Glycated Hemoglobin and Subclinical Atherosclerosis in People Without Diabetes

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ABSTRACT

BACKGROUND The metabolic injury caused by protein glycation, monitored as the level of glycated hemoglobin (HbA1c), is not represented in most risk scores (i.e., Systematic Coronary Risk Estimation or atherosclerotic cardiovascular disease risk scale).

OBJECTIVES The purpose of this study was to assess the association between HbA1c and the extent of subclinical atherosclerosis (SA) and to better identify individuals at higher risk of extensive SA using HbA1c on top of key cardiovascular risk factors (CVRFs).

METHODS A cohort of 3,973 middle-aged individuals from the PESA (Progression of Early Subclinical Atherosclerosis) study, with no history of cardiovascular disease and with HbA1c in the nondiabetic range, were assessed for the presence and extent of SA by 2-dimensional vascular ultrasound and noncontrast cardiac computed tomography.

RESULTS After adjusting for established CVRFs, HbA1c showed an association with the multiterritorial extent of SA (odds ratio: 1.05, 1.27, 1.27, 1.36, 1.80, 1.87, and 2.47 for HbA1c 4.9% to 5.0%, 5.1% to 5.2%, 5.3% to 5.4%, 5.5% to 5.6%, 5.7% to 5.8%, 5.9% to 6.0%, and 6.1% to 6.4%, respectively; reference HbA1c $\leq$4.8%; p < 0.001). The association was significant in all pre-diabetes groups and even below the pre-diabetes cut-off (HbA1c 5.5% to 5.6% odds ratio: 1.36 [95% confidence interval: 1.03 to 1.80]; p = 0.033). High HbA1c was associated with an increased risk of SA in low-risk individuals (p < 0.001), but not in moderate-risk individuals (p = 0.335). Relative risk estimations using Systematic Coronary Risk Estimation or atherosclerotic cardiovascular disease predictors confirmed that inclusion of HbA1c modified the risk of multiterritorial SA in most risk categories.

CONCLUSIONS Routine use of HbA1c can identify asymptomatic individuals at higher risk of SA on top of traditional CVRFs. Lifestyle interventions and novel antidiabetic medications might be considered to reduce both HbA1c levels and SA in individuals without diabetes. (J Am Coll Cardiol 2021;77:2777–91) © 2021 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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Type 2 diabetes mellitus (T2DM), diagnosed as glycated hemoglobin (HbA1c) >6.5%, is a metabolic disorder characterized by insulin resistance that progresses to hyperglycemia (1). According to European Society of Cardiology (ESC) guidelines (2,3), individuals with pre-diabetes (HbA1c 5.7% to 6.4% [4]) but no known cardiovascular disease (CVD) are not necessarily at elevated cardiovascular (CV) risk (5,6) but warrant risk scoring for CVD primary prevention (2,3). Since 2003, ESC guidelines on CVD prevention recommend the use of the Systematic Coronary Risk Estimation (SCORE) (7) to estimate 10-year risk of fatal CVD (3). SCORE automatically considers people with diabetes as high-risk individuals (3); however, pre-diabetes status and HbA1c level are not considered in the risk equation. Conversely, age, sex, systolic blood pressure, and total cholesterol are treated as continuous variables conferring progressively increasing risk. U.S. guidelines encourage the use of race- and sex-specific pooled cohort equations to estimate 10-year atherosclerotic cardiovascular disease (ASCVD) risk for asymptomatic adults (1,8). The ASCVD scale includes diabetes as a predictor, but not HbA1c.

Strategies for the early detection of subclinical atherosclerosis (SA) are attracting interest as tools to refine risk prediction for individuals at low or intermediate CV risk according to classical equations (9,10). Recent ESC guidelines recommend coronary artery calcium scoring (CACS) and carotid and femoral ultrasound assessment of atherosclerotic plaques (recommendation Class II, Level of Evidence: B) (10). There is particular interest in assessing asymptomatic individuals at low risk (<1% risk of CV death according to SCORE) or moderate risk (1% to 5% SCORE risk) (3,7), because despite their low estimated risk, they can have underlying SA (9). In absolute terms, individuals at lower risk account for most CV deaths simply because they are a much larger population than higher-risk individuals (11).

The metabolic injury caused by protein glycation, monitored as the level of HbA1c (12), is not represented in the SCORE or ASCVD equations (13). Unlike fasting plasma glucose, HbA1c is unlikely to be on the causal pathway to SA; however, it might be a better biomarker because it provides a more accurate estimate of midterm glycemic exposure (12). Pre-diabetes is extremely prevalent and thus offers an important window of opportunity for implementing preventive interventions (1,6), such as lifestyle changes or medication (2,14-16). This evidence identifies HbA1c a valuable biomarker that could be used as an adjunct to the SCORE and ASCVD risk estimators.
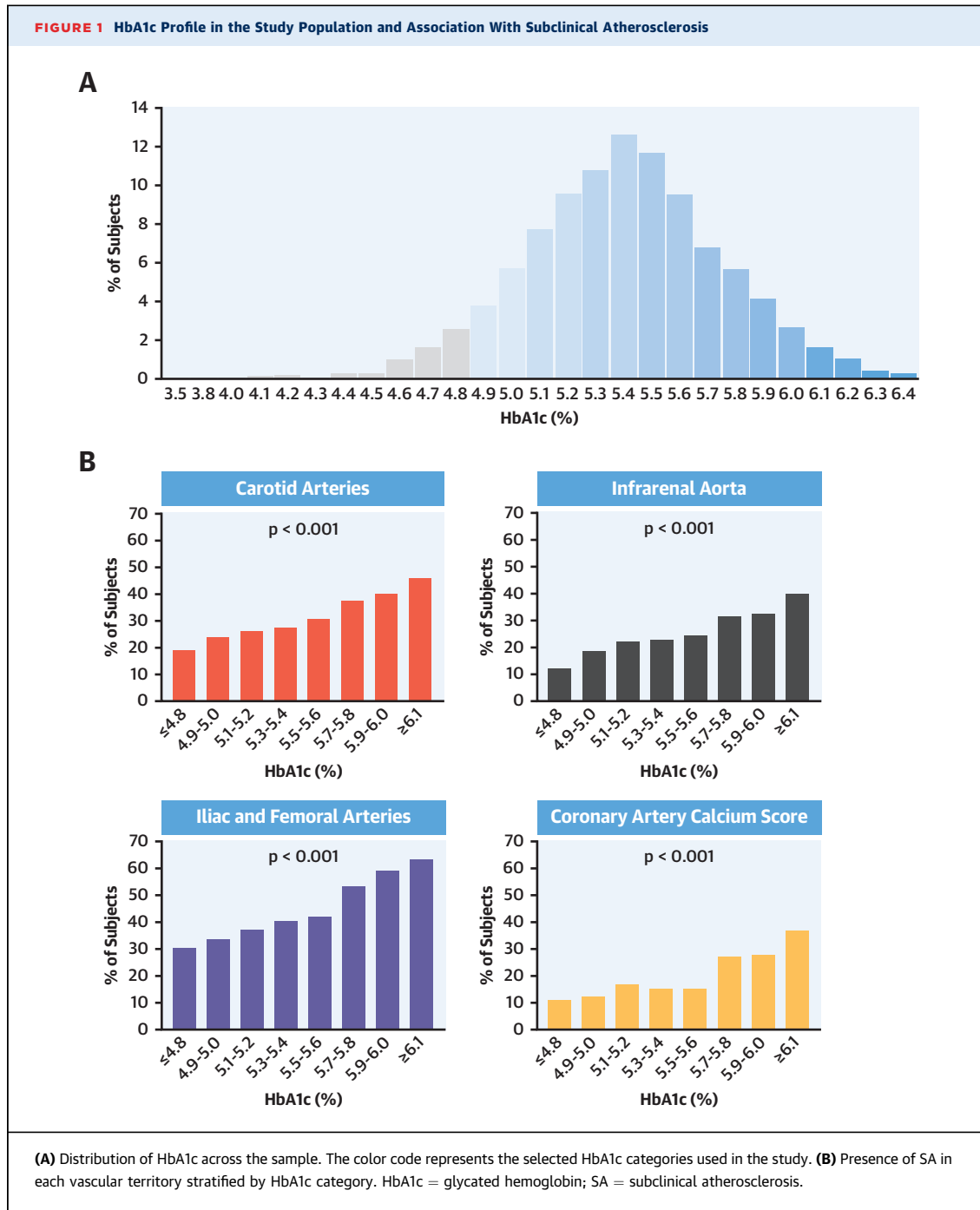
The current study explored the association between HbA1c and SA extent in a large cohort of asymptomatic middle-aged individuals without diabetes and with no known CVD and a low-moderate CV risk on the SCORE index. The additive value of HbA1c to classical risk factors for predicting the presence and extent of SA was also assessed.

METHODS

STUDY OVERVIEW. PESA (Progression of Early Subclinical Atherosclerosis) is an observational study designed to identify determinants of the onset and progression of SA diagnosed by noninvasive vascular imaging at multiple vascular sites in a middle-aged cohort of 4,184 individuals employed at the Santander Bank Headquarters in Madrid, Spain. Full details of the study design and data collection have been reported elsewhere (17). The study protocol was approved by the Ethics Committee of Instituto de Salud Carlos III (Madrid, Spain). All participants provided written informed consent (17).

STUDY PARTICIPANTS. Volunteers between the ages of 40 and 54 years were prospectively included if the baseline examination (between 2010 and 2014) showed that they were free of CV or chronic kidney disease, were not undergoing active treatment for cancer, had no history of organ transplant, had a body mass index (BMI) <40 kg/m², and had no disease that might reduce life expectancy during the originally anticipated follow-up period (6 years). Among the initial participants, 211 (5.0%) were excluded from this analysis (109 lacked imaging or blood tests, 65 lacked SCORE prediction, 18 lacked diabetes status, 16 had Hb1Ac ≥6.5, and 3 were in the high-risk SCORE category). The final sample therefore included 3,973 participants.

HbA1c CATEGORIES. HbA1c (%) categories were chosen to obtain comparable numbers of individuals in each while maintaining clinical meaningfulness (pre-diabetes status is defined by the American Diabetes Association [ADA] as HbA1c 5.7% to 6.4%, whereas it is defined by the National Institute for Health and Care Excellence [NICE] guideline as 6.0% to 6.4% [2,4,18]). Two categorization strategies were used: 1) 8 categories to explore associations (≤4.8% [reference], 4.9% to 5.0%, 5.1% to 5.2%, 5.3% to 5.4%, 5.5% to 5.6%, 5.7% to 5.8%, 5.9% to 6.0%, and 6.1% to 6.4%); and 2) 3 categories (≤5.2%, 5.3% to 5.6%, 5.7% to 6.4%) for further granular analysis. The distribution of HbA1c categories is illustrated in **Figure 1A**.

FIGURE 1 HbA1c Profile in the Study Population and Association With Subclinical Atherosclerosis

(A) Distribution of HbA1c across the sample. The color code represents the selected HbA1c categories used in the study. **(B)** Presence of SA in each vascular territory stratified by HbA1c category. HbA1c = glycated hemoglobin; SA = subclinical atherosclerosis.

SCORE RISK ESTIMATIONS. Predicted probabilities of CV events were estimated individually with the SCORE risk algorithm, which predicts the 10-year risk of CV death) (7,11). This tool is based on large, representative European cohort datasets and has been externally validated (19). Patients were classified at low or moderate risk according to the predicted outcome (<1% and 1% to 5% risk of CV death,

respectively). The predictors included in this score are age, sex, systolic blood pressure, total cholesterol, and smoking status.

SENSITIVITY ANALYSES USING FASTING PLASMA GLUCOSE LEVELS AND ASCVD RISK SCORE. To make our findings more robust, the main analyses were repeated with HbA1c replaced by fasting plasma glucose or the SCORE risk equation replaced by the

TABLE 1 Study Population Clinical Characteristics (N = 3,973) Stratified by HbA1c Category

	Total Population (N = 3,973)	HbA1c ≤5.2% (n = 1,305)	HbA1c 5.3%-5.6% (n = 1,770)	HbA1c ≥5.7% (n = 898)	p Value
Age, yrs	45.7 ± 4.2	45.0 ± 4.0	45.5 ± 4.2	47.1 ± 4.2	<0.001
Female	1,497 (37.7)	573 (43.9)	686 (38.8)	238 (26.5)	<0.001
Hypertension	427 (10.7)	108 (8.3)	176 (9.9)	143 (15.9)	<0.001
Diabetes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Dyslipidemia	1,602 (40.3)	449 (34.4)	676 (38.2)	477 (53.1)	<0.001
Smoking	809 (20.6)	185 (14.3)	365 (20.8)	259 (29.4)	<0.001
BMI, kg/m^2	26 ± 3.7	25.5 ± 3.5	25.8 ± 3.6	27 ± 3.8	<0.001
Obesity, BMI ≥30 kg/m^2	525 (13.2)	148 (11.3)	202 (11.4)	175 (19.5)	<0.001
Central obesity	683 (17.2)	192 (14.7)	288 (16.3)	203 (22.6)	<0.001
Family history of CV disease	623 (15.7)	215 (16.5)	261 (14.7)	147 (16.4)	0.997
Total cholesterol, mg/dl	201 ± 32.9	196 ± 31.7	201 ± 32.5	207 ± 34.6	<0.001
LDL-C, mg/dl	133 ± 29.4	128 ± 28.9	133 ± 28.7	138 ± 30.3	<0.001
HDL-C, mg/dl	49.3 ± 12.2	50.7 ± 12.6	49.5 ± 11.7	47 ± 12.1	<0.001
Triglycerides, mg/dl	79 (59-111)	74 (57-101)	78 (58-111)	91 (65-131)	<0.001
SBP, mm Hg	116 ± 12.3	115 ± 12.1	115 ± 12.2	118 ± 12.6	<0.001
DBP, mm Hg	72.3 ± 9.3	71.6 ± 9.1	71.9 ± 9.2	74.1 ± 9.8	<0.001
Fasting glucose, mg/dl	89.3 ± 8.6	86.4 ± 7.6	89.2 ± 8.1	93.7 ± 9.2	<0.001
HbA1c, %	5.4 ± 0.3	5.0 ± 0.2	5.4 ± 0.1	5.9 ± 0.2	<0.001
SCORE, % of event	0.35 (0.15-0.76)	0.27 (0.10-0.59)	0.34 (0.14-0.73)	0.57 (0.28-1.06)	<0.001

Values are mean ± SD, n (%), or median (first to third quartile).

BMI = body mass index; CV = cardiovascular; DBP = diastolic blood pressure; HbA1c = glycated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; SCORE = Systematic Coronary Risk Estimation.

ASCVD risk score. Each main figure is accompanied by a mirrored figure in the Supplemental Material showing the alternate analysis.

Fasting plasma glucose (mg/dl) was categorized similarly to HbA1c into 8 categories, including 3 categories in the pre-diabetes range according to the ADA criteria (≤79, 80 to 84, 85 to 89, 90 to 94, 95 to 99, 100 to 104, 105 to 109, and 110 to 125) (4). The distribution of glucose categories is shown in Supplemental Figure 1A.

Predicted probabilities of CV events were estimated individually using sex-specific pooled cohort equations to estimate 10-year ASCVD risk for asymptomatic adults (1,8). Patients were classified at low, borderline, or intermediate risk according to their predicted outcome (<5%, 5% to 7.4%, and 7.5% to 19.9%, respectively). The predictors in this risk score are age, sex, systolic blood pressure, total cholesterol, and smoking status (all of which are also included in the SCORE risk) as well as high-density lipoprotein (HDL) and medication for hypertension. The U.S. pooled equation has been validated in some other European populations (20) and has been very recently validated in a Spanish cohort (21).

ASSESSMENT OF SUBCLINICAL ATHEROSCLEROSIS. All participants were assessed by 2-dimensional vascular ultrasound (2DVUS) and noncontrast cardiac computed tomography, as per the PESA study protocol (22). 2DV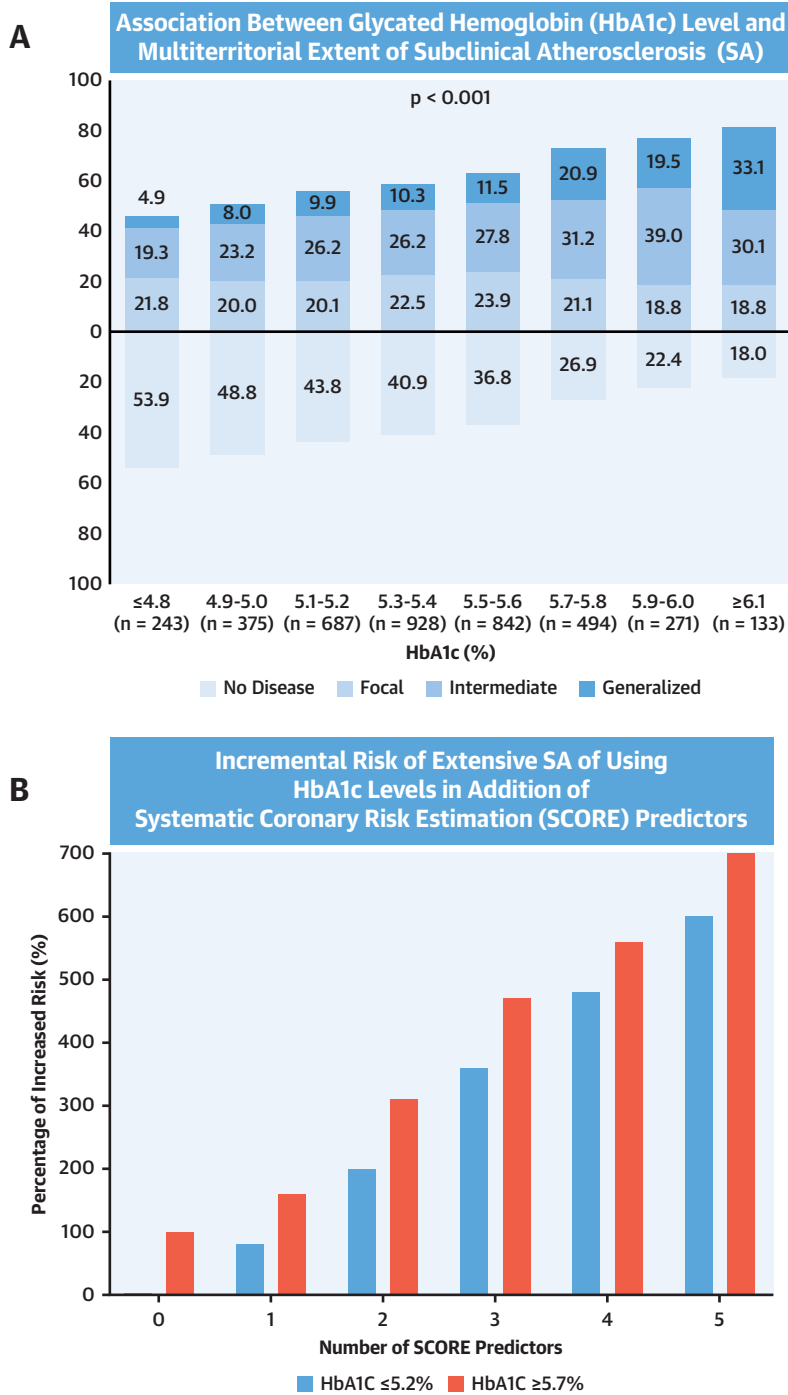US was used to detect the presence of atherosclerotic plaques with cross-sectional sweeps of the carotids, the infrarenal abdominal aorta, and the iliofemoral arteries. Plaques were defined as focal protrusions into the arterial lumen of thickness >0.5 mm or >50% of the surrounding intima-media thickness or as a diffuse intima-media thickening >1.5 mm (23). CACS was estimated from cardiac computed tomography images by the Agatston method (24).

The extent of SA was defined by combining the presence of 2DVUS-detected plaques and CACS scores ≥1 according to a previously described methodology (9). The multiterritorial extent of SA was defined according to the number of vascular territories showing evidence of disease out of the 6 examined: right carotid, left carotid, abdominal aorta, right iliofemoral, left iliofemoral, and coronaries. Participants were classified as disease-free (0 vascular sites affected) or as having focal (1 site), intermediate (2 or 3 sites), or generalized atherosclerosis (4 to 6 sites) (9).

All images were analyzed at a central Imaging Core Laboratory by experienced, blinded operators. Reproducibility assessments between operators have been reported elsewhere (9).

STATISTICAL ANALYSIS. The distribution of continuous variables was assessed with graphical methods. Normally distributed variables are expressed as mean ± SD, whereas non-normally distributed variables are

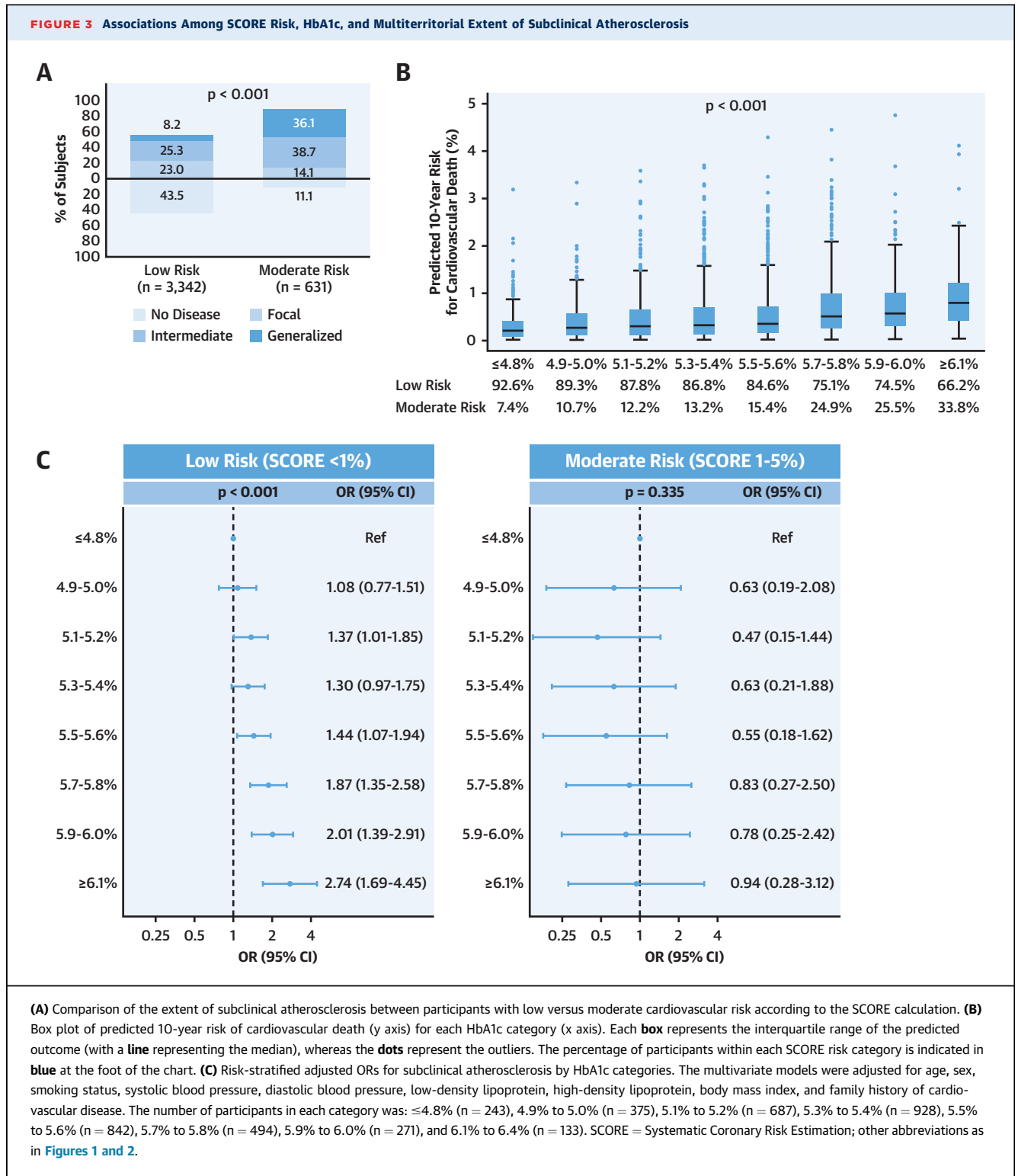CENTRAL ILLUSTRATION Association Among Glycated Hemoglobin, Risk Factors, and Subclinical Atherosclerosis

A Association Between Glycated Hemoglobin (HbA1c) Level and Multiterritorial Extent of Subclinical Atherosclerosis (SA)

B Incremental Risk of Extensive SA of Using HbA1c Levels in Addition of Systematic Coronary Risk Estimation (SCORE) Predictors

Rossello, X. et al. J Am Coll Cardiol. 2021;77(22):2777–91.

(A) Association between categorized glycated hemoglobin (HbA1c) level and the multiterritorial extent of subclinical atherosclerosis (SA) assessed by 2-dimensional vascular ultrasound and noncontrast coronary computed tomography in all participants. Multiterritorial SA extent is defined by combining data from both imaging techniques to classify individuals as disease-free (0 vascular sites affected) or having focal (1 site), intermediate (2 to 3 sites), or generalized atherosclerosis (4 to 6 sites) (9). (B) Incremental risk of extensive SA (intermediate or generalized) of using glycated hemoglobin levels according to the numbers of SCORE (Systematic Coronary Risk Estimation) predictors.

FIGURE 2 Adjusted Multivariate Analysis of the Association Between the Risk of Subclinical Atherosclerosis for Each HbA1c Category
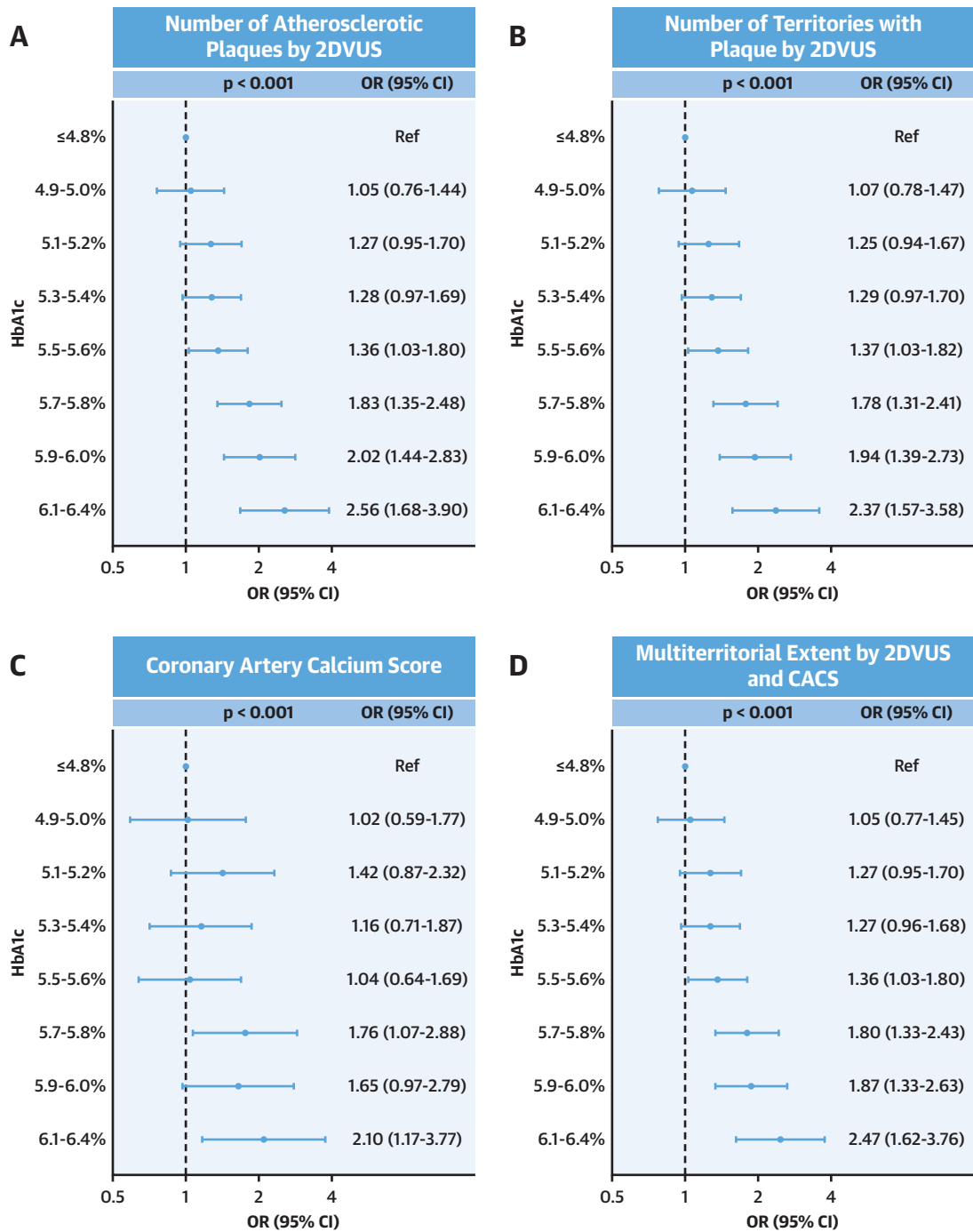
Associations were tested for **(A)** plaque number by 2-dimensional ultrasound (2DVUS), **(B)** number of territories with plaque by 2DVUS, **(C)** coronary artery calcium score by noncontrast coronary computed tomography, and **(D)** multiterritorial SA extent. Multivariate models were adjusted for age, sex, smoking status, systolic blood pressure, diastolic blood pressure, low-density lipoprotein, high-density lipoprotein, body mass index, and family history of cardiovascular disease. Smoking status was missing for 41 participants, and these individuals were therefore excluded from multivariate analysis. The number of patients in each category was: ≤4.8% (n = 243), 4.9% to 5.0% (n = 375), 5.1% to 5.2 % (n = 687), 5.3% to 5.4% (n = 928), 5.5% to 5.6% (n = 842), 5.7% to 5.8% (n = 494), 5.9% to 6.0% (n = 271), and 6.1% to 6.4% (n = 133). CI = confidence interval; OR = odds ratio; other abbreviations as in **Figure 1**.

FIGURE 3 Associations Among SCORE Risk, HbA1c, and Multiterritorial Extent of Subclinical Atherosclerosis

(A) Comparison of the extent of subclinical atherosclerosis between participants with low versus moderate cardiovascular risk according to the SCORE calculation. (B) Box plot of predicted 10-year risk of cardiovascular death (y axis) for each HbA1c category (x axis). Each **box** represents the interquartile range of the predicted outcome (with a **line** representing the median), whereas the **dots** represent the outliers. The percentage of participants within each SCORE risk category is indicated in **blue** at the foot of the chart. (C) Risk-stratified adjusted ORs for subclinical atherosclerosis by HbA1c categories. The multivariate models were adjusted for age, sex, smoking status, systolic blood pressure, diastolic blood pressure, low-density lipoprotein, high-density lipoprotein, body mass index, and family history of cardiovascular disease. The number of participants in each category was: ≤4.8% (n = 243), 4.9% to 5.0% (n = 375), 5.1% to 5.2% (n = 687), 5.3% to 5.4% (n = 928), 5.5% to 5.6% (n = 842), 5.7% to 5.8% (n = 494), 5.9% to 6.0% (n = 271), and 6.1% to 6.4% (n = 133). SCORE = Systematic Coronary Risk Estimation; other abbreviations as in **Figures 1 and 2**.
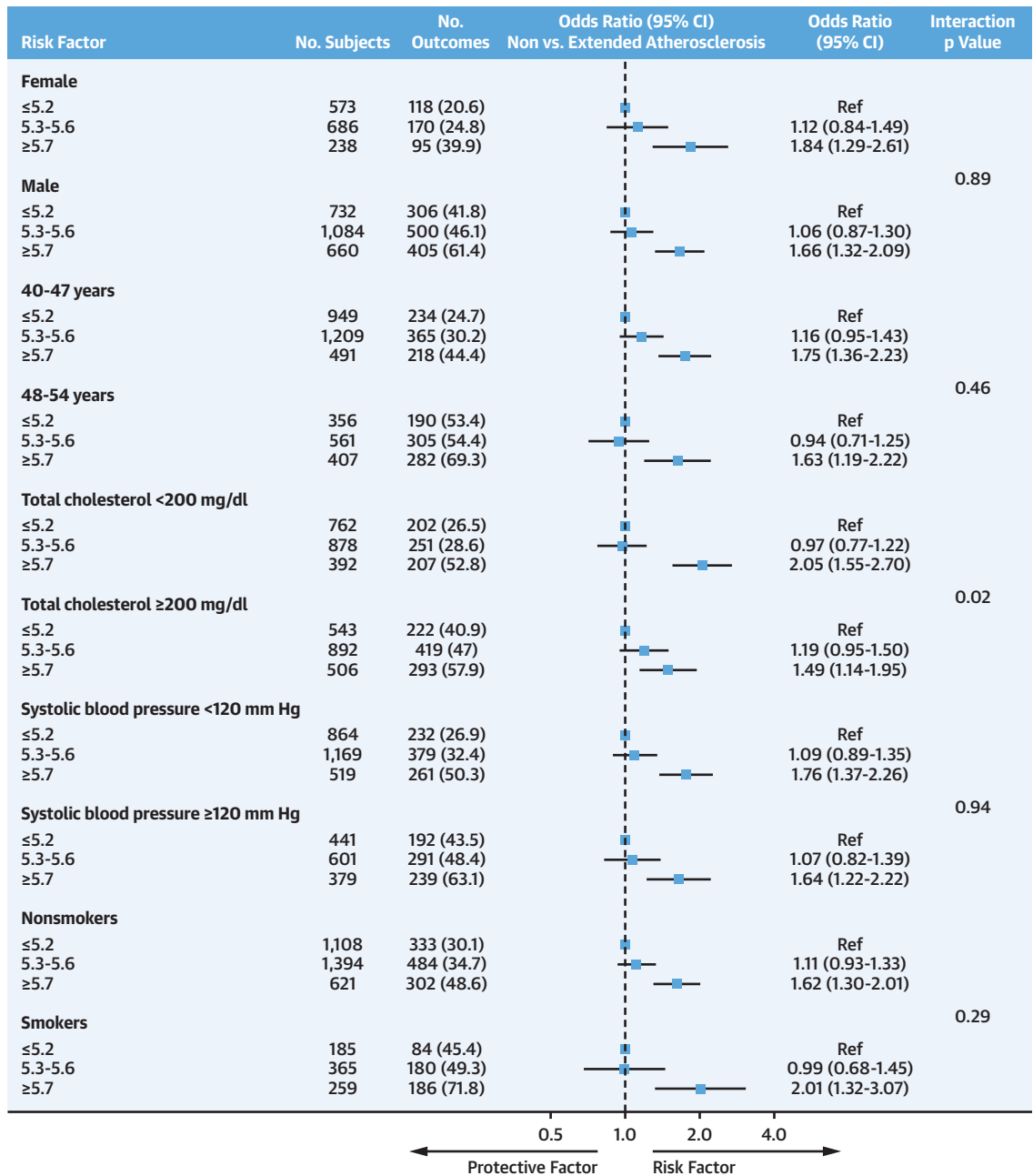
presented as median (first quartile, third quartile). Categorical variables were expressed as absolute frequency (%). Trend tests among HbA1c categories were performed by linear or logistic regression as

appropriate, including HbA1c as a continuous independent variable.

Univariate and multivariate ordinal regression models were used to assess the association between

FIGURE 4 Subgroup Analysis of the Interaction Between Traditional Cardiovascular Risk Factors (SCORE Risk Predictors) and HbA1c Category for the Outcome of No or Focal Subclinical Atherosclerosis Versus Intermediate or Generalized Disease

Risk Factor	No. Subjects	No. Outcomes	Odds Ratio (95% CI) Non vs. Extended Atherosclerosis	Odds Ratio (95% CI)	Interaction p Value
Female					
≤5.2	573	118 (20.6)		Ref	
5.3-5.6	686	170 (24.8)		1.12 (0.84-1.49)	
≥5.7	238	95 (39.9)		1.84 (1.29-2.61)	
Male					0.89
≤5.2	732	306 (41.8)		Ref	
5.3-5.6	1,084	500 (46.1)		1.06 (0.87-1.30)	
≥5.7	660	405 (61.4)		1.66 (1.32-2.09)	
40-47 years					
≤5.2	949	234 (24.7)		Ref	
5.3-5.6	1,209	365 (30.2)		1.16 (0.95-1.43)	
≥5.7	491	218 (44.4)		1.75 (1.36-2.23)	
48-54 years					0.46
≤5.2	356	190 (53.4)		Ref	
5.3-5.6	561	305 (54.4)		0.94 (0.71-1.25)	
≥5.7	407	282 (69.3)		1.63 (1.19-2.22)	
Total cholesterol <200 mg/dl					
≤5.2	762	202 (26.5)		Ref	
5.3-5.6	878	251 (28.6)		0.97 (0.77-1.22)	
≥5.7	392	207 (52.8)		2.05 (1.55-2.70)	
Total cholesterol ≥200 mg/dl					0.02
≤5.2	543	222 (40.9)		Ref	
5.3-5.6	892	419 (47)		1.19 (0.95-1.50)	
≥5.7	506	293 (57.9)		1.49 (1.14-1.95)	
Systolic blood pressure <120 mm Hg					
≤5.2	864	232 (26.9)		Ref	
5.3-5.6	1,169	379 (32.4)		1.09 (0.89-1.35)	
≥5.7	519	261 (50.3)		1.76 (1.37-2.26)	
Systolic blood pressure ≥120 mm Hg					0.94
≤5.2	441	192 (43.5)		Ref	
5.3-5.6	601	291 (48.4)		1.07 (0.82-1.39)	
≥5.7	379	239 (63.1)		1.64 (1.22-2.22)	
Nonsmokers					
≤5.2	1,108	333 (30.1)		Ref	
5.3-5.6	1,394	484 (34.7)		1.11 (0.93-1.33)	
≥5.7	621	302 (48.6)		1.62 (1.30-2.01)	
Smokers					0.29
≤5.2	185	84 (45.4)		Ref	
5.3-5.6	365	180 (49.3)		0.99 (0.68-1.45)	
≥5.7	259	186 (71.8)		2.01 (1.32-3.07)	

0.5   1.0   2.0   4.0

← Protective Factor   Risk Factor →

Interactions were assessed one at a time. The model was adjusted for sex, age, total cholesterol, SBP, and smoking status (all SCORE predictors) and the relevant interaction with HbA1c. Abbreviations as in **Figures 1, 2, and 3**.

HbA1c and: 1) the number of atherosclerotic plaques (0, 1, 2, >3); 2) the number of territories affected (0, 1, 2, >3); and 3) CACS (0 and tertiles) and multiterritorial extent (absence, focal, intermediate, and generalized). All associations were assessed with likelihood ratio tests. Potential confounders were used as adjustment variables to assess the association between subclinical atherosclerosis and HbA1c

TABLE 2 Model Performance Using HbA1c Levels in Addition to SCORE Predictors for Predicting Multiterritorial Extent

	c-Statistic (95% Confidence Interval)	p Value
Risk discrimination of HbA1c		
SCORE (age, sex, smoking status, systolic blood pressure, total cholesterol)	0.732 (0.717–0.748)	<0.001
HbA1c (continuous) + SCORE (age, sex, smoking status, systolic blood pressure, total cholesterol)	0.751 (0.735–0.766)	
Risk discrimination of HbA1c by SCORE risk category		
HbA1c (continuous) + SCORE (age, sex, smoking status, systolic blood pressure, total cholesterol) in low-risk population	0.751 (0.735–0.766)	<0.001
HbA1c (continuous) + SCORE (age, sex, smoking status, systolic blood pressure, total cholesterol) in moderate-risk population	0.736 (0.720–0.752)	

Abbreviations as in **Table 1**.

categories; the adjustment variables were age, sex, smoking status, systolic blood pressure, diastolic blood pressure, low-density lipoprotein levels, HDL levels, BMI, and family history of CVD (11,25). Family history of CVD was defined as a first-degree relative diagnosed with atherosclerosis before 55 years of age in men and 65 years of age in women (9). The same approach was used for the analysis of fasting plasma glucose presented in the Supplemental Appendix.

Predicted probabilities of CV events were estimated individually using the SCORE risk algorithm and were compared across HbA1c categories by linear regression analysis. For the binary outcome of multiterritorial extent (absence or focal vs. intermediate or generalized), potential interactions between SCORE predictors and HbA1c categories were evaluated one at a time in logistic regression models adjusted for all SCORE predictors (sex, age, total cholesterol, SBP, and smoking status) and the relevant interaction. Following ESC guideline recommendations to report relative measures in low- and moderate-risk patients (3), relative risks for the binary outcome of multiterritorial extent were estimated for each combination of predictors (5 from SCORE and HbA1c), with the patient subset with the lowest risk being used as the reference. The same approach was used for the analysis of ASCVD risk presented in the Supplemental Appendix.

Statistical analyses were performed using Stata 15 (StataCorp, College Station, Texas). Differences were considered statistically significant at $p < 0.05$. Some graphs were created in GraphPad Prism version 6.00 (GraphPad Software, La Jolla, California).

RESULTS

STUDY POPULATION. The study included 3,973 participants without diabetes (94.9% of the total PESA cohort). Mean participant age was 45.7 ± 4.2 years, and 37.7% were women. Mean HbA1c was 5.4 ± 0.3% (**Figure 1A**), and the median 10-year risk of CV death

according to the SCORE index was 0.35% (0.15% to 0.76%). Baseline characteristics are detailed further in **Table 1**.

ASSOCIATION BETWEEN HbA1c AND SUBCLINICAL ATHEROSCLEROSIS. HbA1c showed a positive association with the prevalence and multiterritorial extent of SA assessed by 2DVUS and CACS (p for trend <0.001) (**Central Illustration**, panel A). This trend held for individual arteries, with the percentage of participants with SA increasing tracking the HbA1c level in all vascular territories tested (**Figure 1C**, p for trend <0.001 for each territory). These associations remained consistent after considering potential confounders. Adjusted estimates are shown in **Figure 2** for the associations between HbA1c level and plaque number, the number of affected noncoronary territories, coronary CACS, and increasing extent of multiterritorial disease (p < 0.001 for each of the 4 outcomes). Associations were more conspicuous in the pre-diabetes HbA1c range (5.7% to 6.4%), but were also significant for the category below the pre-diabetes cut-off (HbA1c 5.5% to 5.6%: odds ratio: 1.36; 95% confidence interval: 1.03 to 1.80; p = 0.033). This elevated SA risk below the HbA1c pre-diabetes threshold is clinically relevant, and this patient subset is of particular demographic interest because it is a large population; 21.2% of participants had HbA1c 5.5% to 5.6%, and overall, 43.8% of study participants were in the higher-risk categories corresponding to HbA1c between 5.5% and 6.4% (**Figure 1A**).

We also performed a sensitivity analysis for the association between SA and fasting plasma glucose. Unadjusted associations showed similar trends to those observed for HbA1c (Supplemental Figure 1); however, after adjusting for confounders, fasting glucose, including all pre-diabetes categories, showed no association with plaque number, the number of noncoronary affected territories, coronary CACS, or increasing extent of multiterritorial disease (Supplemental Figure 2).

FIGURE 5 Impact of Combining HbA1c With Other Cardiovascular Risk Factors on the Likelihood for Having Extensive Subclinical Atherosclerosis

ASSOCIATIONS BETWEEN SCORE RISK CATEGORIES, HbA1c, AND THE MULTITERRITORIAL EXTENT OF SUBCLINICAL ATHEROSCLEROSIS. Stratification of participants by SCORE risk category revealed that individuals with moderate risk had a greater multiterritorial extent of SA than low-risk individuals (**Figure 3A**). Moreover, top categories of HbA1c showed an association with predicted SCORE risk (10-year risk of CV death) (**Figure 3B**). SCORE stratification of the adjusted association between multiterritorial SA extent and HbA1c category revealed an association between higher HbA1c levels and increased SA risk in low-risk participants, but this association was not observed in moderate-risk individuals (**Figure 3C**) (p for interaction <0.001 for low risk and 0.335 for moderate risk). Similar results were produced after stratification by ASCVD risk category (Supplemental Figure 3).

INTERACTIONS BETWEEN SCORE PREDICTORS AND HbA1c CATEGORIES. Subgroup analyses assessing interaction between SCORE predictors and HbA1c for the outcome "*no SA or focal SA*" versus "*intermediate or generalized SA*" showed that the impact of high HbA1c levels was homogenous across SCORE predictors except for total cholesterol (**Figure 4**). Among participants with total cholesterol ≥200 mg/dl, SA risk increased progressively with each increasing HbA1c category, whereas among those with total cholesterol <200 mg/dl, SA risk was only higher in those with HbA1c in the pre-diabetes range. Similar findings, including the interaction with total cholesterol, were found when these analyses were replicated using the ASCVD risk score (Supplemental Figure 4). In addition to total cholesterol, triglycerides, low-density lipoprotein, and HDL cholesterol levels were also assessed in Supplemental Figure 5.

RELATIVE RISK ESTIMATIONS USING SCORE PREDICTORS: ADDED VALUE OF HbA1c. Relative risks for multiterritorial SA are shown in **Figure 5**. The figure shows the added value of including 3 HbA1c categories in addition to the 5 SCORE risk predictors for the assessment of multiterritorial SA risk. Of note, SCORE predicts 10-year risk of fatal CVD (3), whereas the relative risk that we provide applies to multiterritorial SA and not clinical events. Similar findings were found when the analysis was repeated using the ASCVD risk score (Supplemental Figure 6).

Despite the fact that SCORE was not developed to identify subclinical atherosclerosis, we tested how it discriminates its extension and whether the addition of HbA1 levels improved the discriminative power of the SCORE predictors (**Table 2**).

DISCUSSION

In a large cohort of asymptomatic individuals without diabetes and with a low or moderate CV risk, we have identified an association between HbA1c levels and SA that is maintained after adjusting for potential confounders. The same association was not observed for fasting plasma glucose. Higher blood HbA1c was associated with an increased SA risk in low-risk individuals, whereas there was no association in moderate-risk individuals regardless of the risk score used. Subgroup analyses revealed that the impact of high HbA1c on SA was homogenous across CV risk factors except for total cholesterol. Relative risk estimations using either SCORE or ASCVD predictors demonstrate the additive value of HbA1c for predicting the multiterritorial extent of SA. Viewed in the context of previous evidence, the impact of including HbA1c in risk equations seems to be 3-fold: 1) it identifies individuals at risk of developing T2DM (1,6,26); 2) it indicates a high risk of subclinical and clinical CVD (18), even at levels below the pre-diabetes diagnosis threshold; and 3) it opens the way to interventions to prevent T2DM, SA, and CVD (2,14-16).

HbA1c values reflect mean endogenous exposure to glucose over the preceding 2 to 3 months, including postprandial spikes, and show low intraindividual variability, particularly in people without diabetes (12,27). These features may contribute to the superiority of HbA1c over fasting glucose for SA risk stratification. Although HbA1c might cause some vascular

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FIGURE 5 Continued

(A) Risk of extensive subclinical atherosclerosis increases with both the addition of SCORE predictors (cardiovascular risk factors) and HbA1c levels; **(B)** Individual relative risk of having intermediate or generalized subclinical atherosclerosis (vs. no or focal disease) for each of the 48 potential combinations for the binary categorization of the 4 SCORE predictors (age, smoking status, total cholesterol, and systolic blood pressure) and 3 HbA1c categories in women; and **(C)** Individual relative risk of having intermediate or generalized subclinical atherosclerosis (vs. no or focal disease) for each of the 48 potential combinations for the binary categorization of the 4 SCORE predictors and 3 HbA1c categories in men. **Numbers inside the squares** reflect the relative risk (RR) of having extensive vs. no or focal atherosclerosis. Age is in years, total cholesterol is in mg/dl, and systolic blood pressure (SBP) is in mm Hg. HbA1c = glycated hemoglobin; SCORE = Systematic Coronary Risk Estimation.

damage, its main value is as an index of other gly-cated molecules, such as advanced glycation end-products (12), which are likely drivers of vascular inflammation and SA.

Previous evidence showed that the CV risk entailed by dysglycemia begins at fasting glucose levels in the pre-diabetes range, below the T2DM cut-off, and in-creases with increasing glucose exposure (5,18,27). However, little was known about the potential for SA risk conveyed by increasing HbA1c levels. Our study demonstrates a linear association between HbA1c above 5.4% level and SA in people without diabetes. Moreover, this relationship holds true for plaques detected by 2DVUS, whereas the risk of a higher cor-onary artery calcium score was mainly concentrated in people with HbA1c in the pre-diabetes range. This could be explained by the pathophysiological differ-ences between noncoronary vascular plaques and coronary calcification, the latter occurring at later stages of the disease (28). Fasting glucose showed no association with SA, thus indicating that HbA1c a is a more useful biomarker of SA in people without dia-betes. It is notable that different diagnostic tests for T2DM do not always give matching results for the same individual, reflecting the imperfect correlation between HbA1c and mean plasma glucose in some individuals (4).

The key finding of the present study is that SA is prevalent in the 21.2% of the study population with HbA1c in the band below the pre-diabetes cut-off, and therefore considered not to be at risk. Furthermore, SA prevalence was especially prevalent among par-ticipants in the pre-diabetes HbA1c category who were classified as low-risk by either the SCORE or the ASCVD risk scales (7,8), making this subset of asymptomatic individuals a potential target for in-terventions aimed at preventing progression of sub-clinical disease to clinical events. Most likely, the association between HbA1c and SA was blunted because the presence and extension of SA was already driven by other CV risk factors and, in a way, we might even speculate that HbA1c levels might be a surrogate marker for other concomitant CVRFs.

Although it is acknowledged that individuals with the highest CV risk gain most from interventions addressing CV risk factors, most CVD deaths occur among people at low CV risk, simply because this population is much larger (11). It can therefore be argued that more medical and socioeconomic benefit could be obtained by targeting prevention strategies at low- and moderate-risk populations (3,7) than by maintaining a focus on people at higher risk who are often already receiving treatment (11). Our data indi-cate that directing resources and prevention strategies to people with <5% SCORE risk of CV death or with pre-diabetes would alleviate the societal CVD burden to a greater extent than focusing exclusively in those with >5% risk of CV death or with established diabetes. An estimated 25% of people diagnosed with pre-diabetes according to HbA1c (5.7% to 6.4%) progress to T2DM within 5 years (26). The clear as-sociation between HbA1c and SA suggests that any primary intervention to reduce HbA1c levels could potentially benefit millions of people with HbA1c below the diabetes diagnosis threshold by reducing not only the risk of developing T2DM, but also the extent of SA.

Early intervention to reduce HbA1c might be of interest for the prevention of SA and subsequent CV events. There is already firm evidence that primary prevention can effectively delay and prevent con-version from pre-diabetes to T2DM (2,29,30), and this is expected to translate into improved prognosis (31). Lifestyle interventions targeting dietary habits, physical activity, and body weight (1) are already recommended for people with pre-diabetes (Class IA) (2). In addition to lifestyle interventions, some anti-diabetic medications can be taken by patients without diabetes (2,14-16). ADA guidelines recom-mend consideration of medical treatment with met-formin for individuals with pre-diabetes and who have a high risk of progressing to T2DM, such as older people (age >60 years), people with obesity (BMI ≥35 kg/m²), or women with a history of gesta-tional diabetes (32). The relationship established in the present study between HbA1c level and SA could spur future clinical trials of anti-diabetes medication for pre-diabetes patients not only at a high risk of T2DM progression, but also at low CV risk according to SCORE or ASCVD equations. In addition to met-formin, other antidiabetic medications available for people without diabetes include the GLP-1 inhibitor liraglutide, which, as an adjunct to diet and exercise, helps to reduce body weight and improve metabolic control in obese patients without T2DM (14). The SCALE (Liraglutide Evidence in Individuals with and without Diabetes) trial showed a greater reduction in glycated hemoglobin with liraglutide than with pla-cebo, with a greater benefit in patients with pre-diabetes than in those without (14). Very recently, the SGLT2 inhibitor dapagliflozin has been demon-strated to significantly reduce the risk of worsening heart failure or CVD in heart failure patients with reduced ejection fraction, regardless of diabetes sta-tus (15,33). Ongoing clinical trials are already testing the clinical benefit of other SGLT2 inhibitors in pa-tients with and without diabetes in a broader heart failure patient population (34). It should be noted

that the treatment effect of some antidiabetic medications has not been accompanied by a substantial change in HbA1c levels, hence suggesting a potential mechanism beyond their glucose-lowering capacity (35). Nevertheless, the impact of these medications on HbA1c levels might be different between patients with established T2DM and asymptomatic individuals without T2DM.

Neither the SCORE nor the ASCVD risk scale includes a predictor indicating long-term glycemic exposure in people without diabetes or with pre-diabetes, such as HbA1c (7,8). The relative risk estimations presented here illustrate the additive value of using HbA1c to identify individuals with a higher likelihood of having SA. Although these estimations are not intended for use in routine clinical practice, we nevertheless believe that our findings highlight the benefits of assessing chronic glycemic exposure in people without diabetes and might help in clinical decision-making about lifestyle interventions and antidiabetic medication. Importantly, this approach is a proof-of-concept: both SCORE and ASCVD scales predict 10-year risk of clinical events, whereas these relative risk estimations apply to a surrogate outcome (multiterritorial SA), which happens earlier in the natural history of the disease. This is useful for potential early interventions, but it cannot be interpreted yet as an improvement in the prediction of 10-year risk of clinical events. Moreover, the improvement in terms of risk discrimination should be taken with caution. The use of the c-statistic as a measure of model discrimination has some limitations, given that the c-statistic is a rank-based method that does not take distribution into account (i.e., a difference between 2 individuals who are at very low risk, 0.1% versus 0.2%, have the same impact on the estimate as 2 individuals who are at moderate versus high risk, 4% vs. 10%, if their ranks are the same) and it must be acknowledged that some statistically significant differences across c-statistics might not be translated into clinically meaningful results (36). Nevertheless, there was a formal improvement in risk discrimination of multiterritorial extent of SA when HbA1c was added to SCORE predictors.

STUDY LIMITATIONS. This study should be evaluated in the light of its limitations. First, the association of HbA1c with SA cannot be interpreted as a causal relationship given the observational nature of the study. Second, there may have been residual confounding by unmeasured and measured variables, despite our efforts to adjust for known risk factors with the use of multivariate modeling. Third, HbA1c measurements can be influenced by hemoglobin

variants, genetic hemoglobinopathies, thalassemias, and iron deficiency anemia (26). Nevertheless, HbA1c reflects longer-term glycemic control and shows less intrapersonal variability than impaired glucose tolerance and impaired fasting glucose measurements. Medications were included in the multivariate adjustment, although their inclusion as covariates did not change our conclusions (Supplemental Figure 7). Coronary atherosclerosis was evaluated with the CACS, so we cannot rule out an association between HbA1c levels and prevalence of noncalcified coronary plaque below pre-diabetes levels, similar to that observed when noncoronary plaques were assessed by 2DVUS. Finally, the PESA study cohort is a relatively homogeneous occupational cohort that may not be representative of the general population.

CONCLUSIONS

We have identified an association between HbA1c levels and SA. This association supports available evidence that pre-diabetes confers higher CV risk, but also shows that the risk extends to individuals with HbA1c below the pre-diabetic range. This evidence regarding the association between HbA1c levels and SA in patients without diabetes are expected to translate into similar risk of CV events, though this remains to be elucidated. Higher HbA1c levels were associated with an increased risk of SA in individuals at low CV risk, whereas there was a lack of association in moderate-risk individuals, regardless of the risk scale used. Relative-risk estimations using SCORE or ASCVD predictors showed the additive value of using HbA1c levels for predicting the multiterritorial extent of SA. Lifestyle interventions and novel antidiabetic medications might be considered for individuals without diabetes to both reduce HbA1c levels and prevent SA progression.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In asymptomatic individuals without diabetes and at low or intermediate CV risk, HbA1c levels are associated with the extent of subclinical atherosclerosis. This supports the notion that pre-diabetes increases CV risk and shows that risk extends to those with HbA1c levels below the pre-diabetes threshold.

TRANSLATIONAL OUTLOOK: Further research is needed to delineate the mechanisms linking HbA1c to CV risk and to identify and validate markers of insulin resistance as targets for the development of therapeutic interventions.

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APPENDIX For supplemental figures, please see the online version of this paper.