglyceride levels through an unknown mechanism.[34] Moreover, APOC1 levels are negatively correlated with CETP activity in controls but not in people with hypertriglyceridemia, hypercholesterolemia or T1D or T2D.[36, 38] It is possible that the loss of APOC1 inhibition associated with dyslipidemia is due to a relative redistribution of APOC1 to VLDL.[38] In the case of diabetes-associated loss of CETP inhibition by APOC1, it has been hypothesized to be due to increased glycation of APOC1.[36]

Overall, there is still much we do not know about APOC1's physiological function in different conditions, including in diabetes. Because of its pleiotropic effects, APOC1 may be unlikely to be a promising drug target for combating complications of diabetes.

APOC2

The principal function of APOC2 is much clearer than that of APOC1; APOC2 promotes the hydrolysis of circulating TRLs through the activation of LPL (Figure 2). The exact mechanism for how APOC2 enhances LPL activity is unclear. Most data support a model in which the C-terminus of APOC2 interacts with LPL to promote triglyceride hydrolysis.[39, 40] Consistently, individuals with APOC2 deficiency or *APOC2* loss-of-function mutations have severe hypertriglyceridemia, which can lead to pancreatitis,[41] and APOC2-deficient mice also present with hypertriglyceridemia.[42]

People who have elevated plasma triglycerides also have increased levels of APOC2. Thus, individuals with T2D, who often have hypertriglyceridemia, exhibit elevated plasma APOC2 levels.[43] Similarly, plasma APOC2 levels are elevated in an *Ldlr*$^{-/-}$ mouse model of T1D.[37] The mechanism behind hypertriglyceridemia in the presence of elevated APOC2 levels could be due, in part, to parallel increases in APOC3[43]–an LPL inhibitor–or to excess APOC2 interfering with the access of LPL to its substrates.[16] Accordingly, individuals with

diabetes and increased VLDL have been shown to exhibit an increase in the relative amount of APOC3, and a consequent decrease in the ratio of APOC2/APOC3 in VLDL.[11]

The most promising development in the area of APOC2 in relation to diabetes is the generation of APOC2 mimetic peptides that reduce plasma triglycerides. Consistent with the concept that the ratio of APOC2 to APOC3 is important in controlling plasma triglycerides, the APOC2 mimetic peptide, D6PV, has the ability to reduce plasma triglycerides in transgenic mouse models both via LPL activation and through APOC3 displacement.[42] The displacement of APOC3 is believed to promote TRL metabolism by relieving the LPL-dependent and LPL-independent inhibitory effects of APOC3 on TRL clearance. The mimetic peptide could therefore provide an alternative strategy for reducing diabetes-associated complications by lowering TRLs and their remnants in individuals with diabetes.

APOC3

APOC3 is the most well-studied apolipoprotein in the APOC family (Figures 1–2). Like the other APOCs, the lipoprotein distribution of APOC3 is dependent on the metabolic state of an individual, with elevated levels in individuals with diabetes and elevated levels of TRLs. Moreover, APOC3 can be glycosylated and sialylated in humans, modulating its hepatic clearance,[44] adding another layer of complexity. As with all apolipoproteins, plasma levels are a reflection of both production and clearance rates. Lipoprotein-bound APOC3 is cleared from circulation via receptor-mediated hepatic uptake while non-lipidated APOC3 is cleared via renal filtration.[20, 45] Kinetic studies have shown that individuals with T2D have an increased APOC3 secretion rate that is associated with increased TRL levels.[46] Diabetes also likely reduces the clearance rate of APOC3 by dampening LPL activity and, presumably, hepatic clearance of TRLs.[37, 47]

Early studies in isolated hepatocytes, mice deficient in hepatic insulin receptors, and diabetic mice treated with a bolus dose of insulin suggested that hyperglycemia and lack of insulin contribute to the increased hepatic *Apoc3* mRNA and plasma APOC3 levels associated with diabetes.[48–50] Subsequent studies showed that diabetes-associated hyperglycemia is not the main driver of the elevated plasma levels of APOC3 in a mouse model since normalizing blood glucose with a sodium-glucose co-transporter 2 inhibitor did not reduce plasma APOC3 levels, while intense insulin therapy normalized diabetes-associated increases in APOC3.[51] The reduction in plasma APOC3 in response to insulin treatment of diabetic mice did not appear to occur via a direct decrease in hepatic *Apoc3* transcription. This finding was unexpected because prior studies on mice overexpressing the transcription factor FOXO1 showed increased hepatic *Apoc3* expression consistent with the ability of insulin to induce FOXO1 phosphorylation and nuclear exclusion.[49] To further investigate the role of FOXO transcription factors, we subsequently demonstrated that deletion of all three of the hepatic FOXOs (FOXO1, 3 and 4) failed to suppress hepatic *Apoc3* levels.[51] Thus, the increased hepatic levels of *Apoc3* mRNA in diabetic mice are likely to be explained by increased mRNA stability or other post-transcriptional mechanisms. The conclusion that increased transcription of the *APOC3* gene is not the mechanism whereby T1D can cause to elevated plasma APOC3 levels is also consistent with findings that mutations in an insulin response element (T-455 -> C) in the human *APOC3* promoter in individuals with T1D do not confer

a reduction in either plasma APOC3 or triglycerides.[52] The most consistent observation in humans with either T1D or T2D is that plasma APOC3 concentrations increase in tandem with plasma TRL levels. The mechanism might be related to lack of sufficient insulin signaling in the liver, either because portal vein insulin delivery is not mimicked by exogenous insulin treatment or because insulin resistance exists both in T1D and T2D. In fact, serum APOC3 levels in subjects with T1D associate negatively with insulin sensitivity.[53] Further studies are needed to determine how APOC3 is regulated in diabetes, including transcriptional and post-transcriptional regulation and fractional production and clearance rates.

The seminal study by Pollin and colleagues first demonstrated that individuals with *APOC3* loss-of-function mutations have reduced levels of plasma triglycerides and an apparent protection against CVD.[54] Three separate studies on distinct cohorts of people with T1D in the US and Europe have now shown that serum levels of APOC3 predict cardiovascular events independent of traditional CVD risk factors.[51, 55, 56] Using a mouse model of T1D, we further demonstrated that silencing APOC3 protects against diabetes-accelerated atherosclerosis, both measured as early lesion initiation as well as the expansion of the necrotic cores associated with more advanced atherosclerosis.[51] Importantly, the beneficial effect of APOC3 lowering was observed despite the lack of effects of APOC3 silencing on hyperglycemia. The lesion stabilizing effect of APOC3 reduction was also reported in mice without diabetes, but only in mice in which APOC3 also regulated TRLs,[57] suggesting that the pro-atherogenic effects of APOC3 are mediated by its effects on TRLs and their remnants.

APOC3 causes an elevation of plasma triglyceride levels by reducing the lipolysis and the hepatic clearance of TRLs and their remnants (Figure 2). This interpretation is consistent with the finding that mice expressing a human APOC3 transgene are hypertriglyceridemic and have reduced VLDL clearance.[58] The mechanism behind APOC3-mediated inhibition of triglyceride lipolysis has been proposed to involve inhibition of LPL activity when LPL is bound to glycosylphosphatidylinositol-anchored HDL-binding protein 1 on endothelial cells,[59] perhaps by blocking the access of LPL to TRL triglycerides. When APOC3 levels are very high, it might also work by displacing APOC2 from the surface of the TRLs.[60] In addition, APOC3 inhibits the clearance of TRLs and their remnants through LDLR and LRP1,[20] by a mechanism believed to be due to interference with APOE binding to those receptors. The relative contribution of these mechanisms differ in different conditions. In humans with a loss-of-function mutation in *APOC3*, a 50% reduction in plasma APOC3 levels results in increased fractional clearance rates of VLDL triglycerides and APOB and a higher conversion rate of VLDL remnants to LDL, suggesting increased LPL activity.[61] The fractional clearance rates of both APOC3 and APOC2 were also higher in these subjects. On the other hand, suppression of plasma APOC3 leads to reduced plasma triglycerides in individuals with familial chylomicronemia syndrome and genetic deficiency of LPL, demonstrating that APOC3 also acts through an LPL-independent pathway.[62] This finding, together with the finding that APOC3 can displace APOE from small VLDL[15] supports the conclusion that APOC3 can increase plasma triglycerides and cholesterol independently of LPL, by inhibiting hepatic clearance of TRLs and their remnants.

The mechanism whereby suppression of APOC3 prevents atherosclerosis in the setting of diabetes is most likely due to its inhibition of clearance of TRLs and their remnants. Immunoreactive APOC3 accumulates at a greater extent in lesions in diabetic mice and co-localizes with APOB and APOE, suggesting accumulation of TRLs and/or TRL remnants, and this accumulation is prevented by silencing of APOC3 or by increasing hepatic clearance of TRLs.[37, 51, 63] However, some studies have suggested a role for APOC3 beyond that of TRL lipolysis and clearance. For example, non-lipidated APOC3 can induce alternative NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome activation in isolated human and mouse monocytes.[64, 65] Although it is tempting to speculate that elevated levels of inflammatory markers in humans or mice with diabetes may be due to elevated plasma APOC3, we recently demonstrated that the inflammasome-activating effect of non-lipidated APOC3 is lost when APOC3 is bound to lipid particles, as it is in circulation in people with and without diabetes.[65] Furthermore, diabetic mice in which APOC3 was silenced did not have reduced plasma levels of IL-18 or IL-1β, pro-inflammatory cytokines released through inflammasome pathways, and APOC3 transgenic mice did not exhibit increased plasma levels of IL-1β or IL-18 despite the high levels of APOC3. Although it is possible that some APOC3 exists free of lipoproteins in tissues, these studies are consistent with the proposal that endogenous APOC3 does not act upstream of NLRP3 activation in diabetes. However, human studies show an intriguing interaction between an *NLRP3* intron variant associated with increased NLRP3 activation and APOC3 plasma concentrations.[66] Such a connection might be explained by a mechanism in which plasma APOC3 levels are regulated downstream of NLRP3 because recent mouse studies demonstrated reduced serum APOC3 levels in diabetic mice with hematopoietic NLRP3 deficiency.[67] These results highlight the importance of considering the function of APOC3 in its physiological lipidated state, and support the notion that APOC3 acts to promote atherosclerosis primarily by its effects on TRLs and TRL remnants.

Reduced renal function is associated with increased plasma APOC3 levels,[45, 68] and plasma levels of APOC3 in people with T1D are positively associated with nephropathy and retinopathy.[52, 69] Interestingly, in a study of APOC3 as a predictor of incident CVD in participants with T1D in the FinnDiane cohort, APOC3 only predicted CVD in those who also had markers of diabetic kidney disease,[56] suggesting that the close relationship between diabetic kidney disease and CVD includes APOC3. Plasma levels of APOC3 also predicted renal disease progression in people with T1D. Mechanistically, it is unclear how APOC3, or APOC3-mediated dyslipidemia might accelerate diabetic kidney disease, but APOC3 overexpression appears to worsen mild diabetic kidney disease in part by augmenting glomerular inflammation.[70] It is currently difficult to disentangle the effects of APOC3 from those of elevated triglycerides and insulin resistance, both on CVD and diabetic kidney disease.

Some studies have suggested that APOC3 could play a role in development or worsening of insulin resistance because of the links among plasma APOC3, dyslipidemia, insulin resistance and diabetes.[71, 72] Indeed, a small clinical trial investigating the triglyceride-lowering effects of volanesorsen (an APOC3 antisense oligonucleotide therapeutic) in subjects with T2D indicated that suppression of APOC3 resulted in improved insulin sensitivity.[73] However, a larger randomized clinical trial on volanesorsen failed to detect

improvements in insulin resistance (HOMA-IR) or HbA1c in subjects with T2D.[74] On the other hand, studies in mice and isolated cells suggested that APOC3 is produced by pancreatic islet cells[75] and that reducing APOC3 levels either systemically[76] or locally in the islet increased the survival of beta cells.[75] However, additional studies in which APOC3 was overexpressed demonstrated that APOC3 is insufficient in causing beta cell dysfunction.[77] Although the possible direct or indirect effects of APOC3 in beta cell physiology need further study, current data do not unequivocally support a causative role for APOC3 in insulin resistance or incidence of T2D.

Overall, there is strong evidence that APOC3 is a biomarker and mediator of CVD and atherosclerosis, and likely of kidney disease, in the setting of T1D. Further studies are needed to address whether similar relationships are present in T2D.

APOC4

APOC4 is a relatively understudied member of the APOC family. The *APOC4* gene was discovered and characterized nearly 25 years ago,[78] but so far has not attracted much attention, perhaps because plasma levels of APOC4 are at least an order of magnitude lower than those of APOC1, APOC2 and APOC3 (Figure 1). APOC4 is found on circulating TRLs and HDL, like the other APOCs. Rabbit APOC4 exists in several sialoforms,[79] but the physiological and pathological significance of these isoforms remain unknown.

Endogenous APOC4 is thought to increase plasma triglyceride levels because overexpression of human APOC4 at supraphysiological levels in mice causes elevated levels of VLDL.[18] The mechanism of the elevated VLDL levels in APOC4 transgenic mice was suggested to be due to displacement of APOE, resulting in reduced hepatic clearance of VLDL, rather than to a reduction in lipase activity (Figure 2). Moreover, previous small studies have revealed that polymorphisms in the human *APOC4* gene are associated with plasma triglyceride levels in women, although the contribution of *APOC4* genetic variation to plasma triglycerides was only 2%.[80] *APOC4* polymorphisms have also been associated with mildly elevated triglycerides and an increased coronary artery disease risk in a Chinese Han population.[81] The biological functions of endogenous APOC4 as well as its potential role in diabetes and diabetes complications need further study.

Clinical implications

If APOC3-mediated arterial retention of TRLs and their remnants indeed contributes causally to incident CVD in people with diabetes, APOC3 inhibition (or potentially the APOC2 mimetic peptide described above) might be a promising novel approach to CVD prevention in people with suboptimally controlled T1D, T2D, or insulin resistance. It is important to consider that APOC3 is not universally elevated in these conditions but rather is elevated in those with increased risk for incident CVD.

Several approaches to target APOC3 have been established, including siRNA, liver-targeted antisense oligonucleotides, blocking antibodies and peptides, all with great success in reducing plasma APOC3 levels and triglycerides. Importantly, blocking APOC3's action

would be predicted to increase both TRL lipolysis and hepatic removal of TRLs and TRL remnants, but not to reduce total levels of APOB100-containing lipoproteins.[82]

The necessity of lowering total levels of APOB-containing lipoproteins to achieve cardioprotection was recently highlighted by the PROMINENT trial (NCT03071692).[83] This double-blind, randomized, controlled trial investigated the effect of pemafibrate, a highly selective PPARα modulator, on lipids and cardiovascular outcomes in participants with T2D who had mild-to-moderate hypertriglyceridemia. The results demonstrated that the incidence of CVD events was not lower among those who received pemafibrate than those who received placebo, although pemafibrate lowered triglycerides, VLDL cholesterol, and APOC3 levels by 26–28%.[83] One likely explanation of the study's futility is that the pemafibrate-mediated reductions in TRLs were accompanied by increases in plasma LDL-cholesterol and APOB levels, so that there was no overall benefit in non-HDL cholesterol and total cholesterol levels. The authors suggested that pemafibrate results in an increased conversion of TRLs to LDL, rather than an increased removal of these particles by the liver, which might have negated any benefit of reduction in TRL or remnant levels. This trial suggests that increased lipolysis of TRLs without also increasing hepatic removal of APOB lipoproteins, including TRL remnants and LDL, will not be a successful strategy for CVD prevention in people with diabetes. Therefore, when designing cardiovascular outcome trials on the effect of APOC3 inhibition, including in participants with diabetes and elevated APOC3 levels, strategies to ensure effective hepatic clearance of TRLs, TRL remnants and LDL should be carefully considered.

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Abbreviations

APO	apolipoprotein
CETP	cholesteryl ester transfer protein
CVD	cardiovascular disease
LDLR	LDL receptor
LRP1	LDL receptor related receptor 1
LPL	lipoprotein lipase
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
T1D	type 1 diabetes
T2D	type 2 diabetes
TRL	triglyceride-rich lipoprotein

VLDL	very low-density lipoprotein

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Highlights:

- Apolipoprotein Cs (APOCs) are involved in the regulation of plasma triglycerides and cholesterol, and are increased in individuals who have diabetes and elevated triglyceride levels.
- APOC1 and APOC3 increase plasma triglyceride levels, APOC2 decreases plasma triglycerides, but the role of endogenous APOC4 is largely unknown.
- Elevated levels of serum APOC3 are associated with increased cardiovascular disease risk and kidney disease progression in individuals with type 1 diabetes.
- Targeting the clearance of triglyceride-rich lipoproteins and their remnants via APOC3 inhibition or APOC2 mimetic peptides might be a promising approach for preventing cardiovascular diseases in individuals with diabetes, but such an approach will need to simultaneously increase the hepatic removal of APOB lipoproteins.

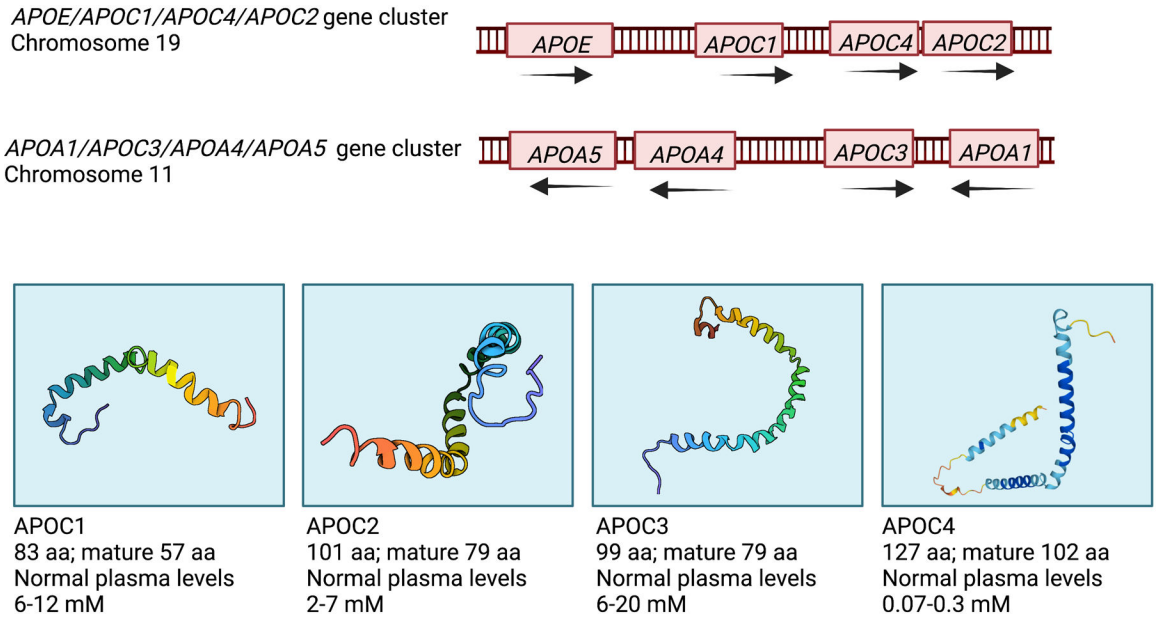

Figure 1. The human APOC genes and proteins.

Two human gene clusters contain the four APOC genes (top). The APOC protein 3D structures (bottom) were retrieved from the US data center for the global Protein Data Bank (PDB) archive. The NMR-derived APOC1 (1IOJ), APOC2 (1O8T) and APOC3 (2JQ3) structures are based on the conformation of human APOCs in complexes with sodium dodecyl sulfate. The human APOC4 structure is predicted by the AlphaFold Protein Structure Database developed by DeepMind and EMBL-EBI (https://alphafold.ebi.ac.uk/). Plasma levels are based on quantification by Hortin et al.[84] Created with BioRender.com

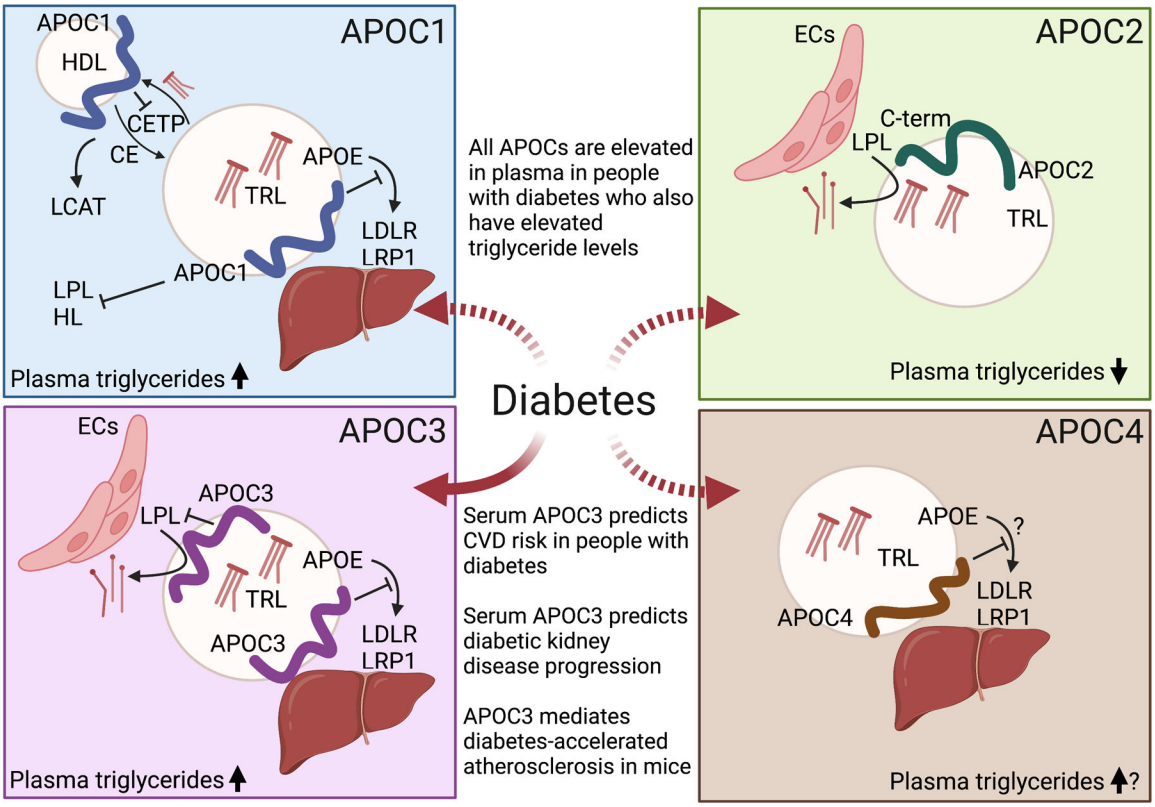

Figure 2. Functions of the four APOCs and their roles in diabetes.

Plasma levels of all APOCs are higher in people and animals with elevated plasma triglycerides, such as often is the case in T2D and poorly controlled T1D. APOC1 (top, left) appears to have a number of functions. APOC1 associated with HDL inhibits the action of CETP. APOC1 also has been shown to inhibit a number of enzymatic activities, including LPL and hepatic lipase (HL) in vitro and when overexpressed, to activate LCAT, and to inhibit hepatic uptake of TRLs by preventing APOE binding to LDLR and LRP1. APOC2 (top, right) promotes LPL activity and therefore is an important mediator of triglyceride lowering. APOC3 (bottom, left) is the most studied APOC with the clearest evidence of an important role in diabetes. Elevated serum levels of APOC3 predict incident CVD and kidney disease progression in people with T1D, and APOC3 is a causal mediator of diabetes-accelerated atherosclerosis in a mouse model of T1D. The likely mechanism of action of APOC3 is through reduced clearance of TRLs and their remnants, leading to increased accumulation of atherogenic remnants in the artery wall. There is little information on the mechanism of action of APOC4 (bottom, right) and its potential role in diabetes. APOC4 overexpression in a mouse model leads to increased plasma triglycerides, likely by interfering with the hepatic uptake of TRLs. The dashed arrows indicate that the link to diabetes is largely unknown. ECs, endothelial cells. Created with BioRender.com