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THE PRESENT AND FUTURE

STATE-OF-THE-ART REVIEW

Genetics and Causality of Triglyceride-Rich Lipoproteins in Atherosclerotic Cardiovascular Disease

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ABSTRACT

Triglycerides represent 1 component of a heterogeneous pool of triglyceride-rich lipoproteins (TGRLs). The reliance on triglycerides or TGRLs as cardiovascular disease (CVD) risk biomarkers prompted investigations into therapies that lower plasma triglycerides as a means to reduce CVD events. Genetic studies identified TGRL components and pathways involved in their synthesis and metabolism. We advocate that only a subset of genetic mechanisms regulating TGRLs contribute to the risk of CVD events. This "omic" approach recently resulted in new targets for reducing CVD events. (J Am Coll Cardiol 2014;64:2525–40) © 2014 by the American College of Cardiology Foundation.

Triglyceride-rich lipoproteins (TGRLs) comprise a vast array of intestinally derived and hepatically secreted particles with distinct compositions and associations with risk for cardiovascular disease (CVD) or pancreatitis. Although the contribution of plasma/serum triglycerides (triacylglycerols [TG]) to increased risk of coronary and cerebrovascular ischemic events was established in multivariate models that adjust for major risk markers, including low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) (1,2), elevated TGRLs alter low-density lipoprotein (LDL) and high-density lipoprotein (HDL) composition and function, which may result in unaccounted risk in observational studies and clinical trials of lipid-modifying therapies. Despite the association of circulating TGRL levels with atherosclerosis, whether abnormal TGRL metabolism and/or TGRL lipolytic products are causal remains uncertain.

The mechanisms underlying TGRLs and atherosclerotic CVD risk are incompletely understood. Mendelian randomization studies provide evidence for causal involvement of TG-mediated pathways in coronary heart disease (CHD); however, the contribution of TGRLs per se was not directly assessed (3). Furthermore, in clinical trials, TG-lowering therapies

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ABBREVIATIONS AND ACRONYMS

Apo = apolipoprotein

CHD = coronary heart disease

CVD = cardiovascular disease

DGAT = diacylglycerol acyltransferase

HDL = high-density lipoprotein

LDL = low-density lipoprotein

PPAR = peroxisome proliferator-activated receptor

TG = triacylglycerols

TGRLs = triglyceride-rich lipoproteins

VLDL = very low-density lipoprotein

not only alter TGRL concentration and composition, but also affect LDL, HDL, and inflammatory pathways.

This state-of-the-art review discusses the complexities of TGRL-associated atherosclerotic CVD risk and new directions in risk assessment and therapeutic responses to TG-lowering therapies from genetic studies. It is not intended to reiterate recent consensus statements on hypertriglyceridemia definitions, diagnosis, and management (4,5).

TGRL, HUMAN ATHEROSCLEROSIS, AND ATHEROSCLEROTIC CARDIOVASCULAR EVENTS

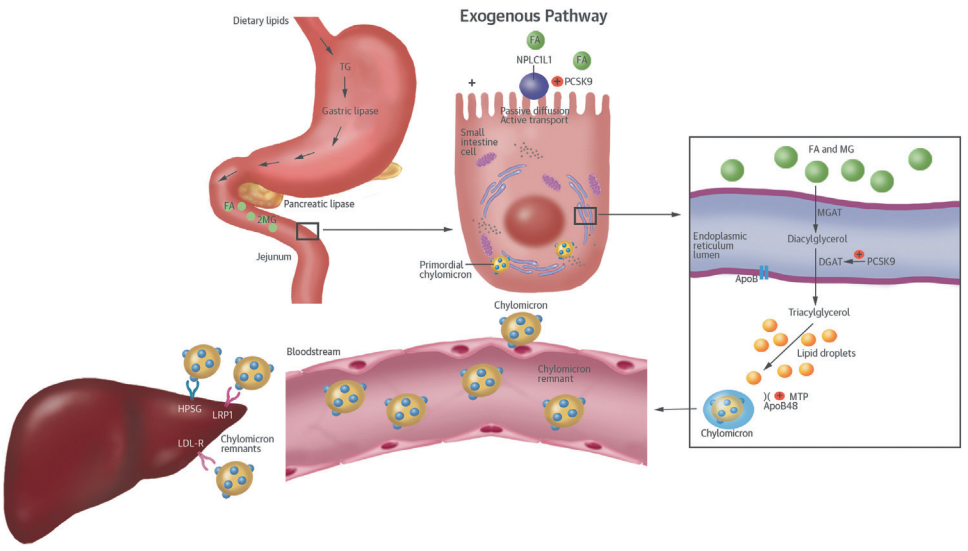
Chylomicron and very low-density lipoprotein (VLDL) remnants rapidly penetrate the arterial wall and contribute cholesterol to atherosclerotic lesions (6–8). VLDL composition is a critical CVD risk determinant. In retrospective and prospective population studies, TG-associated CHD risk was limited to apolipoprotein (Apo) C3-containing VLDL particles and their metabolic remnants, small LDL particles (9–11). VLDL proteome analysis expanded the complexities of VLDL through identification of 33 functional pathways, including 4 related to lipid transport and lipoprotein metabolism, and 8 associated with coagulation, hemostasis, and immunity (12).

METABOLISM OF INTESTINAL AND HEPATIC-DERIVED TGRLS

Chylomicrons are intestinal-specific lipoproteins, formed mainly in the jejunum after a meal. Owing to the large TG core (>90%), chylomicron density is <1.006 g/ml, but they are heterogeneous, ranging from 75 to 1,200 nm in diameter. They also contain a small amount of cholesteryl esters, 1 structural protein, apoB48, and minor exchangeable apolipoproteins.

Dietary TG are hydrolyzed in the stomach and proximal small intestine to form fatty acids and 2-monoacylglycerol (**Central Illustration**, top) (13). Enterocytes absorb these lipids through either passive

CENTRAL ILLUSTRATION **Intestinal Synthesis and Hepatic Synthesis and Metabolism of TGRLs**



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(Top) Key pathways regulating intestinal synthesis and metabolism of triglyceride-rich lipoproteins (TGRLs) are illustrated (see the text for details of this pathway and its genetic regulation). **(Bottom)** Key pathways regulating hepatic synthesis and metabolism of TRGLs are illustrated (see the text for details about this pathway and its genetic regulation). ANGPTL3 = angiopoietin-like protein 3; Apo = apolipoprotein; DGAT = diglyceride acyltransferase; ER = endoplasmic reticulum; FA = fatty acid; HSPG = heparin sulfate proteoglycan; GHIHBP1 = glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; LDL = low-density lipoprotein; LDL-R = low-density lipoprotein receptor; LPL = lipoprotein lipase; LRP1 = low-density lipoprotein receptor-related protein; MG = monoglyceride; MGAT = monoglyceride acyl transferase; MTP = microsomal transfer protein; NPLC1L1 = Niemann-Pick C1 like; PCSK9 = proprotein convertase subtilisin kexin type 9; PCTV = pre-chylomicron transport vesicle; TG = triglyceride; VLDL = very low-density lipoprotein; VTV = very low-density lipoprotein transport vesicle.

diffusion or the action of specific transporters. At the endoplasmic reticulum (ER) membrane, monoacylglycerol:acyl CoA transferase converts monoacylglycerol and fatty acids into diacylglycerol acids, which diacylglycerol:acyl CoA transferase (DGAT) subsequently converts into TGs (14). Lipid droplets (LDs) are transiently formed in the cytosol, and then fuse with apoB48 phospholipid-rich particles in the ER lumen via the action of a chaperone, microsomal transfer protein (MTP), which mediates primordial chylomicron particle formation. Proprotein convertase subtilisin kexin type 9 (PCSK9) enters and stimulates intestinal TGRL production by increasing both apoB48 synthesis and TG production through transcriptional and post-transcriptional mechanisms involved in TRL assembly (MTP messenger ribonucleic acid [mRNA], protein, and lipid transfer activity and Niemann-Pick C1 like protein levels), and sterol response element binding protein 1 (SREBP1) target genes involved in fatty acid/triglyceride biosynthetic pathway (fatty acid synthase, stearoyl-CoA desaturases, and diglyceride acyltransferase-2 [DGAT2]) (15). Chylomicrons exit the ER to the Golgi via prechylomicron transport vesicles (16). After a meal, chylomicrons are exported into mesenteric lymph before entering the circulation, where they contribute to post-prandial TG concentrations (17,18). Lipoprotein lipase (LPL) subsequently hydrolyzes chylomicrons into fatty acids used as an energy supply by the heart and skeletal muscles or stored in adipose tissue. Chylomicron remnants are removed from the circulation after binding to the LDL receptor via the apoE ligand, or by other routes including LDL receptor-related protein 1 and the heparan sulfate proteoglycan pathway (19). Although clearance is the primary mechanism for chylomicron remnants, ApoB48 overproduction increases chylomicron production in fructose-fed, insulin-resistant hamsters (20).

VLDL biogenesis occurs in hepatocytes by a 2-step process involving synthesis of partially lipidated apoB100 to form a primordial VLDL particle (through a process involving MTP), followed by TG addition to the primordial particle in the ER lumen (**Central Illustration**, bottom). However, apoC3 inhibits VLDL assembly independent of MTP (21). After assembly, nascent VLDL particles are transported to the Golgi by a rate-limiting step mediated by a specific ER-derived VLDL transport vesicle (22). Cell death-inducing DFF45-like effector b (cideB) is a VTF-associated protein that mediates VLDL lipidation and maturation (23). CideB silencing results in hepatic secretion of small VLDL particles, which is evidence that VLDL heterogeneity is, in part, genetically regulated.

LPL is the principal enzyme that hydrolyzes TG in TGRLs. LPL is primarily synthesized in the parenchyma of tissues that use fatty acids for energy (cardiac muscle, skeletal muscle) and TG storage (adipose tissue). LPL attaches to heparan sulfide side chains of proteoglycans, and is transported to the capillary lumen by the endothelial protein glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein-1 (GPIHBP1) (24). GPIHBP1 provides a platform for LPL to process TG from TGRL. TGRL hydrolysis at the capillary lumen releases free fatty acids and monoglycerides, resulting in partially catabolized chylomicrons (chylomicron remnants) and VLDL particles (VLDL remnants).

Due to its critical role in lipid homeostasis, LPL activity is highly regulated at the transcriptional, post-transcriptional, translational, and post-translational levels. Various proteins regulate LPL including: apoC1 (25,26), apoC2 (27), apoC3 (26,28), apoA5 (29), angiopoietin-like protein 3 (ANGPTL3) (30), ANGPTL4 (31,32), and ANGPTL8 (33). ANGPTL4 is a secreted protein induced in adipose tissue by fasting (34). TGRL binding stabilizes LPL; however, apoC1 and apoC3 displace LPL from LDs, where ANGPTL4 inactivates it in the subendothelial space. This allows ANGPTL4 to bind LPL's N-terminus, resulting in dissociation of catalytically active LPL homodimers to monomers (26). ANGPTL3 renders LPL more susceptible to proteolytic inactivation by proprotein convertases (35).

Low amounts of LPL are detected in human blood, where it is transported by both apoB48- and apoB100-containing lipoproteins. In humans, apoB-containing lipoproteins with LPL are cleared more rapidly than apoB-containing particles without LPL (36). LPL was more effective in enhancing post-prandial clearance of apoB48- than apoB100-containing lipoproteins. Although apoE facilitates receptor-mediated TGRL clearance pathways, it did not affect TGRL clearance in this study.

The very low-density lipoprotein receptor (VLDLR), an LDL receptor family member (37), is expressed in heart, skeletal muscle, and adipose tissue (38,39). Under most circumstances, it is not detected in the liver; however, fenofibrate activation of the peroxisome proliferator-activated receptor (PPAR) alpha increases hepatic VLDLR translational activity through peroxisome proliferator response element binding to the VLDL promoter (40). After VLDLs bind to VLDLR, VLDL-derived FA are delivered to peripheral tissues, where they are used by heart and muscle for energy and in adipose tissue as an FA storage reservoir. ApoC3 impairs VLDL binding to cellular receptors, resulting in formation of small LDL particles (10,11), thereby counteracting the effects of apoE (41).

INFLAMMATION

TGRLs produced from both the exogenous (chylomicrons) or endogenous (VLDL) pathways are

proinflammatory. Chylomicron accumulation favors pancreatic inflammation and is associated with increased risk of acute pancreatitis. In contrast, VLDL, intermediate-density lipoprotein (IDL), VLDL remnants, and chylomicron remnants increase endothelial inflammation and facilitate arterial wall infiltration of monocytes. After a high-fat meal, hypertriglyceridemic subjects produce large, buoyant chylomicrons and VLDLs. VLDL particles produced post-prandially have a distinct fatty acid composition and increased apoC3 (42,43). TGRLs with high TG content up-regulate tumor necrosis factor-alpha, thereby inducing vascular cell adhesion molecule (VCAM)-1 expression in human aortic endothelial cells and monocyte adhesion. By reducing VCAM-1 expression and monocyte recruitment, TGRLs with low triglyceride content have an atheroprotective effect (42–44) (**Table 1**). The influence of TGRLs on VCAM-1 transcription and expression depends on ER homeostasis. ER stress is the accumulation of unfolded or misfolded proteins in the ER lumen, and is a unifying moniker for metabolic dysregulation linking obesity to insulin resistance and inflammation in diabetes and atherosclerosis (45,46). ER stress is mitigated by the unfolded protein response, which involves dissociation of binding immunoglobulin protein from ER transmembrane sensor proteins and their downstream effectors. In hypertriglyceridemic subjects, TGRLs regulate tumor necrosis factor alpha-induced VCAM-1 expression kinetics through processes involving ER stress and the unfolded protein response (47).

TGRL lipolysis by LPL on the endothelial cell surface elaborates high concentrations of lipolysis products along the blood endothelial interface, which may contribute to atherosclerosis through mechanisms encompassing proinflammatory, procoagulant, and proapoptotic gene activation. TGRL lipolysis releases neutral and oxidized free fatty acids that induce endothelial inflammation, vascular apoptosis (48), and reactive oxygen species production in endothelial cells, and altered lipid raft physiology (49).

TABLE 1 TGRLs and Inflammation

Endothelium	VCAM-1 transcription and expression
	Monocyte infiltration
	Increased permeability facilitates VLDL remnant uptake
Blood	Lipolytic products (oxidized fatty acids)
	ROS-mediated AP-1 up-regulation of IL-1, IL-6, IL-8, VEGF, and PPAR-gamma
Intestinal microflora	LPS transport into blood via dietary lipids

AP = activator protein; IL = interleukin; LPS = lipopolysaccharide; PPAR = peroxisome proliferator-activated receptor; ROS = reactive oxygen species; TGRL = triglyceride-rich lipoprotein; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial cell growth factor; VLDL = very low-density lipoprotein.

The mechanism for vascular inflammation and apoptosis depends upon induction of transcription factor ATF3-related genes in p38 and stress-activated protein kinase/c-Jun N-terminal kinase subset of mitogen-activated protein kinase signaling, and cytokines associated with activator protein 1 signaling, including interleukin-1-alpha, interleukin-6, interleukin-8, vascular endothelial cell growth factor, and PPAR-gamma (50). TGRL lipolysis products increase endothelial permeability and VLDL remnant uptake in the artery wall (51). The presence of VLDLR on macrophages, endothelium, and vascular smooth muscle surfaces of the vessel wall may further facilitate internalization of VLDL remnants.

Intestinal microflora were recently recognized as another source of inflammatory mediators. Gram-negative bacteria in the intestinal microflora release lipopolysaccharide (LPS) after lysis. Dietary lipids facilitate LPS entry into the circulation (52). Upon cell entry via Toll-like receptor 4, LPS activates redox-sensitive transcription factors that regulate proinflammatory responses.

TGRLs increase atherosclerosis, inflammation, and the lipid content of atherosclerotic lesions both directly and indirectly. Causal pathways supporting direct contributions of TGRLs to CVD events are discussed in the following sections.

PHYSIOLOGY OF POST-PRANDIAL LIPEMIA: CARDIOVASCULAR RISK OF FASTING VERSUS NONFASTING TRIGLYCERIDE MEASUREMENT

Current guidelines recommend screening and management of patients with hypertriglyceridemia on the basis of fasting TG measurement (53,54). However, prospective studies suggest that altered post-prandial TG metabolism contributes to atherosclerosis and may predict CVD development. Furthermore, studies demonstrated variable post-prandial metabolic responses in dyslipidemic patients compared with healthy subjects, suggesting that common genetic polymorphisms may underlie this difference (55,56). Nonfasting TG measurement was proposed as a marker for CVD risk stratification; however, further investigation into factors modulating the post-prandial response may prove valuable.

The recommendation for TG measurement in the fasting state arose due to early work demonstrating the unreliability of fat tolerance testing, primarily due to individual differences in gastric emptying, raising concerns regarding serum TG level variability (57–59). The lack of direct LDL-C assays promoted widespread adoption of the Friedewald equation to

estimate LDL-C, which depends on fasting-state measurements (60). Additionally, epidemiological evidence showed that fasting TGs are statistically independent predictors of atherosclerosis and incident CVD events (1,2). Studies also revealed a strong correlation between fasting and nonfasting TG, regardless of glucose status. Thus, fasting TG levels have been used as a predictor of post-prandial TG levels (61,62). However, the recommendation to measure fasting TG is not made on the basis of prospective studies demonstrating its superiority in predicting CVD risk compared with nonfasting TG. Instead, following the implementation of screening guidelines, epidemiologic investigations simply adopted fasting TG level measurements (62).

In contrast to the observed variability with fat tolerance tests, lipoprotein profiles change only minimally in response to normal food consumption in healthy individuals, thereby calling into question concerns over post-prandial serum TG level variability (60,62). In addition, fasting TG measurement may not accurately capture the effect of TGRLs and remnant particles in the post-prandial period on multiple lipoprotein profile components (63). Furthermore, prospective studies suggested that nonfasting TG levels are equal to or, in some populations, better than fasting TG levels for predicting future CVD events (64,65). These studies controlled for variability in post-prandial TG measures by stratifying by time since last meal, in addition to other factors implicated in affecting post-prandial measures including age, sex, body mass index, ethnicity, menopausal status, hormone use, and diabetes (64,65) (**Table 2**).

The post-prandial period is characterized by circulation of potentially atherogenic lipoprotein particles absorbed and processed through the intestine and liver, including chylomicrons, VLDL, and remnant particles (55,66). Their presence is modulated by traditional epidemiologic and environmental factors including sex, age, body mass index, physical activity, and smoking, and by the amount and type of dietary fat in a meal (67,68). Because individuals are in the nonfasting state most of the day (approximately 18 h) (68), the notion that alterations in post-prandial TG concentration and lipoprotein clearance contribute to atherogenesis is plausible. Recent data showed that in normolipidemic control subjects, a post-prandial rise in TG and quantitative changes in other lipoproteins and lipids are negligible in response to dietary fat compared with baseline values (58). However, in patients with dyslipidemia, the rise in TGRL is 3-fold greater than normal, and persists for up to 8 h (55,56). Studies demonstrated the atherogenic potential of remnant particles in these

TABLE 2 Recent Studies Investigating the Use of Nonfasting TG and RC Levels as Predictors of CVD Events

Study/First Author (Ref. #)	Primary Endpoint, Sample Size	Primary Endpoint, Hazard Ratio (95% CI)	Subgroup, Sample Size	Subgroup Primary Endpoint, Hazard Ratio (95% CI)	Fasting/Nonfasting Definition (Limitations)
The Women's Health Study (64)	Incident CVD, nonfatal MI, nonfatal stroke, coronary revascularization, or cardiovascular death (n = 26,509)	Fasting 2.23 (1.82–2.74) Nonfasting 2.53 (1.69–3.79)	HDL-C ≥50 mg/dl	Fasting 1.32 (1.03–1.68) Nonfasting 1.94 (1.21–3.10)	Fasting: ≥8 h since last meal Nonfasting: <8 h since last meal (Only 1 measurement collected from each subject—either a fasting or nonfasting measurement, as determined by self-reporting of time from last meal)
Nordestgaard et al. (65)	Incident MI, CVD, and death with 1 mmol/l increase in nonfasting TG (n = 13,981)	CVD outcome: Women 1.30 (1.22–1.40) Men 1.14 (1.10–1.19)	Adjusted for baseline HDL-C	Women 1.25 (1.14–1.37) Men 1.12 (1.07–1.18)	Fasting: No fasting samples collected Nonfasting: 1–8 h from last reported meal (Nonfasting group consisted of post-prandial measures from subjects within the CCHS and CGPS, and therefore across different time periods and by different methods [self-report and FTT])
Varbo et al. (72)	Incidence of CVD with 1 mmol/l increase in nonfasting RC (n = 73,513)	1.4 (1.3–1.5)	Adjusted by ratio of nonfasting RC to HDL-C	1.23 (1.19–1.27)	Fasting: No fasting samples collected (Nonfasting RC calculated indirectly by subtracting HDL-C and LDL-C from TC)
Jørgensen et al. (73)	Incidence of MI with genetically elevated doubling of nonfasting TG and RC (n = 10,391)	1.87 (1.25–2.81)	Adjustment by nonfasting measure	TG 1.57 (1.32–2.68) RC 1.67 (1.38–2.02)	(Nonfasting group consisted of post-prandial measures from subjects within the CCHS, CGPS, and CIHDS, and therefore with methodological variability in collecting nonfasting TG levels [adjusted for in the Jørgensen et al. (73) study])

1 mmol/l = 88.41 mg/dl.

CCHS = Copenhagen City Heart Study; CGPS = Copenhagen General Population Study; CI = confidence interval; CIHDS = Copenhagen Ischemic Heart Disease Study; CVD = cardiovascular disease events; FTT = fat tolerance test; HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol; MI = myocardial infarction; RC = remnant cholesterol; TC = total cholesterol; TG = triglycerides.

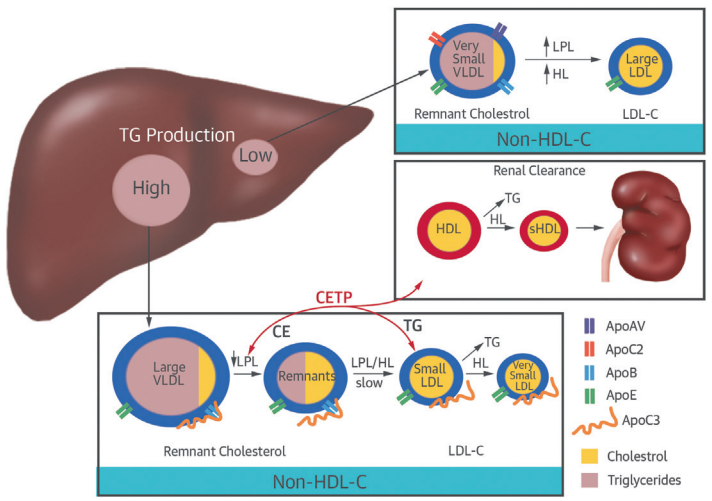
patients, including chylomicron remnants, not previously known to be proatherogenic (69-73); this likely explains the strength of nonfasting TG measurements in identifying patients at greater CVD risk.

Post-prandial TG metabolism abnormalities are also strongly associated with single nucleotide polymorphisms (SNPs) at common genetic loci, suggesting genetic mediation of interindividual variation in post-prandial lipemia (5,70). These associations persist after accounting for effects on other lipid traits, including LDL-C, HDL-C, and lipoprotein concentrations. Among the >150 deoxyribonucleic acid (DNA) sequence variants associated with serum lipids, candidate genes associated with post-prandial lipemia include apolipoprotein genes A1, A4, A5, C3, and E, as well as LPL, fatty acid binding protein 2, MTP, and scavenger receptor B1 (74). The ApoA5 locus has the strongest causal association between nonfasting TG and incident CVD (73,74).

ApoA5 is involved in multiple stages of TGRL metabolism, including VLDL production, TGRL remnant clearance, and regulation of LPL activity (3). Early studies of apoA5 knockout mice reported a 400% increase in serum TG levels (73,75,76). Genome-wide association studies (GWAS) reliably demonstrated that polymorphisms in or near the apoA5 locus are associated with nonfasting TG levels. Furthermore, associations between apoA5 genetic variants and incident CHD in direct relation to the increase in elevated nonfasting TG and remnant cholesterol were recently reported (76-78). Investigations into the additive effect of other SNPs, including glucokinase regulatory protein, apoE, and LPL, further enhanced the predictive ability of SNP-phenotype associations (67,74). Similarly, environmental factors, such as obesity, modify the effect of the apoA5 locus on post-prandial TGRL metabolism and atherosclerosis (77). Other studies demonstrated differential post-prandial responses to therapy on the basis of genetic variants at this locus, suggesting that SNP alterations may predict treatment efficacy (78).

Other gene loci thought to be important regulators of the TG response to dietary fat consumption were reported, including ANGPTL4 and PPAR alpha (67,74). With increasing appreciation of nonfasting TG levels and CVD risk, further investigation into the roles of genetic variants and environmental factors in mediating the post-prandial response may provide promising therapeutic approaches.

FIGURE 1 Effect of apoC3 on VLDL Metabolism



In patients with increased hepatic lipogenesis, enlarged very low-density lipoprotein (VLDL) is secreted with apolipoprotein (apo)C3. ApoC3 slows clearance of these triglyceride-rich lipoproteins (TGRLs), resulting in increased remnant cholesterol and small dense low-density lipoprotein cholesterol (LDL-C), and low high-density lipoprotein cholesterol (HDL-C). CE = cholesterol ester; CETP = cholesterol ester transfer protein; HL = hepatic lipase; LDL = low-density lipoprotein; LPL = lipoprotein lipase; sHDL = small high-density lipoprotein; TG = triglyceride.

ATHEROGENICITY OF TGRL CHOLESTEROL

Non-HDL-C is an aggregate measure of the cholesterol content transported in atherogenic lipoproteins. The greater predictive value of non-HDL-C over LDL-C comes from the premise that VLDL-C (non-HDL-C minus directly-measured LDL-C, also known as remnant cholesterol or TGRL cholesterol) is also atherogenic. Recent data supports the hypothesis that VLDL-C or remnant cholesterol is even more atherogenic than LDL-C. Remnants can cross the endothelial barrier and were identified in human arteries (79). Because of their larger size, TGRL carries 5 to 20 times more cholesterol/particle than LDL. Importantly, unlike native LDL, remnants can be taken up in an unregulated fashion by scavenger receptors expressed by resident macrophages in the subendothelial space, thus promoting foam cell formation. Chylomicron remnants and VLDL remnants rapidly penetrate the arterial wall and contribute to atherogenesis (6,8). VLDL composition is also a critical CVD risk determinant. In retrospective and prospective population studies, TG-associated CHD risk was limited to apoC3-containing VLDL particles and their metabolic remnants, small LDL particles (9-11). ApoC3 impairs VLDL binding to cellular receptors, resulting in small dense LDL particle formation (9,10), thereby counteracting the effects of apoE (11) (**Figure 1**). In 2 prospective cohorts, the Nurses' Health Study and the Health Professionals

Follow-up Study, CHD risk was higher in VLDL and LDL with apoC3 with a low apoE content than with a high apoE content (multivariable-adjusted relative risk for apoE content in LDL with apoC3 for the top vs. lowest quintile: 0.45, 95% confidence interval [CI]: 0.31 to 0.64; and for VLDL with apoC3: 0.50, 95% CI: 0.35 to 0.72; p value for trend <0.001) (11).

More recently, a Mendelian randomization study supports a causative role for remnant cholesterol in CHD (72) (**Table 2**). In this study, a small number of SNPs were strongly associated with remnant cholesterol, remnant cholesterol/HDL-C, LDL-C, and HDL-C. Notably, risk alleles for remnant cholesterol, remnant cholesterol/HDL-C, and LDL-C were even more strongly associated with CHD risk than measured lipid levels. Remnant cholesterol was more strongly linked to CHD than LDL-C (hazard ratio [HR]: 2.82 for remnant cholesterol vs. 1.41 for LDL-C). This concurs with previous genetic studies and likely relates to the genetic determinants of a lipid/lipoprotein trait more accurately representing a subject's lifetime exposure than a single biochemical measure. In contrast, the number of risk alleles for reduced HDL-C showed no relationship to CVD events. The strength of this analysis includes large datasets, consisting of >73,000 Copenhagen participants enrolled in 1 of 2 prospective studies (72), or a case-control study with a total of nearly 14,000 diagnosed with CHD (65).

NON-HDL-C, APOLIPOPROTEIN B, AND LDL PARTICLE CONCENTRATION AS TARGETS OF THERAPY FOR THE PREVENTION OF ATHEROSCLEROSIS AND ATHEROSCLEROTIC CVD

Hypertriglyceridemia is accompanied by elevated non-HDL-C levels (due to increased VLDL-C), IDL, small and total LDL particles, and total apoC3, as well as decreased HDL-C levels due to fewer smaller HDL particles. All of these changes are associated with increased risk, and which parameters are causal is debated. Population studies consistently show that non-HDL-C more strongly correlates with CHD event risk than LDL-C in those with and without hypertriglyceridemia (80). A large meta-analysis of statin outcome trials found that the subgroup of patients on statin therapy with non-HDL-C >130 mg/dl and with LDL-C >100 mg/dl had the highest relative risk (HR: 1.32) compared with patients with both LDL-C and non-HDL-C at the target levels of <100 and <130 mg/dl, respectively. The view expressed in the National Cholesterol Education Program Adult Treatment Panel III report is that TGRLs (remnants of VLDL, IDL, and chylomicron particles) are atherogenic, and that VLDL-C is a strong correlate of TGRL cholesterol (81). Thus, non-HDL-C is a better indicator of the total cholesterol burden carried by atherogenic lipoproteins ("real" LDL + IDL + Lp(a) + VLDL + chylomicron remnants). Although the American College of Cardiology/American Heart Association cholesterol guidelines did not recommend non-HDL-C as a therapeutic target, as it was not a primary efficacy measure in clinical trials of statin therapy (82), a meta-analysis of randomized placebo or active-controlled trials showed that non-HDL-C reduction was associated with a reduction in nonfatal myocardial infarction and CHD death (83).

The superiority of non-HDL-C as a biomarker of risk was suggested to not result from TGRL atherogenicity, but to instead reflect the relationship between non-HDL-C and the number of circulating LDL particles (84). The basis for this argument is that whether or not an individual has hypertriglyceridemia, approximately 90% of apoB is found in LDL particles. In those with an elevated TG concentration, the average LDL particle size is typically smaller, and thus the LDL particle concentration is often higher than would be predicted from the LDL-C level. In the ARIC (Atherosclerosis Risk in Communities) study, small, dense LDL-C was associated with incident CHD (85). In a GWAS performed in the ARIC study, the PCSK7 locus was associated with small, dense LDL-C, suggesting that genetic variants contribute to CHD risk. However, in a prospective study that adjusted for the risk of small LDL versus large LDL particles per total LDL particle concentration, risk relationships for incident CVD were not significantly higher for small LDL particles (86).

In studies including higher proportions of individuals with insulin resistance (obesity, metabolic syndrome, and type 2 diabetes), the discordance between LDL-C and total and small LDL particles increases. After multivariate adjustment, including major CHD risk factors, TG, and HDL-C, LDL particles are associated with a 2-fold higher risk of atherosclerosis and CVD events (reviewed in Rosenson et al. [87]). These prospective studies were the basis for multiple professional society recommendations for a hierarchical approach emphasizing LDL-C, non-HDL-C, and apoB/LDL particles for evaluation of CVD risk (88–91).

Controversy exists regarding whether there is an atherogenicity gradient across apoB-containing lipoproteins and, if so, whether it is incremental, particularly in the general population or in subpopulations with low insulin resistance rates (80). Most dyslipidemia guidelines recognize LDL-C as the major atherogenic lipoprotein and the primary target

of therapy (82,92-94). However, multiple studies indicate that that VLDL is at least as (or more) atherogenic than LDL (73,79). Thus, according to recent professional society guidelines, combining LDL-C and VLDL-C makes non-HDL-C a preferred target in patients with dyslipidemia (93,94). Moreover, a recent analysis of contemporary statin trials demonstrated that on-treatment levels of non-HDL-C are more strongly associated with future risk of CVD events than either apoB or LDL-C (80). In the same analysis, non-HDL-C explained a larger proportion of the atheroprotective effects of statin therapy than either apoB or LDL-C. These findings favor the use of non-HDL-C over LDL-C as a therapy target, particularly in hypertriglyceridemic individuals (93,94). Since apoB is the major apolipoprotein of both LDL and VLDL, some investigators propose total apoB as an alternative to non-HDL-C (95).

TGRLS: LESSONS FROM HUMAN GENETICS

Over the past 15 years, human genetic studies have identified new proteins involved in TGRL metabolism, revealed insights into the genetic architecture of plasma TG, and clarified the contribution of TGRL to human CVD. At least 3 different human genetic approaches—sequencing of biologic candidate genes, genetic analysis of Mendelian TG phenotypes, and GWAS of common DNA sequence variants—have yielded new proteins and novel human DNA variants involved in plasma TGRL regulation. Notable examples include apoA5 (biologic candidate), ANGPTL3 (biologic candidate and Mendelian low TG), LMF1 (biologic candidate), apoC3 (GWAS), GCKR (GWAS), COL18A1 (GWAS), and TRIB1 (GWAS), among many others (96-106).

The genetic architecture for TG in the population appears to be a mosaic comprised of rare large-effect variants, common small-effect variants, and environmental influences (107). Variants associated with common, complex traits like plasma TG range from common (>1:20) frequency to low frequency (1:200 to 1:20) and to rare (<1:200). Roughly 50% of interindividual plasma TG variability is estimated to come from DNA sequence variants. Johansen et al. (108) studied individuals from the extremes of the plasma TG distribution (438 individuals with high TG [mean 1,255 mg/dl] and 327 individuals with low TG [mean 106 mg/dl]) using both GWAS and resequencing of selected genes. In the GWAS, common variants at 7 loci were associated with plasma TG, and there was an excess of rare, nonsynonymous variants across 4 genes in individuals with high TG compared with those with low TG in the resequencing study. A comprehensive logistic regression model including clinical variables and both common and rare genetic variants explained 42% of total variation in hypertriglyceridemia diagnosis: clinical variables explained 20%, common genetic variants in 7 loci explained 21%, and rare genetic variants in 4 loci explained 1%.

Genetics can be used to distinguish causal from reactive processes in humans, and such studies have suggested that plasma TG causally relates to CHD. A common polymorphism in the APOA5 gene's promoter region led to decreased gene expression and was not only associated with higher plasma TG, but also with higher CHD risk (3). GWAS defined >150 polymorphisms associated with plasma lipids (102). Across 185 polymorphisms, the strength of a variant's effect on plasma TG highly correlated with the magnitude of its effect on CHD, even after accounting for each variant's potential effects on LDL-C and/or HDL-C (109). These results support the notion that TGRL causally influences CHD risk.

Common, low frequency, and/or rare DNA sequence variants in 4 TGRL metabolism genes were convincingly associated with CHD risk, and all 4 genes functionally relate to LPL (**Table 3**). Beyond

TABLE 3 DNA Sequence Variants in LPL-Regulating Genes Associated With TG and CHD

Gene	rsID	Protein	Effect of Variant on Protein Function	Effect of Mutation on LPL Activity	Lipid Phenotype Association	CHD Risk	Allele Frequency in Whites	Allele Frequency in Blacks	Ref. #
LPL	rs1801177 rs328	D36N S474X	Loss Gain	Decrease Increase	↑TG ↓TG	↑ ↓	2% 11%	5% 7%	(72)
APOA5	rs662799	Promoter -1131T→C	Loss	Decrease	↑TG	↑	8%	Rare	(3)
ANGPTL4	rs116843064	E40K, T266M	Loss	Increase	↓TG	↓	1.6%	0.1%	(96)
APOC3	multiple	R19X, IVS2+1 G→A, IVS3+1 G→T, A43T	Loss	Increase	↓TG	↓	0.7% (collective for all 4 mutations)	0.7% (collective for all 4 mutations)	(99,110,111)

CHD = coronary heart disease; LPL = lipoprotein lipase; TG = triglycerides.

genes that alter LDL, this is the first set of functionally related genes where naturally occurring genetic variation alters the complex disease of CHD. We highlight 2 recent studies that establish a central role of apoC3 in TG and CHD risk (110,111). In the first study (110), the protein-coding regions (exome) of 18,666 genes were sequenced in 3,734 individuals from the United States, and rare mutations in each gene were tested for association with plasma TG. The top result was for APOC3. Approximately 1 in 150 individuals carried any of 4 protein-altering or splice-site variants of APOC3. Heterozygous carriers of any of these 4 APOC3 mutations had 39% lower plasma TG ($p < 1 \times 10^{-20}$), 46% lower circulating plasma APOC3 ($p = 8 \times 10^{-10}$), and 40% lower risk for CHD (odds ratio: 0.60, 95% CI: 0.47 to 0.75; $p = 4 \times 10^{-6}$).

A second study (111), using data from 75,725 participants in 2 general population studies in Denmark, associated low levels of nonfasting TG with reduced risks of ischemic vascular disease and ischemic heart disease. Participants with nonfasting TG levels <90 mg/dl had a significantly lower incidence of CVD than those with levels ≥350 mg/dl (HR for ischemic vascular disease: 0.43, 95% CI: 0.35 to 0.54; HR for ischemic heart disease: 0.40, 95% CI: 0.31 to 0.52). Sequencing of APOC3 in 10,797 participants identified 3 of the 4 loss-of-function mutations from the first study. Those heterozygous for loss-of-function mutations in APOC3 had lower TG and lower ischemic vascular disease risk, at estimates remarkably similar to the first study (44% nonfasting TG and 41% for ischemic vascular disease) (99). Findings from these 2 studies extend a previous observation that the APOC3 R19X mutation was quite frequent (5%) in the Amish population and associated with lower plasma TG and decreased coronary artery calcium (99). These human genetic data suggest that therapeutic strategies that enhance LPL function, decrease APOC3 function, decrease ANGPTL4 function, or enhance APOA5 function should be cardioprotective.

RECENT ADVANCES IN THE GENETICS AND THERAPEUTIC BENEFITS OF CURRENTLY AVAILABLE TG-LOWERING DRUGS

Four classes of agents are currently available for managing hypertriglyceridemia: fibrates (PPAR-alpha agonists); niacin; omega-3 fatty acids; and medium chain triglyceride (MCT) oil, the latter being mainly used for treatment of chylomicronemia and Mendelian forms of severe hypertriglyceridemia (TG >1,000 mg/dl). Although not TG-lowering agents, statins reduce TG in patients with mild to severe hypertriglyceridemia, and the more effective the statin in decreasing LDL-C, the greater its effect on plasma TG (112). Pooled data from placebo-controlled trials also suggest that 2 to 7 years of statin treatment may reduce the risk of pancreatitis in patients with mild to moderate hypertriglyceridemia. However, because severe hypertriglyceridemia is clinically, physiologically, and metabolically distinct from mild to moderate hypertriglyceridemia (5,113), statins have limited efficacy for management of severe hypertriglyceridemia and the clearance of large, buoyant, TG-rich lipoproteins such as chylomicrons. When the fasting TG concentration is >1,000 mg/dl, chylomicrons are the predominant plasma lipoproteins. At TG levels between 500 and 1,000 mg/dl, chylomicrons, VLDL, and remnant lipoproteins cohabit in variable proportions, whereas minimal fasting chylomicronemia is observed when TG values are <500 mg/dl. The pancreatitis risk is highest in the presence of severe hypertriglyceridemia and chylomicronemia (5,113).

The physiology of hypertriglyceridemia is complex, involving exogenous and endogenous metabolic pathways for 2 distinct lipoproteins and a variety of mechanisms and tissues. Consequently, it is not surprising that most currently available TG-lowering agents, almost all of which influence expression of key lipid metabolism genes, have pleiotropic effects and act on multiple targets. Changes in gene expression induced by TG-lowering drugs contribute to their efficacy, or may exacerbate side effects that limit their therapeutic use. For example, fenofibrate exerts its beneficial hypotriglyceridemic action by activation of PPAR alpha, a nuclear receptor protein that increases the gene transcription, expression, and activity of LPL and apoAI, among others (114,115). Recent genetic analyses in mice suggest that fenofibrate may increase the risk of hepatic steatosis in a dose-dependent manner by acting on the expression of genes associated with lipogenesis and by up-regulating SREBP-1c (116). However, the effect of PPAR alpha agonists on liver steatosis remains controversial (117). In contrast, recent data suggest that niacin has no effect on SREBP-1c mRNA expression but significantly inhibits DGAT-2 mRNA levels, protein expression, and activity, thereby significantly regressing hepatic steatosis in rats with pre-existing steatosis (118). However, high-dose niacin has multiple side effects in humans, including possible induction of chemical hepatitis. Niacin up-regulates a niacin receptor 1 (GPR109A) genetic cascade, which activates arrestin beta 1-mediated ERK1/2 MAP kinase and prostaglandin D_2 release, and (depending on the drug formulation) causes flushing that often limits niacin use. Sustained-release niacin is generally better tolerated, but is more hepatotoxic than

immediate-release niacin. In the Heart Protection Study-2 and AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes) trials, extended-release niacin was associated with increased infection rates (119,120).

OMEGA-3S FOR MANAGEMENT OF HYPERTRIGLYCERIDEMIA

Since the initial observations by Bang et al. (121) in the early 1970s that Greenland Eskimos with a diet rich in omega-3 fatty acids have a low incidence of CVD, clinical benefits of the major omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been investigated extensively. There is a better understanding that the complex mixture of fatty acids found in fish oil contains multiple active components, and that each of the major omega-3 fatty acids (EPA, DHA, and docosapentaenoic acid [DPA]), has overlapping, but distinct, biological activities (122). Health benefits of omega-3 fatty acids are linked to fish intake, but EPA, DHA, and DPA plasma levels are also determined by polymorphisms of the desaturases (delta-5 and -6) that convert short-chain polyunsaturated fatty acids (SC-PUFAs) to long-chain polyunsaturated fatty acids (LC-PUFAs). Not only has the role of omega-3 fatty acids in human health broadened since the initial observations in Greenland Eskimos, but there is also a better appreciation of the pharmacological and biological intricacies of the major fatty acid constituents of fish oil.

Recent advances in "omics" suggest new mechanisms of action for niacin and omega-3 fatty acids (123–129). Clinical trials conducted with GPR109A agonists, studies in mice lacking GPR109A, and analyses of niacin's effect on gene expression profiles in skeletal muscle of obese Zucker rats suggest that niacin's mechanism of action is not mainly driven by adipocyte triglyceride lipolysis, but involves genes and consecutive metabolic effects in hepatocytes, skeletal muscle cells, macrophages, neutrophils, and the endothelial wall (123–125).

Omega-3 fatty acids modify expression of acylglycerol synthesis pathway genes and their polymorphisms (particularly GPAM, AGPAT3, and AGPAT4) modulate the effect of omega-3 fatty acids on plasma TG concentration (126). The EVOLVE (Epanova for Lowering Very High Triglycerides) clinical trial (127), demonstrated the safety and efficacy of the free fatty acid form of omega-3 fatty acids in severe hypertriglyceridemia (plasma TG concentration from 500 to 2,000 mg/dl), confirming that they are effective on post-prandial metabolism and the exogenous (chylomicron-dependent) metabolic pathway. A recent study suggests that the biologic effect of omega-3 fatty acids in enterocytes may be exerted through microribonucleic acids (miRNAs), particularly miR-192 and miR-30c. miRNAs are small, noncoding RNA molecules that play key roles in transcriptional and post-transcriptional regulation of gene expression. Enterocyte targets of miR-192 and miR-30c include genes encoding nuclear receptor corepressor 2, isocitrate dehydrogenase 1, caveolin 1, ATP-binding cassette subfamily G member 4, and retinoic acid receptor β (128).

FADS POLYMORPHISMS AND PLASMA LEVELS OF OMEGA-3 FATTY ACIDS

Until recently, dietary intake of long-chain marine omega-3 fatty acids was thought to be the major determinant of plasma concentrations. Short-chain omega-3 fatty acids, primarily alpha-linolenic acid, are abundant in the diet, but only small amounts are converted to EPA and even less are elongated to DHA by desaturase enzymes (delta-5 and -6) (**Figure 2A**) (129). However, it is now widely recognized that genetic polymorphisms of the desaturase genes, FADS1 to 3, significantly affect LC-PUFA formation. FADS polymorphisms are relatively common and potent, and may explain up to 30% of the variability in LC-PUFA levels (both omega-6 and -3). For example, about 80% of African Americans have a polymorphism (rs174537) associated with more effective conversion of SC-PUFA to LC-PUFA by desaturase-5, resulting in higher levels of both arachidonic acid (AA) and EPA, even though their fish consumption is lower, on average, than that of whites. The "desaturase hypothesis" (130) is that, in populations following a Western diet rich in omega-6 and relatively deficient in omega-3 PUFAs, FADS polymorphisms associated with high desaturase activity may lead to predominant proinflammatory, detrimental effects (131). It is intriguing to hypothesize that the higher levels of disorders such as hypertension, asthma, and aspirin resistance in African Americans are linked to a higher inflammatory state driven by overproduction of prostaglandins and leukotrienes synthesized from AA. In contrast, Hispanic individuals more commonly have a different FADS polymorphism (rs17454), resulting in less conversion of SC-PUFA to LC-PUFA, and they consequently have lower AA and EPA levels (130,132). This FADS polymorphism is associated with dyslipidemia (high TG and HDL-C), modulated, in part, by PUFA intake. This polymorphism may explain the high prevalence of hypertriglyceridemia in the Hispanic

population, whereas the polymorphism found predominantly in African Americans and associated with higher EPA levels may lead to their lower incidence of elevated TG and higher HDL-C levels. Further studies are needed to define the function of FADS1 and FADS2 polymorphisms relative to mechanisms involved in the development of atherosclerosis, dyslipidemia, and other diseases linked to either AA overproduction or marine LC-PUFA deficiencies.

BIOLOGICAL DIFFERENCES IN EPA, DHA, AND DPA

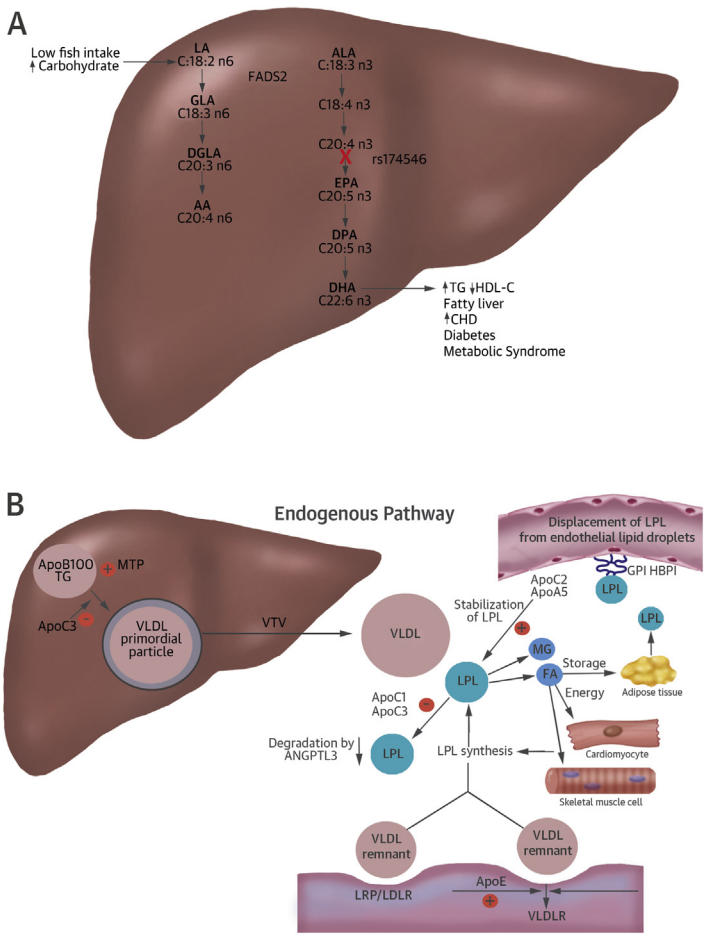
Omega-3 fatty acid treatment lowers the TG level by both reducing hepatic TG secretion and enhancing the rate of TG clearance from the circulation. ApoC3 appears to play an important role in hypertriglyceridemia pathogenesis, particularly with regard to inhibiting lipoprotein lipase and hepatic lipase, which slows TG hydrolysis (133). Apo C3 also interferes with the TGRL interactions with hepatic apoB/E receptors, slowing removal of these particles from circulation (134). ApoC3 is regulated by the hepatic nuclear factor-4 alpha, forkhead box O transcription factor O1, and carbohydrate response element-binding protein in response to insulin (135). Increased apoC3 synthesis may represent a compensatory mechanism to reduce TGRL catabolism and uptake by hepatic receptors in an attempt to cope with a large influx of substrates for TG production. The effect of omega-3 fatty acids on apoC3, unlike that of fibrates, is independent of PPAR-alpha (136). Both EPA and DHA down-regulate SREBP-1c, the transcription factor that controls lipogenesis. EPA is a more potent agonist of PPAR-alpha than DHA, whereas DHA appears to regulate hepatic nuclear factor-4 alpha, forkhead box O transcription factor O1, and carbohydrate response element-binding protein (137).

The severity of hypertriglyceridemia is typically associated with the apoC3 level. Fish oil containing a complex mixture of omega-3 fatty acids lowered apoC3 in patients with hypertriglyceridemia (138). This is of potential clinical importance, because elevated apoC3 levels associated with VLDL + LDL particles were independent predictors of CVD event risk, and loss-of-function apoC3 gene polymorphisms were associated with reduced CVD event risk (110,111) (**Figure 2B**).

EMERGING THERAPIES FOR MANAGEMENT OF HYPERTRIGLYCERIDEMIA

A number of new therapies to address the challenges and unmet needs of patients with high TG and mixed dyslipidemia are being developed. Some are new formulations or improved delivery systems of preexisting drugs, whereas others target new mechanisms for TGRL metabolism and hypertriglyceridemia management. Cell-penetrating peptides or linker technologies combining niacin and omega-3, PCSK9 inhibitors, apoC3-antisense, DGAT1 inhibitors, MTP inhibitors, peptide mimetics, and LPL gene replacement therapy represent the diversity of mechanisms and targets for management of TGRL and severe

FIGURE 2 FADS Polymorphisms and Fatty Acid Metabolism



(A) Long-chain polyunsaturated fatty acid (PUFA) plasma levels are determined by both dietary intake and conversion of shorter-chain PUFAs by the delta-5 and -6 desaturases. Polymorphisms of these fatty acid desaturases (FADS) result in either higher or lower long-chain PUFA levels, possibly explaining differences in hypertriglyceridemia prevalence between Hispanics and African Americans. **(B)** Long-chain omega-3 fatty acids in fish oils decrease hepatic lipogenesis, thereby reducing VLDL-c levels, and enhance the conversion by LPL of VLDL to LDL mediated in part by a decrease in apoC3. AA = arachidonic acid; ALA = alpha-linoleic acid; CHD = coronary heart disease; DGLA = dihomo-gamma-linoleic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; GLA = gamma-linoleic acid; LA = linoleic acid; other abbreviations as in **Figure 1**.

hypertriglyceridemia. These emerging drugs decrease fasting TG concentration in different proportions: some are more effective on post-prandial lipid metabolism and the exogenous (chylomicron) pathway, and others are more effective on the endogenous pathway, on liver fat management (including nonalcoholic fatty liver disease and insulin resistance), or on fat metabolism in adipocytes and peripheral tissues (including lipodystrophies).

Cell-penetrating peptides represent a new mechanism of drug delivery. When conjugated to macromolecules or when used at low concentrations, cell-penetrating peptides enter cells via the endocytic pathway. CAT-2003, a novel niacin and EPA conjugate, is currently in a phase II clinical trial for treatment of severe hypertriglyceridemia of different causes (139). In pre-clinical studies, CAT-2003 synergistically reduced TG superior to niacin and EPA, individually or combined. CAT-2003 inhibits the transcription factor SREBP in vitro and in vivo (140), resulting in reduced PCSK9 expression. SiRNA inhibition of PCSK9 gene expression reduces production and assembly of multiple cholesterol biosynthetic enzymes in the cellular SREBP2 pathway, as well as of several enzymes involved in intestinal TGRL synthesis and assembly (15).

ISIS-APOCIII$_{Rx}$ is a second-generation 2′-O-(2-methoxyethyl) modified antisense inhibitor of apoC3 synthesis. ISIS-APOCIII$_{Rx}$ selectively inhibits hepatic apoC3 protein synthesis by binding to a small sequence of apoC3 mRNA to elicit its degradation by RNase H1, an endogenous ribonuclease expressed ubiquitously in mammalian cells. In phase 2 studies, ISIS-APOCIII$_{Rx}$ was highly effective in lowering apoC3, fasting plasma TG, and non–HDL-C in patients with elevated VLDL-TG or chylomicron-TG due to a variety of conditions, including familial chylomicronemia due to lipoprotein lipase deficiency (LPLD), suggesting that apoC3 might play a key role in a non-LPL-dependent TGRL metabolic pathway (141). Because hepatic apoC3 potentially promotes clearance of excess hepatic TG, ASO inhibition of apoC3 could theoretically exacerbate hepatic lipid accumulation (142,143).

Lomitapide is an MTP inhibitor that interferes with apoB-containing lipoprotein assembly in the apoB100 and apoB48 pathways, thus reducing both chylomicron and VLDL secretion. It is currently available for the management of homozygous FH, and has been used long-term (>13 years) to treat a single patient with extremely severe hypertriglyceridemia due to LPLD (144). Lomitapide 40-mg daily eliminated chronic abdominal pain and prevented pancreatitis; however, the fatty liver present before treatment progressed to steatohepatitis and fibrosis after 12 to 13 years.

DGAT1 catalyzes the final step of TG synthesis and is highly expressed in the gut wall, where it plays a key role in dietary fat absorption as chylomicron-TG (145,146). DGAT1 and DGAT2 are also required for LD formation in adipocytes, they are active in sebaceous glands, and their dysregulation contributes to the imbalance between lipid supply and demand and nonalcoholic fatty liver disease development (147,148). Pradigastat (formerly LCQ908), a selective DGAT1 inhibitor, is being evaluated for treatment of familial chylomicronemia (149). In healthy human volunteers, pradigastat decreases post-prandial chylomicron particle numbers and prevents post-prandial hypertriglyceridemia (150). Gastrointestinal events such as diarrhea, abdominal pain, and nausea are the most commonly reported adverse events, and are related to dose and dietary fat content.

AAV1-LPLS447X gene replacement therapy (alipogene tiparvovec) was developed for treatment of familial chylomicronemia due to LPLD (151–155). LPL gene therapy adds extra copies of the gene encoding a functionally potent enzyme to muscle tissue of affected patients, specifically homozygotes or compound heterozygotes with documented null, LPLD-causing LPL gene mutations. In pivotal clinical trials, intramuscular administration of alipogene tiparvovec was generally well tolerated and associated with signs of clinical improvement and reduction in overall pancreatitis incidence up to 5 years after administration (155).

CONCLUSIONS

Elevated fasting and nonfasting TG levels were associated with incident CVD events in multiple studies; however, they also accompany a multitude of biomarkers (lipoproteins, inflammatory mediators and proteins, and hemostatic measures) and CVD-associated conditions, including type 2 diabetes mellitus. Recent clinical trials failed to demonstrate a reduction in CVD events in statin-treated patients also treated with fibrates and niacin, challenging elevated TG as an independent risk factor. However, clinical trials with fibrates demonstrated risk reduction in subgroups with fasting TG ≥200 mg/dl, particularly those with low HDL-C, indicating a threshold effect that should mandate future TG-lowering therapy trials.

Our understanding of TG-associated CVD risk evolved with the recognition that TG is just 1 component of a vastly heterogeneous TGRL pool. Genetic studies demonstrate a causal relationship of

certain TGRL components and TGRL synthetic and metabolic pathways with atherosclerotic CVD events. Specific genetic mechanisms that increase plasma TGRL and affect CVD include loss of LPL function, loss of APOA5 function, gain of ANGPTL4 function, and gain of APOC3 function.

Importantly, these genetic studies identified specific targets in the causal pathway that may be candidates for pharmacological intervention. Ongoing clinical trials targeting causal pathways in TGRL metabolism should clarify the role of TGRLs and CVD. In the interim, we advocate the use of available TG-lowering therapy for prevention of acute pancreatitis.

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