Original Article

Atherogenic Lipoprotein Subfractions and Carotid Atherosclerosis in Menopausal Women

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Arcangelo Iannuzzi, MD[1], Marco Gentile, PhD[2], Gabriella Iannuzzo, MD[2], Giuseppe Covetti, MD[1], Camilla Panico, MD[2], Amalia Mattiello, MD[2], Ersilia La Fata, MD[2], Lanfranco D'Elia, MD[2], Mario De Michele, MD[3], and Paolo Rubba, MD[2]

Abstract

The aim of the study was to evaluate the relationship between cholesterol contained in very-low-density lipoproteins (VLDL-C), intermediate-density lipoproteins (IDL-C), low-density lipoproteins, high-density lipoproteins, and carotid intima–media thickness (cIMT) and carotid plaques in 228 postmenopausal women (63.1 $\pm$ 8.2 years) who participated in the ATENA Project and underwent clinical, biochemical (including the assay of lipoproteins using the Lipoprint system), and carotid ultrasound tests. Very-low-density lipoprotein cholesterol had a statistically significant linear association with cIMT ($P < .001$), which remained significant after adjustment for age, smoking, systolic blood pressure, glucose, and body mass index ($r^2 = .20$, $P < .05$). Higher concentrations of IDL-C and cholesterol contained in triglyceride-rich lipoproteins (TRL-C, ie, VLDL-C + IDL-C) were associated with plaques in the common carotid (tertile III/tertile I: odds ratio [OR] = 2.52, 95% confidence interval [CI] = 1.21-5.32, $P < .02$; OR = 2.30, 95% CI = 1.05-5.01, $P < .05$, respectively), after adjustment for main cardiovascular risk factors. In conclusion, high concentrations of VLDL-C and TRL-C are independently associated with the presence of carotid plaques. Their assay represents a useful tool for improving our knowledge on the role of different classes of lipoproteins in atherosclerosis.

Keywords

lipoprotein subclass, atherosclerosis, carotid, women, menopause

Introduction

Plasma cholesterol is now established as a risk factor for cardiovascular disease (CVD).[1] A reduction in total cholesterol and low-density lipoprotein cholesterol (LDL-C) is associated with a lower risk of both coronary heart disease and stroke.[2,3] Nevertheless, patients who undergo cholesterol-lowering treatment and reach a specified LDL-C target are still at risk of mortality and morbidity due to CVD (residual risk).[4] A role is played by other known CVD risk factors (eg, diabetes, arterial hypertension, cigarette smoking, and obesity). Nevertheless, it is likely that other lipids, in addition to LDL-C, also play a role in determining this residual risk.

Epidemiological evidence from univariate analyses has highlighted the importance of triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) as risk factors for CVD.[5,6] However, in other studies, TG and HDL-C lost their role as "independent" risk factors owing to the correction for other cardiovascular risk factors (in particular body mass index [BMI] and hyperglycemia) and also since there is a strong inverse association between these 2 lipid variables. Moreover, several trials failed to demonstrate any clinical advantage of increasing HDL-C or reducing TG.[7,8]

Many expert guidelines and other studies have recognized apolipoprotein B (apoB) and non-HDL-C levels as better predictors of cardiovascular risk compared with LDL-C, particularly among postmenopausal women.[9,10] The excess risk of CVD and atherosclerosis captured by apoB or non-HDL-C suggests a need for more precision when measuring TG-rich lipoproteins (TRLs) levels and their association with ultrasound markers of atherosclerosis. In the fasting state, TRLs consist of very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) concentrations and could be estimated with advanced lipoprotein testing.

[1] Department of Medicine and Medical Specialties, Antonio Cardarelli Hospital, Naples, Italy
[2] Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy
[3] Division of Cardiology, Moscati Hospital, Aversa, Italy

Corresponding Author:
Arcangelo Iannuzzi, Department of Medicine and Medical Specialties, Antonio Cardarelli Hospital, Naples, Italy.
Email: lelliann@libero.it

In a recent study, a fraction of VLDL cholesterol ($VLDL_3$-C) plus IDL-C concentrations were associated with a risk of incident coronary heart disease in 2 diverse, prospective, longitudinal observational US cohorts (Jackson Heart Study and Framingham Offspring Cohort Study).[11]

The Lipoprint system is a method based on the prestaining of serum lipid fractions with Sudan black and on the subsequent 3% nondenaturing polyacrylamide gel gradient electrophoresis.[12,13] It provides information on the distribution of lipoproteins and measures the levels of esterified cholesterol in each fraction. This method separates up to 12 lipoprotein fractions (1 VLDL, 3 IDLs, 7 LDLs, and 1 HDL) of cholesterol of decreasing size and increasing density and electrophoretic mobility. The inter- and intra-assay coefficients of variation for the 12 subfractions were <10%.

Carotid intima–media thickness (cIMT) is the most studied and used surrogate marker for systemic atherosclerosis. The cIMT is a predictor of cardiovascular events, and an increase in cIMT in the common carotid (CC) artery is associated with an increased risk of stroke and acute myocardial infarction.[14-16]

Compared with premenopausal women, postmenopausal women have a more atherogenic lipid profile, with higher concentrations of total cholesterol, LDL-C, and TG and lower HDL_2-C.[17-19] In addition to these major lipid abnormalities, modifications in size and density of the lipoproteins happen after menopause. Small and dense LDLs (sdLDLs) are more frequent in postmenopausal women, while larger and buoyant LDLs are decreased.[20] The lack of estrogen after menopause could contribute to hypertriglyceridemia, low HDL-C, and a predominance of sdLDLs.[21] These lipid changes after menopause could partly explain the increased cardiovascular risk in postmenopausal women.[22]

The objective of this study was to evaluate the relationship between cholesterol contained in VLDL, IDL, LDL, and HDL, calculated using the Lipoprint method, and cIMT and carotid plaques in women of postmenopausal age.

Methods

The Progetto ATENA (ATENA project) is an epidemiological investigation with the aim of studying the environmental, biological, and genetic causes of the major chronic degenerative diseases in women.[23] Between 1993 and 1996, a total of 5062 women, aged between 30 and 69 years, and free of any known diseases, participated in the study. Approximately 400 of these women were randomly selected from the City of Naples' registry lists. The remaining women responded voluntarily to the call made through information campaigns. All participants signed a specific informed consent form. The study was approved by the ethics committee of the institutions involved. Approximately 10 years since the first visit, 228 women, randomly chosen from the eldest and all postmenopausal, were recalled and underwent clinical, biochemical, and ultrasound tests. Our study is based on the data deriving from these investigations including the assay of lipoproteins using the Lipoprint system.

The BMI was used as an obesity indicator. Systolic blood pressure (SBP) and diastolic blood pressure at rest were measured 2 times after an interval of $\geq$5 minutes using a random zero sphygmomanometer, and the average of those measurements was used in statistical analyses. A standard questionnaire was used to gather information regarding smoking habits. Eight women were taking hormone therapy at the time of the visit. Twenty-six individuals were taking lipid-lowering drugs.

Blood samples were collected after 12 to 14 hours of fasting, from 8:00 to 9:30 in the morning, and biochemical analyses were performed on fresh blood samples (serum), whereas measurements of the cholesterol content in each lipoprotein subfractions were performed with the Lipoprint system (Quantimetrix Inc, Redondo Beach, California) on frozen serum samples (-80°C) within 5 years from blood collection. The concentrations of total cholesterol and TG were measured using standard enzymatic methods. High-density lipoprotein cholesterol was measured after a precipitation of very-low-density and of low-density lipoproteins with phosphotungstic acid, and LDL-C was calculated using the Friedewald equation.[24] Non-HDL cholesterol was calculated as total cholesterol − HDL-C. Fasting glucose levels were determined enzymatically with the peroxidase method. Fasting insulin levels were determined using an immunoenzymatic assay (Ultrasensitive Insulin Elisa; Mercodia, Uppsala, Sweden). Both ApoB and high-sensitivity C-reactive protein were measured using an automated turbidimetric method (Cobas-Mira, Roche, Italy). The insulin resistance index (homeostatic model assessment) was calculated using the formula by Matthews et al.[25]

The high-resolution B-mode carotid ultrasound examination was performed by an expert sonographer using a Esaote AU4 (Biosound Esaote, Inc, Indianapolis, Indiana). The sonographer was blinded to the participants' biomarker data. Measurements were taken on the anterior and posterior wall of the distal segment (equal to 1 cm) of the CC artery on both sides. At each examination, the sonographer used different scanning angles (anterior, lateral, and posterior) to identify the maximum thickness of the cIMT for each wall, following a standardized protocol. The images were recorded for subsequent analyses.[26] The CC T-max was defined as the maximum cIMT value measured between all CC walls on each side (single value). Differently from other measures of cIMT, CC T-max better reflects the severity than the extension of the arterial disease. In particular, in this study, a CC T-max value $\geq$1.2 mm (CC T-max median) in addition to luminal protrusion of 50% in relation to the neighboring walls was considered as CC plaque. It was possible to take IMT measurements for the CC in all of the 228 participants recruited for the study. Continuously monitored quality control data indicated a coefficient of reproducibility for IMT of the CC of 0.85. This parameter includes the variability of the medical equipment, sonographer, and reader.[27]

Statistical Analysis

This was carried out using version 19.0 of the SPSS program (SPSS, Inc, Chicago, Illinois). The continuous variables were

Table 1. Clinical Characteristics of Participants Without Plaques (n = 129) and With Plaques (n = 99).[a]

Variable	Women Without Plaques	Women With Plaques	P
Age, years, range	60.8 (8.1), 43-78	66.2 (7.2), 48-810	<.001
BMI (kg/m^2)	27.9 (4.6)	28.5 (4.7)	.36
Total cholesterol (mg/dL)	217 (38)	233 (38)	.001
Triglycerides (mg/dL)	103 (53)	122 (61)	.015
HDL-C (mg/dL)	58 (13)	57 (13)	.35
LDL-C (mg/dL)	138 (33)	152 (34)	.002
Apo-B (mg/dL)	1.1 (0.2)	1.2 (0.2)	.001
Non-HDL-C (mg/dL)	158 (38)	177 (40)	.001
Glucose (mg/dL)	103 (18)	109 (32)	.12
HOMA	1.7 (1.2)	1.9 (1.4)	.20
SBP (mm Hg)	139 (20)	149 (22)	<.001
DBP (mm Hg)	81 (9)	82 (8)	.44
Ln-CRP (units)	0.4 (1.1)	0.4 (1.0)	0.62
Smoking, n (%)			
Current	43 (33)	35 (35)	.94
Ex	26 (20)	20 (20)	
Never	60 (47)	44 (44)	

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; Ln-CRP, logarithm of high-sensitivity C-reactive protein; SBP, systolic blood pressure; SD, standard deviation.
[a]Values are means (SD). Smoking is expressed as absolute numbers and percentage (in brackets).

described as mean and standard deviation. Linear regression analysis was used to compare the association among cIMT and lipoprotein subfractions (continuous variables). Subsequently, the HDL-C, LDL-C, IDL-C, and VLDL-C values measured with the Lipoprint system were stratified by tertiles, and logistic regression analyses were used to test the relationship between the tertiles of these lipid parameters and the presence of plaque in the CC. Main cardiovascular risk factors, such as age, smoking, blood pressure, glycaemia, HDL-C, and BMI, were added as covariates. A 2-tailed $P < .05$ was considered significant.

Results

The demographic, clinical, and biochemical characteristics of the studied women are shown in Table 1. It should be remembered that the Friedewald calculation of LDL-C (Table 1) is not only an indirect value but also includes lipoprotein(a) cholesterol and IDL-C values.

Women with carotid plaques were older and had higher concentrations of total cholesterol, TG, apoB, non-HDL-C, and SBP. In a linear regression analysis, VLDL-C (Pearson $r = .26$, $P < .001$), IDL-C ($r = .16$, $P = .021$), VLDL-C + IDL-C ($r = .25$, $P < .001$), and LDL-C ($r = .14$, $P = .036$) had a positive and significant correlation with the continuous variable CC T-max. The association was confirmed for VLDL-C after correction for the principal confounding cardiovascular risk factors (Table 2).

Table 2. Association Between Cholesterol Contained in the Various Lipoprotein Fractions (Lipoprint) and CC T-Max After Adjustment for the Main CV Risk Factors (Linear Regression).[a]

Variable	β	95% CI	P
HDL-C	−.00154	−0.00491 to 0.00183	.37
LDL-C	.00155	−0.00033 to 0.00342	.11
IDL-C	.00138	−0.00154 to 0.00431	.35
VLDL-C	.00336	0.00010 to 0.00662	.043
TRL-C	.00176	−0.00016 to 0.00367	.072

Abbreviations: CC T-max, maximum intima–media thickness measured as a continuous variable between all the common carotid artery walls; CI, confidence interval; CV, cardiovascular; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TRL-C, triglyceride-rich lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol.
[a]Adjusted for age, systolic blood pressure (SBP), glucose, smoking, and body mass index (BMI).

Table 3. Lipoprint Assay of Cholesterol (mg/dL) Contained in Different Lipoprotein Subfractions.[a]

Variable	Mean (SD)	I Tertile	II Tertile	III Tertile
VLDL, mg/dL	29.1 (13.7)	<22	22-31	>31
IDL (C), mg/dL	19.3 (6.2)	<17	17-21	>21
IDL (B), mg/dL	16.4 (7.0)	<14	14-18	>18
IDL (A), mg/dL	21.2 (7.3)	<18	18-23	>23
Total IDL (C + B + A), mg/dL	56.8 (14.4)	<50	50-62	>62
VLDL + IDL, mg/dL	85.9 (23.0)	<75	75-92	>92
Large LDL (1), mg/dL	47.9 (14.4)	<41	41-53	>53
Intermediate LDL (2), mg/dL	25.7 (12.8)	<19	19-32	>32
Small LDL (3-7), mg/dL	3.4 (6.8)	0	0-3	>3
Total LDL (1-7), mg/dL	77.1 (22.6)	<67	67-87	>87
HDL, mg/dL	60.9 (13.5)	<54	54-65	>65

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; IDL (C, B, A), progressively smaller intermediate-density lipoproteins; LDL, low-density lipoproteins; LDL (1, 2, 3-7), progressively smaller low-density lipoproteins; SD, standard deviation; VLDL, very-low-density lipoproteins.
[a]n = 228.

The results of the Lipoprint assay of cholesterol contained in different lipoprotein subfractions are shown in Table 3.[28] The LDL subfraction 1 indicates large LDL particles, 2 represents intermediate, and 3 to 7 means small. In univariate analysis, there was a correlation between the cholesterol contained in TRLs and sdLDLs ($r = .44$, $r^2 = .20$, $P < .001$).

In a logistic regression analysis, higher tertiles of VLDL-C, IDL-C, VLDL-C + IDL-C, and LDL-C were associated with the presence of carotid plaques. After adjustment for the main cardiovascular risk factors, the highest tertile of IDL-C (odds ratio [OR]: 2.52, 95% confidence interval [CI]: 1.24-5.32, $P < .02$) and TRL-C (OR: 2.30, 95% CI: 1.05-5.01, $P < .05$) proved to be significantly correlated with the presence of plaques in the CC artery (Table 4). These results did not substantially change when we added sdLDL concentration in the model: Highest tertiles of IDL-C and TRL-C showed an association with

Table 4. Association Between Tertiles of Lipoprotein Subfractions and Carotid Plaques After Adjustment for the Main CV Risk Factors (Logistic Regression).[a]

	Tertile II (n = 78) vs Tertile I (Reference; n = 73)			Tertile III (n = 77) vs Tertile I (Reference; n = 73)		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
HDL-C	1.41	0.89-3.01	.37	0.83	0.38-1.81	.63
LDL-C	1.99	0.92-4.27	.29	2.11	0.97-4.91	.13
IDL-C	1.29	0.60-2.76	.51	2.52	1.21-5.32	.014
VLDL-C	1.57	0.74-3.33	.19	1.64	0.75-3.57	.26
TRL-C	1.88	0.86-4.11	.08	2.30	1.05-5.01	.037

Abbreviations: CI, confidence interval; CV, cardiovascular; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TRL-C, triglyceride-rich lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol.
[a]Adjusted for age, systolic blood pressure (SBP), glucose, smoking, and body mass index (BMI).

carotid plaques (OR: 2.66, 95% CI: 1.26-5.62, $P < .02$; OR: 2.50, 95% CI: 1.12-5.56, $P < .05$, respectively).

Discussion

The present study demonstrated a linear, statistically significant and positive association between the cholesterol of the VLDL lipoproteins and cIMT, after adjustment for the main CV risk factors. High concentrations of cholesterol contained in IDLs and TRLs were associated with plaques in the CC artery even after correction for the main risk factors for CVD.

A sizeable proportion of CV events occurs in participants who have LDL-C levels in the range of normal values.[29] It has been hypothesized that some of this increased risk is due to other atherogenic lipoproteins (VLDL particles and IDL) or their subfractions.[30] A variety of lipoprotein assays have been developed that subfractionate lipoprotein particles according to their size, density, or charge. The number and nomenclature of lipoprotein subfractions are not uniform across the different techniques and have not been standardized, making it difficult to compare results from various tests.[31]

In the JUPITER trial, baseline LDL-C was not associated with CVD events, in contrast with significant associations for atherogenic particles and subfractions of VLDL and LDL particles.[32] Several lines of evidence demonstrate a significant association of sdLDLs with increased CVD risk.[33,34]

There are few published studies on the association between cholesterol of different lipoprotein fractions and cIMT. The present article focuses on the relationship between TRLs concentration and cIMT; however, our group demonstrated, in the same patients enrolled in the present study, a significant association between sdLDLs, measured using the Lipoprint system, and the cIMT. This association was maintained even after adjustment for age and apoB.[35] The LDL subclass phenotypes may result from interaction of multiple genetic and environmental determinants. In view of the close relationship of change in plasma TG, apoB, VLDL, and IDL concentrations with change in LDL particle size and with generation of sdLDLs, it is likely that both genetic and nongenetic factors could involve effects on metabolism of plasma TRLs and sdLDLs. In the present study, there was a significant correlation between the cholesterol contained in TRLs and sdLDLs. However, the association between carotid plaques and highest tertiles of TRLs did not substantially change after adjustment for sdLDL concentration.

In a Japanese study conducted among apparently healthy men, the only lipoprotein fractions (measured using High Performance Liquid Chromatography) associated with cIMT were the "small" VLDL-C and the "large" and "very small" LDL-C. No association was present for HDL-C.[36] In a study on 156 men in apparent good health, conducted in Milan, the concentrations of cholesterol in TRLs and sdLDLs proved to be independent predictors of cIMT and were associated with monocytic and endothelial pro-inflammatory activation.[37]

In the MARS study, IDLs were the only lipoproteins found to be significantly correlated with cIMT progression.[38] In a recent study, atherogenic lipoprotein particle concentrations were associated with CVD risk when LDL-C was low. Among individuals with the lowest LDL-C on statin therapy, the smallest subclass of VLDL was strongly associated with residual risk, and this risk was related to the cholesterol carried in VLDL.[39] It seems plausible that the cholesterol content of VLDLs was causal for atherosclerosis development, by accumulation in the arterial wall. Finally, in a study conducted, like ours, among postmenopausal women, the calcium content of the coronary arteries was associated with VLDLs and sdLDLs, but not with LDLs, and these results were confirmed even after adjustment for age, smoking, arterial pressure, TG, and HDL-C.[40] Therefore, in the studies in which the different classes of lipoproteins were measured and not calculated, the lipoprotein fractions most often associated with atherosclerosis were VLDLs, IDLs, and sdLDLs and not LDLs or HDLs.

The limitations of our study need to be acknowledged. The study population consists of a relatively small number of postmenopausal women. The cross-sectional nature of the current findings does not allow inference of causality. Additional studies are needed to understand prospectively the role of specific lipoprotein subfractions in the development of carotid atherosclerosis in postmenopausal women.

In conclusion, the present cross-sectional study suggests that in menopausal women the concentrations of VLDL-C are

linearly and directly correlated with cIMT, independently of the main risk factors for CVD and that high concentrations of IDL-C and TRL-C are independently associated with the presence of carotid plaques. Their assay therefore represents a useful tool for improving our knowledge on the role of specific lipoprotein subfractions in the development of carotid atherosclerosis in postmenopausal women.

Authors' Note

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

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ORCID iD

Arcangelo Iannuzzi http://orcid.org/0000-0001-6586-8597

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