Universitat de Girona

# EXPLORATION INTO NON-TRADITIONAL RISK FACTORS OF SUBCLINICAL ATHEROSCLEROSIS IN LOW-RISK ADULTS

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Universitat de Girona

# DOCTORAL THESIS

## Exploration into Non-Traditional Risk Factors of Subclinical Atherosclerosis in Low-risk Adults

Josep Iglesias Grau

2024

**DOCTORAL THESIS. Exploration into Non-Traditional Risk Factors of Subclinical Atherosclerosis in Low-risk Adults**

This Doctoral Thesis includes two appendices with supplementary information about the main research studies.

**Annex 1**. Supplementary Information from Published Article: Subclinical Atherosclerosis in Young, Socioeconomically Vulnerable Hispanic and Non-Hispanic Black Adults.

**Annex 2**. Supplementary Information from Published Article: Early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis.

**Josep Iglesias Grau, 2024**

**This thesis is part of the official Doctoral Program in Molecular Biology, Biomedicine, and Health, directed by Dr. Rodrigo Fernández Jiménez, M.D., Ph.D., and Dr. Ramon Brugada i Terradellas, M.D., Ph.D., submitted to the University of Girona to obtain the degree of doctor, with international mention.**

To all the mentors, family, and friends who have guided me steadfastly,

Your passion and dedication,

Your constant support,

Have been invaluable throughout my journey.

*Cooperando nihil impossibile est*

In pursuit of an earlier, enhanced, and more equitable prevention.

# Acknowledgments

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# List of Publications

This doctoral thesis titled "Exploration into Non-Traditional Risk Factors of Subclinical Atherosclerosis in Low-risk Adults" is a **compendium of already published** research articles:

- Iglesies-Grau, J, Fernandez-Jimenez, R, Diaz-Munoz, R, Jaslow, R, Cos-Gandoy, A, Santos-Beneit, G, Hill, C. A, Turco, A, Kadian-Dodov, D, Kovacic, J. C, Fayad, Zahi A, Fuster, V. Subclinical Atherosclerosis in Young, Socioeconomically Vulnerable Hispanic and Non-Hispanic Black Adults. **J Am Coll Cardiol**. 2022 Jul, 80 (3) 219–229**.** https://doi.org/10.1016/j.jacc.2022.04.054

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- Iglesies-Grau, J, Fuster, V, Fernandez-Jimenez, R. Reply: Carotid Plaque Burden Is a Stronger Predictor of Cardiovascular Risk Than IMT. **J Am Coll Cardiol**. 2022 Nov, 80 (21) e193. https://doi.org/10.1016/j.jacc.2022.09.020

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- Iglesies-Grau, J, Garcia-Alvarez, A, Oliva, B, Mendieta, M, García-Lunar, I, Fuster, José J., Devesa, A, Pérez-Herreras, C, Fernández-Ortiz, A, Brugada, R, Ibanez, B, Fernandez-Jimenez, R, Fuster, V. Early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis. **Cardiovasc Diabetol.** 22, 350 (2023). https://doi.org/10.1186/s12933-023-02090-1

**Impact Factor 9.3** (year 2022)**. Q1 in Cardiac & Cardiovascular Systems**. **D1 in Endocrinology & Metabolism**.

# Abbreviations

| | |
|---|---|
| ASCVD | Atherosclerotic cardiovascular disease |
| CV | Cardiovascular |
| 2DVUS | 2-dimensional vascular ultrasound |
| 3DVUS | 3-dimensional vascular ultrasound |
| T2D | Type 2 diabetes |
| FAMILIA | Family-based approach in a minority community integrating systems-biology for the promotion of health |
| PESA | Progression of Early Subclinical Atherosclerosis |
| HOMA-IR | Homeostatic Model Assessment of Insulin Resistance |
| LDL-C | Low-density lipoproteins cholesterol |
| ApoB | Apolipoprotein-B |
| HDL-C | High-density lipoproteins cholesterol |
| CT | Computed tomography |
| MACE | Major adverse cardiovascular events |
| $PM_{2.5}$ | Small particulate matter, ≤2.5 μm in diameter |
| HbA1c | Glycated hemoglobin |
| CIMT | Carotid intima-media thickness |
| ARIC | Atherosclerosis Risk in Communities Study |
| CARDIA | Coronary Artery Risk Development in Young Adults Study |
| MESA | Multi-Ethnic Study of Atherosclerosis Study |
| CT-CACS | Computed tomography coupled with coronary artery calcium score |
| CAC | Coronary artery calcification |

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# Resum

**Antecedents**: La malaltia cardiovascular (MCV) es manifesta silenciosament com a aterosclerosi subclínica abans d'un esdeveniment clínic. Les disparitats en el risc de patir una MCV, especialment en grups tradicionalment menys investigats, així com l'augment global de factors de risc cardiovascular (FRCV), com ara l'obesitat i la diabetis tipus 2, ressalten la importància d'explorar FRCV no convencionals i entendre millor l'impacte d'indicadors precoços de malaltia, com és el cas de la resistència a la insulina, que podrien contribuir al procés ateroescleròtic subclínic.

**Metodologia**: Aquest resum sintetitza les troballes de dos estudis que investiguen l'impacte de l'etnicitat/raça i de la presència precoç de resistència a la insulina en la prevalença i distribució de l'aterosclerosi subclínica en adults joves, emprant ecografia vascular 2D i 3D, així com tomografia cardíaca. El primer estudi avalua l'aterosclerosi subclínica en 436 adults (edat mitjana 38 anys), vulnerables des del punt de vista socioeconòmicament, de Harlem, Nova York. El segon estudi analitza 3,741 individus de l'estudi PESA (edat Mitjana 46 anys), de Madrid, utilitzant l'índex del model homeostàtic de resistència a la insulina (HOMA-IR) per tal d'explorar la relació entre la resistència a la insulina precoç i l'aterosclerosi subclínica.

**Resultats**: En el primer estudi, després d'ajustar per FRCV tradicionals i factors socioeconòmics, els individus afroamericans mostren una prevalença significativament major d'aterosclerosi subclínica (12.9%) en comparació amb el grup d'origen hispànic (6.6%). En el segon estudi, s'observa que tenir valors de HOMA-IR ≥3 (referència <2) s'associa a un increment d'aterosclerosi subclínica (odds ràtio de 1.41; 95%IC: 1.01 a 1.95, p = 0.041) i la presència de calci coronari >0 (odds ràtio de 1.74; 95%IC: 1.20 a 2.54, p = 0.004), després d'ajustar per FRCV clàssics i hemoglobina glicada.

**Conclusions**: Els resultats del primer estudi demostren una major vulnerabilitat a l'aterosclerosi subclínica en persones joves d'un barri socioeconòmicament desfavorit, especialment entre aquells d'origen afroamericà, ressaltant la necessitat d'intervencions comunitàries que abordin els diferents determinants socials de la salut. Paral·lelament, els resultats del segon estudi subratllen el potencial ús clínic del HOMA-IR com una eina pràctica per a detectar individus de baix risc amb un risc augmentat de malaltia ateroscleròtica subclínica, proporcionant d'aquesta manera al clínic, una nova estratègia preventiva per millorar la salut cardiovascular com més aviat millor.

# Resumen

**Antecedentes**: La enfermedad cardiovascular (ECV) se manifiesta silenciosamente como aterosclerosis subclínica antes de presentarse como un evento clínico. Las disparidades en el riesgo de sufrir ECV, especialmente en grupos menos investigados, y el aumento global de factores de riesgo cardiovascular (FRCV), tales como la obesidad y la diabetes tipo 2, resaltan la importancia de explorar FRCV no convencionales y entender mejor el impacto de indicadores tempranos de enfermedad, como es el caso de la resistencia a la insulina, que podrían contribuir al proceso aterosclerótico subclínico.

**Metodología**: Este resumen sintetiza los hallazgos de dos estudios que investigan el impacto de la etnicidad/raza y de la presencia precoz de resistencia a la insulina en la prevalencia y distribución de la aterosclerosis subclínica en adultos jóvenes, empleando ecografía vascular 2D y 3D, así como tomografía cardíaca. El primer estudio evalúa la aterosclerosis subclínica en 436 personas adultas del estudio FAMILIA (edad media 38 años), vulnerables desde el punto de vista socioeconómico, de Harlem, Nueva York. El segundo estudio analiza a 3,741 individuos del estudio PESA (edad media 46 años) de Madrid, utilizando el índice del modelo homeostático de resistencia a la insulina (HOMA-IR) para explorar la relación entre la resistencia a la insulina precoz y la aterosclerosis subclínica.

**Resultados**: En el primer estudio, después de ajustar por FRCV tradicionales y factores socioeconómicos, los individuos afroamericanos exhibieron una prevalencia significativamente mayor de aterosclerosis subclínica (12.9%) en comparación con el grupo de origen hispánico (6.6%). En el segundo estudio, se observó que los valores de HOMA-IR ≥3 (en referencia a <2) se asociaron con un aumento en la aterosclerosis subclínica (odds ratio 1.41; 95%IC: 1.01 a 1.95, p = 0.041) y la presencia de calcio coronario >0 (odds ratio 1.74; 95%IC: 1.20 a 2.54, p = 0.004), incluso después de ajustar por FRCV clásicos y hemoglobina glicada.

**Conclusiones**: Los resultados del primer estudio destacan la mayor vulnerabilidad de aterosclerosis subclínica en personas jóvenes de un área vulnerable, especialmente entre aquellos de origen afroamericano, resaltando la necesidad de intervenciones comunitarias que aborden los determinantes sociales de la salud. Paralelamente, los resultados del segundo estudio subrayan el potencial uso clínico del HOMA-IR como una herramienta práctica para detectar individuos de bajo riesgo con un riesgo augmentado de enfermedad subclínica, proporcionando así una nueva estrategia preventiva para mejorar la salud cardiovascular de manera precoz.

# Abstract

**Background**: Atherosclerotic cardiovascular disease (ASCVD) manifests silently as subclinical atherosclerosis before a clinical event. Disparities in ASCVD risk, particularly among underrepresented ethnic and racial groups, underscore the need for comprehensive investigations into non-traditional risk factors. Additionally, the rising global burden of CV risk factors (CVRFs), including obesity and type 2 diabetes, highlights the urgency of exploring the effect of early indicators of disease like insulin resistance, which may precede overt metabolic changes and contribute to the subclinical atherosclerotic process.

**Methodology**: This abstract synthesizes findings from two studies exploring the impact of ethnicity/race and early insulin resistance on the presence, burden, and extent of subclinical atherosclerosis in young adults, employing 2D and 3D vascular ultrasound and cardiac computed tomography. Study 1 examined 436 socioeconomically disadvantaged adults from the FAMILIA study (mean age 38 years) in Harlem, New York. Study 2 analyzed 3,741 individuals from the PESA study (mean age 46 years) in Madrid, employing the homeostatic model assessment of insulin resistance index (HOMA-IR) to assess early insulin resistance.

**Results**: In the first study, after adjusting for traditional CVRFs and socioeconomic factors, non-Hispanic Black individuals had a significantly higher subclinical atherosclerosis prevalence (12.9%) than their Hispanic counterparts (6.6%), indicating heightened vulnerability. In Study 2, HOMA-IR values ≥3 (reference < 2) were associated with increased multiterritorial subclinical atherosclerosis (odds ratio 1.41; 95%CI: 1.01 to 1.95, p = 0.041) and coronary calcium scores > 0 (odds ratio 1.74; 95%CI: 1.20 to 2.54, p = 0.004), even after adjusting for key CVRFs and glycated hemoglobin, emphasizing its potential as an early marker to identify low-risk individuals with heightened risk.

**Conclusion**: The revelations from the FAMILIA study underscore the need for targeted interventions, emphasizing urgency in mitigating social determinants and structural inequalities affecting CV health. Concurrently, the revelations from the PESA cohort underscore the transformative potential of HOMA-IR screening as a practical tool, thereby providing a concrete opportunity for clinicians to intervene at earlier stages, contributing to earlier and personalized preventive strategies in CV care.

# Manuscript Overview

## Thesis theme and importance of the study

### Atherosclerosis, the defining disease of the 21st century

Atherosclerotic cardiovascular diseases (ASCVD), predominantly coronary heart disease and stroke, stand as the foremost contributors to global mortality and significant contributors to disability. These conditions are preceded by a slow, progressive, and highly preventable subclinical state known as atherosclerosis, which silently and gradually accumulates, often remaining undetected over time. Social risk factors, such as an elevated body mass index, dietary risks, environmental hazards, tobacco and alcohol consumption, and limited physical activity, contribute significantly to this burden, thriving within our obesogenic environments and the unequal structuring of resources and societies (1).

In the pursuit of predicting and preventing cardiovascular (CV) events, various CV risk scores integrating demographic data and established risk factors have emerged. However, a considerable number of events occur in individuals classified as low or intermediate risk (2). To refine the characterization of populations at higher risk of ASCVD, non-invasive imaging such as 2-dimensional and 3-dimensional vascular ultrasound (2DVUS and 3DVUS) technologies have been developed to detect the presence and quantify the burden of subclinical atherosclerosis. Furthermore, these technologies show promise in capturing and quantifying the silent progression of atherosclerosis, offering valuable insights into the early stages of ASCVD (3).

Despite significant medical advancements over the past decade, achieving uniform progress in risk reduction and the incidence of ASCVD remains a challenge, particularly within underrepresented racial and ethnic groups (4). This disparity underscores a pressing need for a more thorough examination of factors associated with the initiation and progression of subclinical atherosclerosis, particularly within underrepresented communities.

Looking at the broader landscape, there is an alarming surge in CV risk factors such as obesity and type 2 diabetes (T2D). Recent estimates project that by 2050, a staggering 1.31 billion people worldwide could be living with diabetes, disproportionately affecting socio-economically disadvantaged individuals (5). Moreover, while ASCVD typically manifests after a progressive subclinical disease process, early indicators of insulin resistance, characterized by a gradual increase in fasting insulin levels, may precede the onset of glucose intolerance and fasting glucose level alterations associated with the insulin resistance-prediabetes-type two diabetes spectrum. Unfortunately, early insulin resistance, as much as subclinical atherosclerosis, frequently evades detection until reaching more

advanced stages, underscoring the importance of proactive screening and intervention at earlier points in the disease continuum. As a result, the potential for prevention in this area is significant.

**Article 1**, **titled** "Subclinical Atherosclerosis in Young, Socioeconomically Vulnerable Hispanic and Non-Hispanic Black Adults," **published in the Journal of the American College of Cardiology in 2022** (6), explores differences in subclinical atherosclerotic disease in underrepresented communities influenced by ethnicity and race. The study delves into the early prevalence and burden of subclinical atherosclerosis among young adults (mean age: 38 years) from socioeconomically disadvantaged backgrounds in the area of Harlem, New York, utilizing data from the FAMILIA (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) trial, which included 436 cardiovascular disease-free adults (66% Hispanic and 34% non-Hispanic Black; 82% women). The findings reveal a higher prevalence of CV risk factors and subclinical atherosclerosis in the non-Hispanic black population compared to the Hispanic group. This difference persists even after adjusting for the Framingham Risk Score and traditional risk factors, with an adjusted odds ratio of 3.45 (95% CI: 1.44-8.29; P = 0.006). The paper explores potential explanations, citing differences in the prevalence of traditional risk factors such as higher rates of smoking and hypertension, disparities in social determinants of health, and the possibility of distinct disease mechanisms and genetic susceptibility. Notably, it highlights the disparities in social determinants of health, affecting access to care and treatment for individuals with early subclinical atherosclerosis within these communities.

**Article 2, titled** "Carotid Plaque Burden Is a Stronger Predictor of Cardiovascular Risk Than Intima-Media Thickness," published in the **Journal of the American College of Cardiology in 2022** (7), builds upon the prior study's findings. It emphasizes the superior predictive capacity of 3DVUS in assessing CV risk compared to the conventional measurement of intima-media thickness. This traditional measurement is widely considered a surrogate marker of atherosclerosis, but it has shown inconsistencies in validating its improved predictive capacity over conventional risk models (8,9). This paper advocates directly quantifying atherosclerosis burden through plaque area or volume measurements. It underscores the heightened predictive capability of this approach, showcasing its superiority over traditional measurements (10). Additionally, the article notes that measures of plaque burden across multiple vascular territories, not confined to the carotid, have demonstrated associations with future major adverse events.

**Article 3, titled** "Early Insulin Resistance in Normoglycemic Low-Risk Individuals is Associated with Subclinical Atherosclerosis," **published in Cardiovascular Diabetology in 2023** (11), investigates the association between early insulin resistance in normoglycemic low-risk individuals and the presence, burden, and extent of subclinical atherosclerosis, which remains inadequately elucidated in current literature. The study, based on data from the PESA (Progression of Early Subclinical Atherosclerosis) trial with 3,741 CVD-free participants (mean age: 46 years; 39% women), demonstrates that assessing the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ratio, a model that translates how much insulin is needed to keep glucose low, identifies normoglycemic individuals with an increased burden of subclinical atherosclerosis.

Specifically, HOMA-IR values ≥3 are associated with an adjusted odds ratio of 1.41(95%CI: 1.01 to 1.95, p = 0.041) for the multiterritorial extent of subclinical atherosclerosis and 1.74 (95%CI: 1.20 to 2.54, p = 0.004) for the presence of coronary calcium score, compared with the reference HOMA-IR category (<2). By identifying a simple and cost-effective metric like the HOMA-IR, the study provides a practical tool for clinicians to assess the risk of subclinical atherosclerosis in individuals without overt diabetes. This directly impacts the possibility of an earlier implementation of targeted primary CV prevention strategies.

In summary, these studies contribute to advancing CV care by exploring non-traditional CV risk factors, refining risk stratification, advocating for non-invasive imaging techniques, emphasizing early detection of subclinical atherosclerosis, using early easy-to-measure metabolic markers, and recognizing the importance of community-specific interventions.

**Collectively, the three articles in this compendium reflect my professional journey over the past five years**. They resonate with my passion for delving into disparities and seeking unconventional risk factors for early-stage subclinical atherosclerosis. The purpose behind these endeavors is twofold: first, to enable the early identification of subclinical disease, facilitating the implementation of preventive measures to mitigate ASCVD as early as possible; second, to contribute towards reducing health inequalities across diverse populations.

**I hope** the reader will find enjoyment and informative insights within this compendium, ultimately inspiring future generations to pursue earlier, enhanced, and more equitable cardiovascular prevention strategies for all.

# 1. Introduction

## SECTION 1: Atherosclerosis, Subclinical Atherosclerosis, and Cardiovascular Disease

### Definitions

**Atherosclerosis** represents a distinctive pathological progression characterized by the thickening and hardening of arteries, culminating in the vast majority of vascular incidents, encompassing coronary heart disease and stroke (12). This phenomenon arises from a complex interplay of oxidative mechanisms, inflammation-driven processes, and mechanical factors influenced by various internal and external agents. Notably, lipids carriers and high blood pressure are key contributors, exerting chronic effects on the endothelium and the arterial intima (13). As it slowly progresses, atherosclerosis is the underlying cause of one-third of all global deaths and about 50% in Westernized societies (1).

The components of the word have their origins rooted in Greek: 'athere,' denoting a paste or gruel-like substance, alluding to the soft, fatty material found within the core of a plaque; 'skleros,' signifying hardness, referencing the firm scar-like tissue that emerges subsequently, mainly due to the presence of collagen and ectopic calcium deposition; and 'osis,' the Greek suffix denoting a pathological state.

Although it usually becomes clinically apparent later in life, the condition is rooted in a progressive disease process. This process is marked by a prolonged latent and asymptomatic phase that may commence early in life. A range of CV risk factors encompassing genetic, biological, and non-biological factors expedite the advancement of this condition.

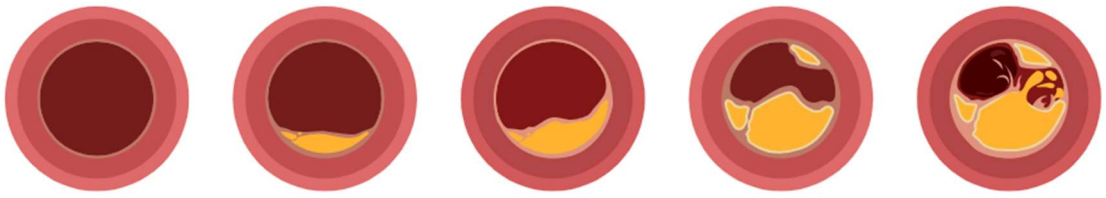


**Figure 1. Different stages of arterial atherosclerotic disease** include the accumulation of lipids in the walls (fatty streaks) and the formation of plaque and plaque rupture. This figure is my original creation and was generated using Biorender.com under a license for publication.

**Subclinical atherosclerosis** refers to the presence of disease in the absence of clinical events. It develops nicely in advance and provides an opportunity for timely detection to hinder or arrest the progression of CV disease. Indeed, identifying the presence and extent of subclinical atherosclerosis early on can serve as a guide for preventive care approaches, which will be delved into further in this thesis work. These approaches include lifestyle adjustments, revaluation of risk factors, and medical interventions such as aspirin usage, antihypertensive treatments, lipid-lowering therapies, and anti-obesity medications. These interventions can potentially prevent the eventual clinical manifestations of atherosclerosis (3).

**The central focus of this doctoral thesis, and likely a key theme in my professional career, will center around the early detection of subclinical atherosclerosis, the contributing factors to its development, and various interventions aimed at halting progression or inducing regression.**

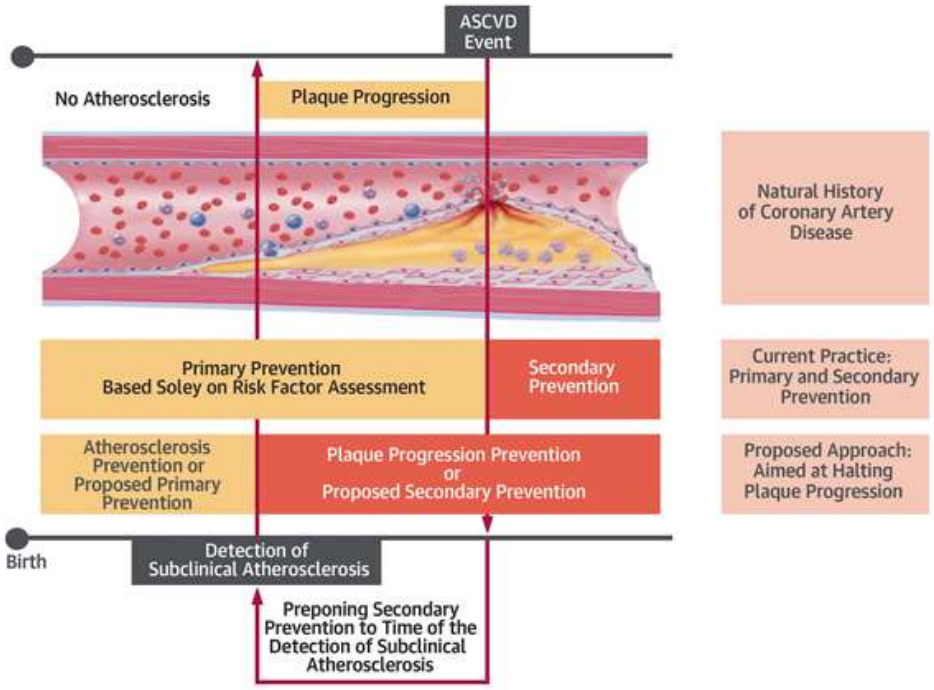


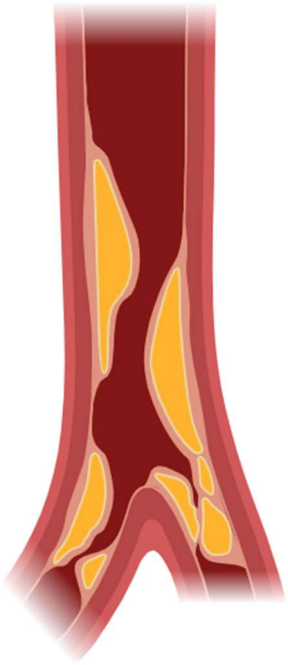

**Figure 2. The natural progression of arterial plaque leads to an atherosclerotic cardiovascular event**. This underscores the current practice strategy, primarily focused on responding after the event has already occurred. It also introduces a novel preventive approach centered around identifying subclinical atherosclerosis and implementing earlier preventive interventions. It is adapted with permission (License Number 5717830206049) from the central illustration of Ahmadi, A. et al. J Am Coll Cardiol—2019 Sep, 74 (12) 1608–1617 (14).

**Clinical manifestations of atherosclerotic cardiovascular disease.** They include conditions such as myocardial infarction, stroke, and peripheral atherosclerotic disease, among others. As patients progress toward developing obstructive disease or a primary vascular event, they not only remain asymptomatic but also typically exhibit normal results on functional tests. A crucial intermediary stage in this process involves plaque progression, connecting early non-obstructive subclinical atherosclerosis disease to the eventual rupture of plaques, triggering acute vascular events. Fortunately, advancements in imaging technology now enable the early detection of subclinical atherosclerosis.

The subsequent paragraphs will summarize the established underlying causes of the initiation and progression of atherosclerosis, the identified risk factors that accelerate atherosclerotic plaque progression, and the epidemiological tools and imaging technologies devised for atherosclerosis assessment, along with their current and potential future applications. The two adult cohorts through which subclinical atherosclerosis was examined via imaging will be delineated subsequently, forming the foundation of this PhD thesis.

## Initiation and progression of atherosclerosis

Atherosclerosis is a slow, lifelong progression of artery changes that may start early in childhood and worsen faster as we age (15), **Figure 3**. Normal endothelial functions include regulating fluid and traffic between blood flow and tissues, serving as an anticoagulant surface, contributing to angiogenesis and vascular repair, and regulating vascular tone and blood flow (16). A healthy endothelium displays a vasodilatory phenotype consisting of high nitric oxide and prostacyclin levels and low levels of reactive oxygen species. A healthy endothelium also has an anticoagulative phenotype and very low levels of inflammation parameters present (17).



Endothelial dysfunction (induced by toxic substances and blood flow patterns)

↓

Lipoprotein and monocyte influx and retention in the artery wall

↓

Lipoprotein oxidation and foam cell formation

↓

Release of cytokines, reactive oxygen species, and other inflammatory mediators

↓

Perpetuation of immune response

**Figure 3. Schematic timeline of underlying causes of the initiation and perpetuation of atherosclerotic plaque.** This figure is my original creation, using Biorender.com under a license for publication and was adapted from Stary H.C. et al., Circulation**,** 1994; 89:2462–2478 (18).

**Endothelial dysfunction**, mainly induced by toxic substances and disturbances of blood flow pattern, increases endothelial permeability to atherogenic low-density cholesterol lipoproteins (LDL-C). That is believed to be the initiating factor for atherosclerotic lesions.

One of the endothelial's primary functions is regulating the selective exchange of solutes between blood flow and tissues. According to the concentration gradient, water and small molecules may pass through the inner vascular layer into the tunica intima. In contrast,

larger molecules and cells can only pass through receptors and vesicles (e.g., endothelial transcytosis of lipoproteins) or when the endothelial junctions are impaired (19).

The glycocalyx is the first regulator of LDL-C endothelial passage, a thick matrix layer that lines the inner wall of healthy blood vessels. Several factors are known to increase the number of oxidative free radicals in the body that can affect, among others, the glycocalyx, including smoking, insulin resistance and high glucose intake, obesity, sleep deprivation, acute microbial infections, and exposure to metals and air pollutants (20,21).

The direction and velocity of the blood flow also affect endothelial cell function and survival (22). In straight arterial segments, shear stress – the force exerted parallel to the surface of the blood vessels as a result of blood flow - is high, and blood flow is uniformly laminar. In contrast, at inner curvatures, aneurysms, bifurcations, and branch points, shear stress decreases, flow is oscillatory and disturbed, and atherosclerotic plaques have been shown to have a preference for these sites (23). Importantly, endothelial cells in disturbed flow regions exhibit altered morphology and differences in gene expression patterns, such as changes in the production of matrix and pro-inflammatory molecules.

The current consensus is that physiologic laminar flow and high shear stress (>15 dyn/cm$^2$) inhibit several endothelial pro-atherogenic genes and are conducive to atherosclerosis protective genes and biologically active products, including vasodilators (nitric oxide and prostacyclin) and antithrombotic agents (thrombomodulin). Oppositely, disturbed flow and low shear stress (<4 dyn/cm$^2$) stimulate endothelial cell turnover and apoptosis and the expression of genes and gene products that promote the initiation and progression of atherosclerosis, including cytokines and growth factors, adhesion molecules, radical oxygen species, and pro-thrombotic factors (24).

**Lipoprotein & monocyte influx and retention in the artery wall**: According to the infiltration theory, the development of atherosclerosis is triggered by the entry and sub-endothelial retention of lipoprotein from the bloodstream, particularly LDL-C and apolipoprotein-B (ApoB)-containing remnants within the arterial wall (**Figure 4**). This sub-endothelial accumulation of pro-atherogenic lipoproteins is pivotal in initiating atherosclerosis (25).

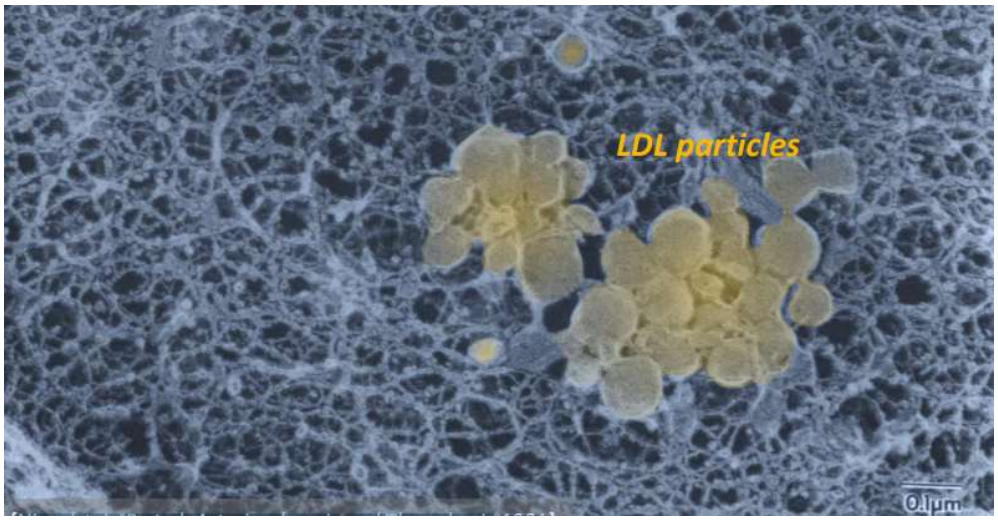



**Figure 4. Lipoprotein retention.** A cluster of LDL particles enmeshed within the extracellular matrix filaments of the aortic intima. Freeze-etch electron photomicrograph adapted from Nievelstein P. et al., Arteriosclerosis and Thrombosis 1991; 11:1795-1805 (26).

In an altered endothelial permeability rich-LDL-C and ApoB ambiance and with increased oxidative stress as the final common pathway of the effects of CV risk factors, the lipoproteins can interact longer and be trapped in the extracellular matrix filaments where molecules can be spontaneously oxidized to form among others, oxidized LDL-C (23). Oxidation, lipid hydrolysis, and other modifications of the retained, aggregated lipoproteins release biologically active byproducts that activate resident macrophages and the recruitment of monocyte-derived cells, which differentiate into mononuclear phagocytes that ingest the deposited lipoproteins to become foam cells. Their accumulation in the intima characterizes the formation of the fatty streak, the earliest morphological change occurring in atherosclerosis (27).

**Release of cytokines, reactive oxygen species, proteases, and other inflammatory mediators leading to plaque progression**

Whereas intimal infiltration and modification of plasma-derived lipoproteins and their uptake by macrophages, with the consequent formation of lipid-filled foam cells, initiate atherosclerotic lesion formation, a balance of pro-inflammatory and inflammation-resolving mechanisms dictate the final clinical outcome (28). In a healthy endothelial, removing cholesterol from the sub-endothelial space to the circulation by high-density lipoproteins (HDL-C)-mediated reverse cholesterol transport represents a relevant anti-atherogenic pathway (29). Atherosclerosis progression is triggered when the fragile

equilibrium between pro-inflammatory and anti-atherogenic factors is misbalanced. A successful resolution of inflammatory disease processes, including inhibition of inflammatory cell recruitment and clearance of apoptotic cells, would be pivotal to halt plaque progression (30).

Indeed, failure to resolve inflammation is part of the etiology of many chronic inflammatory diseases, including rheumatoid arthritis, asthma, chronic obstructive pulmonary disease, inflammatory bowel disease, cancers, Alzheimer's disease, and advanced atherosclerosis.

**Perpetuation of immune response**

The transition from the relatively simple fatty streak to the more complex lesion is mainly mediated by chemokines and cytokines and characterized by 1) A continuous recruitment of macrophages 2) The premature death of foam cells in poorly oxygenated regions of atherosclerotic lesions releasing their contents to the extracellular space, thereby worsening the inflammatory status and the structural stability of the plaque 3) The migration and proliferation of smooth muscle cells from the medial layer of the artery wall past the internal elastic lamina into the intimal/sub-endothelial space (31). Furthermore, in the presence of high levels of pro-inflammatory cytokines and modified lipoproteins, recruited macrophages polarize toward a pro-inflammatory phenotype (32). The ratio between specialized pro-resolving mediators and pro-inflammatory molecules in advanced atherosclerotic lesions is meager, providing a molecular explanation for these lesions' defective inflammation resolution features.

The infiltration and buildup of plasma lipoproteins alongside immune cell subsets collectively instigate atherosclerotic plaque. The absence of a resolution phase, responsible for mending the consequential inflammation-induced harm, propels the expansion of atherosclerotic lesions.

## Is plaque progression essential for plaque rupture?

For numerous years, the prevailing belief was that CV events primarily resulted from the rupture of mildly stenotic plaques. This was attributed to the composition and susceptibility of "soft plaques," which are more prone to rupture and thrombus formation than collagen-rich firm plaques (33). However, over the past two decades, studies have consistently reported an average culprit lesion lumen diameter stenosis of more than 60%, excluding the presence of thrombus (34). Moreover, plaque progression before the acute event has been consistently observed in various studies that were able to assess serial coronary angiograms before the occurrence of the event.

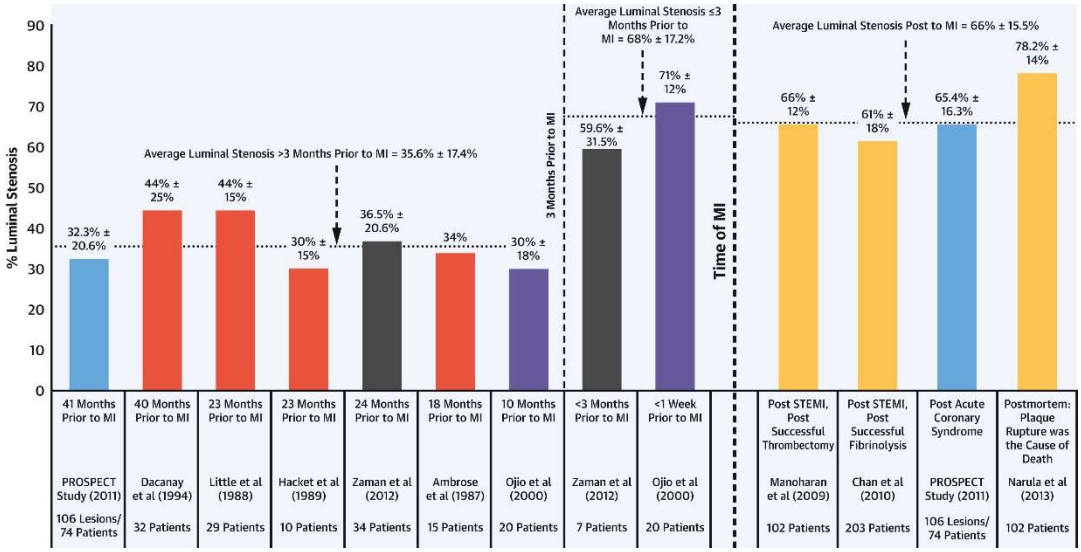



**Figure 5. Plaque progression precedes an acute coronary event**. Studies consistently show an increase in luminal stenosis over the subsequent weeks to months as the transitional phase between non-obstructive subclinical atherosclerosis and the acute coronary event. The mean percentage of lumen stenosis rises from 36±17% to 66±15%. This figure is adapted with permission (License Number 5717840680984) from Ahmadi, A. et al. J Am Coll Cardiol—2019 Sep, 74 (12) 1608–1617 (14).

**Hence, if plaque progression is noticeable before an event, what factors contribute to its advancement, and what chiefly dictates the fate of a lesion?**

Several studies have delved into this question, proposing that an expansion in the necrotic core's volume leads to the fibrous cap's attenuation, positive remodeling, and impaired vasodilatory capacity. This renders the plaque susceptible to rupture, categorizing it as a high-risk plaque commonly known as thin cap fibroatheroma.

Coronary computed tomography (CT) angiography studies are particularly significant in this context. High-risk plaques exhibiting progression over time through these studies were found to carry a 28-fold higher likelihood of triggering an acute coronary event. Conversely, plaques that did not demonstrate progression between the two coronary CT angiography assessments remained free from acute events (35). In a recent study, diabetic patients with non-revascularized fractional flow reserve negative lesions –an absence of ischemia- were divided into two groups based on the presence or absence of thin-cap fibroatheroma lesions. Patients testing positive for thin cap fibroatheroma showcased a five-fold higher incidence of major adverse cardiovascular events (MACE) despite the lack of ischemia (36).

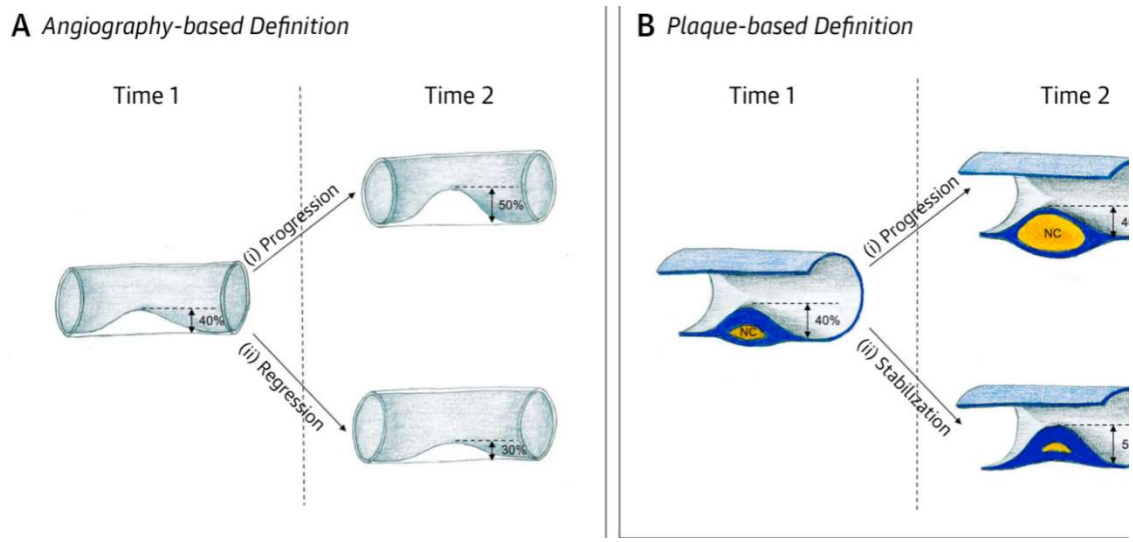



**Figure 6. The angiography-based vs. plaque-based definition of plaque progression.** It shows not only the luminal stenosis but also the morphology and composition of the plaque matter. This figure is adapted with permission (License Number 5717840513475) from Ahmadi, A. et al., and J Am Coll Cardiol—2019 Sep, 74 (12) 1608–1617 (14).

## Is it possible for plaque to regress?

Given that plaque progression, characterized by morphological changes leading to positive remodeling and a decrease in fibrous cap thickness, represents a crucial phase bridging early atherosclerosis and a major CV event, recognizing the presence of atherosclerosis, understanding the mechanisms behind its progression/regression, and preventing plaque advancement can potentially reduce the likelihood of plaque rupture and a MACE.

In this context, interventions targeting at least three pivotal pathways have demonstrated effectiveness:

1) **Lipid-driven mechanisms:** Medical intervention with lipid-lowering agents, such as statins, has shown the ability to reduce total plaque burden by decreasing the volume of the necrotic core while simultaneously increasing fibrous cap thickness, thus halting plaque progression (37,38). This effect is particularly pronounced in patients receiving intensive medical treatment to achieve LDL-C levels below 70 mg/dL, using either statins alone or in combination with proprotein convertase subtilisin/kexin type 9, PCSK9 inhibitors (39,40). Statin use has also been associated with an augmentation in calcium densification within plaques, a pattern believed to confer protection against rupture (41). Notably, the recent REDUCE-IT trial, which explored the impact of triglyceride-lowering omega-3 fatty acid (eicosapentaenoic acid) in addition to statin therapy, revealed a 31% reduction in total ischemic events compared to placebo among at-risk patients (42). Subsequent coronary CT angiography sub-studies indicated decreased high-risk plaques and total plaque volume (43).

**2) Inflammatory-driven mechanisms:** Despite substantial reductions in CV events achieved through intensive lipid-lowering therapy, the residual risk persists among patients with ASCVD, often attributable to various contributors, including inflammation. Recent prospective randomized studies have unveiled that targeting specific inflammatory pathways could offer a novel avenue for mitigating the risk of acute CV events. For example, the Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) study involving canakinumab—an IL-1β monoclonal antibody approved for rare auto-inflammatory syndromes—reported a 15% decrease in MACE compared to placebo, without an accompanying change in LDL-C levels (44). Similarly, using low-dose colchicine, the Colchicine Cardiovascular Outcomes Trial (COLCOT) (45) and the Low-dose Colchicine (LODOCO) 2 (46) trials have yielded increased reductions in CV outcomes and most recently, colchicine's multifaceted anti-inflammatory effects with diverse CV benefits have made it the first anti-inflammatory atheroprotective cardiovascular treatment available for

prescription. Numerous ongoing trials will provide additional insights in the coming years, with the potential to position anti-inflammatory drugs as a promising adjunct to standard medical therapy to prevent ASCVD.

**3) Obesity-driven Mechanisms**: Recent findings on anti-obesity drugs, notably the recent SELECT trial (47) conducted with semaglutide, a glucagon-like peptide-1 receptor agonist among individuals with established ASCVD and obesity with at least two-thirds of patients in the prediabetes range, yielded impressive results in terms of weight reduction, an increasingly recognized risk factor for CVD. This was accompanied by noteworthy decreases in MACE, including myocardial infarction, heart failure, and all-cause mortality. These effects were observed early in the treatment process and were associated with significantly decreased inflammation. This emphasizes a universal connection among risk factors like obesity, early stages of the T2D continuum, inflammation, and atherosclerotic events, supporting the hypothesis and potential impact of addressing root causes at the earliest opportunity.

Based on the mechanisms mentioned above, we can summarize that most acute coronary events originate from plaque rupture, which, in turn, results from plaque progression—a modifiable process that can even show regression. With this comprehension, numerous avenues exist to mitigate residual risk, and various therapeutic investigations are currently underway. However, the question arises: What is the optimal timing for preventing the initiation and progression of atherosclerosis, and who should be the direct targets of these efforts, and by what means? How can lifestyle modifications effectively reduce lipid levels, mitigate inflammation, and lower the risk associated with ectopic fat accumulation? Is there a common underlying factor that links inflammation, ectopic fat buildup, and elevated blood plasma lipid levels, thereby exacerbating the progression of atherosclerosis? Additionally, what role does the early detection of subclinical atherosclerosis play in event prevention? Can we enhance prevention efforts or improve efficiency by categorizing patients based on the absence or presence of subclinical atherosclerosis? Does the burden of atherosclerosis hold significance?

To offer insights and generate hypotheses, the following passages will explore biological and non-biological factors linked to the initiation and advancement of atherosclerosis. Furthermore, the discussion will encompass the significance of early detection of uncomplicated subclinical atherosclerosis, its existing assessment methods, and the potential window of opportunity it presents for efficacious interventions aimed at halting plaque progression in the early stages, thereby reducing the occurrence of clinical events.

## SECTION 2: Imaging subclinical atherosclerosis

### Non-invasive imaging of subclinical atherosclerosis

Given the slow, progressive subclinical phase characteristic of CV disease, using non-invasive imaging techniques has garnered increasing attention. These techniques prove invaluable in assessing direct signs of early subclinical CV disease and signs of progression, offering a promising avenue to optimize preventive strategies at the population and individual levels. As we delve into this arena, the potential for early detection tools becomes even more salient, presenting an unprecedented opportunity to address CV disease at its nascent stages and institute proactive preventive measures as early as possible.

Population-based epidemiological studies investigating subclinical atherosclerosis have incorporated various non-invasive imaging modalities. Chronologically, key cohort studies include the Atherosclerosis Risk in Communities (ARIC) study, the Coronary Artery Risk Development in Young Adults (CARDIA) study, the Multi-Ethnic Study of Atherosclerosis (MESA), the Bioimage study, and more recently, the Progression of Early Subclinical Atherosclerosis (PESA) study.

Commencing in 1986, the ARIC study utilized 2DVUS to examine the carotid and popliteal arteries (48). Simultaneously, the CARDIA study, initiated in the same year with a focus on over 5000 young adults aged 18-35, also employing vascular ultrasound to measure CIMT, along with echocardiography and non-contrast computed tomography coupled with coronary artery calcium score (CT–CACS) (49).

In 2000, the Multi-Ethnic Study of Atherosclerosis (MESA) study was launched, incorporating diverse imaging techniques such as CT–CACS, cardiac magnetic resonance for ventricular mass and function assessment, and 2DVUS for carotid intima-medial wall thickness and distensibility evaluation (50,51).

As part of the High-Risk Plaque initiative, the Bioimage study aimed to determine cost-effective treatment strategies for individuals at intermediate or high risk of developing CV disease. It introduced innovative technology, pseudo-3DVUS, alongside other non-invasive techniques. In contrast to previous studies, vascular ultrasound in Bioimage identified more carotid plaques and established that atherosclerosis is a generalized disease across multiple vascular beds (10).

Commencing in 2010, the PESA study aimed to detect atherosclerotic disease in its earliest phases, involving over 4000 mid-life volunteers from Banco Santander (aged 40 to 55) (52).

This specific cohort will be further developed in Section 5 of the introduction, as it has been one of the cohorts used for this Ph.D. work. In consideration of a summary, the PESA Cohort utilized a 3DVUS approach together with vascular imaging of the aorta and CT-CACS to find that subclinical atherosclerosis was already highly prevalent (63%) in a cohort of CV disease-free participants, even in those with low Framingham 10-year risk (58%), highlighting the value of imaging for early diagnosis and prevention. The study concluded that 3DVUS is a cost-effective, radiation-free, and feasible imaging technique with the potential to be a key large-scale screening tool for identifying at-risk individuals in clinical and longitudinal settings.

When integrated with traditional CV risk factors, the assessment of subclinical atherosclerosis furnishes supplementary insights into the risk of myocardial infarction, stroke, and CVD disease mortality (53). Initially, utilizing 2DVUS to estimate CIMT garnered most of the attention. While certain prospective studies demonstrated that CIMT enhanced the predictive capacity of traditional risk models (54), others showed inconsistency in validating these findings (8,9). Consequently, the 2013 American College of Cardiology/American Heart Association guideline for CVD risk prediction stopped recommending its routine measurement (55).

A more robust predictive value was attributed to the presence of carotid plaques. Noteworthy contributions include a significant net reclassification index in the Framingham Offspring Study cohort (54) and a substantial risk reclassification of up to 23% of the individuals in the ARIC study (56). A meta-analysis encompassing 11 cohort studies involving 54,336 subjects has robustly affirmed the link between plaque presence and CV events (57).

The BioImage study revealed that the carotid plaque burden assessed through 3DVUS was on par with CT-CACS in predicting mortality, myocardial infarction, angina, and coronary revascularization during a mean follow-up of nearly three years (10).

Recent cohorts, exemplified by the PESA study, aim to explore the association between the presence, extent, and burden of subclinical atherosclerosis, including examining coronary artery subclinical disease and the incidence of CV disease events. The potential for individualized preventive actions is immense.

## Definitions of plaque by vascular ultrasound and computed tomography

For this Ph.D. work, three vascular imaging modalities - 2DVUS, 3DVUS, and non-contrast CT-CACS - were employed to evaluate the presence and extent of subclinical atherosclerosis. The subsequent section will delineate the protocols and definitions of plaque, extension, and plaque burden utilized in the study.

a) 2DVUS, cross-sectional sweeps were conducted in the carotid, abdominal aortic, and femoral territories.

b) 3DVUS utilized a standardized 6-cm acquisition for quantifying atheroma plaque volume in the carotid and femoral arteries.

c) non-contrast CT-CACS determined the coronary artery calcification score using the Agatston method (58).

In the context of vascular ultrasound usage, plaque was defined based on the Mannheim consensus criteria as a focal structure encroaching into the arterial lumen, measuring ≥0.5 mm or >50% of the surrounding intima-media thickness, or possessing a diffuse thickness ≥1.5 mm measured from the media–adventitia to the intima–lumen interface in any of the territories (59).

The extent of atherosclerosis was determined by the number of regions with plaque presence, categorized as disease-free (0 vascular sites affected), focal disease (1 territory affected), or multiterritorial disease (>1 territory affected). Plaque burden was quantified as the plaque volume and measured in $mm^3$. Global plaque burden represents the cumulative sum of all plaque areas from all images displaying plaque, encompassing both carotid and femoral arteries.

Examples of images of plaque detected by 2DVUS, 3DVUS, and CT-CACS are depicted in **Figure 7**.

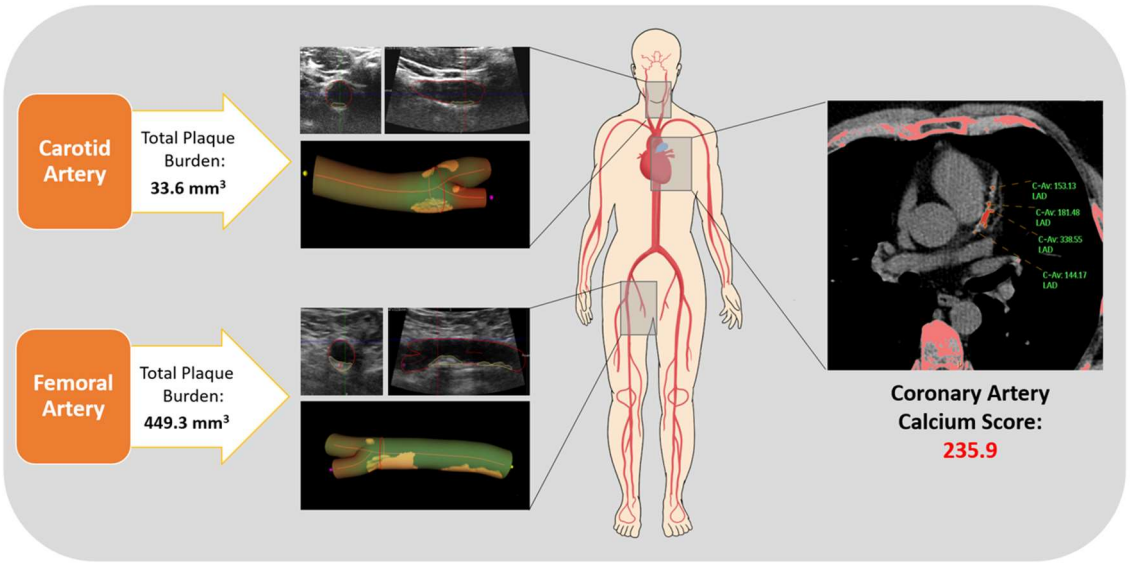



**Figure 7. Assessment of plaque using vascular ultrasound and non-contrast CT-CACS.** This figure is my original creation and was generated using PowerPoint and images from a participant of the Early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis study.

## SECTION 3: Multifactorial Mechanisms of Atherosclerosis

### Traditional CV risk factors

Arterial hypertension, unhealthy dietary habits, smoking, and air pollution are all correlated with early endothelial dysfunction, vascular oxidative stress, inflammation, and vascular remodeling. As previously described, these factors contribute to the initiation and progression of atherosclerosis, ultimately culminating in CV events. The impact of these potentially modifiable risk factors on CV events has been globally recognized, irrespective of the income level of the country of residence (60).

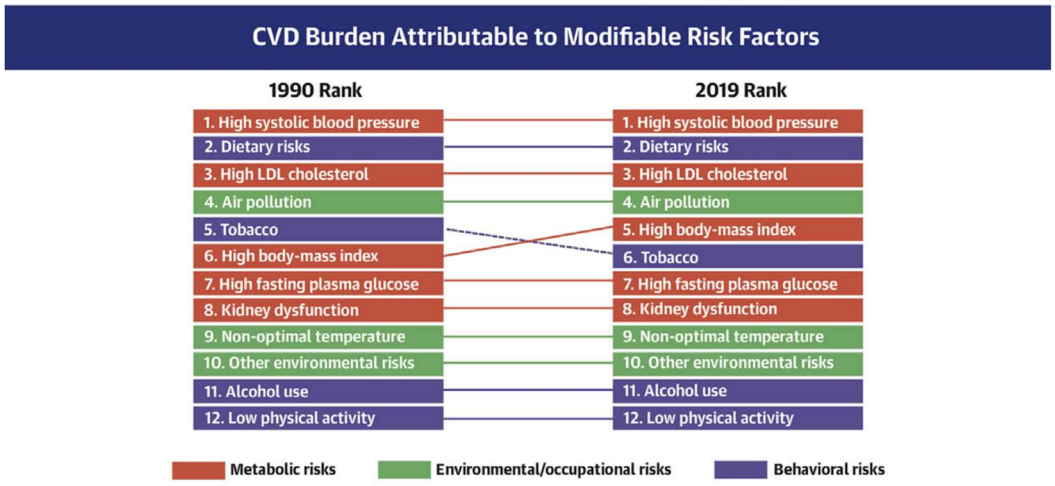



**Figure 8. ASCVD burden attributable to modifiable risk factors** is depicted in this figure. The major contributors include 1) High blood pressure, 2) High fasting plasma glucose, high LDL-C, high body-mass index (globally, dietary risks), and 3) Smoking and air pollution, which have been demonstrated to initiate and contribute to the progression of atherosclerosis. Numerous other risk factors have been identified, although they play relatively minor roles compared to the significant impact of the "big three." The following lines will provide a review of their roles in the development of atherosclerosis. Adapted from the Central Illustration (Open Access). Roth G.A et al. J Am Coll Cardiol. 2020 Dec, 76(25) 2982–3021 (1).

**Arterial hypertension** stands as the most prevalent risk factor correlated with elevated CV morbidity and mortality. Hypertension primarily manifests through morphological alterations in the arterial endothelium and hypertrophy of the smooth muscle within the arterial media. It is connected to heightened trans-endothelial LDL-C permeability in arteries, excessive production of reactive oxygen species, and inflammation (61). The escalation of inflammation corresponds to an increase in endothelial dysfunction,

subsequently leading to elevated hypertension, thereby creating a cyclical pattern. Hypertension's severity directly correlates with the extent of impairment in endothelial function (62). Other factors contributing to hypertension, such as sympathetic nervous system activation, aging, or aldosterone, are likewise associated with inflammation (63). Clinically, lowering blood pressure significantly reduces vascular risk across diverse baseline levels and comorbidities, with a 10-mmHg reduction in systolic blood pressure leading to substantial risk reductions in MACE, coronary heart disease, stroke, and heart failure, resulting in a significant 13% reduction in all-cause mortality (64).

**Unhealthy dietary habits** are closely associated with a higher body mass index, dyslipidemia, early insulin resistance, metabolic syndrome, prediabetes, and T2D. These conditions are interconnected with high blood pressure, ectopic body fat accumulation, inflammation, and elevated LDL-C levels, all widely recognized as primary contributors to atherosclerosis and pivotal in the development of ASCVD.

The ongoing obesity pandemic, coupled with the subsequent surge in T2D and other metabolic issues, is occurring concurrently with an unprecedented level of food processing by the industry. This shift has moved dietary patterns from meals and dishes crafted from unprocessed or minimally processed ingredients to those predominantly centered around ultra-processed food and drink products. These ultra-processed items are linked to excessive calorie consumption, weight gain (65), the presence of subclinical atherosclerosis (66), occurrences of coronary heart and cerebrovascular events (67,68), and even cancer (67). For instance, recent data reveals that ultra-processed foods constitute over 50% of daily caloric intake for both adults and children in certain high-income nations (69,70).

Despite available treatments, each distinct stage along the insulin resistance-prediabetes-T2D continuum is associated with an escalated risk of CV, microvascular, and other complications (71,72). A significant gradient of CV risk is observed across glycated hemoglobin (HbA1c) levels, starting as low as 5.4%, below the threshold for diabetes (73,74). Pre-diabetic levels of HbA1c also show an association with the presence of multi-territorial extent of subclinical atherosclerosis, thereby identifying asymptomatic individuals at high risk (75).

**Smoking and air pollution** contribute to inflaming an already highly combustible environment. Cigarette smoking exhibits a robust correlation with subclinical atherosclerosis across various vascular beds, emerging as a primary driver of non-communicable diseases on a global scale. This habit significantly elevates the risk not only for ASCVD but also for pulmonary conditions and cancer (76). Smoking undermines the

integrity of blood vessel walls by altering endothelial cell function, potentially heightening lipid permeability, and influencing all stages of atherosclerosis, ranging from endothelial dysfunction to acute occlusive clinical events. Smoking is responsible for 50% of all avoidable deaths among smokers, diminishing their lifespan by approximately 10 years on average. Undoubtedly, one of the most impactful approaches to ASCVD prevention is championing a healthy lifestyle throughout one's life. However, if there were one definitive action to reduce CV risk, it would be quitting smoking.

Moreover, over the past two decades, a growing body of evidence has indicated that not only active or passive smoking but also air pollution contributes to the initiation and progression of atherosclerosis, resulting in a reduction in life expectancy comparable to that of smoking (77). Precisely, the adverse health effects of both acute and chronic exposure to gaseous pollutants (ozone, nitrogen dioxide, carbon monoxide) and fine particulate matter with a diameter of ≤2.5 μm ($PM_{2.5}$) have been extensively investigated. These pollutants predominantly originate from fossil fuel combustion, industrial processes, forest fires, and power generation, with no identified safe lower exposure thresholds at the population level (78). As we recently delineated, the pathophysiology of PM2.5-induced CV disease initiates at the pulmonary level (given the inhalational nature of air pollution), inciting pollution-mediated oxidative stress and local inflammation. This cascade advances with the release of biologically active inflammatory intermediates (systemic inflammation) and the translocation of pollutant constituents into the circulation (pollutant translocation), ultimately resulting in endothelial barrier disruption, vascular inflammation, atherosclerosis, heightened coagulation, and thrombosis (79). On average, every 10 μg/m3 increase in $PM_{2.5}$ exposure results in a 1% rise in all-cause mortality. The long-term impact on health is mainly associated with $PM_{2.5}$ exposure, with evidence suggesting that reducing exposure to these particles is linked to improvements in inflammation, thrombosis, oxidative stress, and a decrease in CV death (80).

The influence of air pollution on CV and overall health remains a significant area of scientific interest, particularly in the context of urbanization, widespread migration to urban areas, global warming, climate change, and wildfires, all of which are expected to intensify. Air pollution and climate change share common precipitating factors and exhibit a complex, bidirectional relationship expected to escalate exponentially. This interplay will likely emerge as one of the most significant existential threats to humanity and public health.

## Social determinants of health

Two standardized case-control international studies on modifiable risk factors associated with myocardial infarction (INTERHEART) and stroke (INTERSTROKE) robustly showed that CV risk factors are responsible for more than 80% of CV events (60,81). However, subclinical atherosclerosis and ASCVD, intricate processes with profound health implications, are subject to a nuanced interplay between various biological and non-biological factors. These factors span the spectrum of **social determinants of health**, reflecting the social circumstances under which individuals are born, live, and work (82). Key components within this framework include socioeconomic status, economic stability, education, employment status, access to healthcare and its quality, and neighborhood socioeconomic factors (83). **Socioeconomic status**, in particular, emerges as a pivotal determinant influencing individuals' opportunities for engaging in health-promoting behaviors and accessing essential resources. Socioeconomic inequality significantly contributes to disparities in disease burden, as individuals from lower socioeconomic backgrounds often encounter formidable obstacles to adopting healthier lifestyles and obtaining comprehensive healthcare. In addition, disparities based on sex have been shown in several studies (84).

With limited access exacerbating these disparities, **educational attainment** further compounds individuals' challenges when making health decisions beyond their academic background. For instance, this study conducted on individuals without known CV disease found that lower education level was significantly associated with a higher risk of generalized subclinical atherosclerosis, with lifestyle behaviors, particularly tobacco consumption, explaining 70.5% of the observed effect, highlighting the need for targeted smoking cessation programs, especially in populations with lower education levels (85). Low levels of education are also associated with a higher incidence of and mortality of ASCVD (86).

Unstable employment or job insecurity can contribute to chronic stress, a well-established risk factor for ASCVD (87). **Disparities in healthcare access** contribute to the delayed diagnosis and treatment of CV conditions, further exacerbating the progression of atherosclerosis (88). **Neighborhoods with limited resources**, poor infrastructure, and reduced access to fresh, healthy food can contribute to an unhealthy lifestyle and increase the risk of ASCVD (89).

An illustration of the convergence of the impact of these social determinants of health can be seen in the analysis of the prevalence of CV risk factors and established ASCVD among

**migrants** from various national origins residing in Catalonia—a region with universal healthcare access. Notably, migrant groups exhibited a higher prevalence of most risk factors and established CV disease despite their relatively young age compared to the local population (90).

Finally, it is not solely the influence of individual social determinants of health that is significant, but rather the cumulative impact arising from the aggregation of these determinants. This study, conducted on 6,479 participants from the MESA cohort, investigated the cumulative effects of social determinants of health on cardiovascular health (91). Using a composite score based on 14 components across five domains representing the cumulative number of unfavorable social determinants of health, the research found that increasing social disadvantage was associated with higher odds of prevalent cardiovascular risk factors, systemic inflammation, and incident ASCVD. Smoking was the risk factor most strongly associated. These findings emphasize the crucial role of addressing social determinants of health to enhance CV outcomes.

## Racial and ethnic disparities and their association with ASCVD

Race and ethnicity categorize humans based on their origins, culture, or descent from specific geographical areas and have been routinely employed in epidemiology to explore associations. The categories used in medicine and research align with the recommendations from the Office of Management and Budget, encompassing two ethnicities and a minimum of five races (see **Table 1**).

While these categories genuinely group individuals based on intrinsic, distinct biological factors and underlying population genetics, the ongoing debate centers on whether they inadvertently aggregate people based on non-biological risk factors, such as socioeconomic status or other social determinants of health. A further question remains: does categorizing individuals by race and ethnicity offer a clinical utility that outweighs any potential risks stemming from a long, fraught history of racism in medicine? This remains a topic of active debate within the scientific community (92).

| **Ethnicities** | **Races** |
|---|---|
| "Hispanic or Latino" | "American Indian or Alaska Native" |
| "non-Hispanic or Latino" | "Asian" |
| | "Black or African American" |
| | "Native Hawaiian or Other Pacific Islander" |
| | "White" |

**Table 1. Ethnic and racial categories according to the Office of Management and Budget.** Ethnicity is mandated to be collected independently of race. It classifies individuals as "Hispanic" or "non-Hispanic," signifying whether they trace their origin or descent to Central or South America or other Spanish cultures, encompassing shared cultural values and behaviors. Race comprises a minimum of five categories, with the potential for subcategories. For example, subcategories like Pakistani, Chinese, and Indian may be included under the broader category of "Asian." "American Indian or Alaska Native" identifies a person with origins in any of the Native peoples of North or South America, maintaining cultural identification through tribal affiliation or community attachment. "Black or African American" denotes a person with origins in any of the black racial groups of Africa. At the same time, "White" designates a person with roots in any of the original peoples of Europe, the Middle East, or North Africa.

**What we objectively know**

Despite the ongoing and appropriate debate, the literature extensively documents health outcome disparities based on race/ethnicity. Both Hispanic and non-Hispanic Blacks face a higher risk of CV disease compared to other racial/ethnic groups. Notably, in non-Hispanic Blacks, this risk tends to manifest earlier in life and to a greater extent. Examining life expectancy at birth in the United States, the gap between white persons and non-Hispanic Black persons was 8.5 years for men and 5.8 years for women in 1993. This gap decreased to 5.9 and 3.9 years in 2007, respectively (93). Smoking alone has been attributed to explaining between 23 to 48% of this gap, despite relatively small differences in ever-smoking prevalence between whites and non-Hispanic Black individuals (94). The effect is thought to be notable due to lower access to effective smoking cessation interventions, daily stressors, lower quitting success, and, consequently, longer smoking durations among black persons. This illustrates once more the complex relationship between habits, risk factors, and the impact of social determinants of health, as previously described.

Non-Hispanic Blacks also exhibit a higher prevalence of hypertension across all age groups (95), T2D (96), peripheral artery disease (97), albuminuria, and chronic kidney disease (98), as well as a more common clustering of multiple-risk factors compared to their white counterparts (99). On the other hand, Hispanic individuals have been shown to have lower levels of physical activity and higher rates of unhealthy dietary habits, likely explaining in part the higher proportion of overweight, obesity, and T2D within this community, particularly in children and young adults (100).

**Data on ethnic/racial disparities in subclinical atherosclerosis is limited.**

Although health disparities in CV risk factors based on ethnicity/race are extensively documented, their impact on the early manifestation of subclinical atherosclerosis is less explored, with inconclusive results. Thus, there exists a critical gap in research that necessitates comprehensive investigations into the influence of race and ethnicity on the early stages of subclinical atherosclerosis.

**Table 2** summarizes documented racial disparities among Hispanic and non-Hispanic Black individuals compared to their white counterparts. Ethnic/racial disparities have been identified in aortic wall thickness, indicating increased atherosclerosis, as assessed by magnetic resonance imaging in non-Hispanic Black persons (101) but not in Hispanics, particularly Hispanic women (102). Earlier and more accelerated carotid artery stiffness, measured by pulse wave velocity, has been found to be higher in both non-Hispanic Blacks

and Hispanics individuals across the lifespan (103), a discovery associated with the development of hypertension and a robust predictor of CV outcomes (104). Carotid intima-media thickness, another subclinical marker of CV disease, has consistently been found to be greater in non-Hispanic Blacks in various cohorts compared to their white counterparts. In contrast, Hispanic participants exhibited thinner common carotid artery intima-media thicknesses (105,106). Moreover, coronary artery calcification, a marker indicating the presence and quantity of coronary atherosclerosis, has been reported to be higher in white individuals than in their Hispanic or non-Hispanic Black counterparts. Higher mortality risk has been reported for non-Hispanic Black and Hispanic individuals after adjusting for atherosclerosis burden (107); however, understanding the mechanisms underlying these differences is an area that requires further exploration (108).

| Ethical and racial Disparities | Measure | Implication |
|---|---|---|
| ↑ Aortic wall thickness in non-Hispanic Blacks vs Hispanics (particularly in Hispanic women) | MRI (mm) | Higher subclinical atherosclerosis |
| ↑ Arterial Stiffness in non-Hispanic Blacks and Hispanics compared to their white counterparts | Carotid-femoral pulse wave velocity (m/s) | A 1 m/s increase in pulse wave velocity corresponds to an age-, sex-, and risk factor-adjusted increased risk of 14% and 15% for CV events and mortality, respectively. |
| ↓ CIMT in Hispanics<br><br>↑ CIMT in non-Hispanic Blacks | B-mode ultrasonography (mm) | Each 0.2-mm increase in CIMT is associated with age-adjusted incident ASCVD hazard ratios of 1.4 (95%CI: 1.2 to 1.5) for women and 1.3 (95%CI: 1.1 to 1.6) for men. |
| ↓ CAC Score in non-Hispanic Black and Hispanic individuals but higher mortality vs White Persons | CT Scan (Agatston Score) | A strong predictor of incident coronary heart disease and mortality. |

**Table 2. Documented ethnic and racial disparities.** They reveal higher subclinical atherosclerosis in Hispanic and non-Hispanic Black individuals compared to their white counterparts, with increased hazard ratios reported for arterial stiffness (109), CIMT (110), and CAC Scores (111). MRI = Magnetic resonance imaging, CIMT = Carotid intima-media thickness, ASCVD =atherosclerotic cardiovascular disease, CAC = coronary artery calcification.

## Ethnicity/race in the new era of human genetics

Adaptive traits like skin color often define humans. Yet, the average genetic differences between individuals from different populations are slightly higher than those within a single population. A pivotal study assessing human genetic diversity across seven major geographical regions, examining the distribution of 4,000 alleles (gene variants), revealed that most alleles are shared across regions. Only 7.4% of over 4,000 alleles are exclusive to a geographical region. When region-specific alleles emerge, they appear in only about 1% of the population in that region, challenging the reliability of genetic differences tracking with race (112).

Modern genome-wide genotyping methods and computational algorithms now allow scientists to infer a person's ancestral geographic origin from differences in allele frequencies (113). While ethnicity/race may capture some information about specific alleles, genetic ancestry provides a more accurate prediction. It helps focus categorization on the distinct geographical origins of an individual's lineage rather than merely the adaptive superficial characteristics expressed in one person, particularly given the current genetic admixture or exchange between people of different ancestries. For instance, Hispanic subpopulations exhibit a mixed ancestry of European, Native American, and African origins (114). Thus, genetic ancestry may reflect fixed genome characteristics, while race may serve more as a proxy for socioenvironmental exposures.

## Informed use of race, ethnicity, and genetic ancestry

### Advantages

As long ago as 1985, the Task Force on Black and Minority Health reported that racial and ethnic minorities were underrepresented in health research (115). The report noted that the consequence of this underrepresentation was significant gaps in knowledge about the health of racial and ethnic minority populations and their responses to interventions. The publication of this report was an essential element in triggering the creation of several research cohorts among racial/ethnic minorities that have prompted over a thousand publications in the last decades and contributed to the understanding of the nature and extent of heart disease and CV risk factors for these underrepresented groups.

Consequently, the documentation of health disparities has prompted the development of race-specific clinical algorithms in cardiology and various medical specialties, as well as the identification of clear priorities for highly effective public health interventions (e.g., smoking cessation interventions for non-Hispanic Black subpopulations and interventions

focusing on diet and physical activities for Hispanic children and young adults peers) (92). An exemplary initiative involved a cluster randomized trial in 52 black-owned barbershops, unconventional healthcare settings, where barbers encouraged uncontrolled hypertensive black males to meet specialty-trained pharmacists for prescribed drug therapy. This intervention resulted in a notable reduction in systolic blood pressure, with a decrease of -21.6 mmHg compared to the control group (116).

Despite the National Institutes of Health's concerted efforts to include minority populations in biomedical and clinical studies, inadequate funding persists for these communities. For instance, fewer than 2% of clinical trials from the National Cancer Institute have included non-white participants (117). Clinical and research findings continue to be predominantly generated in populations of European descent and then extrapolated for treating non-European populations—a practice that is neither equitable nor potentially safe.

Furthermore, recent scientific publications have contributed to the ongoing debate, suggesting that race-specific clinical algorithms may exacerbate anti-black racism. A thoughtful review by five black male geneticists discusses the emerging field of genetic ancestry (118). The authors highlight that while genetic ancestry data is becoming available, its routine use in clinical care and clinicians' understanding of its implications has yet to materialize fully. They argue for the advantages of embracing ethnicity/race until a more evidence-based transition to genetic ancestry can be achieved. They argue that disregarding ethnicity/race may enhance equality by providing uniform medical care, but equity remains essential for addressing disparities until better predictive tools are available.

**Disadvantages**

Defining race proves challenging. It often relies on self-reports and is entangled with non-biological social determinants of health, like socioeconomic status. Complicating matters is that some individuals identify with multiple categories when self-reporting.

Researchers frequently employ ethnicity/race in assessing clinical measures and outcomes, integrating these categories into established analytic approaches. Unfortunately, even after adjusting for socioeconomic indicators, environmental exposures, and established risk factors, studies often uncover a higher risk of adverse health outcomes among black Americans, for instance, compared to white Americans. This elevated risk is sometimes presented without context, either as an intrinsic biological difference between races or as an outcome of racialized biological expressions—an embodiment of inequities tied to unmeasured risk factors or exposures, including individual and structural racism.

The complex interplay between race and ethnicity in biomedical research and clinical practice necessitates a thoughtful and nuanced approach (119). While recognizing the limitations of these categories as unreliable proxies for genetic differences, their utility in capturing epidemiologic information essential for understanding health disparities is acknowledged. In the absence of genetic ancestry data, the first article presented as part of this thesis work adopts a pragmatic approach, utilizing self-reported ethnic and racial backgrounds, particularly distinguishing between Hispanic and non-Hispanic Black origin. Well-established socioeconomic determinants of health are also gathered to comprehensively address these categories' multifaceted nature. The ensuing discussion will critically explore the advantages and disadvantages of categorizing by ethnic and racial backgrounds while contemplating the evolving role of genetic ancestry in advancing precision medicine and research to a more nuanced and accurate level.

## Early insulin resistance as an overlooked marker of metabolic status

Taking a broader perspective, there is a concerning rise in CV risk factors, notably obesity and T2D. It is estimated that a minimum of 45% of American adults live with T2D or prediabetes (120), and similar figures are observed in Europe (121,122). A significant portion of these individuals may be unaware of their condition, while many more might have early insulin resistance—a risk factor not only associated with future T2D but also ASCVD (123). Projections indicate that by 2050, an astounding 1.31 billion people worldwide could be grappling with diabetes, disproportionately impacting socio-economically disadvantaged individuals (5). In light of this, recent studies reveal that HbA1c levels are linked to subclinical atherosclerosis, even within the range of HbA1c as low as 5.5% (75) (see **Figure 9**). This is below the prediabetes and T2D thresholds, and incorporating HbA1c as a continuous parameter into the Systematic Coronary Risk Evaluation (SCORE) model significantly enhances the risk assessment for multiterritorial subclinical atherosclerosis.

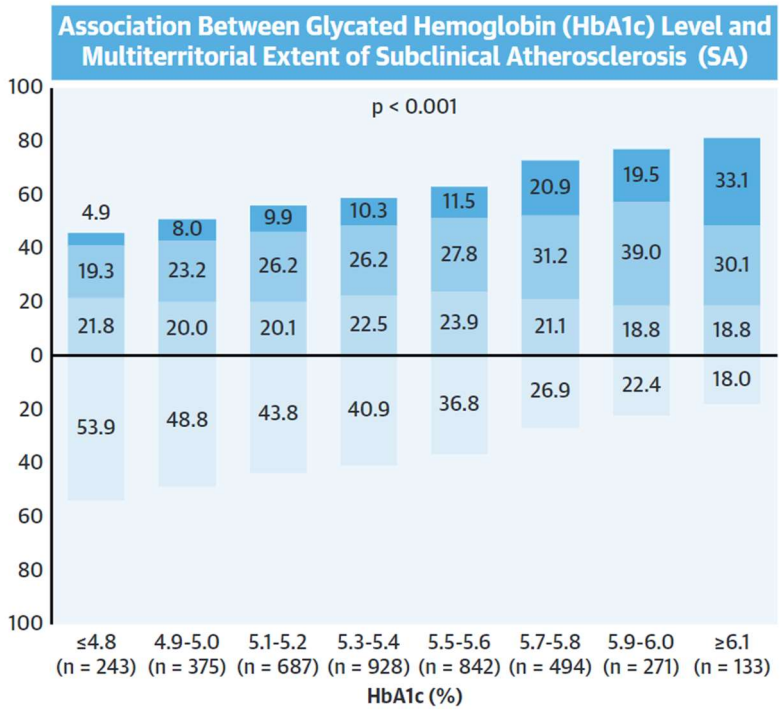



**Figure 9**. **Association between glycated hemoglobin level and the multiterritorial extent of subclinical atherosclerosis**. This analysis sparked the idea of exploring an earlier stage of the insulin resistance-prediabetes-T2D continuum to investigate whether early insulin resistance in normoglycemic individuals is also independently associated with subclinical atherosclerosis, independently of HbA1c levels. Reproduced from Rossello, X. et al. (No license required) Glycated Hemoglobin and Subclinical Atherosclerosis in People Without Diabetes. J Am Coll Cardiol—2021 Jun, 77 (22) 2777–2791 (75).

Furthermore, while ASCVD typically emerges after a gradual subclinical disease process, early signs of insulin resistance—marked by a progressive rise in fasting insulin levels—may precede the onset of glucose intolerance and alterations in fasting glucose levels associated with the insulin resistance-prediabetes-T2D spectrum (124). Unfortunately, akin to subclinical atherosclerosis, early insulin resistance often goes undetected until reaching advanced stages, emphasizing the importance of proactive screening and intervention at earlier points in the disease continuum. This presents a significant opportunity for prevention. Furthermore, the association between the initial insulin resistance stages and subclinical atherosclerosis remains poorly described.

The HOMA-IR ratio serves as a simple surrogate for early insulin resistance. It captures early abnormalities in systemic glucose homeostasis by testing how much insulin is needed to keep fasting glycemia low. For instance, fasting plasma insulin of 5 mU/L is necessary for a healthy individual to maintain fasting plasma glucose at 81 mg/dL, and this results in HOMA-IR = 1 when using the original formula: HOMA- IR = fasting insulin (mU/L) x fasting glucose (mg/dL) / 405. In this highly regulated mechanism, insulin resistance occurs when a higher-than-normal insulin concentration is required to obtain a quantitatively normal response in target tissues, and, among other parameters, HOMA-IR values start to increase (125). Previous evidence indicates metabolic risk increases for values ≥2 (126). During this initial compensatory phase of early insulin resistance, higher HOMA-IR values are associated with markers of ectopic fat accumulation and metabolic syndrome, including elevated circulating triglycerides, adipose tissue dysfunction, increased secretion of free fatty acids and pro-inflammatory adipokines, and elevated liver production of very-low-density lipoprotein particles in the presence of visceral fat and hepatic fat (127).

HOMA-IR is not only straightforward and cost-effective but also easily integrated into medical practice, serving as a robust clinical and epidemiological tool (128).

Identifying individuals in the very early stages of insulin resistance, independent of high glucose levels, studying the association with the initiation of subclinical atherosclerosis, and implementing preventive strategies at this nascent point could significantly delay CV events. Early detection of insulin resistance could serve as both a warning and an opportunity for individuals to make lifestyle changes, preventing the onset of full-blown T2D and ASCVD.

## SECTION 4: The FAMILIA Study

The (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) - FAMILIA study enrolled young families, including 562 preschool children and 635 of their parents or caregivers, with a primary focus on enhancing CV health in children aged three to five years and their caregivers. The targeted communities were those facing elevated CV risks in New York City, particularly in Harlem, where a majority of the population comprises Hispanic and non-Hispanic black individuals from low-income backgrounds. These socio-economically disadvantaged communities experience a disproportionately higher burden of major CV risk factors and a greater prevalence of CV disease compared to their white counterparts but also to other neighborhoods in New York (129,130).

Commencing in the fall of 2015, this four-year project aimed to showcase the efficacy of concentrating efforts on a younger age group (3-5 years) while employing a family-based approach. The overarching goal was to demonstrate that this strategy could be pivotal in promoting CV health throughout the lifespan, including mitigating the childhood obesity epidemic and gaining a deeper understanding of how the interplay between a child's behavior and the family environment might contribute to the development of heart disease. Simultaneously, the project sought to refine future prevention and educational techniques. In this regard, the FAMILIA study represented a comprehensive and forward-thinking endeavor to address health disparities in high-risk communities by fostering CV well-being from an early age.

The FAMILIA Project capitalized on the success of analogous health promotion educational initiatives for preschoolers, such as the SI! Program in Bogota, Colombia, and various programs throughout Spain (131,132). These programs, designed to instill healthy lifestyle behaviors early in life, aimed to establish habits that endure into adulthood, emphasizing preventative measures as early as possible. The targeted schools were participants in The Head Start program, administered by the U.S. Department of Health and Human Services, which delivers comprehensive services to low-income children and their families.

As part of the study, children enrolled in Harlem public preschools and their respective parents or caregivers were recruited. Employing a hierarchical design, the study treated schools as units for randomization, intervention, and analysis. Over four months, 562 children were randomly assigned to two parallel interventions, entailing a minimum of 37 educational hours for children and 12 hours for parents/caregivers, compared to a control group. The interventions were grounded in a multicomponent educational approach

encompassing promoting a healthy diet, increased physical activity, understanding the human body, and emotion management. While most interventions were school-based and integrated into the curriculum, some involved the participating children's parents and other family members. The parents or caregivers of the children were concurrently randomized in a separate controlled trial to undergo either an "individual-focused" or "peer-to-peer-based" lifestyle intervention program for 12 months, or they served as part of the control group.

The complete design and rationale of the FAMILIA Study have been previously documented (133), yielding two primary results. Among children aged 3 to 5 years who underwent a 4-month preschool-based educational intervention to promote health, there was a significant improvement in their knowledge, attitudes, and habits pertaining to a healthy lifestyle (134), measured by the KAH score, first developed in the Colombian Initiative for Healthy Heart Study (131,135). The most significant impact was observed in children who received over 75% of the curriculum. In the adult cohort (136), with a mean age of 38 ± 11 years, comprising 83% women, 57% of Hispanic origin, and 31% of non-Hispanic black origin, the primary outcome measured in this study was the change from baseline to 12 months in a composite health score, encompassing blood pressure, exercise, weight, diet, and tobacco use (The Fuster-BEWAT Score, ranging from 0 to 15, where an ideal health score is 15, (137)). To evaluate the sustainability of the intervention, this study also assessed changes in the score at the 24-month mark. Neither an intensive individual intervention nor a peer-to-peer intervention program, administered over 12 months, significantly impacted basic health metrics compared to control subjects. Although overall intervention adherence was moderate, a discernible dose-response intervention effect emerged. Individuals who attended more than 50% of the intervention program sessions demonstrated healthier changes than their counterparts with lower adherence levels at both evaluated time points, yielding helpful information to tailor future health promotion programs in adults (136).

In the FAMILIA study, 635 adults were invited to provide separate consent for noninvasive 3DVUS to examine the presence and burden of atherosclerosis in the carotid and femoral arteries. Among them, 515 underwent baseline imaging. **The data from these FAMILIA Study adult participants were utilized for one of the primary objectives of this Ph.D. thesis, to describe the prevalence and burden of subclinical atherosclerosis in this cohort of young adults from a socioeconomically disadvantaged community utilizing the innovative 3DVUS Technology (Article 1 and 2 of this compendium).**

## SECTION 5: The PESA Study

The Progression of Early Subclinical Atherosclerosis - PESA study represents an ongoing, forward-looking cohort initiative that explores imaging, biological markers, and behavioral factors associated with early subclinical atherosclerosis's emergence, extent, burden, and progression. This collaboration is a joint effort between the Spanish National Center for Cardiovascular Research (CNIC) and the Santander Bank in Madrid, Spain.

Between 2010 and 2014, the PESA study enrolled 4,184 asymptomatic individuals in their middle-aged years (40 to 54 years, with a mean age of 46 years upon enrollment; 37% women, 99,9% white). Since enrollment, these participants have undergone comprehensive assessments every three years for six years. The assessments have included clinical interviews, lifestyle questionnaires, sampling, and extensive noninvasive imaging evaluations targeting multiterritorial subclinical atherosclerosis. The imaging tests comprise 2DVUS and 3DVUS scans of the aorta, iliofemoral, and carotid arteries and non-contrast CT scans to ascertain the CAC.

In 2019, the PESA study was extended for an additional ten years under the banner of PESA-HEALTH. This extension introduced a modified protocol with two supplementary visits, scheduled at the ten and 15-year marks post-enrollment. Overall, PESA traces the trajectories of atherosclerosis with the ultimate aim of characterizing its presence and progression, spanning from the early stages in apparently healthy middle-aged individuals to the transition into symptomatic phases., spanning a remarkable 16-year period.

The original study design has been documented previously (52). Since its commencement, the PESA study has yielded pivotal insights into CV prevention and the progression of subclinical ASCVD. The noteworthy findings from this study are briefly outlined below.

1) The PESA study is the first to assess carotid and femoral atherosclerosis using a 3DVUS approach within a large cohort, creating an extensive dataset for future reference. Within this cohort of individuals free from clinically established ASCVD, subclinical atherosclerosis manifested prominently in 63% of participants (71% of men, 48% of women), and nearly half of these individuals were categorized as having intermediate or generalized disease (138).

2) The prevalence of plaques was highest in the iliofemoral region (44%), suggesting a possible commencement of atherosclerosis within this region.

3) There was a robust correlation between plaque burden and CV risk factors (139).

4) Notably, most participants (95%) deemed at high CV 10-year risk by the Framingham Heart Study score exhibited subclinical disease. However, among participants with a low 10-year risk, according to the Framingham Heart Study, subclinical disease was detected in 58%, with 36% exhibiting intermediate or generalized disease. This underscores the additional value of imaging in conjunction with CV risk scores for diagnosis and prevention, particularly in the low-risk group. The high rate of disease detection in low-to-intermediate risk groups likely stems from the comprehensive screening of various territories using diverse imaging techniques in PESA. According to the authors, this observation might help explain, to some extent, the disparity between traditional population-based risk-scoring systems and the actual occurrence of clinical events at the individual level.

5) Quantifying plaque burden by 3DVUS proved to reflect estimated CV risk more closely than merely detecting plaques alone, a finding consistent with other imaging cohorts such as the Bioimage study in the HRP (10).

6) In individuals without diabetes, and after adjusting for established CV risk factors, HbA1c demonstrated a linear association with the multiterritorial extent of subclinical atherosclerosis in all pre-diabetes groups, including those below the pre-diabetes cut-off (75).

7) Despite the known progression of atherosclerosis over time, the rate of progression remained unknown. Unexpectedly, at the 3-year follow-up visit, a significant proportion of apparently healthy middle-aged individuals from the PESA cohort exhibited evidence of disease progression (26.4% by 2DVUS, 21.3% by 3DVUS, and 11.5% by CACS), particularly in the iliofemoral vascular territory (140). Over the baseline to 6-year period, progression of subclinical atherosclerosis occurred in 32.7% of the cohort, with 17.5% presenting incident disease and 15.2% progressing from prevalent disease at enrollment (141). Factors such as age, sex, dyslipidemia, hypertension, smoking, and family history of premature cardiovascular disease contributed to progression, with dyslipidemia emerging as the most influential modifiable risk factor.

8) A regression of atherosclerosis was observed in 8.0% of patients with baseline disease (141), representing a noteworthy contribution that propels the research into the mechanisms by which atherosclerosis can not only fail to progress or remain stable but also undergo regression.

Additionally, other notable contributions have emerged, including the identification of new risk factors for atherosclerosis, such as the role of clonal hematopoiesis as a driver of atherosclerosis, and the revelation of lifestyle patterns associated with subclinical atherosclerosis, such as healthy and unhealthy patterns of diet and sleep (142).

**For the current Ph.D. research work, the baseline data from the PESA Study cohort have been employed to investigate the association between the presence of early insulin resistance, as assessed by HOMA-IR, in normoglycemic individuals, and the presence, burden, and multiterritorial extent of subclinical atherosclerosis (Article 3 of the compendium).**

# 2. Objectives

This doctoral thesis encompasses **two primary objectives**:

1) Investigating the prevalence and burden of subclinical atherosclerosis in a cohort of young adults from a socioeconomically disadvantaged community utilizing an innovative non-invasive 3D vascular ultrasound (3DVUS) technology. The study aims to explore the impact of race, ethnicity, and socioeconomic factors on the early stages of atherosclerosis.

2) Examining the association between early insulin resistance in normoglycemic low-risk individuals and the prevalence and burden of subclinical atherosclerosis. This investigation employs the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ratio as a clinically practical metric for identifying at-risk individuals.

**Overall Significance**:

This doctoral thesis strives to contribute substantially to cardiovascular care by examining non-traditional risk factors and refining risk stratification methods. This entails advocating for integrating imaging technologies into risk assessment protocols for targeted primary cardiovascular prevention, providing clinicians with practical tools such as the HOMA-IR ratio, and actively developing strategies to counteract atherosclerotic cardiovascular diseases at their nascent stages.

# 3. Methodology and Results

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# Subclinical Atherosclerosis in Young, Socioeconomically Vulnerable Hispanic and Non-Hispanic Black Adults

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## ABSTRACT

**BACKGROUND** Non-Hispanic Black persons are at greater risk of cardiovascular (CV) events than other racial/ethnic groups; however, their differential vulnerability to early subclinical atherosclerosis is poorly understood.

**OBJECTIVES** This work aims to study the impact of race/ethnicity on early subclinical atherosclerosis in young socioeconomically disadvantaged adults.

**METHODS** Bilateral carotid and femoral 3-dimensional vascular ultrasound examinations were performed on 436 adults (parents/caregivers and staff) with a mean age of 38.0 ± 11.1 years, 82.3% female, 66% self-reported as Hispanic, 34% self-reported as non-Hispanic Black, and no history of CV disease recruited in the FAMILIA (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) trial from 15 Head Start preschools in Harlem (neighborhood in New York, New York, USA). The 10-year Framingham CV risk score was calculated, and the relationship between race/ethnicity and the presence and extent of subclinical atherosclerosis was analyzed with multivariable logistic and linear regression models.

**RESULTS** The mean 10-year Framingham CV risk was 4.0%, with no differences by racial/ethnic category. The overall prevalence of subclinical atherosclerosis was significantly higher in the non-Hispanic Black (12.9%) than in the Hispanic subpopulation (6.6%). After adjusting for 10-year Framingham CV risk score, body mass index, fruit and vegetable consumption, physical activity, and employment status, non-Hispanic Black individuals were more likely than Hispanic individuals to have subclinical atherosclerosis (OR: 3.45; 95% CI: 1.44-8.29; $P = 0.006$) and multiterritorial disease ($P = 0.026$).

**CONCLUSIONS** After adjustment for classic CV risk, lifestyle, and socioeconomic factors, non-Hispanic Black younger adults seem more vulnerable to early subclinical atherosclerosis than their Hispanic peers, suggesting that the existence of emerging or undiscovered CV factors underlying the residual excess risk (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health [FAMILIA (Project 2)]; NCT02481401)
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**ABBREVIATIONS AND ACRONYMS**

**3DVUS** = 3-dimensional vascular ultrasound

**CV** = cardiovascular

Cardiovascular (CV) disease tends to affect non-Hispanic Black persons earlier in life and to a greater extent than is the case with other racial and ethnic groups.[1] This may be attributable in part to an elevated prevalence of CV risk factors such as hypertension, diabetes, peripheral artery disease, and chronic kidney disease, or to a more frequent clustering of multiple risk factors; however, other factors could also play a critical role in generating these disparities.

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Recently developed CV risk scores that consider race and ethnicity together with well-established CV risk factors have proved to be useful tools for predicting CV events and establishing prevention strategies.[2] Nevertheless, a substantial proportion of events occur in individuals classified at low or moderate risk. Because CV disease has a slowly progressing asymptomatic phase, interest has grown in the preventive potential of noninvasive imaging techniques used to detect direct signs of early subclinical CV disease. Progress in this area has been achieved with the recent establishment of 3-dimensional vascular ultrasound (3DVUS) as a safe, inexpensive, and reliable tool for detecting the presence and quantifying the extent or burden of subclinical atherosclerosis.[3] The observed racial and ethnic disparities in CV disease may reflect differential vulnerability to early atherosclerosis; however, few studies have addressed this relationship.

The randomized interventional trial FAMILIA (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) enrolled young families (preschool children and their parents/caregivers) and school staff from a low-income area in the neighborhood of Harlem, New York, New York, USA.[4] A high proportion of FAMILIA participants are of African-American and Hispanic descent. The main goal of the trial was to test the efficacy of a family-based approach to CV health promotion across the lifespan that integrates behavioral and imaging strategies.[5,6] At enrollment, adults (parents/caregivers and staff) underwent a comprehensive assessment of lifestyle and CV health, including bilateral carotid and femoral 3DVUS. Here, we assessed the impact of race and ethnicity on the presence, extent, and distribution of 3DVUS-detected subclinical atherosclerosis through a cross-sectional analysis of the information collected at baseline in adult participants enrolled in the FAMILIA study.

## METHODS

**STUDY DESIGN AND POPULATION.** The FAMILIA trial rationale and design have been described elsewhere.[4] The FAMILIA study recruited a total of 635 adult caregivers and school staff from 15 Head Start preschools in the neighborhood of Harlem, New York, New York. These adult trial participants underwent a complete clinical evaluation at baseline, including lifestyle questionnaires supervised by trained personnel and point-of-care testing to determine blood glucose and lipid profile. All questionnaires were available in English and Spanish, and health counselors were fluent in both languages to accommodate participant language preferences. In addition, all participants were invited to sign a separate consent form to undergo noninvasive 3DVUS to examine for the presence and burden of atherosclerosis in the carotid and femoral arteries.

Non-Hispanic White, Asian, and Native American racial and ethnic groups each formed a small proportion of participants (2.3%, 2.3%, and 0.3%, respectively), and people in these groups were excluded from the present analysis. Also excluded were adults with a history of heart disease or stroke. The Icahn School of Medicine at Mount Sinai Institutional Review Board approved the study (HS#:14-01054), which was conducted in accordance with institutional and federal guidelines involving human participants. The study is registered on ClinicalTrials.gov, identifier number NCT02481401.

**BASELINE CHARACTERISTICS.** The following characteristics were assessed at baseline before initiation of the FAMILIA trial intervention.[5] Ethnic and racial background was self-reported and classified as Hispanic, non-Hispanic Black, or others (including non-Hispanic White, Asian, and Native American). Data were collected on well-established socioeconomic determinants of health, including self-reported employment status and average annual household income, as well as self-reported history of hypertension, diabetes, and dyslipidemia. Family history of CV disease was defined as a self-reported diagnosis of heart attack or stroke in a full parent or sibling by the age of 60 years. In addition, several health metrics (blood pressure, fasting blood glucose, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglycerides, body mass index (BMI), fruit and vegetable consumption, smoking habits, and physical activity) were measured as detailed in the Supplemental Methods.

**PREDICTED 10-YEAR CV RISK AND CATEGORIZATION OF HEALTH METRICS.** The predicted 10-year CV disease risk for each participant at enrollment was calculated with the Framingham Heart Study CV risk score equation using the user-written Stata command "framingham."[7,8] The equation includes the following parameters: age (in years), sex, measured systolic blood pressure (mm Hg), self-reported antihypertensive medication, smoking status, self-reported diabetes status, and measured total cholesterol and high-density lipoprotein-cholesterol (mg/dL). Based on Framingham CV risk scores, 10-year CV disease risk was classified as low (<10%), moderate (≥10-<20%), or high (≥20%).

Fruit and vegetable consumption and moderate/vigorous physical activity were categorized according to thresholds described in the Fuster-BEWAT score (a health metric that includes 5 factors: blood pressure, exercise, weight, alimentation, and tobacco).[9] This risk scale has a demonstrated accuracy for predicting subclinical atherosclerosis in relatively low-risk individuals similar to that of other American Heart Association-approved risk scales, but without the need for laboratory results.[10]

**3DVUS IMAGING PROTOCOL/ANALYSIS AND DEFINITION OF ATHEROSCLEROSIS. Imaging protocol.** Vascular ultrasound imaging to quantify the presence and extent of atherosclerosis in carotid and femoral arteries was performed with the Philips EPIQ-7G ultrasonography system (Philips Healthcare). Transducers used in this study were the 2-dimensional (2D) 9L-D linear array (9-3.1 MHz) and C1-6 curved array (6-1 MHz) transducers and the 3D VL 13-5 linear volume array transducer (13-5 MHz). The imaging protocol was adapted from the one used in the PESA (Progression and Early detection of Subclinical Atherosclerosis) study.[3] The procedure was performed by experienced registered vascular technologists who completed additional specialized training with the Philips ultrasonography system. The scanning protocol included standard imaging of the left and right carotid artery bifurcation and its branches (internal and external carotid arteries) and of the left and right common femoral artery bifurcation and its branches (superficial and deep femoral arteries). These 4 territories were scanned in cross-section with a 2D linear or curved array transducer to detect the presence of plaques and to sum plaque burden. The same vessels were examined by 3D ultrasound with the VL 13-5 linear array volume transducer, which performs a mechanical automated sweep in cross-section, allowing the assessment of plaque volume and estimation of total atherosclerotic burden.

**Image analysis.** All ultrasound recordings and digital images were analyzed at the Zena and Michael A. Wiener Cardiovascular Institute at Icahn School of Medicine at Mount Sinai by 2 observers (raters). Three-dimensional images were analyzed with dedicated software specially engineered by Philips (QStation-VPQ [vascular plaque quantification] 3.5). Each carotid and femoral 2D cross-sectional image was assessed for the presence of plaque. When a plaque was identified, QStation was used to assess the 3D dataset (displayed as multiple transverse slices) and to sum plaque volume in each vascular bed (carotid or femoral) on each side (left or right), as well as the total plaque volume in all vascular beds (bilateral carotid and femoral). Maximum percent stenosis was also estimated.

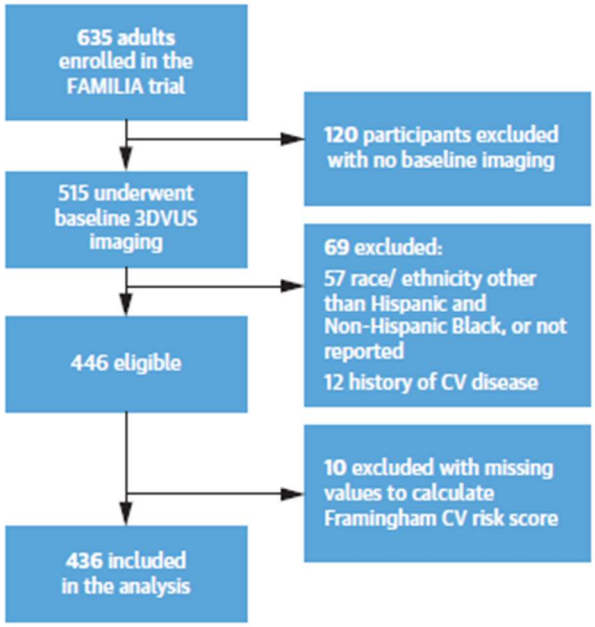
**Definition of atherosclerosis.** Plaque was defined according to the Mannheim consensus criteria as a focal structure encroaching into the arterial lumen and measuring ≥0.5 mm or >50% of the surrounding intima-media thickness or having a diffuse thickness ≥1.5 mm measured from the media-adventitia to the intima-lumen interface in any of the territories.[3]

The extent of atherosclerosis was defined according to the number of regions with presence of plaque (disease-free, 0 vascular sites affected; focal disease, 1 territory affected; multiterritorial disease, >1 territory affected). Plaque burden was defined as the plaque volume ($mm^3$), and global plaque burden corresponded to the sum of all plaque areas from all images showing plaque, including both carotid and femoral arteries.

**STATISTICAL ANALYSIS.** Summary statistics describing baseline characteristics are presented as mean ± SD for continuous variables and as count and frequencies for categorical variables. Unpaired Student's *t*-tests were used to assess crude differences between continuous variables; the chi-square test and Fischer exact test were used to determine crude differences between categorical variables. The Cochran-Mantel-Haenszel test was used to assess crude differences across ordered categorical variables.

To assess the adjusted impact of race and ethnicity on the presence and burden of atherosclerosis, we used multivariable logistic regression models for categorical outcome variables (presence or absence of atherosclerosis) and linear regression models for continuous outcomes (atherosclerosis burden in $mm^3$). Participant race and ethnicity were included in all models as the main independent variable. Other covariates were selected according to their reported

**FIGURE 1** Participant Flow Chart of FAMILIA Participants Included in This Study




The FAMILIA (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) trial enrolled 635 adults, of whom 515 consented to and underwent 3-dimensional vascular ultrasound (3DVUS) imaging. After exclusion of participants with a history of cardiovascular (CV) disease (n = 12), those self-reporting a race and ethnicity other than Hispanic or non-Hispanic Black (n = 57), and those with missing data for calculating the Framingham CV risk score (n = 10), a total of 436 adults were included in this study.

association with atherosclerosis (clinical plausibility) or as potential confounders according to the rules proposed by Kleinbaum and colleagues using the user-written Stata command "confound."[11] The covariates included in the multivariable models were as follows: participant 10-year CV Framingham risk score (categorical variable: low, moderate, high), participant employment status (categorical variable: employed, unemployed, other status, unknown), BMI (continuous variable), physical activity (categorical variable: <10, 10-<75, ≥75-<150, and ≥150 minutes/week of moderate-to-vigorous exercise) and fruit and vegetable consumption (categorical variable: <1, 1-2, 3-4, >4 daily servings). Practical and clinical interpretations are presented as measures of association (OR) and estimated mean differences with 95% CIs. Statistical significance was assigned at $P < 0.05$.

Prevalence-adjusted and bias-adjusted kappa coefficients were calculated in a subsample of FAMILIA participants to assess intraobserver and interobserver reproducibility for plaque detection. For intraobserver and interobserver reproducibility analysis of atherosclerosis burden (plaque volume quantification), the intraclass correlation coefficient and Bland-Altman plots were used for all plaque-positive FAMILIA participants. Methodological details of agreement and reproducibility analysis are presented in the Supplemental Methods. Statistical analyses were performed with STATA (2017, Stata Statistical Software: Release 15, StataCorp LLC).

## RESULTS

The FAMILIA trial enrolled 635 adults, of whom 515 consented to and underwent 3DVUS imaging. Participants with a history of CV disease were excluded, as were those self-reporting a race and ethnicity other than Hispanic or non-Hispanic Black and those with missing data for calculating the Framingham CV risk score. A final total of 436 adults were included in this study (Figure 1).

**BASELINE CHARACTERISTICS, HEALTH METRICS, AND PREDICTED CV RISK.** Participants self-reporting as Hispanic or non-Hispanic Black accounted for approximately two-thirds (n = 289, 66.3%) and one-third (n = 147, 33.7%) of the study population, respectively. The mean age was 38.0 ± 11.1 years, and 82.3% were women. Age and sex profiles did not differ significantly between racial and ethnic groups. Baseline characteristics of adults included in this study are summarized in Table 1, whereas baseline characteristics of all individuals enrolled in the FAMILIA trial grouping subjects by availability of 3DVUS information are presented in Supplemental Table 1.

Compared with their Hispanic counterparts, non-Hispanic Black participants were ~3.5 times more likely to be hypertensive (OR: 3.54; 95% CI: 2.14-5.87; $P < 0.001$). Non-Hispanic Black participants similarly were 3 times more likely to be active smokers (OR: 3.15; 95% CI: 1.83-5.41; $P < 0.001$), and also had a higher BMI (mean between-group difference = 1.45 kg/m$^2$; 95% CI: 0.17-2.74; $P = 0.027$) and reported higher consumption of fruits and vegetables ($P < 0.001$). There was no between-group difference in the prevalence of self-reported diabetes ($P = 0.735$) or determined fasting glucose ($P = 0.402$) and total cholesterol ($P = 0.873$). The mean 10-year Framingham CV risk score for the whole study population was 4.0% ± 5.6%, and there were no significant differences between racial and ethnic groups ($P = 0.104$). Most participants (89%) were classified at low risk, with ~9% and 2% classified at

**TABLE 1** Baseline Characteristics of Enrolled Adults in the FAMILIA Trial Included in This Study

| | Overall (N = 436) | Hispanic (n = 289 [66.3%]) | Non-Hispanic Black (n = 147 [33.7%]) | *P* Value |
|---|---|---|---|---|
| Age, y | 38.0 ± 11.1 | 37.5 ± 11.0 | 39.0 ± 11.3 | 0.191 |
| Female | 359 (82.3) | 232 (80.3) | 127 (86.4) | 0.113 |
| Employment status | | | | |
| Employed | 276 (63.3) | 168 (58.1) | 108 (73.5) | 0.018 |
| Unemployed/unable to work/homemaker | 123 (28.2) | 92 (31.8) | 31 (21.1) | |
| Other (student, retired) | 23 (5.3) | 18 (6.2) | 5 (3.4) | |
| Unknown | 14 (3.2) | 11 (3.8) | 3 (2.0) | |
| Annual household income | | | | |
| <$25,000 | 214 (59.8) | 151 (67.4) | 63 (47.0) | <0.001 |
| ≥$25,000 | 144 (40.2) | 73 (32.6) | 71 (53.0) | |
| Unknown | 78 (17.9) | 65 (22.5) | 13 (8.8) | |
| Cardiovascular risk factors | | | | |
| Self-reported hypertension | 77 (17.7) | 32 (11.1) | 45 (30.6) | <0.001 |
| Active smoking | 63 (14.5) | 27 (9.3) | 36 (24.5) | <0.001 |
| Body mass index, kg/m$^2$ | | | | |
| Low, <18.5 | 4 (0.9) | 2 (0.7) | 2 (1.4) | 0.322 |
| Normal, 18.5–<25 | 74 (17.0) | 49 (17.0) | 25 (17.0) | |
| Overweight, 25–<30 | 156 (35.8) | 113 (39.1) | 43 (29.3) | |
| Obese, ≥30 | 202 (46.3) | 125 (43.3) | 77 (52.4) | |
| Self-reported diabetes | 56 (12.8) | 36 (12.5) | 20 (13.6) | 0.735 |
| Self-reported dyslipidemia | 104 (23.9) | 77 (26.6) | 27 (18.4) | 0.055 |
| Family history of CV disease | 30 (7.7) | 14 (5.5) | 16 (11.9) | 0.026 |
| Mean 10-y Framingham CV risk score | 4.0 ± 5.6 | 3.6 ± 5.1 | 4.6 ± 6.4 | 0.104 |
| Categorized 10-y Framingham CV risk | | | | |
| Low risk, <10% | 388 (89.0) | 261 (90.3) | 127 (86.4) | 0.279 |
| Moderate risk, 10%–<20% | 40 (9.2) | 23 (8.0) | 17 (11.6) | |
| High risk, ≥20% | 8 (1.8) | 5 (1.7) | 3 (2.0) | |
| Physical activity, moderate-to-vigorous activity, min/wk | | | | |
| <10 | 83 (19.9) | 58 (21.2) | 25 (17.4) | 0.753 |
| 10–<75 | 60 (14.4) | 33 (12.0) | 27 (18.8) | |
| 75–<150 | 85 (20.3) | 54 (19.7) | 31 (21.5) | |
| ≥150 | 190 (45.5) | 129 (47.1) | 61 (42.4) | |
| Fruit/vegetable consumption, n daily servings | | | | |
| <1 | 22 (5.1) | 13 (4.5) | 9 (6.1) | <0.001 |
| 1–2 | 239 (54.8) | 173 (59.9) | 66 (44.9) | |
| 3–4 | 139 (31.9) | 86 (29.8) | 53 (36.1) | |
| >4 | 36 (8.3) | 17 (5.9) | 19 (12.9) | |

Values are mean ± SD or n (%). *P* values are derived from unpaired Student's *t*-tests for continuous variables, chi-square test and Fischer exact test for binary categorical variables, and the Cochran-Mantel-Haenszel tests for ordered categorical variables.

CV = cardiovascular; FAMILIA = Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health.

moderate and high risk, respectively. Measured CV risk and health factors of adults included in this study are listed in Supplemental Table 2, whereas those of all individuals enrolled in the FAMILIA trial grouping subjects by availability of 3DVUS information are presented in Supplemental Table 3.

**PREVALENCE, EXTENT, AND DISTRIBUTION OF SUBCLINICAL ATHEROSCLEROSIS.** The overall prevalence of subclinical atherosclerosis assessed by 3DVUS was 8.7%, and the mean global plaque burden was 5.0 ± 27.9 mm$^3$ (**Table 2**). The prevalence of subclinical atherosclerosis was higher in non-Hispanic Black participants across all 10-year Framingham CV risk score categories, and this difference was especially prominent in the high-risk category (**Central Illustration**). Overall, the crude odds of having subclinical atherosclerosis were 2 times higher among non-Hispanic Black persons than in the Hispanic subpopulation (OR: 2.11; 95% CI: 1.09-4.08; $P = 0.026$). Non-Hispanic Black participants had a higher mean disease burden (mean difference in total plaque volume = 6.17 mm$^3$; 95% CI: 0.68-11.66; $P = 0.028$) and a higher prevalence of multiterritorial disease ($P = 0.026$).

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**TABLE 2** Crude Prevalence, Extent, and Distribution of Subclinical Atherosclerosis as Assessed by 3-Dimensional Vascular Ultrasound

| | Overall (N = 436) | Hispanic (n = 289) | Non-Hispanic Black (n = 147) | *P* Value |
|---|---|---|---|---|
| Evidence of atherosclerosis | | | | |
| Any territory | 38 (8.7) | 19 (6.6) | 19 (12.9) | 0.026 |
| Carotids | 33 (7.6) | 14 (4.8) | 19 (12.9) | 0.003 |
| Femorals | 18 (4.1) | 11 (3.8) | 7 (4.8) | 0.635 |
| Extent of atherosclerosis | | | | |
| Disease free | 398 (91.3) | 270 (93.4) | 128 (87.1) | 0.026 |
| Focal | 15 (3.4) | 8 (2.8) | 7 (4.8) | |
| Multiterritorial | 23 (5.3) | 11 (3.8) | 12 (8.2) | |
| Burden of atherosclerosis, $mm^3$ | | | | |
| Carotid and femoral | 5.0 ± 27.9 | 2.9 ± 19.6 | 9.0 ± 38.8 | 0.028 |
| Carotid | 3.7 ± 22.0 | 1.5 ± 13.0 | 7.9 ± 32.8 | 0.004 |
| Femoral | 1.3 ± 7.8 | 1.3 ± 8.0 | 1.2 ± 7.5 | 0.819 |

Values are n (%) or mean ± SD. *P* values are derived from unpaired Student's *t*-test for continuous variables, and chi-square test and Fischer exact test for binary categorical variables.

In the multivariable analysis, race and ethnicity showed an independent association with the presence and extent of subclinical atherosclerosis. Compared with the Hispanic subpopulation, non-Hispanic Black participants had an adjusted OR for having subclinical atherosclerosis of 3.45 (95% CI: 1.44-8.29; $P = 0.006$). The adjusted mean difference between racial and ethnic groups in total plaque volume was 6.94 $mm^3$ (95% CI: 1.43-12.46 $mm^3$; $P = 0.014$). Adjustment for individual risk factors instead of Framingham risk score was performed as sensitivity analysis, and associations of race and ethnicity with the presence and extent of subclinical atherosclerosis remained similar.

Separate analyses of carotid and femoral involvement revealed significant differences by race and ethnicity in the carotid territory, with the adjusted odds of finding plaques in the carotids ~6 times higher in non-Hispanic Black participants than in their Hispanic peers (adjusted OR: 5.94; 95% CI: 2.17-16.26; $P = 0.001$). In contrast, no between-group differences were observed in the femoral arteries (adjusted OR: 1.72; 95% CI: 0.53-5.53; $P = 0.364$). Similar results were observed for the comparison of atherosclerosis burden; the mean adjusted difference in total plaque volume between the non-Hispanic Black and Hispanic groups was 7.06 $mm^3$ (95% CI: 2.65-11.48 $mm^3$; $P = 0.002$) in the carotid region and -0.12 $mm^3$ (95% CI: -1.66 to 1.42 $mm^3$; $P = 0.877$) in the femoral territory.

Intraobserver and interobserver agreement were excellent for the detection of plaque and very good for volume plaque quantification (Supplemental Tables 4 to 6, Supplemental Figure 1).

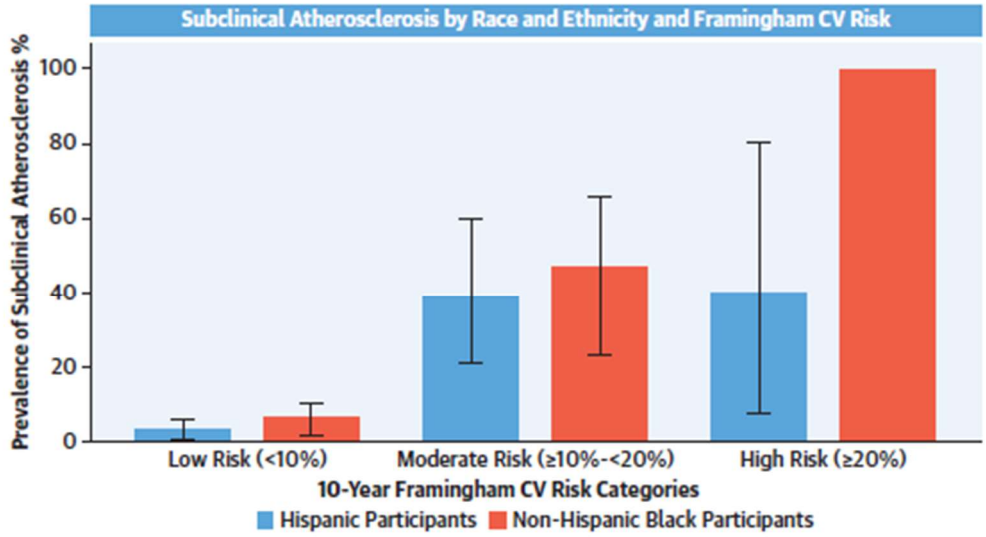
## DISCUSSION

This cross-sectional study of adult FAMILIA trial participants is one of the first to report the presence of 3DVUS-assessed subclinical atherosclerosis in a young cohort of mainly women, from a socioeconomically disadvantaged community. The study generated a number of key findings. 1) Compared with the Hispanic subcohort, non-Hispanic Black participants had higher rates of hypertension, active smoking, BMI, and self-reported fruit and vegetable consumption. 2) There was an overall low prevalence of subclinical atherosclerosis assessed by carotid and femoral 3DVUS (~9%), and plaques were more frequently found in the carotid than in the femoral arteries. 3) Although 10-year Framingham CV risk scores were similar in the 2 study subcohorts, non-Hispanic Black participants had higher odds of having subclinical atherosclerosis, a higher disease burden, and a higher prevalence of multiterritorial disease. 4) These racial and ethnic differences were mainly driven by differences in the carotid arteries (**Figure 2**). These findings may help to explain the observed differences in CV-disease prevalence between racial and ethnic groups.

Hispanic and non-Hispanic Black populations in the United States both have a higher prevalence of subclinical atherosclerosis and a higher CV-disease burden than other ethnic groups.[12] Previous studies have also reported an earlier and more diffuse involvement, particularly in the non-Hispanic Black population, which might reflect the presence of more CV risk factors at an earlier age, poorer CV health habits, or other factors such as different genetic predisposition to atherosclerosis.[13,14] However, data on these underrepresented populations are limited, especially in relation to younger age groups.

In our cohort, non-Hispanic Black individuals had significantly higher blood pressure and a higher prevalence of self-reported smoking, risk factors known to trigger earlier atherosclerotic-plaque formation and adverse epigenetic pathway activations, and subsequent CV disease.[15,16] Furthermore, both groups had high prevalence rates for other risk factors, including overall diabetes and overweight/obesity prevalence rates of 12.8% and 82.1%, respectively, potentially contributing to earlier vascular aging.[17] These observations are consistent with National Health and Nutrition Examination Survey statistics showing that the non-Hispanic Black population has the lowest prevalence of ≥5 ideal CV health metrics, both in children and adults aged ≥20 years.[12] Together, these findings support the notion

**CENTRAL ILLUSTRATION** **Prevalence of Subclinical Atherosclerosis Across Framingham Cardiovascular Risk Categories**

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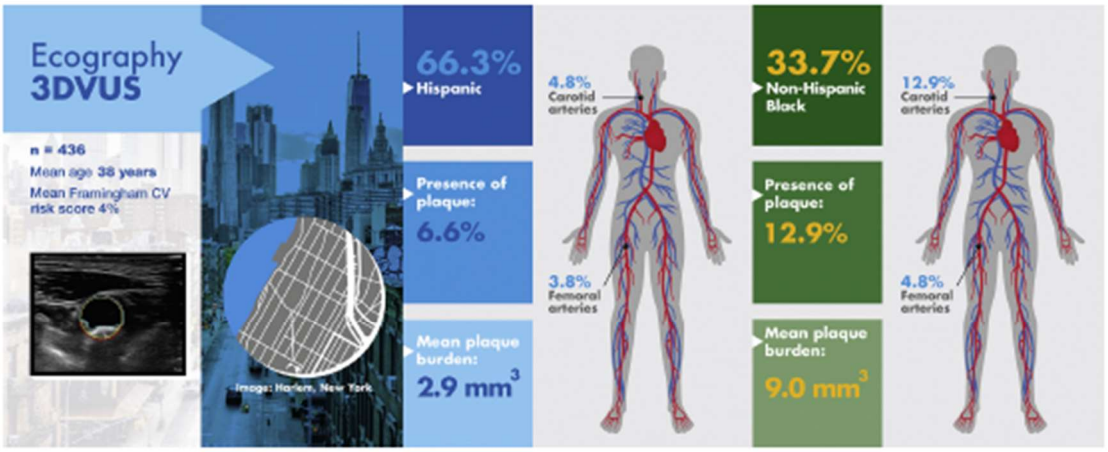
Bars (**blue**, Hispanic participants; **red**, Non-Hispanic Black participants) represent the mean prevalence of subclinical atherosclerosis in the FAMILIA (Family-Based Approach in a Minority Community Integrating Systems-Biology for Promotion of Health) trial adults included in this study stratified by Framingham cardiovascular (CV) risk category. The presence of subclinical atherosclerosis was defined as the detection of any plaque in the explored bilateral carotid and femoral territories by 3-dimensional vascular ultrasound. Error bars corresponding to logit-transformed 95% CIs of the estimated prevalence.

that specific racial and ethnic groups would benefit more from targeted early health promotion programs, CV screening campaigns, and other interventions, such as more intense antismoking health marketing strategies or increased access to cessation treatments, counseling, and medication.[18]

Nevertheless, in our study population, subclinical atherosclerosis prevalence and atherosclerotic burden were both higher in the non-Hispanic Black group than in the Hispanic population, despite both groups having similar 10-year Framingham CV risk scores. These findings were consistent across all Framingham CV risk score categories and suggest that nontraditional or unknown risk factors could potentially work through distinct epigenetic pathways that might explain the earlier and more encroaching progression of subclinical atherosclerosis among the non-Hispanic Black population.[19] The extent to which these differences may be heritable could be addressed through the implementation of genome-wide association study methods to understand the genetic contributions to disease. Nevertheless, evidence of biological differences in disease pathogenesis between racial and ethnic groups remains limited, and previous work has shown that disease development and progression are at least equally influenced by other factors, such as acculturation, socioeconomic status, educational attainment, behavioral and psychological conditions, food environment, access to health care, and other social determinants of health.[20,21] As long ago as 1985, the Task Force on Black and Minority Health reported that noticeable health disparities existed among minority communities and that these communities were underrepresented in health research. This report prompted research initiatives such as the Jackson Heart Study and the creation of the CARDIA (Coronary Artery Risk Development in Young Adults), ARIC (Atherosclerotic Risk In Communities), and MESA (Multi-Ethnic Study of Atherosclerosis) cohorts. These cohorts have since yielded substantial data on the use of noninvasive imaging techniques to assess subclinical atherosclerosis and on the contribution of nonbiological factors to poorer health, including the association between subclinical disease and socioeconomic status.[20,22]

**FIGURE 2** Subclinical Atherosclerosis in Hispanic and Non-Hispanic Black Socioeconomically Vulnerable Young Adults




This study assessed the impact of race and ethnicity on early subclinical atherosclerosis, as assessed by bilateral carotid and femoral 3DVUS, on 436 young Hispanic and non-Hispanic Black adults (parents/caregivers and staff) recruited in the FAMILIA trial from 15 Head Start preschools in Harlem (New York City). After adjustment for traditional CV risk, lifestyle, and socioeconomic factors, non-Hispanic Black individuals were more likely than Hispanic individuals to have subclinical atherosclerosis and higher burden of disease, suggesting the existence of unknown cardiovascular risk factors underlying such residual excess risk. Early detection of atherosclerosis could play an important role in prevention programs in different populations. Abbreviations as in Figure 1.

Although the non-Hispanic Black members of our cohort reported higher employment rates and annual household income than their Hispanic counterparts, the unemployment rate in the whole study population was approximately 28%, and almost 2 of 3 participants reported an annual household income <$25,000, just below the $25,465 2018 U.S. Census Bureau Poverty Threshold for a family of 4 members including 2 children.[23] When we additionally included annual household income in multivariable models, the effect of race and ethnicity remained significant for plaque presence and disease burden (total plaque volume). These findings suggest that household income was not the main driver of the racial and ethnic differences observed in this study.

Although data on subclinical disease in vulnerable communities remain limited, there are numerous noninvasive imaging tests available to assess early atherosclerosis and their use for CV prevention and health promotion is steadily expanding.[24] Moreover, some of these techniques have a low-cost; thus, they offer economic savings and the opportunity for large-scale implementation in a clinical setting, including screening and follow-up studies. In younger populations, noninvasive imaging also offers opportunities to reclassify risk given that traditional risk scores, mathematically dominated by age and sex, provide insufficient accuracy for the assessment of individual risk. Although 2D imaging has long been used to identify plaques and measure plaque thickness, a major advantage of 3DVUS is that it visualizes and quantifies plaques in the longitudinal and cross-sectional planes, providing a more accurate volumetric assessment of disease than 2D ultrasound.[25] The analysis of global plaque burden in our cohort revealed a higher disease burden among non-Hispanic Black participants (Table 2). When only individuals with plaque were considered, the median plaque volume overall, including both the carotid and the femoral territories, was 40.3 $mm^3$ (IQR: 13.7-63.0 $mm^3$), whereas for the non-Hispanic Black group the value was 53.5 $mm^3$ (IQR: 13.7-71.2 $mm^3$) and for the Hispanic group 36.8 $mm^3$ (IQR: 10.3-45.7 $mm^3$). In the PESA study, 3DVUS was used to quantify subclinical disease burden in 3,860 adults (mean age: 45 years; 63% male; 99.9% Caucasian).[26] This analysis detected extensive atherosclerosis in a considerable number of low-risk individuals. Median global plaque burden was 50.8 $mm^3$ (IQR: 18.7-121.5 $mm^3$) among participants with atherosclerosis and 31.2 $mm^3$ (IQR: 12.7-78.2 $mm^3$) among those aged 40-44 years. These findings suggest possible applications for noninvasive imaging in the more accurate individual diagnosis, intervention, and prevention.[27]

Our results also show that non-Hispanic Black participants tended to have more extensive multiterritorial involvement. Nevertheless, separate analyses of the carotid and femoral territories revealed significant between-group differences in atherosclerosis prevalence and disease burden only in the carotid arteries. The disease burden was also generally higher in the carotid than in the femoral arteries. These findings diverge from studies of larger populations such as PESA, in which the iliofemoral territory was more extensively affected.[3] The PESA study cohort consisted of office workers at the Banco de Santander headquarters in Madrid, and the higher femoral involvement may have been driven by the presumably longer working hours per day spent in a sitting posture, which is associated with low oscillatory shear stress, high hydrostatic pressure, and disturbed flow caused by vessel curvature.[28] However, historically, iliofemoral involvement in atherosclerosis has received comparatively little attention, and the differences from the FAMILIA population could be explained by other factors, such as differences in the age profile or in the proportion of male and female participants. Exploration of the impact of socioeconomic status in the PESA population revealed no significant association between the presence of subclinical atherosclerosis and economic status but did find an association with lower education level, mainly related to higher tobacco consumption, a relationship well-described in the literature and also seen in our study.[29,30] These results highlighted once again the critical importance of implementing health promotion and targeted CV prevention strategies, such as smoking cessation and blood pressure control. Efforts in this area should be targeted at the more affected vulnerable communities, where the largest net gains are likely to be made.

**STUDY LIMITATIONS.** This is a cross-sectional study; therefore, causal inference cannot be evaluated. Most study participants were at low CV risk, and most were women who are known to have a low prevalence of CV disease, particularly at a younger age.[12] These factors explain the overall low presence of identifiable plaques. However, this is one of the first studies to assess the presence of subclinical atherosclerosis by 3DVUS in an underrepresented younger population.

Given the heterogeneity among racial and ethnic groups, assessing associations between self-reported racial or ethnic identity and disease is complex and is vulnerable to confounding due to the effects of socioeconomic inequality, environmental disparity, unequal access to care, and other possible emerging or unknown CV risk factors.[31,32] Although racial and ethnic identity may track the existence of certain alleles, these terms tend to denote superficial physical and sociocultural characteristics, and a more precise categorization of the distinct geographical origins in an individual's lineage can be obtained through analysis of genetic ancestry, especially given the history of genetic admixture and exchange between people of different ancestry. For example, the term Hispanic usually denotes a mixed European, Native American, and African ancestry.[33] Thus, whereas race is more a proxy for socioenvironmental exposure, genetic ancestry examines fixed characteristics in the genome and may help to improve understanding of health disparities and improve precision medicine in the future.

The population included in this study was from a specific area (Harlem, New York City) with known intrinsic health disparities compared with other areas in New York City. This could, to some extent, limit our results' generalizability. Nevertheless, the population studied was predominantly low-income, with participants mainly of Hispanic or non-Hispanic Black origin. Although data on other potential nontraditional risk factors such as glycated hemoglobin or lipoprotein(a) levels was not determined, this study controlled for a battery of risk factors, lifestyle habits, and socioeconomic status for the primary analysis, yielding relevant information on these underrepresented communities.

## CONCLUSIONS

For the same predicted CV risk, non-Hispanic Black individuals appear to be more vulnerable than people of Hispanic origin to early subclinical atherosclerosis (particularly in the carotid arteries), potentially placing them at increased risk of clinical CV disease. Despite its limitations, including intrinsic socioenvironmental exposures partially captured by self-reported race and ethnicity, this study contributes to the understanding of higher rates of CV disease observed at an early age in disadvantaged communities. Until underlying biological factors and other undiscovered CV risk factors are better understood and can be addressed by precision medicine, affordable noninvasive imaging techniques such as the 3DVUS can provide valuable information about population disparities and increase the precision of health promotion and prevention programs.

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## PERSPECTIVES

**COMPETENCY IN SYSTEMS-BASED PRACTICE:** Atherosclerosis affects non-Hispanic Black individuals earlier in life and more severely than members of other racial and ethnic groups, and may contribute to racial and ethnic disparities in cardiovascular outcomes.

**TRANSLATIONAL OUTLOOK:** Further research is warranted to identify risk factors that explain the excess risk of cardiovascular disease in certain racial and ethnic groups.

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**KEY WORDS** cardiovascular disease, ethnicity, prevention, race, subclinical atherosclerosis, vascular ultrasound

**APPENDIX** For supplemental methods, tables, and figures, please see the online version of this paper.

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## REPLY: Carotid Plaque Burden Is a Stronger Predictor of Cardiovascular Risk Than IMT

We appreciate Dr Spence's interest in our recent paper[1] and the accompanying editorial.[2] Dr Spence provides valuable comments on the added predictive value conferred by carotid plaque burden in comparison with carotid intima-media thickness (IMT).

The predictive capacity of population risk equations is limited. The addition of IMT measurements, considered a surrogate marker of atherosclerosis by some investigators, has been shown to improve the prediction of a first cardiovascular event on top of traditional risk factors; however, such improvement is of small magnitude and unlikely to be clinically significant.[3] This is not surprising, as IMT may echo a vascular wall aging process rather than the actual atherosclerotic process itself.

Vascular ultrasound, among other noninvasive imaging techniques, allows not only measurement of IMT but also quantification of the burden of atherosclerosis developing in the arteries in both 2-dimensional and 3-dimensional ways. Considering that plaque formation is the macroscopic expression of atherosclerosis, it is not surprising that directly measured plaque burden (the sum of the area or volume of atherosclerotic plaques) is a stronger predictor of cardiovascular events than IMT. It is also worth considering that not only carotid but other multiterritorial measurements of plaque burden have been shown to be associated with future major adverse events.[4]

In conclusion, from our point of view, atherosclerosis detection and progression require direct quantification of plaque burden rather than IMT. This may offer a significant improvement in the prediction of future cardiovascular events in addition to conventional risk factors, particularly when integrating multiple vascular territories. Furthermore, it provides valuable individual information with implications for preventive and precision medicine.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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# Early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis

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## Abstract

**Background** Elevated glycated hemoglobin (HbA1c) is associated with a higher burden of subclinical atherosclerosis (SA). However, the association with SA of earlier insulin resistance markers is poorly understood. The study assessed the association between the homeostatic model assessment of insulin resistance index (HOMA-IR) and SA in addition to the effect of cardiovascular risk factors (CVRFs) in individuals with normal HbA1c.

**Methods** A cohort of 3,741 middle-aged individuals from the Progression of Early Subclinical Atherosclerosis (PESA) study with basal HbA1c < 6.0% (< 42 mmol/mol) and no known CV disease underwent extensive imaging (multiterritorial vascular ultrasound and coronary artery calcium score, CACS) to assess the presence, burden, and extent of SA.

**Results** Individuals with higher HOMA-IR values had higher rates of CVRFs. HOMA-IR showed a direct association with the multiterritorial extent of SA and CACS ($p < 0.001$) and with global plaque volume measured by 3-dimensional vascular ultrasound ($p < 0.001$). After adjusting for key CVRFs and HbA1c, HOMA-IR values ≥ 3 were associated with both the multiterritorial extent of SA (odds ratio 1.41; 95%CI: 1.01 to 1.95, $p = 0.041$) and CACS > 0 (odds ratio 1.74; 95%CI: 1.20 to 2.54, $p = 0.004$), as compared with the HOMA-IR < 2 (the reference HOMA-IR category). In a stratified analysis, this association remained significant in individuals with a low-to-moderate SCORE2 risk estimate (75.6% of the cohort) but not in high-risk individuals.

**Conclusions** The use of HOMA-IR identified low-risk individuals with a higher burden of SA, after adjusting for the effects of key traditional CVRFs and HbA1c. HOMA-IR is a simple measure that could facilitate earlier implementation of primary CV prevention strategies in clinical practice.

**Keywords** Imaging, Insulin resistance, Atherosclerosis, Cardiovascular disease, Primary disease prevention

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## Introduction

Cardiovascular disease (CVD) is preceded by a slow, progressive subclinical disease that can start as early as childhood. Similarly, early insulin resistance, defined as the slow progressive need for higher fasting insulin to maintain normal fasting glucose concentrations, can be seen years or decades before the appearance of increased fasting glucose, glucose intolerance, and elevated glycated hemoglobin (HbA1c) that are characteristic of the insulin resistance–prediabetes–type 2 diabetes spectrum [1].

Elevated HbA1c is an established index of cumulative exposure to high blood glucose that has already been shown to be associated with subclinical atherosclerosis (SA), even at values below the thresholds for prediabetes and type 2 diabetes. Moreover, combining HbA1c with classical cardiovascular risk factors (CVRFs) significantly improves the prediction of the multiterritorial extent of SA [2]. However, there is no previously published information about the potential added value of identifying earlier stages of insulin resistance when HbA1c is still "normal" or the association with the presence of SA. Insulin is rarely measured in routine clinical practice, precluding identification of early insulin resistance until later, more advanced stages of the type 2 diabetes disease spectrum. Similarly, SA frequently goes undetected until the first clinical event.

The homeostatic model assessment of insulin resistance (HOMA-IR) ratio is an easy-to-measure surrogate of early insulin resistance. The higher the insulin concentration needed to maintain low fasting glucose levels, the higher the HOMA-IR value, indicating the early development of insulin resistance [3]. HOMA-IR also has the advantage of being a simple, feasible, and economical measure that can be easily integrated into medical practice and is a proven robust clinical and epidemiological tool [4]. The present study explored the association of HOMA-IR with SA in a large cohort of middle-aged individuals with normal HbA1c and no established CVD.

## Methods

### Study overview and population

The PESA (Progression of Early Subclinical Atherosclerosis)-CNIC-Santander study (NCT01410318) uses extensive noninvasive vascular imaging modalities to characterize the presence and progression of SA in a prospective cohort of 4,184 middle-aged asymptomatic employees of Santander Bank in Madrid. The adult participants were between 40 and 55 years old at recruitment and were prospectively included if the baseline examination showed no cardiovascular or chronic kidney disease or any other condition that might reduce life expectancy or affect study adherence. The Instituto de Salud Carlos III Ethics Committee approved the study protocol, and all eligible participants provided written informed consent. The study rationale, design, and data collection details have been described elsewhere [5].

For the present study, 367 participants with prediabetes, type 2 diabetes, or under hypoglycemic treatment at baseline were excluded. Type 2 diabetes was defined as fasting plasma glucose ≥ 126 mg/dL, baseline HbA1c ≥ 6.5% (≥ 48 mmol/mol), or treatment with insulin or oral hypoglycemic medication. Prediabetes was defined according to National Institute for Health and Care Excellence [NICE] guidelines as baseline HbA1c between 6.0% (42 mmol/mol) and 6.4% (46 mmol/mol). Another 76 participants were excluded due to incomplete imaging test information (carotid, femoral, aorta, or CACS). The final sample for the present analysis, therefore, included 3,741 participants.

### Variables collected

CVRFs, other than prediabetes and diabetes, were determined from blood samples, study visits, and interviews. (1) Systemic arterial hypertension: systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure (DBP) ≥ 90 mm Hg, or use of antihypertensive medication; (2) Dyslipidemia: total cholesterol ≥ 240 mg/dL, low-density lipoprotein cholesterol ≥ 160 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL, or use of lipid-lowering drugs; (3) Smoking: current smoking status and lifetime consumption of > 100 cigarettes; (4) Sedentary lifestyle: reporting > 8 h per day in a sitting position; (5) CVD family history: having a first-degree relative diagnosed with atherosclerosis below the age of 55 years in men and 65 years in women; (6) Metabolic syndrome, defined according to the modified ATP III Criteria as meeting at least three of the following conditions: central obesity (waist circumference ≥ 88 cm in women and ≥ 102 cm in men), elevated plasma triglycerides (≥ 150 mg/dL), low plasma HDL-C (< 40 mg/dL in men or < 50 mg/dL in women), elevated fasting plasma glucose (≥ 100 mg/dL), and high blood pressure (SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg).

To study the association between HOMA-IR and biochemical evidence of hepatic steatosis, the non-alcoholic fatty liver disease (NAFLD) score was calculated for each participant based on routinely available clinical data and three serological markers: fasting insulin, alanine transaminase (ALT), and aspartate transaminase (ALT) [6]. The presence of hepatic steatosis was defined as predicted intrahepatic fat ≥ 5% of liver weight [7].

### HOMA-IR categories

Basal insulin resistance was calculated using the original HOMA-IR formula: HOMA-IR = fasting insulin (mU/L) x fasting plasma glucose (mg/dL) / 405 [3]. Based on previous evidence indicating that metabolic risk increases for

values ≥ 2, HOMA-IR values were grouped into three categories, chosen to study associations and dose-response effects while maintaining clinical meaningfulness [8]: <2 (reference category), 2 to <3, and ≥3.

### Estimating participant CV risk

The 10-year risk of first-onset CVD (fatal and non-fatal) was calculated for each participant using the updated regional and sex-specific SCORE2 risk prediction models for 10-year risk in European populations. As all participants were employees of Santander Bank in Madrid, Spain, the equation for populations with a low CVD risk was selected [9]. This equation includes the following parameters: age (in years), sex, measured SBP (mmHg), smoking status, measured total cholesterol (mg/dL), and measured HDL-C (mg/dL). Based on the SCORE2 risk and using different numerical cutoffs depending on age group (<50 years and 50–69 years), 10-year CVD risk was reclassified into two categories: low-to-moderate CVD risk (<2.5% for participants < 50 years and <5% for those ≥ 50 years) and high CVD risk (≥2.5 to 7.5% for participants < 50 years and ≥5–10% for those ≥ 50 years). Three other CV risk scores were also calculated: the 10-year Framingham Risk Score [10], the 30-year Framingham Risk Score [11], and the Regicor Risk Score [12].

### Imaging protocol, analyses, and definition of atherosclerosis

The presence and extent of SA were evaluated using three imaging modalities: (a) 2-dimensional ultrasound (2DVUS) cross-sectional sweeps were made of the carotid, abdominal aortic, and femoral territories; (b) the coronary artery calcification score (CACS) was obtained by non-contrast computed tomography using the Agatston method; and (c) 3-dimensional vascular ultrasound (3DVUS) with standardized 6-cm acquisition was used to quantify atheroma plaque volume in the carotid and femoral arteries. The presence of plaque by 2DVUS was defined according to the Mannheim consensus. Plaque was defined as a focal structure encroaching into the arterial lumen, measuring ≥ 0.5 mm or greater than 50% of the surrounding intima-media thickness. Alternatively, it was considered as plaque if it exhibited a diffuse thickness ≥ 1.5 mm, measured from the media–adventitia to the intima–lumen interface in any of the territories analyzed. Complete PESA study imaging protocol details have been reported elsewhere [5, 13].

A total of 6 vascular territories were defined: right carotid, left carotid, abdominal aorta, right femoral, left femoral, and the coronary arteries. The extent of atherosclerosis was defined by the number of vascular territories showing the presence of plaque identified by 2DVUS or CACS ≥ 1 in the case of the coronary arteries (disease-free if 0 vascular sites affected; multiterritorial extent of disease if ≥ 1 territory was affected). Global plaque volume (mm3) was defined as the sum of right and left 3DVUS-determined plaque volumes in each vascular territory as previously described [14]. All ultrasound recordings and digital images were analyzed at the CNIC Core Imaging Laboratory by experienced evaluators blinded to participant data. Interoperator reproducibility assessments have been reported elsewhere [13].

### Statistical analysis

The distribution of continuous variables was assessed with graphical methods. Summary statistics describing baseline characteristics are presented as means ± standard deviation (SD) or median and [interquartile range] as appropriate for continuous variables and as count and frequencies for categorical variables. Crude pairwise comparisons among HOMA-IR category groups were performed by Student's t-test or Wilcoxon signed-rank test and $\chi 2$ or Fisher exact test, for continuous and categorical variables, as appropriate, and differences were considered statistically significant in these cases at p-value < 0.025 = (0.05/ number of comparisons).Trend tests among HOMA-IR categories were performed by linear or ordered/binary logistic regression as appropriate. For multivariable models, covariates were selected according to their reported association with atherosclerosis (clinical plausibility) [2]. Thus, estimates were adjusted for age, sex, smoking status, SBP, DBP, low-density lipoprotein cholesterol (LDL-C), HDL-C, body mass index (BMI), family history of CVD, and HbA1c. Statistical analyses were performed with STATA (2017, Stata Statistical Software: Release 17.0, StataCorp, College Station, TX, USA).

## Results

### Study population

The study included 3,741 CVD-free participants without prediabetes or type 2 diabetes and with complete imaging test information (89.4% of the total PESA cohort). The mean participant age was 45.5 ± 4.2 years, and 38.7% were women. Median HbA1c was 5.4% [5.2–5.6%] or 36 mmol/mol [33 to 38]. The median HOMA-IR value was 1.08 [0.74 to 1.60], and in most participants (~85.0%) HOMA-IR was <2 (reference), in ~11% between 2 and 3, and in ~4% ≥3. The full distribution of HOMA-IR values is shown in Supplementary Fig. 1. Observed correlation between HbA1c and HOMA-IR values was weak (Spearman's *rho* = 0.14; 95%CI: 0.11–0.17). The median 10-year risk of combined fatal and nonfatal CVD events, according to the SCORE2 risk equation was 2.0% [1.2–3.1%]. Most participants (~75.6%) were classified as having

low-to-moderate CVD risk, the rest being at high CVD risk, with none at very-high CVD risk. The baseline characteristics of the study participants are summarized in Table 1. Baseline characteristics according to risk category are presented in Supplementary Tables 1 and 2.

The participant groups with HOMA-IR > 2 had significantly higher rates of hypertension, dyslipidemia, a family history of CV disease, and metabolic syndrome ($p < 0.001$) and had higher CVRF measures, including for SBP, DBP, body mass index, waist circumference, as well as indicators of poorer metabolic health, including values for total cholesterol, HDL-C, LDL-C,

**Table 1** Baseline study population clinical characteristics (N = 3,741) stratified by HOMA-IR category

| | Total population (N = 3,741) | HOMA-IR < 2 (reference) (n = 3,181) | HOMA-IR 2 to < 3 (n = 410) | HOMA-IR ≥ 3 (n = 150) | P value |
|---|---|---|---|---|---|
| **Demographic** | | | | | |
| Age, years | 45.5 ± 4.2 | 45.4 ± 4.2 | 46.4 ± 4.3 | 46.9 ± 4.2 | < 0.001[a,b] |
| Sex, male | 2,295 (61.3) | 1,815 (57.1) | 349 (85.1) | 131 (87.3) | < 0.001[a,b] |
| Race/ethnicity, Caucasian | 3,736 (99.9) | 3,176 (99.8) | 410 (100) | 150 (100) | 0.808 |
| **Risk factors** | | | | | |
| Hypertension | 389 (10.4) | 244 (7.7) | 95 (23.2) | 50 (33.3) | < 0.001[a,b] |
| Dyslipidemia | 1,468 (39.2) | 1,096 (34.4) | 271 (66.1) | 101 (67.3) | < 0.001[a,b] |
| Smoking | 743 (20.0) | 638 (20.2) | 82 (20.3) | 23 (15.3) | 0.190 |
| Sedentary lifestyle | 3,642 (98.5) | 3,099 (98.6) | 395 (97.8) | 148 (99.3) | 0.875 |
| Family history of CV disease | 585 (15.6) | 474 (14.9) | 79 (19.3) | 32 (21.3) | < 0.001[a] |
| Metabolic syndrome | 279 (7.5) | 94 (3.0) | 111 (27.1) | 74 (49.3) | < 0.001[a,b] |
| **Measured risk and health factors** | | | | | |
| SBP, mmHg | 115.7 ± 12.5 | 114.3 ± 12.0 | 123.0 ± 12.3 | 125.3 ± 11.8 | < 0.001[a,b] |
| DBP, mmHg | 72.1 ± 9.4 | 71.0 ± 8.9 | 78.2 ± 9.2 | 79.6 ± 9.3 | < 0.001[a,b] |
| Total cholesterol, mg/dL | 199.8 ± 32.7 | 198.0 ± 32.0 | 209.3 ± 35.1 | 212.2 ± 32.3 | < 0.001[a,b] |
| HDL-C, mg/dL | 49.5 ± 12.2 | 50.8 ± 12.1 | 42.3 ± 9.4 | 40.2 ± 8.7 | < 0.001[a,b] |
| LDL-C, mg/dL | 131.9 ± 29.2 | 130.3 ± 28.8 | 141.2 ± 30.1 | 140.0 ± 27.6 | < 0.001[a,b] |
| Non-HDL-C, mg/dL | 150.4 ± 33.1 | 147.2 ± 32.1 | 167.0 ± 33.6 | 172.0 ± 31.6 | < 0.001[a,b] |
| Triglycerides, mg/dL | 78 [59–109] | 73 [56–99] | 117 [85–151] | 132 [103–197] | < 0.001[a,b] |
| BMI, kg/m$^2$ | 25.9 ± 3.6 | 25.2 ± 3.2 | 29.3 ± 3.2 | 30.9 ± 3.6 | < 0.001[a,b] |
| Waist, cm | 88.5 ± 11.5 | 86.4 ± 10.5 | 99.4 ± 9.1 | 103.5 ± 9.3 | < 0.001[a,b] |
| Basal insulin, mU/L | 5 [3.5–7.1] | 4.5 [3.3-6.0] | 10.1 [9.3–11] | 14.8 [13.4–17.6] | < 0.001[a,b] |
| Basal glucose, mg/dL | 88 [83–94] | 87 [82–93] | 95 [90–100] | 99 [94–104] | < 0.001[a,b] |
| HbA1c, % | 5.4 [5.2–5.6] | 5.4 [5.1–5.6] | 5.4 [5.2–5.7] | 5.5 [5.4–5.7] | < 0.001[a,b] |
| Ratio total-cholesterol/HDL | 4.1 [3.4–4.9] | 3.9 [3.3–4.7] | 5.0 [4.2–5.8] | 5.3 [4.6–6.4] | < 0.001[a,b] |
| Ratio triglycerides/HDL | 1.6 [1.1–2.5] | 1.5 [1.0-2.2] | 2.7 [1.9-4.0] | 3.6 [2.4–5.2] | < 0.001[a,b] |
| TyG index | 4.4 [4.3–4.6] | 4.4 [4.2–4.5] | 4.6 [4.5–4.8] | 4.7 [4.6–4.9] | < 0.001[a,b] |
| Mod/Vig activity, min/week | 241 [160–346] | 244 [162–349] | 233 [152–335] | 223 [142–357] | < 0.001 |
| **Inflammatory markers** | | | | | |
| hs-CRP, mg/dL | 0.09 [0.05–0.18] | 0.08 [0.04–0.16] | 0.15 [0.09–0.28] | 0.16 [0.10–0.30] | < 0.001[a,b] |
| Ferritin, ng/mL | 55.4 [25.0-126.0] | 50.5 [23.6-110.7] | 135.1 [57.2-213.3] | 125.4 [46.6-221.2] | < 0.001[a,b] |
| 1–hour ESR, mm | 5 [4–8] | 5 [4–8] | 5 [4–8] | 6 [4–9] | 0.572 |
| Fibrinogen, mg/dL | 258.6 [231.9-288.7] | 257.5 [231.4-287.7] | 262.6 [233.7-296.4] | 268.5 [248.6-301.5] | < 0.001[a,b] |
| **Risk scores** | | | | | |
| SCORE2, % | 2.0 [1.2–3.1] | 1.9 [1.1–2.9] | 2.8 [2.1–3.9] | 3.1 [2.4–4.1] | < 0.001[a,b] |
| Framingham risk score 10y, % | 5.5 [3.4-9.0] | 5.1 [3.1–8.2] | 8.5 [6.0-13.2] | 10.7 [7.1–14.4] | < 0.001[a,b] |
| Framingham risk score 30y, % | 14.4 [8.4–22.5] | 13.0 [7.7–20.7] | 22.1 [15.5–31.8] | 26.0 [18.8–33.3] | < 0.001[a,b] |
| Regicor risk score, % | 1.6 [0.9–2.5] | 1.4 [0.8–2.3] | 2.5 [1.7–3.5] | 2.9 [2.1–3.9] | < 0.001[a,b] |

Values are mean ± SD, n (%), or median [first quartile, third quartile]. Indicated p-value derived from trend tests among HOMA-IR categories

*HOMA-IR* homeostatic model assessment of insulin resistance index, *CV* cardiovascular, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *BMI* body mass index, *HbA1c* glycated hemoglobin, *TyG* triglyceride to glucose, *Mod/Vig activity min/week* moderate to vigorous minutes of physical activity per week, *hs-CRP* high-sensitivity C-reactive protein, *1-hour ESR* erythrocyte sedimentation rate, *SCORE2* systematic coronary risk estimation 2

[a]Indicates statistically significant differences ($p < 0.025$) between HOMA-IR < 2 and HOMA-IR 2 to < 3 groups

[b]Indicates statistically significant differences ($p < 0.025$) between HOMA-IR < 2 and HOMA-IR ≥ 3 groups

non-HDL-C, plasma triglycerides, HbA1c, and inflammatory parameters ($p<0.001$). These participants also had higher liver enzymes, a higher calculated NAFLD score, and a higher proportion of hepatic steatosis ($p<0.001$, Supplementary Table 3).

### Association between HOMA-IR category and subclinical atherosclerosis

Figure 1 provides visual examples illustrating the association between HOMA-IR and imaging findings of subclinical atherosclerosis. High HOMA-IR values showed a positive association with the multiterritorial extent of SA assessed by 2D vascular ultrasound and non-contrast cardiac computed tomography ($p<0.001$) (Fig. 2A). This trend held for three individual territories: the carotid, femoral, and coronary (CACS) (Fig. 2B–D). In a sensitivity analysis, the association was also observed in individuals with HbA1c below and greater than or equal to the median value of 5.4% (36 mmol/mol) ($p<0.001$) (Supplementary Figs. 2 and 3). Similar trends were observed when categorizing HOMA-IR values by tertiles, with significant differences found between the lowest and the highest tertile of HOMA-IR values (HOMA-IR<0.8 and HOMA-IR≥1.4, respectively) for all outcomes analyzed (data not shown).

In the multivariable regression analyses after adjusting for key CVRFs and HbA1c, the higher HOMA-IR categories maintained an association both with multiterritorial SA extent (odds ratio for HOMA-IR≥3=1.41 [95%CI: 1.01 to 1.95, $p=0.041$]) and with CACS (odds ratio for HOMA-IR≥3=1.74 [95CI: 1.20 to 2.54, $p=0.004$)], as compared with the HOMA-IR<2 (reference category).

Of the 3,741 participants, 3,592 (~96.0%) underwent 3DVUS imaging. After adjusting for key CVRFs and HbA1c, higher HOMA-IR categories showed a significant association with elevated global plaque burden ($p<0.001$) and with the presence of SA in the carotid ($p=0.009$) and femoral arteries ($p<0.001$) when analyzed separately (Fig. 3A–C). Sensitivity analysis, including further adjustment for non-HDL cholesterol and high-sensitivity C-reactive protein (hs-CRP), showed similar results (data not shown).

### Associations between SCORE2 risk, HOMA-IR, and SA

Stratification of participants by SCORE2 risk category revealed that individuals with high CVD risk had higher HOMA-IR values, a greater multiterritorial extent of SA ($p<0.001$), and a higher global plaque volume ($p<0.001$) than individuals with low-to-moderate CVD risk (Fig. 4A–C). SCORE2 risk stratification of the adjusted association between HOMA-IR categories, the multiterritorial extent of SA, and global plaque volume revealed an independent association between higher HOMA-IR and SA burden in low-to-moderate risk participants, but this association was not observed in high-risk individuals (Fig. 4D, E).

## Discussion

To our knowledge, this cross-sectional analysis of the PESA cohort study is the first to report an independent association between HOMA-IR-estimated early insulin resistance and SA measured by 2D vascular ultrasound, non-contrast cardiac computed tomography, and 3D vascular ultrasound in CVD-free individuals without prediabetes or type 2 diabetes (HbA1c<6.0%, <42 mmol/mol).

The assessment of the HOMA-IR ratio in this population with normal HbA1c conferred added value in at least 3 respects. First, it identified individuals with higher measured values for CVRFs, including SBP, DBP, body mass index, and waist circumference, as well as indicators of poorer metabolic status, including values for HbA1c, lipid profile, inflammatory parameters, liver parameters, and hepatic steatosis. Second, it identified a group of individuals with an elevated prevalence and extent of SA in the carotid, femoral, and coronary arterial beds, as well as a higher overall disease burden quantified by 3DVUS global plaque volume; notably, this association was found regardless of whether HbA1c was above or below the median value of 5.4% (36 mmol/mol) ($p<0.001$). Finally, after adjusting for CVRFs and HbA1c, the odds of multiterritorial SA and CACS were higher among participants with HOMA-IR≥3, particularly those with a low-to-moderate SCORE2 CVD risk estimate. Moreover, these odds decreased with the HOMA-IR category, being lower for participants with HOMA-IR=2–3 and lowest for those with HOMA-IR≤2.

Our findings have important implications, considering that HOMA-IR is a simple clinical parameter that identifies the onset of insulin resistance before the rise of HbA1c and only requires a regular blood test. HOMA-IR screening could be used to facilitate early identification of individuals with a high risk of SA and help refine and further individualize primary prevention measures. By serving as an early indicator of susceptibility to the development of advanced atherosclerosis, HOMA-IR screening could prompt the early implementation of prevention strategies, particularly among individuals with low-to-moderate CVD risk (Fig. 5- Central Illustration).

### Value of HOMA-IR as an early marker of poorer metabolic health

Prediabetes and type 2 diabetes are advanced stages of the dysglycemia-based chronic disease continuum associated with an increased risk of CVD [1, 15]. Yet both the development of SA and frequently the first clinical events, occur earlier and at HbA1c levels below the

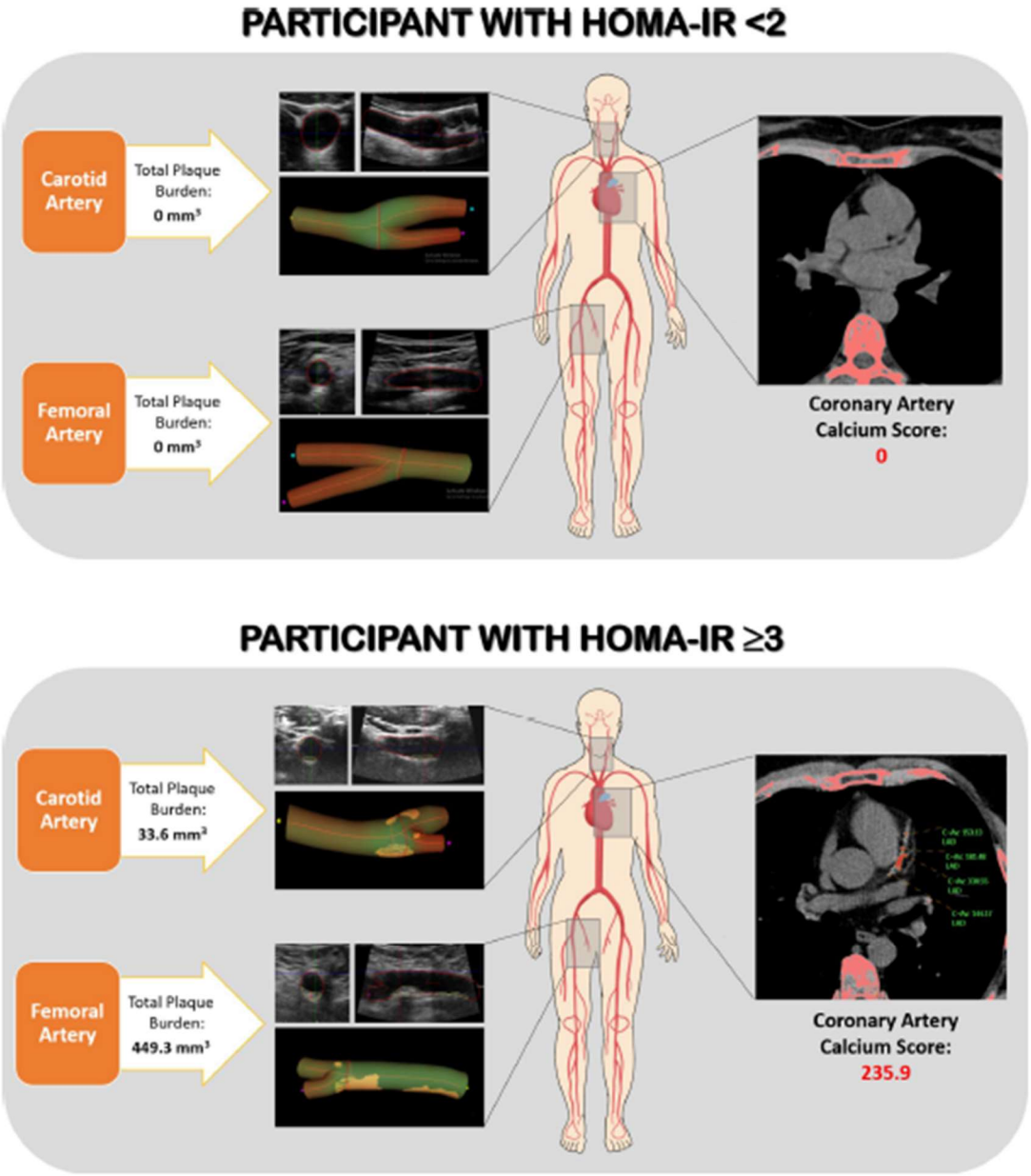



**Fig. 1** 3-dimensional vascular ultrasound and computed tomography images exemplifying the study findings. (**A**) Visual example illustrating imaging findings in a participant with HOMA-IR < 2 (reference group), depicting the absence of subclinical atherosclerosis affecting vascular sites. (**B**) Visual example illustrating imaging findings in a participant with HOMA-IR ≥ 3 (category with the highest HOMA-IR values), revealing a multiterritorial extent of subclinical atherosclerosis in the carotid and femoral arteries and an elevated coronary artery calcium score. HOMA-IR = homeostatic model assessment of insulin resistance

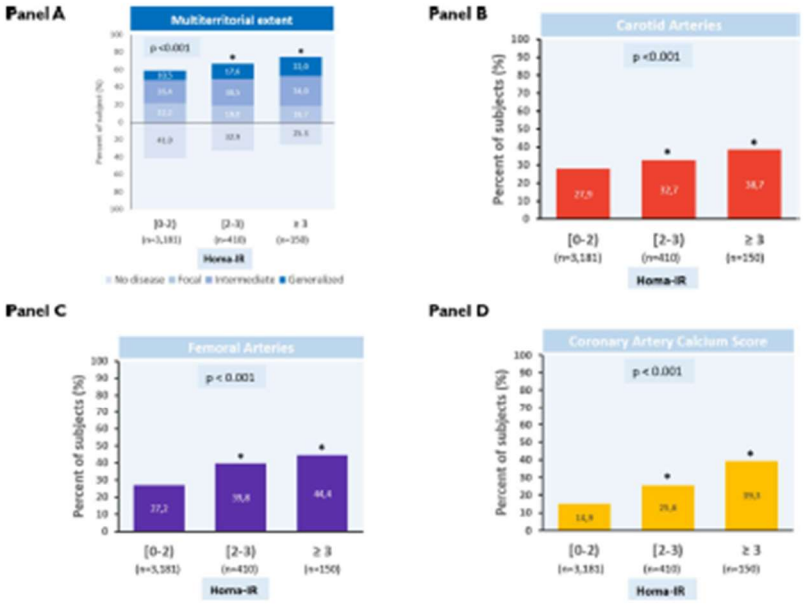



**Fig. 2** HOMA-IR categories, and association with the multiterritorial extent of subclinical atherosclerosis in different vascular territories. (**A**) Multiterritorial extent of subclinical atherosclerosis (SA), assessed by 2-dimensional vascular ultrasound and non-contrast coronary computed tomography, stratified by HOMA-IR category. Multiterritorial SA extent for this figure is defined by combining data from both imaging techniques to classify individuals as having no disease (0 vascular sites affected) or having focal (1 site), intermediate (2 to 3 sites), or generalized atherosclerosis (4 to 6 sites). (**B**–**D**) Presence of SA in different vascular territories stratified by HOMA-IR category. *Indicates statistically significant differences ($p < 0.025$) as compared to the lowest HOMA-IR category (HOMA-IR < 2, reference group). HOMA-IR = homeostatic model assessment of insulin resistance

diagnostic thresholds [16–18]. The question thus remains of whether we are properly addressing primary prevention of CVD by only screening for and treating diabetes and hyperlipidemia while ignoring the early onset of insulin resistance.

The HOMA-IR ratio captures early abnormalities in systemic glucose homeostasis by testing how much insulin is needed to keep fasting glycemia low. For instance, a fasting plasma insulin of 5 mU/L is needed for a healthy individual to maintain fasting plasma glucose at 81 mg/dL., and this results in HOMA-IR=1 when using the original formula: HOMA- IR=fasting insulin (mU/L) x fasting plasma (mg/dL) / 405. In this highly regulated mechanism, insulin resistance occurs when a higher than normal insulin concentration is required to obtain a quantitatively normal response in target tissues, and, among other parameters, HOMA-IR values start to increase [19]. During this initial compensatory phase of early insulin resistance, higher HOMA-IR values, among other insulin resistance surrogates such as the triglyceride-glucose index [20], are associated with markers of ectopic fat accumulation and metabolic syndrome, including elevated circulating triglycerides, adipose tissue dysfunction, increased secretion of free fatty acids (FFAs) and pro-inflammatory adipokines, and elevated liver production of very-low-density lipoprotein (VLDL) particles in the presence of visceral fat and hepatic fat [21]. Additionally, HOMA-IR levels are elevated in patients with heart failure, a condition often accompanied by comorbidities such as obesity and hypertension [22], and have been identified as predictor of adverse clinical events in non-diabetic individuals experiencing decompensated heart failure [23]. In this regard, it is not surprising that in our study, higher HOMA-IR values were associated with a cluster of unfavorable anthropometric, metabolic, and inflammatory markers ($p<0.001$, Table 1) in individuals with low-to-moderate and high CVD risk (Supplementary Tables 1 and 2).

### Association of HOMA-IR with subclinical atherosclerosis

Our analysis establishes an association between higher HOMA-IR values and the multiterritorial extent of SA and CACS after adjusting for key CVRFs and HbA1c (Fig. 2A). Similar results have been previously documented in other cohorts and with different imaging methodologies in adults, children, and adolescents, although these analyses used less robust markers of SA, like carotid intima-media thickness (cIMT) [24]. Studies of populations without type 2 diabetes have reported associations of HOMA-IR with CAC prevalence and


**Fig. 3** HOMA-IR categories and plaque volume measured by 3-dimensional vascular ultrasound (3DVUS). (**A**) Global plaque volume of subclinical atherosclerosis (no disease vs. volume in tertiles) assessed by 3DVUS and stratified by HOMA-IR category. (**B**, **C**) The presence of carotid and femoral plaque was assessed by 3DVUS (plaque volume > 0 $mm^3$) and stratified by the HOMA-IR category. *Indicates statistically significant differences as compared to the lowest HOMA-IR category (HOMA-IR < 2, reference group)

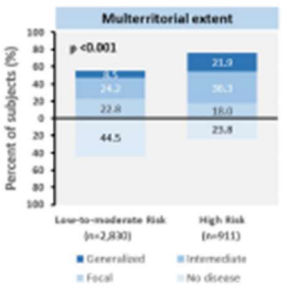
progression for some ethnicities [25, 26] and with the severity of multivessel coronary artery disease [27]. Our results, obtained with 2DVUS and CACS imaging techniques and including assessment of global plaque volume by 3DVUS, support previous findings in individuals with less advanced disease status (HbA1c<6.0% (<42 mmol/mol) and suggest that early insulin resistance may be an indicator of higher risk for the development of SA, independently of other CVRFs and HbA1c. These findings highlight the importance of early screening to identify at-risk individuals before clinical CVD events occur and to reduce the risk of future type 2 diabetes.

The relationship between HOMA-IR and SA described here was particularly striking for the coronary territory. In the reference HOMA-IR group (<2), only 14.9% had a CACS>0; in other words, 85.1% were free of coronary artery calcium (Fig. 2D). The proportion of participants with CACS increased with the insulin-resistance category, to 25.6% for HOMA-IR=2 to 3 and 39.3% for HOMA-IR≥3. When further studying the participant subgroup with the highest values of HOMA-IR≥4 (n=49), the proportion with CACS>0 increased to 49% (more than 3 times the rate in the reference HOMA-IR group). It is important to note that all the study participants are asymptomatic individuals considered to be in primary prevention, with mainly low-to-moderate SCORE2 risk estimates. HOMA-IR thus appears to identify the risk of coronary artery calcification in this overtly disease-free population, even after adjusting for other CVRFs and HbA1c. It is unclear why the association between HOMA-IR and SA was only observed in individuals with a low-to-moderate SCORE2 risk estimate and not in those with high CVD risk. It may be that SCORE2, designed to predict the risk of clinical events, is less able to predict SA. The fact that a notable occurrence of multi-territorial SA within the low-to-moderate risk group is observed could be attributed to the comprehensive examination of various territories, including vascular areas more susceptible to disease, such as the iliofemoral arteries, which were not explored in earlier studies. Another possibility is that the high CVD-risk subgroup had a higher prevalence of traditional CVRFs that heavily influenced the SCORE2 equations or that the sample size for this risk category was too small, leaving our study underpowered to detect differences in this subgroup. These results emphasize the importance of exploring the relationship between the early onset of insulin resistance and SA, especially in individuals with low-to-moderate CVD risk, who often receive less attention in clinical practice but who may have a substantial burden of atherosclerotic disease.

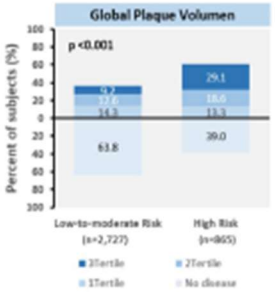
**Panel A**

| HOMA-IR | SCORE2 Risk Categories Low-to-Moderate | High |
|---|---|---|
| <2 | 2,522 (89.1) | 659 (72.3) |
| 2 to <3 | 237 (8.4) | 173 (19.0) |
| ≥ 3 | 71 (2.5) | 79 (8.7) |
| | 2,830 | 911 |

**Panel B**




**Panel C**




**Panel D**

2DVUS — Multi-territorial Extent

| OR (95% CI) P value | Low-to-moderate SCORE2 | High SCORE2 |
|---|---|---|
| HOMA-IR (continuous) | 1.29 (1.17; 1.42) P <0.001 | 0.91 (0.81; 1.02) P 0.121 |
| HOMA-IR categories | | |
| <2 (n=3,181) | 1. Ref | 1. Ref |
| 2 to <3 (n=410) | 1.38 (1.07; 1.77) P 0.012 | 0.93 (0.69; 1.26) P 0.636 |
| ≥3 (n=150) | 2.08 (1.36; 3.19) P 0.001 | 1.02 (0.66; 1.57) P 0.943 |

**Panel E**

3DVUS — Plaque Volume by 3DVUS

| OR (95% CI) P value | Low-to-moderate SCORE2 | High SCORE2 |
|---|---|---|
| HOMA-IR (continuous) | 1.28 (1.15-1.42) P 0.001 | 0.92 (0.82-1.04) P 0.182 |
| HOMA-IR categories | | |
| <2 (n=3,061) | 1. Ref | 1. Ref |
| 2 to <3 (n=387) | 1.30 (1.00-1.70) P 0.052 | 1.09 (0.80-1.48) P 0.597 |
| ≥3 (n=144) | 2.20 (1.38-3.51) P 0.001 | 0.89 (0.57-1.37) P 0.591 |

**Fig. 4** HOMA-IR, multiterritorial extent of subclinical atherosclerosis, and global plaque volume, stratified by SCORE2 risk category. (**A**) Number of participants in each SCORE2 risk category, stratified by HOMA-IR category. (**B**) Extent of subclinical atherosclerosis stratified by SCORE2 risk category. (**C**) Global plaque volume (absence of disease or plaque volume in tertiles) stratified by SCORE2 risk category. (**D**) SCORE2 risk-adjusted odds ratios for the multiterritorial extent of subclinical atherosclerosis stratified by HOMA-IR category. (**E**) Adjusted odds ratios for global plaque volume (absence of disease or plaque volume in tertiles) stratified by HOMA-IR category and SCORE2 risk category. The multivariable models were adjusted for age, sex, smoking status, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, family history of CVD, and HbA1c. OR = odds ratio, SCORE2 = systematic coronary risk evaluation score 2, HOMA-IR = homeostatic model assessment of insulin resistance, 2DVUS = 2-dimensional vascular ultrasound, 3DVUS = 3-dimensional vascular ultrasound

### Potential of HOMA-IR as a marker of metabolic syndrome and NAFLD

In clinical practice, HOMA-IR is often used as an indicator of metabolic syndrome, a condition characterized by a clustering of risk factors indicative of poor metabolic health but for which clinical utility beyond its individual components is still disputed [28]. In our study population, the HOMA-IR category (<2, 2 to 3, and ≥3) showed a direct correlation with the prevalence of metabolic syndrome (3.0%, 27.1%, and 49.3%) (Table 1) and hepatic steatosis (0.8%, 21.9%, and 59.3%) (p<0.001, Supplementary Tables 3 and Supplementary Fig. 4). An exploratory analysis showed that individuals meeting NAFLD diagnostic criteria had increased odds of multiterritorial SA (OR=1.45 (95%CI: 1.05 to 2.00), p=0.023) and CACS (OR=2.32 (95%CI: 1.59 to 3.40), p<0.001) independently of all other CVRFs evaluated, including HOMA-IR. Although all these parameters are highly related and share common pathophysiological pathways, in clinical practice, HOMA-IR offers the potential to detect and monitor a common root cause of both conditions - insulin resistance and insulin hypersecretion - with a convenient measure obtained from a blood test. HOMA-IR could be particularly useful for the early detection of NAFLD, a condition whose prevalence is projected to increase in the coming decades, often goes undetected until its later stages, and for which there is currently no approved effective treatment [29].

### Measuring HOMA-IR in individuals with low-to-moderate CVD risk: an opportunity to initiate prevention

Almost 60% of PESA participants have SA despite being classified as low CVD risk in the baseline evaluation [13]. In this substudy of individuals with HbA1c<6.0% (<42 mmol/mol), measuring insulin resistance with the HOMA-IR ratio added clinical value by detecting individuals at higher risk of developing SA, particularly among the subgroup with a low-to-moderate SCORE2 risk estimate.

Beginning intervention at an early disease stage is typically less costly, less risky, and more effective. Identifying individuals in the early stages of insulin resistance is likely to increase the potential to significantly reduce the occurrence of cardiovascular events over time. However, it is important to note that further research is needed to establish this effect with greater certainty. Furthermore, repeated measures of HOMA-IR could be used to monitor changes in individual risk and health status. For instance, the inclusion of fasting insulin in addition to fasting glucose or HbA1c in asymptomatic individuals

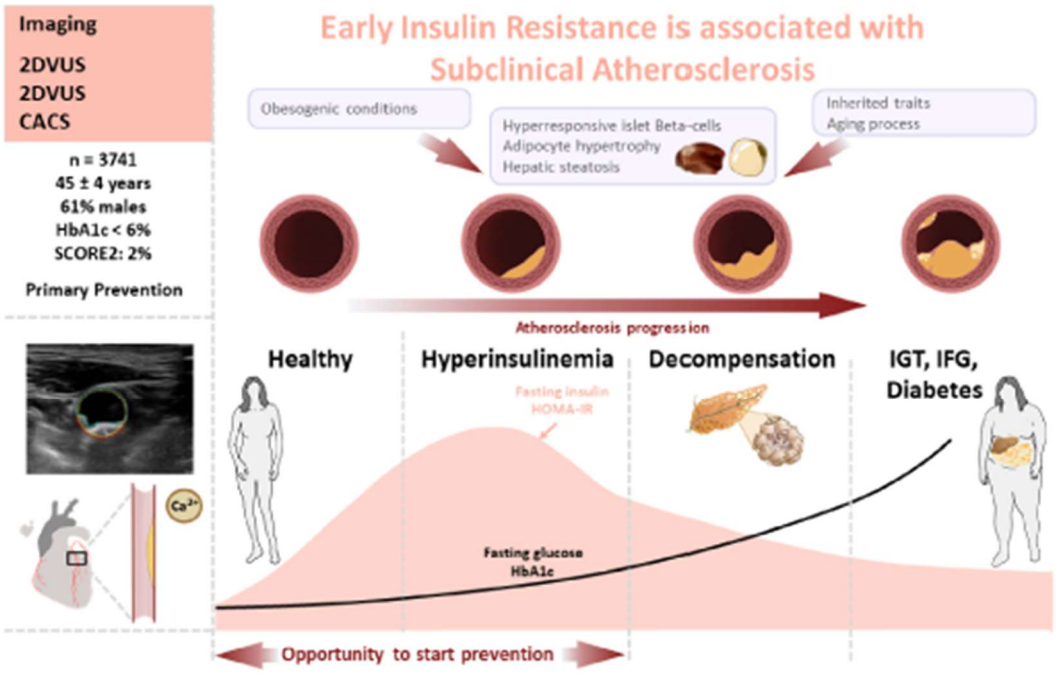



**Fig. 5 Central illustration**: Early insulin resistance and subclinical atherosclerosis. This study examined 3,741 individuals with normal HbA1c < 6.0% <42 mmol/mol) and no known cardiovascular disease. Higher homeostatic model assessment of insulin resistance (HOMA-IR) values were associated with higher subclinical atherosclerosis (SA) burden as measured by extensive imaging after adjusting for traditional CVRFs and HbA1c. The HOMA-IR index offers a simple and cost-effective way to detect the onset of insulin resistance and SA and could be used to support the implementation of early prevention strategies in clinical practice. 2DVUS, 2-dimensional vascular ultrasound; 3DVUS, 3-dimensional vascular ultrasound; CACS, coronary artery calcium score; IGT, impaired glucose tolerance; IFG, impaired fasting glucose

could potentially identify individuals at risk of type 2 diabetes years before disease initiation. Although there are currently no medications specifically approved to treat insulin resistance [30], HOMA-IR provides an effective way to monitor lifestyle interventions designed to improve insulin sensitivity, such as weight loss, exercise, and diet replacement interventions. In addition, our results indicate that HOMA-IR monitoring may help to mitigate the risk of developing metabolic syndrome and NAFLD, as well as the overall SA disease burden.

While the HOMA-IR index has been widely utilized in epidemiological settings, we acknowledge that the simplicity and cost-effectiveness of measuring HOMA-IR may be subject to variability, particularly given the non-standardized nature of insulin determinations and variations among different populations. We recognize that its suitability in clinical practice is a topic of debate, and further considerations are warranted, especially considering the projected increase in CVRFs and CVD prevalence [31].

### Study limitations

Several study limitations need to be acknowledged. First, this was a cross-sectional study, and the association detected between HOMA-IR and SA, therefore, cannot be considered causal. In addition, any residual confounding resulting from unmeasured or inadequately controlled variables cannot be ruled out. It should be noted that the PESA study cohort is composed of individuals from a specific occupation and living in Spain, and therefore, caution should be exercised when generalizing the results. The prevalence of multiterritorial disease in this cohort is relatively high among subjects considered at low-to-moderate risk. While the SCORE2 scale was designed to evaluate the risk of CV events stemming from atherosclerosis, it was not intended to estimate the presence of SA. Our study suggests the added value of imaging for diagnosis and prevention beyond traditional CV risk scores. Furthermore, it underscores the potential to identify easily measurable markers, as demonstrated in this study with early insulin resistance markers, to predict the presence of SA. Lastly, further studies are needed

to define the relationship between the HOMA-IR ratio and other CVRFs, including genetics, lifestyle, and environmental factors. This could lead to a more comprehensive understanding of the underlying mechanisms of CVD and facilitate the development of more effective prevention strategies.

## Conclusions

The results of this cross-sectional study suggest that assessment of the HOMA-IR ratio in populations without prediabetes or type 2 diabetes can provide valuable information for identifying individuals at a higher risk of developing subclinical atherosclerosis. Through a simple and economical blood test, HOMA-IR informs on the onset of insulin resistance and may help to identify the need for earlier implementation of prevention strategies. These findings highlight the importance of including the HOMA-IR ratio in the assessment of cardiovascular risk and underscore the potential benefits of early intervention in primary prevention.

### List of abbreviations

| | |
|---|---|
| CVD | Cardiovascular disease |
| HbA1c | Glycated hemoglobin |
| SA | Subclinical atherosclerosis |
| CVRFs | Cardiovascular risk factors |
| HOMA-IR | Homeostatic Model Assessment of Insulin Resistance |
| PESA | Progression of Early Subclinical Atherosclerosis |
| SBP | Systolic blood pressure |
| DBP | Diastolic blood pressure |
| HDL-C | High-density lipoprotein cholesterol |
| LDL-C | Low-density lipoprotein cholesterol |
| TyG | Triglyceride to glucose |
| NAFLD | Non-alcoholic fatty liver disease |
| ALT | Alanine transaminase |
| ALT | Aspartate transaminase |
| 2DVUS | 2-dimensional vascular ultrasound |
| CACS | Coronary artery calcium score |
| 3DVUS | 3-dimensional vascular ultrasound |
| SD | Standard deviation |
| BMI | Body mass index |
| hs-CRP | high-sensitivity C-reactive protein |
| FFAs | Free fatty acids |
| VLDL | Very-low-density lipoprotein |
| MAFLD | Metabolic (dysfunction)-associated fatty liver disease |
| cIMT | Carotid intima-media thickness |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-023-02090-1.

Supplementary Material 1

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### Author contributions

Conception and design of the research: J.I.G, A.G.A, B.O, R.B, B.I, R.F.J, V.F; Principal investigators: R.F.J, B.I, V.F; Co-investigators: J.I.G, A.G.A, B.O, G.M, I.G.L, J.F, A.D, C.P.H, A.F.O, R.B; Data analysis: B.O; Drafting and revision of the manuscript: J.I.G wrote the first version of the manuscript, all authors revised it and contributed significantly to write the final version that was accepted.

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### Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

The Instituto de Salud Carlos III Ethics Committee approved the study protocol, and all eligible participants provided written informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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## Publisher's Note

# 4. Synthesis and Discussion of Key Findings

The following summaries present the pivotal outcomes of the studies, each offering unique insights into the domain of CV health.

**In the first investigation**, a cross-sectional analysis of participants from the FAMILIA trial sheds light on the presence and burden of 3DVUS-assessed subclinical atherosclerosis in a predominantly female, young cohort (mean age: 38 years) of 436 adults from a socioeconomically disadvantaged community in Harlem, New York. The study's key revelations encompass significant ethnic and racial disparities, with non-Hispanic Black participants exhibiting higher rates of cardiovascular risk factors and a greater burden of subclinical atherosclerosis, particularly in the carotid arteries, compared to their Hispanic counterparts, even after adjusting for 10-year Framingham CV risk and other lifestyle and socioeconomic factors. These findings have far-reaching implications for understanding and addressing variations in CV disease prevalence among different racial and ethnic groups generally unrepresented in research. **Figure 10 (Central Illustration 1)** provides a comprehensive study overview, encompassing details about the population, methodology, and key findings.

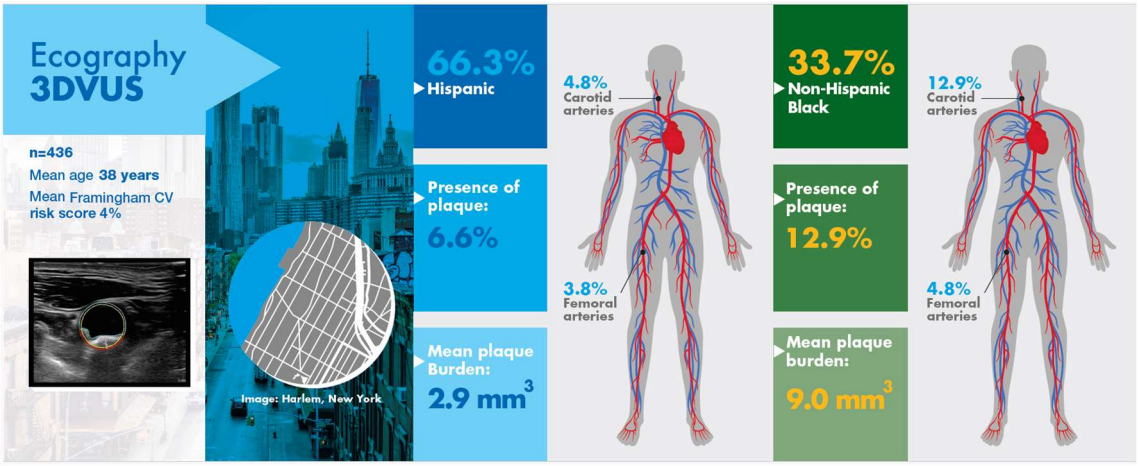



**Figure 10. Subclinical atherosclerosis in Hispanic and non-Hispanic Black socioeconomically vulnerable young adults, Central Illustration 1.** This study assessed the impact of race and ethnicity on early subclinical atherosclerosis, as assessed by bilateral carotid and femoral 3-dimensional vascular ultrasound (3DVUS), on 436 young Hispanic and non-Hispanic Black adults (parents/caregivers and staff) recruited in the FAMILIA trial from 15 Head Start preschools in Harlem (New York). After adjustment for traditional cardiovascular risk, lifestyle, and socioeconomic factors, non-Hispanic Black individuals were more likely than Hispanic individuals to have subclinical atherosclerosis and a higher

burden of disease, suggesting the existence of unknown cardiovascular risk factors underlying such residual excess risk. Early detection of atherosclerosis could play an important role in prevention programs in different populations. This figure was crafted by a professional designer following the instructions provided.

**The second investigation**, a cross-sectional analysis of the PESA cohort, establishes for the first time an independent association between early insulin resistance, measured by the HOMA-IR ratio, and subclinical atherosclerosis through comprehensive assessments using 2D- and 3D-vascular ultrasound, and non-contrast cardiac CT in 3,741 ASCVD-free individuals (mean age: 46 years) without prediabetes or T2D (HbA1c < 6.0%). The study highlights the added value of employing HOMA-IR, a surrogate of early insulin resistance, on top of traditional CV risk factors and HbA1c, identifying those with heightened prevalence of poorer metabolic status and heightened prevalence and extent of subclinical atherosclerosis across various vascular beds. Notably, the association persists irrespective of HbA1c levels. This breakthrough implies that HOMA-IR screening, a straightforward clinical parameter detectable before HbA1c elevation by measuring how much fasting insulin is required to maintain blood glucose in the normal range, holds substantial promise for the early identification of individuals at high risk of subclinical atherosclerosis. This screening method, requiring only a routine blood test, could facilitate the timely implementation of personalized prevention measures in clinical practice, especially among individuals with low-to-moderate cardiovascular risk. **Figure 11 (Central Illustration 2)** provides a comprehensive study overview, encompassing details about the population, methodology, and key findings.

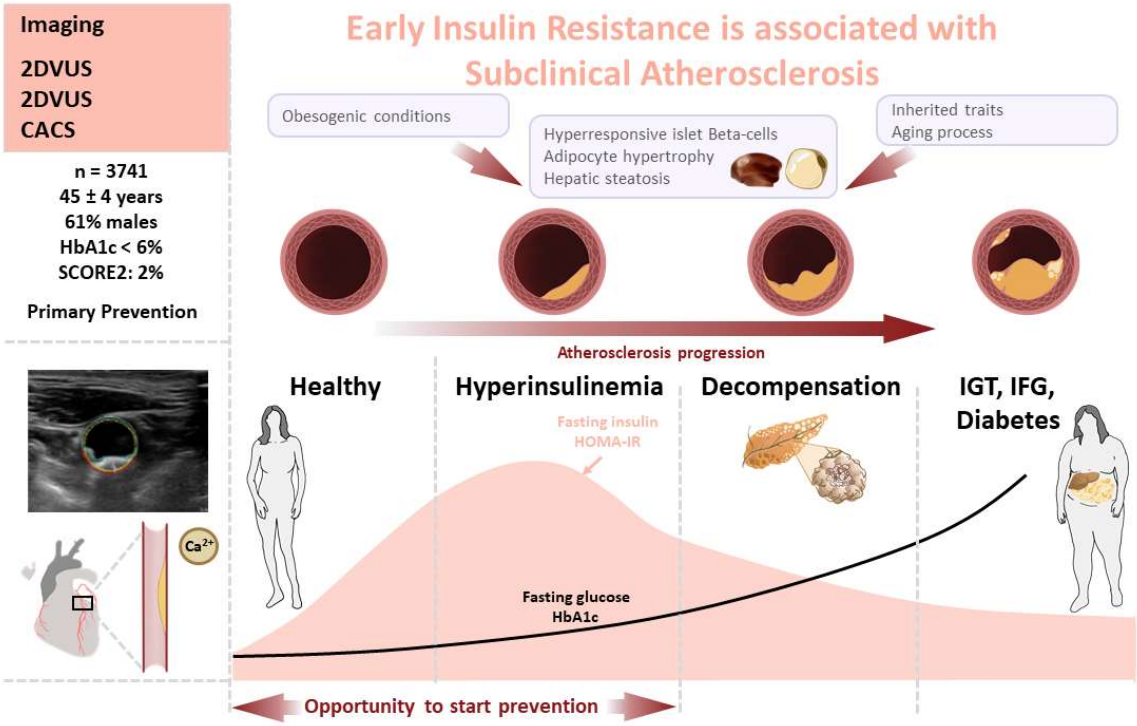



**Figure 11. Early insulin resistance and subclinical atherosclerosis, Central Illustration 2.** This study examined 3,741 individuals with normal HbA1c <6.0% and no known cardiovascular disease. Higher homeostatic model assessment of insulin resistance (HOMA-IR) values were associated with a higher burden of subclinical atherosclerosis as measured by extensive imaging after adjusting for traditional CV risk factors and HbA1c. The HOMA-IR index offers a simple and cost-effective way to detect the onset of insulin resistance and subclinical atherosclerosis. It could be used to support the implementation of early prevention strategies in clinical practice. 2DVUS, 2-dimensional vascular ultrasound; 3DVUS, 3-dimensional vascular ultrasound; CACS, coronary artery calcium score; IGT, impaired glucose tolerance; IFG, impaired fasting glucose. This figure is my original creation and was generated using Biorender.com under a license for publication.

**Global Significance**

Collectively, these findings significantly advance our understanding of CV health, risk factors, and the association with subclinical atherosclerosis within different populations. The revelations from the FAMILIA study underscore the importance of addressing ethnic and racial disparities in subclinical atherosclerosis, providing a foundation for targeted community interventions. On the other hand, the insights from the PESA study underscore the potential of HOMA-IR screening as a practical tool for identifying individuals at risk of subclinical atherosclerosis, enabling the early implementation of tailored preventive strategies. These studies collectively contribute to the ongoing pursuit of more precise and personalized approaches to CV care, emphasizing early detection and intervention to reduce the global burden of CV diseases.

### Insights from the FAMILIA study on ethnicity/race and subclinical atherosclerosis.

Achieving satisfactory progress in reducing the risk and incidence of ASCVD has proven challenging, particularly within certain ethnic and racial groups (143). The lack of substantial advancement could be in part attributed to various factors, including disparities in the prevalence of traditional CV risk factors, differences in social determinants of health such as education, household income, and environmental exposure, as well as inequalities in healthcare delivery, racial discrimination, and the potential existence of fundamental distinctions in disease mechanisms and genetic susceptibility. The intricate interplay between these factors has not been thoroughly examined, and the under-representation of these ethnic and racial groups in clinical trials has most likely contributed to this significant knowledge gap (144).

The results of the FAMILIA study shed light on the prevalence and burden of subclinical atherosclerosis in a socioeconomically disadvantaged community comprising individuals of non-Hispanic Black and Hispanic origins. This study presents unique data derived from bilateral carotid and femoral 3DVUS examinations conducted on 436 young adults, including parents, caregivers, and staff from 15 Head Start preschools in Harlem, New York, with no history of ASCVD. Several strengths characterize this investigation, including a well-defined population from the FAMILIA study, meticulously measured outcomes, and covariables. Notably, the study's distinct feature lies in including individuals from different ethnic and racial backgrounds, all sharing similar neighborhood resources and low/very low economic status backgrounds, effectively minimizing the impact of socioeconomic confounding. Despite this, notable differences emerged in comparing non-Hispanic Black participants to their Hispanic counterparts. Non-Hispanic Black individuals exhibited higher rates of classic CV risk factors such as hypertension, active smoking, and body mass index. On the other hand, they reported higher consumption of fruits and vegetables than their Hispanic counterparts. Furthermore, non-Hispanic Black subjects displayed a higher prevalence of subclinical atherosclerosis (12.9% in non-Hispanic Black individuals vs. 6.6% in Hispanic individuals) despite having similar 10-year Framingham CV risk scores. They also presented with an increased disease burden and a higher prevalence of multi-territorial disease, primarily attributable to disparities in the carotid arteries.

Upon adjusting for classic CV risks, lifestyle factors, and socioeconomic variables, it became evident that young adults of non-Hispanic Black descent may be more susceptible to early subclinical atherosclerosis than their Hispanic counterparts. This observation suggests the

existence of emerging or undiscovered cardiovascular factors contributing to the residual excess risk.

Within our cohort, non-Hispanic Black individuals demonstrated significantly higher blood pressure and a higher prevalence of self-reported smoking, both recognized risk factors associated with early atherosclerotic plaque formation and subsequent cardiovascular disease (145), consistent with the National Health and Nutrition Examination Survey (NHANES) statistics, highlighting the lowest prevalence of ≥5 ideal CV health metrics among the non-Hispanic Black population, particularly in children and adults aged ≥20. Additionally, both ethnic groups exhibited high prevalence rates for other risk factors, including overall diabetes and overweight/obesity, potentially contributing to premature vascular aging (146). These findings underscore the need for targeted early health promotion programs. Tailored interventions, such as CV screening campaigns and intensified anti-smoking health marketing, along with improved access to cessation treatments, counseling, and medication, could significantly benefit specific racial and ethnic groups. This underscores the imperative for more equitable initiatives within these communities.

### Ethnicity/race is probably a social circumstance but also a macro determinant of health: a call for precision.

The incorporation of ethnicity and race as variables in biomedical research and clinical practice has long been a subject of debate, with documented advantages and disadvantages. Recognizing ethnicity and race as master status impacting every aspect of life and influencing social interactions, opportunities, and resources has proven essential for documenting health disparities and designing interventions that demonstrably improve outcomes (114). However, this approach raises concerns regarding its limited biological precision, the potential for bias, and inadequate representation of diverse experiences within ethnical and racial categories. On the one hand, ethnicity/race serves as a critical tool for identifying disparities and targeting interventions. By capturing epidemiologic information, it sheds light on social determinants like racism, discrimination, research underrepresentation, socioeconomic position, and environmental exposures. This approach allows researchers to set specific targets to improve disparities, employing frameworks such as the social-ecological model (147). In essence, ethnicity/race aids in aggregating higher-prevalent risk factors associated with atherosclerosis, offering valuable insights for tailored health strategies.

While acknowledging the advantages, it is crucial to recognize the limitations and potential pitfalls of utilizing ethnicity/race (148). The lack of biological precision, dependence on self-reported and socially constructed criteria, and the risk of perpetuating biases and stereotypes are prominent concerns. Ethnical/racial categories often overlook the diverse experiences within groups, potentially leading to generalizations that do not accurately reflect individual health risks.

Upon careful consideration, instead of outright abandonment, a nuanced approach is necessary to continue utilizing ethnicity and race. These variables offer valuable epidemiologic data crucial for comprehending health disparities and, notably, shaping targeted interventions. However, the current discourse emphasizes a transition toward more precise tools, such as genetic ancestry, directing attention to the geographical origins of one's forebears—parents, grandparents, and beyond, providing a clearer insight into individual genetic backgrounds (114). In contrast to the term "race," the notion of "ancestry" redirects attention away from fixed human categorization to the evolving narrative of an individual's history. This standpoint recognizes the adaptable nature of human heritage and emphasizes the significance of comprehending the intricate and unique paths that contribute to the formation of individual ancestral backgrounds. Simultaneously, prioritizing unbiased interventions that consider the social determinants of health becomes imperative. Completely disregarding race may not alleviate health disparities but could perpetuate and exacerbate them. A conscientious shift toward precision and sensitivity is essential for advancing biomedical research and clinical practice, aiming for improved health outcomes and equity for all.

### Insights from the PESA study on early insulin resistance and subclinical atherosclerosis

It is estimated that at least 45% of American adults are living with T2D or prediabetes (149), and similar numbers are seen in Europe (150,151). Many of these persons are unaware of it, and potentially many more have early insulin resistance to some degree, a risk factor relevant to developing future T2D and ASCVD (125).

As much as ASCVD is preceded by a slow, progressive subclinical disease that may start as early as childhood (152), early insulin resistance, defined by the slow progressive need for higher fasting insulin to maintain normal fasting glucose concentrations, can be seen years or decades before the increase in fasting glucose, glucose intolerance, or HbA1c levels that are characteristic of the insulin resistance-prediabetes-T2D continuum (153).

In this regard, increasing levels of HbA1c have already been shown to be associated with subclinical atherosclerosis, even within values below the prediabetes and T2D thresholds (75). Adding HbA1c to classical CV risk factors has also significantly improved the prediction of the multi-territorial extent of subclinical atherosclerosis.

Nevertheless, the added value of identifying individuals with earlier stages of insulin resistance when HbA1c is still "normal" and the association with subclinical atherosclerosis was poorly described. In fact, insulin levels are rarely looked at in clinical practice, and early insulin resistance frequently comes under the radar until it reaches further advanced stages. A similar scenario is observed with subclinical atherosclerosis, often not detecting the presence of atherosclerosis until a first clinical event strikes, which can happen prematurely (154), even at HbA1c levels below established diagnostic thresholds (155,156). Unfortunately, clinical practice often involves waiting for a diagnosis of prediabetes, T2D, hyperlipidemia, hypertension, or other risk factors before acting. This situation raises a crucial question about the adequacy of our current approach to preventing ASCVD while ignoring the early onset of insulin resistance.

This specific question underpinned our hypothesis: Can the evaluation and finding of early insulin resistance in normoglycemic adults facilitate earlier intervention? Furthermore, we sought to explore potential connections between the presence or absence of insulin resistance, increasing levels of insulin resistance, and the occurrence, burden, and extent of subclinical atherosclerosis. To study this, we used the HOMA-IR ratio as an easy-to-measure surrogate of early insulin resistance. Among others, it has the advantage of being a simple, feasible, and economical measure that can be easily integrated into most clinical practices. Moreover, it has proven to be a robust clinical and epidemiological tool (128).

This cross-sectional baseline analysis of the PESA cohort, comprising 3,741 participants free of established ASCVD with a median HbA1c of 5.4% [5.2% to 5.6%], revealed an independent association between early insulin resistance in normoglycemic adults and subclinical atherosclerosis. Notably, higher HOMA-IR values were found to correlate with adverse cardiovascular risk profiles and an increased presence and burden of subclinical atherosclerosis across different vascular beds. Specifically, participants with HOMA-IR >2 exhibited significantly higher rates of hypertension, dyslipidemia, hepatic steatosis, and metabolic syndrome ($p<0.001$). Elevated HOMA-IR values demonstrated a positive association with multiterritorial subclinical atherosclerosis assessed by 2DVUS and non-contrast cardiac CT ($p<0.001$) across carotid, femoral, and coronary territories, as well as a

higher overall disease burden quantified by 3DVUS global plaque volume (p<0.001) and after adjusting for key CV risk factors and HbA1c, higher HOMA-IR categories maintained associations with multiterritorial subclinical atherosclerosis (odds ratio= 1.41; 95%CI: 1.01 to 1.95, p = 0.041) and CAC score (odds ratio= 1.74; 95%CI: 1.20 to 2.54, p = 0.004). These associations were particularly evident in individuals with low-to-moderate CV risk. In a sensitivity analysis, the association persisted in individuals with HbA1c <5.4% or ≥5.4% (p< 0.001). Similar trends emerged when categorizing HOMA-IR values by tertiles, revealing significant differences between the lowest and highest tertile of HOMA-IR values (HOMA-IR < 0.8 and HOMA-IR ≥ 1.4, respectively). These findings indicated a continuous association with increasing values of HOMA-IR, emphasizing the robustness of the observed relationships across various stratifications. **Figure 12 (Central Illustration 3)** is an example of the study findings, illustrating two distinct participants: one from the reference group with HOMA-IR <2 and the other from the higher category with HOMA-IR >3. The figure visually captures subclinical atherosclerosis's presence, distribution, and burden in these contrasting individuals and groups.

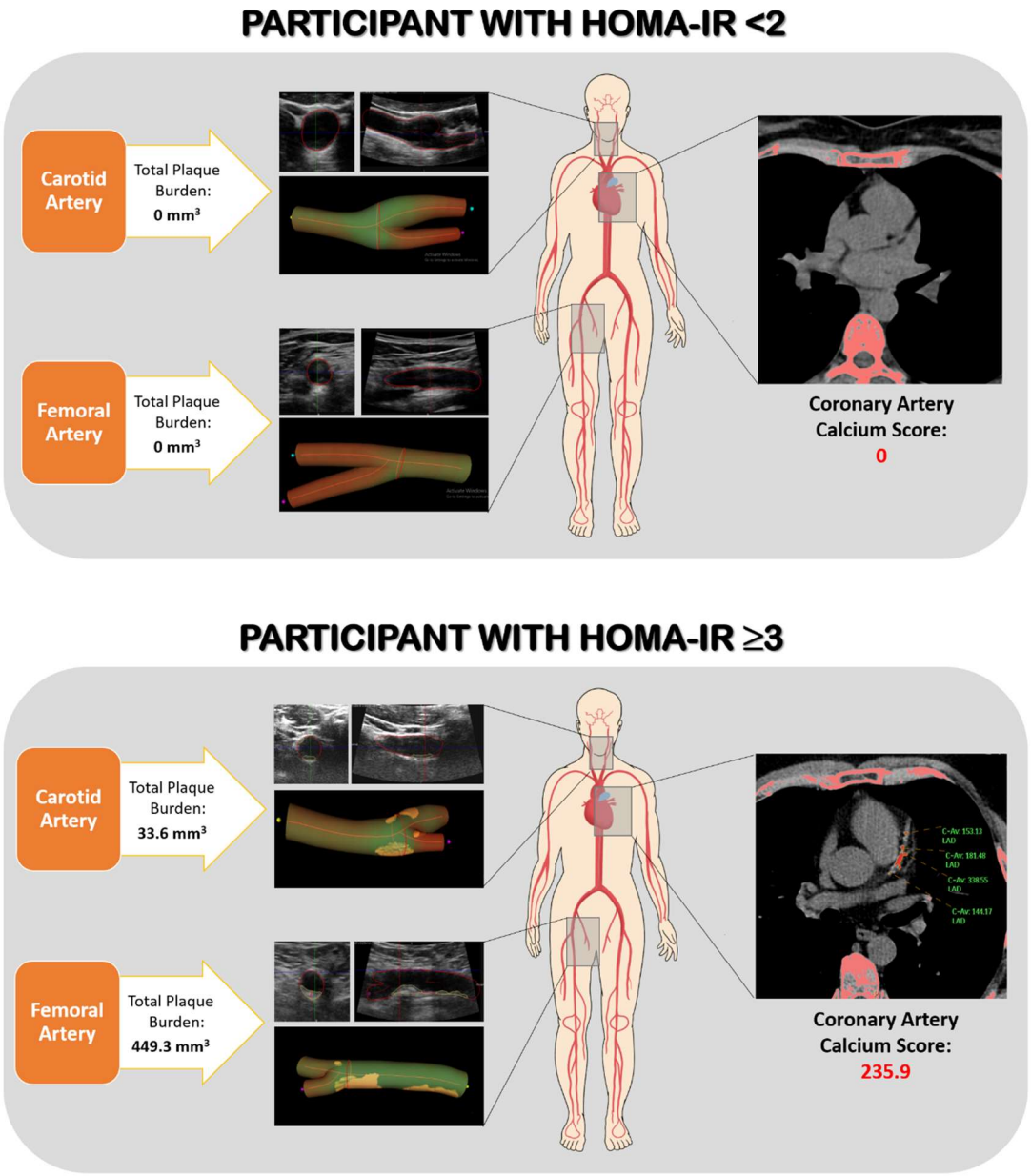



**Figure 12. Two- and three-dimensional vascular ultrasound and computed tomography images exemplify the study findings, Central Illustration 3**. **A)** Visual example illustrating imaging findings in a participant with HOMA-IR < 2 (reference group), depicting the absence of subclinical atherosclerosis affecting vascular sites. **B)** Visual example illustrating imaging findings in a participant with HOMA-IR ≥3 (category with the highest HOMA-IR values), revealing a multiterritorial extent of subclinical atherosclerosis in the carotid and femoral arteries and an elevated coronary artery calcium score. HOMA-IR = homeostatic model assessment of insulin resistance. This figure is my original creation and was generated using PowerPoint with images from two participants of the early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis study.

Combined, this study's clinical implications are significant. To our knowledge, this cross-sectional analysis is the first to report an independent association between HOMA-IR-estimated early insulin resistance and subclinical atherosclerosis measured by 2DVUS, 3DVUS, and non-contrast cardiac CT in ASCVD-free individuals without prediabetes or T2D. Recognizing this independent association emphasizes HOMA-IR's potential as a valuable marker for risk stratification in normoglycemic individuals. Integrating it into routine assessments could bolster primary prevention measures.

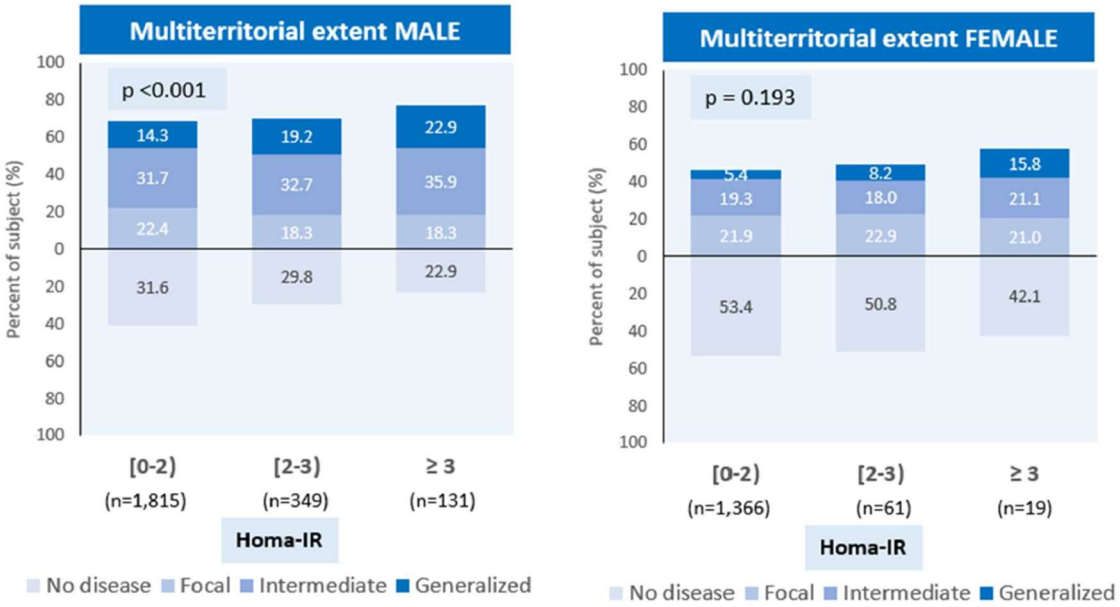
One of the study's strengths lies in its substantial sample size, encompassing 3,741 participants from the PESA cohort. The comprehensive assessment, utilizing various imaging techniques such as 2D- and 3DVUS and non-contrast cardiac CT, enhances the depth of the findings. Notably, the relationship between HOMA-IR and subclinical atherosclerosis was especially pronounced in the coronary territory. In the reference HOMA-IR group (HOMA-IR <2), only 14.9% had a CACS> 0; thus, 85.1% were devoid of coronary artery calcium. The proportion of participants with CACS increased with the insulin-resistance category, reaching 25.6% for HOMA-IR = 2 to 3 and 39.3% for HOMA-IR ≥ 3. Examining the subgroup with the highest values of HOMA-IR ≥ 4 (n = 49) revealed a substantial increase to 49%, more than three times the prevalence in the reference HOMA-IR group. HOMA-IR appeared thus to identify the risk of coronary artery calcification in this ostensibly disease-free population, even after adjusting for several other CV risk factors, including age, sex, smoking status, systolic and diastolic blood pressure, LDL-C, HDL-C, body mass index, and family history of ASCVD, as well as HbA1c. An in-depth study of the mechanisms explaining this relationship could reveal interesting insights between the onset of insulin resistance and the finding of already calcified plaques in the coronary arteries. Another notable strength of the study is the mean age of the individuals (46 years). Although not as young as in the FAMILIA study (38 years), young individuals remain insufficiently protected by existing guidelines despite their heightened vulnerability to risk factors (157). Intervening early in this demographic could yield the greatest impact, as we will hypothesize in the last chapter of the discussion. In this context, as part of the PESA cohort project, the study contributes with new data underscoring the importance of not passively waiting until risk factors or even clinical disease are established but instead emphasizing the significance of acting as soon as possible to attain the maximum benefit.

### Exploring insulin resistance in diverse subgroups: challenges and insights from the FAMILIA and the PESA studies

The concept of evaluating insulin resistance using the HOMA-IR ratio emerged later in the trajectory of the Ph.D. program. In this regard, efforts were undertaken to explore the possibility of retrieving measurements of fasting insulin from the initial assessment of FAMILIA study participants to investigate the association between HOMA-IR values and subclinical atherosclerosis in different ethnic and racial subgroups from a slightly younger age and with a higher ratio of women (83% vs. 39%). Unfortunately, this measure was not included in the protocol when the FAMILIA trial was established, and obtaining the measurement of basal insulin was not feasible. However, a subsequent analysis was conducted in the PESA cohort to examine sex differences among participants. This initiative stemmed from literature reviews indicating that men generally exhibit higher visceral and intrahepatic fat accumulation levels, particularly at younger ages, correlating with increased insulin resistance (158). Notably, this pattern changes, particularly after menopause in women, as fat stores become more centralized, leading to metabolic risk profiles resembling those observed in men (159).

When conducting this analysis (data not presented in the associated published manuscript or supplemental material), elevated HOMA-IR values exhibited a positive association with multiterritorial subclinical atherosclerosis, as assessed by 2DVUS and non-contrast cardiac CT ($p<0.001$) in men but not in women. However, a noticeable trend was also observed in the female subgroup ($p=0.193$), **Figure 13**. After adjusting for key CV risk factors and HbA1c, higher HOMA-IR categories sustained trends associations with multiterritorial subclinical atherosclerosis, albeit without statistical significance likely due to reduced sample size in subgroup analysis (odds ratio= 1.33; 95%CI: 0.93 to 1.90, $p=0.118$), and odds ratio= 1.60; 95%CI: 0.66 to 3.91, $p=0.302$) for HOMA-IR ≥ 3 compared to the reference group, for males and females, respectively).

PANEL A




PANEL B

| OR (95%CI), p Value | MALE | FEMALE |
|---|---|---|
| **HOMA-IR (continuous)** | 0.99 (0.89; 1.10) p=0.840 | 0.95 (0.76; 1.17) p=0.609 |
| **<2** | 1. Ref | 1. Ref |
| **2 to <3** | 0.93 (0.73; 1.17) p=0.531 | 0.83 (0.49; 1.41) p=0.498 |
| **≥3** | 1.33 (0.93; 1.90) p=0.118 | 1.60 (0.66; 3.91) p=0.302 |

**Figure 13**. **Exploring sex differences in multiterritorial extent of subclinical atherosclerosis. A)** Multiterritorial extent of subclinical atherosclerosis, assessed by 2-dimensional vascular ultrasound and non-contrast coronary computed tomography, stratified by HOMA-IR category. The multiterritorial SA extent in this figure is defined by combining data from both imaging techniques to categorize individuals as having no disease (0 vascular sites affected), focal (1 site), intermediate (2 to 3 sites), or generalized atherosclerosis (4 to 6 sites). **B)** Sex-adjusted odds ratios for the multiterritorial extent of subclinical atherosclerosis stratified by the HOMA-IR category. The multivariable models were adjusted for age, smoking status, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, body mass index, family history of ASCVD, and HbA1c. OR = odds ratio, HOMA-IR = homeostatic model assessment of insulin resistance.

Sex hormones exert differential effects on adipogenesis, influencing the development of fat cells. Testosterone has been identified as inhibiting adipogenesis, while estrogen has a stimulatory role in promoting preadipocyte activity (160). Additionally, progestins have been found to stimulate the differentiation of adipocytes (161). Apart from hormonal influences, both genetic and environmental factors play pivotal roles in determining fat distribution patterns (162). Notably, variations in visceral fat distribution have been observed among different ethnic and racial groups. For instance, South Asian individuals tend to exhibit greater central obesity compared to those of European descent. At the same time, differences in visceral fat are noted between non-Hispanic White individuals and non-Hispanic Black and Hispanic individuals (159).

Age is another crucial factor influencing fat distribution. As individuals age, there is a preferential increase in abdominal fat, particularly visceral adipose tissue, coupled with a concurrent decrease in subcutaneous adipose tissue in the lower body. Moreover, aging is associated with ectopic fat deposition in organs such as the heart, liver, and skeletal muscle. Fat accumulation in ectopic sites with age poses an elevated risk for insulin resistance and ASCVD (163).

Taken together, future lines of research could involve a more in-depth exploration of the mechanisms underlying these sex-specific associations and considering additional factors that may influence the observed trends. Further investigations could also explore the potential impact of other metabolic and lifestyle factors on the relationship between insulin resistance and subclinical atherosclerosis in diverse populations. Expanding the study to include a longitudinal perspective may provide valuable insights into the temporal aspects of insulin resistance and its association with atherosclerosis over time.

## Reimagining cardiovascular health

**Cardiovascular disease is largely preventable.** As stated in the Victoria Declaration on Heart Health more than 30 years ago, "We have the scientific knowledge to create a world in which heart disease and stroke are rare (164)." Yet, ASCVD continues to stand as the foremost cause of mortality and disability across the majority of nations. Approximately 30% of initial acute events culminate in a fatality, with survivors frequently grappling with lingering sequelae and a curtailed life expectancy (165). Furthermore, a substantial number of CV events occur among individuals with low or intermediate risk, highlighting a critical gap in our current preventive measures (2,166). And despite notable medical advances, our interventions often unfold late in the disease continuum. This underscores a pressing necessity for enhanced CV detection tools and a suitable avenue for refining detection mechanisms and preventive strategies.

In this regard, **Figure 14 (Central Illustration 4)** illustrates a model elucidating different hypothetical vascular aging trajectories throughout the lifespan and the potential benefits of early or late preventive interventions. **Ideally** (**green line**), the primary focus should be preventing CV risk factor development throughout life (avoidable threshold 1). Promoting a healthy lifestyle encompasses essential elements such as maintaining proper nutrition, adopting a tobacco-free way of life, engaging in regular physical exercise, and fostering supportive built environments. The crucial role of education and the continual practice of healthy habits throughout an individual's entire life should be emphasized without question. To effectively implement an optimal life course policy for the prevention of ASCVD, it is imperative to bring together individuals and their communities, healthcare professionals, scientists, industry stakeholders, and policymakers. Collaboration among these diverse groups is crucial for developing comprehensive strategies that address the multifaceted aspects of ASCVD prevention and promote overall well-being.

The **average vascular life** course is illustrated by a **blue line**, representing the accumulation of risk factors and aging throughout an individual's lifespan, thereby increasing the likelihood of a clinical event with time. Efforts in this trajectory should be focused on preventing the onset of subclinical atherosclerosis (best preventive effort, avoidable threshold 2) and, if not reached, at least halting the progression of the subclinical disease toward a clinical event (early preventive efforts: avoidable threshold 3). Subsequently, the early identification of subclinical atherosclerosis becomes crucial for prompt action (**precision medicine through vascular imaging of subclinical atherosclerosis**), enabling personalized and potentially more effective utilization of

existing CVrisk assessment methods, such as CVrisk scores. Within this trajectory, both studies in this Ph.D. thesis aim to provide insights into the prevalence, extent, and burden of subclinical atherosclerosis in different subpopulations and contemplate possibilities for early targeted interventions to facilitate the detection of disease emergence. This would also involve allocating more resources where the maximal gain can be obtained.

Finally, **accelerated vascular aging**, illustrated by a **red line**, occurs in approximately 34% of individuals worldwide (avoidable threshold 4) (154), making it highly conducive to prevention, early diagnosis, or treatment using the numerous safe and effective interventions available today. In this context, late-stage interventions would become imperative upon detecting progressing subclinical atherosclerosis, given its association with the highest risk of imminent CV events. However, it is noteworthy that the predominant focus of current efforts remains largely on preventing recurring events after the disease has taken hold.

This proposed model of CV prevention opportunities elucidates that efforts should be redirected to prioritize preventive measures at earlier stages, fostering a more proactive approach to CV health (167). Moreover, due to its lasting impact and the possible development of an early intervention paradigm, the consideration of cost-effectiveness further emphasizes the importance of adopting proactive measures.

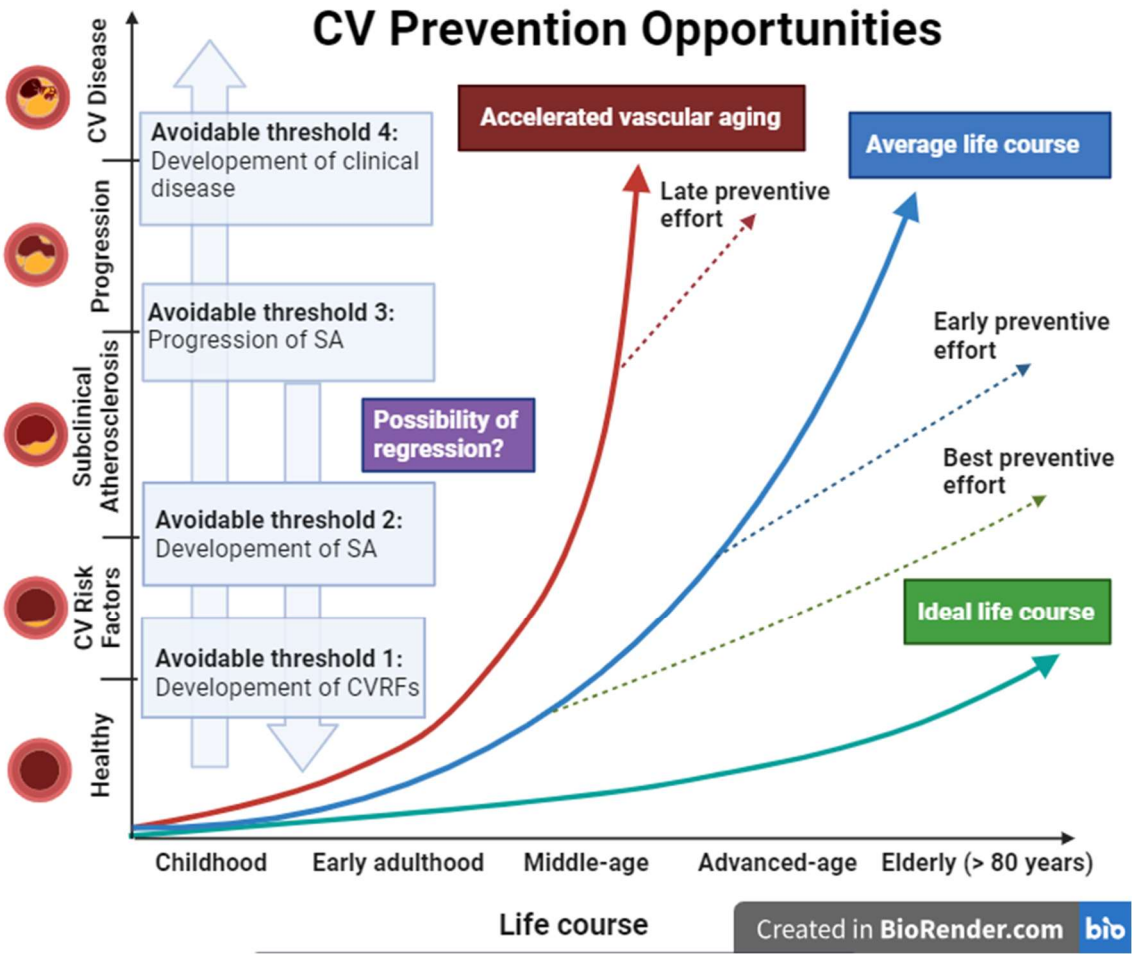



**Figure 14. Vascular aging throughout a lifespan and potential gain with early vs. late preventive efforts, Central Illustration 4**. The life course approach to preventing ASCVD is depicted in the figure. It illustrates three trajectories of health and disease over a lifetime: the life course of individuals with accelerated vascular aging, the average life course, and the ideal life course. Preventive efforts should concentrate on four avoidable thresholds, aiming to optimize the life-course trajectory as much as possible. The possibility of reversing CV risk factors or regressing subclinical atherosclerosis has been minimally explored (arrow indicating a shift from disease to health). The figure was created with Biorender.com with a license to publish and was inspired from Olsen MH. et al. A call to action and a life course strategy to address the global burden of raised blood pressure on current and future generations: the Lancet Commission on hypertension. Lancet. 2016 (168). CV = Cardiovascular, SA = Subclinical Atherosclerosis.

## Future research avenues in remission strategies for ASCVD and subclinical atherosclerosis

While existing strategies to halt progression to clinical events predominantly focus on managing cardiometabolic risk factors through lifestyle modifications, recurrent medical checkups, or pharmacotherapy, only a handful of studies have delved into the prospect of achieving remission for the underlying causes of ASCVD. This novel and underexplored concept is illustrated in **Figure 14**, with an arrow indicating a shift from disease to health. A significant development in this realm recently occurred in 2021 with the publication of the ADA/EASD/DUK consensus statement on the definition of T2D remission (169). The statement precisely outlines remission as maintaining a stable HbA1c level below the diagnostic threshold for at least three months without pharmacological therapy. It underscores the significant role of weight loss, particularly the excess intra-organ fat, in attaining this objective (170). Unfortunately, there is currently no universally accepted terminology for describing remission from prediabetes to normal glucose levels or the remission of early insulin resistance, hypertension, or metabolic syndrome, and also not yet an entirely clear rationale for seeking this endpoint. This research area holds substantial potential implications, aligning with my current interests, clinical practice, and future research endeavors.

Additionally, atherosclerosis, once considered incurable, has demonstrated the potential for regression if treated early. These challenges prevail in dogma and practices, as evidenced by the six-year follow-up of the PESA cohort (141), where regression of subclinical atherosclerosis was observed in 8.0% of patients with baseline disease rather than showcasing stability or progression. Factors associated with regression in this cohort included nonsmoking, female sex, low inflammation levels, followed by younger age, and lower LDL-C (all $p<0.05$). The concept that **atherosclerosis is a disease that can be cured if treated early** is groundbreaking and has the potential to revolutionize current perspectives on disease prevention. This shift from mere management to the active regression of subclinical atherosclerosis holds excellent promise for future research. The trajectory ahead envisions a combination of preventive strategies, early identification of metabolic markers, and integrated screening with imaging. This holistic approach has the transformative potential to redefine medical practices, empowering individuals to attain earlier, enhanced, and more equitable preventive measures. As we embark on this transformative journey, the pursuit of understanding and implementing these revolutionary concepts is vital to shaping a new era in CV health.

# 5. Conclusions

1) Subclinical atherosclerosis, a highly preventable state that precedes ASCVD, can be conveniently detected and measured through noninvasive imaging techniques such as two- and three-dimensional vascular ultrasound or computed tomography.

2) In the FAMILIA study, among 436 young adults from Harlem, New York, subclinical atherosclerosis, as evaluated by 3DVUS, was more prevalent in self-identified non-Hispanic Black individuals compared to their Hispanic counterparts. Such prevalence difference persisted after adjusting for traditional and socioeconomic risk factors and the Framingham risk score.

3) Whether this discrepancy reflects intrinsic biological differences or non-biological factors remains to be elucidated. However, it underscores the possibility of community-targeted interventions to mitigate social determinants and structural inequalities affecting CV health.

4) In the PESA study, among 3,741 CVD-free adult participants, early insulin resistance, as indicated by HOMA-IR values, was directly associated with subclinical atherosclerosis's presence, burden, and extent in normoglycemic low-risk individuals.

5) HOMA-IR is a simple measure that could facilitate earlier implementation of primary CV prevention strategies in clinical practice.

In summary, these studies advance cardiovascular care by investigating non-traditional risk factors in underrepresented communities, advocating for the clinical application of non-invasive imaging in CV prevention, refining risk stratification, and emphasizing early detection of subclinical atherosclerosis using easily measurable metabolic markers in clinical practice. These collective efforts hold the potential to enhance primary cardiovascular prevention and reduce the overall burden of cardiovascular diseases.

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# 6. Annexes

The following appendices are attached to the main manuscript of the Ph.D. thesis. These include the two following documents:

**Annex 1**. Supplementary Information from Publish Article: Subclinical Atherosclerosis in Young, Socioeconomically Vulnerable Hispanic and Non-Hispanic Black Adults.

**Annex 2**. Supplementary Information from Publish Article: Early insulin resistance in normoglycemic low-risk individuals is associated with subclinical atherosclerosis.