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Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors

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ABSTRACT

BACKGROUND Absence of cardiovascular risk factors (CVRFs) is traditionally considered low risk for atherosclerosis; however, individuals without CVRFs, as currently defined, still have events.

OBJECTIVES This study sought to identify predictors of subclinical atherosclerosis in CVRF-free individuals.

METHODS Participants from the PESA (Progression of Early Subclinical Atherosclerosis) study (n = 4,184) without conventional CVRFs were evaluated (n = 1,779; 45.0 ± 4.1 years, 50.3% women). CVRF freedom was defined as no current smoking and untreated blood pressure <140/90 mm Hg, fasting glucose <126 mg/dl, total cholesterol <240 mg/dl, low-density lipoprotein cholesterol (LDL-C) <160 mg/dl, and high-density lipoprotein cholesterol ≥40 mg/dl. A subgroup with optimal CVRFs (n = 740) was also defined as having blood pressure <120/80 mm Hg, fasting glucose <100 mg/dl, glycosylated hemoglobin <5.7%, and total cholesterol <200 mg/dl. We evaluated ultrasound-detected carotid, iliofemoral, and abdominal aortic plaques; coronary artery calcification; serum biomarkers; and lifestyle. Adjusted odds ratios (with 95% confidence interval) and ordinal logistic regression models were used.

RESULTS Subclinical atherosclerosis (plaque or coronary artery calcification) was present in 49.7% of CVRF-free participants. Together with male sex and age, LDL-C was independently associated with atherosclerosis presence and extent, in both the CVRF-free and CVRF-optimal groups (odds ratio [×10 mg/dl]: 1.14 to 1.18; $p < 0.01$ for all). Atherosclerosis presence and extent was also associated in the CVRF-free group with glycosylated hemoglobin levels.

CONCLUSIONS Many CVRF-free middle-aged individuals have atherosclerosis. LDL-C, even at levels currently considered normal, is independently associated with the presence and extent of early systemic atherosclerosis in the absence of major CVRFs. These findings support more effective LDL-C lowering for primordial prevention, even in individuals conventionally considered at optimal risk. (Progression of Early Subclinical Atherosclerosis [PESA] Study; NCT01410318) (J Am Coll Cardiol 2017;70:2979-91) © 2017 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

CAC = coronary artery calcification

CVRF = cardiovascular risk factor

eGFR = estimated glomerular filtration rate

HbA_{1c} = glycosylated hemoglobin

hs-CRP = high-sensitivity C-reactive protein

HDL-C = high-density lipoprotein cholesterol

LDL-C = low-density lipoprotein cholesterol

VCAM = vascular cell adhesion molecule

Cardiovascular risk in asymptomatic individuals is assessed on the basis of conventional cardiovascular risk factors (CVRFs), and most cardiovascular events are linked to elevated CVRFs (1). However, atherosclerosis and cardiovascular events are common even among individuals with a low CVRF burden (2–4). This is especially the case among younger adults and women, who can experience cardiovascular events despite being considered at low short-term risk (5–7). According to current preventive recommendations (8), healthy individuals without CVRFs (as presently defined) are usually not considered a target for prevention strategies despite the possible presence of atherosclerosis.

Subclinical atherosclerosis underlies most cardiovascular events, and its detection can improve risk stratification (9,10). However, a mismatch has been reported between low conventional risk and the presence of subclinical atherosclerosis detected by coronary artery calcification (CAC) or carotid ultrasound (11,12). Our group identified subclinical atherosclerosis in nearly 60% of middle-aged individuals classified at low risk according to traditional risk scales, with multiple vascular sites affected in 41% (13). These findings demonstrate a disparity between conventional CVRFs and the presence of atherosclerosis, suggesting that other factors play a role in atherogenesis. In this study, we aimed to explore and identify potential predictors of the presence and multiterritorial extent of subclinical atherosclerosis in the absence of major CVRFs.

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METHODS

STUDY DESIGN. This study was conducted in a subset of individuals from the PESA (Progression of Early Subclinical Atherosclerosis) study (13–15) with CVRF levels below current thresholds. The PESA study uses noninvasive imaging to prospectively evaluate the presence and progression of subclinical atherosclerosis in a middle-aged population of 4,184 adults aged between 40 and 54 years. The main exclusion criteria were known cardiovascular disease, active treatment for cancer, or any disease expected to decrease life expectancy or protocol adherence. Participants underwent clinical interviews, physical activity and lifestyle evaluations, physical examination, electrocardiogram, laboratory analysis, and imaging studies at baseline, with repeat evaluations scheduled for 3- and 6-year follow-up visits. The study protocol was approved by the Instituto de Salud Carlos III Ethics Committee and all participants provided written informed consent.

DEFINITION OF THE CVRF-FREE POPULATION AND THE CVRF-OPTIMAL SUBGROUP. This study included nonsmokers with no hypertension, diabetes, or dyslipidemia according to Adult Treatment Panel III CVRF definitions (16,17): 1) untreated systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg; 2) untreated fasting plasma glucose <126 mg/dl; 3) untreated total cholesterol <240 mg/dl, low-density lipoprotein cholesterol (LDL-C) <160 mg/dl, and high-density lipoprotein cholesterol (HDL-C) ≥40 mg/dl; and 4) no current smoking status. This subpopulation represents 42.5% of the total PESA study population (**Figure 1**).

Within the conventional CVRF-free population, we also defined a subgroup of individuals with optimal modifiable CVRFs (3,18): systolic blood pressure <120 mm Hg, diastolic blood pressure <80 mm Hg, total cholesterol <200 mg/dl, fasting plasma glucose <100 mg/dl, and glycosylated hemoglobin (HbA_{1c}) <5.7%.

ASSESSMENT OF CVRFs, SERUM BIOMARKERS, AND LIFESTYLE PARAMETERS. CVRFs were prospectively collected through questionnaires (smoking, family history) or objective quantification (hypertension, diabetes, dyslipidemia) as previously described (13). Family history of cardiovascular disease was defined as having a first-degree relative diagnosed with clinical atherosclerosis below 55 years of age in men and 65 years of age in women (16). Obesity was defined as body mass index ≥30 kg/m^2 (2,16). The 10-year risk of atherosclerotic cardiovascular disease was calculated using the Pooled Cohort Equations and cutoffs were defined as <5%, 5% to <7.5%, and ≥7.5% for low, intermediate, and high risk, respectively (19). The 30-year Framingham risk score was also measured and classified as low (<10%), moderate (10% to 20%), or high (<20%) risk (20).

Venous blood was collected after 8 h of fasting and samples were tested for total cholesterol, HDL-C, LDL-C, oxidized LDL-C, triglycerides, lipoprotein (a), glucose, insulin, HbA_{1c}, cystatin C, and creatinine by standard methods (14). LDL-C was calculated by the Friedewald method except for participants with triglycerides >300 mg/dl, where it was measured directly. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation (21). The baseline PESA study protocol also included the following inflammation markers: high-sensitivity C-reactive protein (hs-CRP), fibrinogen, vascular cell

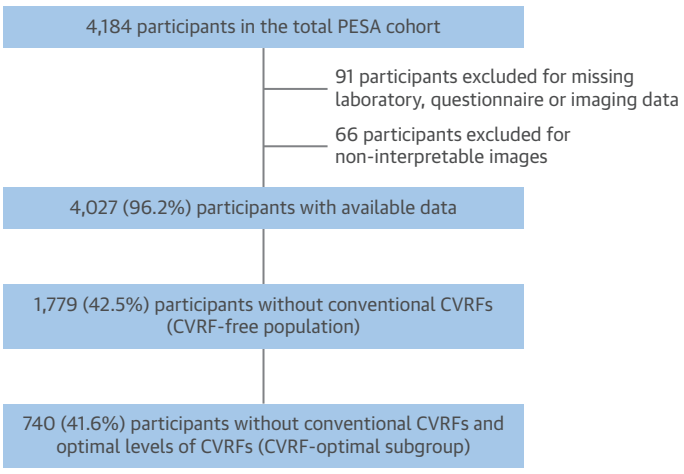
adhesion molecule (VCAM)-1, and P-selectin. Physical activity was assessed by triaxial accelerometry with ActiTrainer accelerometers (ActiGraph, Pensacola, Florida) placed on each participant's waist for 7 consecutive days, including sleep time. Moderate and vigorous physical activity were defined according to standard Troiano cutoffs (22). We also calculated the PREDIMED (PREvencion con DIeta MEDiterranea) score, which reflects increasing adherence to Mediterranean diet (23,24). In addition, 7 ideal cardiovascular health metrics were quantified, as recently proposed (3).

ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS. Two-dimensional vascular ultrasound and noncontrast cardiac computed tomography were performed in all participants as previously described (13). In brief, presence of atherosclerotic plaques by ultrasound was assessed by cross-sectional sweep of carotids, infrarenal abdominal aorta, and iliofemoral arteries. Plaques were defined as focal protrusions into the arterial lumen of thickness >0.5 mm or >50% of the surrounding intima-media thickness, or as a diffuse intima-media thickness >1.5 mm (25). The CAC score was calculated from computed tomography images by the Agatston method (26). All images were analyzed at a central Imaging Core Laboratory by experienced, blinded operators.

Subclinical atherosclerosis was defined as the presence of atherosclerotic plaques by vascular ultrasound or CAC score ≥1. The multiterritorial extent of subclinical atherosclerosis was defined according to the number of vascular sites with evidence of disease, including right carotid, left carotid, abdominal aorta, right iliofemoral, left iliofemoral, and coronary arteries. Participants were classified as disease free (0 vascular sites affected) or having focal (1 site), intermediate (2 to 3 sites), or generalized atherosclerosis (4 to 6 sites) (13).

STATISTICS. The distribution of continuous variables was analyzed using graphical methods. Log transformation was performed before analyses to normalize the distribution as appropriate. Comparisons between participants with and without atherosclerosis were performed using a chi-square test for categorical variables and the Student's *t*-test for continuous variables. Linear trends across groups according to multiterritorial extent were evaluated with an extension of the nonparametric Wilcoxon rank sum test (27). Logistic and ordinal regression models with forward stepwise variable selection were used to analyze the associations of multiple covariates with the presence and extent of atherosclerosis in the CVRF-free and CVRF-optimal groups. Analyses were then repeated with inclusion restricted to participants with LDL-C <130 mg/dl. Candidate variables with a clinical rationale explored in the multivariate analyses included age, sex, body mass index, systolic blood pressure, diastolic blood pressure, family history of premature cardiovascular disease, fasting glucose, insulin, HbA_{1c}, triglycerides, HDL-C, LDL-C, oxidized LDL-C, lipoprotein (a), eGFR, cystatin C, hs-CRP, VCAM-1, P-selectin, and fibrinogen. Weight, height, obesity, total cholesterol, and risk scores were excluded due to multicollinearity, defined as a correlation r ≥0.8 between variables. To better describe the association between the identified independent risk factors and the multiterritorial extent of atherosclerosis, ordinal logistic regression models were replicated after categorizing the index variable into quintiles or 3 groups for age (40 to 44, 45 to 49, and 50 to 54 years of age). Associations were expressed as odds ratio (OR) and standardized OR with 95% confidence interval (CI). Statistical analyses were conducted using Stata version 12 (StataCorp, College Station, Texas). A p value < 0.05 was considered statically significant.

FIGURE 1 Study Population Flowchart



Flow diagram of the selection process of both the cardiovascular risk factor (CVRF)-free and CVRF-optimal groups from the total PESA (Progression of Early Subclinical Atherosclerosis) study population.

RESULTS

CHARACTERIZATION OF THE CVRF-FREE PESA STUDY POPULATION: MISMATCH WITH ATHEROSCLEROSIS. Our study population consisted of 1,779 individuals (50.3% women, 45.0 ± 4.1 years of age), with most in the 40 to 44 years of age subgroup (51.5% vs.

TABLE 1 Baseline Characteristics of the CVRF-Free PESA Study Population Based on Presence of Atherosclerosis

	CVRF-Free Population (n = 1,779)	No Atherosclerosis (n = 899)	Atherosclerosis (n = 880)	p Value
Baseline characteristics				
Age, yrs	45.0 ± 4.1	44.0 ± 3.7	46.0 ± 4.2	<0.001
Male	884 (49.7)	349 (38.8)	535 (60.8)	<0.001
10-yr ASCVD	0.99 (0.50–2.10)	0.73 (0.40–1.50)	1.40 (0.68–2.70)	<0.001
30-yr FHS	0.09 (0.06–0.15)	0.07 (0.05–0.12)	0.12 (0.07–0.17)	<0.001
Family history of CVD*	248 (13.9)	120 (13.3)	128 (14.5)	0.466
Weight, kg	71.8 ± 13.4	69.6 ± 12.9	73.9 ± 13.6	<0.001
Height, cm	169.3 ± 8.7	168.2 ± 8.7	170.3 ± 8.7	<0.001
BMI, kg/m^2	24.9 ± 3.3	24.5 ± 3.3	25.3 ± 3.4	<0.001
Obesity	138 (7.8)	57 (6.3)	81 (9.2)	0.024
SBP, mm Hg	112.6 ± 10.4	110.9 ± 10.3	114.4 ± 10.2	<0.001
DBP, mm Hg	69.8 ± 7.7	68.7 ± 7.5	70.8 ± 7.8	<0.001
Biomarkers				
Fasting glucose, mg/dl	87 (82–93)	86 (81–92)	89 (83–94)	<0.001
HbA_{1c}, %	5.3 (5.1–5.6)	5.3 (5.1–5.5)	5.4 (5.2–5.6)	<0.001
Insulin, μU/ml	4.3 (3.2–6.0)	4.3 (3.2–5.9)	4.4 (3.3–6.2)	0.058
Total cholesterol, mg/dl	190.7 ± 24.0	187.0 ± 24.4	194.6 ± 22.9	<0.001
LDL-C, mg/dl	121.5 ± 21.3	117.4 ± 21.7	125.7 ± 20.1	<0.001
Oxidized LDL-C, mg/dl	46.0 ± 13.4	44.8 ± 12.8	47.2 ± 13.9	<0.001
HDL-C, mg/dl	54.4 ± 10.4	55.4 ± 10.6	53.5 ± 10.1	<0.001
Triglycerides, mg/dl	65 (52–87)	63 (50–83)	68 (53–92)	<0.001
Lipoprotein (a), mg/dl	16.3 (6.4–42.0)	16.2 (6.4–44.5)	16.3 (6.5–38.3)	0.980
eGFR, ml/min/1.73 m^2	100.8 ± 8.9	101.7 ± 8.8	100.0 ± 9.0	<0.001
Cystatin C, mg/l	0.72 ± 0.1	0.71 ± 0.1	0.72 ± 0.1	0.137
hs-CRP, mg/dl	0.08 (0.04–0.15)	0.08 (0.04–0.15)	0.07 (0.04–0.15)	0.459
VCAM-1, ng/ml	619.8 (500.4–760.9)	609.9 (485.9–739.8)	628.5 (512.8–778.7)	0.018
Fibrinogen, mg/dl	258.1 ± 43.0	258.1 ± 42.4	258.1 ± 43.7	0.999
P-selectin, ng/ml	125.2 ± 38.7	122.0 ± 37.7	128.5 ± 39.5	<0.001
Lifestyle				
PREDIMED score†	5.0 ± 1.4	4.9 ± 1.4	5.1 ± 1.4	0.003
MVPA, min/day†	47.4 ± 21.0	46.1 ± 20.9	48.8 ± 21.0	0.007

Values mean ± SD, n (%), or median (interquartile range). *n = 1,749. †n = 1,768.

ASCVD = atherosclerotic cardiovascular disease; BMI = body mass index; CVD = cardiovascular disease; CVRF = cardiovascular risk factor; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; FHS = Framingham Heart Study; HbA_{1c} = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; MVPA = moderate and vigorous physical activity; PREDIMED = PREvencion con DIeta MEDiterranea; SBP = systolic blood pressure; VCAM = vascular cell adhesion molecule.

31.4% and 17.1% in the 45 to 49 and 50 to 54 years of age subgroups, respectively). As expected, the majority of individuals (94.6%) had low 10-year cardiovascular risk; intermediate and high risk were observed in 56 (3.1%) and 10 (0.6%) participants, respectively. The corresponding long-term risk proportions were 54.6%, 35.6% and 9.8%. Online Table 1 shows baseline characteristics of the study population (CVRF free) and of the PESA participants with CVRFs.

Tables 1 and 2 summarize baseline characteristics, serum biomarkers, and lifestyle parameters of study participants stratified according to the presence and extent of atherosclerosis. All CVRFs, risk scores, and lifestyle measurements except for family history differed significantly according to atherosclerosis status. Similarly, significant differences were found in all serum biomarkers except for lipoprotein (a), cystatin C, hs-CRP, and fibrinogen. Ideal cardiovascular health metrics are shown in Online Table 2. As expected in this CVRF-free population, ideal metrics were prevalent, although only 121 (6.8%) participants met all 7 ideal criteria. Significant differences in these health metrics between those with and without atherosclerosis were found for blood pressure, total cholesterol, glucose, and body mass index, but not for smoking, physical activity, and diet.

Despite the absence of conventional CVRFs, subclinical atherosclerosis was highly prevalent (49.7%). Overall, 46.7% had peripheral atherosclerotic plaques: 22.7% in the carotid arteries, 17.2% in the infrarenal aorta, and 30.1% in the iliofemoral arteries,

TABLE 2 Baseline Characteristics of the CVRF-Free PESA Study Population Based on Multiterritorial Extent of Atherosclerosis

	No Disease (n = 899)	Focal (n = 401)	Intermediate (n = 372)	Generalized (n = 107)	p Value for Trend (All)	p Value for Trend (Disease Only)
Baseline characteristics						
Age, yrs	44.0 ± 3.7	44.8 ± 3.9	46.4 ± 4.1	48.6 ± 4.0	<0.001	<0.001
Male	349 (38.8)	226 (56.4)	230 (61.8)	79 (73.8)	<0.001	0.001
10-yr ASCVD*	0.73 (0.40–1.51)	1.05 (0.60–2.04)	1.52 (0.73–2.91)	2.56 (1.27–4.21)	<0.001	<0.001
30-yr FHS	0.07 (0.05–0.12)	0.10 (0.06–0.14)	0.12 (0.08–0.17)	0.16 (0.10–0.22)	<0.001	<0.001
Family history of CVD	120 (13.3)	56 (14.0)	49 (13.2)	23 (21.5)	0.136	0.172
Weight, kg	69.6 ± 12.9	72.8 ± 14.2	73.9 ± 12.9	78.1 ± 13.0	<0.001	<0.001
Height, cm	168.2 ± 8.7	170.0 ± 9.2	170.5 ± 8.5	171.3 ± 7.4	<0.001	0.153
BMI, kg/m^2	24.5 ± 3.3	25.0 ± 3.5	25.3 ± 3.2	26.5 ± 3.2	<0.001	<0.001
Obesity	57 (6.3)	36 (9.0)	29 (7.8)	16 (15.0)	0.011	0.228
SBP, mm Hg	110.9 ± 10.3	113.1 ± 9.9	114.5 ± 10.2	118.7 ± 10.3	<0.001	<0.001
DBP, mm Hg	68.7 ± 7.5	69.9 ± 7.8	71.0 ± 7.7	73.6 ± 7.5	<0.001	<0.001
Biomarkers						
Fasting glucose, mg/dl	86 (81–92)	88 (83–93)	89 (83–94)	91 (86–97)	<0.001	0.001
HbA_{1c}, %	5.3 (5.1–5.5)	5.4 (5.1–5.6)	5.4 (5.2–5.6)	5.4 (5.2–5.7)	<0.001	0.017
Insulin, μU/ml	4.3 (3.2–5.9)	4.3 (3.1–6.1)	4.3 (3.3–5.9)	5.4 (3.7–7.2)	0.007	0.009
Total cholesterol, mg/dl	187.0 ± 24.4	192.1 ± 23.4	195.3 ± 22.6	201.1 ± 20.7	<0.001	<0.001
LDL-C, mg/dl	117.4 ± 21.7	123.3 ± 20.4	126.4 ± 19.9	132.4 ± 17.7	<0.001	<0.001
Oxidized LDL-C, mg/dl	44.8 ± 12.8	45.7 ± 13.3	48.0 ± 14.4	50.3 ± 14.1	<0.001	<0.001
HDL-C, mg/dl	55.4 ± 10.6	54.3 ± 9.8	53.3 ± 10.5	51.3 ± 9.7	<0.001	<0.001
Triglycerides, mg/dl	63 (50–83)	65 (51–87)	70 (54–92)	77 (60–106)	<0.001	<0.001
Lipoprotein (a), mg/dl	16.2 (6.4–44.5)	15.2 (6.4–37.9)	17.4 (6.6–37.1)	16.3 (5.9–43.7)	0.796	0.551
eGFR, ml/min/1.73 m^2	101.7 ± 8.8	100.2 ± 9.5	100.1 ± 8.8	98.7 ± 7.7	<0.001	0.017
Cystatin C, mg/l	0.71 ± 0.1	0.72 ± 0.1	0.72 ± 0.1	0.72 ± 0.1	0.132	0.962
hs-CRP, mg/dl	0.08 (0.04–0.15)	0.08 (0.04–0.14)	0.07 (0.04–0.16)	0.08 (0.05–0.14)	0.828	0.445
VCAM-1, ng/ml	609.9 (485.9–739.8)	622.0 (491.3–760.7)	617.0 (517.1–767.3)	689.0 (565.2–870.8)	<0.001	0.004
Fibrinogen, mg/dl	258.1 ± 42.4	255.8 ± 42.9	260.5 ± 44.3	258.4 ± 44.8	0.333	0.178
P-selectin, ng/ml	122.0 ± 37.7	127.3 ± 39.4	127.8 ± 39.9	135.2 ± 38.0	<0.001	0.165
Lifestyle						
PREDIMED score†	4.9 ± 1.4	5.1 ± 1.3	5.1 ± 1.4	5.3 ± 1.4	0.001	0.192
MVPA, min/day†	46.1 ± 20.9	49.1 ± 22.0	48.2 ± 19.5	49.5 ± 22.1	0.012	0.979

Values are mean ± SD, n (%), or median (interquartile range). The p values were calculated for all participants and also for those with atherosclerosis. *n = 1,749. †n = 1,768.
Abbreviations as in Table 1.

CAC was detected in 11.1% of participants, the majority of them with mild calcification (183 individuals with a CAC score <100, 14 with a score of 100 to 399, and 1 with a score ≥400). Analysis of the extent of atherosclerosis revealed focal disease in 22.6% of participants, intermediate disease in 20.9%, and generalized disease in 6.0%. Among participants with optimal CVRFs (n = 740) (Online Tables 3 and 4), 280 (37.8%) had atherosclerosis, with peripheral plaques in 268 individuals and CAC in 43. In this subgroup, focal, intermediate, and generalized atherosclerosis was present in 20.8%, 13.8%, and 3.2% of participants, respectively.

PREDICTORS OF ATHEROSCLEROSIS PRESENCE AND MULTITERRITORIAL EXTENT. In the CVRF-free population, univariable analyses showed significant associations between disease presence and extent and all measured variables except for family history of cardiovascular disease, lipoprotein (a), cystatin C, hs-CRP, and fibrinogen. VCAM-1 was associated with the extent but not the presence of atherosclerosis (Online Tables 5 and 6). In multivariable models, male sex, age, LDL-C, and HbA_{1c} were associated with the presence of disease (**Table 3**). Age and sex showed the strongest associations with atherosclerosis presence, followed by LDL-C (**Figure 2**). The same variables, and additionally VCAM-1 and cystatin C, were also associated with multiterritorial extent of atherosclerosis (**Table 3**). **Figure 3** shows the stratification of these associations according to age intervals (40 to 44, 45 to 49, and 50 to 54 years) by sex, and to quintiles for LDL-C, HbA_{1c}, VCAM-1, and cystatin C. Again, age and sex demonstrated the strongest associations with atherosclerosis multiterritorial extent, followed by LDL-C (**Figure 3**). When restricting analyses to participants with atherosclerosis, age, LDL-C, VCAM-1, and systolic blood pressure were associated with

TABLE 3 Multivariable Analysis for the Presence and Multiterritorial Extent of Atherosclerosis in the CVRF-Free PESA Study Population and in the CVRF-Optimal Subgroup

	CVRF-Free Population				CVRF-Optimal Subgroup			
	Presence of Atherosclerosis		Multiterritorial Extent		Presence of Atherosclerosis		Multiterritorial Extent	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Age, ×1 yr	1.11 (1.08–1.13)	<0.001	1.14 (1.11–1.16)	<0.001	1.11 (1.06–1.15)	<0.001	1.13 (1.08–1.17)	<0.001
Male	2.03 (1.66–2.47)	<0.001	2.20 (1.80–2.69)	<0.001	2.00 (1.45–2.77)	<0.001	2.13 (1.57–2.91)	<0.001
LDL-C, ×10 mg/dl	1.14 (1.08–1.19)	<0.001	1.14 (1.09–1.20)	<0.001	1.16 (1.05–1.28)	0.003	1.18 (1.07–1.30)	0.001
HbA_{1c}, ×1%	1.77 (1.31–2.40)	<0.001	1.79 (1.36–2.36)	<0.001				
Cystatin C, ×1 mg/l			0.20 (0.08–0.52)	0.001				
VCAM-1, ×300 ng/ml			1.15 (1.06–1.26)	0.002				

Abbreviations as in Table 1.

increasing disease extent in the CVRF-free group, and only age and triglycerides in the CVRF-optimal group; however, sample size in the latter analysis was too small (Online Table 7).

In the subgroup with optimal CVRFs, age, male sex, and LDL-C were the only variables significantly associated with both disease presence and multiterritorial extent (Table 3). Similar to the overall population, age and male sex had the strongest associations with the presence and extent of atherosclerosis (Figure 2).

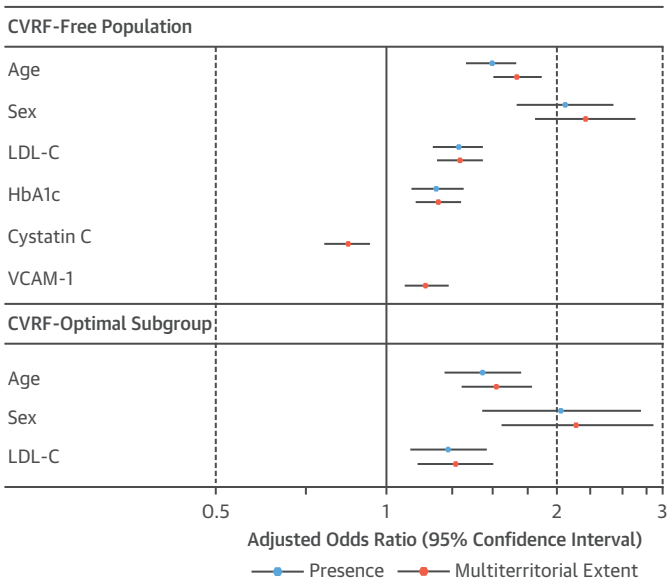
In both the CVRF-free and CVRF-optimal groups, results were similar when LDL-C was replaced by non-HDL-C (Online Table 8).

PREDICTORS OF ATHEROSCLEROSIS IN PARTICIPANTS WITH LDL-C <130 MG/DL. Similar results were obtained in a further multivariable analysis restricted to individuals with LDL-C <130 mg/dl (1,107 CVRF free and 682 CVRF optimal). Age, male sex, and LDL-C were the only variables associated with atherosclerosis presence and multiterritorial extent in both groups. In the CVRF-free group only, HbA_{1c} was also associated with both atherosclerosis presence and extent, and fibrinogen with extent (Table 4).

"NORMAL" LDL-C VALUES ARE INDEPENDENTLY ASSOCIATED WITH SUBCLINICAL ATHEROSCLEROSIS. The relationship between LDL-C and atherosclerosis in the absence of dyslipidemia, hypertension, diabetes, and smoking is illustrated in the Central Illustration and Figure 4. As LDL-C levels increased, there was a linear and significant increase in the prevalence of atherosclerosis, ranging from 11% in the 60 to 70 mg/dl category to 64% in the 150 to 160 mg/dl subgroup ($p < 0.001$) (Central Illustration). This progressive increase was noted in both men and women (Online Figure 1). A similar pattern was observed for the number of vascular sites affected (Central Illustration) and for each vascular bed analyzed separately (Figure 4). Indeed, in a secondary analysis by each vascular territory, LDL-C remained associated with atherosclerosis presence in each territory for the total CVRF-free population (Online Table 9).

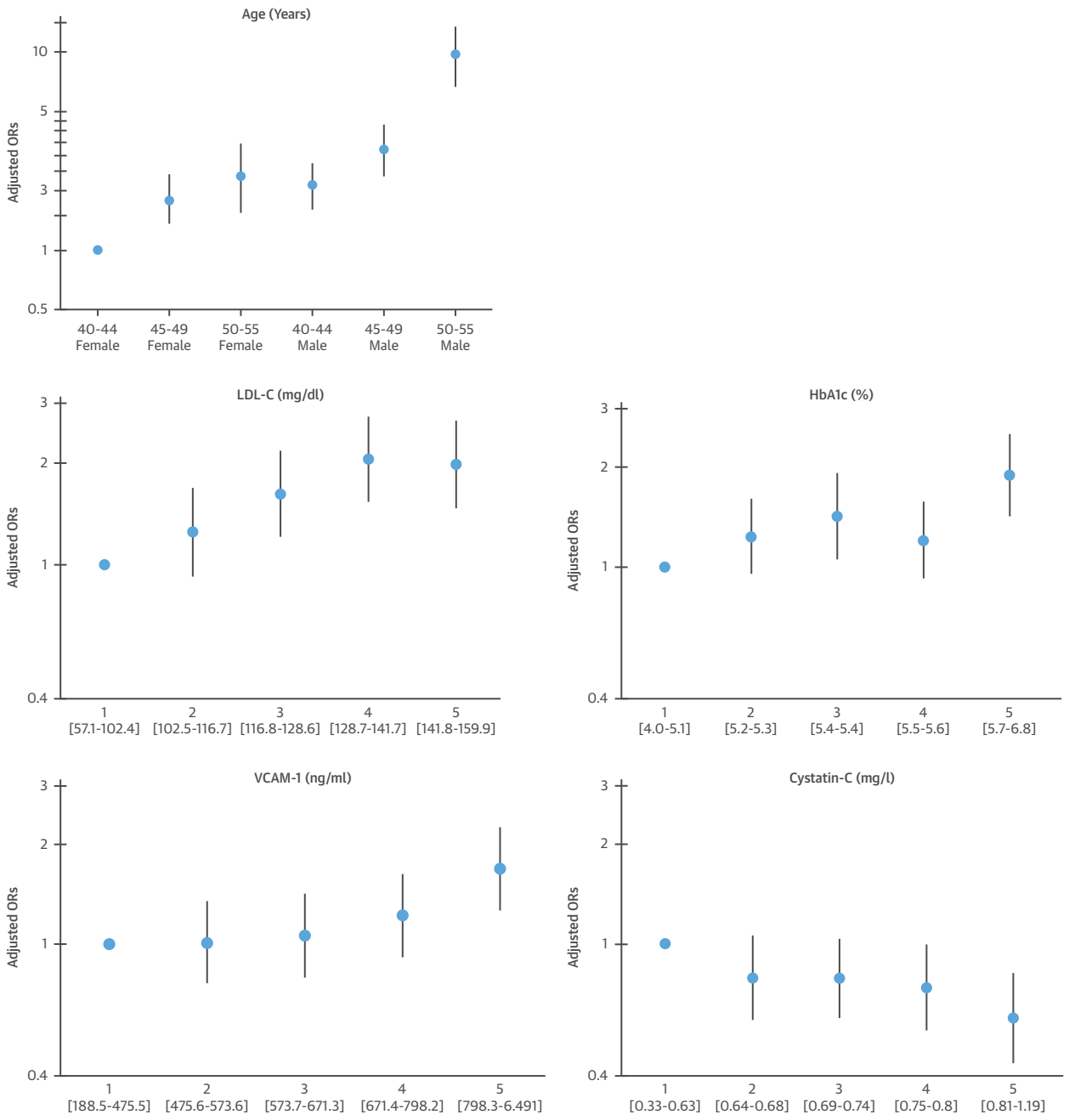
Finally, we also assessed whether LDL-C tracked atherosclerosis similarly across 30-year Framingham risk score categories. Whereas there was influence in

FIGURE 2 Comparison of Adjusted Odds Ratios and 95% Confidence Intervals for the Presence and Multiterritorial Extent of Atherosclerosis in the CVRF-Free Population and in the CVRF-Optimal Subgroup



Age and sex showed the strongest associations with atherosclerosis presence and multiterritorial extent, followed by low-density lipoprotein cholesterol (LDL-C) in both the cardiovascular risk factor (CVRF)–free population and in the CVRF-optimal subgroup. Standardized odds ratios are expressed per SD increase in each continuous risk factor. Odds ratios for sex are for men versus women. HbA1c = glycosylated hemoglobin; VCAM = vascular cell adhesion molecule.

FIGURE 3 **Adjusted Odds Ratios for the Association of Atherosclerosis Multiterritorial Extent With Progressive Increases in Age, for Both Men and Women, and in Those Modifiable Biomarkers That Remained Significantly Associated With Disease in Multivariate Models**



Age and male sex demonstrated the strongest associations, followed by LDL-C. Odds ratios (ORs) were adjusted for age, sex, LDL-C, HbA1c, cystatin C, and VCAM-1. Quintile 1 served as the reference category. **Vertical bars** represent 95 percent confidence intervals. Abbreviations as in Figure 2.

the CVRF-free population for presence and multiterritorial atherosclerosis (interaction test $p = 0.035$ and $p = 0.005$, respectively), this was absent in the CVRF-optimal subgroup ($p = 0.217$ and $p = 0.344$, respectively). To observe the effects of this interaction, we performed a stratified multivariable analysis by each 30-year Framingham risk score category. LDL-C remained significantly associated with subclinical atherosclerosis only in the low-risk group (Online Table 10).

TABLE 4 Multivariable Analysis for the Presence and Multiterritorial Extent of Atherosclerosis in the CVRF-Free PESA Population and in the CVRF-Optimal Subgroup With LDL-C Levels <130 mg/dl

	CVRF-Free Population (n = 1,107)				CVRF-Optimal Subgroup (n = 682)			
	Presence of Atherosclerosis		Multiterritorial Extent		Presence of Atherosclerosis		Multiterritorial Extent	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Age, ×1 yr	1.12 (1.08–1.15)	<0.001	1.15 (1.12–1.19)	<0.001	1.12 (1.07–1.17)	<0.001	1.13 (1.09–1.18)	<0.001
Male	2.06 (1.60–2.65)	<0.001	1.92 (1.50–2.46)	<0.001	1.90 (1.35–2.67)	<0.001	2.03 (1.46–2.81)	<0.001
LDL-C, ×10 mg/dl	1.18 (1.08–1.29)	<0.001	1.19 (1.09–1.29)	<0.001	1.21 (1.08–1.35)	0.001	1.23 (1.10–1.37)	<0.001
HbA_{1c}, ×1%	1.79 (1.21–2.64)	0.003	1.79 (1.25–2.59)	0.002				
Fibrinogen, ×100 mg/dl			0.66 (0.49–0.90)	0.008				

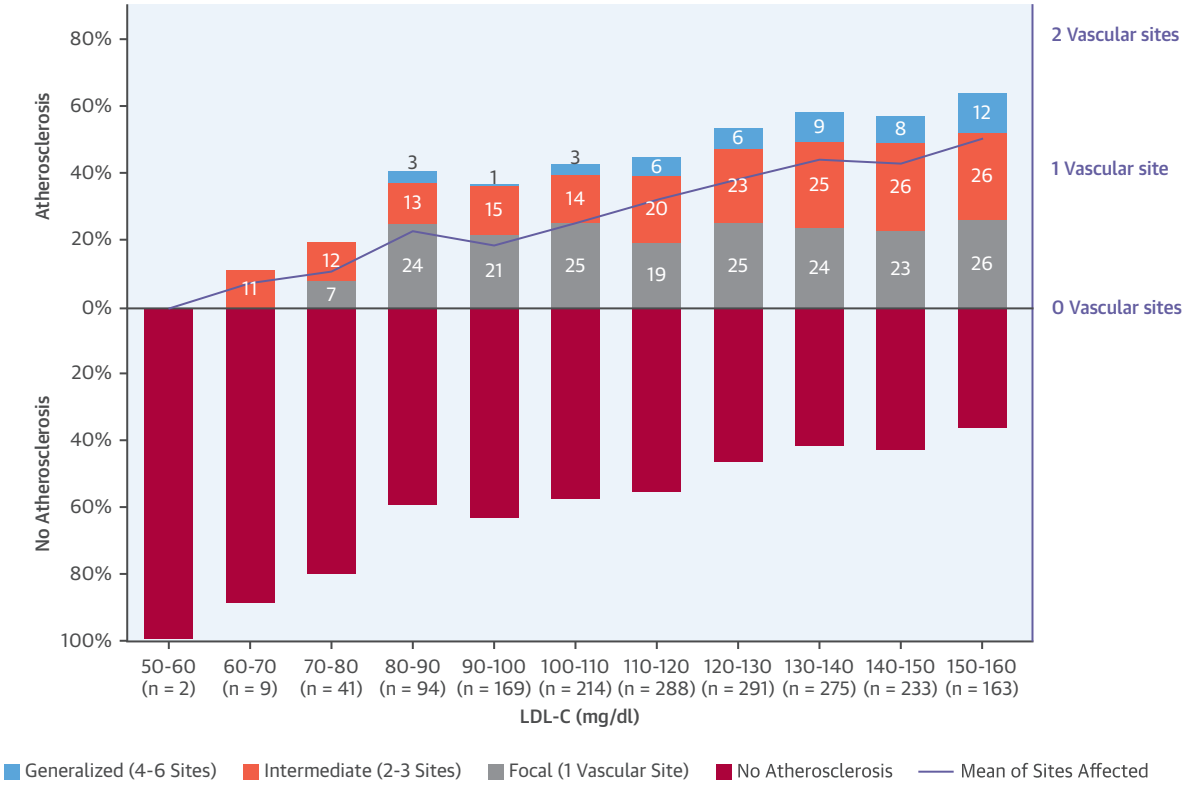
Abbreviations as in Table 1.

DISCUSSION

Absence of conventional CVRFs is traditionally considered a reliable indicator of low atherosclerosis risk. However, we identified subclinical atherosclerosis in one-half of a CVRF-free middle-aged population, with multiple vascular sites affected in nearly 30%. This finding expands on previous studies examining the association of risk with subclinical disease in single vascular territories (4,12), characterizing the extent of systemic atherosclerosis through multiterritorial assessment in low-risk individuals. Moreover, more than one-third of individuals with optimal CVRF levels and an ostensibly healthy status had subclinical atherosclerosis, suggesting that additional, poorly defined factors play a role in early atherogenesis. In the absence of conventional CVRFs, the presence and extent of atherosclerosis were associated with age, male sex, LDL-C, and HbA_{1c}. Even with CVRF levels considered to be optimal, atherosclerotic burden remained independently associated with age, male sex, and LDL-C, highlighting the crucial role of LDL-C in early disease stages.

The LDL-C hypothesis considers LDL-C as a causal factor in atherosclerosis. Although this hypothesis is generally accepted, controversy remains regarding its validity (28,29). Evidence supporting this hypothesis stems from experimental models, epidemiological cohorts, and cholesterol-lowering (mainly statin-based) clinical trials (30). However, potential remaining confounders should be considered. Animals used in experimental models typically develop much higher concentrations of plasma cholesterol than seen clinically (31), and results need to be extrapolated to humans. Clinical studies have typically enrolled participants with either clearly abnormal lipid levels or coexisting CVRFs, which may have synergistic or additive effects on disease development (32). In addition, some benefits of statin therapy may be related to pleiotropic effects beyond cholesterol lowering (30). In this study of apparently healthy individuals without conventional CVRFs, we demonstrated an independent and direct link between LDL-C levels and atherosclerotic burden. In fact, LDL-C was the strongest modifiable factor associated with atherosclerosis. Furthermore, even when all other risk factors were at optimal levels, this association persisted. Although association does not equate with causation, in the context of extensive prior data, we believe that these unique data from a large human cohort eliminate some of the potential confounders mentioned previously and provide indirect but solid evidence for the central role of LDL-C in early human atherogenesis. This is further highlighted by our findings of associations between LDL-C and disease in participants with low 30-year risk. These results also support the notion that cholesterol alone, in the absence of other known conventional CVRFs, may be enough to drive the development of atherosclerosis in humans (33). Multivariable analysis yielded similar results when LDL-C was replaced with non-HDL-C, suggesting no advantage of using one lipid variable over the other. Conversely, the multivariable models showed no link to subclinical atherosclerosis for other apolipoprotein B-containing particles, specifically oxidized LDL-C and lipoprotein (a). Although these particles are in principle more atherogenic, the absence of association may be related to their low concentrations and the low between-group variability in this CVRF-free population, and their role may be more important in the setting of higher cardiovascular risk (34). Importantly, LDL-C levels in our population were well within the range considered normal, reinforcing the concept that desirable LDL concentrations are probably much lower than those currently recommended (35). If confirmed, the results shown in the **Central Illustration and Figure 4**

CENTRAL ILLUSTRATION **Relation Between LDL-Cholesterol Levels and Atherosclerosis**



Fernández-Friera, L. et al. J Am Coll Cardiol. 2017;70(24):2979–91.

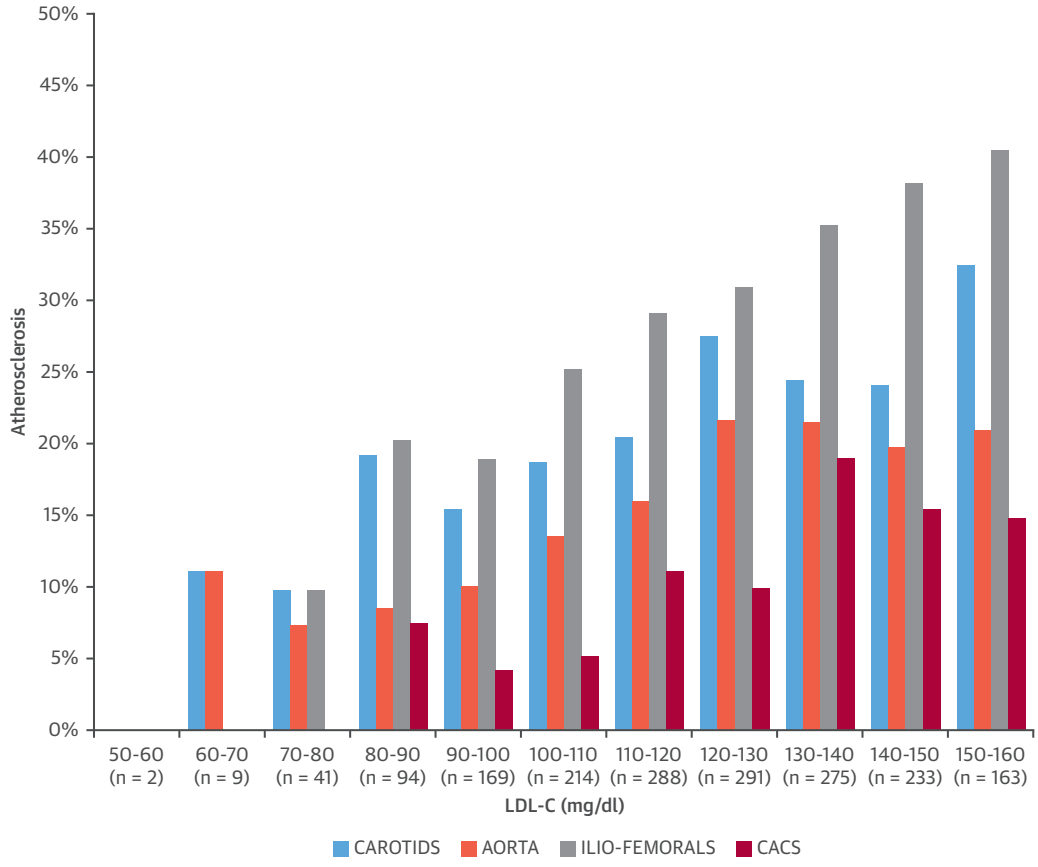
As LDL-cholesterol levels rise, there is a linear and significant increase in the prevalence of atherosclerosis, ranging from 11% in the 60 to 70 mg/dl category to 64% in the 150 to 160 mg/dl subgroup ($p < 0.001$). A similar pattern is observed for the multiterritorial extent of atherosclerosis (focal, intermediate or generalized disease) as well as for the mean number of vascular sites affected (blue line). LDL-C = low-density lipoprotein cholesterol.

would indicate that atherosclerosis in both men and women develops above an LDL-C threshold concentration of approximately 50 to 60 mg/dl, similar to the level associated with disease regression (36). This hypothesis is consistent with recent lipid-lowering trials, in which adverse clinical outcomes were significantly reduced when LDL-C levels were lowered below current targets (37). Thus, our findings may have important implications for primordial prevention strategies and for establishing cutoff values to define lipid disorders.

Additional independent (and nonmodifiable) predictors of atherosclerosis included age and sex. It is known that age is the most significant risk factor for cardiovascular events (8), and we also showed a strong association with subclinical atherosclerosis. This is likely related to the longer exposure to a variety of atherosclerotic risk determinants (e.g., LDL-C) as well as other aging-associated phenomena such as increased nucleic acid damage, apoptosis, or reduced regenerative capacity (38). Sex-based differences in atherosclerosis prevalence and severity are also well described, with women experiencing their first clinical event a decade later than men on average (39). Underlying mechanisms are incompletely understood but are probably multifactorial, including estrogen levels as well as differences in risk factor prevalence and susceptibility, psychosocial factors, and vascular biology (40).

In our study, HbA_{1c} was also independently associated with the presence and extent of subclinical atherosclerosis in the absence of CVRFs, both for participants with LDL-C <160 and <130 mg/dl, but not in those participants with optimal CVRF levels. HbA_{1c} reflects average glucose levels in the previous 2 to 3 months and therefore provides an index of glucose

FIGURE 4 Relation Between LDL-Cholesterol Levels and Atherosclerosis by Vascular Territory



Similar to the **Central Illustration**, as LDL-C levels rises, there is a significant increase in the prevalence of disease in each evaluated territory: carotid, aortic, iliofemoral and coronary. CACS = coronary artery calcium score; LDL-C = low-density lipoprotein cholesterol.

metabolism. Several previous reports have demonstrated an association between HbA_{1c} and subclinical atherosclerosis. In 2,340 nondiabetic individuals, higher HbA_{1c} concentrations (between 5.7% and 6.4%) were independently associated with increased CAC and carotid intima-media thickness (41). In another nondiabetic prospective series (n = 2,652), the upper 2 quartiles of HbA_{1c} levels (>5.7%) were linked to both carotid intima-media thickness progression and cardiovascular events (42). Notably, in our nondiabetic population we also observed a relative increase in systemic disease burden at HbA_{1c} levels >5.7% (**Figure 3**). These findings suggest that slightly increased HbA_{1c} levels are linked to subclinical atherosclerosis, particularly in combination with other risk factors (43), possibly explaining the increased cardiovascular risk associated with prediabetes (44). Although again our data do not establish a causal role, extensive prior evidence indicates detrimental vascular effects of chronic hyperglycemia through a variety of mechanisms (43).

Other serum markers possibly associated with the multiterritorial extent of atherosclerosis included VCAM-1 (an endothelial adhesion protein), cystatin C (an endogenous marker of renal function) (45), and fibrinogen (a hemostatic and inflammatory marker). Although each of these markers has potential mechanistic links to atherosclerosis, we did not find consistent associations in our models, and their link to atherosclerosis in the absence of CVRFs needs further investigation. Interestingly, blood pressure was not associated with systemic atherosclerosis in our sample, although we found a link with carotid disease (Online Table 9). We observed a paradoxical response between subclinical disease and healthy lifestyle parameters, such as diet and physical

activity. Whether this unexpected finding reflects a real biological process or the methodology used to measure these complex and multifactorial parameters is beyond the scope of this paper, and the influence of lifestyle behaviors has been addressed in recent PESA publications (24). We hypothesize that early atherogenesis in the absence of CVRFs and even with optimal risk factors levels is driven by age, sex, and LDL-C levels currently considered normal. Mild elevations in HbA_{1c} and advanced glycation products (associated with glucose metabolic dysregulation and aging) may cause further vascular injury. Although our data support the central role of LDL-C in early atherosclerosis, they do not exclude potential contributions from other multiple factors, which might be demonstrated with large sample sizes. Thus, early control of all risk factors should be taken into consideration for primordial prevention.

Our findings need to be placed in the context of the CVRF definitions used here. Specifically, the definition of dyslipidemia is not universally accepted and different lipid thresholds may slightly alter the results. However, we employed a commonly used definition from the National Cholesterol Education Program guidelines (16), in line with previous PESA study publications (13). To test the consistency of our results, we performed a secondary analysis including LDL-C levels <130 mg/dl, obtaining comparable results. Similarly, there is no consensus definition of an optimal risk profile. Our definition of optimal risk individuals is largely based on widely accepted factors of ideal cardiovascular health (3); however, our selection criteria did not include ideal cardiovascular behaviors other than smoking because only 121 of 1,779 individuals in our cohort reached the ideal levels for all 7 metrics. This very low ideal-health prevalence, which is consistent with previous reports (46), precluded meaningful statistical analysis. Recent U.S. guidelines (19) propose different optimal CVRF thresholds, but only 97 (5.5%) participants in our study fulfilled these criteria (not shown), again precluding meaningful multivariable analysis. Similarly, we could not perform multivariable analysis using a more restrictive LDL-C cutoff (<100 mg/dl) because of insufficient power (n = 235, not shown). In any case, our findings strongly suggest that thresholds to define elevated LDL-C should be lower than recommended across current guidelines.

STUDY LIMITATIONS. In the PESA study, diabetes was diagnosed based on glucose levels and not on HbA_{1c} levels; the study population therefore included a small proportion (0.17%) of participants with HbA_{1c} concentrations >6.4 mg/dl who could qualify as having diabetes but were not excluded. However, a sensitivity analysis excluding these participants yielded similar results (not shown). The small number of participants with LDL-C <70 mg/dl precludes reaching strong conclusions about a potential LDL-C threshold below which disease does not develop; however, the linear trend observed across high LDL-C levels supports the possibility of such a threshold. We did not evaluate other nonmodifiable risk factors (e.g., second-hand smoking or air pollution) and did not explore the potential roles of diet and exercise in greater detail because this was not the focus of this study. However, diet and physical activity showed no significant associations with atherosclerosis in the main models used (not shown), probably due to the homogeneity of these variables in our sample. Similarly, we did not evaluate all possible serum biomarkers because they were not included in the baseline PESA study examination (4). Finally, we did not evaluate the genetic contribution to disease development (47,48), which can be independent of CVRFs and could thus play an important role in our population.

CONCLUSIONS

Subclinical atherosclerosis is present in one-half of middle-aged PESA study individuals without major CVRFs and in one-third of those in the CVRF-optimal subgroup, suggesting that additional factors are involved in its development. LDL-C, at levels currently considered normal, is independently associated with the presence and extent of atherosclerosis in this setting, including in those participants with optimal risk profile. Thus, these data provide strong evidence of a unique, independent role of LDL-C in early human atherogenesis. These findings have important implications for guiding primordial prevention and understanding the mechanisms underlying early atherosclerosis.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Subclinical atherosclerosis can be detected in about one-half of otherwise healthy, middle-aged individuals without conventional cardiovascular risk factors. Serum LDL-C levels, even within the range currently considered normal, is independently associated with the presence and extent of subclinical atherosclerosis in multiple vascular territories.

TRANSLATIONAL OUTLOOK: The high global cardiovascular burden of cardiovascular disease makes effective primordial prevention a health care priority. Prospective studies are needed to evaluate the efficacy of more aggressive LDL-C lowering strategies at both the individual and population levels to reduce the incidence of clinical ischemic events.

REFERENCES

1. Khot UN, Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003;290:898–904.

2. Yusuf S, Rangarajan S, Teo K, et al. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. N Engl J Med 2014;371:818–27.

3. Lloyd-Jones DM, Hong Y, Labarthe D, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation 2010;121:586–613.

4. Silverman MG, Blaha MJ, Krumholz HM, et al. Impact of coronary artery calcium on coronary heart disease events in individuals at the extremes of traditional risk factor burden: the Multi-Ethnic Study of Atherosclerosis. Eur Heart J 2014;35:2232–41.

5. Michos ED, Nasir K, Braunstein JB, et al. Framingham risk equation underestimates subclinical atherosclerosis risk in asymptomatic women. Atherosclerosis 2006;184:201–6.

6. Berry JD, Liu K, Folsom AR, et al. Prevalence and progression of subclinical atherosclerosis in younger adults with low short-term but high lifetime estimated risk for cardiovascular disease: the coronary artery risk development in young adults study and multi-ethnic study of atherosclerosis. Circulation 2009;119:382–9.

7. Sibley C, Blumenthal RS, Merz CN, Mosca L. Limitations of current cardiovascular disease risk assessment strategies in women. J Womens Health (Larchmt) 2006;15:54–6.

8. Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2016;37:2315–81.

9. Peters SA, den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart 2012;98:177–84.

10. Baber U, Mehran R, Sartori S, et al. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BioImage study. J Am Coll Cardiol 2015;65:1065–74.

11. Naqvi TZ, Mendoza F, Rafii F, et al. High prevalence of ultrasound detected carotid atherosclerosis in subjects with low Framingham risk score: potential implications for screening for subclinical atherosclerosis. J Am Soc Echocardiogr 2010;23:809–15.

12. Pen A, Yam Y, Chen L, Dennie C, McPherson R, Chow BJ. Discordance between Framingham Risk Score and atherosclerotic plaque burden. Eur Heart J 2013;34:1075–82.

13. Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort: the PESA (Progression of Early Subclinical Atherosclerosis) study. Circulation 2015;131:2104–13.

14. Fernandez-Ortiz A, Jimenez-Borreguero LJ, Penalvo JL, et al. The Progression and Early detection of Subclinical Atherosclerosis (PESA) study: rationale and design. Am Heart J 2013;166:990–8.

15. Lopez-Melgar B, Fernandez-Friera L, Sanchez-Gonzalez J, et al. Accurate quantification of atherosclerotic plaque volume by 3D vascular ultrasound using the volumetric linear array method. Atherosclerosis 2016;248:230–7.

16. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and the Treatment of High Blood Cholesterol Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.

17. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871–81.

18. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2010;56:e50–103.

19. Goff DC Jr., Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;63:2935–59.

20. Pencina MJ, D'Agostino RB Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study. Circulation 2009;119:3078–84.

21. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–12.

22. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc 2008;40:181–8.

23. Agudo A, Cabrera L, Amiano P, et al. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). Am J Clin Nutr 2007;85:1634–42.

24. Penalvo JL, Fernandez-Friera L, Lopez-Melgar B, et al. Association between a social-business eating pattern and early asymptomatic atherosclerosis. J Am Coll Cardiol 2016;68:805–14.

25. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness and plaque consensus (2004–2006–2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis 2012;34:290–6.

26. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–32.

27. Cuzick J. A Wilcoxon-type test for trend. Stat Med 1985;4:87–90.

28. Ravnskov U, Diamond DM, Hama R, et al. Lack of an association or an inverse association between low-density-lipoprotein cholesterol and mortality in the elderly: a systematic review. BMJ Open 2016;6:e010401.

29. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;38:2459–72.

30. Jarcho JA, Keaney JF Jr. Proof that lower is better–LDL cholesterol and IMPROVE-IT. N Engl J Med 2015;372:2448–50.

31. Fuster JJ, Castillo AI, Zaragoza C, Ibanez B, Andres V. Animal models of atherosclerosis. Prog Mol Biol Transl Sci 2012;105:1–23.

32. Jackson R, Lawes CM, Bennett DA, Milne RJ, Rodgers A. Treatment with drugs to lower blood pressure and blood cholesterol based on an individual's absolute cardiovascular risk. Lancet 2005;365:434–41.

33. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001;104:503–16.

34. Homma Y. Predictors of atherosclerosis. J Atheroscler Thromb 2004;11:265–70.

35. O'Keefe JH Jr., Cordain L, Harris WH, Moe RM, Vogel R. Optimal low-density lipoprotein is 50 to 70 mg/dl: lower is better and physiologically normal. J Am Coll Cardiol 2004;43:2142–6.

36. Tsujita K, Sugiyama S, Sumida H, et al. Impact of dual lipid-lowering strategy with ezetimibe and atorvastatin on coronary plaque regression in patients with percutaneous coronary intervention: the multicenter randomized controlled PRECISE-IVUS trial. J Am Coll Cardiol 2015;66:495–507.

37. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 2017;376:1713–22.

38. Wang JC, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ Res 2012;111:245–59.

39. Anand SS, Islam S, Rosengren A, et al. Risk factors for myocardial infarction in women and men: insights from the INTERHEART study. Eur Heart J 2008;29:932–40.

40. Mathur P, Ostadal B, Romeo F, Mehta JL. Gender-related differences in atherosclerosis. Cardiovasc Drugs Ther 2015;29:319–27.

41. Xing FY, Neeland IJ, Gore MO, et al. Association of prediabetes by fasting glucose and/or haemoglobin A1c levels with subclinical atherosclerosis and impaired renal function: observations from the Dallas Heart Study. Diab Vasc Dis Res 2014;11:11–8.

42. Sander D, Schulze-Horn C, Bickel H, Gnahn H, Bartels E, Conrad B. Combined effects of hemoglobin A1c and C-reactive protein on the progression of subclinical carotid atherosclerosis: the INVADE study. Stroke 2006;37:351–7.

43. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur Heart J 2013;34:2436–43.

44. Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. J Am Coll Cardiol 2010;55:1310–7.

45. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049–60.

46. Ford ES, Li C, Zhao G, Pearson WS, Capewell S. Trends in the prevalence of low risk factor burden for cardiovascular disease among United States adults. Circulation 2009;120:1181–8.

47. Khera AV, Emdin CA, Drake I, et al. Genetic risk, adherence to a healthy lifestyle, and coronary disease. N Engl J Med 2016;375:2349–58.

48. Natarajan P, Young R, Stitziel NO, et al. Polygenic risk score identifies subgroup with higher burden of atherosclerosis and greater relative benefit from statin therapy in the primary prevention setting. Circulation 2017;135:2091–101.

KEY WORDS atherosclerosis, LDL-cholesterol, risk factors

APPENDIX For supplemental tables and a figure, please see the online version of this paper.