Published in final edited form as:

Atherosclerosis. 2009 December ; 207(2): 559–566. doi:10.1016/j.atherosclerosis.2009.05.016.

Altered Composition of Triglyceride-Rich Lipoproteins and Coronary Artery Disease in A Large Case-Control Study

Paul N. Hopkins, MD, MSPH, M. Nazeem Nanjee, PhD, Lily L. Wu, PhD, Michael G. McGinty, Eliot A. Brinton, MD, Steven C. Hunt, PhD, and Jeffrey L. Anderson, MD

Cardiovascular Genetics, Department of Internal Medicine, Cardiology Division, University of Utah School of Medicine, (PNH, EAB, LLW, SCH, MNN) and the Department of Pathology, University of Utah School of Medicine, Associated Regional and University Pathologists (LLW), and Intermountain Healthcare, Salt Lake City, UT (JLA)

Abstract

Background—Traditional beta-quantification of plasma lipoproteins by ultracentrifugation separates triglyceride-rich lipoproteins (TGRL) from higher density lipoproteins. The cholesterol in the TGRL fraction is referred to as measured very low-density lipoprotein cholesterol (VLDL-C) recognizing that other TGRL may be present. The measured VLDL-C to total plasma triglyceride (VLDL-C/TG) has long been considered an index of average TGRL composition with abnormally high VLDL-C/TG ratios (≥ 0.30 with TG > 150 mg/dL) indicative of atherogenic remnant accumulation (type III hyperlipidemia). However, virtually no reports are available which examine potential associations between CAD and VLDL-C/TG at the lower end of the spectrum.

Methods and Results—We performed ultracentrifugation in 1170 cases with premature-onset, familial CAD and 1759 population-based controls and examined the VLDL-C/TG ratio as an index of TGRL composition. As expected, we found very high CAD risk associated with severe type III hyperlipidemia (OR 10.5, $p = 0.02$). Unexpectedly, however, we found a robust, graded, and independent association between CAD risk and *lower* than average VLDL-C/TG ratios ($p < 0.0001$ as ordered categories or as a continuous variable). Among those in the lowest VLDL-C/TG category (a ratio < 0.12), CAD risk was clearly increased (OR 4.5, 95% CI 2.9–6.9) and remained significantly elevated in various subgroups including those with triglycerides below 200 mg/dl, in males and females separately, as well as among those with no traditional CAD risk factors (OR 5.8, 95% CI 1.5–22). Significant compositional differences by case status were confirmed in a subset whose samples were re-spun with measurement of lipids and apolipoprotein B (apo B) in each subfraction.

Conclusions—We found a strong, graded, independent, and robust association between CAD and lower VLDL-C/TG ratios. We consider this a novel, hypothesis-generating observation which will hopefully generate additional future studies to provide confirmation and further insight into potential mechanisms.

Corresponding author: Paul N. Hopkins, MD, MSPH, University of Utah, Cardiovascular Genetics Research, 420 Chipeta Way, Room 1160, Salt Lake, City, UT 84108, Phone: 801-581-3888 ext 228, FAX: 801-581-6862, email: paul.hopkins@utah.edu.

Disclosures: The authors have no conflicts of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

plasma triglycerides; VLDL; lipids; ultracentrifugation; type III hyperlipidemia; dysbetalipoproteinemia; coronary artery disease; HDL cholesterol

Introduction

Triglyceride-rich lipoproteins (TGRL) include a complex array of particles with potentially different cardiovascular risk associations. Ultracentrifugation at a solvent density (D) of plasma (being 1.006 g/ml) yields a $D < 1.006$ supernatant fraction containing essentially all TGRL (chylomicrons, very low density lipoprotein (VLDL), and β -VLDL) having diameters ranging from approximately 30-80 nm for the VLDL particles to considerably larger than this for chylomicrons [1]. In the $D > 1.006$ infranatant fraction there are intermediate density- (IDL), low density- (LDL) and high density lipoproteins (HDL). In traditional "beta-quantification," cholesterol in the top fraction is measured (referred to as VLDL-C, although cholesterol from all TGRL is included) and the ratio of VLDL-C to total plasma triglycerides (TG) is considered an index of the composition of TGRL. The average VLDL-C/TG ratio in normolipemic individuals ranges from approximately 0.18 to 0.22 and is the basis of the Friedewald equation used for estimation of LDL cholesterol (LDL-C) in clinical laboratories worldwide [2-5]. The VLDL-C/TG ratio varies substantially from this range in the presence of either TGRL remnants (which are enriched in cholesterol) [5-8] or chylomicrons or very large VLDL (which are relatively enriched in triglyceride) [6].

Cholesterol-enriched TGRL remnants are considered to be highly atherogenic [1,6,7,9-21]. Such TGRL remnants include the highly cholesterol-enriched and relatively large " β -VLDL" seen in type III hyperlipidemia, as well as the smaller, more common "slow pre- β " particles which are reported to be less cholesterol-enriched than β -VLDL [22,23]. Type III hyperlipidemia has been traditionally defined as a $\text{VLDL-C/TG} \geq 0.30$ with total triglycerides > 150 mg/dl [13,16,24-26]. We have previously reported the only population-based estimate of coronary artery disease (CAD) risk associated with this traditional definition of type III hyperlipidemia [27], but there has been virtually no published consideration of the possible associations between CAD and TGRL with VLDL-C/TG values below the normal range.

In this study we examine the entire range of observed VLDL-C/TG for association with CAD utilizing the largest series of CAD cases and controls of which we are aware with ultracentrifuged lipids measured in all.

Methods

Study Participants

Cases included 1104 persons age 30-75 when examined who had experienced premature CAD defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting by age 60 for men or age 70 for women. All cases were recruited from families in which two or more first-degree relatives (parent, sibling or child) had similarly early onset of clinical CAD as previously described [27]. To minimize possible artifactual effects of acute coronary syndrome on lipid levels, patients were seen at least 6 months after their acute event. Controls were also age 30-75 when screened and were representative of the general Utah population as previously described for the original group 1104 [27]. For this report, we added 655 newly recruited controls with the same age range who were identified through randomly ascertained driver's license records, and thus also considered to represent the general population. This study was approved by the Institutional Review Board of the University of

Utah Medical Center and LDS Hospital. All subjects signed informed consent prior to participating.

A participant was considered to have hypertension if taking antihypertensive medication for a prior physician diagnosis of hypertension, or if at exam the mean of two sitting blood pressures (taken with a Critikon Dinamap automated blood pressure machine) was greater than or equal to 140 mm Hg systolic or 90 mm Hg diastolic. Diabetes was considered present if a prior physician diagnosis had been made or if the fasting serum glucose from their exam was greater than or equal to 126 mg/dl. Cigarette smoking was dichotomized into “ever” or “never” with ever smoking defined as having smoked daily for one year or more. Many patients had quit after onset of their CAD, hence the designation as ever smoking rather than current and former.

Laboratory Methods

EDTA-anticoagulated blood samples were collected in the morning after 10-12 hours of fasting and prepared according to guidelines of the Lipid Research Clinic's program *Manual of Laboratory Operations* [29]. Lipid and lipoprotein concentrations were measured by a microscale ultracentrifugation procedure developed in our laboratory [30]. HDL-C was measured in the supernatant after removal of non-HDL using a magnetized solid-phase dextran sulfate / MgCl_2 precipitation reagent [31]. Cholesterol and triglycerides in total plasma and ultracentrifugal subfractions were measured with automated analyzers (FARA II (Roche Diagnostics) or PolyChem (Polymedco)). TGRL were separated from IDL, LDL and HDL by ultracentrifugation (4 h, $157\,000 \times g$, 20°C) in a Beckman TL100 ultracentrifuge and tube slicing. The value for VLDL-C was taken as the measured cholesterol in the $d < 1.006 \text{ g/mL}$ fraction. LDL-C was taken as the cholesterol measured in the bottom fraction minus HDL-C. Type III hyperlipidemia was defined as present if the ratio of VLDL-C/TG was ≥ 0.30 and plasma total TG were $> 150 \text{ mg/dl}$ [24]. Our laboratory participates in the CDC Standardization Program and consistently achieves excellent agreement with CDC standards (coefficients of variation in the range of 0.5-3.3% for total cholesterol, triglyceride and HDL-C, with $< 3\%$ difference in means). Concentrations of apolipoprotein B (using a polyclonal antibody recognizing both B-100 and B-48) was measured in both total plasma and plasma in the $D < 1.006 \text{ g/ml}$ fraction were quantified by liquid-phase, double-antibody radioimmunoassays with the difference taken as LDL-apo B [32]. Estimated β -VLDL-C levels were calculated by an algebraic method using VLDL-C, total triglycerides, LDL-C, and HDL-C as previously described [33-35].

Statistical Analysis

The SAS Statistical Software Package (version 9.2 for the PC) was used for data analysis (SAS Institute, Inc. Cary, NC). Statistical analyses on triglycerides were done after logarithmic transformation. Statistical tests included Student's t test, χ^2 , Pearson's correlation, and stepwise multiple logistic regression using SAS PROC LOGISTIC. We used the Cochran-Armitage trend test with the exact option (two-sided) to compare the difference in TGRL composition distribution in selected cases and controls (using SAS PROC FREQ). Analysis of covariance was performed using PROC GLM. Because some CAD cases came from the same family, effects of subject relatedness in logistic regression analyses were tested using generalized estimating equations with an exchangeable correlation matrix in PROC GENMOD in SAS. As results were nearly identical with the two methods, p-values obtained with PROC LOGISTIC are reported.

Results

Clinical characteristics of familial CAD cases are compared with controls in table 1. Highly significant differences by univariate analysis were apparent for standard CAD risk factors as shown.

Our focus in this study was to examine the association of CAD risk with differences in the composition of TGRL. As expected (see figure 1), increasing levels of estimated β -VLDL-C were associated with a graded increase in CAD risk. Results are given for logistic regression using a minimally adjusted model (adjusted for age and gender only) and a full model (age, gender, smoking history, hypertension, diabetes, measured LDL-C, log of plasma triglycerides, and HDL-C). In several prior studies, we had found that over 90% of persons meeting traditional cut-points for type III hyperlipidemia (VLDL-C/TG ≥ 0.30 and triglycerides >150 mg/dl) had estimated β -VLDL-C levels over 35-40 mg/dl [33-35]. Similar results were seen in the current study (all 44 subjects meeting traditional type III criteria had estimated β -VLDL-C above 32 mg/dl, with 36 of the 44 having levels above 40 mg/dl). While the gradient of risk was steep, confidence intervals were wide and only those with the highest estimated β -VLDL-C levels had risk entirely independent of standard risk factors including total plasma triglycerides and HDL cholesterol with no elevation of risk at modest elevations in VLDL-C/TG ratio (i.e. in the range of 0.22 to <0.30) (see figure 2). In all subsequent multiple logistic regression models we adjusted for the presence of type III hyperlipidemia as a risk factor unless otherwise specified.

We then examined CAD risk associated with TGRL having lower than usual cholesterol content as shown in figure 2. Surprisingly, we found a marked, graded, and statistically highly significant increase in risk associated with *lower* ratios of VLDL-C/TG ($p < 0.0001$ for trend using categories shown in figure 2; p -values were <0.0001 for both a linear and a quadratic term using VLDL-C/TG as a continuous variable, also shown in figure 2). The distribution of cases and controls in each category of VLDL-C/TG is shown in table 2.

The association between with low VLDL-C/TG and CAD was remarkably robust in subgroup analyses. As shown in table 2, CAD risk estimates for low levels of VLDL-C/TG were similar among males, females, those not taking lipid-lowering medication, persons with very early onset CAD (males by age 45, females by age 55), those with plasma TG <200 mg/dl, and even among those with expected low CAD risk based on standard risk factors (never smoked, no hypertension or diabetes, measured LDL-C <130 mg/dl, HDL-C 40 mg/dl or higher, plasma TG <200 mg/dl, and no use of lipid-lowering medication). In none of these analyses was VLDL-C alone significant once log plasma TG was in the model.

Other lipid-related variables potentially associated with lower VLDL-C/TG ratios are shown in table 3. Generally, lipids shown in the multivariable logistic regression models to be independently related to risk (including LDL-C, HDL-C, and plasma TG) were only modestly related to the VLDL-C/TG ratio. Surprisingly, plasma TG tended to be slightly *lower* in those with the lowest VLDL-C/TG ratios. Indeed, risk associated with low VLDL-C/TG was clearly not driven by high plasma TG levels. While odds ratios for CAD associated with plasma TG over 500 mg/dl (OR = 6.2, 95%CI 3.2-12 $p < 0.0001$) or over 800 mg/dl (OR = 16.7, 95%CI 2.2-129, $p = 0.0003$ by Fisher's exact test) were statistically significant, the number of affected individuals was quite small. Nevertheless, even after eliminating all subjects with plasma TG over 200 mg/dl, the odds ratios for VLDL-C/TG shown in figure 2 were essentially unchanged, as noted above. Furthermore, risk associated with VLDL-C/TG was entirely independent of plasma TG and HDL-C in logistic regression as illustrated by the 2-way classification models shown in figure 3 (with adjustment for all other risk factors).

We observed several significant associations between potential confounding variables and VLDL-C/TG (see table 3). Most importantly, however, was the observation that a low VLDL-C/TG ratio was *not* associated with high plasma TG. Use of lipid-lowering medication was significantly greater among those with lower VLDL-C/TG in both cases and controls. Nevertheless, neither this nor other potential confounding factors affected the association between CAD risk and VLDL-C/TG when included in multivariate logistic regression models or in subgroup analyses in which all persons using lipid lowering drugs were excluded (see table 2).

To further define the composition of VLDL, we retested TGRL, LDL, and HDL lipids and apoprotein B after ultracentrifugation in a selected, relatively homogeneous subset of our CAD cases and controls. The targeted subset of samples included only males with CAD by age 55 and male controls. All were between ages 40 and 70 at the time of screening, not taking any lipid drugs, with TG <200 mg/dl, never smokers, and without diabetes. We selected only those with a previous VLDL-C/TG <0.18 (eliminating 70% of controls and 59% of cases and thus, conservatively limiting some of the difference in VLDL composition between the cases and controls). Laboratory personnel were blinded to the case/control identity during subsequent testing. Because prior studies had utilized many of our stored CAD samples, of the 58 CAD cases selected only 22 had sufficient sample for testing, while all 54 selected controls had sample. As shown in figure 4, distribution of VLDL-C/VLDL-TG was found to differ greatly between cases and controls (p for chi-square and exact trend <0.0001). In multiple logistic regression including age, measured LDL-C, HDL-C, and triglycerides, the odds ratio for VLDL-C/VLDL-TG <0.34 (the lowest quartile in controls) was 79 (95% CI 7.1 – 881, p = 0.0004). We consider these findings preliminary, but they are strongly confirmatory.

Upon further examination of VLDL composition in the retested subset, cholesterol per VLDL particle (VLDL-C/VLDL-apo B) was significantly lower in cases (1.91 ± 0.379 versus 2.26 ± 0.334 , p = 0.0002) while triglyceride per VLDL particle (VLDL-TG/VLDL-apo B) was significantly higher (8.61 ± 2.130 versus 6.26 ± 1.549 , p <0.0001), making VLDL-C/VLDL-TG the best variable tested to distinguish cases versus controls. Neither VLDL-apo B nor LDL-apo B correlated with VLDL-C/VLDL-TG. Furthermore, among these selected cases and controls the correlation between total TG and VLDL-TG was $r = 0.96$ (p <0.0001) and between VLDL-C/TG and VLDL-C/VLDL-TG the correlation was $r = 0.64$ (p <0.0001). We therefore expect the VLDL-C/TG ratio to be a reasonable estimate of VLDL composition for the entire population. Finally, there was a strong correlation between VLDL-C/VLDL-TG and LDL-C/LDL-apo B, an index of LDL particle size ($r = 0.63$, p <0.0001). In analysis of covariance, both total triglyceride and VLDL-C/VLDL-TG independently correlated significantly with LDL-C/LDL-apo B (both p <0.0001). However, when both VLDL-C/VLDL-TG and LDL-C/LDL-apo B were entered as continuous variables in a multiple logistic regression with age and other lipids, only VLDL-C/VLDL-TG remained significantly associated with CAD.

Discussion

The major finding in this study was a strong, graded, highly significant increase in risk of early onset CAD associated with lower VLDL-C/TG ratios. This observation was unexpected and ran counter to our original hypothesis, and, as far as we are aware, has not been previously reported. We confirmed and extended our findings in a homogeneous subgroup of our cases and controls using a more detailed assessment of VLDL composition. These novel findings may provide new insights into potential mechanisms of atherosclerosis risk and will generate new hypotheses that may prove useful, but we recognize the need for further confirmation. However, because ultracentrifugation is generally reserved for more severe cases of hyperlipidemia rather than being applied to an entire population, there are remarkably few population-based studies available to confirm our findings [3,36-38]. A re-examination of these

studies would be very useful as a means to provide confirmation (or refutation) of our findings. Based on the prevalence and associated odds ratios, we calculate that approximately 20% of premature CAD could be attributed to VLDL-C/TG ratios less than 0.18. Interestingly, this risk occurred primarily in those with average or lower plasma total TG and was particularly strong in those lacking standard coronary risk factors.

Our findings of excess risk associated with high estimated β -VLDL-C level are consistent with a number of prior studies, but few of these studies provided any risk estimate compared to a control population. Persons with type III hyperlipidemia and those with increased levels of TGRL remnants have long been known to be at increased risk [12-20,23,27,39-42]. Those who were more severely affected, as estimated by our calculated β -VLDL-C level, would be expected to be at the highest risk, though the lack of a quantitative index has generally limited such estimates. Admittedly, confidence intervals were wide in this study, owing to the infrequency of type III hyperlipidemia. Furthermore, our estimated β -VLDL-C is limited by being unable to distinguish between classical β -VLDL particles and reportedly more common “slow pre- β ” particles. Nevertheless, both particles have been associated previously with higher CAD risk.

In contrast, our finding of elevated risk associated with TGRL having low cholesterol content (manifested by low VLDL-C/TG) was unexpected and, at first glance, seemed counterintuitive. Indeed, we had initially hypothesized that excluding such particles (initially assumed to represent very large TGRL) in risk models might increase the calculated risk associated with the remaining particles since large TGRL (greater than 75 nm in diameter which includes large VLDL and chylomicrons, both having lower VLDL-C/TG ratios) previously have been reported not to be atherogenic [43]. Note, however, that higher plasma triglycerides remained a strong, independent risk factor in our study, confirming our prior observations [27]. We recognize that one limitation of this study is that we did not perform preparative ultracentrifugation to separate and uniquely characterize the various subfractions of chylomicrons, VLDL, intermediate density lipoprotein (IDL), Lp(a), LDL or HDL and their respective apoproteins. However, the analysis in our substudy using more detailed characterization of TGRL and LDL was strongly supportive of our overall findings. Certainly, an increase in IDL (found in the D >1.006 g/ml fraction along with LDL) could not explain our findings as these particles have higher cholesterol per particle (or per apo B) and we found that lower LDL-C/LDL-apo B ratios were strongly associated with lower VLDL-C/VLDL-TG. Thus, while beta-quantification is not the definitive means of characterizing lipoproteins, it provides considerably more information than the standard Friedewald calculation. Furthermore, our current findings are compelling, and raise important issues that could not have been appreciated without ultracentrifugation (or a comparable method to examine composition). We hope to provide more detailed lipoprotein characterization in future studies.

Because this study was not designed to explore possible mechanisms of the evidently increased CAD risk in those with low VLDL-C/TG, we can only speculate in this regard. Many TGRL are smaller than the theoretical 75 nm diameter limit for atherogenicity noted above [1]. Based on our subset analyses, we would not expect that the particles with low VLDL-C/VLDL-TG were necessarily larger than other VLDL. Therefore, size alone cannot exclude the possibility of direct atherogenicity of these particles. Furthermore, prior studies suggest that larger, more triglyceride-rich VLDL₁ particles are taken up more avidly by macrophages and lead to greater lipid accumulation than smaller VLDL₂, though these were in vitro observations [44,45]. Additionally, there is evidence that chylomicron remnants are avidly taken up by macrophages without the need for modification and can promote endothelial dysfunction or activation [46].

Others have noted increased CAD risk associated with impaired lipolysis or increased postprandial lipemia [47-49]. However, we would not expect impaired lipolysis to explain our

observation of increased risk in those with low VLDL-C/TG since, (1) the association was independent of plasma triglycerides and unaffected by eliminating all those with triglycerides of 200 mg/dl and higher, and (2) our subjects were sampled after a 10-12 hour fast. Also, impaired lipolysis with resulting increased plasma transit time of TGRL would be expected to lead to increased, not decreased, VLDL-C/TG ratios. Nevertheless, because we did not perform a dietary fat challenge in these subjects, we cannot completely rule out a contribution of decreased postprandial clearance of TGRL to our findings.

Complete apoprotein content of the various subfractions was not measured and may have differed by VLDL-C/TG and contributed to CAD risk. Of particular interest may be apo CIII. Apo CIII is known to inhibit lipolysis by lipoprotein lipase and hepatic lipase (at higher levels) and delay clearance of TGRL, possibly by inhibiting binding to cell-surface glycosaminoglycans, thereby making the particles less accessible to the lipases and for binding to cell-surface receptors [50]. Higher plasma levels of VLDL apo CIII and several apo CIII gene variants have been associated hypertriglyceridemia in humans [51-53], as well as increased risk of CAD [53-58]. A null mutation in apo CIII may offer protection from both [59]. Interestingly, apo CIII appears to impair insulin-induced stimulation of endothelial nitric oxide production and increase endothelial inflammation and expression of adhesion molecules [60,61]. Recently, certain genetic variants of apo CIII were found to be associated with manifestation of type III hyperlipidemia in those with apo E 2-2 [62]. Thus, while impaired lipolysis might lead to increased triglyceride on TGRL, the lengthened residence time would be expected to promote exchange of triglyceride for cholesterol ester via the CETP reaction, leading to accumulation of cholesterol-enriched remnants. It would thus seem unlikely that apo CIII would provide an explanation for our findings in relation to low VLDL-C/TG. Nevertheless, future studies should include measurement of apo CIII.

Nascent VLDL appear to be secreted from hepatocytes with substantially less cholesterol ester (CE) than that of VLDL typically found in plasma, suggesting much or most of the CE in VLDL is acquired in the intravascular compartment from HDL through the action of cholesteryl ester transfer protein (CETP) [63,64]. This seems to be the case even though hepatocyte CE content can be a strong determinant of the rate of VLDL production, an effect likely mediated in part by various intracellular transcription factors [65-72]. Furthermore, a substantial portion of so-called reverse cholesterol transport from the periphery to the liver appears dependent on CE transfer from HDL to TGRL via CETP, as suggested by studies of HDL lipid turnover and other data in humans [73-75]. The VLDL-C/TG ratio might therefore be an indirect index of reverse cholesterol transport through VLDL or of remodelling in the plasma compartment. A similar process could also result in LDL particles with reduced cholesterol content per particle as was seen by the strong correlation in our substudy between LDL-C/LDL-apo B and VLDL-C/VLDL-TG (independent of plasma triglyceride level). Future studies might examine activities or gene variants of phospholipid transfer protein, lecithin-cholesterol acyl transferase, and ABCA1, as well as CETP. Furthermore, composition of other lipid moieties, such as phospholipids, may also be important in cholesterol transport [76]. These considerations certainly raise the possibility that further examination of composition of TGRL and other lipoproteins may be a fruitful avenue for future investigations beyond mere particle numbers and size distributions. and may therefore provide important insights into recent controversies regarding the atheroprotective role of this pathway in atherogenesis [77-94].

We recognize that case-control studies have inherent limitations. We cannot provide absolute risks associated with the various lipid levels nor can we demonstrate temporal sequence (i.e., that risk factors were present before the onset of CAD). We have no data regarding possible longitudinal changes in the VLDL-C/TG ratio prior to or after the development of clinical CAD. Certainly, detailed measurement of VLDL composition in a large prospective follow-up study would provide important confirmation. While use of lipid medications appeared to

be associated with somewhat lower VLDL-C/TG ratio in both cases and controls, excluding all those taking these medications had virtually no effect on our CAD risk estimates.

Potential limitations of case-control studies in general also include recall bias and selection bias. Because all cases and controls were examined in our clinic with blood testing performed by a single laboratory, various forms of information bias were minimized. In particular, recall bias, a common problem in case-control studies as opposed to prospective studies, was minimized in this study since all subjects were interviewed in our clinic and cases were not asked to recall factors present before the onset of their CAD other than lifetime use of cigarettes. Inappropriate selection of cases and controls can inadvertently introduce bias into a case-control study. Sampled cases should be representative of all cases arising from a given population and controls should be representative of the general population. Our CAD cases were defined as premature and familial, thus potentially limiting generalization of our findings to non-familial or older-onset CAD. Genetic causes of CAD, including familial dyslipidemias may be over-represented in familial CAD cases. However, all risk factors tend to be somewhat higher among familial cases and generally reflect risk factors seen in non-familial cases with similar age of onset [95]. Furthermore, a large proportion of premature CAD cases do show evidence for a positive family history of CAD [96]. As our premature, familial CAD cases are the result of an intensive search for such cases among the major urban and suburban areas of Utah, we believe these to be representative of all such cases in Utah. We have previously presented detailed evidence regarding the representative nature of our control group [27]. As survivors of clinically apparent CAD could represent less severely affected patients as compared to those that had died, bias may be introduced but this would diminish the observed odds ratios (as more severe CAD would be expected to have more severe risk factors). This limitation is common to all case-control studies of CAD.

In conclusion, we found a strong, graded increase in risk for premature CAD associated with decreasing levels of the VLDL-C/TG ratio as determined by ultracentrifugation. In addition, we confirmed and extended prior observations regarding excess risk associated with accumulation of CE-rich TGRL remnants at the opposite end of the VLDL-C/TG spectrum. All these relationships were independent of plasma total TG levels, which were also independently and positively associated with increased CAD risk. These findings underscore the complexity of the association of TGRL with atherosclerosis and point to a need for further detailed observational, mechanistic, and interventional studies of this relationship.

Acknowledgments

This study was supported in part by grants HL47651, HL21088, HL47466, and HL71878 from the National Heart, Lung, and Blood Institute, Bethesda, Md., grant AG18734 from the National Institute on Aging, and a prior grant from Myriad Genetics.

References

1. Shen BW, Scanu AM, Kezdy FJ. Structure of human serum lipoproteins inferred from compositional analysis. *Proc Natl Acad Sci U S A* 1977;74(3):837–841. [PubMed: 265578]
2. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502. [PubMed: 4337382]
3. Wilson PW, Abbott RD, Garrison RJ, Castelli WP. Estimation of very-low-density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clin Chem* 1981;27(12):2008–2010. [PubMed: 6946880]
4. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* 1990;36(1):15–19. [PubMed: 2297909]

5. Tremblay AJ, Morrisette H, Gagne JM, Bergeron J, Gagne C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. *Clin Biochem* 2004;37(9):785–790. [PubMed: 15329317]
6. Wilson PW, Zech LA, Gregg RE, Schaefer EJ, Hoeg JM, Sprecher DL, Brewer HB Jr. Estimation of VLDL cholesterol in hyperlipidemia. *Clin Chim Acta* 1985;151(3):285–291. [PubMed: 3863729]
7. Ishibashi S, Yamada N, Shimano H, Takaku F, Akanuma Y, Murase T. Composition of very-low-density lipoproteins in non-insulin-dependent diabetes mellitus. *Clin Chem* 1989;35(5):808–812. [PubMed: 2720974]
8. Senti M, Pedro-Botet J, Nogues X, Rubies-Prat J. Influence of intermediate-density lipoproteins on the accuracy of the Friedewald formula. *Clin Chem* 1991;37(8):1394–1397. [PubMed: 1868600]
9. Gofman JW, DeLalla O, Glazier F, Freeman NK, Lindgren FT, Nichols AV, Strisower B, Tamplin AR. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis, and coronary disease. *Plasma* 1954;2:413–484.
10. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34(9):1345–1353. [PubMed: 13252080]
11. Chapman MJ, Goldstein S, Lagrange D, Laplaud PM. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. *J Lipid Res* 1981;22(2):339–358. [PubMed: 6787159]
12. Borrie P. Type 3 hyperlipoproteinaemia. *Br Med J* 1969;2(658):665–667. [PubMed: 5783124]
13. Morganroth J, Levy RI, Fredrickson DS. The biochemical, clinical, and genetic features of type III hyperlipoproteinemia. *Ann of Intern Med* 1975;82:158–174. [PubMed: 163608]
14. Hazzard W, Goldstein J, Schrott H. Hyperlipidemia in coronary heart disease. III. Evaluation of lipoprotein phenotypes of 156 genetically defined survivors of myocardial infarction. *J Clin Invest* 1973;52:1569–1577. [PubMed: 4352458]
15. Hazzard WR, O'Donnell TF, Lee YL. Broad-beta disease (type III hyperlipoproteinemia) in a large kindred. Evidence for a monogenic mechanism. *Ann Intern Med* 1975;82(2):141–149. [PubMed: 163606]
16. Mishkel MA, Nazir DJ, Crowther S. A longitudinal assessment of lipid ratios in the diagnosis of type III hyperlipoproteinaemia. *Clin Chim Acta* 1975;58(2):121–136. [PubMed: 164305]
17. Stuyt PM, Van 't Laar A. Clinical features of type III hyperlipoproteinaemia. *Neth J Med* 1983;26(4):104–111. [PubMed: 6855990]
18. Feussner G, Wagner A, Kohl B, Ziegler R. Clinical features of type III hyperlipoproteinemia: analysis of 64 patients. *Clin Invest* 1993;71(5):362–366.
19. Marz W, Feussner G, Siekmeier R, Donnerhak B, Schaaf L, Ruzicka V, Gross W. Apolipoprotein E to B ratio: a marker for type III hyperlipoproteinaemia. *Eur J Clin Chem Clin Biochem* 1993;31(11):743–747. [PubMed: 8305617]
20. Dobmeyer J, Lohrmann J, Feussner G. Prevalence and association of atherosclerosis at three different arterial sites in patients with type III hyperlipoproteinemia. *Atherosclerosis* 1996;119(1):89–98. [PubMed: 8929260]
21. Koba S, Hirano T, Sakaue T, Takeuchi H, Adachi M, Katagiri T. An increased number of very-low-density lipoprotein particles is strongly associated with coronary heart disease in Japanese men, independently of intermediate-density lipoprotein or low-density lipoprotein. *Coron Artery Dis* 2002;13(5):255–262. [PubMed: 12394649]
22. Cohn JS, Giroux LM, Fortin LJ, Davignon J. Prevalence of double pre-beta lipoproteinemia in hyperlipidemic patients is influenced by gender, menopausal status, and apoE phenotype. *Arterioscler Thromb Vasc Biol* 1997;17:2630–2637. [PubMed: 9409236]
23. Cohn JS, Marcoux C, Davignon J. Detection, quantification, and characterization of potentially atherogenic triglyceride-rich remnant lipoproteins. *Arterioscler Thromb Vasc Biol* 1999;19(10):2474–2486. [PubMed: 10521378]
24. Fredrickson DS, Morganroth J, Levy RI. Type III hyperlipoproteinemia: an analysis of two contemporary definitions. *Ann Intern Med* 1975;82:150–157. [PubMed: 163607]
25. Patsch JR, Jackson RL, Gotto AM Jr. Evaluation of the classical methods for the diagnosis of type III hyperlipoproteinemia. *Klin Wochenschr* 1977;55(21):1025–1030. [PubMed: 200792]

26. Albers JJ, Warnick GR, Hazzard WR. Type III hyperlipoproteinemia: a comparative study of current diagnostic techniques. *Clin Chim Acta* 1977;75(2):193–204. [PubMed: 191218]
27. Hopkins PN, Wu LL, Hunt SC, Brinton EA. Plasma triglycerides and type III hyperlipidemia are independently associated with premature familial coronary artery disease. *J Am Coll Cardiol* 2005;45(7):1003–1012. [PubMed: 15808755]
28. Hopkins PN, Stephenson S, Wu LL, Riley WA, Xin Y, Hunt SC. Evaluation of coronary risk factors in patients with heterozygous familial hypercholesterolemia. *Am J Cardiol* 2001;87(5):547–553. [PubMed: 11230837]
29. Lipid Research Clinics. Manual of laboratory operations: Lipid Research Clinics Program. Vol. 1. National Institutes of Health; Bethesda, MD: 1974.
30. Wu LL, Warnick GR, Wu JT, Williams RR, Lalouel JM. A rapid micro-scale procedure for determination of the total lipid profile. *Clin Chem* 1989;35:1486–1491. [PubMed: 2758594]
31. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem* 1982;28:1379–1388. [PubMed: 7074948]
32. Nanjee MN, Crouse JR, King JM, Hovorka R, Rees SE, Carson ER, Morgenthaler JJ, Lerch P, Miller NE. Effects of intravenous infusion of lipid-free apo A-I in humans. *Arterioscler Thromb Vasc Biol* 1996;16(9):1203–1214. [PubMed: 8792776]
33. Hopkins PN, Wu LL, Schumacher MC, Emi M, Hegele RM, Hunt SC, Lalouel JM, Williams RR. Type III dyslipoproteinemia in patients heterozygous for familial hypercholesterolemia and apolipoprotein E2. Evidence for a gene-gene interaction. *Arterioscler Thromb* 1991;11:1137–1146. [PubMed: 1680391]
34. Hopkins, PN.; Wu, LL.; Williams, RR. Dyslipidemias. In: Noe, DA.; Rock, RC., editors. Laboratory medicine The selection and interpretation of clinical laboratory studies. Baltimore: Williams & Wilkins; 1994. p. 476-511.
35. Hopkins PN, Wu LL, Williams RR, Leary ET, Wang T, Nakajima K. Type III hyperlipidemia and lipoprotein remnants in early onset familial coronary artery disease. *Circulation* 1998;98(suppl I):I–791.
36. Hulley SB, Rhoads GG. The plasma lipoproteins as risk factors: comparison of electrophoretic and ultracentrifugation results. *Metabolism* 1982;31(8):773–777. [PubMed: 6954343]
37. Williams OD, Stinnett S, Chambless LE, Boyle KE, Bachorik PS, Albers JJ, Lippel K. Populations and methods for assessing dyslipoproteinemia and its correlates. The Lipid Research Clinics Program Prevalence Study. *Circulation* 1986;73(1 Pt 2):I4–11. [PubMed: 3940682]
38. LaRosa JC, Chambless LE, Criqui MH, Frantz ID, Glueck CJ, Heiss G, Morrison JA. Patterns of dyslipoproteinemia in selected North American populations. The Lipid Research Clinics Program Prevalence Study. *Circulation* 1986;73(1 Pt 2):I12–29. [PubMed: 3940679]
39. Mahley, RW.; Rall, SC. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. The metabolic and molecular bases of inherited disease. Vol. 8th. New York: McGraw Hill, Inc; 2001. p. 2835-2862.
40. Schaefer EJ, Audelin MC, McNamara JR, Shah PK, Tayler T, Daly JA, Augustin JL, Seman LJ, Rubenstein JJ. Comparison of fasting and postprandial plasma lipoproteins in subjects with and without coronary heart disease. *Am J Cardiol* 2001;88(10):1129–1133. [PubMed: 11703957]
41. Björkegren J, Silveira A, Boquist S, Tang R, Karpe F, Bond MG, de Faire U, Hamsten A. Postprandial enrichment of remnant lipoproteins with apoC-I in healthy normolipidemic men with early asymptomatic atherosclerosis. *Arterioscler Thromb Vasc Biol* 2002;22(9):1470–1474. [PubMed: 12231568]
42. Onat A, Hergenc G, Sansoy V, Fobker M, Ceyhan K, Toprak S, Assmann G. Apolipoprotein C-III, a strong discriminant of coronary risk in men and a determinant of the metabolic syndrome in both genders. *Atherosclerosis* 2003;168(1):81–89. [PubMed: 12732390]
43. Nordestgaard BG, Zilversmit DB. Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. *J Lipid Res* 1988;29:1491–1500. [PubMed: 3241125]
44. Milosavljevic D, Griglio S, Le Naour G, Chapman MJ. Preferential reduction of very low density lipoprotein-I particle number by fenofibrate in type IIB hyperlipidemia: consequences for lipid

- accumulation in human monocyte-derived macrophages. *Atherosclerosis* 2001;155(1):251–260. [PubMed: 11223449]
45. Milosavljevic D, Kontush A, Griglio S, Le Naour G, Thillet J, Chapman MJ. VLDL-induced triglyceride accumulation in human macrophages is mediated by modulation of LPL lipolytic activity in the absence of change in LPL mass. *Biochim Biophys Acta* 2003;1631(1):51–60. [PubMed: 12573449]
 46. Botham KM, Bravo E, Elliott J, Wheeler-Jones CP. Direct interaction of dietary lipids carried in chylomicron remnants with cells of the artery wall: implications for atherosclerosis development. *Curr Pharm Des* 2005;11(28):3681–3695. [PubMed: 16305504]
 47. Ebenbichler CF, Kirchmair R, Egger C, Patsch JR. Postprandial state and atherosclerosis. *Curr Opin Lipidol* 1995;6(5):286–290. [PubMed: 8520851]
 48. Ginsberg HN, Illingworth DR. Postprandial dyslipidemia: an atherogenic disorder common in patients with diabetes mellitus. *Am J Cardiol* 2001;88(6A):9H–15H.
 49. Sposito AC, Lemos PA, Santos RD, Hueb W, Vinagre CG, Quintella E, Carneiro O, Chapman MJ, Ramires JA, Maranhao RC. Impaired intravascular triglyceride lipolysis constitutes a marker of clinical outcome in patients with stable angina undergoing secondary prevention treatment: a long-term follow-up study. *J Am Coll Cardiol* 2004;43(12):2225–2232. [PubMed: 15193684]
 50. Ebara T, Ramakrishnan R, Steiner G, Shachter NS. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. *J Clin Invest* 1997;99(11):2672–2681. [PubMed: 9169497]
 51. Cohn JS, Tremblay M, Batal R, Jacques H, Rodriguez C, Steiner G, Mamer O, Davignon J. Increased apoC-III production is a characteristic feature of patients with hypertriglyceridemia. *Atherosclerosis* 2004;177(1):137–145. [PubMed: 15488876]
 52. 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(3):590–596. [PubMed: 16410456]
 53. Atzmon G, Rincon M, Schechter CB, Shuldiner AR, Lipton RB, Bergman A, Barzilai N. Lipoprotein genotype and conserved pathway for exceptional longevity in humans. *PLoS Biol* 2006;4(4):e113. [PubMed: 16602826]
 54. Blankenhorn DH, Alaupovic P, Wickham E, Chin HP, Azen SP. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors. *Circulation* 1990;81(2):470–476. [PubMed: 2404631]
 55. Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Krams DM, Blankenhorn DH. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation* 1994;90(1):42–49. [PubMed: 8026027]
 56. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pfeffer MA, Braunwald E. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation* 2000;102(16):1886–1892. [PubMed: 11034934]
 57. Olivieri O, Stranieri C, Bassi A, Zaia B, Girelli D, Pizzolo F, Trabetti E, Cheng S, Grow MA, Pignatti PF, Corrocher R. ApoC-III gene polymorphisms and risk of coronary artery disease. *J Lipid Res* 2002;43(9):1450–1457. [PubMed: 12235176]
 58. Olivieri O, Bassi A, Stranieri C, Trabetti E, Martinelli N, Pizzolo F, Girelli D, Friso S, Pignatti PF, Corrocher R. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. *J Lipid Res* 2003;44(12):2374–2381. [PubMed: 14563827]
 59. 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(5908):1702–1705. [PubMed: 19074352]
 60. Kawakami A, Osaka M, Tani M, Azuma H, Sacks FM, Shimokado K, Yoshida M. Apolipoprotein CIII links hyperlipidemia with vascular endothelial cell dysfunction. *Circulation* 2008;118(7):731–742. [PubMed: 18663085]

61. Bobik A. Apolipoprotein CIII and atherosclerosis: beyond effects on lipid metabolism. *Circulation* 2008;118(7):702–704. [PubMed: 18695202]
62. Henneman P, van der Sman-de Beer F, Moghaddam PH, Huijts P, Stalenhoef AF, Kastelein JJ, van Duijn CM, Havekes LM, Frants RR, van Dijk KW, Smelt AH. The expression of type III hyperlipoproteinemia: involvement of lipolysis genes. *Eur J Hum Genet* 2009;17(5):620–628. [PubMed: 19034316]
63. Nicolosi RJ, Hayes KC. Composition of plasma and nascent very low density lipoprotein from perfused livers of hypercholesterolemic squirrel monkeys. *Lipids* 1980;15(8):549–554. [PubMed: 7432101]
64. Lin Y, Havinga R, Verkade HJ, Moshage H, Slooff MJ, Vonk RJ, Kuipers F. Bile acids suppress the secretion of very-low-density lipoprotein by human hepatocytes in primary culture. *Hepatology* 1996;23(2):218–228. [PubMed: 8591844]
65. Avramoglu RK, Cianflone K, Sniderman AD. Role of the neutral lipid accessible pool in the regulation of secretion of apoB-100 lipoprotein particles by HepG2 cells. *J Lipid Res* 1995;36(12):2513–2528. [PubMed: 8847478]
66. Thompson GR, Naumova RP, Watts GF. Role of cholesterol in regulating apolipoprotein B secretion by the liver. *J Lipid Res* 1996;37:439–447. [PubMed: 8728309]
67. Burnett JR, Wilcox LJ, Telford DE, Kleinstiver SJ, Barrett PH, Newton RS, Huff MW. Inhibition of HMG-CoA reductase by atorvastatin decreases both VLDL and LDL apolipoprotein B production in miniature pigs. *Arterioscler Thromb Vasc Biol* 1997;17(11):2589–2600. [PubMed: 9409231]
68. Burnett JR, Wilcox LJ, Telford DE, Kleinstiver SJ, Barrett PH, Newton RS, Huff MW. The magnitude of decrease in hepatic very low density lipoprotein apolipoprotein B secretion is determined by the extent of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition in miniature pigs. *Endocrinology* 1999;140(11):5293–5302. [PubMed: 10537160]
69. Isusi E, Aspichueta P, Liza M, Hernandez ML, Diaz C, Hernandez G, Martinez MJ, Ochoa B. Short- and long-term effects of atorvastatin, lovastatin and simvastatin on the cellular metabolism of cholesteryl esters and VLDL secretion in rat hepatocytes. *Atherosclerosis* 2000;153(2):283–294. [PubMed: 11164417]
70. Kang S, Davis RA. Cholesterol and hepatic lipoprotein assembly and secretion. *Biochim Biophys Acta* 2000;1529(13):223–230. [PubMed: 11111091]
71. Blasiole DA, Davis RA, Attie AD. The physiological and molecular regulation of lipoprotein assembly and secretion. *Mol Biosyst* 2007;3(9):608–619. [PubMed: 17700861]
72. van der Veen JN, Havinga R, Bloks VW, Groen AK, Kuipers F. Cholesterol feeding strongly reduces hepatic VLDL-triglyceride production in mice lacking the liver X receptor alpha. *J Lipid Res* 2007;48(2):337–347. [PubMed: 17088263]
73. Nestel PJ, Reardon M, Billington T. In vivo transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins in man. *Biochim Biophys Acta* 1979;573(2):403–407. [PubMed: 221036]
74. Fielding PE, Fielding CJ, Havel RJ. Cholesterol net transport, esterification, and transfer in human hyperlipidemic plasma. *J Clin Invest* 1983;71:449–460. [PubMed: 6826717]
75. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. *J Lipid Res* 2004;45(9):1594–1607. [PubMed: 15145983]
76. Fournier N, Paul JL, Atger V, Cogny A, Soni T, de la Llera-Moya M, Rothblat G, Moatti N. HDL phospholipid content and composition as a major factor determining cholesterol efflux capacity from Fu5AH cells to human serum. *Arterioscler Thromb Vasc Biol* 1997;17(11):2685–2691. [PubMed: 9409243]
77. Borggreve SE, Hillege HL, Dallinga-Thie GM, de Jong PE, Wolffenbuttel BH, Grobbee DE, van Tol A, Dullaart RP. High plasma cholesteryl ester transfer protein levels may favour reduced incidence of cardiovascular events in men with low triglycerides. *Eur Heart J* 2007;28(8):1012–1018. [PubMed: 17409111]
78. de Vries R, Perton FG, Dallinga-Thie GM, van Roon AM, Wolffenbuttel BH, van Tol A, Dullaart RP. Plasma cholesteryl ester transfer is a determinant of intima-media thickness in type 2 diabetic and nondiabetic subjects: role of CETP and triglycerides. *Diabetes* 2005;54(12):3554–3559. [PubMed: 16306375]

79. Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest* 1996;97(12):2917–2923. [PubMed: 8675707]
80. Hirano, Ki; Yamashita, S.; Nakajima, N.; Arai, T.; Maruyama, T.; Yoshida, Y.; Ishigami, M.; Sakai, N.; Kameda-Takemura, K.; Matsuzawa, Y. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol* 1997;17:1053–1059. [PubMed: 9194754]
81. Agerholm-Larsen B, Tybjaerg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations, decreased HDL cholesterol, and possible decreased risk of ischemic heart disease: The Copenhagen City Heart Study. *Circulation* 2000;102(18):2197–2203. [PubMed: 11056092]
82. Borggreve SE, Hillege HL, Wolffenbuttel BH, de Jong PE, Zuurman MW, van der Steege G, van Tol A, Dullaart RP. An increased coronary risk is paradoxically associated with common cholesteryl ester transfer protein gene variations that relate to higher high-density lipoprotein cholesterol: a population-based study. *J Clin Endocrinol Metab* 2006;91(9):3382–3388. [PubMed: 16684835]
83. Zheng K, Zhang S, Zhang L, He Y, Liao L, Hou Y, Huang D. Carriers of three polymorphisms of cholesteryl ester transfer protein gene are at increased risk to coronary heart disease in a Chinese population. *Int J Cardiol* 2005;103(3):259–265. [PubMed: 16098387]
84. Moriyama Y, Okamura T, Inazu A, Doi M, Iso H, Mouri Y, Ishikawa Y, Suzuki H, Iida M, Koizumi J, Mabuchi H, Komachi Y. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. *Prev Med* 1998;27(5 Pt 1):659–667. [PubMed: 9808796]
85. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AVG, Lie KI, Kastelein JJP, Regression Growth Evaluation Statin Study Group. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. *N Engl J Med* 1998;338:86–93. [PubMed: 9420339]
86. Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, Lahoz C, Coltell O, Wilson PW, Schaefer EJ. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. *Arterioscler Thromb Vasc Biol* 2000;20(5):1323–1329. [PubMed: 10807749]
87. Brousseau ME, O'Connor JJ Jr, Ordovas JM, Collins D, Otvos JD, Massov T, McNamara JR, Rubins HB, Robins SJ, Schaefer EJ. Cholesteryl ester transfer protein TaqI B2B2 genotype is associated with higher HDL cholesterol levels and lower risk of coronary heart disease end points in men with HDL deficiency: Veterans Affairs HDL Cholesterol Intervention Trial. *Arterioscler Thromb Vasc Biol* 2002;22(7):1148–1154. [PubMed: 12117730]
88. Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R, Cheng S, Shuldiner AR. Unique lipoprotein phenotype and genotype associated with exceptional longevity. *JAMA* 2003;290(15):2030–2040. [PubMed: 14559957]
89. Blankenberg S, Rupprecht HJ, Bickel C, Jiang XC, Poirier O, Lackner KJ, Meyer J, Cambien F, Tiret L. Common genetic variation of the cholesteryl ester transfer protein gene strongly predicts future cardiovascular death in patients with coronary artery disease. *J Am Coll Cardiol* 2003;41(11):1983–1989. [PubMed: 12798569]
90. Anderson JL, Carlquist JF. Genetic polymorphisms of hepatic lipase and cholesteryl ester transfer protein, intermediate phenotypes, and coronary risk. Do they add up yet? *J Am Coll Cardiol* 2003;41(11):1990–1993. [PubMed: 12798570]
91. Curb JD, Abbott RD, Rodriguez BL, Masaki K, Chen R, Sharp DS, Tall AR. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. *J Lipid Res* 2004;45(5):948–953. [PubMed: 14967821]
92. Thompson JF, Durham LK, Lira ME, Shear C, Milos PM. CETP polymorphisms associated with HDL cholesterol may differ from those associated with cardiovascular disease. *Atherosclerosis* 2005;181(1):45–53. [PubMed: 15939053]
93. Tall AR. CETP Inhibitors to Increase HDL Cholesterol Levels. *N Engl J Med*. 2007
94. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B. Effects

- of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007;357(21):2109–2122. [PubMed: 17984165]
95. Jomini V, Oppliger-Pasquali S, Wietlisbach V, Rodondi N, Jotterand V, Paccaud F, Darioli R, Nicod P, Mooser V. Contribution of major cardiovascular risk factors to familial premature coronary artery disease. The GENECARD project. *J Am Coll Cardiol* 2002;40(4):676. [PubMed: 12204497]
96. Williams RR, Hunt SC, Heiss G, Province MA, Bensen JT, Higgins M, Chamberlain RM, Ware J, Hopkins PN. Usefulness of cardiovascular family history data for population-based preventive medicine and medical research (The Health Family Tree Study and the NHLBI Family Heart Study). *Am J Cardiol* 2001;87(2):129–135. [PubMed: 11152826]

Figure 1.

Odds ratios (and lower 95% CI) for premature coronary artery disease (CAD) by estimated β -VLDL cholesterol. In model 1 additional covariates included only age and gender (both significant at $p < 0.0001$). In model 2 risk factors included age, gender, history of smoking (ever / never), hypertension (present / absent), diabetes (present / absent), measured LDL cholesterol, log triglycerides, and HDL cholesterol (all significant at $p < 0.0001$). In model 2, the odds ratio for very high β -VLDL cholesterol remained significant with OR 10.5, $p = 0.02$.

Figure 2.

Odds ratios for premature CAD by measured VLDL-C/TG ratio. Model 3 and 4 as in figure 1, models 1 and 2, with the addition in model 4 of type III hyperlipidemia (VLDL-C/TG ≥ 0.30 with triglycerides >150 mg/dl) (present / absent). The dashed line represents the solution of a logistic regression model using all risk factors in model 4 but excluding type III subjects.

VLDL-C/TG was entered as a continuous variable including both a linear and quadratic term. The regression coefficient for the linear term was -18.5 ($p < 0.0001$) and for the quadratic term (the ratio squared) it was 23.9 ($p < 0.0001$).

Figure 3.

Odds ratios of premature CAD by combinations of VLDL-C/TG ratio and a. total plasma triglycerides or b. HDL-C. Adjusted for age, gender, history of smoking (ever / never), hypertension (present / absent), diabetes (present / absent), measured LDL cholesterol, and HDL cholesterol (for a.) or triglycerides (for b.).

Figure 4.
VLDL-C/VLDL-TG in a selected subgroup of male cases and controls.

Clinical characteristics. Cases with premature coronary artery disease (CAD) are compared with population controls. Differences were tested by Chi-square analysis or Student's t-test. For triglycerides and VLDL-C, p-values are for log-transformed variables.

Table 1

Variable	CAD Cases (n = 1170)			Controls (n = 1759)		
	Mean	S.D.		Mean	S.D.	p-value
Age (years)	56.7	8.4		54.0	9.7	<.0001
Male (%)	79.1			44.9		<.0001
BMI	29.1	5.0		27.8	5.1	<.0001
Hypertension (%)	56.5			24.4		<.0001
Diabetes (%)	20.5			6.1		<.0001
Ever smoked (%)	43.5			15.6		<.0001
Taking lipid lowering medication (%)	14.1			13.9		0.86
Total cholesterol (mg/dL)	209	54		187	35	<.0001
Triglycerides (mg/dL) *	173	1.8		124	1.7	<.0001
HDL cholesterol (mg/dL)	40.8	12.5		50.4	14.9	<.0001
LDL cholesterol, measured (mg/dL)	128	46		109	30	<.0001
VLDL cholesterol, measured (mg/dL)	39.6	33.6		28.4	19.2	<.0001
Estimated β-VLDL cholesterol (mg/dl)	9.4	19.6		8.9	9.7	0.46
Type III hyperlipidemia (%)	2.7			0.7		<.0001

* shown is the geometric mean and SD. The median and range in cases and controls was 164 (37-2134), 123 (31-1615) respectively.

Table 2

Distribution of VLDL-C/TG in 1170 cases and 1759 controls and associated odds ratios (OR) from multiple logistic regression analysis (adjusted for age, gender, smoking, hypertension, diabetes, type III hyperlipidemia, measured LDL-C, HDL-C, and log triglycerides) in the entire group and subgroups shown. Very premature CAD was defined as onset by age 45 for males and 55 for females. The “low CAD risk” subgroup was defined as subjects who never smoked, had no history of hypertension or diabetes, and who had measured LDL-C under 130 mg/dl, HDL-C 40 mg/dl or more, plasma triglycerides <200 mg/dl without lipid medications and did not meet criteria for type III hyperlipidemia.

	Ratio of measured VLDL-C / plasma triglycerides				
	<0.120	0.120 – 0.139	0.140 – 0.159	0.160 – 0.179	≥ 0.180
All cases and controls					
number of cases (%)	83 (7.1)	92 (7.9)	150 (12.8)	159 (13.6)	686 (58.6)
number of controls (%)	56 (3.2)	87 (5.0)	149 (8.5)	228 (13.0)	1239 (70.4)
OR for CAD (95% CI) ^a	4.5 (2.9-6.9)	2.1 (1.4-3.1)	1.8 (1.3-2.5)	1.2 (0.9-1.6)	1.0
OR for subgroups (cases, controls)					
Men (926, 790) ^d	5.1 (2.9-9.1)	2.3 (1.5-3.8)	2.0 (1.4-3.0)	0.98 (0.7-1.4)	1.0
Women (244, 969) ^c	3.6 (1.8-7.4)	1.6 (0.7-3.4)	1.5 (0.8-2.5)	1.8 (1.1-3.0)	1.0
Very premature CAD (457, 1759) ^d	4.4 (2.5-7.7)	2.4 (1.5-4.0)	1.8 (1.2-2.8)	1.3 (0.9-1.9)	1.0
No lipid medication (1005, 1515) ^d	5.0 (3.0-8.4)	2.4 (1.5-3.7)	2.0 (1.4-2.9)	1.3 (0.9-1.8)	1.0
Triglycerides < 200 (735, 1449) ^d	4.7 (2.9-7.6)	2.2 (1.4-3.4)	2.0 (1.4-2.9)	1.0 (0.7-1.4)	1.0
Low CAD risk (37, 535) ^d	5.8 (1.5-22)	3.6 (1.0-13)	1.5 (0.4-5.8)	0.6 (0.2-2.0)	1.0

^a p-values for VLDL-C/TG as a class variable with 4 degrees of freedom: p <0.0001,

^b p-values for VLDL-C/TG as a class variable with 4 degrees of freedom: p <0.001;

^c p-values for VLDL-C/TG as a class variable with 4 degrees of freedom: p <0.01;

^d p-values for VLDL-C/TG as a class variable with 4 degrees of freedom: p <0.05

Table 3

Shown are least squares mean $\pm$ SE from analysis of covariance. Age and gender was included in all models. Chylomicron TG was estimated as described in Methods. Unadjusted percentages are presented for medication use. For statistical analyses, case/control status was treated as a class variable and VLDL-C/TG as an ordered variable with values 1-5 according to categories below.

		Ratio of measured VLDL-C / plasma triglycerides				
		< 0.120	0.120 – 0.139	0.140 – 0.159	0.160 – 0.179	≥ 0.180
BMI (kg/m ²) ^{a,b}						
in cases		28.6 $\pm$ 0.6	28.8 $\pm$ 0.6	29.2 $\pm$ 0.4	29.6 $\pm$ 0.4	28.5 $\pm$ 0.2
in controls		29.8 $\pm$ 0.7	29.3 $\pm$ 0.5	29.1 $\pm$ 0.4	27.9 $\pm$ 0.3	27.6 $\pm$ 0.1
Triglycerides (mg/dl) ^a						
in cases		144 $\pm$ 13	196 $\pm$ 13	222 $\pm$ 10	216 $\pm$ 10	200 $\pm$ 4.8
in controls		137 $\pm$ 16	148 $\pm$ 13	171 $\pm$ 10	151 $\pm$ 7.9	140 $\pm$ 3.4
HDL cholesterol (mg/dl) ^{a,b}						
in cases		49.6 $\pm$ 1.4	46.8 $\pm$ 1.3	44.0 $\pm$ 1.1	43.8 $\pm$ 1.0	43.3 $\pm$ 0.5
in controls		51.8 $\pm$ 1.7	51.2 $\pm$ 1.4	49.2 $\pm$ 1.0	49.1 $\pm$ 0.8	49.9 $\pm$ 0.4
LDL cholesterol (mg/dl) ^{a,b}						
in cases		124 $\pm$ 4.1	120 $\pm$ 3.9	122 $\pm$ 3.1	131 $\pm$ 3.0	133 $\pm$ 1.5
in controls		96.3 $\pm$ 4.9	109 $\pm$ 4.0	108 $\pm$ 3.0	108 $\pm$ 2.5	109 $\pm$ 1.1
Use of lipid medication (%) ^{a,b}						
in cases		24.1	21.7	15.3	13.2	11.8
in controls		26.8	19.5	23.5	18.9	10.8

^a p < 0.001 for case effect;

^b p < 0.001 for VLDL-C/TG category effect.