Apolipoprotein C-III: understanding an emerging cardiovascular risk factor

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ABSTRACT

The concurrence of visceral obesity, insulin resistance and dyslipidaemia comprises the concept of the metabolic syndrome. The metabolic syndrome is an escalating problem in developed and developing societies that tracks with the obesity epidemic. Dyslipidaemia in the metabolic syndrome is potently atherogenic and, hence, is a major risk factor for CVD (cardiovascular disease) in these subjects. It is globally characterized by hypertriglyceridaemia, near normal LDL (low-density lipoprotein)-cholesterol and low plasma HDL (high-density lipoprotein)-cholesterol. ApoC-III (apolipoprotein C-III), an important regulator of lipoprotein metabolism, is strongly associated with hypertriglyceridaemia and the progression of CVD. ApoC-III impairs the lipolysis of TRLs [triacylglycerol (triglyceride)-rich lipoproteins] by inhibiting lipoprotein lipase and the hepatic uptake of TRLs by remnant receptors. In the circulation, apoC-III is associated with TRLs and HDL, and freely exchanges among these lipoprotein particle systems. However, to fully understand the complex physiology and pathophysiology requires the application of tracer methodology and mathematical modelling. In addition, experimental evidence shows that apoC-III may also have a direct role in atherosclerosis. In the metabolic syndrome, increased apoC-III concentration, resulting from hepatic overproduction of VLDL (very-LDL) apoC-III, is strongly associated with delayed catabolism of triacylglycerols and TRLs. Several therapies pertinent to the metabolic syndrome, such as PPAR (peroxisome-proliferator-activated receptor) agonists and statins, can regulate apoC-III transport in the metabolic syndrome. Regulating apoC-III metabolism may be an important new therapeutic approach to managing dyslipidaemia and CVD risk in the metabolic syndrome.

INTRODUCTION

Compelling evidence from a large number of clinical studies has established that elevated triacylglycerols (triacylglycerides) are an independent risk factor for atherosclerotic CVD (cardiovascular disease) [1–5]. The finding of TRLs (triacylglycerol-rich lipoproteins) and TRL remnants in atheromous plaques provides critical evidence supporting their direct role in atherogenesis. In the metabolic syndrome, hypertriglyceridaemia commonly co-exists with

Key words: apolipoprotein C-III, cardiovascular disease, dyslipidaemia, hypertriglyceridaemia, metabolic syndrome, risk factor.

Abbreviations: apo, apolipoprotein; apoC-III-LpB, apoC-III associated with apoB-containing lipoproteins; apoC-III-non-LpB, apoC-III associated with non-apoB-containing lipoproteins; CARE, Cholesterol and Recurrent Events; CETP, cholesteryl ester transfer protein; CLAS, Cholesterol-Lowering Atherosclerosis Study; CVD, cardiovascular disease; ECTIM, Etude Cas-Temoin de l’Infarctus du Myocarde; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HL, hepatic lipase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; ICAM-1, intracellular adhesion molecule-1; IEF, isoelectric focusing; IRE, insulin-response element; LCAT, lecithin cholesterol acyltransferase; LDL, low-density lipoprotein; LDLR, LDL receptor; LpB:C-III, concentration of apoB-containing particles that contain apoC-III; LPL, lipoprotein lipase; LSR, lipolysis-stimulated receptor; MARS, Monitored Atherosclerosis Regression Study; MUFA, monosaturated fatty acid; NF-κB, nuclear factor κB; PPAR, peroxisome-proliferator-activated receptor; RXR, retinoid X receptor; TRL, triacylglycerol-rich lipoprotein; VCAM-1, vascular cell adhesion molecule-1; VLDL, very-low-density lipoprotein.

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ApoC-III is associated with apoB-containing lipoproteins and HDL, and exchanges rapidly between these particles (Figure 1). Various nomenclatures have been used to describe lipoproteins containing apoC-III. Alaupovic et al. [18] first defined the term LpB:C to describe the concentration of apoB-containing lipoproteins enriched with apoC-III. The development of methods to measure apoC-III concentrations, described later in this review, have led to additional terminologies. For example, apoC-III-LpB and apoC-III-non-LpB describes the concentration of apoC-III associated with apoB-containing lipoproteins and non-apoB-containing lipoproteins respectively [19]. This nomenclature has been used throughout this review.

During the hydrolysis of VLDL triacylglycerols by LPL, apoC-III redistributes from VLDL to HDL, and is subsequently transferred back to newly synthesized TRL particles [20,21]. In vitro and in vivo radiotracer studies have demonstrated rapid exchange and equilibration between TRLs and HDL particles [22–24]; however, other radiotracer studies have suggested non-equilibrating pools of apoC-III that do not exchange between VLDL and HDL [25,26]. Recent endogenous stable isotope tracer studies suggest that the kinetics of apoC-III in VLDL and HDL are similar, supporting the concept of a kinetically homogeneous plasma apoC-III pool [27]. Methodological differences may explain the discrepancies among these findings. In subjects with normolipidaemia, the majority of plasma apoC-III is bound to HDL [23]. By contrast, in subjects with hypertriglyceridaemia, the majority is bound to TRLs [22]. The mechanism regulating the putative apoC-III exchange between TRLs and HDL, however, remains unknown.

The apoC-III gene is located on human chromosome 11 between the apoA-I and apoA-IV genes (Figure 2) [28]. The exact evolutionary biology of apoC-III is unknown.
We know that apoCs are involved in regulating fat transport in plasma and, hence, in maintaining energy flux in vivo; apoC-III could mediate some of the effects of insulin on triacylglycerol clearance in the fasting state, and we speculate that its increase in acute inflammation may confer protection against infective organisms by increasing the plasma lipid pool that counteracts lipopolysaccharide. ApoC-III shares a common apoA-I-like ancestral gene with other apoCs, apoAs and apoEs [29].

Several pathways have been proposed for the regulation of apoC-III gene expression. ApoC-III expression is regulated, in part, by insulin via the promoter IRE (insulin-response element) on the apoC-III gene [30]. Transcription of the apoC-III gene is down-regulated by insulin [31]. The transcription of the apoC-III gene is also mediated by PPARs (peroxisome-proliferator-activated receptors) [32]. The induction of PPARα, principally the PPARα form, reduces apoC-III gene expression [33,34]. These experimental findings suggest that, in insulin-resistant states, such as the metabolic syndrome and Type 2 diabetes, the expression and secretion of apoC-III by the liver and intestines are likely to be dysregulated.

**APOC-III: BIOCHEMICAL ANALYSES**

**Measuring plasma apoC-III concentrations in plasma**

Sensitive, accurate and specific methods for routine measurements of apoC-III are available. The most widely used is an immunoturbidimetric assay available from Wako Pure Chemicals Industries and Daiichi Chemicals. The principle of this assay is that, when a serum or plasma sample containing apoC-III is mixed with a buffer containing an anti-(human apoC-III) antibody, an insoluble turbid aggregate is formed. The degree of turbidity is measured optically and is proportional to the amount of apoC-III. ApoC-III can also be measured by ELISA [35] and by electroimmunodiffusion (Hydragel LP CIII; Sebia). Both fresh and frozen (−80°C) sera or plasma samples can be used to measure total apoC-III concentrations. The reference values for total apoC-III concentrations have been quoted as 92.5 ± 33.2 mg/l [36] and 112.2 ± 31 mg/l [37].

**Measuring apoC-III concentrations in non-apoB-containing subfractions**

The Hydragel LP CIII kit allows for the quantification of total plasma apoC-III and apoC-III-non-LpB (after precipitation of apoB-containing lipoproteins) in human plasma by electroimmunodiffusion on mildly alkaline-buffered agarose gels [38]. These gels contain anti-(apoC-III) mono-specific antibodies. Following protein migration, the resulting rockets are stained with Acid Violet solution. The excess stain is removed with an acid/alcohol mixed solution, and the height of the resulting immunoprecipitation rockets is proportional to the apoC-III concentration. Total plasma apoC-III and apoC-III-non-LpB concentrations are then calculated from the standard curve, and apoC-III-LpB concentrations are calculated as total apoC-III–apoC-III-non-LpB.

**Extraction of apoC-III from VLDL and HDL subfractions**

The specific laboratory methodologies associated with the extraction of apoC-III from VLDL and HDL...
are described in detail elsewhere [27,39]. In brief, lipoprotein fractions are isolated by sequential ultracentrifugation, and apoC-III is isolated from other apoproteins present in VLDL and HDL using preparative IEF (isoelectric focusing). Intralipid®, an artificial triacylglycerol/phospholipid emulsion, can also be applied prior to IEF separation to isolate apoC-III [27]. The principle of this methodology is that Intralipid® preferentially and rapidly extracts apoC proteins, thus minimizing the interference of other apoproteins that migrate with or near apoC-III by IEF separation [27].

**MECHANISTIC MODELS OF APOC-III METABOLISM**

Several mechanistic models have been developed to better understand the metabolism of apoC-III [40]. The apoC-III model shown in Figure 3(A) includes the secretion of apoC-III into the VLDL and HDL fractions, the exchange of apoC-III between VLDL and HDL, and the removal of apoC-III from plasma via both VLDL and HDL. This general compartment model is most consistent with our present understanding of apoC-III metabolism. From a theoretical standpoint, there is a unique solution or set of model parameters that can be determined for this model. Although the compartment model fits the apoC-III tracer results, the model parameters cannot be estimated with any degree of precision because of the rapid exchange of apoC-III between the VLDL and HDL fractions. The model shown in Figure 3(B) is the simplest model that describes apoC-III kinetics in the VLDL and HDL fractions. This model describes apoC-III kinetics using a single homogeneous plasma compartment that fits the VLDL- and HDL-apoC-III tracer results separately and estimates FCR (fractional catabolic rate) with high precision. As described below, the application of this model has elucidated the regulation of apoC-III metabolism in the metabolic syndrome [40,41].

**APOC-III AND THE PATHOPHYSIOLOGY OF DYSLIPIDAEMIA IN THE METABOLIC SYNDROME**

In the general population, plasma total and TRL apoC-III concentrations are positively correlated with plasma triacylglycerol concentrations. Accordingly, variation in the expression of apoC-III has been associated with varying severity of hypertriglyceridaemia.

The primary role of apoC-III is as a regulator of lipolysis through non-competitive inhibition of endothelial-bound LPL. This enzyme hydrolyses triacylglycerols in TRLs, releasing fatty acids into the plasma and transforming large triacylglycerol-rich particles into smaller triacylglycerol-depleted remnant lipoproteins [42,43] (Figure 4). Individuals lacking apoC-III have low TRL levels, coupled with highly efficient lipolysis of triacylglycerols [44]. Furthermore, mice in which the apoC-III gene is genetically deleted also have low plasma triacylglycerol levels and efficient TRL catabolism [45]. ApoC-III at high concentrations may also inhibit HL (hepatic lipase), a lipolytic enzyme with triacylglycerol lipase and phospholipase A1 activity that is synthesized in the liver [46] (Figure 4). The inhibitory effect of apoC-III on HL reduces further the lipolysis and uptake of TRL remnants by the liver [47].

More recently, apoC-III has been shown to stimulate VLDL synthesis in cultured cells [48,49]. The underlying mechanisms associated with this effect of apoC-III is unclear, but may relate to the inhibition of proteosome-mediated degradation of apoB, resulting in increased apoB synthesis and secretion [49], and increased synthesis of VLDL triacylglycerols [48]. ApoC-III may therefore play a key role in regulating VLDL output by the liver.

Cellular studies report that apoC-III may interfere with TRL and remnant binding to hepatic lipoprotein
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Figure 4 ApoC-III regulates lipoprotein metabolism by multiple mechanisms
ABCA1, ATP-binding cassette A1; CE, cholesterol ester; FC, free cholesterol; SR-B1, scavenger receptor B-1.

receptors. ApoC-III can abolish apoB- and apoE-mediated binding of lipoproteins to LDLR [LDL (low-density lipoprotein) receptor], either by masking or altering the conformation of apoB and apoE [50,51] (Figure 4). The binding of chylomicrons and VLDL particles to the LSR (lipolysis-stimulated receptor) is also significantly inhibited by apoC-III [52].

For the mechanistic reasons referred to above, we consider that elevated plasma apoC-III concentrations, and specifically its accumulation in TRLs and their remnants, is causally related to hypertriglyceridaemia in the metabolic syndrome.

LCAT (lecithin cholesterol acyltransferase) and CETP (cholesterol ester transfer protein) are involved in TRL and HDL metabolism. The regulatory effect of apoC-III on LCAT activity, an enzyme that catalyses the maturation of nascent to mature HDL, remains conflicting [53,54]. It is thought that apoC-III may regulate LCAT activity indirectly through apolipoproteins that activate LCAT, although further studies are required. Little has been published concerning the effects of apoC-III on CETP, a glycoprotein that facilitates the exchange of neutral lipids between TRL and HDL particles. A preliminary finding from a study using recombinant HDL particles suggests that apoC-III may stimulate CETP activity [55]. Further studies are required to determine the relationship between apoC-III with LCAT and CETP and its implications on TRL and HDL metabolism in the metabolic syndrome.

Kinetic studies using tracer methodology have provided valuable insights into the potential regulatory effects of apoC-III in the metabolic syndrome with hypertriglyceridaemia. Elevated rates of plasma and VLDL-apoC-III production explain the higher plasma and VLDL-apoC-III levels these subjects [40,41]. The increased production rate and concentration of VLDL-apoC-III were both significantly associated with elevated VLDL triacylglycerols [56,57], consistent with several studies showing that elevated apoC-III concentrations are associated with increased VLDL-apoB secretion and decreased VLDL-apoB catabolism [58–60]. Elevated plasma apoC-III concentrations were also associated with increased plasma concentrations of remnant-like protein cholesterol and apoB-48, consistent with the contribution of apoC-III to postprandial hypertriglyceridaemia in subjects with the metabolic syndrome [61]. The delayed catabolism of remnant-like particles was also reported to be related to elevated apoC-III, again supporting the notion that apoC-III inhibits lipolysis and hepatic update of TRL remnants [61]. These findings collectively support the concept that apoC-III is a key determinant in the clearance of TRLs and its remnants in
hypertriglyceridaemic states, including visceral obesity, insulin resistance and the metabolic syndrome.

ApoC-III AND CVD: EVIDENCE FROM CLINICAL, EXPERIMENTAL AND GENETIC STUDIES

Clinical studies
Results from several studies have demonstrated the importance of apoC-III as a predictor of CVD outcomes (Table 1). Both CLAS (Cholesterol-Lowering Atherosclerosis Study) and MARS (Monitored Atherosclerosis Regression Study) demonstrated that plasma apoC-III predicted progression of angiographic coronary artery disease [62,63]. CLAS also reported that a decrease in apoC-III-non-LpB was a significant independent predictor for increased progression of coronary lesions. In MARS, apoC-III-LpB predicted the risk of progression, independent of LDL-cholesterol concentrations. The ECTIM (Etude Cas-Témoin de l’Infarctus du Myocarde) study reported that subjects post-myocardial infarction had increased apoC-III-LpB levels compared with healthy control subjects [19]. The CARE (Cholesterol and Recurrent Events) trial demonstrated that apoC-III-LpB was a stronger predictor of coronary heart disease events than plasma triacylglycerols [64] (Figure 5). In a substudy of the CARE trial, the concentration of LDL particles containing apoC-III was an independent risk factor for coronary events in patients with diabetes [65]. This indicates that the atherogeneity of apoB particles may be conditional on the presence of apoC-III. In three cross-sectional analyses, higher apoC-III concentrations were associated with increased severity of CVD in subjects with angiographically defined coronary artery disease [66], with the metabolic syndrome [67] and with Type 2 diabetes [68]. Collectively, with the exception of one study [69], elevated apoC-III, in particular apoC-III-LpB, was a significant predictor of coronary events and progression of CAD [19,62–64,66–68,70].

Experimental and transgenic animal studies
The potential effects of apoC-III in atherogenesis have been examined in experimental studies. These suggest that apoC-III activates PKC (protein kinase C) and NF-κB (nuclear factor κB) expression of endothelial VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intracellular adhesion molecule-1), and the recruitment of monocytes to the vascular wall [71–73] (Figure 6). Hence the apoC-III pathway may promote a diverse inflammatory response through monocyte-endothelial interactions and play a specific role in the development of atherogenesis.

A large body of evidence supporting the role of apoC-III in atherogenesis has come from transgenic and gene-targeted mouse studies. Overexpression of human apoC-III in wild-type mice enhances the development of atherosclerosis [74], in association with elevated triacylglycerol and low HDL-cholesterol and apoA-I levels. Elevated apoC-III concentrations and gene expression were also associated with an increase in hepatic VLDL triacylglycerol production rate, decreased VLDL and remnant catabolic rates [75,76], and reduced TRL binding to proteoglycan matrix on the surface of endothelial cells where LPL is located. Overexpression of apoC-III in apoE-knockout mice resulted in the accumulation of TRLs and hypertriglyceridaemia [77], whereas overexpression of apoC-III in LDLR-knockout mice was associated with severe hypercholesterolaemia and atherosclerosis [78]. By contrast, targeted disruption of the apoC-III gene in mice results in rapid catabolism of TRLs, consistent with decreased inhibition of LPL and increased lipolysis [79], and protection from both fasting and postprandial hypertriglyceridaemia [45], implying protection against atherogenesis.

ApoC-III deficiencies and genetic polymorphisms
The consequence of a complete lack of apoC-III in human subjects on the changes in plasma lipoprotein levels and CVD is unclear. In most studies, apoC-III deficiency was coupled with an apoA-I deficiency; however, one genetic variant of apoC-III (Lys58→Glu) was associated with lower plasma apoC-III and triacylglycerol levels, and higher HDL-cholesterol and apoA-I concentrations [80]. Animal knockout studies provide some insight into the significance of apoC-III deficiency. ApoC-III-knockout mice had normal intestinal lipid absorption and hepatic VLDL triacylglycerols secretion, but a rapid clearance of VLDL triacylglycerols and VLDL cholesteryl esters from plasma that may explain the observed hypolipidaemia [81,82]. The enhanced LPL-mediated lipolysis of VLDL triacylglycerols in apoC-III-knockout mice was also shown to be independent of apoE [82]. Furthermore, experimental apoC-III deficiency may prevent hyperlipidaemia associated with the overexpression of apoE [81]. These studies support the concept that apoC-III is an effective inhibitor of VLDL triacylglycerols hydrolysis and a potential target for reducing CVD progression.

Polymorphisms in the apoC-III gene may have implications for hypertriglyceridaemia and susceptibility to CVD. Carriers of the C3238G gene variant have higher plasma apoC-III and triacylglycerol concentrations [83–85]. However, whether this gene variant confers increased CVD risk is conflicting [86]. Homozygotes for the T→455C variant are resistant to insulin-mediated down-regulation of apoC-III gene transcription and have elevated triacylglycerol levels and increased CVD.
### Table 1  Clinical studies showing direct associations between apoC-III levels and CAD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Author</th>
<th>Subjects</th>
<th>Study design</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[62]</td>
<td>Blankenhorn et al. (1990)</td>
<td>162 men with CAD in the CLAS trial</td>
<td>Randomized placebo-controlled angiographic trial</td>
<td>↑ apoC-III risk factor for angiographic progression of CAD in the placebo group ↓ apoC-III-LpB/apoC-III-LpB is associated with the progression of CAD in the placebo group ↓ in apoC-III-LpB is a significant independent predictor for ↑ progression of CAD in the drug group</td>
</tr>
<tr>
<td>[70]</td>
<td>Chivot et al. (1990)</td>
<td>74 men with CAD and 78 control men</td>
<td>Case-control study</td>
<td>↑ apoC-III and apoC-III-LpB in subjects with CAD ↑ apoC-III-LpB predicted CAD independent of triacylglycerols</td>
</tr>
<tr>
<td>[69]</td>
<td>Genest et al. (1991)</td>
<td>145 men with CAD and 135 control men</td>
<td>Case-control study</td>
<td>↓ apoC-III in subjects with CAD LpB:C-III not different between CAD and control groups</td>
</tr>
<tr>
<td>[63]</td>
<td>Hodis et al. (1994)</td>
<td>220 subjects with CAD and moderate dyslipidaemia in MARS trial</td>
<td>Randomized double-blind placebo-controlled angiographic trial</td>
<td>↑ apoC-III-LpB is an independent predictor of progression of CAD apoC-III-LpB &gt; 5.10 mg/dl, RR for progression of CAD = 5.10 (1.4–17.1)</td>
</tr>
<tr>
<td>[19]</td>
<td>Luc et al. (1996)</td>
<td>360 post-myocardial infarction and 489 healthy subjects in the ECTIM study</td>
<td>Case-control study</td>
<td>↓ apoC-III-LpB/apoC-III-LpB ratio associated with CAD</td>
</tr>
<tr>
<td>[68]</td>
<td>Gervaise et al. (2000)</td>
<td>188 subjects with Type 2 diabetes</td>
<td>Cross-sectional study</td>
<td>↑ apoC-III and apoC-III-LpB in subjects with Type 2 diabetes ↑ apoC-III-LpB associated with CAD apoC-III-LpB &gt; 17 mg/L, OR for macroangiopathy = 2.73 (1.33–5.60) apoC-III-LpB &gt; 17 mg/L, OR for CAD = 3.95 (1.73–9.04)</td>
</tr>
<tr>
<td>[64]</td>
<td>Sacks et al. (2000)</td>
<td>788 men with myocardial infarction</td>
<td>Prospective nested, case-control study</td>
<td>↑ apoC-III-LpB predicted CAD independent of triacylglycerols, HDL-cholesterol and LDL-cholesterol apoC-III-LpB &gt; 10.2 mg/dl, RR for recurrent coronary events = 2.25 (1.4–3.6)</td>
</tr>
<tr>
<td>[67]</td>
<td>Onat et al. (2003)</td>
<td>875 subjects with metabolic syndrome</td>
<td>Cross-sectional study</td>
<td>↑ apoC-III-LpB associated with CAD apoC-III-LpB &gt; 7.6 mg/dl, OR for CAD in women = 3.22 (1.29–8.01) apoC-III-LpB ≥ 8.0 mg/dl, OR for CAD in men = 8.87 (2.64–29.8) ↑ apoC-III and apoC-III-LpB associated with the metabolic syndrome apoC-III-LpB &gt; 7.0 mg/dl, OR for metabolic syndrome = 4.66 (3.43–6.32)</td>
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</table>
risk [87]. The C1100T variant is associated with increased triacylglycerol levels, predominantly in homozygotes [88]; however, this genetic variant was not associated with variations in apoC-III levels.

**THERAPEUTIC REGULATION OF APOC-III**

**Statins**

A rate-limiting step in cholesterol biosynthesis is the conversion of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) into mevalonic acid by HMG-CoA reductase [89]. Statins competitively inhibit this enzyme, decreasing the intracellular pool of cholesterol and reciprocally up-regulating LDLR activity. Independent of its effects on LDL and LDLR activity, statins lower the plasma concentration and cellular mRNA levels of apoC-III [64,90–92] (Table 2). Although the precise mechanism for the effects of statins on apoC-III is not fully understood, it may relate to the activation of hepatic PPARα protein [93] and, hence, a reduction in the expression and secretion of apoC-III. Furthermore, statins improve insulin action in subjects with hypertriglyceridaemia [94]. This may translate to an insulin-dependent down-regulation of apoC-III gene transcription via the IRE in the apoC-III promoter.

**PPAR agonists**

PPARs are nuclear transcription factors that regulate the expression of genes involved in lipid and lipoprotein metabolism. There are three PPAR isoforms (PPARα, PPARγ and PPARδ), each representing an intracellular ligand-induced receptor known to heterodimerize with RXR (retinoid X receptor) for transcriptional promotion of various enzymes [95].

PPARα agonists, also known as fibrates, regulate lipid metabolism and may diminish CVD events [96]. Reduction in the expression and levels of apoC-III is the most consistent effect of PPARα agonists [33]. A 36% reduction in plasma apoC-III levels was reported with fenofibrate treatment in the metabolic syndrome [97] (Table 2). Fibrates also increase acyl CoA synthase and fatty acid transporter protein; this facilitates intracellular transport, acylation and β-oxidation of fatty acids, with the net effect of decreasing fatty acid availability for triacylglycerol synthesis and VLDL-apoB secretion [98].

In vitro, fibrates can induce the expression of genes encoding LPL, apoA-I, apoA-II and ABCA1 (ATP-binding cassette A1).

PPARγ agonists, such as pioglitazone and rosiglitazone, improve insulin sensitivity in muscle, liver and fat, and are efficacious therapies in treating diabetic dyslipidaemia [99]. Pioglitazone was shown to significantly decrease apoC-III concentrations by reducing its production rate [100]. The mechanism whereby
Mean percentage changes in total plasma apoC-III and lipid and lipoprotein concentrations in subjects with dyslipidaemia following lipid-regulating interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Reference</th>
<th>ApoC-III</th>
<th>Triacylglycerols</th>
<th>LDL-cholesterol</th>
<th>HDL-cholesterol</th>
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<tr>
<td>Statis</td>
<td>[64,90–92]</td>
<td>↓ 27%</td>
<td>↓ 7–30%</td>
<td>↓ 18–55%</td>
<td>↑ 5–15%</td>
</tr>
<tr>
<td>Fibrates</td>
<td>[33,97]</td>
<td>↓ 36%</td>
<td>↓ 20–50%</td>
<td>↓ 5–20%</td>
<td>↑ 10–20%</td>
</tr>
<tr>
<td>Thiazolidinomedones</td>
<td>[100]</td>
<td>↓ 20%</td>
<td>↓ 16–30%</td>
<td>↑ 0–8%</td>
<td>↑ 14–15%</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>[112]</td>
<td>↓ 21%</td>
<td>↓ 6–11%</td>
<td>↓ 12–14%</td>
<td>↑ 1–5%</td>
</tr>
<tr>
<td>Niacin</td>
<td>[119]</td>
<td>↓ 31.5%</td>
<td>↓ 20–50%</td>
<td>↓ 5–25%</td>
<td>↑ 15–35%</td>
</tr>
<tr>
<td>Fish oil</td>
<td>[113,114]</td>
<td>↓ 0–5%</td>
<td>↓ 25–44%</td>
<td>↓ 5–25%</td>
<td>↑ 0–8%</td>
</tr>
<tr>
<td>Weight loss</td>
<td>[123]</td>
<td>↓ 9%</td>
<td>↓ 5–15%</td>
<td>↓ 5–18%</td>
<td>↑ 0–9%</td>
</tr>
</tbody>
</table>

The effects may be attributed to EPA (eicosapentaenoic acid), rather than DHA (docosahexaenoic acid). EPA regulates hepatic fatty acid oxidation and has a direct effect on TRL synthesis, assembly and secretion [115,116]. The mechanism underlying the apoC-III-lowering effect of fish oils is unclear, but may relate to the activation of PPARα [117] (Figure 2). Future studies are required to clarify the regulatory effects of fish oil on apoC-III metabolism and their role in managing dyslipidaemia in the metabolic syndrome.

FXR (farnesoid X receptor) agonists

FXR is a member of the nuclear receptor superfamily that is expressed in the liver, intestines and kidneys [104]. Following heterodimerization with RXR, FXR binds and activates the transcription of genes via positive FXR-response elements [105]. FXR may also regulate lipid and lipoprotein metabolism [106]. Previous studies have reported that FXR agonists decrease plasma triacylglycerol and apoC-III concentrations [107,108]. The mechanism is unclear, but may relate to the down-regulation of apoC-III mRNA and protein expression [107], as suggested for PPARα in Figure 2.

Ezetimibe

Ezetimibe inhibits dietary and biliary cholesterol absorption at the brush border of the intestine by binding to the NCP1L1 (Niemann–Pick C1-like 1) transporter [109]. It has been shown to reduce LDL-cholesterol by 12–14% [110]. The LDL-cholesterol-lowering effect of ezetimibe is predominantly associated with increases in catabolism of VLDL, IDL (intermediate-density lipoprotein) and LDL-apoB [111]. A recent trial demonstrated further that ezetimibe significantly reduced apoC-III and triacylglycerol concentrations in dyslipidaemic subjects [112] (Table 2); however, the effect of ezetimibe on apoC-III metabolism has not been elucidated and warrants further study.

Fish oils

n-3 Polyunsaturated fatty acids contained in fish oils may exert triacylglycerol- and apoC-III-lowering effects [113,114] (Table 2). The effects may be attributed to EPA (eicosapentaenoic acid), rather than DHA (docosahexaenoic acid). EPA regulates hepatic fatty acid oxidation and has a direct effect on TRL synthesis, assembly and secretion [115,116]. The mechanism underlying the apoC-III-lowering effect of fish oils is unclear, but may relate to the activation of PPARα [117] (Figure 2). Future studies are required to clarify the regulatory effects of fish oil on apoC-III metabolism and their role in managing dyslipidaemia in the metabolic syndrome.

Niacin

Nicotinic acid (or niacin) confers beneficial effects on all major plasma lipid and lipoprotein fractions, particularly by increasing HDL-cholesterol concentrations and decreasing both LDL-cholesterol and triacylglycerol concentrations [118]. In addition, niacin has been shown to reduce VLDL-apoC-III concentrations in subjects with hyperlipidaemia [119]. Niacin activates the G-protein-coupled nicotinic acid receptor [120] and inhibits diacylglycerol acyltransferase 2, the key enzyme in triacylglycerols synthesis [121] and hormone-sensitive triacylglycerol lipase, a lipolytic enzyme [122]. It is through the combination of these actions that niacin exerts a potent effect upon lipid and lipoprotein metabolism; however, the exact regulatory effect of niacin on apoC-III metabolism is unclear and warrants further study.

Lifestyle modifications

Weight loss has been associated with a reduction in plasma apoC-III concentrations in postmenopausal women [123], with the decrease in apoC-III being associated with the decrease in plasma triacylglycerol concentrations. In a 16-week randomized controlled dietary intervention study, weight loss was associated with an 18% reduction in apoC-III concentrations in men with the metabolic syndrome (Ng, T.W.K., Barrett, P.H.R., Chan, D.C. and Watts, G.F., unpublished work). The reduction in plasma apoC-III with weight loss was associated with decreases in insulin, triacylglycerols, apoB-48, VLDL-apoB and
RLP (remnant-like protein)-cholesterol. The underlying mechanism of action of weight loss on apoC-III metabolism requires further study, but it is likely to be associated with improved insulin sensitivity and reduced hepatic secretion of apoC-III. Dietary composition has also been demonstrated to have varying effects on plasma apoC-III concentrations [124–126] (Table 2). Consumption of diets high in MUFAs (monosaturated fatty acids) resulted in a significant reduction in plasma apoC-III and fasting triacylglycerol levels [57]. By contrast, a low-fat and high-carbohydrate diet was associated with increased apoC-III and triacylglycerol concentrations [127]. The hypotriglyceridaemic effect of a high-MUFA diet may be attributable, in part, to reduced hepatic apoC-III production [57]. Studies also suggest that alcohol consumption increased total and apoC-III non-LpB and decreased apoC-III LpB [128]. Further studies are required to elucidate the regulatory effect of alcohol on apoC-III metabolism. The effect of exercise on apoC-III metabolism has yet to be examined and warrants further study.

**Other therapies**

The use of siRNA (small interfering RNA) directed at inhibiting the translation of apoB mRNA has attracted much recent attention as a highly effective approach for treating dyslipidaemia [129]. Further investigations are warranted to determine whether this antisense technology may be applied to inhibit apoC-III synthesis and to treat the associated dyslipidaemia.

**CONCLUSIONS**

Elevated plasma triacylglycerol concentrations have been recently identified as powerful predictors of CVD in diverse populations. Abnormal metabolism of apoC-III, a small-molecular-mass peptide, may form the basis for this relationship. Recent studies also provide evidence for a direct role of apoC-III in atherogenesis, supporting the findings in clinical studies that elevated apoC-III are independent predictors of coronary events in high risk individuals. ApoC-III metabolism is a complex physiological system that needs to be elucidated further using tracer methods and systems analysis. In time, apoC-III may become a new target for interventions, particularly in subjects with insulin resistance, the metabolic syndrome and Type 2 diabetes. Future studies are required to establish the therapeutic target for apoC-III and the practical aspects of its routine assay in the laboratory.

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