"Subclinical atherosclerosis burden in midlife: carotid and femoral three-dimensional vascular ultrasound in the PESA study"

Authors: Beatriz López-Melgar[a,b] MD, PhD; Leticia Fernández-Friera[a,b,c] MD, PhD; Belén Oliva[a] MSc; José Manuel García-Ruiz[a,d,c] MD; José Luis Peñalvo[a,e] PhD; Sandra Gómez-Talavera[a,f] MD; Javier Sánchez-González[g] PhD; José María Mendiguren[h] MD; Borja Ibáñez[a,c,f] MD, PhD; Antonio Fernández-Ortiz[a,c,i] MD, PhD; Javier Sanz[a,j] MD; Valentín Fuster[a,j] MD, PhD.

Authors affiliation: [a]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [b]HM Hospitales–Centro Integral de Enfermedades Cardiovasculares HM CIEC, Madrid, Spain; [c]CIBER de enfermedades CardioVasculares (CIBERCV), Spain; [d]Hospital Universitario Central de Oviedo, Asturias, Spain; [e]Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA; [f]IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain; [g]Philips Healthcare, Spain; [h]Banco de Santander, Madrid, Spain; [i]Hospital Clínico San Carlos, Madrid, Spain; [j]The Zena and Michael A. Wiener Cardiovascular Institute/Marie-Josée and Henry R. Kravis Center for Cardiovascular Health, Mount Sinai School of Medicine, New York, USA.

Address for Correspondence: Valentín Fuster, MD. PhD. Physician-in-Chief, Mount Sinai Medical Center. Director, Mount Sinai Heart. Icahn School of Medicine at Mount Sinai. One Gustave L. Levy. Place, Box 1030 New York City, NY 10029. General Director, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC). Melchor Fernandez Almagro, 3. 28029. Madrid Spain. Tel +3491 4531200. Email: vfuster@cnic.es

SOURCES OF FUNDING: The PESA study is co-funded equally by the Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, and Banco Santander, Madrid, Spain. The study also receives funding from the Institute of Health Carlos III (PI15/02019) and the European Regional Development Fund (ERDF). The CNIC is supported by the Ministry of Economy, Industry and Competitiveness (MINECO) and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505).

CONFLICTS OF INTEREST: Javier Sánchez-González is an employee of Philips Healthcare. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Trial registration: Progression of Early Subclinical Atherosclerosis (PESA) Study. Identifier: NCT01410318, https://clinicaltrials.gov/ct2/show/NCT01410318

BRIEF TITLE: Atherosclerotic burden by 3D vascular ultrasound

TEXT COUNT:
3759 (text) + 1303 (ref) + 295 (figure legends) = 5.357
Abstract words 247; Condensed Abstract words 89

Number of figures and tables:

Main figures 5, Main tables 5, online tables 7 and online figures 2

ABSTRACT

Background: Detection of subclinical atherosclerosis improves risk prediction beyond cardiovascular risk factors (CVRF) and risk scores, but quantification of plaque burden may improve it further. Novel three-dimensional vascular ultrasound (3DVUS) provides accurate volumetric quantification of plaque burden.

Objectives: To evaluate associations between 3DVUS-based plaque burden and CVRFs and explore potential added value over simple plaque detection.

Methods: We included 3860 (92.2%) PESA (Progression of Early Subclinical Atherosclerosis) participants (age 45.8±4.3; 63% males). Bilateral carotid and femoral territories were explored by 3DVUS to determine the number of plaques and territories affected, and to quantify global plaque burden defined as the sum of all plaque volumes. Linear regression and proportional odds models were used to evaluate associations of plaque burden with CVRFs and estimated 10-year cardiovascular risk.

Results: Plaque burden was higher in men (63.4 mm^3 [23.8-144.8] vs 25.7 mm^3 [11.5-61.6] in women, $p<0.001$), in the femoral territory (64 mm^3 [27.6-140.5] vs 23.1 mm^3 [9.9-48.7] in the carotid territory, $p<0.001$), and with increasing age ($p<0.001$). Age, sex, smoking, and dyslipidemia were more strongly associated with femoral than with carotid disease burden, whereas hypertension and diabetes showed no territorial differences. Plaque burden showed a direct association with estimated cardiovascular risk independently of the number of plaques or territories affected ($p<0.01$).

Conclusions: 3DVUS quantifies higher plaque burden in men, in the femoral territory, and with increasing age during midlife. Plaque burden correlates strongly with CVRFs, especially at the femoral level, and reflects estimated cardiovascular risk more closely than plaque detection alone.

KEY WORDS: subclinical atherosclerosis, three-dimensional ultrasound, plaque volume, carotid plaque, femoral plaque.

CONDENSED ABSTRACT

Subclinical atherosclerotic burden evaluation by novel 3D vascular ultrasound (3DVUS) may improve cardiovascular risk prediction beyond cardiovascular risk factors (CVRF). We evaluated carotid and femoral atherosclerotic burden by 3DVUS in 3860 asymptomatic middle-aged participants of the PESA (Progression of Early Subclinical Atherosclerosis) study. 3DVUS quantifies higher plaque burden in men, in the femoral territory, and with increasing age (all $p<0.001$). Plaque burden correlates strongly with CVRFs, especially at the femoral level, and reflects estimated cardiovascular risk more closely than plaque detection alone, showing potential for improving risk stratification strategies.

ABBREVIATIONS:

2DVUS: 2 dimensional vascular ultrasound
3DVUS: 3 dimensional vascular ultrasound
ASCVD: Atherosclerotic cardiovascular disease
BMI: Body mass index
CACS: Coronary artery calcium score
CV: Cardiovascular
CVRF: Cardiovascular risk factors
IMT: Intima-media thickness
PB: plaque burden
PESA: Progression of Early Subclinical Atherosclerosis study

INTRODUCTION

Detection of subclinical atherosclerosis improves cardiovascular risk stratification (1,2). A direct relationship between atherosclerotic lesions and conventional cardiovascular risk factors (CVRF) has been previously established, first with necropsy findings (3) and subsequently using different noninvasive imaging techniques. Coronary artery calcium score (CACS) and intima-media thickness (IMT) have been more widely explored in their relationship with CVRFs and cardiovascular risk than direct evaluation of atheromas. Nonetheless, increasing evidence reinforces the potential value for risk assessment of detecting atherosclerotic lesions (4-6) including multi-territorial evaluation since atherosclerosis is a systemic process (7,8). Two-dimensional vascular ultrasound (2DVUS) is well suited not only for detecting but also for grading the extent of atherosclerosis in different territories. However, methods for quantifying atherosclerotic plaque burden with 2DVUS have differed between studies and no consensus exists on which measurement should be used or which arterial territory is more suitable for cardiovascular risk prediction (9).

Recently introduced semiautomated three-dimensional vascular ultrasound (3DVUS) has been proposed as a better method for quantifying peripheral atherosclerotic burden (10). Plaque volume may be a more comprehensive measure that incorporates disease presence and amount, and is thus more likely to reflect the cumulative effect of chronic CVRF exposure and individual susceptibility. However, 3DVUS has not been used to quantify multi-territorial plaque burden in large population studies, and the associations of plaque volume with CVRFs have not been explored. Our goals were to: 1) evaluate feasibility and reproducibility, and provide reference values of carotid and femoral plaque burden distribution by 3DVUS in asymptomatic middle-aged individuals; 2) determine the

relationship between CVRFs and plaque burden; and 3) explore the potential additive value of quantifying plaque volume versus plaque detection alone.

METHODS

The Progression of Early Subclinical Atherosclerosis (PESA-CNIC-Santander) study is an ongoing observational prospective cohort study aimed at characterizing early subclinical atherosclerotic burden and determinants of atherosclerosis presence and progression using different noninvasive image techniques. The study rationale and design have been reported in detail (11). Briefly, between June 2010 and February 2014, PESA enrolled 4184 volunteers between 40 and 54 years without prior cardiovascular disease who were employees at the Banco de Santander headquarters in Madrid (Spain). Participants completed a baseline visit that included 3DVUS in the carotid and femoral territories. In addition, the visit included clinical interviews, standardized lifestyle questionnaires, physical examination, fasting blood draw, urine sample collection, and 12-lead ECG. Participants are being prospectively followed up with repeat visits at 3 and 6 years. The study protocol has been approved by the *Instituto de Salud Carlos III* Ethics Committee and complies with the Declaration of Helsinki. All eligible participants have provided written informed consent. The definitions of CVRFs, including dyslipidemia, smoking, hypertension, diabetes, obesity, and family history of premature cardiovascular disease, are as reported previously (7). Cumulative smoking exposure was determined in terms of pack-years by multiplying the number of years smoked by the average number of packs per day. The ACC/AHA atherosclerotic cardiovascular disease (ASCVD) risk score was quantified, and classified according to 10-year risk as low (<5%), intermediate (5 to <7.5%), or high (≥7.5%) (12).

Imaging protocol

The 3DVUS examinations were performed with a Philips iU22 ultrasound system equipped with a VL13-5 3D volume-linear array transducer (Philips Health Care, Andover, MA, USA). Detailed 3DVUS acquisition and analysis methodology have been reported previously, together with experimental validation of this novel 3D vascular technique (13). The PESA 3DVUS methodology included evaluation of carotid and femoral arterial segments adjacent to the bifurcation in a standardized fashion. The protocol for the carotid arteries consisted of a 30º automatic sweep (explored vessel segment ≈6 cm long) centered at the carotid bulb to include the distal common carotid artery, the bulb, the bifurcation, and the proximal internal and external carotid artery segments. For the femoral arteries, the 30º and ≈6cm acquisition was centered at the bifurcation and included the mid-distal common femoral artery, the bifurcation, and the proximal superficial and deep femoral artery segments *(Online Figure 1)*. The acquired images were analyzed off-line using the Vascular Plaque Quantification (VPQ) tool in QLAB 10.2 (Philips Health Care, Andover, MA, USA) at the Centro Nacional de Investigaciones Cardiovasculares (CNIC) Imaging Core Laboratory. For analysis, three contours were defined: the media-adventitia boundary, the lumen-intima boundary, and the plaque-lumen boundary *(Online Figure 1)*. Ultrasound readers can then review and manually correct the contours that the software semi-automatically propagates throughout the imaged vessel. In this study, 3DVUS acquisitions were analyzed by four trained technicians blinded to other test results. There is currently no standard definition of atherosclerosis plaque using 3DVUS, and plaque was therefore defined using the Mannheim criteria for 2DVUS as a focal protrusion into the arterial lumen of thickness >0.5 mm or >50% of the IMT or IMT >1.5 mm (14). Plaque burden was quantified by measuring the volumes of all atherosclerotic plaques visualized within the standardized

6-cm acquisition. Global plaque burden was defined as the sum of plaque volumes in the right and left carotid and femoral arteries. We also recorded plaque presence (yes/no), the number of arteries affected, and the total number of plaques present in the 3DVUS acquisitions. Participants without plaques in the analyzed segment were assigned a plaque burden of 0 mm^3. Plaque volume was not adjusted for artery size because we analyzed a fixed 6-cm segment regardless of total vessel size.

Image quality was classified as "good" (diagnostic acoustic window without significant artifacts), "acceptable" (suboptimal window or artifacts but analysis could still be performed), and "inadequate" (reliable analysis not possible). Additionally, we identified specific features that may limit plaque analysis like "low-echodensity" (echogenicity similar to blood), "calcification" (causing significant acoustic shadowing), and "complex morphology" (highly irregular surface and possible surface defects).

To assess the reproducibility of 3DVUS for plaque detection and volume measurement, a random sample of 32 participants was drawn from the entire study cohort, providing a total of 128 3DVUS studies (64 carotid and 64 femoral). Pairs of technicians analyzed all selected studies to evaluate interobserver agreement. To evaluate intraobserver agreement, the same reader repeated the initial analysis after 1 month.

Statistical methods

Baseline characteristics are presented as mean ± standard deviation or median and interquartile range (IQR) for continuous variables and counts and proportions for categorical variables. Differences between continuous variables and categorical variables were tested with unpaired t tests and chi-square tests, respectively. Variables with non-normal distribution were log-transformed before comparison. For reproducibility analyses, intraclass correlation coefficient (ICC) and Bland-Altman plots were employed.

Kappa coefficients were used to assess agreement of plaque detection between techniques. For ICC and *Kappa*, good agreement was defined as >0.70 and excellent agreement as >0.90.

Global plaque burden by 3DVUS is a right-skewed variable. The relationship between CVRFs and global plaque burden was explored by ordinal logistic regression, with plaque volume tertiles (outcome) used to classify atherosclerotic burden as none, mild, moderate, or severe. The candidate variables in the multivariable model were sex, age, smoking in pack-years, dyslipidemia, diabetes, hypertension, obesity, and family history of cardiovascular disease. To compare the effects of different CVRFs on plaque burden in our cohort we calculated the "adequacy" index (15). When excluding participants without plaques, linear regression on log-transformed plaque burden was used to investigate sex and age-related changes in the extent of carotid and femoral subclinical atherosclerosis. In addition, we used linear regression on log-transformed data to explore the association between global plaque burden and ASCVD risk score stratified by number of plaques and affected territories. A p for trend <0.05 was considered significant. Statistical analyses were conducted with Stata 12 (Stata Corp, College Station, TX).

RESULTS

The PESA cohort consists of 4184 participants. A total of 33 participants withdrew and 167 were excluded due to incomplete 3DVUS studies in at least one of the 4 territories, resulting in 3984 participants available for analysis. A further 8 participants were excluded due to inadequate image quality in one or more territories and 116 were excluded due to missing information about CVRFs. The final study population thus comprised 3860 (92.2%) PESA participants (Figure 1). Baseline demographic, clinical, and laboratory characteristics are summarized in Table 1. In brief, there was a relatively

low prevalence of diabetes, hypertension, obesity, and family history of premature cardiovascular disease; in contrast, there was a non-negligible prevalence of dyslipidemia and cigarette smoking. Overall, the PESA participants are largely a low-risk population (median ASCVD risk 2.17% [IQR 0.95-4.37%]), with most (79.4%) in the low-risk category.

A total of 15 936 arteries (99% of the 3DVUS studies) were successfully analyzed, with good image quality in 93% *(Online Table 1).* Acceptable quality window was slightly more frequent in the carotid territory than in the femoral territory (7.6% vs 6.2%). Low-echogenic plaques (4.4%) or plaques with complex morphologies (2.3%) did not generally prevent adequate plaque volume analysis; and severe calcification was rare (0.03%). Interobserver and intraobserver agreement was good for the detection of plaque (*kappa* ranging from 0.87 to 0.97 and 0.94 to 1, respectively) and for global plaque burden quantification [ICC 0.89 (95%CI 0.86-0.91) and 0.87 (95%CI 0.83-0.90), respectively], with similar good results for carotid and femoral territories when analyzed separately (*Online Tables 2 and 3*). Bland-Altman plots for global and regional plaque volume quantification are shown in *Online Figure 2*.

Sex, age, and territorial distribution of plaque burden by 3DVUS

Plaque detection and plaque burden quantification by age, sex and territory is shown in Table 2. Median global plaque burden was 50.8 mm^3 [IQR 18.7-121.5], being more pronounced in men than in women (63.4 mm^3 [IQR 23.8-144.8] vs 25.7 mm^3 [IQR 11.5-61.6], $p<0.001$). For both sexes, plaque burden was higher in the femoral than the carotid territory ($p<0.001$) and increased significantly with age (Table 2). Men tended to have a higher age-related plaque burden increase for the femoral territory (Figure 2); nevertheless, this did not reach statistical significance when compared to the carotid

territory or to women (p=0.1 for both). Plaque burden values by percentiles are shown in *Online Table 4*, and percentile curves for each sex as a function of age are shown in Figure 3.

Similar results were observed for plaque presence (Table 2). Atherosclerosis was more prevalent in men (55.8%) than in women (30.5%). Overall, plaques were more frequent in the femoral than the carotid territory (32.1% vs 27.2%, p<0.001); however, while this remained true in men (42% vs 30.8%, p<0.001), the opposite was observed for women (15.1% vs 20.9%, p<0.001).

Association of CVRFs and plaque burden

The cardiovascular risk profile of participants without plaque and across tertiles of global plaque burden is showed in Table 3, and separately for carotid and femoral arteries in *Online Tables 5 and 6*. As expected, participants in the highest tertile were older and had a higher prevalence of all CVRFs except for family history of premature cardiovascular disease. The univariate and multivariate predictors of plaque burden are summarized in Table 4. Age, male sex, and all CVRFs demonstrated independent associations with plaque burden, but not family history and obesity. The strongest associations were noted for age, sex, and smoking exposure, followed by dyslipidemia, and hypertension, whereas diabetes was the weakest independent predictor in our cohort, and was not significant for carotid plaque burden. When comparing association of risk factors with either carotid or femoral plaque burden, the multivariate associations of age, male sex, smoking and dyslipidemia were stronger for the femoral than the carotid territory, while diabetes and hypertension showed no significant territorial differences. The magnitudes of these differences are shown in Figure 4.

Relationship between plaque volume *versus* plaque detection and cardiovascular risk scales

Most participants were disease-free (53.6%). Involvement of 1, 2, 3, or 4 territories was noted in 22.9%, 14.2%, 5.6%, and 3.6% participants, and 1, 2, 3, or ≥4 plaques were detected in 19.8%, 11.6%, 6.3%, and 8.7% participants, respectively (Table 5 and Figure 5; Central Illustration). Figures 5A and 5C display the distribution of disease presence across ASCVD risk categories. Plaque presence increased across risk categories whether assessed by the number of territories affected or the number of plaques. However, a non-negligible proportion of participants classified at high-risk had only localized disease (19.1% and 15.4% of participants with 1 territory or plaque, respectively) and participants with extensive disease were found among the low-risk subgroup (1.8% and 4.6% of participants with 4 territories or ≥4 plaques). Detailed data on the number of plaques and affected territories as well as plaque burden across ASCVD risk categories are shown in Table 5.

Global plaque burden showed a significant correlation with cardiovascular risk categories, and this relationship held after stratification by the number of affected territories (Figure 5B) or plaque number (Figure 5D). Plaque volume correlated significantly with higher cardiovascular risk category independently of the number of affected territories or the number of plaques, with a p trend <0.01 for all comparisons.

DISCUSSION

In the present study, we evaluated 3DVUS in a large population. We provide evidence that 3DVUS is a feasible and reproducible approach to not only detect but also quantify early atherosclerotic burden in the carotid and femoral arteries. In addition, we report the reference values of atherosclerotic plaque volume for a middle-aged, asymptomatic

population. The key messages derived from our observations are as follows: 1) Carotid and femoral 3DVUS identifies higher plaque burden in men, in the femoral arteries, and with increasing age; moreover, the pattern of disease changes with age is sex- and artery-dependent; 2) 3DVUS-detected plaque burden is strongly associated with CVRFs, and this association is more significant for the femoral than for the carotid territory; 3) The evaluation of plaque burden in addition to plaque presence provides a closer match with global cardiovascular risk.

Prevalence and distribution of plaque burden by 3DVUS

A "pseudo-3DVUS" (freehand-2D-sweep/3D-like reconstruction) method was used previously for the carotid territory in the BioImage Study (10); however, the present study is the first study to evaluate carotid and femoral atherosclerosis with a true 3DVUS approach in a large cohort. In agreement with previous 2DVUS studies, we found that the plaque burden in men is almost double that in women within the middle-age range (16,17). However, we also found a higher plaque volume in the femoral territory in both sexes, whereas plaque presence was more frequent in the femoral arteries in men but in the carotid arteries in women. Furthermore, age-related changes in plaque volume tended to be more rapid in the femoral arteries in men. Thus, compared with plaque detection, plaque volume quantification unveils novel sex and artery-related differences in the early development of atherosclerotic disease. Prevalence of carotid atherosclerosis has been reported to be low in women before menopause (18,19), subsequently becoming similar to the prevalence in men (20). Our data suggest that the same pattern may occur with femoral atherosclerosis evaluated by 3DVUS. These observations are in accordance with the later development of cardiovascular disease in women until menopause worsens their cardiovascular risk profile. In addition, there is an increasing awareness of the importance of femoral artery evaluation for the detection of early subclinical atherosclerosis (7,8,21-

23). Our results confirm that this territory is more extensively diseased in both sexes, suggesting a potential to improve early disease evaluation in the young.

The availability of reference values in the population has been extremely valuable for image-based strategies for cardiovascular risk stratification. In the literature on CACS and carotid IMT, values above the 75th percentile are considered pathological in the general population (24,25). There are no previous reports on true 3DVUS-volumetric data in population-based cohorts, and this study provides a large dataset for future reference. Ongoing long-term PESA follow-up will help to identify associations with cardiovascular events.

Relationship between plaque burden and CVRFs

Many previous reports have associated CVRFs with carotid IMT and CACS (26,27). More recently, the Aragon Workers' Heart Study (AWHS) assessed the association between CVRFs and carotid and femoral atherosclerosis in 1423 asymptomatic men between 40 and 59 years old (22). However, this study only evaluated the presence of plaque with 2DVUS and not its burden. Only a few groups have used quantitative 2DVUS measurements of peripheral atherosclerosis, like plaque area or plaque thickness, in the general population (28,29). Our study confirms the strong association between multiple CVRFs and atherosclerotic plaque burden as measured with 3DVUS. Age was the strongest predictor of plaque burden, followed by sex and the modifiable risk factors, as demonstrated by the adequacy index. Among the modifiable risk factors, smoking exposure in pack-years was strongly associated with atheroma burden, followed by dyslipidemia, hypertension and, to a lesser extent, diabetes. Although this is in apparent contradiction with the well-documented relation between diabetes and increased atherosclerosis, it probably reflects the low prevalence of diabetes in our cohort and the

likely short disease exposure among those who are affected. In a further analysis (data not shown), being a smoker also showed an independent association with plaque burden (Adequacy index = 0.16 $p<0.001$). However, pack-years of smoking was more strongly related than the simple smoking status to baseline plaque burden. Contrasting with our results, AWHS did not find dyslipidemia and diabetes to be significant predictors of the presence of carotid plaques even though these risk factors were more prevalent than in our study, highlighting the difference between simple plaque detection versus burden quantification. In this regard, Yerly et al. evaluated the link between CVRFs and 2DVUS-quantified carotid and femoral atherosclerosis in a middle-aged cohort (n=496, age 45-64 years) (29). CVRFs were more strongly associated with quantitative plaque measures than IMT. Notably, the relationship between CVRFs and carotid plaque thickness was inconsistent and become clearer when plaque area was measured. The authors attributed this to high variability in 2DVUS measurements, something that 3DVUS has partially overcome (30). Together, the results of these studies and ours suggest that more comprehensive measures of atherosclerotic burden such as plaque volume may better reflect long-term CVRF exposure.

Territorial differences in the relationship between plaque burden and CVRFs

Beyond the age- and sex-based differences discussed above, the associations between CVRFs and plaque burden tended to be stronger for the femoral than the carotid territory. Similar observations were reported in AWHS (22) and by Yerly et al. (29), both using 2DVUS, although these studies did not make formal comparisons. Our analyses confirm these observations, showing statistical significant differences for sex, age, smoking, and dyslipidemia. Smoking has been linked to peripheral artery disease and intermittent claudication (31), and early atherosclerotic changes are more significant in the femoral compared to the carotid territory in patients with familial hypercholesterolemia (32). The

apparent lack of territorial differences in hypertension contrasts with previous reports that have tended to link high blood pressure with carotid atherosclerosis and stroke (16,33). However, these studies did not include assessment of the femoral territory and usually enrolled older populations. In accordance with our results, the few available reports in middle-aged participants show a relationship between hypertension and both carotid and femoral subclinical atherosclerosis (22,29,34). The mechanisms of these artery-related differences are unclear, and are likely related to both histological and hemodynamic factors (35). Overall, our findings support multi-territorial imaging for comprehensive evaluation of subclinical atherosclerosis and the combined effect of CVRFs in early atheroma formation.

Relationship of plaque volume and plaque presence with cardiovascular risk

Our results confirm the well-established mismatch between cardiovascular risk profile determined by risk scales and the presence of subclinical atherosclerosis (7,36), which has been attributed to variable individual susceptibility. However, adding plaque burden quantification to the detection of plaque presence provides a clearer picture of the relationship between risk and subclinical disease: among individuals with the same number of plaques or affected territories, global plaque burden was independently and positively associated with estimated ASCVD risk. Global plaque volume, by integrating plaque presence, number, and plaque size, is a more comprehensive index of disease burden that may better reflect individual susceptibility, and thus partially explain the mismatch between risk profile and plaque presence. The difference between disease presence and volume is also illustrated by the discrepancy between these two methods in comparing disease severity between the femoral and carotid arteries in women; the number of plaques was similar or even lower in the femoral territory, but femoral plaque volume was almost double that in the carotid territory. The potential of plaque burden

quantification to improve cardiovascular risk stratification is suggested by the BioImage study, which showed that quantifying carotid burden as the sum of consecutive plaque areas in mm^2 improves risk prediction, risk reclassification, and statin eligibility for primary prevention strategies with comparable results to CACS (37,38). Also, previous studies using 2D plaque area measurements demonstrated significant improvement in risk prediction of myocardial infarction, stroke, and even cardiovascular death (39-41). Our results suggest that the ability of global plaque burden to predict cardiovascular risk is likely to be improved by adding evaluation of the femoral arteries at early stages of cardiovascular disease.

Feasibility and reproducibility of 3DVUS PESA protocol

Assessment of the 3DVUS method in PESA demonstrated good feasibility for screening early atherosclerosis, providing a unique opportunity to study subclinical disease in large populations. Previous 3DVUS approaches reported drop-out rates that varied depending on the clinical context, ranging from 23% in patients undergoing revascularization of peripheral artery disease (42) to 33% in patients with recent ischemic stroke or transient ischemic attack (43). The main reported cause of dropout in these studies was a high percentage of severely calcified plaques, suggesting that 3DVUS performs worse at advanced stages of atherosclerosis compared with our sample of individuals in the early stages of disease. Also, the 3DVUS protocol developed in PESA, by centering the acquisition at bifurcations without probe displacement, is simple and has good reproducibility, ensuring the re-evaluation of the same segment during follow-up to detect small changes and facilitating the generalizability of our results across sites.

Study limitations

This is a cross-sectional study and so does not allow causal inference about the relationships between CVRFs and plaque volume. Similarly, whether 3DVUS plaque volume is associated with increased number of events is currently unknown, and we can only infer a potential role to predict cardiovascular risk. Future follow-up data from the PESA cohort will help confirm this hypothesis by evaluating associations with cardiovascular outcomes. Finally, our sample represents a middle-aged, white-collar working population, which may limit the generalizability of the results, and our findings need further validation in other cohorts.

CONCLUSIONS

3DVUS is a feasible, reproducible and novel imaging technique for quantifying early carotid and femoral atherosclerotic burden in large populations. Quantitative plaque burden, especially in the femoral arteries, is associated with conventional CVRFs. Additionally, 3DVUS offers incremental value over the presence of plaque alone in its association with cardiovascular risk. This inexpensive and radiation-free technology thus has the potential to become a key large-scale screening tool for identifying at-risk individuals.

CORE CLINICAL COMPETENCIES: Atherosclerosis is the leading cause of death and disability worldwide, and the introduction of effective strategies for primary prevention is a healthcare priority. Atherosclerosis burden quantification by carotid and femoral 3DVUS has potential as a primary measure of individual cardiovascular risk, complementing scales based on traditional risk factors and improving previous 2DVUS-based atherosclerotic risk markers. In addition, the standardized 3DVUS PESA protocol for carotid and femoral plaque burden quantification is a feasible and reliable imaging method for screening the general population.

TRANSLATIONAL OUTLOOK IMPLICATIONS: Extending the use of imaging techniques to the general population requires a technique that is simple, reliable and cost-effective. 3DVUS using the volumetric-linear array method is valid for imaging superficial peripheral atherosclerosis burden from early to advanced stages. 3DVUS has the potential to become a key population screening tool for identifying at-risk individuals, targeting preventive therapy or monitoring the response to treatment. Moreover, this bedside method is simple and radiation-free, encouraging its uptake in clinical practice and large-scale trials.

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FIGURE TITLES AND CAPTURES

Figure 1. Flow diagram on the 3DVUS study population

Figure 2. Distribution of femoral and carotid atherosclerotic plaque volume by sex and age

Box-plots represent median and interquartile range, and dots represent the outlier values of femoral and carotid plaque burden in mm^3 across age categories in those participants with evidence of atherosclerotic disease (i.e. excluding participants with a plaque burden of 0 mm^3). The p values for trend of the age-related increases in carotid and femoral plaque burden are shown.

Figure 3. Percentiles of global plaque burden stratified by age and sex

Each plot shows the curves for the percentiles of global plaque burden in mm^3 across age in the study cohort, including healthy participants (plaque burden of 0 mm^3) and diseased participants. Disease appears later in women than in men, and plaque burden in men is strikingly higher than in women of the same age.

Figure 4. Multivariable analysis for the prediction of carotid and femoral atherosclerotic plaque burden

Smoking, dyslipidemia, diabetes and hypertension were predictors of atherosclerotic plaque burden after adjustment for sex, age, and all evaluated CVRFs. The strength of the relationship between sex, age, and CVRFs tends to be higher in the femoral territory, except for hypertension.

95% CI: 95% confidence interval. CV: cardiovascular. PB: plaque burden.

CENTRAL ILUSTRATION Figure 5. Relationship between global plaque burden and the estimated cardiovascular risk stratified by plaque presence-based atherosclerosis markers

A and **C** show the distribution of disease among ASCVD risk categories stratified by the number of territories affected and the number of plaques (excluding no disease). **B** and **D** show mean global plaque burden across risk strata adjusted by the number of territories affected and number of plaques. The p values for the trend between plaque burden and ASCVD risk are shown.

TABLES

Table 1. Characteristics of the study population

	Total (n=3860)	Men (n=2434)	Women (n=1426)	p value
Age (years)	45.8 ± 4.3	46.2 ± 4.4	45.0 ± 3.9	<0.001
CV risk factors				
Dyslipidemia	1600 (41.5)	1303 (53.5)	297 (20.8)	<0.001
Diabetes	71 (1.8)	64 (2.6)	7 (0.5)	<0.001
Hypertension	447 (11.6)	378 (15.5)	69 (4.8)	<0.001
Smoking	794 (20.6)	467 (19.2)	327 (22.9)	0.005
Pack-years of smoking	14.5 [7.2-24.3]	15.8 [7.4-26.6]	13.0 [7.1-26.2]	0.182
Obesity	573 (14.8)	470 (19.3)	103 (7.2)	<0.001
Family history of CV disease	600 (15.5)	370 (15.2)	230 (16.1)	0.443
CV risk factors therapy				
Lipid-lowering	270 (7.0)	225 (9.2)	45 (3.2)	<0.001
Antihypertensive	287 (7.4)	246 (10.1)	41 (2.9)	<0.001
Antidiabetic	54 (1.4)	48 (2.0)	6 (0.4)	<0.001
Number of CV risk factors				
0	1699 (44)	840 (34.5)	859 (60.2)	<0.001
1	1506 (39)	1060 (43.5)	446 (31.3)	<0.001
2	564 (14.6)	454 (18.7)	110 (7.7)	<0.001
>2	91 (2.4)	80 (3.3)	11 (0.8)	<0.001
Conventional risk scales				
10-year ASCVD risk*	2.17 [0.95 – 4.37]	3.40 [1.92 – 5.77]	0.79 [0.44 – 1.59]	<0.001

Categorical variables are presented as n (%) and quantitative variables as mean ± SD or median [interquartile range; IQR]. ASCVD: atherosclerosis cardiovascular disease risk. CV: cardiovascular. *Log-transformed for the analysis.

Table 2. Prevalence of atherosclerosis and plaque burden distribution by 3DVUS

	TOTAL (n=3860)		MEN (n=2434)		WOMEN (n=1426)		*p value	
GLOBAL PLAQUE BURDEN	N (%)	Median (IQR)	N (%)	Median (IQR)	N (%)	Median (IQR)	N	Median
All	1792 (46.4)	50.8 [18.7 – 121.5]	1357 (55.8)	63.4 [23.8 – 144.8]	435 (30.5)	25.7 [11.5 – 61.6]	<0.001	<0.001
40-44 yo	584 (34.5)	31.2 [12.7 – 78.2]	421 (43.4)	42.0 [16.3 – 99.3]	163 (22.5)	16.8 [9.1 – 37.7]	<0.001	<0.001
45-49 yo	612 (47.6)	55.0 [19.7 -121.2]	445 (55.5)	66.7 [24.3 – 144.8]	167 (34.4)	30.0 [14.4 – 72.8]	<0.001	<0.001
50-54 yo	596 (67.9)	78.9 [27.7 -176.6]	491 (74.3)	86.2 [32.1 – 207.6]	105 (48.4)	38.6 [16.0 – 89.3]	<0.001	<0.001
†p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
CAROTID PLAQUE BURDEN								
All	1048 (27.2)	23.1 [9.9 – 48.7]	750 (30.8)	25.5 [11.4 – 54.9]	298 (20.9)	16.3 [7.6 – 33.6]	<0.001	<0.001
40-44 yo	342 (20.2)	17.9 [8.2 – 37.3]	225 (23.2)	20.5 [9.3 – 43.4]	117 (16.2)	13.2 [6.9 – 25.1]	0.001	<0.001
45-49 yo	338 (26.3)	22.6 [10.3 – 50.2]	234 (29.2)	25.2 [12.1 – 55.0]	104 (21.4)	17.3 [7.7 – 38.8]	0.002	0.019
50-54 yo	368 (41.9)	28.7 [12.7 – 58.1]	291 (44.0)	29.7 [13.6 – 64.1]	77 (35.5)	23.1 [9.9 – 46.4]	0.027	0.065
†p value	<0.001	<0.001	<0.001	0.002	<0.001	<0.001		
FEMORAL PLAQUE BURDEN								
All	1238 (32.1)	64.0 [27.6 – 140.5]	1023 (42.0)	72.5 [30.8 – 157.1]	215 (15.1)	36.7 [18.2 –78.7]	<0.001	<0.001
40-44 yo	331 (19.5)	48.2 [23.8 – 107.5]	271 (27.9)	56.7 [26.8 – 118.4]	60 (8.3)	28.2 [15.0 – 45.2]	<0.001	<0.001
45-49 yo	441 (34.3)	63.3 [25.9 – 131.2]	346 (43.1)	67.6 [30.9 – 151.2]	95 (19.6)	36.7 [17.9 – 90.5]	<0.001	<0.001
50-54 yo	466 (53.1)	82.2 [35.2 – 177.0]	406 (61.4)	86.0 [37.8 –194.2]	60 (27.6)	47.2 [19.9 – 95.8]	<0.001	<0.001
†p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.018		
‡p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

*p value of the differences between men and women. †p value of the differences among age strata. ‡ p value of the differences between carotid and femoral territory. Plaque volume data only include participants with plaques (values of 0mm^3 were excluded for the calculation).

Table 3. Participant's characteristics according to global plaque burden tertiles

	Global Plaque Burden				
	Volume 0 mm^3	**Tertile 1 1 – 26.4 mm^3**	**Tertile 2 26.4 – 88.1 mm^3**	**Tertile 3 > 88.1 mm^3**	p value
Age (years)	44.6 ± 3.9	45.8 ± 4.3	46.9 ± 4.3	48.3 ± 4.1	<0.001
Sex (male)	991 (47.9)	221 (37.2)	146 (24.5)	68 (11.3)	<0.001
CV risk factors					
Dyslipidemia	648 (31.3)	258 (43.4)	310 (52)	384 (63.8)	<0.001
Diabetes	17 (0.8)	8 (1.4)	16 (2.7)	30 (5)	<0.001
Hypertension	153 (7.4)	60 (10.1)	94 (15.8)	140 (23.3)	<0.001
Smoking	298 (14.4)	123 (20.7)	150 (25.2)	223 (37)	<0.001
Pack-years of smoking	9.2 [5.1-16.8]	13.0 [7.0-21.7]	17.3 [9.5-25.0]	22.8 [11.6-32.5]	<0.001
Obesity	251 (12.1)	77 (13)	119 (20)	126 (20.9)	<0.001
Family history of CV disease	308 (14.9)	94 (15.8)	96 (16.1)	102 (16.9)	0.191
CV risk factors therapy					
Lipid-lowering	74 (3.6)	34 (5.7)	70 (11.7)	92 (15.3)	<0.001
Antihypertensive	87 (4.2)	42 (7.1)	58 (9.7)	100 (16.6)	<0.001
Antidiabetic	14 (0.7)	8 (1.4)	12 (2)	20 (3.3)	<0.001
Number of CV risk factors					
0	1144 (55.3)	254 (42.8)	183 (30.7)	118 (19.6)	<0.001
1	741 (35.8)	240 (40.4)	278 (46.6)	247 (41)	<0.001
2	174 (8.4)	91 (15.3)	114 (19.1)	185 (30.7)	<0.001
>2	9 (0.4)	9 (1.5)	21 (3.5)	52 (8.6)	<0.001
Conventional risk scales					
10-year ASCVD risk	1.44 [0.65 - 2.87]	2.16 [1.07 – 4.04]	3.38 [1.71 – 5.55]	5.05 [2.92 – 8.19]	<0.001

ASCVD: atherosclerosis cardiovascular disease. CV: cardiovascular.

Table 4. Univariate and multivariate predictors of global, carotid, and femoral plaque burden

Univariate Proportional Odds

	Global Plaque Burden		Carotid Plaque Burden		Femoral Plaque Burden		
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	‡ p value
Sex (male)	3.19 (2.79 to 3.65)	<0.001	1.76 (1.51 to 2.05)	<0.001	4.28 (3.63 to 5.05)	<0.001	<0.001
Age (5 years)	2.07 (1.92 to 2.22)	<0.001	1.73 (1.59 to 1.88)	<0.001	2.19 (2.02 to 2.38)	<0.001	<0.001
Smoking	2.43 (2.10 to 2.81)	<0.001	1.83 (1.56 to 2.16)	<0.001	2.59 (2.22 to 3.01)	<0.001	0.003
Smoking (10 pack-years)	1.77 (1.65 to 1.90)	<0.001	1.45 (1.35 to 1.55)	<0.001	1.76 (1.64 to 1.89)	<0.001	<0.001
Dyslipidemia	2.60 (2.30 to 2.94)	<0.001	1.86 (1.61 to 2.14)	<0.001	2.97 (2.59 to 3.40)	<0.001	<0.001
Diabetes	4.06 (2.63 to 6.25)	<0.001	2.80 (1.78 to 4.41)	<0.001	4.34 (2.85 to 6.60)	<0.001	0.181
Hypertension	2.69 (2.24 to 3.23)	<0.001	2.15 (1.76 to 2.62)	<0.001	2.69 (2.23 to 3.24)	<0.001	0.129
Family history of CV disease	1.12 (0.95 to 1.31)	0.191	1.12 (0.93 to 1.36)	0.242	1.16 (0.97 to 1.39)	0.102	0.796
Obesity	1.67 (1.41 to 1.97)	<0.001	1.43 (1.19 to 1.72)	<0.001	1.81 (1.52 to 2.16)	<0.001	0.074

†***Multivariate Proportional Odds***

	OR (95% CI)	AI	p value	OR (95% CI)	p value	OR (95% CI)	p value	‡ p value
Sex (male)	2.63 (2.27 to 3.04)	0.32	<0.001	1.38 (1.17 to 1.62)	<0.001	3.55 (2.97 to 4.26)	<0.001	<0.001
Age (5 years)	1.79 (1.66 to 1.93)	0.41	<0.001	1.53 (1.40 to 1.67)	<0.001	1.87 (1.71 to 2.03)	<0.001	<0.001
Smoking (10 pack-years)	1.75 (1.62 to 1.88)	0.28	<0.001	1.36 (1.26 to 1.46)	<0.001	1.73 (1.60 to 1.87)	<0.001	<0.001
Dyslipidemia	1.55 (1.35 to 1.77)	0.24	<0.001	1.33 (1.14 to 1.55)	<0.001	1.68 (1.44 to 1.95)	<0.001	<0.001
Diabetes	1.91 (1.21 to 3.02)	0.04	0.006	1.58 (0.98 to 2.53)	0.059	1.84 (1.17 to 2.89)	0.008	0.185
Hypertension	1.47 (1.21 to 1.80)	0.12	<0.001	1.44 (1.16 to 1.78)	<0.001	1.39 (1.13 to 1.71)	0.002	0.116
Family history of CV disease	1.05 (0.88 to 1.25)	0.00	0.621	1.05 (0.87 to 1.28)	0.605	1.09 (0.90 to 1.32)	0.395	0.744
Obesity	0.98 (0.82 to 1.18)	0.04	0.843	1.01 (0.82 to 1.23)	0.961	1.03 (0.85 to 1.24)	0.798	0.060

AI: adequacy index. CI: confidence interval. CV: cardiovascular. OR: odds ratio.

*Unadjusted odds ratios for conventional cardiovascular risk factors (CVRF). †Adjusted model by sex, age, and CVRFs (pack-years of smoking, dyslipidemia, diabetes, hypertension, family history of CV disease, and obesity).

‡ p value of the difference between carotid and femoral OR.

Table 5. Relationship with ASCVD risk of the number of affected territories, the total number of plaques, and plaque burden

	10-year ASCVD risk							
	TOTAL	LOW ASCVD <5% (n=3066)		INTERMEDIATE ASCVD 5-7.5% (n=423)		HIGH ASCVD ≥7.5% (n=371)		*p value
TERRITORIES AFFECTED	N (%)	N (%)	Median [IQR] mm3	N (%)	Median [IQR] mm3	N (%)	Median [IQR] mm3	Median (mm^3)
NO PLAQUE	2068 (53.6)	1869 (60.9)	0	127 (30.02)	0	72 (19.4)	0	-
1 TERRITORY	886 (22.9)	698 (22.7)	18.8 [9.1-40.1]	117 (27.6)	27.7 [11.8-49.6]	71 (19.1)	27.5 [12.0-55.4]	<0.001
2 TERRITORIES	549 (14.2)	346 (11.2)	76.9 [35.6-121.1]	105 (24.8)	88.4 [49.7-158.5]	98 (26.4)	119.2 [56.6-188.3]	<0.001
3 TERRITORIES	217 (5.6)	97 (3.1)	131.9 [74.7-206.0]	48 (11.3)	162.9 [89.5-248.5]	72 (19.4)	185.3 [92.9-274.5]	0.005
4 TERRITORIES	140 (3.6)	56 (1.8)	246.5 [125.1-352.8]	26 (6.1)	260.7 [182.9-359.8]	58 (15.6)	310.2 [195.5-510.2]	0.014
	10-year ASCVD risk							
		LOW ASCVD <5% (n=3066)		INTERMEDIATE ASCVD 5-7.5% (n=423)		HIGH ASCVD ≥7.5% (n=371)		*p value
NUMBER OF PLAQUES	N (%)	N (%)	Median [IQR] mm^3	N (%)	Median [IQR] mm^3	N (%)	Median [IQR] mm^3	Median (mm^3)
NO PLAQUE	2068 (53.6)	1869 (61.0)	0	127 (30.0)	0	72 (19.4)	0	-
1 PLAQUE	763 (19.8)	606 (19.8)	17.6 [8.7 – 36.7]	100 (23.6)	27.1 [11.1 – 47.3]	57 (15.4)	25.7 [9.7 – 46.2]	0.001
2 PLAQUES	450 (11.6)	301 (9.8)	49.3 [24.0 – 95.6]	82 (19.6)	67.6 [37.2 – 153.7]	66 (17.8)	64.7 [33.7 – 130.9]	<0.001
3 PLAQUES	242 (6.3)	149 (4.9)	89.8 [51.0 – 146.2]	44 (10.4)	112.2 [74.7 – 177.2]	49 (13.2)	121.3 [73.5 – 216.7]	<0.001
≥4 PLAQUES	337 (8.7)	141 (4.6)	156.3 [84.5 – 262.7]	69 (16.3)	183.6 [92.3 – 304.4]	127 (34.2)	238.5 [150.5 – 376.2]	<0.001

ASCVD: atherosclerosis cardiovascular disease. IQR: interquartile range.

*p of trend in the association between disease burden and estimated ASCVD risk

Figure 1. Flow diagram on the 3DVUS study population

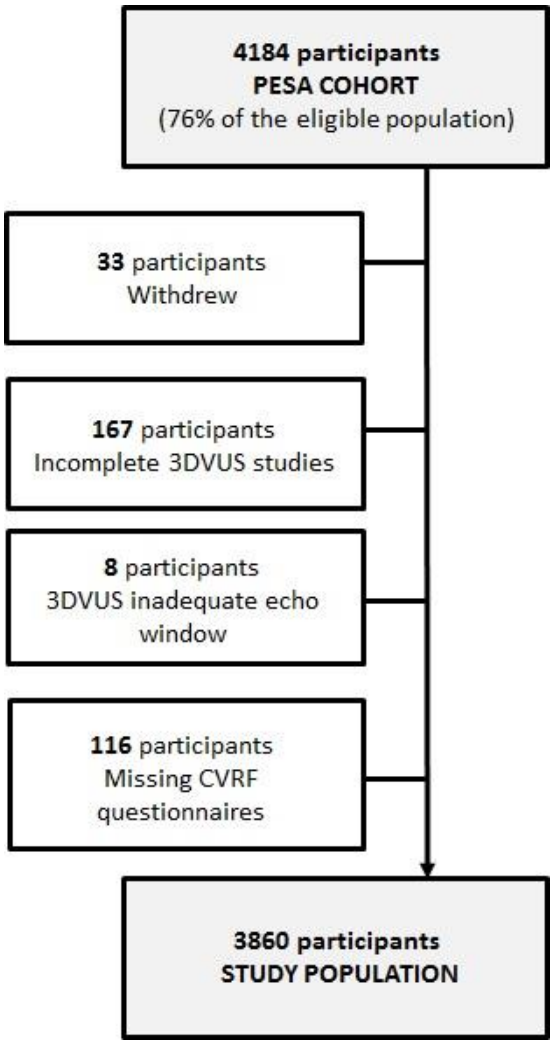

Figure 2. Distribution of femoral and carotid atherosclerotic plaque volume by sex and age

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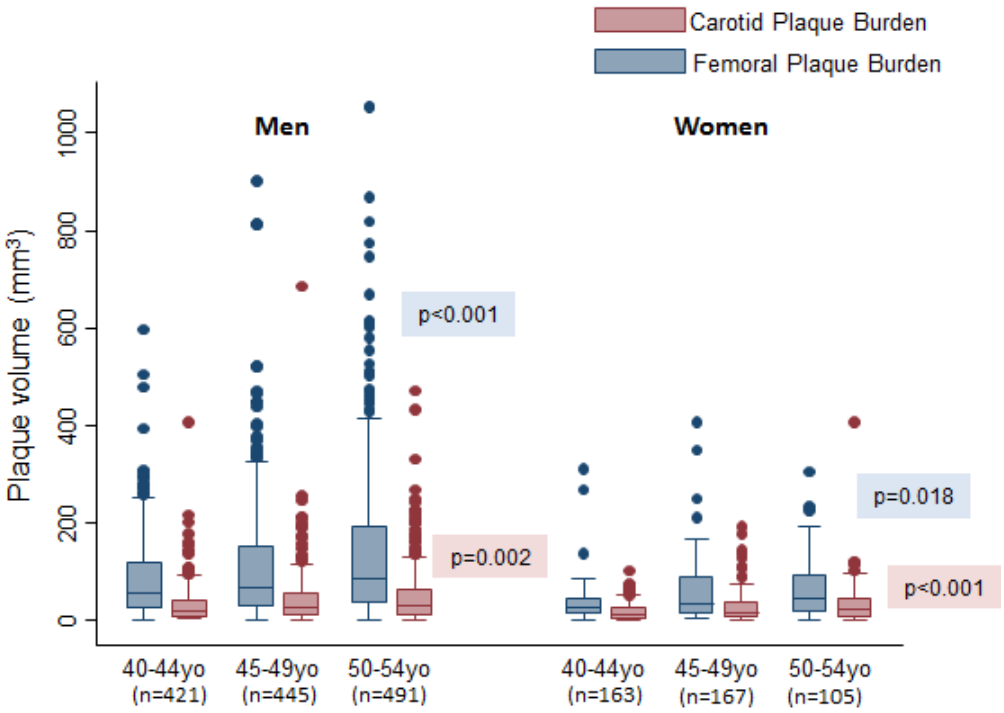

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Each plot shows the curves for the percentiles of global plaque burden in mm^3 across age in the study cohort, including healthy participants (plaque burden of 0 mm^3) and diseased participants. Disease appears later in women than in men, and plaque burden in men is strikingly higher than in women of the same age.

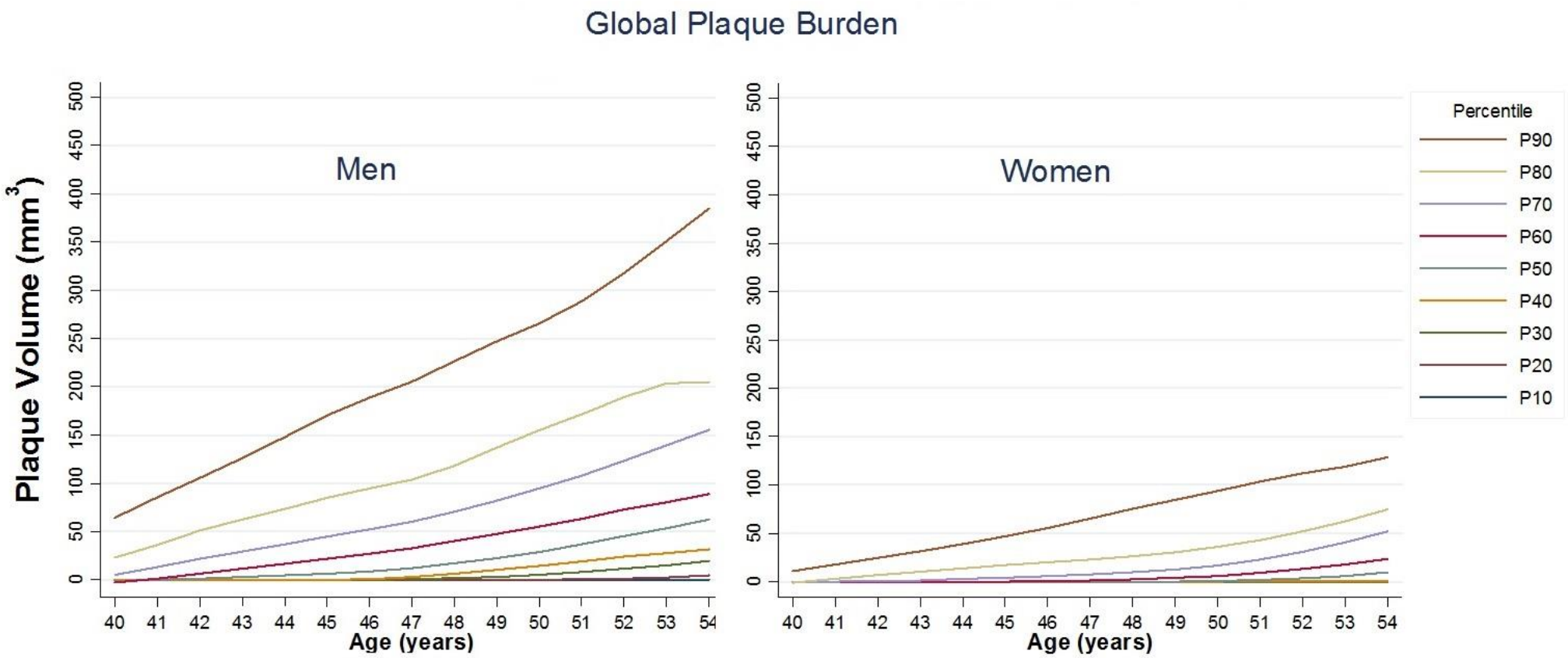
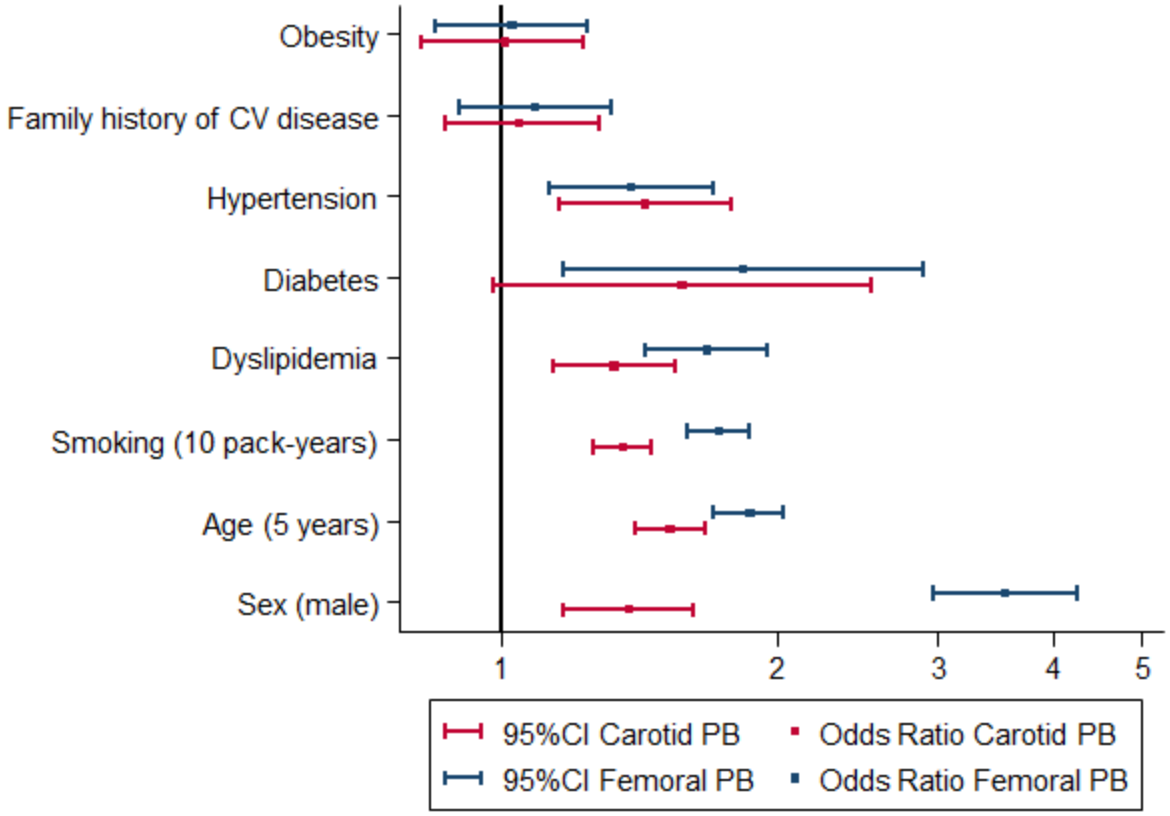


Figure 4. Multivariable analysis for the prediction of carotid and femoral atherosclerotic plaque burden

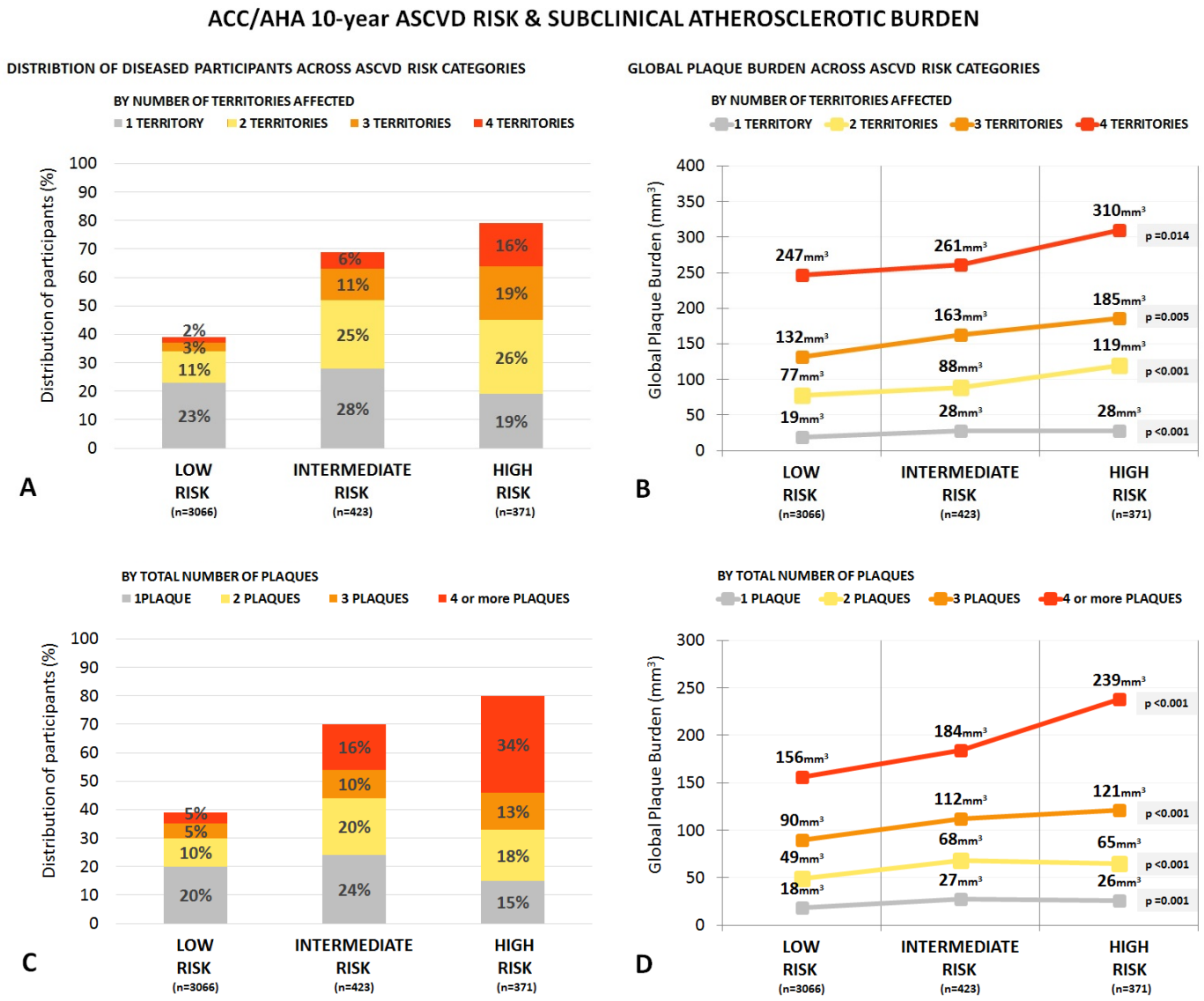
Smoking, dyslipidemia, diabetes and hypertension were predictors of atherosclerotic plaque burden after adjustment for sex, age, and all evaluated CVRFs. The strength of the relationship between sex, age, and CVRFs tends to be higher in the femoral territory, except for hypertension.

95% CI: 95% confidence interval. CV: cardiovascular. PB: plaque burden.



CENTRAL ILUSTRATION Figure 5. Relationship between global plaque burden and the estimated cardiovascular risk stratified by plaque presence-based atherosclerosis markers

A and **C** show the distribution of disease among ASCVD risk categories stratified by the number of territories affected and the number of plaques (excluding no disease). **B** and **D** show mean global plaque burden across risk strata adjusted by the number of territories affected and number of plaques. The p values for the trend between plaque burden and ASCVD risk are shown.



SUPPLEMENTAL MATERIAL

Online table 1. Feasibility analysis of 3DVUS in the PESA cohort

	3DVUS		
Feasibility analysis in PESA cohort	Total	Carotid	Femoral
Missing 3DVUS studies	167	35	136
Nº arteries analyzed	15 936	7968	7968
Nº arteries with atherosclerosis	3293 (20.6%)	1416 (17.7%)	1877 (23.5%)
Echo quality:			
Good	14 821 (93.0%)	7355 (92.3%)	7466 (93.6%)
Acceptable	1108 (6.9%)	610 (7.6%)	498 (6.2%)
Inadequate	7 (0.04%)	3 (0.04%)	4 (0.05%)
Limiting plaque features:			
Low-echogenic plaques	145 (4.4%)	35 (2.4%)	110 (5.8%)
Sever calcification with echo-shadows	1 (0.03%)	0	1 (0.05%)
Complex plaque morphologies	78 (2.3%)	29 (2.0%)	49 (2.6%)

Online Table 2. *Kappa* values for agreement in plaque detection with 3DVUS

Interoserver and intraobserver *kappa* coefficients.

Interobserver Kappa for plaque detection on 3DVUS						
n=69	All territories		Carotid territory		Femoral territory	
	Observer 2	Observer 3	Observer 2	Observer 3	Observer 2	Observer 3
Observer 1	0.95	0.89	0.94	0.84	0.97	0.93
Observer 2		0.87		0.84		0.90
Intraobserver Kappa for plaque detection on 3DVUS						
	All territories		Carotid territory		Femoral territory	
Observer 1	1		1		1	
Observer 2	1		1		1	
Observer 3	0.95		0.94		0.97	

Online Table 3. Reproducibility of 3DVUS atherosclerotic plaque volume measurement in all territories and in carotid and femoral arteries separately

Interobserver and intraobserver intraclass correlation coefficient (ICC) and 95% confidence interval.

Interobserver ICC for 3DVUS Volume measurement						
All territories			Carotid territory		Femoral territory	
n=69	Observer 2	Observer 3	Observer 2	Observer 3	Observer 2	Observer 3
Observer 1	0.81 (0.70-0.88)	0.85 (0.77-0.91)	0.89 (0.76-0.95)	0.80 (0.58-0.91)	0.77 (0.59-0.87)	0.83 (0.70-0.91)
Observer 2		0.86 (0.77-0.91)		0.76 (0.54-0.88)		0.85 (0.93-0.92)
Global	0.89 (0.86-0.91)		0.81 (0.67-0.91)		0.82 (0.71-0.89)	
Intraobserver ICC for 3DVUS Volume measurement						
n=69	All territories		Carotid territory		Femoral territory	
Observer 1	0.97 (0.94-0.98)		0.90 (0.77-0.95)		0.97 (0.95-0.99)	
Observer 2	0.96 (0.94-0.98)		0.96 (0.91-0.98)		0.95 (0.91-0.97)	
Observer 3	0.88 (0.81-0.93)		0.88 (0.76-0.94)		0.85 (0.73-0.92)	
Global	0.87 (0.83-0.90)		0.91 (0.86-0.94)		0.93 (0.90-0.95)	

Online Table 4. Percentiles of global, carotid, and femoral plaque burden by age and sex

	GLOBAL PLAQUE BURDEN (mm^3)							CAROTID PLAQUE BURDEN (mm^3)							FEMORAL PLAQUE BURDEN (mm^3)					
	Men			Women				Men			Women				Men			Women		
Age	40-45	45-50	50-55	40-45	45-50	50-55	Age	40-45	45-50	50-55	40-45	45-50	50-55	Age	40-45	45-50	50-55	40-45	45-50	50-55
n	971	802	661	724	485	217	n	971	802	661	724	485	217	n	971	802	661	724	485	217
p5th	0	0	0	0	0	0	p5th	0	0	0	0	0	0	p5th	0	0	0	0	0	0
p10th	0	0	0	0	0	0	p10th	0	0	0	0	0	0	p10th	0	0	0	0	0	0
p25th	0	0	0	0	0	0	p25th	0	0	0	0	0	0	p25th	0	0	0	0	0	0
p50th	0	9.9	45.3	0	0	0	p50th	0	0	0	0	0	0	p50th	0	0	28.4	0	0	0
p75th	31.0	75.2	147.4	0	16.3	34.4	p75th	0	7.6	25.5	0	0	11.2	p75th	12.6	58.4	128.1	0	0	11.9
p90th	106.3	200.4	304.4	21.5	62.6	116.7	p90th	25.0	39.3	69.7	9.6	19.4	45.5	p90th	89.7	163.5	260.6	0	36.3	74.3
p95th	160.7	272.1	434.1	42.2	108.9	152.5	p95th	48.0	73.2	118.3	21.9	45.8	76.0	p95th	143.2	247.7	378.1	23.4	90.3	120.7

Online Table 5. Participant´s characteristics according to carotid plaque burden tertiles

	Carotid Plaque Burden				
	Volume 0 mm^3	**Tertile 1 1 – 13.5 mm^3**	**Tertile 2 13.5 – 35.7 mm^3**	**Tertile 3 > 35.7 mm^3**	p value
Age (years)	45.2 ± 4.1	46.3 ± 4.5	47.3 ± 4.3	47.9 ± 4.2	<0.001
Sex (male)	1128 (40.1)	126 (36.3)	102 (29.3)	70 (19.9)	<0.001
CV risk factors					
Dyslipidemia	1054 (37.5)	164 (47.3)	172 (49.3)	210 (59.7)	<0.001
Diabetes	37 (1.3)	8 (2.3)	7 (2)	19 (5.4)	<0.001
Hypertension	266 (9.5)	42 (12.1)	54 (15.5)	85 (24.1)	<0.001
Smoking	501 (17.8)	86 (24.8)	88 (25.2)	119 (33.8)	<0.001
Pack-years of smoking	12.0 [6.2-21.0]	14.7 [7.7-25.2]	17.7 [8.8-31.0]	22.5 [10.5-32.4]	<0.001
Obesity	383 (13.6)	55 (15.9)	63 (18.1)	72 (20.5)	<0.001
Family history of CV disease	428 (15.2)	43 (12.4)	70 (20.1)	59 (16.8)	0.242
CV risk factors therapy					
Lipid-lowering	154 (5.5)	32 (9.2)	34 (9.7)	50 (14.2)	<0.001
Antihypertensive	163 (5.8)	29 (8.4)	36 (10.3)	59 (16.8)	<0.001
Antidiabetic	29 (1)	7 (2)	6 (1.7)	12 (3.4)	0.001
Number of CV risk factors					
0	1,364 (48.5)	128 (36.9)	123 (35.2)	84 (23.9)	<0.001
1	1,072 (38.1)	147 (42.4)	143 (41)	144 (40.9)	0.077
2	342 (12.2)	64 (18.4)	72 (20.6)	86 (24.4)	<0.001
>2	34 (1.2)	8 (2.3)	11 (3.2)	38 (10.8)	<0.001
Conventional risk scales					
10-year ASCVD risk	1.88 [0.84 - 3.72]	2.41 [1.09 – 5.06]	3.19 [1.55 – 5.80]	4.19 [2.46 – 7.63]	<0.001

ASCVD: atherosclerosis cardiovascular disease risk. CV: cardiovascular.

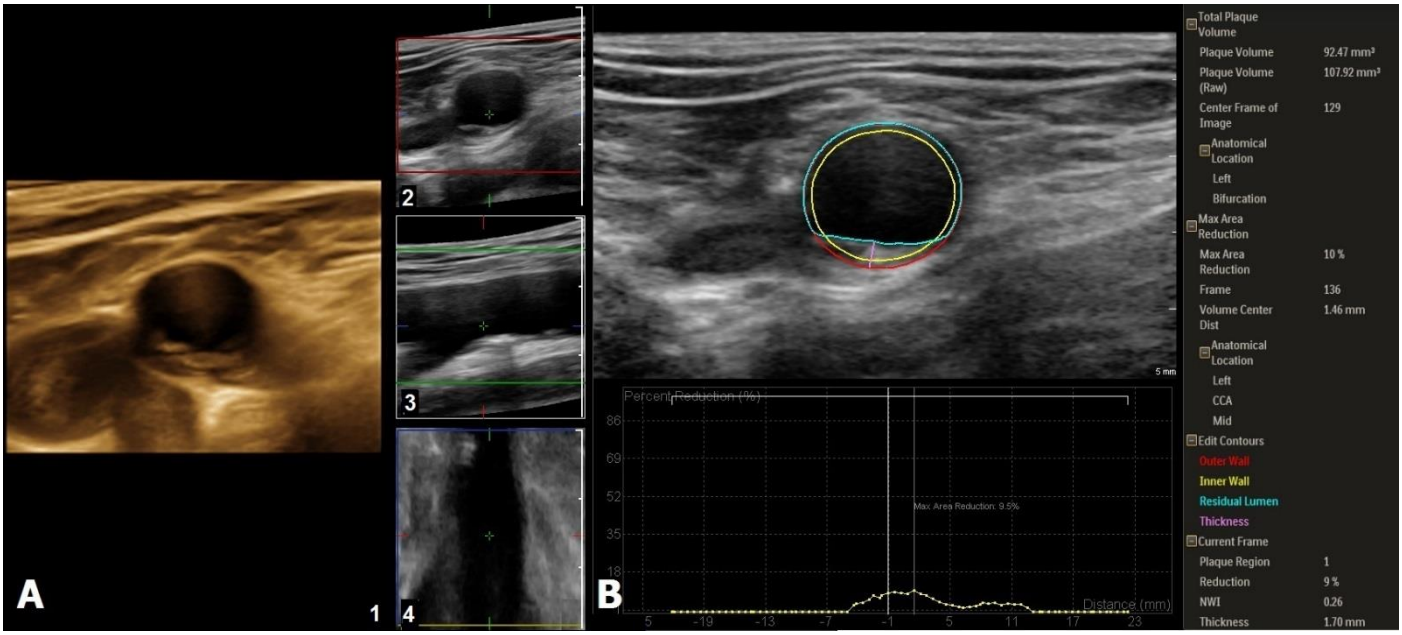
Online Table 6. Participant´s characteristics according to femoral plaque burden tertiles

	Femoral Plaque Burden				
	Volume 0 mm^3	**Tertile 1 1 – 37.7 mm^3**	**Tertile 2 37.7 – 107.4 mm^3**	**Tertile 3 > 107.4 mm^3**	p value
Age (years)	44.9 ± 4.0	46.7 ± 4.4	47.6 ± 4.1	48.5 ± 4.1	<0.001
Sex (male)	1211 (46.2)	110 (26.8)	72 (17.4)	33 (7.9)	<0.001
CV risk factors					
Dyslipidemia	863 (32.9)	229 (55.9)	237 (57.4)	271 (65.3)	<0.001
Diabetes	22 (0.8)	11 (2.7)	16 (3.9)	22 (5.3)	<0.001
Hypertension	214 (8.2)	57 (13.9)	76 (18.4)	100 (24.1)	<0.001
Smoking	403 (15.4)	108 (26.3)	122 (29.5)	161 (38.8)	<0.001
Pack-years of smoking	9.9 [5.2-18.0]	16.6 [9.7-25.4]	18.0 [10.5-28.0]	24.0 [11.7-33.0]	<0.001
Obesity	323 (12.3)	65 (15.9)	96 (23.2)	89 (21.4)	<0.001
Family history of CV disease	392 (15)	65 (15.9)	68 (16.5)	75 (18.1)	0.102
CV risk factors therapy					
Lipid-lowering	102 (3.9)	46 (11.2)	59 (14.3)	63 (15.2)	<0.001
Antihypertensive	125 (4.8)	37 (9)	54 (13.1)	71 (17.1)	<0.001
Antidiabetic	19 (0.7)	10 (2.4)	11 (2.7)	14 (3.4)	<0.001
Number of CV risk factors					
0	1397 (53.3)	122 (29.8)	107 (25.9)	73 (17.6)	<0.001
1	965 (36.8)	182 (44.4)	188 (45.5)	171 (41.2)	<0.001
2	243 (9.3)	96 (23.4)	93 (22.5)	132 (31.8)	<0.001
>2	17 (0.6)	10 (2.4)	25 (6.1)	39 (9.4)	<0.001
Conventional risk scales					
10-year ASCVD risk	1.56 [0.71-3.11]	3.14 [1.71 – 5.29]	4.03 [2.26 – 6.22]	5.46 [3.30 – 9.01]	<0.001

ASCVD: atherosclerosis cardiovascular disease risk. CV: cardiovascular.

Online Figure 1. 3DVUS atherosclerosis assessment

(A) 3D automatic sweep acquisition of a subclinical atherosclerotic plaque in the left femoral artery. Panels **2, 3,** and **4** show the *X*, *Y*, and *Z* axis views of the vessel, and panel **1** shows the reconstructed 3D volume image in an axial view of the distal common femoral artery. **(B)** Semiautomatic delineation of consecutive axial views of the atherosclerotic plaque: calculated atherosclerotic volume, 92.47 mm^3; maximum plaque thickness, 1.7 mm; and maximum percentage stenosis, 10%. The yellow line marks the intima-lumen boundary, red line the media-adventitia interface, and green line the plaque boundary.



Online Figure 2. Reproducibility of 3DVUS evaluation of global, carotid, and femoral atherosclerotic plaque burden

Bland-Altman plots for interobserver and intraobserver agreement between the three readers for global atherosclerotic plaque volume quantification **(A** and **B)** and interobserver agreement separately for carotid and femoral arteries **(C** and **D)**.

Legends: +2SD: superior limit of agreement. -2SD: inferior limit of agreement. OBS1: observer 1.