Relation of the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio to the remainder of the lipid profile: The Very Large Database of Lipids-4 (VLDL-4) study

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Abstract

Background: High levels of the triglycerides to high-density lipoprotein cholesterol (TG/HDL-C) ratio are associated with obesity, metabolic syndrome, and insulin resistance.

Objectives: We evaluated variability in the remaining lipid profile, especially remnant lipoprotein particle cholesterol (RLP-C) and its components (very low-density lipoprotein cholesterol subfraction 3 and intermediate-density lipoprotein cholesterol), with variability in the TG/HDL-C ratio in a very large study cohort representative of the general U.S. population.

Methods: We examined data from 1,350,908 US individuals who were clinically referred for lipoprotein cholesterol ultracentrifugation (Atherotech, Birmingham, AL) from 2009 to 2011. Demographic information other than age and sex was not available. Changes to the remaining lipid profile across percentiles of the TG/HDL-C ratio were quantified, as well as by three TG/HDL-C cut-off points previously proposed in the literature: 2.5 (male) and 2 (female), 3.75 (male) and 3 (female), and 3.5 (male and female).

Results: The mean age of our study population was 58.7 years, and 48% were men. The median TG/HDL-C ratio was 2.2. Across increasing TG/HDL-C ratios, we found steadily increasing levels of RLP-C, non-HDL-C and LDL density. Among the lipid parameters studied, RLP-C and LDL density had the highest relative increase when comparing individuals with elevated TG/HDL-C levels to those with lower TG/HDL-C levels using established cut-off points. Approximately 47% of TG/HDL-C ratio variance was attributable to RLP-C.

Conclusions: In the present analysis, a higher TG/HDL-C ratio was associated with an increasingly atherogenic lipid phenotype, characterized by higher RLP-C along with higher non-HDL-C and LDL density.

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1. Introduction

Increased visceral adiposity promotes the development of insulin resistance and hypertriglyceridemia [1]. Hypertriglyceridemia in the setting of insulin resistance is the result of increased hepatic secretion of very low-density lipoproteins and impaired lipolysis in serum of triglyceride-enriched lipoproteins.
Hypertriglyceridemia stimulates the activity of cholesteryl ester transfer protein (CEPT), which exchanges triglycerides (TG) from TG-rich lipoproteins for cholesteryl esters from high-density lipoprotein (HDL) and low-density lipoprotein (LDL) [2]. Triglyceride enrichment of HDL and LDL particles renders them better substrates for lipolysis by hepatic lipase, resulting in HDL catabolism and elimination and the formation of more numerous, denser LDL particles.

Likely as a result of this relationship, high levels of the triglycerides to high-density lipoprotein cholesterol (TG/HDL-C) ratio have been associated not only with insulin resistance, but also with obesity and metabolic syndrome [3,4]. Since the TG/HDL-C ratio has been shown to independently predict cardiovascular events [5–8], its clinical relevance in quantifying atherogenic risk grows as the epidemic of these disorders continues to worsen. Moreover, the simultaneous use of triglyceride and HDL-C in a ratio is more useful than isolated lipid values as it more closely reflects the complex interactions of lipoprotein metabolism and can better predict plasma atherogenicity [9].

The atherogenic “lipid triad” is characterized by an excess of small, dense, low-density lipoprotein (sLDL), low serum levels of HDL-C, and the overproduction of triglyceride-rich remnant lipoproteins (TRL), including serum remnants lipoproteins such as lipoprotein (a) [10–12]. Some studies have evaluated the distribution of a few basic lipid measures across TG/HDL-C levels [13–20]. However, no prior studies have evaluated the association between TG/HDL-C ratio and an extended lipid profile, that includes these and other lipid measures such as RLP-C, which would help better understand what information is carried by the TG/HDL-C ratio.

Several cut-off points of TG/HDL-C ratio have been proposed in the literature. For instance, the dyslipidemia of metabolic syndrome as defined by ATP-III criteria uses cut-off points of TG <150 mg/dL and HDL-C >40 mg/dL (men) and >50 mg/dL (women) [21], with calculated TG/HDL-C ratios of 3.75 and 3, respectively. However, more aggressive cut-off points have also been recommended, such as the one proposed by Miller et al. (2.5 for men, 2 for women) [6]. Recent studies by McLaughlin et al. indicate that a level of 3.5 may also be useful in predicting individuals who are at increased risk of developing CVD [22,23].

In the Very Large Database of Lipids, a big cross-sectional study, we aimed to assess the correlations of TG/HDL-C with lipid parameters, particularly RLP-C, and to compare lipid profiles between subpopulations categorized by different TG/HDL-C ratio cut-off points.

2. Methods

2.1. Study population

We examined 1,350,908 consecutive lipid profiles from the Very Large Database of Lipids [24,25], an investigator-initiated database managed by the Ciccarone Center for the Prevention of Heart Disease and made possible through collaboration with Atherotech Diagnostic Laboratory. Some of its publications can be found elsewhere [25,26]. Samples were sent for clinical indications to Atherotech Laboratory (Birmingham, AL) from 2009 to 2011. Second and later samples on the same subject were excluded. Lipid profiles included in the analysis were unique for each individual and were the first available lipid profile. There were no other exclusions. The majority of samples were obtained at primary clinics, whereas the remaining samples were from specialized clinics such as lipid clinics, as well as from inpatients at both university-based and private hospitals. Lipid distributions were similar to the National Health and Nutrition Examination Survey (NHANES) population-based survey [25], although it is important to recognize that, based on our lack of demographic information, caution is needed in extrapolating to the general population.

2.2. Lipid measurements

Cholesterol concentrations of lipoprotein classes: intermediate-density lipoprotein cholesterol (IDL-C), LDL-C, very low-density lipoprotein cholesterol (VLDL-C), HDL-C and subclasses were measured by the Vertical Auto Profile (VAP) method (Atherotech) [27–29]. The VAP method separates lipoproteins based on their density using single vertical-spin density gradient ultracentrifugation, and then quantifies cholesterol content using an enzymatic reaction and spectrophotometric absorbance. The accuracy of VAP lipid parameters was cross-validated with reference laboratory as previously described [25]. Triglycerides were directly measured using the ARCHITECT C-8000 system (Abbott) [24,25]. We defined RLP-C as the sum of VLDL subtraction 3 cholesterol (VLDL3-C) and IDL-C [30]. However, RLP-C can also be detected by a previously described immunological technique. Briefly, RLP are separated from other lipoproteins using immunoaffinity mixed gels containing anti-α1B-100 and anti-apo A-I, which lets concentration of remnant-lipoprotein particles cholesterol (RLP-C) and triglycerides (RLP-TG) be measured with a sensitive enzymatic assay [31].

2.3. Data management

Raw data was compiled at Atherotech and subsequently cleaned of duplicate samples, and then de-identified and transferred to the senior investigators. The Very Large Database of Lipids is maintained at The Johns Hopkins Hospital (Baltimore, Maryland) and registered at clinicaltrials.gov (NCT01698489). The Johns Hopkins Institutional Review Board declared this study exempt.

2.4. Statistical analysis

Non-HDL-C levels were calculated as: total cholesterol – HDL-C. LDL particle distribution was characterized as LDL density ratio (LLDR) using the following formula: LLDR = ln[(LDL3-C + LDL4-C) / (LDL1-C + LDL2-C)], where LDL subclass density increases from least dense LDL1 to most dense LDL4. This ratio has previously been shown to strongly correlate with ultracentrifugation-determined modal LDL density (R² = 0.80) [32].

We assigned population percentiles to TG/HDL-C levels and divided our population into 25 groups, corresponding to a 4-percentile increment between each group. Within these groups, we examined several lipid parameters, including RLP-C and its components, TG, non-HDL-C, directly-measured LDL-C, LDLr-C (real LDL, defined as: LDL-C – Lp(a) – IDL-C) and LLDR (Table S1). Additionally, we classified the study population by TG/HDL-C quintiles and examined lipid parameters by TG levels (<150, 150–400 and >400). TG-related variables are known to deviate significantly from a normal distribution; therefore these values are reported as medians (25th–75th percentiles).

After selecting three proposed cut-off points from the literature [6,21,22], we identified those individuals whose TG/HDL-C ratio were above and below those cut-off points, and described lipid parameters for each subgroup. Additionally, we proposed two cut-off points to define high RLP-C levels: ≥75th and ≥90th percentiles, corresponding to 32 and 41 mg/dL respectively. Receiver-operating characteristic (ROC) curves were obtained at 50 TG/HDL-C quintiles. Diagnostic performance was ascertained from the area under the ROC curve (AUC). Optimal sensitivity and specificity were determined by the Youden index.

We evaluated correlations between TG/HDL-C ratio and lipid parameters by using Spearman’s rank correlation coefficient in our
overall study population, and by subgroups classified according to TG/HDL-C ratio cut-off points. We performed a multiple linear regression with log-transformed TG/HDL-C ratio as outcome, and log-transformed (VLDL_{1,2}-C), log-transformed (RLP-C), LDL-C, log-transformed [Lp(a)-C], LDL density, age, and sex as covariates. Statistical analyses were performed in STATA Version 12.0 (College Station, Texas). Logarithmically scaled pseudocolor encoded data density plot were generated in R (http://r-project.org), version 3.0.1 to evaluate concordance between TG/HDL-C ratio and RLP-C.

3. Results

3.1. Study sample characteristics

The study included 1,350,908 subjects with a mean age of 58.7 (48% male). Median values in overall population were: TG/HDL-C ratio, 2.2; IDL-C, 12 mg/dL; VLDL_{3}-C, 13 mg/dL; RLP-C, 24 mg/dL; and non-HDL-C, 133 mg/dL.

3.2. TG/HDL-C ratio population percentiles

We found median values for RLP-C of 17 and 34 mg/dL, and non-HDL-C of 119 and 151 mg/dL in subpopulations of the TG/HDL-C ratio below 25th and above 75th percentiles, respectively. LDL density and non-HDL-C showed a clear increasing trend towards higher TG/HDL-C ratio percentiles, whereas Lp(a)-C showed a decreasing trend (Table 1). A full table with 100 percentiles is provided as an Online Appendix (Table S2). After further stratifying our data by triglyceride levels in TG/HDL-C quintiles, we found that increasing levels of RLP-C and its components (VLDL_{3}-C and IDL-C) were seen with higher triglyceride levels (Table 2).

3.3. TG/HDL-C ratio cut-off points

We found that 49.9%, 28.6% and 26.9% of the overall population had TG/HDL-C ratio levels above those cut-off points proposed by Miller [2.5 (M) and 2 (F)] [6], ATP-III [3.75 (M) and 3 (F)] [21] and McLaughlin [3.5 (M and F)] [22,23]. Among individuals with TG/HDL-C ratios above these cut-off points, proportions of men were higher (50.9%, 52.2%, and 61.6%, respectively), compared with women. Age tended to be lower in those above compared to below cut-off points. We found that individuals in the higher TG/HDL-C groups had higher levels of RLP-C and its components as well as non-HDL-C. LDL-C levels (both “real” and directly-measured) showed only small relative differences between the groups, though LDL density levels were two-fold higher in the groups with higher TG/HDL-C (Table 4).

Diagnostic performance of TG/HDL-C ratio quantiles as determined by AUC was 82% and 85% for ≥75th and ≥90th RLP-C percentile, respectively. The optimal threshold for discriminating high RLP-C levels using TG/HDL-C ratio was 2.62 and 3.07 for ≥75th and ≥90th percentiles, respectively (Online Appendix: Figures S1 and S2).

3.4. TG/HDL-C ratio correlations

The modest concordance of TG/HDL-C ratio and RLP-C is represented visually in Fig. 1A. We found a graphically lower concordance between TG/HDL-C and IDL-C (Fig. 1B), compared with VLDL_{3}-C (Fig. 1C). Additionally, there was clear discordance in TG/HDL-C vs. non-HDL-C (Fig. 1D) and LDL-C (Fig. 1E).

Correlations between TG/HDL-C and TC, TRIG, non-HDL-C and LDL density were 0.9274 (86% of variance), 0.6814 (~47% of variance), 0.2898 (~9% of variance) and 0.3434 (~8% of variance), respectively. Higher correlation was found with VLDL_{1,2}-C
component (0.8622, ~75% of variance), and the VLDL-C subcomponent (0.8243, ~66% of variance), compared with IDL-C (0.5373, ~30% of variance). All correlations found were statistically significant (p < 0.0001) (Table 5) and consistent across age and sex (Table S3 A-C and Table S4 A-B). Correlations between TG/HDL-C ratio and RLP-C were lower among individuals with TG/HDL-C ratio levels above compared with below all of the cut-off points (Table S5). A multivariable linear regression model showed that,
Fig. 1. A. TG/HDL-C vs. RLP-C percentiles. B. TG/HDL-C vs. IDL percentiles. C. TG/HDL-C vs. VLDL₃-C percentiles. D. TG/HDL-C vs. Non-HDL-C. E. TG/HDL-C vs. LDL-C (directly measured).
based on t values, log-transformed (VLDL₁₂-C) had the strongest association with TG/HDL-C ratio, followed by LDLr-C (Table S6).

4. Discussion

In the present analysis, a higher TG/HDL-C ratio was associated with a more atherogenic lipid phenotype, characterized by higher levels of non-HDL-C, LDL density, and especially RLP-C and its components (VLDL₂-C and IDL-C). This finding remained consistent when using TG/HDL-C ratio cut-off points. Compared to other lipid parameters, RLP-C and LDL density were noticeably higher in the groups above TG/HDL-C ratio cut-off points.

4.1. TG/HDL-C ratio and RLP-C

A number of studies have suggested that RLP-C is associated with coronary artery disease (CAD) [33], even after adjusting for other cholesterol measures such as total cholesterol, HDL-C, triglycerides, and LDL-C [34–37]. In our study population, increasing RLP-C was associated with increasing TG/HDL-C. However, this increase was mostly driven by TG rather than HDL-C levels, as seen in Tables 2 and 3. The median RLP-C of individuals above the three TG/HDL-C cut-off points evaluated in this study was 50–57% higher than those below these cut-off points. Only ~47% of variance in TG/HDL-C ratio was explained by RLP-C; however, RLP-C was more closely correlated with TG/HDL-C than non-HDL-C, LDL density, and LDL-C. It is also important to recognize that correlation between RLP-C and TG was stronger than with TG/HDL-C ratio. We hypothesize that the association between increased TG/HDL-C ratio and the development of ASCVD may be explained in part by increased RLP-C levels. RLP-C consists of the cholesterol carried in dense subfractions of VLDL (VLDL₂-C) – which explained ~2/3 of variance in TG/HDL-C ratio in this study – and IDL-C [30]. These remnant particles have been associated with development of atherosclerosis by a number of molecular mechanisms including smooth muscle cell proliferation, macrophage-derived foam cell formation, modulation of monocyte–endothelial interactions, inhibition of endothelial progenitor cell development, and endothelial dysfunction, and the augmentation of systemic inflammation [28,29,38,39]. Additional evidence suggests that RLP-C levels, although measured by other techniques than VAP test, may be more strongly correlated with severity of coronary atherosclerosis than LDL-C, and in one study, RLP-C was superior to non-HDL-C in predicting ASCVD events in patients with known CAD and LDL-C <100 mg/dL [40,41].

4.2. TG/HDL-C ratio and other lipid measures

We found only a modest increasing trend in both “real” and directly measured LDL-C levels with increased TG/HDL-C ratio. Notably, we found a much more marked increase in LDL density. In hypertriglyceridemic settings, triglyceride-enriched LDL particles undergo hydrolysis via lipoprotein lipase or hepatic lipase, reducing LDL particle size. Therefore, TG/HDL-C ratio is a strong predictor of the presence of small dense LDL particles [18,22,42–46], which have been shown to be highly atherogenic [47].

Higher non-HDL-C levels were also associated with increased TG/HDL-C ratio levels, although both correlation and concordance were relatively weak. Non-HDL-C is composed of LDL-C and VLDL-C; while LDL-C levels slightly increased towards higher TG/HDL-C ratio levels, increment in VLDL-C levels was much more marked (Table 1). These findings may be explained by the higher concentration of VLDL-C present in hypertriglyceridemic settings [48], compared with normotriglyceridemic settings.

In this study we also measured Lp(a)-C, an additional risk factor for CVD [49,50]. Interestingly, TG/HDL-C and Lp(a)-C were shown to have an inverse relationship (ρ = −0.3243) and similarly Lp(a)-C levels were lower in individuals with levels of TG/HDL-C ratio above cut-off points. Although prior studies have shown an association between Lp(a) and risk of type 2 diabetes mellitus [51,52], recent evidence suggests that Lp(a) levels decrease in response to hyperinsulinemia [53,54], and that there is a negative relationship between Lp(a) and incidence of type 2 diabetes [55,56]. A previous study suggests that Lp(a), TG and HDL may have a shared metabolic pathway for hepatic clearance of cholesterol resident on these lipoprotein species [57], which may be supported by recent findings that both Lp(a) and HDL bind to the SR-B1 receptor, suggesting a common pathway for hepatic clearance of cholesterol resident on these lipoprotein species [58].

4.3. TG/HDL-C ratio cut-off points

Relative differences in RLP-C and its components, non-HDL-C and LDL density between above vs. below cut-off points were similar between all cut-off points evaluated. Among lipid parameters, the highest increments were found in LDL density (100%), as well as RLP-C (~50%) and its components; however, LDL-C and non-HDL-C did not show marked increments. These findings show that TG/HDL-C ratio cut-off points may reflect atherogenic risk mediated by high RLP-C and high LDL density. Furthermore, in this study, high RLP-C levels were better identified by TG/HDL-C ratios of 2.62 and 3.07 for RLP-C ≥75th and ≥90th percentiles, respectively. However, more evidence evaluating clinical outcomes is needed in order to better assess the clinical impact these specific TG/HDL-C ratios may have.

4.4. Study limitations

The limitations of this study include the use of a single time point for lipid data collection. Besides age and sex, we did not have access to data on extended demographics such as race or biometric data, past medical history, or use of lipid-modifying therapies. Since we do not have clinical outcomes data, such as cardiovascular events or mortality, it is not possible for us to determine which TG/HDL-C cut-off point performs better in classifying populations into...
high or low risk of developing a CV event. Additionally, VAP testing was used for our analysis and may differ from the subfractionation methods of other studies [59,60].

5. Conclusion

In the present analysis, a higher TG/HDL-C ratio was associated with an increasingly atherogenic lipid phenotype, characterized by primarily higher RLP-C and LDL density. Previously proposed TG/ HDL-C ratio cut-off points discriminate among individuals with significantly elevated serum levels of RLP-C and LDL density, which may be the main mediators of cardiovascular risk conferred by high levels of TG/HDL-C ratio.

Conflict of interest

Dr. Blaha served on an Advisory Board for Pfizer. Dr. Toth reports consulting for Amgen, AstraZeneca, Atherotech, GlaxoSmithKline, Kowa, Liposcience, and Merck; serving on the speakers bureau for Amgen; grants from the University of Alabama at Birmingham. Dr. Nasir serves on an Advisory Board for Quest Diagnostic. Dr. Jones reports consulting on the medical advisory board for, and receiving grant funding from, Atherotech. Drs. Quispe, Manalac, Faridi, Virani, Banach, Blumenthal, and Martin have nothing to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.06.057.

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