ESC European Society of Cardiology

Cardiovascular Research (2023) **119**, 2843–2857
https://doi.org/10.1093/cvr/cvad177

INVITED SPOTLIGHT REVIEW

Exploring apolipoprotein C-III: pathophysiological and pharmacological relevance

Chris J. Packard [1], Angela Pirillo [2,3], Sotirios Tsimikas[4], Brian A. Ference[5], and Alberico L. Catapano [3,6]*

[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [2]Center for the Study of Atherosclerosis, E. Bassini Hospital, Milan, Italy; [3]Center for the Study of Dyslipidaemias, IRCCS MultiMedica, Sesto S. Giovanni, 20099 Milan, Italy; [4]Division of Cardiovascular Medicine, Sulpizio Cardiovascular Center, University of California San Diego, La Jolla, CA, USA; [5]Centre for Naturally Randomized Trials, University of Cambridge, Cambridge, UK; and [6]Department of Pharmacological and Biomolecular Sciences, University of Milan, via Balzaretti 9, 20133 Milan, Italy

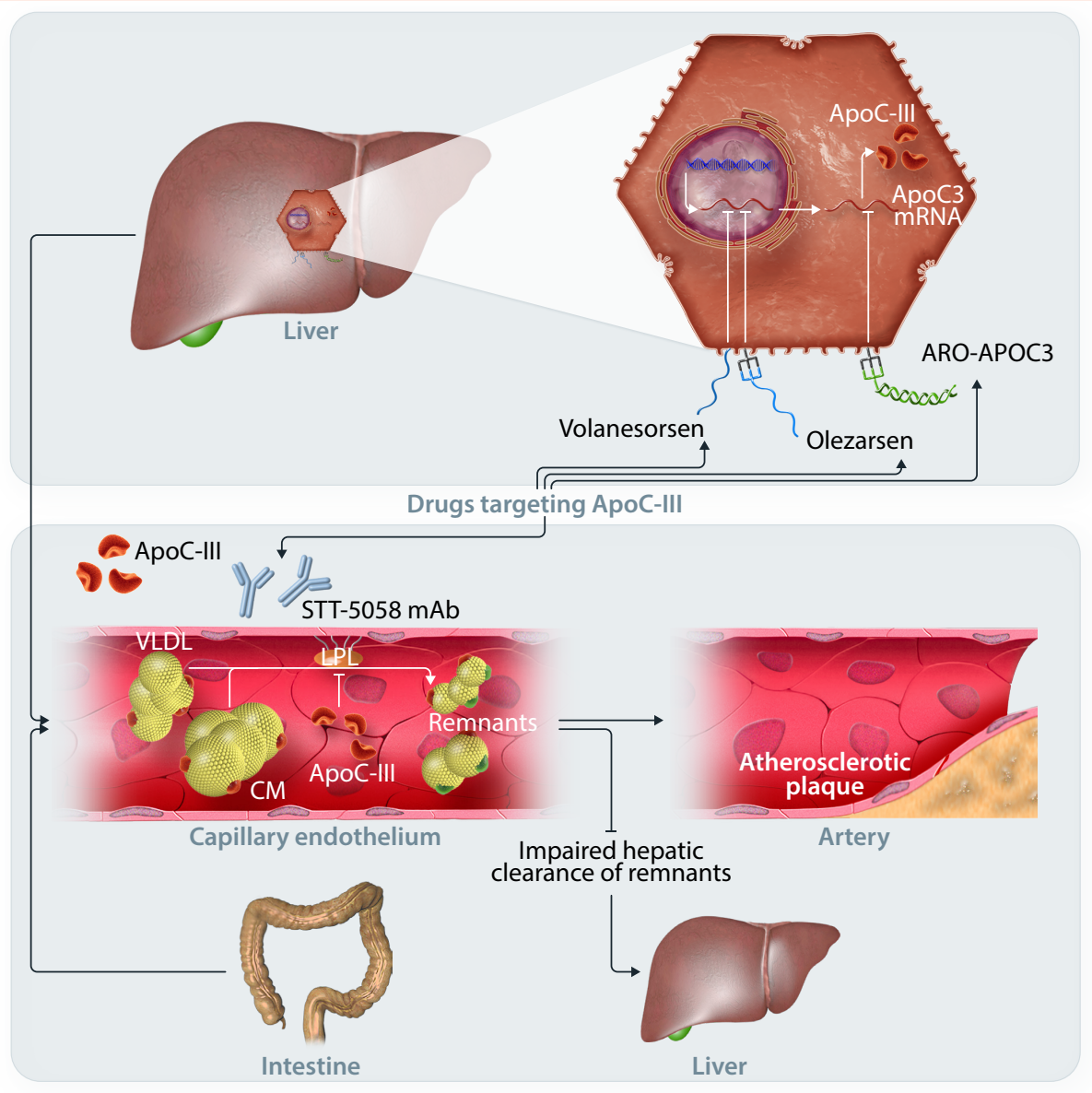
Received 13 October 2022; revised 21 December 2022; accepted 7 February 2023; online publish-ahead-of-print 1 December 2023

Abstract

The availability of pharmacological approaches able to effectively reduce circulating LDL cholesterol (LDL-C) has led to a substantial reduction in the risk of atherosclerosis-related cardiovascular disease (CVD). However, a residual cardiovascular (CV) risk persists in treated individuals with optimal levels of LDL-C. Additional risk factors beyond LDL-C are involved, and among these, elevated levels of triglycerides (TGs) and TG-rich lipoproteins are causally associated with an increased CV risk. Apolipoprotein C-III (apoC-III) is a key regulator of TG metabolism and hence circulating levels through several mechanisms including the inhibition of lipoprotein lipase activity and alterations in the affinity of apoC-III-containing lipoproteins for both the hepatic receptors involved in their removal and extracellular matrix in the arterial wall. Genetic studies have clarified the role of apoC-III in humans, establishing a causal link with CVD and showing that loss-of-function mutations in the *APOC3* gene are associated with reduced TG levels and reduced risk of coronary heart disease. Currently available hypolipidaemic drugs can reduce TG levels, although to a limited extent. Substantial reductions in TG levels can be obtained with new drugs that target specifically apoC-III; these include two antisense oligonucleotides, one small interfering RNA and an antibody.

* Corresponding author. Tel: +390249637591, Email: alberico.catapano@unimi.it

Graphical Abstract



The enrichment in apolipoprotein C-III (apoC-III) content impairs the hepatic clearance of triglyceride-rich lipoprotein remnants and may contribute to the formation of atherosclerotic plaque. Several options targeting specifically apoC-III are under development, including antisense oligonucleotides (volanesorsen and olezarsen), a siRNA (ARO-APOC3) and an antibody (STT-5058).

Keywords Apolipoprotein C-III • Triglycerides • Triglyceride-rich lipoproteins • Cardiovascular disease • Genetics

This article is part of the Spotlight Issue on Obesity, Metabolism, and Diabetes.

1. Introduction

Taking for granted that elevated levels of LDL cholesterol (LDL-C) are a major risk factor for atherosclerosis-related cardiovascular disease (ASCVD),[1,2] it is now clear that LDL-C levels cannot explain all cardiovascular (CV) events and the residual CV risk observed in patients with optimally treated LDL-C levels. Although the role of triglycerides (TGs) in atherosclerosis has been long debated, a large body of epidemiological, preclinical, and clinical trial evidence has suggested an association between TG, TG-rich lipoproteins (TRLs), and their remnants with inflammation, ASCVD, and all-cause mortality.[3–5] In support of these observations, genetic studies have indicated a causal link between elevated levels of TG and the risk of ASCVD.[6–10]

Apolipoprotein C-III (apoC-III) has received remarkable attention as a key regulator of TG metabolism. ApoC-III resides on the surface of TRLs, which include very-low-density lipoproteins (VLDL), chylomicrons (CMs), and their remnants, as well as high-density lipoproteins (HDL). The presence of apoC-III modulates the levels of these lipoproteins by influencing both the conversion rate to smaller and denser lipoproteins and their direct clearance from circulation. In some studies, high plasma levels

of apoC-III were shown to predict CV mortality independently of fasting TG and other traditional risk factors in the general population;[11] apoC-III levels are significantly associated with incident coronary artery disease (CAD) risk;[12] elevated levels of remnant lipoproteins, small dense low-density lipoproteins, and low-grade inflammation may explain, at least in part, this association.

In this review, we summarize the available knowledge on apoC-III synthesis and regulation, discuss the pathogenetic role of apoC-III in hypertriglyceridaemia and CV disease (CVD), and review data on currently available or under-development pharmacological approaches for the inhibition of apoC-III.

2. ApoC-III synthesis and regulation

Apoc-III is a low molecular weight apolipoprotein mainly produced by hepatic cells and to a lesser extent by enterocytes.[13] In plasma, the majority of apoC-III is transported by TRLs and HDL and plays a key role in the metabolism of TRLs.[14] The distribution of apoC-III among different lipoproteins depends on the metabolic state and varies between the fasting and postprandial conditions as well as between subjects with normal and elevated plasma TG levels.[15–18] In normolipidaemic subjects, the majority of apoC-III is carried by HDL, while in hypertriglyceridaemic subjects, the majority circulates with TRLs.[19] ApoC-III is rapidly and freely interchangeable between these two lipoprotein classes; it has been shown that simply mixing VLDL and HDL leads to an enrichment of HDL particles with apoC-III by an aqueous diffusion mechanism.[20,21] Overall, during lipolysis, when TG levels decrease, apoC-III is transferred from TRLs to HDL; conversely, after a meal, when plasma TG levels raise, apoC-III content in TRL increases while decreasing in HDL,[22] suggesting a highly dynamic process. Furthermore, as recently reinforced by the publication of the results with pemafibrate in the PROMINENT trial,[23] a reduction of TG and/or apoC-III alone may not suffice, and a decrease of apoB may be necessary to produce a clinically relevant benefit as we have previously shown at least for TG.[24]

ApoC-III can be present in multiple copies on some lipoproteins but is not present uniformly on all lipoproteins of the same class. Thus, only a proportion of lipoproteins contains apoC-III and thereby has enhanced pro-inflammatory and pro-atherogenic features. Plasma levels of VLDL particles containing apoC-III (with or without apoE) are increased in hypertriglyceridaemic patients compared with hypercholesterolaemic or normolipidaemic individuals.[25] Thus, TRL metabolism shifts from an apolipoprotein E (apoE)-governed system in normotriglyceridaemic individuals, exhibiting a rapid clearance of TRLs from the circulation, to an apoC-III-governed system in hypertriglyceridaemic patients, exhibiting a reduced clearance of TRLs and formation of dense LDL.[26] Of note, overexpression and/or accumulation of apoE may contribute to hypertriglyceridaemia by stimulating VLDL–TG production and by impairing VLDL lipolysis, at least in animal models, suggesting that an optimal expression of apoE is just as critical for normal metabolism of TRLs.[27]

Nearly all VLDL containing apoC-III undergo intravascular TG lipolysis, producing large-sized LDL with apoC-III which are then further catabolized to smaller LDL.[26,28,29] VLDL and LDL containing apoC-III, but not VLDL and LDL without apoC-III, induce endothelial activation and monocyte adhesion.[30,31] The presence of apoC-III in LDL is associated with changes in lipoprotein composition that favour its binding to extracellular the matrix, thus increasing lipoprotein retention.[32] A subfraction of circulating LDL with increased electronegative charge [LDL(-)] is characterized not only by apoB misfolding, which increases the binding affinity to proteoglycans, but also by the presence of apoC-III, which may contribute to the negative particle charge through its sialic acid content.[33] Furthermore, LDL containing apoC-III, which represents only 10–20% of plasma LDL, is an independent risk factor for coronary events.[34,35] Finally, epidemiological studies have shown that apoC-III drives the relationship between HDL and atherosclerosis, with HDL containing apoC-III being directly associated with CV risk and HDL lacking apoC-III having an inverse association.[36–40]

ApoC-III is an inhibitor of lipoprotein lipase (LPL); elevated levels of apoC-III are associated with hypertriglyceridaemia, which, however, derives from both the inhibition of LPL activity, resulting in a reduced catabolic rate of CM and VLDL, and the impairment of apoE-mediated hepatic receptor clearance of TRL remnants.[14] As a result, the plasma residence time of TRLs and their remnants increases. In normolipidaemic conditions, the liver produces VLDL and IDL containing both apoC-III and apoE, which are rapidly lipolysed and cleared from the circulation; under hypertriglyceridaemic conditions, the liver generates mainly VLDL particles containing apoC-III but not apoE, thus reducing their likelihood of being cleared and promoting their conversion into small, denser LDL particles.[14]

Although enterocytes produce apoC-III, the role of intestinal apoC-III has been only recently investigated. Transgenic mice overexpressing apoC-III have reduced lymphatic lipid transport compared with wild-type mice due to both a delayed dietary lipid uptake into enterocytes and impaired esterification to form TG in the mucosa;[41] primary murine enteroids overexpressing apoC-III show a reduced secretion of triacylglycerol (likely due to reduced incorporation of free fatty acids into triacylglycerol) along with the secretion of smaller CMs, containing significantly less dietary triacylglycerol.[42]

Several metabolic and nutritional factors modulate the transcription rate of *APOC3*, the gene encoding apoC-III, including inhibiting factors, such as insulin and polyunsaturated fatty acids,[43,44] and stimulating factors, such as glucose and saturated fatty acids (*Figure 1*).[45,46] By promoting the phosphorylation of the nuclear transcription factor Forkhead box O1 (FOXO1), insulin prevents its nuclear translocation, resulting in the down-regulation of *APOC3* transcription;[47,48] glucose induces the expression of apoC-III by activating the carbohydrate response element binding protein (ChREBP) and hepatic nuclear factor-4a (HNF4a).[45] Under physiological conditions, glucose-mediated induction and insulin-mediated suppression of hepatic *APOC3* expression balance each other to regulate the total amount of apoC-III secreted from the liver. In insulin-resistant conditions, such as obesity, metabolic syndrome, and type 2 diabetes, the sensitivity of *APOC3* to insulin is lost, and a hyperglycaemic condition may lead to a dysregulated transcription of *APOC3* and an increased content of apoC-III in TRLs. Earlier studies showed that in subjects carrying a common genetic variant in the promoter of *APOC3*, insulin cannot down-regulate the transcription of the gene, thus resulting in an overexpression of apoC-III and consequent hypertriglyceridaemia;[49] accordingly, two *APOC3* promoter variants were significantly more common among individuals with metabolic syndrome than in healthy individuals.[50] Peroxisome proliferator-activated receptor α (PPARα) appears to be involved in the effect of polyunsaturated fatty acids on apoC-III, with a possible indirect mechanism involving the displacement of HNF4α from the *APOC3* promoter region.[51] Saturated fatty acids act by activating mainly PPARγ coactivator-1 β (PGC-1β).[52]

ApoC-III is secreted as a unique glycoprotein having 1 *O*-*N*-acetylgalactosamine (GalNAc) in a β−3 linkage to galactose (Gal) (Galβ1–3GalNAc) attached to Thr^{74} (*Figure 1*).[53] The terminal GalNAc can present a variable number of molecules of sialic acid, resulting in three major glycoforms, referred to as apoC-III_{0b}, apoC-III_1, and apoC-III_2 containing 0, 1, and 2 molecules of sialic acid per molecule of protein, respectively (*Figure 1*).[54] Almost all circulating apoC-III is in glycosylated forms (both non-sialylated and sialylated).[55] It is still unclear whether different apoC-III glycoforms have different inhibitory activities towards LPL.[12,55–57] However, they impact TLR metabolism with TRL containing apoC-III_1 being rapidly cleared by LDLR and LRP1 and those containing apoC-III_2 being preferentially and more slowly cleared by the heparan sulfate proteoglycan (HSPG) syndecan-1.[57–59] Several pathological conditions (including obesity, metabolic syndrome, diabetes, hyperlipidaemia, and CAD) present with alterations in the relative abundance of apoC-III glycoforms.[12,55–57] Overweight and obese individuals have higher apoC-III_0/apoC-III_2 and apoC-III_1/apoC-III_2 ratios compared with healthy weight subjects, which correlated directly with TG levels and inversely with insulin sensitivity.[55] Accordingly, apoC-III_{0a} (non-glycosylated, non-sialylated apoC-III), apoC-III_{ob}, and apoC-III_1 levels, but not apoC-III_2, correlate with fasting TG levels,[55] and higher apoC-III_2 levels and apoC-III_2/apoC-III_1 ratio were associated with lower plasma levels of TG and small dense LDL particles in diabetics.[60] In patients with CAD, the relative abundance of apoC-III_0 decreased, whereas that of apoC-III_1 increased across

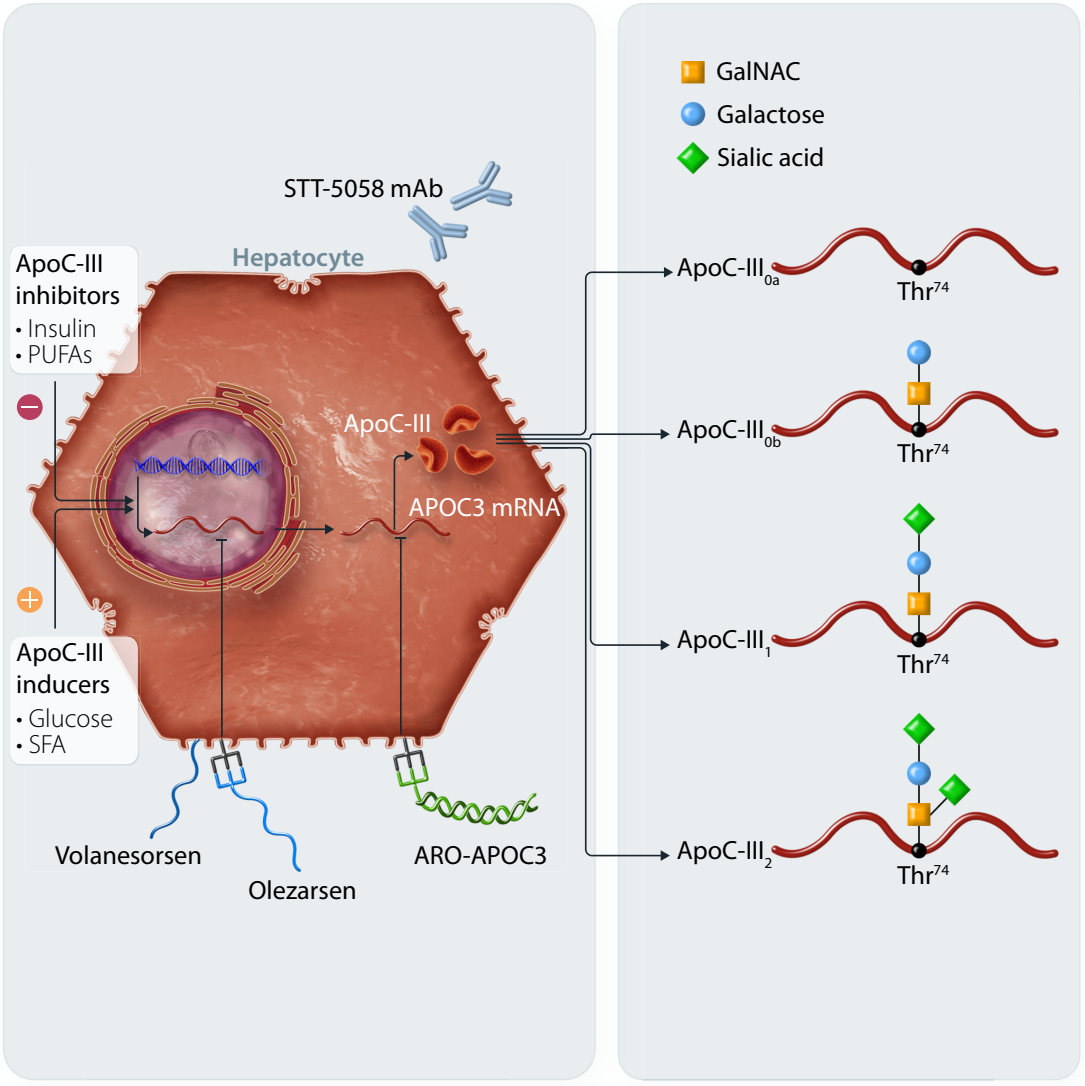


Figure 1 ApoC-III synthesis, regulation, and glycosylation; drugs targeting apoC-III. Apoc-III is mainly produced by hepatic cells (and to a lesser extent by enterocytes). Metabolic and nutritional factors modulate the transcription rate of the gene encoding apoC-III (*APOC3*) and include inhibiting factors (insulin and polyunsaturated fatty acids) and stimulating factors (glucose and saturated fatty acids). ApoC-III is secreted as a unique glycoprotein having one GalNAc linked to galactose and attached to Thr^{74}. A variable number of molecules of sialic acid can be present, resulting in three major glycoforms, referred to as apoC-III_{0b}, apoC-III_1, and apoC-III_2 containing 0, 1, and 2 molecules of sialic acid per molecule of protein, respectively. Drugs specifically targeting apoC-III include two ASOs (volanesorsen and olezarsen), a siRNA (ARO-APOC3), and a monoclonal antibody (STT-5058). GalNAC, *O-N*-acetyl-galactosamine; ASO, antisense oligonucleotide; siRNA, small interfering RNA.

apoC-III quartiles, and higher levels of apoC-III_1 correlated with a detrimental lipid profile.[56] A recent study has shown that apoC-III proteoform composition is associated with age, sex, race, and ethnicity in participants of Multi-Ethnic Study of Atherosclerosis, an observational community-based cohort.[61] In this cohort, apoC-III_2 levels were higher in older individuals, in males, and in Black and Chinese participants and were associated with a more favourable cardiometabolic profile [lower body mass index (BMI), fasting plasma glucose and TG levels, and higher plasma HDL cholesterol (HDL-C)].[61] Increased relative amount of apoC-III_{ob} was associated with a lower CV risk.[61] All these associations were independent of total plasma apoC-III levels,[61] suggesting that post-translational modifications of apoC-III may be influenced by clinical characteristics and may play distinct roles in regulating plasma lipids levels and future CV risk.

Heterozygous carriers of a rare loss-of-function mutation in *GALNT2*, encoding an enzyme involved in the glycosylation of apoC-III, show a 6.6-fold increase in the levels of apoC-III_0, a ~30% reduction in apoC-III_1, and similar levels of apoC-III_2 compared with non-carriers; an improved post-prandial TG clearance was observed, likely due to reduced glycosylation of apoC-III.[62] Plasma from homozygous patients carrying other *GALNT2* loss-of-function mutations contained only non-glycosylated apoC-III (apoC-III_0).[63] In hypertriglyceridaemic patients, treatment with an antisense oligonucleotide (ASO) targeting *APOC3* mRNA reduced total apoC-III and apoC-III_1 and increased apoC-III_2 relative abundance.[58] Two genetic variants of apoC-III have been described, one being oversialylated,[64] the other preventing O-glycosylation;[65] carriers of these variants are normolipidaemic, suggesting that the degree of apoC-III sialylation does not affect lipoprotein metabolism.

3. *APOC3* genetic variants and role in CVD

Earlier observations in transgenic mice expressing human *APOC3* reported mild hypertriglyceridaemia in animals with 1–2 copies of the human gene to severe hypertriglyceridaemia in animals containing ~100 gene copies, associated with an increase in plasma VLDL and a decrease in plasma HDL levels.[66] Conversely, targeted disruption of *APOC3* resulted in low levels of TGs and a reduced post-prandial response in mice.[67] In humans, the rare apoC-III Gln38Lys variant (a gain-of-function mutation that promotes VLDL1 production) was identified in a large kindred of Mexican origin; compared with unaffected relatives, heterozygous carriers showed a significant 32% increase in plasma TGs.[68,69] Loss-of-function mutations in the *APOC3* gene, on the other hand, are associated with a favourable lipid profile. Heterozygous carriers of the null mutation R19X had a lifelong 50% reduction in apoC-III associated with lower levels of TG compared with non-carriers, a blunted post-prandial TG dietary response, lower LDL-C levels, and higher HDL-C.[70] These subjects had a higher conversion rate of VLDL to LDL, with little effect on the direct hepatic uptake of VLDL.[71] This favourable lipid profile[70,72] was associated with a reduced incidence of subclinical atherosclerosis.[70] A study enrolling 10 000 Pakistanis identified 14 individuals carrying the R19X in homozygosity.[73] They had near-absent plasma apoC-III (−89%), lower TG levels (−60%), higher HDL-C, and unchanged LDL-C compared with non-carriers; the post-prandial rise in plasma TG was markedly blunted, suggesting that the almost complete lack of apoC-III not only is well tolerated but is also associated with lower fasting TGs and a substantial acceleration of the clearance of TG after a fatty meal.[73] Another two mutations were then identified in individuals being associated with lower apoC-III and TG levels.[74]

Mendelian randomization studies have established that loss-of-function mutations in the *APOC3* gene are associated with lower levels of plasma TG and with reduced risk of coronary heart disease (CHD) (*Figure 2*).[75,76] Among 110 970 study participants from 14 studies, subjects carrying rare mutations in *APOC3* had 39% lower plasma TG levels and 40% lower risk of CHD compared with non-carriers.[75] Similarly, data from 72 725 participants in 2 general populations showed that heterozygosity for loss-of-function mutations in the *APOC3* gene was associated with a 44% mean reduction in plasma TG levels and 41 and 36% reductions in the risk of ischaemic vascular disease and ischaemic heart disease, respectively.[76] A meta-analysis of ~137 000 subjects from 8 study cohorts showed that the lower risk of ischaemic vascular disease observed in carriers of *APOC3* loss-of-function mutations is mainly mediated by the associated low remnant (VLDL) cholesterol rather than by low LDL-C.[77]

Of note, ethnic differences in apoC-III plasma levels and the risk of CVD have been observed. The loss-of-function *APOC3* variants associated with reduced CV risk in European populations[75] did not show cardioprotective effects in other ethnic groups, thus challenging the generalizability of a beneficial effect from apoC-III inhibition.[78,79]

4. The link between hypertriglyceridaemia and apoC-III

Plasma TG levels vary widely in the population; a recent consensus statement from the European Atherosclerosis Society (EAS) defined as 'optimal' a TG level < ~100 mg/dL (<1.2 mmol/L) and classified various degrees of hypertriglyceridaemia from 'borderline' to 'moderately elevated', 'severe', and 'extreme'. As TG levels rise, there is a continuous increase in the risk of CHD and also a high risk of acute pancreatitis in those with extreme hypertriglyceridaemia (>880 mg/dL, 10.0 mmol/L).[4] While TG is found in all lipoprotein classes, the vast majority of TG is transported in CMs released from the intestine following a meal and in VLDL released from the liver (*Figure 3*). The main physiological role of these TRLs is to transport TGs to muscle cells for energy production and adipocytes for storage.

Liver-derived VLDL particles are 30–70 nm in diameter and contain a single copy of the very large protein apolipoprotein B100 (apoB100) as a key structural element. Newly secreted VLDL are metabolized in a delipidation cascade to VLDL remnants, intermediate-density lipoproteins (IDL), and LDL. The intestine, on the other hand, releases CMs, which are larger (>100 nm in diameter) and contain a single apoB48 protein, a truncated form of apoB100 made exclusively in the intestine. CMs are metabolized rapidly to remnant particles via the same LPL-mediated pathway used by VLDL. While CMs are produced mainly in the post-prandial state, there is a continuous release of apoB48-containing VLDL during a typical day.[80]

4.1 TRL assembly, secretion, and intravascular processing

The liver assembles and secretes a range of VLDL particles differing in TG content, size, and density. VLDL assembly is a multi-stage process that is initiated in the rough endoplasmic reticulum (RER) where apoB100 is synthesized and immediately lipidated by the action of microsomal triglyceride transfer protein (MTP) that adds TG to the growing apoB100–phospholipid complex,[81,82] forming VLDL precursors. This primordial particle is retained within the RER and either degraded (if insufficient lipid is

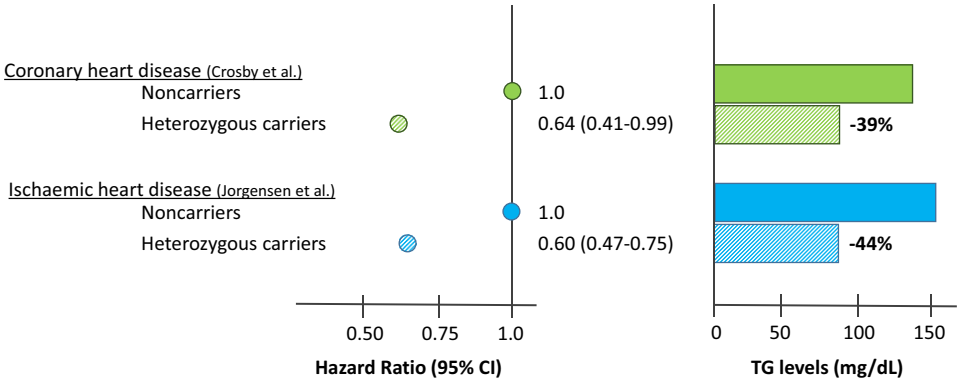


Figure 2 Loss-of-function mutations in *APOC3*, TG levels, and coronary disease. Risk of coronary disease (*left panel*) and mean plasma levels of triglycerides (*right panel*) in heterozygous carriers of loss-of-function mutations in *APOC3* and non-carrier individuals from two independent studies. Data were adapted from Crosby *et al.*[75] and Jorgensen *et al.*[76]

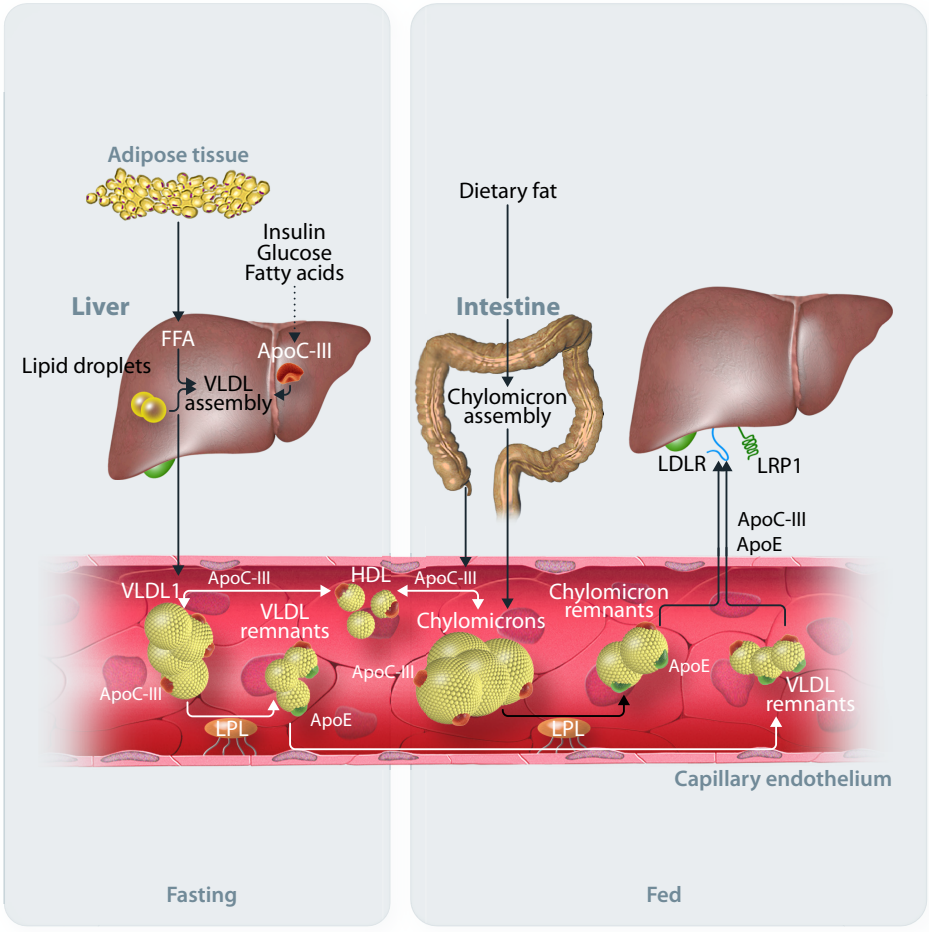


Figure 3 Role of apoC-III in TRL metabolism. ApoC-III is secreted on VLDL and may play a part in the assembly of large TG-rich VLDL (VLDL1). Upon entering the bloodstream, VLDL released by the liver and CM of intestinal origin acquire further apoC-III by transfer from HDL (which acts as a reservoir for the apoprotein). The rate of lipolysis of TRL via lipoprotein lipase present on capillary endothelium is governed in part by the ratio of apoC-II (a positive cofactor) and apoC-III (an inhibitor) on the particle's surface. After TG has been removed from newly secreted TRL, remnants are formed which are cleared by the liver. Interaction of these lipoprotein particles with hepatic receptors such as the LDLR and LDL receptor-related protein 1 (LRP1) is influenced by the presence of apoE (a ligand for these receptors) and apoC-III (which has an inhibitory effect on clearance) on these particles.

available) or further lipidated to become a VLDL2-sized particle[83] (*Figure 3*). These particles then pass to the smooth ER (SER) and the Golgi apparatus. VLDL2 can be secreted as it is or converted to larger VLDL1 by the addition of a further, major quantum of TGs in the form of lipid droplets in the lumen of the SER/Golgi.[83] The major determinant of VLDL particle size is the intrahepatic availability of TGs,[84] which mainly derives from the uptake by the liver of circulating fatty acids or from the lipolytic mobilization of hepatic storage pools[85] rather than *de novo* lipogenesis of TGs.[86] VLDL metabolism is governed by several factors including particle size and lipid and apoprotein composition. ApoC-III may play a role in hepatic VLDL assembly and secretion. Heterozygous carriers of the loss-of-function mutation K58E exhibit lower levels of VLDL and VLDL–apoC-III in circulation.[87] In model systems, this mutation, located at the C-terminal domain, impairs the lipid binding capacity of apoC-III and so hampers the formation of luminal lipid droplets, resulting in reduced VLDL1 assembly.[88] Conversely, the missense mutation A23T located at the N-terminal domain does not impair the binding of apoC-III to lipid droplets; rather, its presence is associated with the accumulation of droplets in the microsomal lumen, suggesting impaired fusion between lipid droplets and VLDL2 precursor particles.[88] These findings suggest a 'two-domain' model for apoC-III, with the C-terminal domain being a prerequisite for lipid droplet formation and binding and the N-terminal promoting the fusion between lipid droplets and pre-VLDL.[88] However, care must be taken in translating these *in vitro* observations into the complex dynamic system that is human hepatocyte physiology; in fact, carriers of *APOC3* loss-of-function mutations have normal VLDL–apoB100 and VLDL–TG production rates.[71,89] In the circulation, HDL particles can transfer apoproteins to the VLDL particle surface, which promotes lipolysis (apoC-II) and clearance (apoE). ApoC-III and apoE are not distributed uniformly among VLDL particles (but also IDL and LDL): in normolipidaemic people, 35–60% of VLDL possess apoE, 40–80% possess apoC-III, and some particles have both apoC-III and apoE.[14] Under hypertriglyceridaemic conditions, apoC-III secretion is higher, which translates into a higher secretion of light and dense VLDL E–/C-III+ and less of VLDL E+/C-III+.[14] In humans with hypertriglyceridaemia, a shift in the secretion of VLDL from the apoE subspecies to the apoC-III subspecies or subspecies having neither apoE nor apoC-III can be observed.[14] Compared with their nascent precursors, TRL remnants are depleted in TG, phospholipids, and apoCs and are enriched in cholesterol esters and apoE.[90] Conceptually, apoC-III and apoE have opposing effects on the processing of apoB-containing lipoproteins

and thus play a crucial role in determining their metabolic fate.[14] ApoE acts as a high-affinity ligand for the LDL receptor (LDLR) and other hepatic receptors and proteoglycans. Thus, VLDL containing apoE are cleared much faster than VLDL not containing apoE,[91] and kinetic studies in humans showed that VLDL and IDL containing apoE but not apoC-III were cleared rapidly from the circulation before they can be metabolized to smaller lipoproteins. Thus, the presence of apoC-III on VLDL particles appears to retard their clearance.

Enterocytes use apoB48-containing precursor particles for CM assembly during the post-prandial state in a pathway that is analogous to the VLDL secretory mechanism in the liver.[92] At the inner ER membrane, apoB48 is lipidated by MTP leading to the formation of pre-CMs, which are transferred from the ER to the Golgi apparatus, further lipidated to form mature CMs, and then secreted.[92]

Newly secreted VLDL and CM enter the bloodstream where they acquire several important regulatory apoproteins (apoC-II, apoC-III, and apoE) mainly from HDL. At the luminal surface of capillaries in adipose tissue and skeletal muscle, TGs within these TRLs are hydrolysed by the action of LPL in a process dependent on the presence of apoC-II on the particle's surface. Lipolysis generates free fatty acids, which are then absorbed by the tissues.[93] Lipolysis is tightly regulated by several proteins, including apoC-III; ANGPTL3, 4, and 8; and apoA-V.[94,95]

Following the hydrolysis of TGs, CMs are converted into smaller, cholesterol-enriched remnant lipoproteins. Since apoB48 lacks the main LDLR-binding domain, which resides in the C-terminal portion of the protein, apoB48-containing lipoproteins need to acquire apoE from other lipoproteins in the bloodstream to interact with the hepatic lipoprotein receptors responsible for their clearance. CM remnants can also be cleared following the binding to HSPG on hepatocytes.[96,97] Likewise, after TG hydrolysis, VLDL are converted into cholesterol-rich VLDL remnants and IDL, which can be removed by the liver through the binding of apo B100 and/or acquired apoE to LDLR or further lipolysed and converted into LDL. The TRL remnant removal pathways in the liver appear to be high capacity, and, once TG delivery has been accomplished, it facilitates the rapid clearance of the residual particles.

Remnants are a heterogeneous spectrum of lipoproteins. As proposed in the recent EAS consensus statement, remnants could be divided conceptually into 'transient remnants', which are capable of further lipolysis, and 'end-product remnants', which are resistant to further lipolysis and remain in circulation until their removal by the liver.[4] In people with a highly efficient lipolytic system (high LPL, low apoC-III), there is a rapid conversion of VLDL to LDL and little opportunity for remnant formation. If lipolysis is impaired (low LPL activity, increased apoC-III), the VLDL particle residence time in circulation increases, leading to a remodelling characterized by the acquisition of cholesteryl esters and apoE and the generation of a lipolysis-resistant particle.[98] The level of TRL remnants in the circulation is the net result of the balance between (i) TRL production, (ii) the rate of lipolysis, (iii) the rate of formation of VLDL remnants, (iv) the rate of further lipolysis to LDL involving hepatic lipase, and (v) the efficiency of remnant hepatic removal. Likely, remnants differ widely in their atherogenic potential linked to the variation in size and composition. Having a reduced size compared with large, newly secreted TRLs, remnant particles can enter and be retained in the subendothelial space and contribute to the process of atherosclerotic lesion formation and progression.[93]

4.2 Role of apoC-III in the regulation of triglyceride transport and development of hypertriglyceridaemia

In humans, apoC-III is a major regulator of TG levels. Plasma apoC-III concentration is elevated in hypertriglyceridaemia, is strongly inversely related to VLDL clearance rates, and is an independent determinant of TG and VLDL levels.[99] Individuals carrying a loss-of-function variant in *APOC3* exhibit accelerated CM metabolism, VLDL–TG lipolysis, VLDL–apoB100 fractional catabolic rate, and conversion to LDL even in the heterozygous state (where apoC-III concentration is reduced by 50–60%).[71,100] The possible mechanisms by which apoC-III inhibits LPL-mediated lipolysis appear to include both the displacement of the LPL activator apoC-II from the TRL surface and reduced binding of TRLs to the capillary endothelial sites where LPL is located.[101] The levels of apoC-III and apoC-II on TRLs, through their opposing actions on LPL, are thus major determinants of LPL-mediated lipolytic activity, with deficiency of plasma apoC-II and overexpression of apoC-III being associated with marked hypertriglyceridaemia and TRL elevation.[102]

While apoC-III is believed to increase plasma TG levels primarily by inhibiting LPL activity, LPL-independent activities have also been suggested. Suppressing apoC-III synthesis with an ASO significantly reduced TG levels in mice lacking LPL.[103] Further, the ASO was found to reduce TG levels in mice lacking not only LPL but also HSPG, LDLR, or LRP1. However, in mice lacking both LDLR and LRP1, TG was not decreased, indicating that apoC-III action required the presence of at least one of these receptors. These findings suggest that apoC-III on TRLs can modulate TRL metabolism by inhibiting the hepatic clearance of remnants mediated by LDLR and LRP1. In these animal models, the administration of an ASO against *APOC3* did not affect hepatic VLDL production, heparin-induced TG reduction, or uptake of lipids into the heart and skeletal muscle.[103] Accordingly, in patients with familial chylomicronaemia syndrome (FCS) due to inactivating mutations in the *LPL* gene, treatment with the ASO volanesorsen reduced TG levels substantially,[104] and in individuals with *APOC3* loss-of-function variants, the rates of direct VLDL particle clearance were higher than in control subjects.[100] Taken together, these findings reveal that apoC-III retards hepatic TRL clearance via hepatic LDLR and LRP1 in an apoE-dependent manner, but it is still unclear the reason why syndecan1-mediated TRL clearance is not altered by apoC-III.[105]

ApoC-III plays a central role in the development of hypertriglyceridaemia in cardiometabolic conditions such as obesity and type 2 diabetes. Elevated blood glucose and insulin resistance/deficiency are associated with dysregulated synthesis and secretion of apoC-III leading to overproduction.[106,107] In centrally obese subjects, the production of large TG-rich VLDL (VLDL1) and apoC-III increases, due possibly to hepatic insulin resistance. Circulating VLDL particles in these conditions contain more apoC-III, with consequences for the rates of lipolysis and clearance of TRLs.[108] Individuals with visceral obesity had higher secretion rates and lower catabolic rates for VLDL apoB and reduced conversion of VLDL to IDL and LDL compared with lean controls.[109] Furthermore, plasma levels of apoC-III were associated positively with VLDL–apoB secretion rate and inversely with VLDL–apoB FCR and percent conversion of VLDL to LDL–apoB.[109] Accordingly, plasma levels of VLDL–apoC-III significantly and independently predicted delayed catabolism of VLDL–TG and VLDL–apoB in men with a wide range of BMI and mild dyslipidaemia.[110] The altered metabolism of apoC-III in type 2 diabetic patients is amenable to intervention; improvement in the glycaemic control with liraglutide significantly reduced the apoC-III secretion rate but not its fractional catabolic rate.[111]

In addition, insulin resistance locally up-regulates apoC-III expression within pancreatic islets, with a negative autocrine effect on ß-cell survival[112–114] and a paracrine effect on neighbouring islet cells. ApoC-III can induce dysglycaemia by several mechanisms, including apoptosis of pancreatic β-cells, enhancement of insulin resistance, impairment of the antidiabetogenic properties of HDL particles, and development of obesity and hyperglycaemia through the inhibition of LPL in the hypothalamus.[107] Accordingly, recent studies showed that obese adolescents and lean adolescents with insulin resistance have higher levels of apoC-III (and ANGPTL3), together with TRL dyslipoproteinaemia, compared with lean adolescents without insulin resistance.[115,116]

4.3 TRLs, apoC-III, and atherosclerosis

A large body of evidence suggests that TRLs and their remnants, as well as specific players involved in TG metabolism, such as LPL and apoC-III, contribute to the process of atherogenesis, both directly and indirectly. Most attention to date has focussed on remnant lipoproteins deriving from delipidation (with loss of the TG core) of CM and VLDL. Compared with 'parent' newly secreted particles, they are enriched in cholesterol,

cholesteryl ester, apoE, and apoC-III.[97,117,118] Being smaller than newly secreted TRLs, TRL remnants can more easily enter the arterial intima, bind to extracellular matrix proteoglycans, and be taken up by local macrophages, leading to foam cell formation and inflammation in the arterial wall.[119,120]

The apoC-III content of apoB-containing lipoproteins (VLDL, IDL, and LDL) modulates the interaction of these lipoproteins with arterial proteoglycans, with apoC-III-enriched lipoproteins showing the highest affinity for proteoglycans.[117] Small, dense LDL, purportedly the most atherogenic LDL species, have an increased affinity for arterial proteoglycans and a higher content of apoC-III,[32,117,121] which also renders the lipoprotein much more susceptible to hydrolysis and aggregation by sphingomyelinases.[122] ApoC-III-enriched apoB-containing lipoproteins can increase the expression of cell adhesion molecules, thus activating endothelial cells, and favour the adhesion of monocytes.[30,31] It is interesting to note that volanesorsen therapy substantially reduces the content of apoC-III on all apoB-containing lipoproteins, Lp(a), and HDL in hypertriglyceridaemic patients.[123]

The pro-atherogenic effects of apoC-III can also be extended to HDL. HDL enriched with apoC-III exhibit unfavourable alterations in their composition and function, such as a reduced capacity to promote cholesterol efflux and diminished ability to inhibit endothelial cell apoptosis.[124–126] Classifying HDL species according to apoC-III content allows the identification of two subclasses exhibiting opposing associations with the risk of CHD.[127] In healthy people with normal body weight, about 7% of total plasma apoA-I in HDL is associated with apoC-III, but this percentage doubles in obese subjects (who also present with lower HDL-C and higher TG levels).[128] A meta-analysis of four prospective studies of adults free of CHD has shown that HDL containing apoC-III was associated with an increased risk of CHD, whereas HDL lacking apoC-III was associated with a lower risk.[37] In a multi-ethnic population, higher baseline apoC-III levels were positively associated with coronary artery calcium (CAC) in men and with carotid plaque and IMT in both men and women; HDL lacking apoC-III was inversely associated with all measures of subclinical atherosclerosis, whereas HDL containing apoC-III was positively associated with CAC and carotid plaque.[36] According to this observation, low apoE-HDL-C is strongly associated with early coronary plaque development, an association even stronger when low apoE-HDL-C and high apoC-III in HDL are combined.[40] Of note, treatment with the cholesteryl ester transfer protein (CETP) inhibitors evacetrapib and torcetrapib increased apoA-I in HDL subspecies containing apoC-III; furthermore, both drugs increased HDL-containing apoE but only the subtype that also contained apoC-III, which is associated with higher CV risk.[129] These changes in HDL particle composition may help explain the lack of clinical benefit of CETP inhibition in major outcome trials. We must also highlight that an enrichment of HDL with apoC-III was observed in subjects with lower estimated glomerular filtration rate (eGFR),[130] that HDL containing apoC-III is associated with a reduction in insulin sensitivity (being HDL lacking apoC-III associated with an increase),[131] that HDL lacking apoC-III is associated with a lower incidence of diabetes (while those not containing apoC-III did not),[132] and that higher levels of apoE in HDL lacking apoC-III (but not in HDL containing apoC-III) were associated with a lower risk of dementia or Alzheimer's disease and better cognitive function,[133] suggesting that, overall, the presence of apoC-III exerts a detrimental impact on protective HDL functions.

Most of the day is spent in the post-prandial state, and lipoproteins that accumulate in the circulation during post-prandial periods (CMs and their remnants, large VLDL1 and their remnants, and apoB48-containing VLDL released during fat absorption) may be especially atherogenic.[100] Furthermore, compositional changes occur in other lipoprotein classes, particularly HDL, in response to the high levels of TRLs in the post-prandial state. Post-prandial hypertriglyceridaemia (PPT), a condition in which TRLs and remnants rise to excess levels following dietary fat ingestion, is associated with an increased risk of CHD and type 2 diabetes. Subjects with PPT have higher levels of fasting and post-prandial apoC-III, which is an independent risk factor for PPT.[134] Whereas in normotriglyceridaemic subjects the levels of apoC-III carried by VLDL may be not sufficient to inhibit markedly LPL activity, in hypertriglyceridaemic subjects, elevated levels of apoC-III can both inhibit substantially LPL-mediated lipolysis and interfere with the binding of apoB/apoE to hepatic receptors, thus delaying TRL clearance. The *APOC3 SstI* polymorphism, which has been correlated with hypertriglyceridaemia,[135] has been associated with higher PPT and post-prandial CM TG levels in patients with metabolic syndrome.[136]

A recent study assessed the role of apoC-III-HDL in the post-prandial response. After a meal, apoC-III content in HDL increased while decreasing in non-HDL particles (total apoC-III was unchanged); this was accompanied by a proportional increase in post-prandial TG levels. The enrichment of apoC-III in HDL might retard the transfer of apoC-II to nascent VLDL and CM, thus interfering with both the TG lipolysis process and the anti-atherogenic functions of HDL.[124] As more is learned about the nature and function of lipoproteins in the post-prandial state, the cardinal role of apoC-III in regulating TG metabolism will become clearer, as will the mechanisms by which elevated levels of apoC-III increase CHD risk.

5. ApoC-III as a pharmacological target

The observation that lifelong low levels of TG due to loss-of-function mutations in *APOC3* are associated with reduced risk of ischaemic vascular disease has suggested apoC-III as a crucial drug target for reducing residual CV risk.[76] All commonly used lipid-modulating drugs have modest effects on apoC-III levels, with reductions ranging from 10 to 30% for fibrates, fish oils, niacin, statins, and ezetimibe, which in many cases are not sufficient to achieve optimal TG levels. To address this unmet need, drugs have been developed to inhibit specifically and substantially apoC-III (*Figure 1*).

5.1 Volanesorsen

Volanesorsen is a second-generation ASO that specifically targets *APOC3* mRNA, inducing its degradation through RNAse H1-mediated cleavage. In a dose-ranging phase 2 study, volanesorsen produced dose-dependent reductions up to 80% in apoC-III and up to 71% in TG levels when used as monotherapy or added to stable fibrate therapy in patients with hypertriglyceridaemia (*Table 1*).[137] VLDL apoB was decreased by 62.7% from baseline in the 300 mg dose group in monotherapy; apoB48 was also substantially decreased (61.1%), whereas LDL-C and LDL–apoB increased and non-HDL-C and total apoB were relatively unchanged.[137] Volanesorsen reduced significantly apoC-III carried by all classes of lipoproteins.[123] Volanesorsen also reduced apoC-III and TG levels in patients with type 2 diabetes and hypertriglyceridaemia by 87.5 and 69.1%, respectively, accompanied by a 57% improvement in insulin sensitivity.[138] This marked reduction in TG did not associate with increases in LDL-C; non-HDL-C and apoB decreased non-significantly.[138] The observed significant improvement in insulin sensitivity might produce a beneficial impact following volanesorsen therapy independently of its lipid-modifying effects, at least in diabetic patients.

The efficacy of volanesorsen has been then assessed in patients with FCS, a rare (1:1 000 000) autosomal recessive genetic disease mainly caused by mutations in the *LPL* gene, although mutations in other genes encoding proteins required for LPL activity can also cause this condition.[142,143] FCS is characterized by extreme elevations in blood TG due to severe impairment of CM metabolism and clearance; patients with FCS have a high risk of acute and recurrent pancreatitis.[143] Conventional TG-lowering drugs are largely ineffective in these patients, who require a substantial reduction of TG levels below the threshold for pancreatitis (500–885 mg/dL) to relieve symptoms and reduce the risk of pancreatitis. Three patients with FCS received 300 mg volanesorsen once weekly for 13 weeks in an open-label study, resulting in reductions of 71–90% in apoC-III and 56–86% in TG levels compared with baseline (*Table 1*).[104] The results of this small study paved the road for the APPROACH trial, in which 66 patients with FCS received 300 mg volanesorsen or placebo once weekly for 52 weeks (*Table 1*).[139] Volanesorsen reduced apoC-III and TG levels by 84 and 77%, respectively, whereas placebo increased by 6 and 18%; a mean

Table 1 Effect of volanesorsen on lipid and lipoprotein profiles in patients with hypertriglyceridaemia

Studies	Patients	Mean percent changes	Mean percent changes in other parameters
Phase 2[137] Dose-ranging Cohort (a): monotherapy Cohort (b): added to fibrate	Severe or uncontrolled HTG	ApoC-III: ↓39.2–79.9% (a); ↓60.5–71.7% (b) TG: ↓31.3–70.9% (a); ↓51–64% (b)	VLDL-C: ↓40.0–69.2% (a); ↓54.3–63.2% (b) VLDL–apoB: ↓7.5–62.7% (a); ↓44.1–64.4% (b) LDL-C: ↑48.3–118.3% (a); ↑3.5–21.0% (b) LDL–apoB: ↑21.0–46.4% (a); ↑4.0–12.0% (b) HDL-C: ↑28.1–46.0% (a); ↑47.5–49.4% (b)
Phase 2[138]	T2D + HTG (TG > 200 mg/dL, <500 mg/dL)	ApoC-III: ↓87.5% TG: ↓69.1%	VLDL–apoC-III: ↓90.2% HDL-C: ↑42.5%
Open-label study[104]	3 patients with FCS	ApoC-III: ↓71–90% TG: ↓56–86%	CM–TG: ↓58–91% VLDL–TG: ↓44–79% Non-HDL-C: ↓46–74% ApoB-48: ↓15–82% TC: ↓40–69% LDL-C: ↑3–173% HDL-C: ↑21–163%
APPROACH[139] Phase 3	66 patients with FCS	ApoC-III: ↓84.2% TG: ↓76.5%	CM–TG: ↓82.7% ApoB48: ↓75.9% Non-HDL-C: ↓45.9% VLDL-C: ↓58.3% ApoB: ↑19.5% LDL-C: ↑135.6% HDL-C: ↑46.1%
COMPASS[140] Phase 3	Patients with FCS or MCS	ApoC-III: ↓76.1% TG: ↓71.2%	CM–TG: ↓78.1% ApoB-48: ↓71.1% Non-HDL-C: ↓27.3% VLDL-C: ↓71.5% LDL-C: ↑95.5% HDL-C: ↑61.2%
BROADEN[141] Phase 3	Patients with FPLD	TG: ↓65.4%	HFF: ↓53.3% VAT: no change HbA_{1c}: no change

apoC-III, apolipoprotein C-III; TG, triglycerides; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; apoB, apolipoprotein B; apoB-48, apolipoprotein B-48; CM, chylomicron; T2D, type 2 diabetes; HTG, hypertriglyceridaemia; FCS, familial chylomicronaemia syndrome; MCS, multifactorial chylomicronaemia syndrome; FPLD, familial partial lipodystrophy; HFF, hepatic fat fraction; VAT, visceral adipose tissue; HbA1c, glycated haemoglobin

absolute decrease of 1712 mg/dL in TG levels was observed in the volanesorsen group (+92 mg/dL in the placebo group).[139] Patients receiving volanesorsen had reductions in CM TG (−83%), apoB48 (−76%), non-HDL-C (−46%), and VLDL-C (−58%); of note, total apoB increased by 20% and LDL-C levels increased even more substantially (+136%), but the baseline LDL-C was very low (28 mg/dL) and reached 61 mg/dL at 3 months and thus was considered to not be of concern.[139] Among patients who experienced multiple previous episodes of pancreatitis, no episodes of acute pancreatitis were reported during treatment with volanesorsen; three patients in the placebo group had four episodes of acute pancreatitis, whereas one patient in the volanesorsen group had one episode (9 days after receiving the final dose).[139] A major concern with the use of ASO is the development of thrombocytopoenia, defined as a platelet count <140 000/μL; this may be even more relevant in patients with FCS, who suffer from important asymptomatic fluctuations in platelet counts over time.[144] In the APPROACH trial, 76% of the patients treated with volanesorsen showed a platelet count reduction <140 000/μL (vs. 24% in the placebo group); since two patients had platelet count <25 000/μL, more stringent monitoring of platelet count was initiated, allowing no further platelet-related discontinuations of volanesorsen therapy.[139]

The phase 3 COMPASS trial assessed the safety and efficacy of volanesorsen in patients with multifactorial chylomicronaemia syndrome (MCS) or FCS (*Table 1*).[140] Based on the concerns on thrombocytopoenia raised in the APPROACH trial, patients received 300 mg volanesorsen once a week for 13 weeks and then every 2 weeks for the rest of the trial period. After 3 months, volanesorsen reduced mean plasma apoC-III by 76.1% and TG levels by 71.2% (compared with −3.3 and −0.9%, respectively, in the placebo group).[140] Mean absolute lowering of TGs was 869 mg/dL in the volanesorsen group compared with an increase of 74 mg/dL in the placebo group.[140] In line with the TG reduction, apoB-48 was reduced by 71.1% (compared with a 5.3% increase in placebo). Importantly, the efficacy of volanesorsen was independent of the cause of severe hypertriglyceridaemia. A significant increase in LDL-C levels was observed in patients receiving volanesorsen compared with placebo (+95.5 and +4.7%, respectively), despite apoB being unchanged, suggesting that the overall number of atherogenic particles did not change.[140] Contrarily to patients enrolled in the APPROACH trial, LDL-C levels were higher at baseline (64 mg/dL) and reached 111 mg/dL after 3 months of treatment with volanesorsen.[140] The increase in LDL-C appears to be a re-equilibration to ambient levels with the normalization of apoC-III metabolism and can be effectively treated with traditional lipid-modifying measures, including statin therapy. During the study, one patient in the placebo group and nine in the volanesorsen group showed platelet counts <100 000/μL.[140] The five acute pancreatitis events observed during the trial occurred in three patients in the placebo

group.[144] In the APPROACH-OLE (open-label) study, patients with FCS enrolled in the two previous studies (APPROACH and COMPASS) have received volanesorsen for an additional 52-week treatment period and a 13-week post-treatment evaluation period.[145] The treatment with volanesorsen reduced substantially TG levels in patients with FCS, both in those who were treatment naïve and those who received treatment in the index studies.[145] The safety profile was consistent with those observed in the index studies.[145] The risk of thromobocytopoenia (associated with no or mild bleeding) appears to be manageable by platelet monitoring, dose adjustment, or treatment pause.[145] A pooled analysis of randomized controlled trials has shown that volanesorsen reduces significantly TG, VLDL-C, apoB48, and non-HDL-C while increasing in patients with severe HTG, with an acceptable safety profile.[146]

The BROADEN study assessed the effect of volanesorsen in patients with familial partial lipodystrophy (FPLD), a rare genetic disorder characterized by abnormal fat distribution across the body and metabolic abnormalities including hypertriglyceridaemia, insulin resistance, and hepatic steatosis.[141] Patients with FPLD often have insulin resistance, greater risk of acute pancreatitis, premature CVD, and severe liver disease.[147] After 3 months, volanesorsen reduced TG by 67% compared with placebo, a reduction that persisted up to 12 months during the study and up to Week 123 in the open-label extension period.[141] Reductions in TG levels were associated with significant reduction in hepatic steatosis and no increase in visceral adipose tissue.[141] This study reported a consistent long-term safety profile, with no signs of hepatic renal and cardiac safety.[141]

The analysis of hepatic fat fraction in patients with severe hypertriglyceridaemia participating in COMPASS, APPROACH, and BROADEN studies has shown that volanesorsen significantly reduced the absolute hepatic fat fraction compared with placebo, suggesting a favourable effect in patients with HTG of different origin.[148]

5.2 Olezarsen

Olezarsen (AKCEA-APOCIII-L_{Rx}) is an *N*-acetylgalactosamine-conjugated ASO that targets selectively the hepatic synthesis of apoC-III. The nucleic acid sequence is identical to volanesorsen, but it differs from volanesorsen in its GalNAc moiety and the linker, ensuring a less frequent administration of significantly lower doses for a longer duration of action. In a phase 1/2a trial, olezarsen induced dose-dependent reductions in apoC-III (up to 80%) and TG (up to 77%) levels, with an overall improvement in lipid profile in healthy volunteers with TG levels ≥90 or ≥200 mg/dL (*Table 2*).[149] Contrarily to what was observed with volanesorsen, the treatment with olezarsen produced a broad improvement in the atherogenic lipid profile.[149] When administered to patients with moderate hypertriglyceridaemia (200–500 mg/dL; 2.26–5.65 mmol/L) at high risk for or with established CVD, olezarsen dose-dependently reduced TG levels, ranging from 23% with 10 mg every 4 weeks up to 60% with 50 mg every 4 weeks or 10 mg every week, compared with a 6% increase in the pooled placebo group (*Table 2*).[150] ApoC-III levels were reduced up to 74%.[150] Overall, olezarsen treatment improved the lipoprotein profile, with reductions in TRLs, remodelling to larger LDL particles, and an increase in small HDL particle number.[150] Olezarsen reduced other atherogenic lipoproteins including VLDL-C and non-HDL-C and increased HDL-C; the effects on apoB reduction were modest and LDL-C was unchanged;[150] a more

Table 2 Other drugs targeting apoC-III

Drug	Type of drug	Studies	Patients	ApoC-III and TG mean percent changes	Changes in other parameters
Olezarsen	ASO + GalNAC	Dose escalation Phase 1/2a Cohorts (a): single-dose Cohorts (b): multiple-dose[149]	Healthy volunteers (TG ≥ 90 or ≤200 mg/dL)	ApoC-III: ↓0–92% (a); ↓65.4–84.3% (b) TG: ↓7–77% (a); ↓60.7–70.5% (b)	VLDL-C: ↓2.0–81.2% (a); ↓40.1–72.9% (b) Non-HDL-C: ↓4.5–25.6% (a); ↓21.8–30.7% (b) ApoB: ↓15.9–26% (a); ↓15.4–30.2% (b) HDL-C: ↑7.3–63.3% (a); ↑49.6–75.8% (b)
		Dose-ranging Phase 2[150]	Moderate HTG (TG 200–500 mg/dL) at high risk for or with established CVD	ApoC-III: ↓29–74% TG: ↓23–60%	VLDL-C: ↓27–58% Non-HDL-C: ↓6–24% ApoB: ↓7–17% HDL-C: ↑11–40%
		Study of Olezarsen in Adults with FCS-Phase 3-NCT05130450 (BALANCE)			
		Study of Olezarsen in Adults with FCS Previously Treated with Volanesorsen-Phase 3-NCT05185843 (BALANCE OLE)			
		Study of Olezarsen in Adults with Severe HTG (TG ≥ 500 mg/dL)-Phase 3-NCT05079919 (CORE)			
		Study of Olezarsen Administered Subcutaneously to Participants with Severe HTG-Phase 3-NCT05552326			
ARO-APOC3	siRNA	Dose escalation Phase 1[151]	Healthy volunteers	ApoC-III: ↓94% TG: ↓74%	HDL-C: ↑42–84%
		Dose escalation Phase 1[152]	FCS, MCS	ApoC-III: ↓98.2%, ↓96% TG: ↓91.3%, ↓89.8%	Non-HDL-C: ↓58.3%, ↓48.6% HDL-C: ↑152.4%, 110.8%
		Study of ARO-APOC3 in Adults with Dyslipidemia-Phase 2-NCT05413135			
		Study of ARO-APOC3 in Adults with Mixed Dyslipidemia-Phase 2-NCT04998201 (MUIR)			
		Study to Evaluate ARO-APOC3 in Adults with Severe Hypertriglyceridemia-Phase 2-NCT04720534 (SHASTA-2)			
		Study of ARO-APOC3 in Adults with Familial Chylomicronemia Syndrome (FCS)-Phase 3-NCT05089084 (PALISADE)			
STT-5058	Humanized mAb	A First in Human Study of STT-5058, an Antibody That Binds APOC3-Healthy volunteers-Phase 1-NCT04419688			

ASO, antisense oligonucleotide; GalNAC, *N*-acetylgalactosamine; siRNA, small interfering RNA; mAb, monoclonal antibody; TG, triglycerides; apoC-III, apolipoprotein C-III; VLDL-C, very-low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; apoB, apolipoprotein B; HTG, hypertriglyceridaemia; FCS, familial chylomicronaemia syndrome; MCS, multifactorial chylomicronaemia syndrome

detailed analysis showed that TRL particle concentrations were largely reduced (51%), large LDL particles increased (+186%), small LDL particles reduced (39%), and HDL particles increased (15%), with an overall net benefit on atherogenic lipoproteins.[153]

So far, olezarsen shows more favourable tolerability and safety profile than volanesorsen, with no evidence of thrombocytopoenia.[149] Several phase 3 clinical trials are currently ongoing for a further assessment of the efficacy and safety of olezarsen (*Table 2*), which include the BALANCE study in FCS (NCT05130450), an open-label extension of BALANCE (NCT04568434), and the CORE study in ~540 patients with severe hypertriglyceridaemia (NCT05079919), as well as several other supporting studies.

5.3 siRNA

ARO-APOC3 is a siRNA that provides a durable inhibition of *APOC3* synthesis in the liver. Preliminary data have been presented in abstract form. In healthy volunteers, the subcutaneous administration of ARO-APOC3 on Days 1 and 29 at different doses (10, 25, and 50 mg) dose-dependently reduced apoC-III (up to 94%) and TG levels (up to 74%); such reductions persisted for at least 10 weeks after the second dose, and good tolerability over 16 weeks was reported (*Table 2*).[151] A modest LDL-C reduction was observed; the most common adverse events were mild reactions at the injection site and headache.[151] In a subsequent study in 4 genetically confirmed FCS patients and 26 MCM patients, ARO-APOC3 reduced apoC-III by 98 and 96% and TG by 91 and 90%, respectively, and non-HDL-C was substantially reduced in both groups (58.3 and 48.6%, respectively), with comparable safety profile (*Table 2*).[152] Injection sites reactions were mild and present in 19.5% of subjects. Liver function test (LFT) abnormalities were noted in 7/41 (17%) of subjects on active drug. Ongoing trials (*Table 2*) are evaluating the efficacy and safety of ARO-APOC3 in adults with mixed dyslipidaemia (NCT04998201), severe hypertriglyceridaemia (NCT04720534), and FCS (NCT05089084); a phase 2 open-label extension study to will assess the long-term safety and efficacy of ARO-APOC3 in adults with dyslipidaemia (NCT05413135).

Recently, two siRNAs targeting apoC-III have been developed and used as a mix with the aim of reducing the dose of each siRNA and thereby reducing the molecule-specific side effect.[154] In an obese mouse model of diabetes, intravenous injections of the siRNA mix encapsulated in lipid nanoparticles resulted in the selective and liver-specific inhibition of apoC-III expression.[154] Metabolic improvements (including cessation of weight gain, improved insulin sensitivity and glucose tolerance, lowering of liver and circulating TGs) have been observed during a 9-week treatment period.[154]

5.4 STT-5058

STT-5058 is a human antibody targeting plasma apoC-III that lowers TG and increases TRL clearance.[155] STT-5058 is characterized by a high affinity to apoC-III at pH 7.4; at acidic pH, it dissociates from the antigen and can be recycled from intracellular endosomes, enabling multiple clearance cycles of apoC-III-containing lipoproteins.[155] A phase 1 double-blind, randomized, placebo-controlled trial is currently evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple ascending intravenous doses and ascending subcutaneous doses of STT-5058 in healthy volunteers with elevated TG levels (>150 mg/dL, >1.7 mmol/L) or patients with moderate hypertriglyceridaemia (TG >200 mg/dL, 2.2 mmol/L) (NCT04419688) (*Table 2*).

5.5 Other approaches

The TG-lowering activity of an apoC-II mimetic peptide (D6PV) has been assessed in a mouse model of hypertriglyceridaemia; D6PV increased TG lipolysis both activating LPL and antagonizing apoC-III, resulting in a substantial reduction in TG levels.[156] DP6V also reduced plasma apoC-III by ~85%, as well as apoC-III bound to VLDL, LDL, and HDL. Of note, DP6V showed a high affinity for HDL and potentiated HDL-C efflux capacity.[156] Despite its effectiveness in reducing TG levels, D6PV had several limitations in terms of drug development, including a potential immunogenicity and lack of modifications that can improve its resistance to proteolysis. To overcome these issues, next-generation apoC-II mimetic peptides have been developed, which exhibit a greater resistance to proteolysis while retaining the TG-lowering activity[157]

6. Conclusions

The prevalence of hypertriglyceridaemia is increasing worldwide especially in Western countries, but to date, there are only a few approaches that can substantially reduce TG levels. Fibrates, the agents with the most effective TG-lowering properties, have been shown recently to be ineffective in reducing CV risk. While there is clear evidence that targeting apoC-III is a valuable tool to reduce severe hypertriglyceridaemia-related pancreatitis, the proof that lowering apoC-III levels may provide a CV benefit is still lacking. Similarly, alternative approaches that reduce substantially TG levels by inhibiting ANGPTL3 (which also reduces significantly LDL-C levels) still lack evidence of CV benefit.[158–160]

Although genetic studies support the concept that TRLs are causal factors for ASCVD, we must emphasize that the effect on TGs in Mendelian randomization studies is relatively small and could reflect a change in the levels of 'normal' VLDL. As the heterogeneity of apoC-III-containing lipoproteins is quite large and the possibility of a disconnection between TG reduction and apoB reduction exists, the question of whether the Mendelian randomization findings will translate into a definitive beneficial effect of TG-lowering on ASCVD in clinical trials remains crucial. Further, while contemporary guidelines suggest that non-HDL-C (LDL-C + VLDL-C) measured under fasting conditions may be a good surrogate for apoB, this may not always be the case, as demonstrated by the PROMINENT trial. This study was stopped for futility, as pemafibrate, a selective PPARα modulator with TG-lowering activity, did not reduce the risk of the primary endpoint (a composite of non-fatal myocardial infarction, non-fatal ischaemic stroke, coronary revascularization, and CV death) in high-risk patients with type 2 diabetes, in spite of a 26% reduction in plasma TG associated with improvements in other markers for TRL metabolism, including decreases in apoB-48, apoC-III, and remnant cholesterol but not apoB.[23] Whether the lack of change in apoB is due to a reduction of VLDL and increase of LDL particle number or a change in the composition of VLDL with accumulation of smaller VLDL remains to be addressed and will be key to better frame future studies involving apoC-III silencing. Finally, the observation that loss-of-function variants of *APOC3* are associated with lower CV risk in Europeans, but not in other ethnic groups, calls for specific trials deeply assessing the biological mechanisms beyond these differences to test potential limitations of apoC-III inhibition strategies.

Authors' contributions

All authors contributed to the design, literature review, writing of the manuscript, and revising it critically for important intellectual content.

Acknowledgements

S.T. is supported by NHLBI R01HL159156 and the Fondation Leducq. The work of A.L.C. is supported in part by the Ricerca Corrente from the Italian Ministry of Health.

Conflict of interest: C.J.P. reports grant funding/honoraria from Amarin, Amgen, Daiichi-Sankyo, SalCor, MSD, and Novartis. A.P. has nothing to disclose. S.T. is a co-inventor and receives royalties from patents owned by University of California San Diego (UCSD) and is a co-founder and has an equity interest in Oxitope, LLC, and its affiliates, Kleanthi Diagnostics, LLC, and Covicept Therapeutics, Inc., and has a dual appointment at UCSD and Ionis Pharmaceuticals. Although these relationships have been identified for conflict of interest management based on the overall scope of the project, the research findings included in this particular publication may not necessarily relate to the interests of the above companies. The terms of this arrangement have been reviewed and approved

by the UCSD in accordance with its conflict of interest policies. B.A.F. reports research grants from Merck, Novartis, Amgen, and Esperion Therapeutics and consulting fees, advisory boards, or lecture honoraria from Novartis, Merck, Amgen, Regeneron, Sanofi, Novartis, Pfizer, Eli Lilly, Novo Nordisk, Daiichi-Sankyo, Viatris, Ionis Pharmaceuticals, New Amsterdam Pharmaceuticals, dalCOR, The Medicines Co, Mylan, CiVi Pharma, KrKa Phamaceuticals, American College of Cardiology, EAS, and European Society of Cardiology. A.L.C. reports grant(s)/support from Akcea, Amarin, Amgen, Menarini, Mylan, Sanofi, and Sanofi/Regeneron and is a consultant for Akcea, Amgen, Amarin, Daiichi-Sankyo, Eli Lilly, Esperion, Kowa, Ionis Pharmaceuticals, Menarini, MSD, Mylan, Novartis, Recordati, Regeneron, and Sanofi, outside the submitted work.

Data availability

No new data were generated or analysed in this paper.

References

1. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, Watts GF, Boren J, Fazio S, Horton JD, Masana L, Nicholls SJ, Nordestgaard BG, van de Sluis B, Taskinen MR, Tokgozoglu L, Landmesser U, Laufs U, Wiklund O, Stock JK, Chapman MJ, Catapano AL. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017;**38**:2459–2472.
2. Boren J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, Daemen MJ, Demer LL, Hegele RA, Nicholls SJ, Nordestgaard BG, Watts GF, Bruckert E, Fazio S, Ference BA, Graham I, Horton JD, Landmesser U, Laufs U, Masana L, Pasterkamp G, Raal FJ, Ray KK, Schunkert H, Taskinen MR, van de Sluis B, Wiklund O, Tokgozoglu L, Catapano AL, Ginsberg HN. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2020;**41**:2313–2330.
3. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014;**384**:626–635.
4. Ginsberg HN, Packard CJ, Chapman MJ, Boren J, Aguilar-Salinas CA, Averna M, Ference BA, Gaudet D, Hegele RA, Kersten S, Lewis GF, Lichtenstein AH, Moulin P, Nordestgaard BG, Remaley AT, Staels B, Stroes ESG, Taskinen MR, Tokgozoglu LS, Tybjaerg-Hansen A, Stock JK, Catapano AL. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies-a consensus statement from the European Atherosclerosis Society. *Eur Heart J* 2021;**42**:4791–4806.
5. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res* 2016;**118**:547–563.
6. Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkila K, Hypponen E, Isaacs A, Jackson AU, Johansson A, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikainen LP, Magnusson PK, Mangino M, Mihailov E, Montasser ME, Muller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney AS, Doring A, Elliott P, Epstein SE, Eyjolfsson GI, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJ, Khaw KT, Kim E, Klopp N, Komulainen P, Kumari M, Langenberg C, Lehtimaki T, Lin SY, Lindstrom J, Loos RJ, Mach F, McArdle WL, Meisinger C, Mitchell BD, Muller G, Nagaraja R, Narisu N, Nieminen TV, Nsubuga RN, Olafsson I, Ong KK, Palotie A, Papamarkou T, Pomilla C, Pouta A, Rader DJ, Reilly MP, Ridker PM, Rivadeneira F, Rudan I, Ruokonen A, Samani N, Scharnagl H, Seeley J, Silander K, Stancakova A, Stirrups K, Swift AJ, Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild SH, Willemsen G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL, Bandinelli S, Bennett F, Bochud M, Boehm BO, Boomsma DI, Borecki IB, Bornstein SR, Bovet P, Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YD, Collins FS, Cooper RS, Danesh J, Dedoussis G, de Faire U, Feranil AB, Ferrieres J, Ferrucci L, Freimer NB, Gieger C, Groop LC, Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A, Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Jarvelin MR, Jula A, Kahonen M, Kaprio J, Kesaniemi A, Kivimaki M, Kooner JS, Koudstaal PJ, Krauss RM, Kuh D, Kuusisto J, Kyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, Marz W, McCarthy MI, McKenzie CA, Meneton P, Metspalu A, Moilanen L, Morris AD, Munroe PB, Njolstad I, Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quertermous T, Rauramaa R, Saleheen D, Salomaa V, Sanghera DK, Saramies J, Schwarz PE, Sheu WH, Shuldiner AR, Siegbahn A, Spector TD, Stefansson K, Strachan DP, Tayo BO, Tremoli E, Tuomilehto J, Uusitupa M, van Duijn CM, Vollenweider P, Wallentin L, Wareham NJ, Whitfield JB, Wolffenbuttel BH, Altshuler D, Ordovas JM, Boerwinkle E, Palmer CN, Thorsteinsdottir U, Chasman DI, Rotter JI, Franks PW, Ripatti S, Cupples LA, Sandhu MS, Rich SS, Boehnke M, Deloukas P, Mohlke KL, Ingelsson E, Abecasis GR, Daly MJ, Neale BM, Kathiresan S. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet* 2013;**45**:1345–1352.
7. Rosenson RS, Davidson MH, Hirsh BJ, Kathiresan S, Gaudet D. Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease. *J Am Coll Cardiol* 2014;**64**:2525–2540.
8. Holmes MV, Asselbergs FW, Palmer TM, Drenos F, Lanktree MB, Nelson CP, Dale CE, Padmanabhan S, Finan C, Swerdlow DI, Tragante V, van Iperen EP, Sivapalaratnam S, Shah S, Elbers CC, Shah T, Engmann J, Giambartolomei C, White J, Zabaneh D, Sofat R, McLachlan S, UCLEB Consortium, Doevendans PA, Balmforth AJ, Hall AS, North KE, Almoguera B, Hoogeveen RC, Cushman M, Fornage M, Patel SR, Redline S, Siscovick DS, Tsai MY, Karczewski KJ, Hofker MH, Verschuren WM, Bots ML, van der Schouw YT, Melander O, Dominiczak AF, Morris R, Ben-Shlomo Y, Price J, Kumari M, Baumert J, Peters A, Thorand B, Koenig W, Gaunt TR, Humphries SE, Clarke R, Watkins H, Farrall M, Wilson JG, Rich SS, de Bakker PI, Lange LA, Davey Smith G, Reiner AP, Talmud PJ, Kivimäki M, Lawlor DA, Dudbridge F, Samani NJ, Keating BJ, Hingorani AD, Casas JP. Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J* 2015;**36**:539–550.
9. Allara E, Morani G, Carter P, Gkatzionis A, Zuber V, Foley CN, Rees JMB, Mason AM, Bell S, Gill D, Lindström S, Butterworth AS, Di Angelantonio E, Peters J, Burgess S; INVENT Consortium. Genetic determinants of lipids and cardiovascular disease outcomes: a wide-angled Mendelian randomization investigation. *Circ Genom Precis Med* 2019;**12**:e002711.
10. Lee SH, Lee JY, Kim GH, Jung KJ, Lee S, Kim HC, Jee SH. Two-sample Mendelian randomization study of lipid levels and ischemic heart disease. *Korean Circ J* 2020;**50**:940–948.
11. Scheffer PG, Teerlink T, Dekker JM, Bos G, Nijpels G, Diamant M, Kostense PJ, Stehouwer CD, Heine RJ. Increased plasma apolipoprotein C-III concentration independently predicts cardiovascular mortality: the Hoorn study. *Clin Chem* 2008;**54**:1325–1330.
12. van Capelleveen JC, Bernelot Moens SJ, Yang X, Kastelein JJP, Wareham NJ, Zwinderman AH, Stroes ESG, Witztum JL, Hovingh GK, Khaw KT, Boekholdt SM, Tsimikas S. Apolipoprotein C-III levels and incident coronary artery disease risk: the EPIC-Norfolk prospective population study. *Arterioscler Thromb Vasc Biol* 2017;**37**:1206–1212.
13. Ramms B, Gordts P. Apolipoprotein C-III in triglyceride-rich lipoprotein metabolism. *Curr Opin Lipidol* 2018;**29**:171–179.
14. Sacks FM. The crucial roles of apolipoproteins E and C-III in apoB lipoprotein metabolism in normolipidemia and hypertriglyceridemia. *Curr Opin Lipidol* 2015;**26**:56–63.
15. Kohan AB. Apolipoprotein C-III: a potent modulator of hypertriglyceridemia and cardiovascular disease. *Curr Opin Endocrinol Diabetes Obes* 2015;**22**:119–125.
16. Taskinen MR, Borén J. Why is apolipoprotein CIII emerging as a novel therapeutic target to reduce the burden of cardiovascular disease? *Curr Atheroscler Rep* 2016;**18**:59.
17. Wyler von Ballmoos MC, Haring B, Sacks FM. The risk of cardiovascular events with increased apolipoprotein CIII: a systematic review and meta-analysis. *J Clin Lipidol* 2015;**9**:498–510.
18. Norata GD, Tsimikas S, Pirillo A, Catapano AL. Apolipoprotein C-III: from pathophysiology to pharmacology. *Trends Pharmacol Sci* 2015;**36**:675–687.
19. Fredenrich A, Giroux LM, Tremblay M, Krimbou L, Davignon J, Cohn JS. Plasma lipoprotein distribution of apoC-III in normolipidemic and hypertriglyceridemic subjects: comparison of the apoC-III to apoE ratio in different lipoprotein fractions. *J Lipid Res* 1997;**38**:1421–1432.
20. Yamazaki A, Ohkawa R, Yamagata Y, Horiuchi Y, Lai SJ, Kameda T, Ichimura N, Tohda S, Tozuka M. Apolipoprotein C-II and C-III preferably transfer to both high-density lipoprotein (HDL)2 and the larger HDL3 from very low-density lipoprotein (VLDL). *Biol Chem* 2021;**402**:439–449.
21. Boyle KE, Phillips MC, Lund-Katz S. Kinetics and mechanism of exchange of apolipoprotein C-III molecules from very low density lipoprotein particles. *Biochim Biophys Acta* 1999;**1430**:302–312.
22. Barr SI, Kottke BA, Mao SJ. Postprandial exchange of apolipoprotein C-III between plasma lipoproteins. *Am J Clin Nutr* 1981;**34**:191–198.
23. Das Pradhan A, Glynn RJ, Fruchart JC, MacFadyen JG, Zaharris ES, Everett BM, Campbell SE, Oshima R, Amarenco P, Blom DJ, Brinton EA, Eckel RH, Elam MB, Felicio JS, Ginsberg HN, Goudev A, Ishibashi S, Joseph J, Kodama T, Koenig W, Leiter LA, Lorenzatti AJ, Mankovsky B, Marx N, Nordestgaard BG, Páll D, Ray KK, Santos RD, Soran H, Susekov A, Tendera M, Yokote K, Paynter NP, Buring JE, Libby P, Ridker PM, PROMINENT Investigators. Triglyceride lowering with pemafibrate to reduce cardiovascular risk. *N Engl J Med* 2022;**387**:1923–1934.
24. Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, Laufs U, Oliver-Williams C, Wood AM, Butterworth AS, Di Angelantonio E, Danesh J, Nicholls SJ, Bhatt DL, Sabatine MS, Catapano AL. Association of triglyceride-lowering LPL variants and LDL-C-lowering LDLR variants with risk of coronary heart disease. *JAMA* 2019;**321**:364–373.
25. Campos H, Perlov D, Khoo C, Sacks FM. Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia. *J Lipid Res* 2001;**42**:1239–1249.
26. Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. *Circulation* 2010;**121**:1722–1734.

27. Huang Y, Liu XQ, Rall SC Jr, Taylor JM, von Eckardstein A, Assmann G, Mahley RW. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. *J Biol Chem* 1998;**273**:26388–26393.
28. Zheng C, Khoo C, Ikewaki K, Sacks FM. Rapid turnover of apolipoprotein C-III-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions. *J Lipid Res* 2007;**48**:1190–1203.
29. Mendivil CO, Zheng C, Furtado J, Lel J, Sacks FM. Metabolism of very-low-density lipoprotein and low-density lipoprotein containing apolipoprotein C-III and not other small apolipoproteins. *Arterioscler Thromb Vasc Biol* 2010;**30**:239–245.
30. Kawakami A, Aikawa M, Libby P, Alcaide P, Luscinskas FW, Sacks FM. Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. *Circulation* 2006;**113**:691–700.
31. Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule-1 in vascular endothelial cells and increases adhesion of monocytic cells. *Circulation* 2006;**114**:681–687.
32. Hiukka A, Stahlman M, Pettersson C, Levin M, Adiels M, Teneberg S, Leinonen ES, Hulten LM, Wiklund O, Oresic M, Olofsson SO, Taskinen MR, Ekroos K, Boren J. ApoCIII-enriched LDL in type 2 diabetes displays altered lipid composition, increased susceptibility for sphingomyelinase, and increased binding to biglycan. *Diabetes* 2009;**58**:2018–2026.
33. Ke LY, Law SH, Mishra VK, Parveen F, Chan HC, Lu YH, Chu CS. Molecular and cellular mechanisms of electronegative lipoproteins in cardiovascular diseases. *Biomedicines* 2020;**8**:550.
34. Lee SJ, Campos H, Moye LA, Sacks FM. LDL containing apolipoprotein CIII is an independent risk factor for coronary events in diabetic patients. *Arterioscler Thromb Vasc Biol* 2003; **23**:853–858.
35. Mendivil CO, Rimm EB, Furtado J, Chiuve SE, Sacks FM. Low-density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease. *Circulation* 2011;**124**: 2065–2072.
36. Aroner SA, Koch M, Mukamal KJ, Furtado JD, Stein JH, Tattersall MC, McClelland RL, Jensen MK. High-density lipoprotein subspecies defined by apolipoprotein C-III and subclinical atherosclerosis measures: mESA (the Multi-Ethnic Study of Atherosclerosis). *J Am Heart Assoc* 2018;**7**:e007824.
37. Jensen MK, Aroner SA, Mukamal KJ, Furtado JD, Post WS, Tsai MY, Tjønneland A, Polak JF, Rimm EB, Overvad K, McClelland RL, Sacks FM. High-density lipoprotein subspecies defined by presence of apolipoprotein C-III and incident coronary heart disease in four cohorts. *Circulation* 2018;**137**:1364–1373.
38. Yamamoto R, Sacks FM, Hu FB, Rosner B, Furtado JD, Aroner SA, Ferrannini E, Baldi S, Kozakova M, Balkau B, Natali A, Jensen MK, RISC Investigators. High density lipoprotein with apolipoprotein C-III is associated with carotid intima-media thickness among generally healthy individuals. *Atherosclerosis* 2018;**269**:92–99.
39. Morton AM, Koch M, Mendivil CO, Furtado JD, Tjønneland A, Overvad K, Wang L, Jensen MK, Sacks FM. Apolipoproteins E and CIII interact to regulate HDL metabolism and coronary heart disease risk. *JCI Insight* 2018;**3**:e98045.
40. Sorokin AV, Patel N, Abdelrahman KM, Ling C, Reimund M, Graziano G, Sampson M, Playford MP, Dey AK, Reddy A, Teague HL, Stagliano M, Amar M, Chen MY, Mehta NN, Remaley AT. Complex association of apolipoprotein E-containing HDL with coronary artery disease burden in cardiovascular disease. *JCI Insight* 2022;**7**:e159577.
41. Wang F, Kohan AB, Dong HH, Yang Q, Xu M, Huesman S, Lou D, Hui DY, Tso P. Overexpression of apolipoprotein C-III decreases secretion of dietary triglyceride into lymph. *Physiol Rep* 2014;**2**:e00247.
42. Jattan J, Rodia C, Li D, Diakhate A, Dong H, Bataille A, Shroyer NF, Kohan AB. Using primary murine intestinal enteroids to study dietary TAG absorption, lipoprotein synthesis, and the role of apoC-III in the intestine. *J Lipid Res* 2017;**58**:853–865.
43. Dallinga-Thie GM, Groenendijk M, Blom RN, De Bruin TW, De Kant E. Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin. *J Lipid Res* 2001;**42**: 1450–1456.
44. Chen YJ, Chen CC, Li TK, Wang PH, Liu LR, Chang FY, Wang YC, Yu YH, Lin SP, Mersmann HJ, Ding ST. Docosahexaenoic acid suppresses the expression of FoxO and its target genes. *J Nutr Biochem* 2012;**23**:1609–1616.
45. Caron S, Verrijken A, Mertens I, Samanez CH, Mautino G, Haas JT, Duran-Sandoval D, Prawitt J, Francque S, Vallez E, Muhr-Tailleux A, Berard I, Kuipers F, Kuivenhoven JA, Biddinger SB, Taskinen MR, Van Gaal L, Staels B. Transcriptional activation of apolipoprotein CIII expression by glucose may contribute to diabetic dyslipidemia. *Arterioscler Thromb Vasc Biol* 2011;**31**:513–519.
46. Pavlic M, Valéro R, Duez H, Xiao C, Szeto L, Patterson BW, Lewis GF. Triglyceride-rich lipoprotein-associated apolipoprotein C-III production is stimulated by plasma free fatty acids in humans. *Arterioscler Thromb Vasc Biol* 2008;**28**:1660–1665.
47. Altomonte J, Cong L, Harbaran S, Richter A, Xu J, Meseck M, Dong HH. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J Clin Invest* 2004;**114**:1493–1503.
48. Chen M, Breslow JL, Li W, Leff T. Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: correlation with changes in plasma triglyceride levels. *J Lipid Res* 1994;**35**: 1918–1924.
49. Li WW, Dammerman MM, Smith JD, Metzger S, Breslow JL, Leff T. Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. *J Clin Invest* 1995;**96**:2601–2605.
50. Miller M, Rhyne J, Chen H, Beach V, Ericson R, Luthra K, Dwivedi M, Misra A. APOC3 promoter polymorphisms C-482T and T-455C are associated with the metabolic syndrome. *Arch Med Res* 2007;**38**:444–451.
51. Morton AM, Furtado JD, Lee J, Amerine W, Davidson MH, Sacks FM. The effect of omega-3 carboxylic acids on apolipoprotein CIII-containing lipoproteins in severe hypertriglyceridemia. *J Clin Lipidol* 2016;**10**:1442–1451.e4.
52. Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, Castrillo A, Wilpitz DC, Mangelsdorf DJ, Collins JL, Saez E, Tontonoz P. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc Natl Acad Sci U S A* 2003;**100**:5419–5424.
53. Roghani A, Zannis VI. Mutagenesis of the glycosylation site of human ApoCIII. O-linked glycosylation is not required for ApoCIII secretion and lipid binding. *J Biol Chem* 1988;**263**: 17925–17932.
54. Pirillo A, Svecla M, Catapano AL, Holleboom AG, Norata GD. Impact of protein glycosylation on lipoprotein metabolism and atherosclerosis. *Cardiovasc Res* 2021;**117**:1033–1045.
55. Yassine HN, Trenchevska O, Ramrakhiani A, Parekh A, Koska J, Walker RW, Billheimer D, Reaven PD, Yen FT, Nelson RW, Goran MI, Nedelkov D. The association of human apolipoprotein C-III sialylation proteoforms with plasma triglycerides. *PLoS One* 2015;**10**: e0144138.
56. Olivieri O, Chiariello C, Martinelli N, Castagna A, Speziali G, Girelli D, Pizzolo F, Bassi A, Cecconi D, Robotti E, Manfredi M, Conte E, Marengo E. Sialylated isoforms of apolipoprotein C-III and plasma lipids in subjects with coronary artery disease. *Clin Chem Lab Med* 2018;**56**:1542–1550.
57. Catapano AL. The distribution of apo C-II and apo C-III in very low density lipoproteins of normal and type IV subjects. *Atherosclerosis* 1980;**35**:419–424.
58. Kegulian NC, Ramms B, Horton S, Trenchevska O, Nedelkov D, Graham MJ, Lee RG, Esko JD, Yassine HN, Gordts P. ApoC-III glycoforms are differentially cleared by hepatic TRL (triglyceride-rich lipoprotein) receptors. *Arterioscler Thromb Vasc Biol* 2019;**39**:2145–2156.
59. Catapano AL. Activation of lipoprotein lipase by apolipoprotein C-II is modulated by the COOH terminal region of apolipoprotein C-III. *Chem Phys Lipids* 1987;**45**:39–47.
60. Koska J, Yassine H, Trenchevska O, Sinari S, Schwenke DC, Yen FT, Billheimer D, Nelson RW, Nedelkov D, Reaven PD. Disialylated apolipoprotein C-III proteoform is associated with improved lipids in prediabetes and type 2 diabetes. *J Lipid Res* 2016;**57**:894–905.
61. Sinari S, Koska J, Hu Y, Furtado J, Jensen MK, Budoff MJ, Nedelkov D, McClelland RL, Billheimer D, Reaven P. Apo CIII proteoforms, plasma lipids, and cardiovascular risk in MESA. *Arterioscler Thromb Vasc Biol* 2023;**43**:1560–1571.
62. Holleboom AG, Karlsson H, Lin RS, Beres TM, Sierts JA, Herman DS, Stroes ES, Aerts JM, Kastelein JJ, Motazacker MM, Dallinga-Thie GM, Levels JH, Zwinderman AH, Seidman JG, Seidman CE, Ljunggren S, Lefeber DJ, Morava E, Wevers RA, Fritz TA, Tabak LA, Lindahl M, Hovingh GK, Kuivenhoven JA. Heterozygosity for a loss-of-function mutation in GALNT2 improves plasma triglyceride clearance in man. *Cell Metab* 2011;**14**:811–818.
63. Zilmer M, Edmondson AC, Khetarpal SA, Alesi V, Zaki MS, Rostasy K, Madsen CG, Lepri FR, Sinibaldi L, Cusmai R, Novelli A, Issa MY, Fenger CD, Abou Jamra R, Reutter H, Briuglia S, Agolini E, Hansen L, Petaja-Repo UE, Hintze J, Raymond KM, Liedtke K, Stanley V, Musaev D, Gleeson JG, Vitali C, O'Brien WT, Gardella E, Rubboli G, Rader DJ, Schjoldager KT, Moller RS. Novel congenital disorder of O-linked glycosylation caused by GALNT2 loss of function. *Brain* 2020;**143**:1114–1126.
64. Jabs HU, Assmann G. Characterization of an apolipoprotein C-III mutant by high-performance liquid chromatography and time-of-flight secondary ion mass spectrometry. *J Chromatogr* 1987;**414**:323–333.
65. Maeda H, Hashimoto RK, Ogura T, Hiraga S, Uzawa H. Molecular cloning of a human apoC-III variant: Thr 74—-Ala 74 mutation prevents O-glycosylation. *J Lipid Res* 1987; **28**:1405–1409.
66. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science* 1990;**249**:790–793.
67. Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J Biol Chem* 1994;**269**:23610–23616.
68. Sundaram M, Curtis KR, Amir Alipour M, LeBlond ND, Margison KD, Yaworski RA, Parks RJ, McIntyre AD, Hegele RA, Fullerton MD, Yao Z. The apolipoprotein C-III (Gln38Lys) variant associated with human hypertriglyceridemia is a gain-of-function mutation. *J Lipid Res* 2017;**58**:2188–2196.
69. Pullinger CR, Malloy MJ, Shahidi AK, Ghassemzadeh M, Duchateau P, Villagomez J, Allaart J, Kane JP. A novel apolipoprotein C-III variant, apoC-III(Gln38–>Lys), associated with moderate hypertriglyceridemia in a large kindred of Mexican origin. *J Lipid Res* 1997;**38**: 1833–1840.
70. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, Mitchell BD, Miller M, O'Connell JR, Shuldiner AR. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science* 2008;**322**:1702–1705.
71. Reyes-Soffer G, Sztalryd C, Horenstein RB, Holleran S, Matveyenko A, Thomas T, Nandakumar R, Ngai C, Karmally W, Ginsberg HN, Ramakrishnan R, Pollin TI. Effects of APOC3 heterozygous deficiency on plasma lipid and lipoprotein metabolism. *Arterioscler Thromb Vasc Biol* 2019;**39**:63–72.
72. Crawford DC, Dumitrescu L, Goodloe R, Brown-Gentry K, Boston J, McClellan B Jr, Sutcliffe C, Wiseman R, Baker P, Pericak-Vance MA, Scott WK, Allen M, Mayo P, Schnetz-Boutaud N, Dilks HH, Haines JL, Pollin TI. Rare variant APOC3 R19X is associated with cardio-protective profiles in a diverse population-based survey as part of the Epidemiologic Architecture for Genes Linked to Environment Study. *Circ Cardiovasc Genet* 2014;**7**:848–853.

73. Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, Won HH, Karczewski KJ, O'Donnell-Luria AH, Samocha KE, Weisburd B, Gupta N, Zaidi M, Samuel M, Imran A, Abbas S, Majeed F, Ishaq M, Akhtar S, Trindade K, Mucksavage M, Qamar N, Zaman KS, Yaqoob Z, Saghir T, Rizvi SNH, Memon A, Hayyat Mallick N, Ishaq M, Rasheed SZ, Memon FU, Mahmood K, Ahmed N, Do R, Krauss RM, MacArthur DG, Gabriel S, Lander ES, Daly MJ, Frossard P, Danesh J, Rader DJ, Kathiresan S. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. *Nature* 2017;**544**:235–239.
74. Bochem AE, Van Capelleveen JC, Dallinga-Thie GM, Schimmel AW, Motazacker MM, Tietjen I, Singaraja RR, Hayden MR, Kastelein JJ, Stroes ES, Hovingh GK. Two novel mutations in apolipoprotein C3 underlie atheroprotective lipid profiles in families. *Clin Genet* 2014;**85**:433–440.
75. Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 2014;**371**:22–31.
76. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med* 2014;**371**:32–41.
77. Wulff AB, Nordestgaard BG, Tybjærg-Hansen A. APOC3 loss-of-function mutations, remnant cholesterol, low-density lipoprotein cholesterol, and cardiovascular risk: mediation- and meta-analyses of 137 895 individuals. *Arterioscler Thromb Vasc Biol* 2018;**38**:660–668.
78. Rout M, Lerner M, Blackett PR, Peyton MD, Stavrakis S, Sidorov E, Sanghera DK. Ethnic differences in ApoC-III concentration and the risk of cardiovascular disease: no evidence for the cardioprotective role of rare/loss of function APOC3 variants in non-Europeans. *Am Heart J Plus* 2022;**13**:100128.
79. Goyal S, Tanigawa Y, Zhang W, Chai JF, Almeida M, Sim X, Lerner M, Chainakul J, Ramiu JG, Seraphin C, Apple B, Vaughan A, Muniu J, Peralta J, Lehman DM, Ralhan S, Wander GS, Singh JR, Mehra NK, Sidorov E, Peyton MD, Blackett PR, Curran JE, Tai ES, van Dam R, Cheng CY, Duggirala R, Blangero J, Chambers JC, Sabanayagam C, Kooner JS, Rivas MA, Aston CE, Sanghera DK. APOC3 genetic variation, serum triglycerides, and risk of coronary artery disease in Asian Indians, Europeans, and other ethnic groups. *Lipids Health Dis* 2021;**20**:113.
80. Bjornson E, Packard CJ, Adiels M, Andersson L, Matikainen N, Soderlund S, Kahri J, Sihlbom C, Thorsell A, Zhou H, Taskinen MR, Boren J. Investigation of human apoB48 metabolism using a new, integrated non-steady-state model of apoB48 and apoB100 kinetics. *J Intern Med* 2019;**285**:562–577.
81. Gordon DA, Wetterau JR, Gregg RE. Microsomal triglyceride transfer protein: a protein complex required for the assembly of lipoprotein particles. *Trends Cell Biol* 1995;**5**:317–321.
82. Rustaeus S, Stillemark P, Lindberg K, Gordon D, Olofsson SO. The microsomal triglyceride transfer protein catalyzes the post-translational assembly of apolipoprotein B-100 very low density lipoprotein in McA-RH7777 cells. *J Biol Chem* 1998;**273**:5196–5203.
83. Olofsson SO, Borèn J. Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *J Intern Med* 2005;**258**:395–410.
84. Wu X, Shang A, Jiang H, Ginsberg HN. Low rates of apoB secretion from HepG2 cells result from reduced delivery of newly synthesized triglyceride to a "secretion-coupled" pool. *J Lipid Res* 1996;**37**:1198–1206.
85. Gibbons GF, Islam K, Pease RJ. Mobilisation of triacylglycerol stores. *Biochim Biophys Acta* 2000;**1483**:37–57.
86. Gibbons GF, Bartlett SM, Sparks CE, Sparks JD. Extracellular fatty acids are not utilized directly for the synthesis of very-low-density lipoprotein in primary cultures of rat hepatocytes. *Biochem J* 1992;**287**(Pt 3):749–753.
87. von Eckardstein A, Holz H, Sandkamp M, Weng W, Funke H, Assmann G. Apolipoprotein C-III(Lys58—-Glu). Identification of an apolipoprotein C-III variant in a family with hyper-alphalipoproteinemia. *J Clin Invest* 1991;**87**:1724–1731.
88. Qin W, Sundaram M, Wang Y, Zhou H, Zhong S, Chang CC, Manhas S, Yao EF, Parks RJ, McFie PJ, Stone SJ, Jiang ZG, Wang C, Figeys D, Jia W, Yao Z. Missense mutation in APOC3 within the C-terminal lipid binding domain of human ApoC-III results in impaired assembly and secretion of triacylglycerol-rich very low density lipoproteins: evidence that ApoC-III plays a major role in the formation of lipid precursors within the microsomal lumen. *J Biol Chem* 2011;**286**:27769–27780.
89. Taskinen MR, Bjornson E, Matikainen N, Soderlund S, Ramo J, Ainola MM, Hakkarainen A, Sihlbom C, Thorsell A, Andersson L, Bergh PO, Henricsson M, Romeo S, Adiels M, Ripatti S, Laakso M, Packard CJ, Boren J. Postprandial metabolism of apolipoproteins B48, B100, C-III, and E in humans with APOC3 loss-of-function mutations. *JCI Insight* 2022;**7**:160607.
90. Nakajima K, Nakano T, Tokita Y, Nagamine T, Inazu A, Kobayashi J, Mabuchi H, Stanhope KL, Havel PJ, Okazaki M, Ai M, Tanaka A. Postprandial lipoprotein metabolism: VLDL vs chylomicrons. *Clin Chim Acta* 2011;**412**:1306–1318.
91. Tomiyasu K, Walsh BW, Ikewaki K, Judge H, Sacks FM. Differential metabolism of human VLDL according to content of ApoE and ApoC-III. *Arterioscler Thromb Vasc Biol* 2001;**21**:1494–1500.
92. Xiao C, Stahel P, Lewis GF. Regulation of chylomicron secretion: focus on post-assembly mechanisms. *Cell Mol Gastroenterol Hepatol* 2019;**7**:487–501.
93. Dallinga-Thie GM, Kroon J, Borén J, Chapman MJ. Triglyceride-rich lipoproteins and remnants: targets for therapy? *Curr Cardiol Rep* 2016;**18**:67.
94. Basu D, Goldberg IJ. Regulation of lipoprotein lipase-mediated lipolysis of triglycerides. *Curr Opin Lipidol* 2020;**31**:154–160.
95. Wu SA, Kersten S, Qi L. Lipoprotein lipase and its regulators: an unfolding story. *Trends Endocrinol Metab* 2021;**32**:48–61.
96. Behbodikhah J, Ahmed S, Elyasi A, Kasselman LJ, De Leon J, Glass AD, Reiss AB. Apolipoprotein B and cardiovascular disease: biomarker and potential therapeutic target. *Metabolites* 2021;**11**:690.
97. Mahley RW, Huang Y. Atherogenic remnant lipoproteins: role for proteoglycans in trapping, transferring, and internalizing. *J Clin Invest* 2007;**117**:94–98.
98. Packard CJ, Boren J, Taskinen MR. Causes and consequences of hypertriglyceridemia. *Front Endocrinol (Lausanne)* 2020;**11**:252.
99. Borén J, Watts GF, Adiels M, Soderlund S, Chan DC, Hakkarainen A, Lundbom N, Matikainen N, Kahri J, Verges B, Barrett PH, Taskinen MR. Kinetic and related determinants of plasma triglyceride concentration in abdominal obesity: multicenter tracer kinetic study. *Arterioscler Thromb Vasc Biol* 2015;**35**:2218–2224.
100. Boren J, Taskinen MR, Bjornson E, Packard CJ. Metabolism of triglyceride-rich lipoproteins in health and dyslipidaemia. *Nat Rev Cardiol* 2022;**19**:577–592.
101. Borén J, Packard CJ, Taskinen MR. The roles of ApoC-III on the metabolism of triglyceride-rich lipoproteins in humans. *Front Endocrinol (Lausanne)* 2020;**11**:474.
102. Wolska A, Dunbar RL, Freeman LA, Ueda M, Amar MJ, Sviridov DO, Remaley AT. Apolipoprotein C-II: new findings related to genetics, biochemistry, and role in triglyceride metabolism. *Atherosclerosis* 2017;**267**:49–60.
103. Gordts PL, Nock R, Son NH, Ramms B, Lew I, Gonzales JC, Thacker BE, Basu D, Lee RG, Mullick AE, Graham MJ, Goldberg IJ, Crooke RM, Witztum JL, Esko JD. ApoC-III inhibits clearance of triglyceride-rich lipoproteins through LDL family receptors. *J Clin Invest* 2016;**126**:2855–2866.
104. Gaudet D, Brisson D, Tremblay K, Alexander VJ, Singleton W, Hughes SG, Geary RS, Baker BF, Graham MJ, Crooke RM, Witztum JL. Targeting APOC3 in the familial chylomicronemia syndrome. *N Engl J Med* 2014;**371**:2200–2206.
105. Ramms B, Patel S, Nora C, Pessentheiner AR, Chang MW, Green CR, Golden GJ, Secrest P, Krauss RM, Metallo CM, Benner C, Alexander VJ, Witztum JL, Tsimikas S, Esko JD, Gordts P. ApoC-III ASO promotes tissue LPL activity in the absence of apoE-mediated TRL clearance. *J Lipid Res* 2019;**60**:1379–1395.
106. Hiukka A, Fruchart-Najib J, Leinonen E, Hilden H, Fruchart JC, Taskinen MR. Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in type 2 diabetes. *Diabetologia* 2005;**48**:1207–1215.
107. Christopoulou E, Tsimihodimos V, Filippatos T, Elisaf M. Apolipoprotein CIII and diabetes. Is there a link? *Diabetes Metab Res Rev* 2019;**35**:e3118.
108. Chan DC, Nguyen MN, Watts GF, Barrett PH. Plasma apolipoprotein C-III transport in centrally obese men: associations with very low-density lipoprotein apolipoprotein B and high-density lipoprotein apolipoprotein A-I metabolism. *J Clin Endocrinol Metab* 2008;**93**:557–564.
109. Chan DC, Watts GF, Redgrave TG, Mori TA, Barrett PH. Apolipoprotein B-100 kinetics in visceral obesity: associations with plasma apolipoprotein C-III concentration. *Metab Clin Exp* 2002;**51**:1041–1046.
110. Chan DC, Watts GF, Nguyen MN, Barrett PH. Apolipoproteins C-III and A-V as predictors of very-low-density lipoprotein triglyceride and apolipoprotein B-100 kinetics. *Arterioscler Thromb Vasc Biol* 2006;**26**:590–596.
111. Adiels M, Taskinen MR, Bjornson E, Andersson L, Matikainen N, Soderlund S, Kahri J, Hakkarainen A, Lundbom N, Sihlbom C, Thorsell A, Zhou H, Pietilainen KH, Packard C, Boren J. Role of apolipoprotein C-III overproduction in diabetic dyslipidaemia. *Diabetes Obes Metab* 2019;**21**:1861–1870.
112. Avall K, Ali Y, Leibiger IB, Leibiger B, Moede T, Paschen M, Dicker A, Dare E, Kohler M, Ilegems E, Abdulreda MH, Graham M, Crooke RM, Tay VS, Refai E, Nilsson SK, Jacob S, Selander L, Berggren PO, Juntti-Berggren L. Apolipoprotein CIII links islet insulin resistance to beta-cell failure in diabetes. *Proc Natl Acad Sci U S A* 2015;**112**:E2611–E2619.
113. Juntti-Berggren L, Refai E, Appelskog I, Andersson M, Imreh G, Dekki N, Uhles S, Yu L, Griffiths WJ, Zaitsev S, Leibiger I, Yang SN, Olivecrona G, Jörnvall H, Berggren PO. Apolipoprotein CIII promotes Ca2+-dependent beta cell death in type 1 diabetes. *Proc Natl Acad Sci U S A* 2004;**101**:10090–10094.
114. Shi Y, Yang G, Yu J, Yu L, Westenbroek R, Catterall WA, Juntti-Berggren L, Berggren PO, Yang SN. Apolipoprotein CIII hyperactivates beta cell CaV1 channels through SR-BI/beta1 integrin-dependent coactivation of PKA and Src. *Cell Mol Life Sci* 2014;**71**:1289–1303.
115. Paola Gutierrez Castroi K, Patricia Gonzalez A, Caccavello R, Garay-Sevilla ME, Gugliucci A. Lean adolescents with insulin resistance display higher angiopoietin like protein 3, ApoC-III and chylomicron remnant dyslipidemia. *Clin Chim Acta* 2022;**526**:43–48.
116. Rodríguez-Mortera R, Caccavello R, Garay-Sevilla ME, Gugliucci A. Higher ANGPTL3, apoC-III, and apoB48 dyslipidemia, and lower lipoprotein lipase concentrations are associated with dysfunctional visceral fat in adolescents with obesity. *Clin Chim Acta* 2020;**508**:61–68.

117. Olin-Lewis K, Krauss RM, La Belle M, Blanche PJ, Barrett PH, Wight TN, Chait A. ApoC-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan. *J Lipid Res* 2002;**43**:1969–1977.
118. Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, Boren J. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* 2002;**417**:750–754.
119. Peng X, Wu H. Inflammatory links between hypertriglyceridemia and atherogenesis. *Curr Atheroscler Rep* 2022;**24**:297–306.
120. Toth PP. Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. *Vasc Health Risk Manag* 2016;**12**:171–183.
121. Davidsson P, Hulthe J, Fagerberg B, Olsson BM, Hallberg C, Dahllöf B, Camejo G. A proteomic study of the apolipoproteins in LDL subclasses in patients with the metabolic syndrome and type 2 diabetes. *J Lipid Res* 2005;**46**:1999–2006.
122. Schissel SL, Jiang X, Tweedie-Hardman J, Jeong T, Camejo EH, Najib J, Rapp JH, Williams KJ, Tabas I. Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH. Implications for atherosclerotic lesion development. *J Biol Chem* 1998;**273**:2738–2746.
123. Yang X, Lee SR, Choi YS, Alexander VJ, Digenio A, Yang Q, Miller YI, Witztum JL, Tsimikas S. Reduction in lipoprotein-associated apoC-III levels following volanesorsen therapy: phase 2 randomized trial results. *J Lipid Res* 2016;**57**:706–713.
124. Zhang T, Tang X, Mao L, Chen J, Kuang J, Guo X, Xu D, Peng D, Yu B. HDL-associated apoCIII plays an independent role in predicting postprandial hypertriglyceridemia. *Clin Biochem* 2020;**79**:14–22.
125. Luo M, Liu A, Wang S, Wang T, Hu D, Wu S, Peng D. ApoCIII enrichment in HDL impairs HDL-mediated cholesterol efflux capacity. *Sci Rep* 2017;**7**:2312.
126. Riwanto M, Rohrer L, Roschitzki B, Besler C, Mocharla P, Mueller M, Perisa D, Heinrich K, Altwegg L, von Eckardstein A, Lüscher TF, Landmesser U. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. *Circulation* 2013;**127**:891–904.
127. Jensen MK, Rimm EB, Furtado JD, Sacks FM. Apolipoprotein C-III as a potential modulator of the association between HDL-cholesterol and incident coronary heart disease. *J Am Heart Assoc* 2012;**1**:jah3-e000232.
128. Talayero B, Wang L, Furtado J, Carey VJ, Bray GA, Sacks FM. Obesity favors apolipoprotein E- and C-III-containing high density lipoprotein subfractions associated with risk of heart disease. *J Lipid Res* 2014;**55**:2167–2177.
129. Furtado JD, Ruotolo G, Nicholls SJ, Dullea R, Carvajal-Gonzalez S, Sacks FM. Pharmacological inhibition of CETP (cholesteryl ester transfer protein) increases HDL (high-density lipoprotein) that contains ApoC3 and other HDL subspecies associated with higher risk of coronary heart disease. *Arterioscler Thromb Vasc Biol* 2022;**42**:227–237.
130. Lamprea-Montealegre JA, McClelland RL, Otvos JD, Mora S, Koch M, Jensen MK, de Boer IH. Association of high-density lipoprotein particles and high-density lipoprotein apolipoprotein C-III content with cardiovascular disease risk according to kidney function: the Multi-Ethnic Study of Atherosclerosis. *J Am Heart Assoc* 2019;**8**:e013713.
131. Yamamoto R, Jensen MK, Aroner S, Furtado JD, Rosner B, Hu FB, Balkau B, Natali A, Ferrannini E, Baldi S, Sacks FM. HDL containing apolipoprotein C-III is associated with insulin sensitivity: a multicenter cohort study. *J Clin Endocrinol Metab* 2021;**106**:e2928–e2940.
132. Aroner SA, Furtado JD, Sacks FM, Tsai MY, Mukamal KJ, McClelland RL, Jensen MK. Apolipoprotein C-III and its defined lipoprotein subspecies in relation to incident diabetes: the Multi-Ethnic Study of Atherosclerosis. *Diabetologia* 2019;**62**:981–992.
133. Koch M, DeKosky ST, Goodman M, Sun J, Furtado JD, Fitzpatrick AL, Mackey RH, Cai T, Lopez OL, Kuller LH, Mukamal KJ, Jensen MK. Association of apolipoprotein E in lipoprotein subspecies with risk of dementia. *JAMA Netw Open* 2020;**3**:e209250.
134. Guan Y, Hou X, Tian P, Ren L, Tang Y, Song A, Zhao J, Gao L, Song G. Elevated levels of apolipoprotein CIII increase the risk of postprandial hypertriglyceridemia. *Front Endocrinol (Lausanne)* 2021;**12**:646185.
135. Chhabra S, Narang R, Krishnan LR, Vasisht S, Agarwal DP, Srivastava LM, Manchanda SC, Das N. Apolipoprotein C3 SstI polymorphism and triglyceride levels in Asian Indians. *BMC Genet* 2002;**3**:9.
136. Clemente-Postigo M, Queipo-Ortuño M, Valdivielso P, Tinahones FJ, Cardona F. Effect of apolipoprotein C3 and apolipoprotein A1 polymorphisms on postprandial response to a fat overload in metabolic syndrome patients. *Clin Biochem* 2010;**43**:1300–1304.
137. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, Geary RS, Hughes SG, Viney NJ, Graham MJ, Crooke RM, Witztum JL, Brunzell JD, Kastelein JJ. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med* 2015;**373**:438–447.
138. Digenio A, Dunbar RL, Alexander VJ, Hompesch M, Morrow L, Lee RG, Graham MJ, Hughes SG, Yu R, Singleton W, Baker BF, Bhanot S, Crooke RM. Antisense-mediated lowering of plasma apolipoprotein C-III by volanesorsen improves dyslipidemia and insulin sensitivity in type 2 diabetes. *Diabetes Care* 2016;**39**:1408–1415.
139. Witztum JL, Gaudet D, Freedman SD, Alexander VJ, Digenio A, Williams KR, Yang Q, Hughes SG, Geary RS, Arca M, Stroes ESG, Bergeron J, Soran H, Civeira F, Hemphill L, Tsimikas S, Blom DJ, O'Dea L, Bruckert E. Volanesorsen and triglyceride levels in familial chylomicronemia syndrome. *N Engl J Med* 2019;**381**:531–542.
140. Gouni-Berthold I, Alexander VJ, Yang Q, Hurh E, Steinhagen-Thiessen E, Moriarty PM, Hughes SG, Gaudet D, Hegele RA, O'Dea LSL, Stroes ESG, Tsimikas S, Witztum JL; COMPASS Study Group. Efficacy and safety of volanesorsen in patients with multifactorial chylomicronaemia (COMPASS): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol* 2021;**9**:264–275.
141. Oral EA, Garg A, Tami J, Huang EA, O'dea LSL, Schmidt H, Tiulpakov A, Mertens A, Alexander VJ, Watts L, Hurh E, Witztum JL, Geary RS, Tsimikas S. Assessment of efficacy and safety of volanesorsen for treatment of metabolic complications in patients with familial partial lipodystrophy: Results of the BROADEN study: Volanesorsen in FPLD; The BROADEN Study. *J Clin Lipidol* 2022;**16**:833–849.
142. Dron JS, Wang J, Cao H, McIntyre AD, Iacocca MA, Menard JR, Movsesyan I, Malloy MJ, Pullinger CR, Kane JP, Hegele RA. Severe hypertriglyceridemia is primarily polygenic. *J Clin Lipidol* 2019;**13**:80–88.
143. Baass A, Paquette M, Bernard S, Hegele RA. Familial chylomicronemia syndrome: an under-recognized cause of severe hypertriglyceridaemia. *J Intern Med* 2020;**287**:340–348.
144. Gaudet D, Baass A, Tremblay K, Brisson D, Laflamme N, Paquette M, Dufour R, Bergeron J. Natural history (up to 15 years) of platelet count in 84 patients with familial hyperchylomicronemia due to lipoprotein lipase deficiency. *J Clin Lipidol* 2017;**11**:797–798.
145. Witztum JL, Gaudet D, Arca M, Jones A, Soran H, Gouni-Berthold I, Stroes ESG, Alexander VJ, Jones R, Watts L, Xia S, Tsimikas S. Volanesorsen and triglyceride levels in familial chylomicronemia syndrome: long-term efficacy and safety data from patients in an open-label extension trial. *J Clin Lipidol* 2023;**17**:342–355.
146. Calcaterra I, Lupoli R, Di Minno A, Di Minno MND. Volanesorsen to treat severe hypertriglyceridaemia: a pooled analysis of randomized controlled trials. *Eur J Clin Invest* 2022;**52**:e13841.
147. Bagias C, Xiarchou A, Bargiota A, Tigas S. Familial partial lipodystrophy (FPLD): recent insights. *Diabetes Metab Syndr Obes* 2020;**13**:1531–1544.
148. Prohaska TA, Alexander VJ, Karwatowska-Prokopczuk E, Tami J, Xia S, Witztum JL, Tsimikas S. APOC3 inhibition with volanesorsen reduces hepatic steatosis in patients with severe hypertriglyceridemia. *J Clin Lipidol* 2023;**17**:406–411.
149. Alexander VJ, Xia S, Hurh E, Hughes SG, O'Dea L, Geary RS, Witztum JL, Tsimikas S. N-acetyl galactosamine-conjugated antisense drug to APOC3 mRNA, triglycerides and atherogenic lipoprotein levels. *Eur Heart J* 2019;**40**:2785–2796.
150. Tardif JC, Karwatowska-Prokopczuk E, Amour ES, Ballantyne CM, Shapiro MD, Moriarty PM, Baum SJ, Hurh E, Bartlett VJ, Kingsbury J, Figueroa AL, Alexander VJ, Tami J, Witztum JL, Geary RS, O'Dea LSL, Tsimikas S, Gaudet D. Apolipoprotein C-III reduction in subjects with moderate hypertriglyceridaemia and at high cardiovascular risk. *Eur Heart J* 2022;**43**:1401–1412.
151. Schwabe C, Scott R, Sullivan D, Baker J, Clifton P, Hamilton J, Given B, San Martin J, Melquist S, Watts GF, Goldberg I, Knowles JW, Hegele R, Ballantyne C. RNA interference targeting apolipoprotein C-III with ARO-APOC3 in healthy volunteers mimics lipid and lipoprotein findings seen in subjects with inherited apolipoprotein C-III deficiency. *Eur Heart J* 2020;**41**(Suppl 2):ehaa946.3330.
152. Clifton P, Sullivan D, Baker J, Schwabe C, Thackwray S, Scott R, Hamilton J, Pineda AJL, Given B, Melquist S, Chen I, Martin JS, Watts GF, Goldberg IJ, Knowles J, Gaudet D, Hegele RA, Ballantyne CM. Abstract 10357: ARO-APOC3, an investigational RNAi therapeutic, shows similar efficacy and safety in genetically confirmed FCS and non-FCS participants with severe hypertriglyceridemia. *Circulation* 2021;**144**:A10357–A10357.
153. Karwatowska-Prokopczuk E, Tardif JC, Gaudet D, Ballantyne CM, Shapiro MD, Moriarty PM, Baum SJ, Amour ES, Alexander VJ, Xia S, Otvos JD, Witztum JL, Tsimikas S, Olezarsen Trial I. Effect of olezarsen targeting APOC-III on lipoprotein size and particle number measured by NMR in patients with hypertriglyceridemia. *J Clin Lipidol* 2022;**16**:617–625.
154. Recio-Lopez P, Valladolid-Acebes I, Hadwiger P, Hossbach M, Krampert M, Prata C, Berggren PO, Juntti-Berggren L. Treatment of the metabolic syndrome by siRNA targeting apolipoprotein CIII. *Biofactors* 2023;**49**:153–172.
155. Khetarpal SA, Zeng X, Millar JS, Vitali C, Somasundara AVH, Zanoni P, Landro JA, Barucci N, Zavadoski WJ, Sun Z, de Haard H, Toth IV, Peloso GM, Natarajan P, Cuchel M, Lund-Katz S, Phillips MC, Tall AR, Kathiresan S, DaSilva-Jardine P, Yates NA, Rader DJ. A human APOC3 missense variant and monoclonal antibody accelerate apoC-III clearance and lower triglyceride-rich lipoprotein levels. *Nat Med* 2017;**23**:1086–1094.
156. Wolska A, Lo L, Sviridov DO, Pourmousa M, Pryor M, Ghosh SS, Kakkar R, Davidson M, Wilson S, Pastor RW, Goldberg IJ, Basu D, Drake SK, Cougnoux A, Wu MJ, Neher SB, Freeman LA, Tang J, Amar M, Devalaraja M, Remaley AT. A dual apolipoprotein C-II mimetic-apolipoprotein C-III antagonist peptide lowers plasma triglycerides. *Sci Transl Med* 2020;**12**:eaaw7905.
157. Sviridov D, Dasseux A, Reimund M, Pryor M, Drake SK, Jarin Z, Wolska A, Pastor RW, Remaley AT. Short hydrocarbon stapled ApoC2-mimetic peptides activate lipoprotein lipase and lower plasma triglycerides in mice. *Front Cardiovasc Med* 2023;**10**:1223920.
158. Ahmad Z, Banerjee P, Hamon S, Chan KC, Bouzelmat A, Sasiela WJ, Pordy R, Mellis S, Dansky H, Gipe DA, Dunbar RL. Inhibition of angiopoietin-like protein 3 with a monoclonal antibody reduces triglycerides in hypertriglyceridemia. *Circulation* 2019;**140**:470–486.
159. Ahmad Z, Pordy R, Rader DJ, Gaudet D, Ali S, Gonzaga-Jauregui C, Ponda MP, Shumel B, Banerjee P, Dunbar RL. Inhibition of angiopoietin-like protein 3 with evinacumab in subjects with high and severe hypertriglyceridemia. *J Am Coll Cardiol* 2021;**78**:193–195.
160. Raal FJ, Rosenson RS, Reeskamp LF, Hovingh GK, Kastelein JJP, Rubba P, Ali S, Banerjee P, Chan KC, Gipe DA, Khilla N, Pordy R, Weinreich DM, Yancopoulos GD, Zhang Y, Gaudet D; ELIPSE HoFH Investigators. Evinacumab for homozygous familial hypercholesterolemia. *N Engl J Med* 2020;**383**:711–720.