Carotid Intima-Media Thickness and Cardiovascular Disease Risk in African Populations

Engelbert A. Nonterah

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De wanddikte van de halsslagader als indicator voor het hartvaatziekte risico in de Afrikaanse bevolking

(met een samenvatting in het Nederlands)

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door

Engelbert Adamwaba Nonterah

geboren op 10 februari 1984 te Navrongo, Ghana

Promotoren:

Prof. dr. D.E. Grobbee

Prof. dr. N.J. Crowther

Copromotoren:

Dr. K. Klipstein-Grobusch

Dr. A.R. Oduro

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Chapter 1

General introduction

Global burden of cardiovascular disease

The global burden of cardiovascular disease (CVD) and associated mortality is increasing due to ageing and population growth (1-3). Particularly in low- and middle-income countries (LMIC) urbanization and globalization of communities with resultant socio-economic development, has led to lifestyle changes that increase the risk for CVDs. The current Global Burden of Disease study reports that the total CVD cases such as ischemic heart disease, myocardial infarction, cerebrovascular accidents (stroke), heart failure, cardiomyopathies and peripheral vascular diseases nearly doubled from 271 million in 1990 to 523 million in 2019. The number of CVD deaths experienced a steady increase from 12.1 million to 18.6 million in the same period (2). The 2019 estimates ranked ischaemic heart disease and stroke as the top cause of disability adjusted-life years (DALYs) in the older age groups (50-74 years and above) (2, 3). Presented in Figure 1 is the data from Roth et al (2020), presenting age standardised change in CVD deaths from 2010-2019, number of deaths from 1990-2019 and proportions of CVD deaths by cause in 2019 (3).

From 1990 to 2013 there was a 40% increase in global CVD deaths of which ageing accounted for 55% while population growth accounted for 25% of these deaths (1). The World Health Organization (WHO) suggests that by 2030 there would be substantial increases in CVD globally and especially in the African region.

Aetiologically, CVDs arise from a complex interaction between genetic, lifestyle and metabolic risk, access to treatment in acute phase of CVD and advanced management or secondary prevention for established CVD. Over the years several longitudinal studies using clinical endpoints of CVD identified the major risk factors for these diseases. Starting with the Framingham study which began 73 years ago (4) to the updated 2018 Pooled Cohort Equation (PCE) study (5), the following have been established as the main risk factors for CVD: age, sex, smoking, elevated blood pressure, increased body mass index, unfavourable lipid profile, glucose intolerance and physical inactivity. The American Heart Association and the Global Burden of Disease Study further identified these risk factors as well as high alcohol intake as the main contributors to the burden of CVD (6). The development of the PCE was based on data from the ARIC study, Cardiovascular Health Study, Coronary Artery Risk Development in Young Adults, the Framingham Heart Study offspring cohort, the Jackson Heart Study and the Multi-Ethnic study of Atherosclerosis (5).
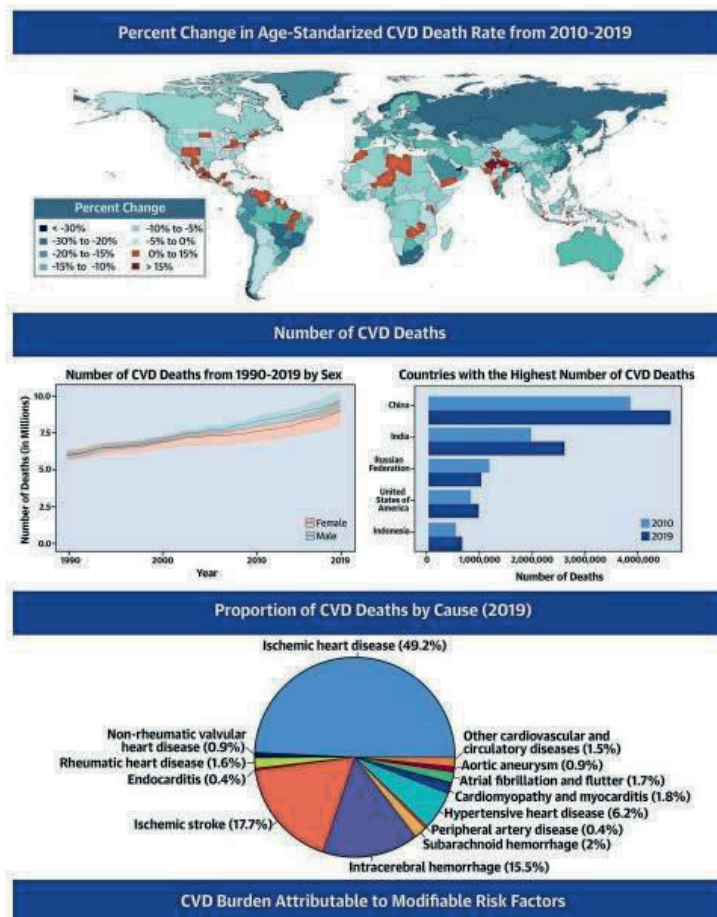
Figure 1: Age standardised change in CVD deaths from 2010-2019, number of deaths from 1990-2019 and proportions of CVD deaths by cause in 2019 (Roth et al 2020 (3))

Despite the high global burden of disease there are observed regional differences in the levels of CVD morbidity and mortality. These differences are attributed to several factors broadly categorized as:

- Factors that affect risk of CVD and are targets for prevention, mainly lifestyle and metabolic risk factors.
- Those that affect survival in the acute phase hinging on the social determinants of health such as access to health care in the acute phase and available treatment options.
- Factors that affect secondary prevention such as access to advanced pharmacological and non-pharmacological treatment options.

The contribution of genetics and novel risk factors such as microalbuminuria and inflammation as well as socio-cultural factors is also important.

The projected global increase in the prevalence of CVD (1, 2) will have significant clinical and public health implications for many countries, especially those with poor healthcare infrastructure. Formulation of context specific interventions is therefore essential to mitigate the rising burden of CVD.

Cardiovascular Disease in sub-Saharan Africa
Low- and middle-income countries such as those in sub-Saharan Africa (SSA) suffer a disproportionately higher burden of CVD and premature mortality due to CVD. For example, nearly 80% of global CVD deaths occur in these countries (3). These countries are rapidly undergoing a transition from communicable diseases to non-communicable diseases (7). However, the co-occurrence of infectious diseases such as HIV, tuberculosis and malaria creates a double burden of diseases (7) further posing huge challenges to healthcare delivery through rising healthcare cost as well as cost related to integrated chronic disease care.

The epidemiology of CVD in SSA is different from that in other regions of the world. CVD risk factor levels in SSA are generally increasing but still remain low compared to high income countries (8). For instance, obesity and hypertension levels are high in most African countries (9-12). CVD mortality is higher in younger age groups when compared to other regions of the world (8). This leads to a loss of persons in the economically viable age groups, and therefore has effects on the economic status of the country.

African populations are experiencing an increase in the proportion of older subjects due to improved life expectancies (13). In addition, increased socio-economic growth and movements of populations to urban centers within these countries has resulted in changes in lifestyles which have led to the increased consumption of processed, energy-dense foods and high levels of physical inactivity (14).

The co-occurrence of rising CVD risk with persistently high chronic infectious diseases (such as HIV, tuberculosis and malaria) further escalates the unfolding CVD burden (15, 16). These diseases are strongly linked to CVD risk. Immune activation from active HIV is known to induce sustained inflammation which can exacerbate atherosclerotic processes (17, 18). With the advent of antiretroviral therapy (ART), the HIV population is also undergoing the normal ageing process due to reduced HIV/AIDS related deaths, exposing them to greater risk for CVD. In addition, the early generations of ARTs were known to cause several lipid

abnormalities that increased CVD risk (17). The newer generation of medication has reduced this occurrence and further improved life expectancies. However, newer drugs such as dolutegravir used commonly in SSA are reported to be associated with obesity and hyperglycaemia (19, 20). The ART care cascade has also served as a good avenue for the screening and management of CVD and their risk (21, 22).

Other factors that account for differences in CVD among SSA and high-income countries include genetics contributions to disease occurrence. The genetic makeup and subsequent gene-environment and risk factor interactions may result in a differential magnitude of CVD and confer increased susceptibility in particular population groups (23).

Finally, social determinants of health are established as an indirect or proximate cause of CVD burden. Inadequate access to effective primary and secondary prevention measures due to inadequate funding results in ineffective screening, identification and management of CVDs and their risk factors (24).

Despite the presence of many studies measuring the prevalence of CVD risk factors in SSA, the absence of cardiovascular event data and pathological confirmation of CVD deaths creates a huge literature vacuum, thus the need for further studies to establish the much needed evidence. While the INDEPTH (International Network for the Demographic Evaluation of Populations and Their Health in low- and middle-income countries) network through WHO, has tried to fill this gap by using verbal autopsies to determine cause of death, much still remains to be done (7). The absence of clinical cardiovascular events data has led to the reliance on proxy makers, such as carotid intima-media thickness (CIMT), for CVD risk assessment and the identification of associated risk factors (25, 26).

The Human Heredity and Health Africa (H3A) consortium (https://h3africa. org) through the National Institute of Health (NIH) and Welcome Trust have funded seven studies of which the African-Wits-INDEPTH Genomic (AWI-Gen) study was one. The AWI-Gen study set out to identify the genomic and environmental risk factors for cardiometabolic diseases in SSA populations specifically Eastern, Western and Southern SSA regions (27, 28). This large pan-African epidemiological and genetic study has collected socio-demographic, behavioral, anthropometric, metabolic, and genetic data on close to 12 000 participants. The current thesis nested in the H3Africa AWI-Gen study used CIMT as a marker of sub-clinical atherosclerosis to investigate CVD risk in four SSA populations (Burkina Faso, Ghana, Kenya and South Africa). The participating countries and sites are shown in Figure 2. These countries are in different

sub-regional blocks of SSA and are at different stages of the epidemiological transition and therefore present varying levels of CVD risk factors and environmental and genetic exposure. For comparison with other race-ethnic groups the AWI-Gen (27) and USE-IMT (29) individual participant data meta-analyses collaboration was formed as part of this thesis and results reported accordingly.
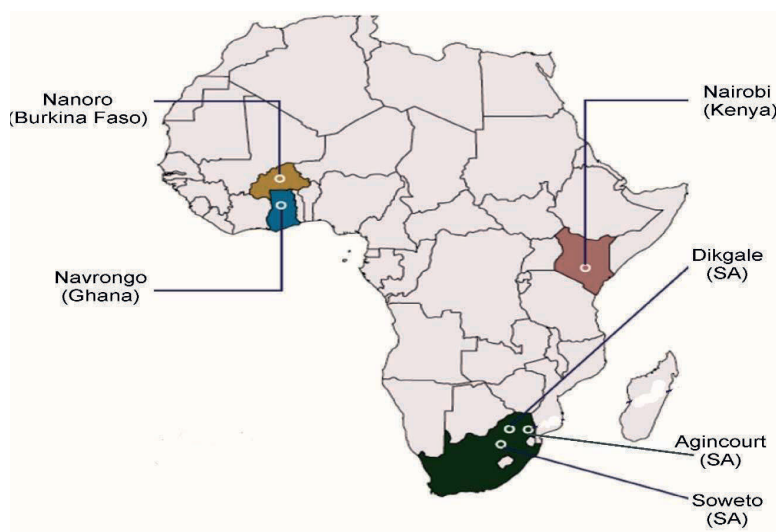
Figure 2: Map showing the six sites and four countries constituting the H3Africa AWI-Gen study; SA, South Africa

Carotid intima-media thickness as a marker for Cardiovascular Disease
A major precursor of CVD is atherosclerosis, a continuous process which often starts early in life, progresses with age and accelerates in the presence of risk factors (30). Several clinical and epidemiological studies have established CIMT measured using B-mode ultrasound scan as a marker for atherosclerosis and an alternative endpoint for identifying CVD risk factors (25, 26, 30-32). CIMT (see Figure 3) is associated with progression of atherosclerosis and has proven to be useful in clinical trials for identifying anti-hypertensive and anti-lipid drugs that reduce progression of atherosclerosis (25, 26, 32). This makes a strong case for the use of CIMT as a proxy for cardiovascular disease in settings where data on clinical events are absent. The strong association between CIMT and cardiovascular events and mortality has been reported in African Americans (33, 34). There is, however, a paucity of data from Africa on the link between CIMT and CVD and associated risk factors. The few studies conducted in Africa are mainly in subjects with established cardiometabolic diseases or in people living with HIV, hypertensive and diabetic patients and mostly have small sample sizes (35-42). The associated selection bias and small sample sizes make it difficult to

apply findings to the general population. The link between CIMT and CVD risk in HIV and CVD free general population from SSA is scare in literature. In addition, a number of novel risk factors have been reported to be associated with CIMT and CVD, including microalbuminuria (43, 44). This is of interest in resource limited regions because of ease of measurement in urine samples.
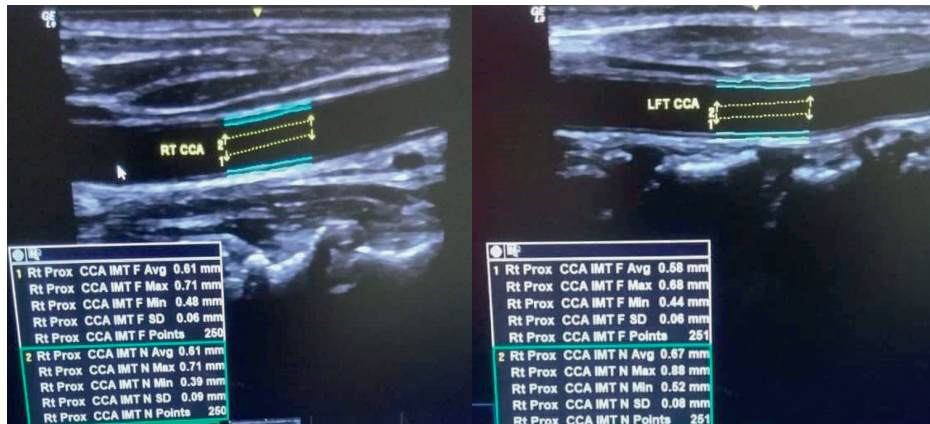
Figure 3: An image depicting common carotid intima media thickness showing automatic selection of the intima-media thickness interface (original image from the AWI-Gen study); the yellow lines represent the position of the cursor (10mm apart) to measure the carotid intima-media thickness (in blue lines); the displayed data give details on minimum, average and maximum intima-media thickness (IMT) of the common carotid artery.

Thesis aims and objectives

This thesis aimed to determine which CVD risk factors associate with CIMT in SSA populations, how these associations differ from those seen in other population groups and to assess the association of CIMT with cardiovascular risk.

This was approached through the following objectives:

I. Determining the association of classical CVD risk factors (age, sex, smoking, physical inactivity, poor diet, body mass index, blood pressure, hyperglycaemia and hypercholesterolemia) and HIV with CIMT (chapter 2).

II. Investigating the association of obesity phenotypes (body mass index, waist and hip circumference, visceral and subcutaneous adipose tissue) with CIMT in SSA (chapter 3).

III.   Describing race-ethnic differences in the association of classical CVD risk factors with CIMT in African, African American, Asian, European and Hispanic populations (chapter 4)

IV.   Establishing the link between cardiovascular health using the American heart Association's (AHA) Cardiovascular Health Index (CVHI) and CIMT in SSA (chapter 5).

V.   Investigating the relationship of CIMT and microalbuminuria with 10-year predicted CVD risk using the 2018 Pooled Cohort Equation (chapter 6).

The results from this thesis would contribute knowledge to priority areas for the primary prevention of atherosclerotic cardiovascular disease.

Thesis outline
Following this chapter the thesis is sub-sectioned into 2 parts (see Figure 4). Part 1 contains 3 chapters (chapters 2 to 4). Chapter 2 looks at the association of the classical CVD risk factors with carotid intima thickness in Burkina Faso, Ghana, Kenya and South Africa. Due to the high level of obesity in certain regions of SSA, chapter 3 investigates the association of adiposity phenotypes with CIMT. Chapter 4 of the thesis explores the race-ethnic differences in the factors associated with CIMT in African, African American, Asian, European and Hispanic populations. Part 2 contains chapters 5 and 6. Chapter 5 describes the association of common screening tools (the cardiovascular health index often referred to as life's simple 7) with CIMT in SSA while Chapter 6 determines association of carotid atherosclerosis and microalbuminuria with predicted high 10-year CVD risk in SSA. The thesis is concluded by discussing the similarities and differences in the risk factors associated with CIMT between African and other populations in Chapter 7. This chapter also examines the implications of our findings for policy on primary prevention, clinical care and further research (chapter 7). The thesis is completed by a summary of the key findings of this study in Chapter 8.
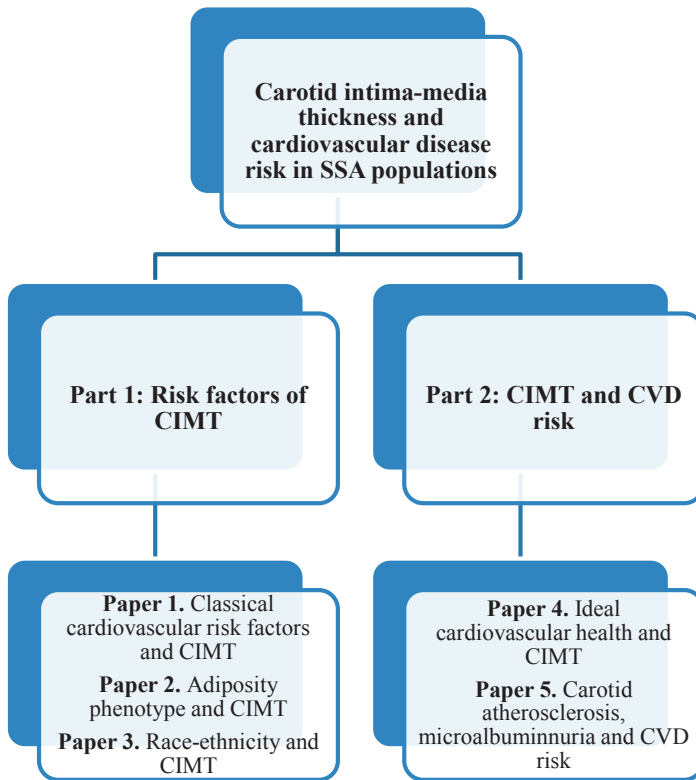
Figure 4: Overall thesis outline. Papers 1 to 3 represent chapters 2 to 4 of the thesis, whilst papers 4 and 5 are chapters 5 and 6. CVD, cardiovascular disease; CIMT, common carotid intima-media thickness and SSA, sub-Saharan African

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Part 1:

Risk factors for CIMT

Chapter 2

Classical cardiovascular risk factors and HIV are associated with Carotid intima-media thickness in adults from sub-Saharan Africa: findings from H3Africa AWI-Gen study

Nonterah EA, Boua PR, Klipstein-Grobusch K, Asiki G, Micklesfield LK, Agongo G, Ali SA, Mashinya F, Sorgho H, Nakanabo-Diallo S, Debpuur C, Kyobutungi C, Alberts M, Norris S, Tollman S, Tinto H, Soo CC, Mukomana F, Hazelhurst S, Wade AN, Kahn K, Oduro AR, Grobbee DE, Sankoh O, Ramsay M, Bots ML, Crowther NJ, as members and collaborators of AWI-Gen and the H3Africa Consortium*

Abstract

Background: Studies on the determinants of carotid intima-media thickness (CIMT), a marker of sub-clinical atherosclerosis, mostly come from white, Asian, and diasporan black populations. We present CIMT data from sub-Saharan Africa, which is experiencing a rising burden of cardiovascular diseases and infectious diseases.

Methods and Results: The H3 (Human Hereditary and Health) in Africa's AWI-Gen (African-Wits-INDEPTH partnership for Genomic) study is a cross-sectional study conducted in adults aged 40 to 60 years from Burkina Faso, Kenya, Ghana, and South Africa. Cardiovascular disease risk and ultrasonography of the CIMT of right and left common carotids were measured. Multivariable linear and mixed-effect multilevel regression modeling was applied to determine factors related to CIMT. Data included 8872 adults (50.8% men), mean age of 50±6 years with age- and sex-adjusted mean (±SE) CIMT of 640±123 µm. Participants from Ghana and Burkina Faso had higher CIMT compared with other sites. Age (β = 6.77, 95%CI [6.34, 7.19]), body mass index (17.6 [12.5, 22.8]), systolic blood pressure (7.52 [6.21, 8.83]), low-density lipoprotein cholesterol (5.08 [2.10, 8.06]) and men (10.3[4.75, 15.9]) were associated with higher CIMT. Smoking was associated with higher CIMT in men. High-density lipoprotein cholesterol (-12.2 [-17.9, -6.41]), alcohol consumption (-13.5 [-19.1, -7.91]) and HIV (-8.86 [-15.7, -2.03]) were inversely associated with CIMT.

Conclusions: Given the rising prevalence of cardiovascular diseases risk factors in sub-Saharan Africa, atherosclerotic diseases may become a major pan-African epidemic unless preventive measures are taken particularly for prevention of hypertension, obesity, and smoking. HIV-specific studies are needed to fully understand the association between HIV and CIMT in sub-Saharan Africa.

Key Words: cardiovascular disease; carotid intima-media thickness; epidemiological transition; prevention and sub-Saharan Africa

Clinical Perspective

What Is New?
- This the first study involving indigenous African populations drawn from 4 countries at different stages of epidemiological transition, to demonstrate that classical cardiovascular risk factors such as age, male sex, systolic blood pressure, serum cholesterol, and obesity are major drivers of increased carotid intima-media thickness.
- HIV infection was not associated with higher carotid intima-media thickness levels.

What Are the Clinical Implications?
- Interventions focused on classical, modifiable cardiovascular disease risk factors will attenuate atherosclerotic risk in sub-Saharan African populations.
- HIV infection may not be a risk factor for atherosclerotic diseases in sub-Saharan African.
- Results from recent studies in sub-Saharan African have reported a lower prevalence of cardiovascular disease risk factors in subjects living with HIV and this new paradigm suggests that the HIV care cascade may be an effective resource for the prevention and control of cardiovascular diseases among people living with HIV.

Introduction

Global morbidity and mortality attributable to noncommunicable diseases are increasing with cardiovascular diseases (CVD) being a significant contributor (1). A greater proportion of the annual CVD-related deaths occur between 30 to 70 years of age and 85% of these deaths occur in low- and middle-income countries, and are projected to increase further (2). A major contributor to the burden of cardiovascular morbidity and mortality is the development of atherosclerosis, a continuous process which often starts early in life and progresses with age (3).

Exposure to unfavorable levels of established cardiovascular risk factors such as high systolic blood pressure, smoking, and dyslipidemia leads to the accelerated development of atherosclerosis. The measurement of sub-clinical atherosclerosis by the assessment of carotid intima-media thickness (CIMT) may provide information about the cardiovascular status of a population (4-7). However, epidemiological data on the prevalence and determinants of high CIMT arise largely from studies in whites, Asians, and Africans in the diaspora or blacks, which may not reflect the situation among Africans living in sub-Saharan Africa (SSA) (4, 8, 9). Africa is currently engulfed in a wave of complex epidemiological transition that is characterized by extensive urbanization with concomitant lifestyle changes such as consumption of calorie dense diets and a decrease in physical activity (10, 11). The surge in CVD risk factors, such as obesity and hypertension, adds to the complex milieu of a high burden of infectious diseases such as HIV, tuberculosis, and malaria (12).

Despite this double burden of non-communicable and infectious diseases in Africa, large cohort studies with harmonized data on CVDs are lacking (10). To formulate appropriate interventions to decrease CIMT as a proxy for subclinical atherosclerosis, it is important to identify the specific risk factors associated with CIMT in SSA populations.

Therefore, the aim of the study was to measure CIMT and identify risk factors associated with CIMT in a large SSA population. This was achieved by measuring CIMT levels within the AWI-Gen (African-Wits-INDEPTH [International Network for the Demographic Evaluation of Populations and their Health in low- and middle-income countries] Partnership for Genomic studies). This is a large pan-African epidemiological and genetic study that has collected sociodemographic, behavioral, anthropometric, metabolic, and genetic data on close to 12 000 participants from 4 countries within SSA (13, 14).

Materials and Methods

All data and materials for the AWI-Gen study that support the findings in this paper will be made available in the European Genome-phenome Archive under the set of projects related to the Human Heredity and Health in Africa (H3Africa) Consortium. Details about access to data can be found in the document titled "H3Africa Data and Biospecimen Access Committee Guidelines," available in the consortium documents section of the H3Africa website (www.h3africa.org).

Study Design and Study Population

We conducted a population-based cross-sectional study as part of the AWI-Gen partnership, a National Institutes of Health-funded Collaborative Centre of the Human Heredity and Health in Africa (H3Africa) Consortium. Six study sites in 4 sub-Saharan African (SSA) countries were involved in the AWI-Gen study (Figure 1). Three of the sites were in South Africa of which two are rural and one is urban. The rural sites were the Dikgale health and demographic surveillance site (HDSS) (15) affiliated with the Department of Pathology and Medical Science, University of Limpopo, and the Agincourt Health and Demographic Surveillance System Site (HDSS) (16) managed by the Medical Research Council/Wits Rural Public Health and Health Transitions Research Unit, University of the Witwatersrand. The urban South African site is the MRC/Wits Developmental Pathways for Health Research Unit, University of the Witwatersrand, in Soweto (17). There was 1 urban site in Nairobi, Kenya: the African Population and Health Research Center HDSS (18). Finally, there were 2 rural sites in West Africa: the Navrongo HDSS hosted by the Navrongo Health Research Center in Ghana (19), and the Nanoro HDSS hosted by the Institut de Recherché en Sciences de la Santé Clinical Research Unit of Nanoro in Burkina Faso (20).

Nanoro Health and Demographic Surveillance System (HDSS), Clinical Research Unit of Nanoro, Burkina Faso

Navrongo Health and Demographic Surveillance System (NHDSS), Navrongo Health Research Centre (NHRC), Ghana

Dikgale HDSS, South Africa

Nairobi Urban Health and Demographic Surveillance System (NUHDSS), Kenya

Agincourt HDSS site, MRC/Wits Rural Public Health and Health transitions Unit, South Africa

Developmental Pathways to Health Research Unit (DPHRU), **Soweto**, South Africa

Figure 1. The sites constituting the H3Africa AWI-Gen study. Reprinted from Ramsay et al (13) with permission. Copyright ©2016, Cambridge University Press

Included in the study were adults aged 40 to 60 years resident in the various sites. Exclusion criteria were current pregnancy and inability to complete the prescribed study procedures. Similar numbers of women and men were randomly sampled from each of the sites using existing sampling frames for the respective HDSS sites (13, 14). Only men from Soweto were included in these analyses as women contributing to the study population in Soweto, were the caregivers of the Birth to 20+ Cohort[17] and did not have CIMT measurements. Our study population covers 3 of the 5 sub-continental blocks of Africa and therefore represents a large proportion of the geographical (covering both rural and urban areas) and social variability of the SSA region.

Ethical Considerations
The AWI-Gen study received overall ethical approval from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa (approval identification numbers: M121029; M170880), as well as from the appropriate ethics committees covering the Dikgale, Navrongo, Nanoro, and Nairobi sites. Community engagement activities were completed at each site to introduce the study to community leaders before commencement of the field work. Written and signed or thumb-printed informed consent was obtained from each participant before performing prescribed study procedures. In compliance with good clinical practice, participants who were found to have clinically overt CVDs were linked to health care by issuing referral letters in accordance with the healthcare system in the particular study site.

Data Collection

Details of data collection methods and procedures have been described elsewhere (13, 14, 21). Briefly, a paper-based questionnaire was used to collect information from 5 of the sites while a Computer-Assisted Personalized Interview was used to collect information from the Agincourt site in South Africa. Information collected included demography, family ethnicity, education, household attributes, substance use (tobacco, alcohol, and drugs), infectious disease history (HIV, TB, and malaria), history of cardiovascular and metabolic diseases (diabetes mellitus, stroke, hypertension, angina, heart attack, congestive heart failure, obesity, and high cholesterol), thyroid disease, kidney disease, and physical activity. Data were then entered into the REDCap (Research Electronic Data Capture) system hosted at the University of the Witwatersrand, Johannesburg (22, 23). Data entry quality control to identify outliers, duplicate information, and missing data were completed on 10% of the data per site.

Carotid Ultrasonography

Training

To maintain uniformity across the sites, carotid ultrasonography procedures were standardized, and technicians trained centrally by a certified sonographer at the MRC/Wits Developmental Pathways for Health Research Unit at the Chris Hani Baragwanath Hospital, Soweto, South Africa. The identified technicians from the study sites were clinicians, nurses, or biomedical scientists. To ensure reproducibility and reduce CIMT measurement variability, masked repeated measurements of 15 volunteers were conducted by each trainee and the lead trainer. The coefficient of variation between and within trainees was calculated and maintained at <2%. Subsequently, the same settings and calibrations of the ultrasound equipment (linear-array 12L-RS transducer with a B-mode LOGIQ e ultrasound machine, GE Healthcare, CT, USA) were used for data collection at all sites throughout the entire recruitment period.

Image acquisition

To measure the right carotid, the participant was asked to lie down in a supine position with a pillow underneath the neck for slight extension, head turned towards the left at a 45º angle and gel applied to the exposed neck area. Using the 2 sternocleidomastoid muscles as landmarks, the exposed area was scanned along the longitudinal plane until the common carotid artery (CCA) was found, and an image frozen. The operator then identified a continuous 1-cm segment (10 mm) of the CCA far wall. The operator then placed a cursor between 2 points (10 mm apart) on this identified segment of the far wall with the proximal starting point 1 cm from the bulb of the CCA. The ultrasound machine software then automatically detected the intima-lumen and the

media adventitia interfaces and calculated the minimum, maximum, and mean common CIMT in millimeters and to 2 decimal places. To measure the left carotid, the participant's head was turned to the opposite side, and the process was repeated. This approach was selected over measuring "multiple carotid segments" because it was easier to measure and more reproducible enabling its widespread use at all study sites. A recent study demonstrates that measuring CCA IMT is a good alternative compared with multiple segments in terms of prediction of risk of CVD events (24). Additional quality control (QC) before analyses included the exclusion of CCA IMT >1.5 mm as this is indicative of plaque. Images with >50% differences between minimum and maximum CCA IMT were also excluded from the analytical data set.[5] The far walls of both the left and right common carotid artery were averaged to determine mean CIMT thickness in millimeters as the main outcome variable. This was then converted to micrometers for the regression analyses.

Assessment of Sociodemographic, Behavioral, Anthropometric, Blood Pressure, Biochemical and HIV-Related Variables

Age at the time of data collection and sex of participants were self-reported. Highest level of education attained was self-reported and categorized as no formal education, completion of primary, secondary or tertiary education. Household socioeconomic status (SES) was assessed using the INDEPTH health equity tool which is an asset index generated by using principal component analysis to combine data on household possessions (http://indepth-network. org/resources/indepth-health-equity-tool-measuring-socio-economic-status). The asset score generated was categorized into quintiles (Q1=poorest, Q2=poorer, Q3=poor, Q4=less poor and Q5=least poor) and this was computed separately for each of the sites. Smoking status was assessed by asking subjects if they had ever smoked any tobacco products such as cigarettes, cigars or pipes, and if they were current or past users of such products, Smoking was then categorized as "never," "current," and "past." For the purpose of these analyses current and past were combined as "smokers." Self-reported alcohol use was assessed using the 4-item CAGE (cut-annoyed-guilty-eye) questionnaire[25] and subsequently categorized as current or never/previous use. The Global Physical Activity Questionnaire was used to compute moderate-to-vigorous intensity physical activity (MVPA) in minutes per week. Participants were said to be active if their MVPA was ≥150 min/week or inactive if their MVPA was <150 min/week (26).

Standing height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured without shoes and in light clothes using a Harpenden stadiometer (Holtain, Crymych, Wales) fixed to the wall and a digital calibrated weighing scale, respectively. Resting systolic blood pressure (SBP) and diastolic blood pressure were measured using a digital sphygmomanometer (Omron M6,

Omron, Kyoto, Japan) with the participant seated with their arm at the level of the chest and with an appropriate-sized cuff. Three readings were taken at 2-minute intervals with the first reading discarded and the average of the final 2 readings taken as the current blood pressure reading.

Overnight fasting serum lipids and glucose were measured using an automated chemistry analyzer (Randox RX Daytona+, Crumlin, Northern Ireland). All serum samples were analyzed at the University of the Witwatersrand Developmental Pathways for Health Research Unit laboratory, Chris Hani Baragwanath Academic Hospital, Soweto, South Africa. The low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (27).

Self-reported HIV status was determined at all sites and in addition HIV testing (using locally-available rapid-test kits) was offered to all participants from Kenya and South Africa. In South Africa all known HIV-positive participants were asked whether they were receiving antiretroviral therapy (ART). In Agincourt, HIV status was determined by use of Vironostika Uniform 11 [Biomeriuex, France] screening assay as part of the HAALSI (Health and Ageing in Africa: a Longitudinal Study of an INDEPTH Community in South Africa) study (28), several months before performing measurements on a subset of these participants for the AWI-Gen study (14). The HIV status could therefore have changed at the time of recruitment into the AWI-Gen study. The prevalence of HIV infection at the Burkina Faso and Ghana sites is known to be between 1% to 2% (29, 30).

Statistical Analysis
Continuous data are presented as means ±SD while categorical data are presented as proportions. Age- and sex-adjusted mean (with standard error of the mean) CIMT was computed and presented for the various sites stratified by sex. One-way analysis of variance was used to determine the differences in mean CIMT levels between the sites whilst the Student t-test was used to determine differences between women and men within the sites. We used various multivariable linear regression models to determine the factors associated with CIMT. In model 1 we adjusted for the classical CVD risk factors. These factors are often used in risk prediction equations (31) to determine the risk of dying from atherosclerotic cardiovascular diseases and include age, sex, current smoking, SBP, fasting glucose, HDL-C, and LDL-C. We replaced total cholesterol with LDL-C because of its key role in the pathogenesis of atherosclerosis and treatment monitoring (3, 32). In model 2, we adjusted for body mass index (BMI), physical activity and alcohol consumption in addition to factors from model 1. In model 3, since household SES and education are a proxy for urbanization and social determinants of health (33, 34), we adjusted for these in addition to factors from model 2. Finally, considering the high burden

of HIV infection in SSA, we adjusted for HIV status in model 4 in addition to the variables included in model 3. We also adjusted for the effect of site in all 4 of the multiple linear regression models. The final model (model 4) therefore included age, sex, smoking, SBP, fasting glucose, HDL-C, LDL-C, BMI, alcohol consumption, physical activity, education status, household SES, HIV status and site.

We checked this model for multi-collinearity using the variance inflation factor ensuring that the variance inflation factor for each variable was <10. We added multiplicative interaction terms to this model to evaluate whether relations between risk factors and common CIMT differed by sex and between sites. If interaction terms were statistically significant (P<0.05), findings were presented for separate strata (men and women; across sites). In a further sub-analysis involving only the South African sites and Kenya we adjusted for objectively measured HIV status and for the South African sites only we adjusted for the effect of ART. This was because only at these sites was HIV status measured objectively and ART status was assessed only in the South African sites.

To fully account for within site differences, we further conducted mixed-effect multi-level (ML) regression using site as a random effect in the final model (model 4) of the multiple linear regression analysis. We also checked for the role of interaction of HIV with age, sex, and household SES in the mixed-effect ML model. A likelihood ratio test was then used to compare the best fitting model between the single multivariable linear regression model, the mixed-effect ML regression model and the mixed-effect ML regression model that included interaction terms. Results are reported as unstandardised β-coefficients with corresponding 95% CIs. Statistical significance was set at a 2-sided P<0.05. For educational status and household SES, which had >2 categories, a post estimation test was used to obtain a single P value for the estimate in the models. All data were analyzed using STATA 14.2 (College Station, TX 77845, USA) software.

Results

Data were available for 10 363 participants drawn from all 6 sites. However, 1491 of these were excluded from the data analysis because they either had no CIMT data or did not meet quality control criteria. The final analytical data set therefore consisted of 8872 participants.

Sociodemographic Characteristics
The mean (±SD) age of the total study population was 49.9±5.83 years and men comprised 50.8% of study participants (Table 1). Nairobi, Kenya had the youngest (48.5±5.43 years) while Navrongo, Ghana had the oldest (51.1±5.75

years) population. The two West African sites, Nanoro in Burkina Faso (83.0%) and Navrongo in Ghana (70.5%), had the highest number of participants with no formal education, while all three South African sites (8.45% Agincourt, 3.56% Dikgale and 14.4% among men in Soweto) and Nairobi (3.69%) had the highest number of participants with tertiary education. More than two-thirds of participants in all countries were in the 2 highest quintiles (less and least poor) of household socio-economic status (Table 1).

Table 1. Basic characteristics of study paticipants by site

	All sites	Nanoro, BF	Navrongo, GH	Agincourt, SA	Dikgale, SA	'Soweto, SA	Nairobi, KE
	N=8872	n=2081	n=1976	n=795	n=1152	n=936	n=1932
Age (years)	49.9±5.82	49.8±5.81	51.1±5.75	50.3±5.79	50.4±5.97	49.4±5.99	48.5±5.43
Men	4506 (50.8)	1043 (50.1)	908 (46.0)	384 (48.3)	355 (30.8)	936 (100)	880 (45.6)
Educational Status							
No formal	3578 (40.5)	1716 (83.0)	1389 (70.6)	226 (28.5)	93 (8.07)	8 (0.85)	146 (7.55)
Primary	2468 (27.9)	239 (11.5)	376 (19.1)	262 (33.0)	381 (33.1)	105 (11.2)	1105 (57.2)
Secondary	2481 (28.0)	95 (4.63)	168 (8.54)	238 (29.9)	637 (55.3)	688 (73.5)	655 (33.9)
Tertiary	324 (3.69)	18 (0.87)	36 (1.82)	68 (8.56)	41 (3.56)	135 (14.4)	26 (1.34)
Household SES							
Poorest	1242 (14.0)	342 (16.4)	366 (18.5)	130 (16.3)	148 (12.8)	24 (2.57)	232 (12.0)
Very poor	1696 (19.1)	404 (19.4)	356 (18.0)	201 (25.3)	259 (22.5)	45 (4.83)	431 (22.3)
Poor	1676 (18.9)	404 (19.4)	383 (19.4)	101 (12.7)	152 (13.2)	189 (20.2)	447 (23.1)
Less poor	1923 (21.7)	386 (18.5)	462 (23.4)	181 (22.8)	251 (21.8)	248 (26.5)	395 (20.4)
Least poor	2335 (26.3)	548 (26.2)	409 (20.7)	182 (22.9)	342 (29.7)	430 (45.9)	427 (22.1)
Current smoking	1622 (18.3)	142 (6.83)	406 (20.6)	98 (12.4)	249 (29.7)	495 (53.0)	232 (12.0)
Current alcohol consumption	4139 (46.7)	1323 (63.8)	1287 (65.3)	187 (23.5)	318 (27.7)	665 (71.1)	359 (18.6)
Insufficient MVPA (<150 mins/wk)	1302 (14.7)	402 (19.3)	293 (14.8)	176 (22.1)	43 (3.73)	256 (27.4)	132 (6.82)
BMI (kg/m^2)	23.9±6.09	20.9±3.45	21.6±3.60	26.8±7.69	27.9±8.19	24.9±5.67	25.4±5.76
Systolic blood pressure (mmHg)	123±21.3	116±18.2	124±21.5	131±21.6	126±20.9	132±21.1	120±21.2
Diastolic blood pressure (mmHg)	78.4±13.1	73.5±10.6	76.9±12.7	78.6±12.8	81.1±12.8	89.2±13.2	78.4±12.9
Fasting glucose (mmol/l)	5.07±1.69	5.05±1.25	4.54±0.81	5.12±1.83	5.21±2.49	5.28±1.54	5.43±1.99

Table 1. Basic characteristics of study paticipants by site (continued)

	All sites	Nanoro, BF	Navrongo, GH	Agincourt, SA	Dikgale, SA	¹Soweto, SA	Nairobi, KE
	N=8872	n=2081	n=1976	n=795	n=1152	n=936	n=1932
HDL-C (mmol/l)	1.18±0.42	1.12±0.37	1.14±0.38	1.19±0.41	1.19±0.41	1.20±0.46	1.26±0.47
LDL-C (mmol/l)	2.21±0.87	1.92±0.76	1.85±0.71	2.23±0.79	2.49±0.89	2.48±0.91	2.58±0.87
HIV+	978 (11.0)	9 (0.43)	16 (0.81)	278 (34.9)	250 (21.0)	188 (20.1)	237 (12.3)
Self-reported ART use	695 (84.7)	-	-	268 (96.4)	138 (55.2)	84 (44.6)	193 (81.4)
Left mean CIMT (µm)	649±0.136	669±132	703±145	611±112	632±0.126	637±142	601±121
Right mean CIMT (µm)	627±130	659±123	667±132	581±107	640±130	601±129	576±119
Average CIMT µm	640±123	667±118	689±129	598±100	638±117	618±118	590±107

Data presented as absolute numbers and percentages (%) or as means ± standard deviations (SD); BF=Burkina Faso, GH=Ghana, SA=South Africa and KE=Kenya; SES=socio-economic status; MVPA=moderate-to-vigorous physical activity; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; ART=antiretroviral therapy and CIMT=carotid intima-media thickness.
¹Data presented are for men only since there was no CIMT measurements for women

Behavioral Factors

The prevalence of smoking was higher among men in Soweto (53%) and Dikgale (29.7%) in South Africa and Navrongo, Ghana (20.6%) compared with Agincourt, South Africa (12.4%), Nairobi, Kenya (12.0%) and Nanoro, Burkina Faso (6.83%). Men in Soweto (71.1%) and the 2 West African sites (65.3% in Ghana and 63.8% in Burkina Faso) recorded higher current alcohol consumption than Dikgale (27.7%), Agincourt (23.5%), and Nairobi (18.6%). The total population was fairly active with more than two-thirds of adults across all countries meeting physical recommendations of ≥150 minutes of moderate-to-vigorous intensity physical activity per week (see Table 1).

Anthropometric, Blood Pressure, Biochemical Variables, and HIV Infection

The average BMI was 23.9±6.09 kg/m2, with the rural sites of Burkina Faso (20.9±3.45 kg/m2) and Ghana (21.6±3.6 kg/m2) in West Africa recording lower BMIs than the urban sites in Kenya (25.4±5.76 kg/m2) and the urban (25.0±5.67 kg/m2 in Soweto) and rural (26.8±7.69 kg/m2 in Agincourt and 27.9±8.19 kg/m2 in Dikgale) sites in South Africa (Table 1). The average SBP and diastolic blood pressure were highest in all 3 South African sites and Kenya compared with the West African sites. Fasting blood glucose was highest in Kenya (5.43±1.99 mmol/L), followed by the South African sites (5.12±1.83 mmol/L in Agincourt, 5.21±2.49 mmol/L in Dikgale and 5.28±1.54 mmol/L in Soweto), Burkina Faso (5.05±1.25 mmol/L) and Navrongo (4.54±0.81 mmol/L). A similar pattern was observed for HDL-C and LDL-C. The prevalence of HIV was highest in South Africa (34.9% in Agincourt, 21% in Dikgale and 20% in Soweto) followed by Kenya (12.3%) and <1% in Burkina Faso and Ghana (Table 1). Self-reported use of ART among HIV positive participants was lowest at Soweto (44.6%) followed by Dikgale with 55.2% and as high as 81.4% in Nairobi and 96.4% in Agincourt.

CIMT Measurements

Mean CIMT of the left CCA was higher than the right CCA (Table 1) in all 6 sites (P<0.001 in all sites). Age and sex adjusted mean (±standard errors) levels of the average CIMT for each sample at the different sites are presented in Figure 1. The average CIMT for the entire population was 640±113 µm. Mean CIMT was significantly different between all sites (P<0.001) with Ghana (689±129 µm) and Burkina Faso (667±118 µm) recording higher common CIMT levels than the South African sites (598±100 µm in Agincourt, 638±117 lm in Dikgale and 0.64±0.002 µm in Soweto) and Kenya (590±107 µm). Women in Kenya had higher CIMT compared with men (P=0.022) while the reverse was observed in Burkina Faso (P<0.001) (Figure 2). In Ghana (P=0.657) and South Africa (Agincourt, P=0.501 and Dikgale, P=0.935), women and men had similar levels of common CIMT.
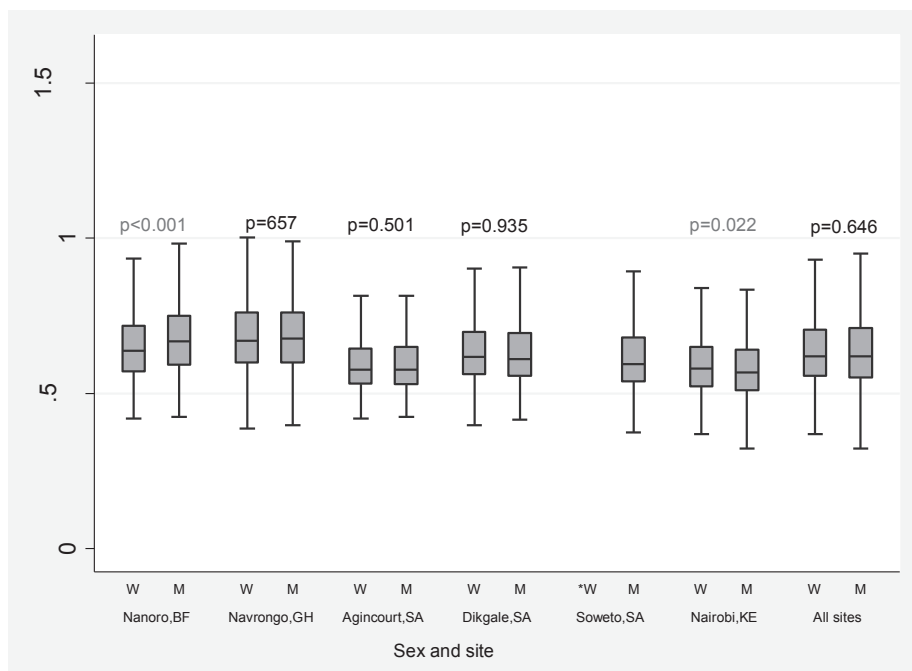
Figure 2: Distribution of mean levels of common CIMT (in mm) across 4 SSA countries stratified by sex. Differences between women (W) and men (M) were generated using sample t-test with equal variance; *Data not available for women in Soweto. BF, Burkina Faso; GH, Ghana; KE, Kenya; SA, South Africa.

Factors Associated With Common CIMT

The factors associated with CIMT in the combined population within univariate analyses are shown in Table S1. Adjusted regression models were then built sequentially and the factors associated with CIMT in the various models are displayed in Table 2. These analyses show that of the variables included in model 1, age, SBP, HDL-C and LDL-C remained significant through to model 4, whilst the effect of sex became significant from model 2 onwards. Smoking status was not significantly associated with CIMT in any of the models but its effect did strengthen across the models, whilst the effect of glucose weakened. Among the variables added in model 2, BMI and alcohol use both were significant and remained so, whilst MVPA was not significantly associated with CIMT in any of the models. Educational status and household SES included in model 3 were not significantly associated with CIMT, whilst HIV status, which was added in model 4, was significant. Model 1 explained 23.4% of the variance in CIMT whilst model 4 explained 24.4% of the variance. To further understand the effect of HIV infection on CIMT, we conducted a sensitivity analysis, where we included objectively assessed HIV status from the South African sites and Kenya only. In

this model we observed an even stronger inverse association with CIMT (–15.3 [–24.2, –6.31]). We conducted a further sub-analysis on HIV participants who used ART and observed that ART was associated with lower CIMT levels (–70.1 [–165, –25.6] in Agincourt; –5.05 [–9.52, –1.29] in Dikgale and –48.6 [–82.9, –14.2] in Soweto). This model included only participants from South Africa, which was the only country in which ART use was assessed.

To determine the within site variation, we conducted a mixed-effect ML regression analyses. Presented in Table 3 is a comparison of multivariable linear (model 4) and mixed-effect ML regression analyses (model 5) and mixed-effect ML regression analyses including interaction terms (model 6). The likelihood ratio test (LR χ2=498.02; *P*<0.001) indicated that the mixed–effect ML model (model 5) had a better fit than the multivariable linear regression model from Table 2 (model 4). In model 5, the association between classical cardiovascular risk factors and CIMT were similar to the multivariable linear regression model. However, HIV, although showing a similar direction of effect (–5.81 [–13.82, 2.21]), the observed effect was no longer significant (P=0.156). Further to this, physical activity was associated with lower CIMT levels (–8.17 [–14.81, –1.53]; *P*=0.016). When interaction terms were introduced into this model (model 6), we noted that HIV did not interact with age (*P*=0.306), sex (*P*=0.252) or household SES (*P*=0.877). The likelihood ratio test (LR χ2=47.88; P=0.318), showed a better fit for model 5 than for model 6.

Table 2. Factors associated with common CIMT, presented as change in common CIMT in μm per increase of the risk factors.

Risk factors	Model 1		Model 2		Model 3		Model 4	
	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value
Age per year	6.66 (6.24, 7.07)	<0.001	6.79 (6.37, 7.21)	<0.001	6.80 (6.37, 7.23)	<0.001	6.77 (6.34, 7.19)	<0.001
Men vs women	4.89 (−0.31, 10.09)	0.065	10.67 (5.25, 16.08)	<0.001	10.62 (5.04, 16.20)	<0.001	10.32 (4.75, 15.90)	<0.001
Current vs Never/previous smoking	−3.04 (−9.82, 3.73)	0.379	6.22 (−0.86, 13.29)	0.085	6.44 (−0.65, 13.53)	0.075	6.26 (−0.83, 13.35)	0.084
SBP per 10 mmHg	8.26 (6.98, 9.53)	<0.001	7.59 (6.29, 8.89)	<0.001	7.62 (6.32, 8.92)	<0.001	7.52 (6.21, 8.83)	<0.001
Glucose per 1mmol/l	1.51 (−0.13, 3.16)	0.072	1.01 (−0.60, 2.62)	0.219	0.98 (−0.63, 2.59)	0.232	0.94 (−0.67, 2.56)	0.252
HDL-C per 1mmol/l	−16.93 (−22.57, −11.28)	<0.001	−11.83 (−17.55, −6.11)	<0.001	−11.99 (−17.73, −6.25)	<0.001	−12.15 (−17.88, −6.41)	<0.001
LDL-C per 1mmol/l	7.85 (4.94, 10.77)	<0.001	5.04 (2.08, 7.99)	0.014	5.08 (2.10, 8.07)	0.001	5.08 (2.10, 8.06)	0.001
BMI per 10 kg/m²	–	–	18.08 (12.99, 23.16)	<0.001	18.21 (13.05, 23.37)	<0.001	17.61 (12.46, 22.76)	<0.001
MVPA in mins/week	–	–	−5.68 (−12.46, 1.10)	0.101	−5.50 (−12.29, 1.29)	0.112	−5.58 (−12.37, 1.21)	0.107
Current vs Never/previous alcohol use	–	–	−13.47 (−19.05, −7.89)	<0.001	−13.46 (−19.06, −7.87)	<0.001	−13.51 (−19.11, −7.91)	<0.001
Educational status								
No formal	–	–	–	–	Ref (value 0)	Ref	Ref	Ref
Primary	–	–	–	–	−4.19 (−11.46, 3.08)	0.589	−4.03 (−11.29, 3.24)	0.625
Secondary	–	–	–	–	−0.65 (−8.54, 7.24)		−7.77 (−8.66, 7.11)	
Tertiary	–	–	–	–	−3.33 (−18.12, 11.46)		−3.81 (−18.60, 10.98)	
Household SES								
Poorest	–	–	–	–	Ref		Ref	Ref
Poorer	–	–	–	–	−0.26 (−8.42, 7.90)	0.092	−0.11 (−8.27, 8.04)	0.096

Table 2. Factors associated with common CIMT, presented as change in common CIMT in μm per increase of the risk factors. (continued)

Risk factors	Model 1		Model 2		Model 3		Model 4	
	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value	β-coefficient [95%CI]	p-value
Poor	-	-	-	-	-2.73 (-10.94, 5.49)	-	-2.77 (-10.98, 5.44)	-
Less poor	-	-	-	-	-7.17 (-15.20, 0.86)	-	-7.18 (-15.20, 0.85)	-
Least poor	-	-	-	-	1.92 (-6.15, 10.0)	-	1.78 (-6.29, 9.85)	-
HIV+ vs HIV-	-	-	-	-	-	-	-8.86 (-15.70, -2.03)	0.011
	-	-	-	-	-	-	-	-
Variance (R²)	0.234	-	0.242	-	0.243	-	0.244	-

Multivariable linear regression analyses of association between risk factors and CIMT. Model 1 = age, sex, current smoking, SBP, fasting glucose, HDL-C, LDL-C and site; Model 2 = model 1 + BMI, alcohol use, physical activity; Model 3 = model 2 + educational status, SES; and Model 4 = model 3 + HIV infection. CI=confidence interval; SES=socio-economic status; MVPA=moderate-to-vigorous physical activity; SBP=systolic blood pressure; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; ART=antiretroviral therapy and CIMT=carotid intima-media thickness.

Table 3. Comparison of linear regression model and mixed-effect multilevel (ML) model depicting the association between risk factors of CIMT in SSA

Risk factors	Model 4 β-coefficients (95%CI)	Model 4 p-value	Model 5 β-coefficients (95%CI)	Model 5 p-value	Model 6 β-coefficients (95%CI)	Model 6 p-value
Age in years	6.77 (6.34, 7.19)	<0.001	6.71(6.29, 7.13)	<0.001	6.78(6.34, 7.22)	<0.001
Men vs women	10.32 (4.75, 15.90)	<0.001	13.11(7.54, 18.69)	<0.001	13.95(8.22, 19.68)	<0.001
Current vs Never/previous smoking	6.26 (−0.83, 13.35)	0.084	3.69(−3.46, 10.85)	0.312	3.75(−3.41, 10.91)	0.304
SBP per 10 mmHg	7.52 (6.21, 8.83)	<0.001	7.78(6.60, 8.95)	<0.001	7.77(6.59, 8.94)	<0.001
Glucose per 1mmol/l	0.94 (−0.67, 2.56)	0.252	0.79(−0.62, 2.21)	0.269	0.82(−0.59, 2.23)	0.256
HDL per 1mmol/l	−12.15 (−17.88, −6.41)	<0.001	−12.71(−18.42, −6.99)	<0.001	−12.77(−18.49, −7.06)	<0.001
LDL per 1mmol/l	5.08 (2.10, 8.06)	0.001	4.35(1.42, 7.27)	0.004	4.34 (1.42, 7.27)	0.004
BMI per 10 kg/m^2	17.61 (12.46, 22.76)	<0.001	16.86(12.09, 21.62)	<0.001	16.71(11.94, 21.48)	<0.001
MVPA in mins/week	−5.58 (−12.37, 1.21)	0.107	−8.17(−14.81, −1.53)	0.016	−8.06(−14.70, −1.42)	0.017
Current vs Never/previous alcohol use	−13.51 (−19.11, −7.91)	<0.001	−13.95(−19.49, −8.41)	<0.001	−13.92(−19.46, −8.38)	<0.001
Educational status						
No formal education	Ref (0)	Ref (0)	Ref (0)	Ref (0)	Ref (0)	Ref (0)
Primary	−4.03 (−11.29, 3.24)	0.625	−7.96(−15.13, −0.79)	0.175	−8.17 (−15.33, −0.99)	0.156
Secondary	−7.77 (−8.66, 7.11)		−7.17(−15.36, 1.02)		−7.42 (−15.61, 0.78)	
Tertiary	−3.81 (−18.60, 10.98)		−5.97(−20.28, 8.33)		−6.27(−20.57, 8.04)	
Household SES						
Poorest	Ref (0)	Ref (0)	Ref (0)	Ref (0)	Ref (0)	Ref (0)
Poorer	−0.11 (−8.27, 8.04)	0.096	−0.62(−8.72, 7.48)	0.142	−0.81(−9.42, 7.79)	0.090

Table 3. Comparison of linear regression model and mixed-effect multilevel (ML) model depicting the association between risk factors of CIMT in SSA (continued)

Risk factors	Model 4		Model 5		Model 6	
	β-coefficients (95%CI)	p-value	β-coefficients (95%CI)	p-value	β-coefficients (95%CI)	p-value
Poor	-2.77 (-10.98, 5.44)		-2.93(-11.11, 5.25)		-3.78 (-12.39, 4.82)	
Less poor	-7.18 (-15.20, 0.85)		-6.68(-14.68, 1.32)		-7.84 (-16.2, 0.60)	
Least	1.78 (-6.29, 9.85)		1.83(-6.25, 9.92)		1.74(-6.71, 10.20)	
HIV+ vs HIV-	-8.86(-15.70, -2.03)	0.011	-5.81(-13.82, 2.21)	0.156	26.80(-41.40, 95.01)	0.441
Interaction terms						
HIV x Sex					-8.82 (-23.91, 6.27)	0.252
HIV x Age					-0.69 (-2.01, 0.630)	0.306
HIV x SES						
Poorest vs poorer					2.25 (-23.27, 27.76)	0.877
Poorest vs poor					8.54 (-18.27, 35.34)	
Poorest vs less poor					10.88 (-14.53, 36.29)	
Poorest vs least poor					2.24 (-23.42, 27.89)	
Constant	215.8(188.6, 242.9)	<0.001	174.4(132.5, 216.3)	<0.001	171.6 (129.3, 214.0)	<0.001
Observations	8,872		8,872		8,872	
Number of groups	-	-	6	-	6	-
Likelihood ratio (LR) test	-	-	Model 2 vs Model 1 LR chi²=498.02	<0.001	Model 3 vs Model 2 LR chi²=47.88	0.318

Model 1 = Multivariable linear regression model; Model 2 = Mixed effect ML regression analyses; Model 3 = Mixed effect ML regression analyses + interaction terms (interactions term, HIV and sex = HIV x sex, HIV and age = HIV x age and HIV vs socio-economic status = HIV x SES). CI=confidence interval; SES= socio-economic status; MVPA=moderate-to-vigorous physical activity; SBP=systolic blood pressure; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; ART=antiretroviral therapy and CIMT=carotid intima-media thickness.

In sex stratified analyses (Tables 4 and 5), the observed association of age, SBP, HDL-C, current alcohol consumption and HIV infection with CIMT was similar between men and women. In the pooled analysis (Table 2), the association between smoking and CIMT had a trend towards a positive association (6.26 µm [-0.83, 13.35]; P=0.084). However, in men only current smoking was positively associated with higher CIMT (10.7 µm [2.93, 18.53]). Also in men but not women, a unit elevation in LDL-C (mmol/L) was likely to present with a 6.05 µm [1.97, 10.13] higher CIMT, whilst higher household SES among men but not women was associated with a lower CIMT (P=0.042).

We added multiplicative interaction terms to the final model to evaluate whether relations between risk factors and common CIMT differed by sites (Table S2). We observed some significant interactions between site and certain risk factors. We therefore conducted sub-analyses for each site stratified by sex. In these site stratified analyses (Tables 4 and 5), differential effects of some of the independent variables on CIMT were observed. Significant associations of plasma glucose levels with CIMT were observed in women in Nanoro (-7.90 [-13.8, -2.04]) and Dikgale (3.68 [0.75, 6.62]) but the effect was negative in the former and positive in the latter group.

In women, BMI correlated significantly and negatively with CIMT in Navrongo (-28.7 [-52.9, -4.56]) but positively in Dikgale (17.8 [7.34, 28.2]). Men with primary, secondary, and tertiary education in Soweto were likely to have higher CIMT than men with no formal education. Alcohol consumption was inversely associated with CIMT in men from Nanoro, Burkina Faso. Similarly, high HDL was associated with lower CIMT in women from Dikgale, South Africa and Nairobi, Kenya. The MVPA had similar inverse associations with CIMT in men from Navrongo and Burkina Faso (see Tables 4 and 5).

Table 4. Factors associated with common CIMT in women, presented as change in common CIMT in μm per increase of the risk factor and stratified by sites.

Factors	All β-coefficient [95%CI]	Nanoro, BF β-coefficient [95%CI]	Navrongo, GH β-coefficient [95%CI]	Agincourt, SA β-coefficient [95%CI]	Dikgale, SA β-coefficient [95%CI]	Soweto, SA	Nairobi, KE β-coefficient [95%CI]
Age	6.48 (5.85, 7.11)	7.92 (6.72, 9.12)	6.15 (4.83, 7.48)	5.18 (3.48, 6.88)	6.54 (4.86, 8.22)	-	4.67 (3.37, 5.97)
*Smoking	1.66 (-20.2, 23.6)	-	29.9 (-9.47, 69.2)	123 (81.5, 165)	-6.31 (-47.2, 34.6)	-	-11.1 (-46.6, 24.5)
SBP	8.55 (6.67, 10.4)	6.57 (2.43, 10.7)	9.57 (6.19, 12.9)	12.3 (3.84, 20.7)	5.27 (1.59, 8.95)	-	11.4 (7.81, 15.0)
Glucose	1.87 (-0.27, 4.01)	-7.90 (-13.8, -2.04)	2.16 (-6.92, 11.2)	1.53 (-4.89, 7.94)	3.68 (0.75, 6.62)	-	1.69 (-1.96, 5.35)
HDL-C	-11.2 (-20.2, -2.19)	-1.22 (-20.0, 17.6)	-11.9 (-34.9, 11.2)	-12.4 (-33.7, 8.98)	-18.1 (-46.6, 10.3)	-	-7.11 (-20.3, 6.04)
LDL-C	2.09 (-2.26, 6.44)	3.13 (-7.35, 13.6)	-2.18 (-13.4, 9.08)	10.0 (-1.92, 21.9)	-5.38 (-14.3, 3.56)	-	7.53 (-0.24, 15.3)
BMI	4.52 (-1.32, 10.4)	8.30 (-12.7, 29.3)	-28.7 (-52.9, -4.56)	-0.09 (-9.32, 9.14)	17.8 (7.34, 28.2)	-	1.99 (-9.25, 13.2)
MVPA	-3.26 (-13.3, 6.77)	-9.73 (-31.2, 11.7)	-6.92 (-26.0, 12.2)	-7.99 (-30.6, 14.6)	18.2 (-29.0, 65.3)	-	-13.3 (-31.9, 5.43)
*Alcohol use	-8.99 (-17.5, -0.50)	-10.8 (-23.6, 2.05)	-1.69 (-17.2, 13.8)	-6.29 (-41.5, 28.9)	-22.1 (-43.9, -0.15)	-	-26.9 (-53.5, -0.27)
Educational status							
No formal	Ref	Ref	Ref	Ref	Ref	-	Ref
Primary	-4.97 (-14.5, 4.60)	5.52 (-19.6, 30.7)	-4.78 (-24.3, 14.7)	-24.8 (-45.1, -4.58)	-0.57 (-28.1, 26.9)	-	-18.8 (-37.6, -0.01)
Secondary	4.21 (-7.01, 15.4)	8.74 (-52.8, 70.3)	-22.5 (-53.4, 8.46)	-10.7 (-34.1, 12.7)	-5.98 (-34.2, 22.2)	-	-8.76 (-30.1, 12.5)
Tertiary	1.09 (-29.7, 31.9)	-15.9 (-36.7, 4.81)	61.6 (-1.68, 124)	10.1 (-49.3, 69.6)	-31.4 (-73.5, 10.7)	-	-7.4 (-79.8, 64.9)
Household SES							
Poorest	Ref	Ref	Ref	Ref	Ref	-	Ref
Poorer	0.17 (-13.2, 11.3)	6.86 (-14.2, 27.9)	-10.6 (-35.6, 14.3)	3.01 (-27.7, 33.7)	0.87 (-23.5, 25.3)	-	1.67 (-19.9, 23.3)
Poor	-3.48 (-14.6, 7.63)	-3.07 (-23.2, 17.1)	-8.54 (-33.3, 16.2)	-16.6 (-48.4, 15.1)	-3.06 (-31.8, 25.6)	-	1.66 (-19.6, 22.9)

Table 4. Factors associated with common CIMT in women, presented as change in common CIMT in µm per increase of the risk factor and stratified by sites. (continued)

Factors	All β-coefficient [95%CI]	Nanoro, BF β-coefficient [95%CI]	Navrongo, GH β-coefficient [95%CI]	Agincourt, SA β-coefficient [95%CI]	Dikgale, SA β-coefficient [95%CI]	Soweto, SA β-coefficient [95%CI]	Nairobi, KE β-coefficient [95%CI]
Less poor	−0.12 (−10.9, 10.7)	8.50 (−12.5, 29.5)	−9.16 (−32.7, 14.4)	2.14 (−28.3, 32.6)	12.9 (−13.7, 39.5)	–	−7.79 (−29.2, 13.6)
Least poor	5.64 (−5.63, 16.9)	6.03 (−13.9, 26.0)	7.27 (−18.5, 33.1)	−5.78 (−35.4, 23.9)	29.6 (2.44, 56.7)	–	−2.82 (−27.2, 21.6)
†HIV+	−9.07 (−18.9, 0.83)	−30.4 (−85.0, 24.3)	−6.71 (−38.2, 24.8)	−16.2 (−31.3, 63.8)	−86.8 (−110, −63.6)	–	−9.94 (−26.6, 6.69)
†HIV+ART+	–	–	–	−8.13 (−47.0, 63.3)	−14.5 (−38.6, −8.78)	–	–
Variance (R²)	0.233	0.197	0.136	0.232	0.211	–	0.174

Multivariable linear regression analyses of factors associated with CIMT among women in the various sites; *Current vs previous or never and †HIV+ versus HIV negative. CI=confidence interval; SES=socio-economic status; MVPA=moderate-to-vigorous physical activity; SBP=systolic blood pressure; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; ART=antiretroviral therapy and CIMT=carotid intima-media thickness.

Table 5. Factors associated with common CIMT in men, presented as change in common CIMT in μm per increase of the risk factor and stratified by sites.

Factors	All β-coefficient [95%CI]	Nanoro, BF β-coefficient [95%CI]	Navrongo, GH β-coefficient [95%CI]	Agincourt, SA β-coefficient [95%CI]	Dikgale, SA β-coefficient [95%CI]	Soweto, SA β-coefficient [95%CI]	Nairobi, KE β-coefficient [95%CI]
Age	7.08 (6.49, 7.68)	8.50 (7.31, 9.69)	7.63 (6.12, 9.14)	6.11 (4.31, 7.91)	6.91 (4.65, 9.17)	7.01 (5.76, 8.26)	4.82 (3.57, 6.08)
*Smoking	10.7 (2.93, 18.5)	14.2 (-5.78, 34.2)	-3.66 (-21.0, 13.9)	6.24 (-21.1, 33.6)	38.3 (2.61, 73.9)	0.45 (-14.8, 15.7)	13.7 (-3.69, 31.0)
SBP	6.03 (4.23, 7.83)	3.78 (0.29, 7.83)	4.69 (0.26, 9.11)	5.47 (0.41, 10.5)	6.07 (-0.44, 12.6)	6.63 (2.96, 10.3)	10.2 (6.35, 14.1)
Glucose	-0.47 (-2.76, 1.82)	-2.84 (-6.75, 1.07)	-4.23 (-14.5, 6.00)	-2.95 (-7.43, 1.53)	4.01 (-6.48, 14.5)	0.04 (-4.21, 4.29)	-2.38 (-7.51, 2.74)
HDL-C	-11.9 (-19.6, -4.38)	1.34 (-17.1, 19.8)	-23.8 (-46.5, -1.14)	-9.19 (-29.3, 10.9)	-12.4 (-36.8, 12.1)	-16.0 (-29.6, -2.40)	-9.21 (-25.8, 7.41)
LDL-C	6.05 (1.97, 10.1)	9.10 (-0.36, 18.6)	9.86 (-2.98, 22.7)	12.0 (-0.53, 24.6)	-1.72 (-17.9, 14.5)	0.24 (-7.09, 7.57)	5.91 (-2.29, 14.1)
BMI	38.1 (28.9, 47.3)	46.4 (22.7, 70.1)	12.1 (-20.8, 44.9)	45.9 (19.6, 72.3)	85.9 (39.2, 132)	48.7 (34.1, 63.2)	30.5 (11.2, 49.8)
MVPA	-3.93 (-13.3, 5.45)	-19.2 (-35.9, -2.40)	-11.5 (-40.1, 17.1)	-3.72 (-28.6, 21.2)	-7.65 (-77.5, 62.2)	6.83 (-9.18, 22.8)	-9.95 (-46.3, 26.4)
*Alcohol use	-14.9 (-22.5, -7.27)	-36.9 (-52.9, -20.9)	-12.4 (-31.2, 6.38)	5.48 (-20.1, 31.0)	-22.2 (-60.1, 15.7)	-14.7 (-30.4, -0.99)	-12.2 (-29.1, 4.69)
Educational status							
No formal	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Primary	-4.21 (-15.1, 6.68)	-15.9 (-34.3, 2.37)	5.06 (-17.4, 27.5)	5.18 (-23.8, 34.2)	-20.2 (-66.2, 25.7)	72.3 (35.2, 109)	-23.3 (-61.8, 15.1)
Secondary	-3.10 (-14.3, 8.11)	-31.7 (-59.6, -3.77)	-6.85 (-29.3, 15.6)	19.9 (-11.8, 51.5)	-14.3 (-57.7, 29.2)	47.4 (14.5, 80.3)	-19.3 (-57.9, 19.4)
Tertiary	-7.74 (-25.7, 10.2)	2.92 (-37.5, 43.3)	-15.5 (-63.3, 32.3)	14.3 (-32.9, 61.6)	-51.1 (-117, 14.8)	43.7 (5.92, 81.5)	1.73 (-86.5, 89.9)
Household SES							
Poorest	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Poorer	-2.11 (-14.1, 9.91)	18.1 (-4.85, 40.9)	-8.45 (-35.4, 18.5)	-26.0 (-58.2, 6.17)	-19.8 (-51.1, 11.5)	22.8 (-18.8, 64.4)	0.77 (-26.6, 28.2)
Poor	-3.43 (-15.6, 8.70)	5.67 (-18.3, 29.6)	-6.30 (-34.8, 22.2)	-42.9 (-75.9, -9.82)	-24.8 (-63.1, 13.4)	23.8 (-9.47, 56.9)	7.85 (-18.2, 33.9)

chapter 2

Table 5. Factors associated with common CIMT in men, presented as change in common CIMT in µm per increase of the risk factor and stratified by sites. (continued)

Factors	All β-coefficient [95%CI]	Nanoro, BF β-coefficient [95%CI]	Navrongo, GH β-coefficient [95%CI]	Agincourt, SA β-coefficient [95%CI]	Dikgale, SA β-coefficient [95%CI]	Soweto, SA β-coefficient [95%CI]	Nairobi, KE β-coefficient [95%CI]
Less poor	-15.3 (-27.1, -3.38)	0.01 (-25.2, 25.2)	-10.9 (-38.3, 16.4)	-40.5 (-73.3, -7.64)	-20.6 (-62.8, 21.6)	5.91 (-26.5, 38.3)	-17.5 (-43.7, 8.73)
Least poor	-4.52 (-16.27, 7.23)	7.88 (-16.4, 32.2)	1.49 (-26.4, 29.4)	-38.6 (-74.7, -2.39)	-15.8 (-54.9, 23.3)	13.6 (-18.8, 46.0)	1.29 (-24.9, 27.5)
†HIV+	-11.7 (-21.0, -2.37)	-79.2 (-148, -9.37)	-60.0 (-110, -9.98)	-42.6 (-137, -31.7)	-92.2 (-162, -22.0)	-38.2 (-181, -10.5)	-3.91 (-26.0, 18.2)
†HIV+ART+	-	-	-	-161 (-214, -108)	-69.3 (-86.9, -11.8)	-49.5 (-83.1, -15.9)	-
Variance (R2)	0.279	0.223	0.144	0.238	0.287	0.269	0.157

Multivariable linear regression analyses of factors associated with CIMT among women in the various sites; *Current vs previous or never and †HIV+ versus HIV negative. CI=confidence interval; SES=socio-economic status; MVPA=moderate-to-vigorous physical activity; SBP=systolic blood pressure; BMI=body mass index; LDL-c=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; ART=antiretroviral therapy and CIMT=carotid intima-media thickness.

Discussion

The AWI-Gen study is the first large African study with harmonized data collection in 4 countries across 3 sub-continental African regions to report on the measurement of CIMT and its associated cardiometabolic risk factors. Our study shows both sex and regional differences in CIMT levels and risk factor associations. In pooled analyses, our findings show that the major factors that were consistently associated with higher CIMT were age, men, SBP, BMI, and LDL-C while a high HDL-C, current alcohol consumption, and HIV infection were associated with lower CIMT. In a sex-stratified analysis, smoking was associated with a higher CIMT in men.

The mean CIMT of the left CCA was higher than the right and this has been reported previously in an adult Pakistani population (35). This could be because of the fact that the left CCA arises directly from the aortic arch and is therefore exposed to greater hemodynamic stress and intimal damage from the systolic pressure from the left ventricles. It may therefore be clinically relevant to use right CIMT for screening purposes. The mean CIMT of 0.64±0.003 mm in our study population was lower than 0.71±0.19 mm reported in adult populations from North America (36) and 0.71±0.12 mm in Europe (36). The difference is likely explained by the relatively older ages of the North American and European cohorts (mean ages 60.2±8.7 and 58.8±7.6, respectively) compared with our population that had a mean age of 49.9±5.8 years. The observed CIMT in our population was, however, higher than reported in studies from India (38), China (39), Pakistan (35), and South America (40, 41) which had a similar age to our population. These observed variations in mean CIMT between white, Asian, and African populations support the notion that CIMT varies by ethnicity4 and may therefore have a substantive genetic contribution, but it must also be noted that differences in sampling methods, sample size, ratio of men to women and CIMT measurement techniques across these studies may also play a part.

In the combined analysis, mean CIMT levels were similar for men and women. In Nanoro, Burkina Faso where women and men had similar ages, men had higher CIMT than women, whereas despite being younger, women had higher CIMT than men in Nairobi, Kenya. In other studies where women were observed to have higher CIMT compared with men, the differences were observed in women over the age of 45 years (42, 43). However, when we adjusted for other risk factors including age, men were more likely to present with higher CIMT compared with women. Differences in CIMT levels between men and women have been attributed to differences in exposure to CVD risk factors.[44] In addition, physiological factors, such as lumen diameter, have been suggested as

explanations for sex differences, with women generally presenting with smaller blood vessels (45).

The association between classical CVD risk factors and CIMT has been established in various populations, predominantly white and Asian (46-49) and only in a few studies, with small sample sizes, from African populations (50, 51). Classical CVD factors that have been observed to be associated with CIMT in the literature were similarly associated in the present study, and this provides some level of external validity to our data. Addition of other potential risk factors into a regression model that included these traditional CVD risk factors had a minimal effect on the variance in CIMT explained by the model. These risk factors included BMI, alcohol intake, and HIV, all of which did contribute significantly to the final model but slightly attenuated the association of the other risk factors with CIMT. Our findings are in agreement with the INTERHEART study that showed that, although the magnitude of the relations differed somewhat, established risk factors were associated with an increased risk of acute myocardial infarction across the world (52). These findings therefore suggest that interventions aimed at reducing the classical risk factors will help prevent the development of atherosclerosis and reduce CVDs and associated mortality in SSA populations.

The association of SBP with high CIMT levels is of particular interest as this presents a worrying phenomenon since a previous AWI-Gen study demonstrated a high prevalence of hypertension across all sites with an associated low awareness and control of high blood pressure (21). While we advocate for preventive measures that should be targeted to the local situation, early detection and management of hypertension may particularly benefit these SSA populations. Increased awareness, screening, early detection and subsequent management of CVD risk factors should be integrated into current public health systems in SSA.

We observed that current alcohol consumption was associated with lower CIMT and this is contrary to findings from the USE Intima-Media Thickness (USE-IMT) 8 cohorts collaboration conducted among North Americans and Europeans, which reported lower CIMT among participants who consumed little or no alcohol compared with those who consumed >10 g of alcohol daily (53). A longitudinal study further clarified this by reporting that sustained heavy drinking in midlife was associated with higher CIMT (54). The observed inverse association of alcohol consumption with CIMT has also been documented in a Korean study (55). One possible explanation for this relationship comes from a randomized cross-over feeding trial among men which showed that alcohol improves lipid profiles and reduces atherosclerosis related inflammatory

markers in plasma. The phenolic content of alcoholic drinks, which is high in sorghum-based beers, which are consumed widely in the study settings, was found to reduce leukocyte adhesion molecules and inflammatory biomarkers (56, 57). Furthermore, the ARIC (Atherosclerosis Risk in Communities) study, using candidate gene analyses with a Mendelian Randomization methodology, demonstrated that low-to-moderate alcohol consumption improves serum lipid levels (58). However, it should be noted that in the present study, the inverse association between alcohol intake and CIMT occurred independently of lipid levels. It is possible that the crude assessment of alcohol intake used in the current study may not be sensitive enough to allow more complex investigations of these relationships, and longitudinal studies using more objective measures of alcohol intake may be required.

In our population and according to the mixed-effect ML model, HIV infection showed a non-significant inverse association with lower CIMT after adjusting for age, education status, household SES, and the classical cardiovascular risk factors. This inverse association was shown to be significant in multivariable regression analyses. Both findings are contrary to studies from Brazil (59) and Uganda (60) reporting higher CIMT among HIV-positive compared with HIV-negative individuals. In line with these, a recent meta-analysis based on 17 cohort and case-control studies indicates a 2-fold higher risk ratio for cardiovascular diseases in people living with HIV in comparison with HIV-negative subjects. However, this meta-analysis included only 1 case-control study from Africa including 200 stroke cases and 398 controls.[61] The non-significant association observed in the current study is similar to findings from rural South Africa (62), North America (63), and Brazil (39, 40). As HIV was not assessed objectively across all sites, with the exception of the South African and Kenyan sites, we conducted (multivariable linear regression) sub-analyses on data from these sites to assess the effect of HIV on CIMT. These showed a negative association between HIV and CIMT. It is interesting to note that a recent study conducted in rural South Africa among (50, 59) participants aged ≥40 years has shown that HIV-positive subjects had a lower prevalence of classical CVD risk factors when compared with HIV-negative subjects, in both men and women (64). This has further been corroborated by more recent studies from South Africa, which demonstrated lower prevalence levels of hypertension and diabetes mellitus among HIV-infected subjects (65, 66). Considerably increased access to ART in SSA in the past decade, particularly so in South Africa, which has the largest ART roll-out program in the world (67), and application of treatment guidelines to screen people living with HIV for CVD risk (32, 68), may have prompted diagnosis and consequent treatment of identified CVD risk factors, or changes in lifestyle, in these subjects. These findings may well be indicative of an emerging paradigm reflecting an improved HIV care cascade.

Further to this, studies have reported that the effects of HIV on CIMT are more pronounced in younger than older population because of active viral replication and immune activation and a greater level of high-CVD risk behaviours (69). Thus, potentially long-term use of ART as noted in our older population may result in chronic viral suppression leading to lower inflammation and reduced CIMT among HIV participants. However, it is possible that the negative association between HIV infection and CIMT observed in our study may be explained by other factors and thus, be attributed to residual confounding. Furthermore, misclassification of HIV status, particularly at sites that did not offer formal HIV testing could be possible, however, the low prevalence of HIV infection observed at such sites is supported by data from the literature (29, 30). It should also be noted that the level of ART coverage varied across the sites ranging from 44.6% in Soweto to 96.4% in Agincourt. Although data on ART regimens were not collected, treatment guidelines in Kenya (68) and South Africa (32) at the time of this study were similar with first line therapies consisting of tenofovir+lamivudine (3TC) + efavirenz/nevirapine and zidovudine (AZT)+lamivudine (3TC) + nevirapine/efavirenz. Despite the heterogeneity across the sites, the negative relationship between HIV infection and CIMT was observed at all study sites in both men and women, suggesting that this is a robust association. The paucity of data on the role of HIV and atherosclerotic CVDs in Africa therefore requires further studies with a wide range of HIV-defining markers, such as viral load, and a larger sample size of subjects with confirmed HIV and ART status.

Several variables demonstrated differential association with CIMT across the sites. Possible reasons for this may include differences across sites in: genetic variance, distribution of the particular effector variables and level of influence of modifying variables. These differential site-specific effects are important to understand but are beyond the scope of the current study and require in depth analysis at individual sites using larger sample sizes and a broader array of input variables.

Limitations to our study include a lack of information on the duration of HIV infection, the ART regimens in use and the duration of therapy, and the absence of measurements of viral load and CD4 counts, all of which may play a role in explaining the association between HIV infection and CIMT. Furthermore HIV status determined in the Agincourt site may have potentially resulted in measurement bias as participants' status may have changed from the time of initial HIV diagnosis to the time of actual recruitment for the AWI-Gen study. The main strength of our study relates to the fact that this is the first large scale study to determine CIMT levels and their association with cardiovascular risk factors in 3 sub-continental blocks in Africa. Measurements of most variables

and CIMT were harmonized across all sites minimising variability and making pooled analysis and cross-site comparisons feasible. We were also able to cover the geographical and social variability across these regional blocks by recruiting in both rural and urban sites thus providing representation of the different stages of the epidemiological transition within Africa.

Conclusions

To our knowledge, this is the first large scale study from SSA to report on the levels of, and factors associated with, CIMT. We observed that the main drivers for higher CIMT were the same CVD risk factors associated with CIMT in white and Asian populations. Given the rising prevalence of these CVD risk factors in SSA, atherosclerotic diseases may become a major pan-African epidemic unless preventive measures are taken particularly targeted at prevention of hypertension and reduction in obesity. In addition, differential effects of certain factors on CIMT were observed across the SSA sites. We advocate for HIV-specific studies to fully understand the true association between HIV and CIMT in SSA.

Appendix
*H3Africa AWI-Gen Consortium Members Conception and design of the original AWI-Gen study: Michele Ramsay (SBIMB, Wits), Osman Sankoh (INDEPTH), Alisha Wade, Stephen Tollman and Kathleen Kahn (Agincourt), Marianne Alberts (Dikgale), Catherine Kyobutungi (Nairobi), Halidou Tinto (Nanoro), Abraham Oduro (Navrongo), Shane Norris (Soweto), and Scott Hazelhurst, Nigel Crowther, Himla Soodyall and Zane Lombard (Wits).

Site specific Investigators: Agincourt, South Africa (Frances Xavier Gomez-Olive Casas and Ryan Wagner), Dikgale, South Africa (Felistas Mashinya, Ian Cook and Sam Ntuli), Nairobi, Kenya (Christopher Khayeka-Wandabwa, Tilahun Nigatu Haregu, Shukri F. Mohammed and Stella Muthuri), Nanoro, Burkina Faso (Palwendé R. Boua, Herman Sorgho, Seydou Nakanabo-Diallo, Toussaint Rouamba), Navrongo, Ghana (Godfred Agongo, Cornelius Debpuur, Engelbert A. Nonterah, Eric Fato and Immaculate Anati and Lucas Amenga-Etego), Soweto, South Africa (Nomses Baloyi, Juliana Kagura, Richard Munthali and Yusuf Guman) and Wits AWI-Gen Collaborative Centre at SBIMB (Cassandra C. Soo, Freedom Mukomana, Stuart A. Ali, Ananyo Choudhury).

Disclosures

None

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Supplementary material

Table S1. Univariable estimates for exposure variables and CIMT in the final combined model

Risk factors	β-coefficient [95%CI]	P-value
Age (years)	7.91 [7.49,8.33]	<0.001
Men vs women	0.99 [−4.12,6.11]	0.704
Current vs Never/previous smoking	−11.93 [−18.45,−5.46]	<0.001
SBP per 10 mmHg	10.49 [9.22,11.75]	<0.001
Glucose per 1mmol/l	0.50 [−1.17,2.18]	0.556
HDL-C per 1mmol/l	−20.24 [−26.34,−14.15]	<0.001
LDL-C per 1mmol/l	−4.04 [−7.05,−1.04]	0.008
BMI per 10 kg/m^2	−2.30 [−6.40,1.79]	0.271
MVPA in mins/week	−17.85 [−25.42,−10.270	<0.001
Current vs Never/previous alcohol use	15.03 [9.91,20.15]	<0.001
Educational status		
No formal education	Ref	
Primary	−49.29 [−55.52,−43.03]	<0.001
Secondary	−58.97 [−64.99,−52.96]	
Tertiary	−49.92 [−64.52,−35.33]	
Household SES		
Poorest	Ref	
Poorer	−8.37 [−17.37,0.63]	0.052
Poor	−8.93 [−17.94,0.08]	
Less poor	−11.11 [−19.93,−2.29]	
Least poor	−2.63 [−11.26,6.00]	
HIV+ vs HIV-	−57.31 [−63.95,−50.68]	<0.001

CI=confidence interval; SES= socio-economic status; MVPA=moderate-to-vigorous physical activity; SBP=systolic blood pressure; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol.

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Table S2. Multiplicative interaction terms between sex and site with the risk factors

Site and risk factor interaction terms	p-value
Site with Age	<0.001
Site with Sex	<0.001
Site with Current smoking	0.844
Site with Systolic blood pressure	0.043
Site with Glucose	0.079
Site with HDL-C	0.619
Site with LDL-C	0.078
Site with BMI	<0.001
Site with MVPA	0.299
Site with Current alcohol	0.281
Site with Educational status	0.003
Site with Household SES	0.108
Site with HIV status	0.113

SES=socio-economic status; MVPA=moderate-to-vigorous physical activity; BMI=body mass index; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol

Chapter 3

Adiposity phenotypes and subclinical atherosclerosis in adults from sub–Saharan Africa: an H3Africa AWI–Gen Study

Nonterah EA, Bots ML, Oduro A, Agongo G, Soo CC, Micklesfield LK, Mashinya F, Boua PR, Mohamed SF, Wade AN, Kyobutungi C, Tinto H, Norris SA, Tollman SM, Ramsay M, Grobbee DE, Klipstein-Grobusch K, Crowther NJ on behalf of the AWI–Gen and the H3Africa Consortium.

Abstract

Background: Obesity and adipose tissue distribution contribute to an increased risk of cardiovascular disease (CVD) by promoting atherosclerosis. This association has been poorly studied in sub–Saharan Africa (SSA) despite the rising prevalence of cardiovascular disease.

Objectives: We determined the association between various adiposity phenotypes and carotid intima–media thickness (CIMT), a proxy of subclinical atherosclerosis, in a large SSA population.

Methods: A population–based cross–sectional study was performed from 2013–2016 in Burkina Faso, Ghana, Kenya and South Africa. Body mass index (BMI), waist (WC), hip circumferences (HC), visceral (VAT) and subcutaneous adipose tissue (SCAT) using B-mode ultrasound were measured. Ultrasonography of left and right far wall CIMT of the common carotid artery was used as an indicator of subclinical atherosclerosis. Individual participant data meta–analyses were used to determine the associations between adiposity phenotypes and CIMT in the pooled sample while adjusted multivariable linear regression analyses were used for site specific analyses.

Results: Data were obtained from 9,010 adults (50.3% women and a mean age of 50 ± 6 years). Men had higher levels of visceral fat than women while women had higher BMI, waist and hip circumference and subcutaneous fat than men at all sites except Burkina Faso. In the pooled analyses, BMI (β–value [95% CIs]: 19.5 [16.8, 22.3] µm) showed the strongest relationship with CIMT followed by VAT (5.86 [4.65, 7.07] µm), SCAT (5.00 [2.85, 7.15] µm), WC (1.27 [1.09, 1.44] µm) and HC (1.23 [1.04, 1.42] µm). Stronger associations were observed in men than in women.

Conclusion: Obesity within SSA will likely result in higher levels of atherosclerosis and promote the occurrence of cardio- and cerebrovascular events, especially in males, unless addressed through primary prevention of obesity in both rural and urban communities across Africa. The inverse association of VAT with CIMT in Burkina Faso and Ghana requires further investigation.

Keywords: Carotid intima-media thickness; subclinical atherosclerosis; obesity; adiposity; cardiovascular disease; sub-Saharan Africa

Highlights

- All adiposity phenotypes were positively associated with common carotid intima–media thickness (CIMT) in the entire cohort (pooled analyses).
- BMI had the strongest association with CIMT compared to other phenotypes.
- The magnitude of association between adiposity phenotypes and CIMT was higher in men than in women.
- Subcutaneous adipose tissue was inversely associated with CIMT only in women.
- An unexpected finding was the inverse association of visceral adipose tissue with CIMT in Burkina Faso and Ghana.

Introduction

Obesity is a major epidemic that occurs not only in the western world. Recent data from sub-Saharan Africa (SSA) demonstrate an equally high burden (1, 2), with south and north Africa having the highest prevalence levels (3). Projections from the World Health Organization (WHO) suggest that in 2025, 75% of the world's obese population will be in low– and middle–income countries such as those in SSA (4, 5). The recent Non-Communicable Disease (NCD) Risk Factor Collaboration study of 112 million adults further observed that the rising prevalence of obesity in rural communities contributes significantly to the global obesity epidemic (6). This is a major health concern, because obesity promotes the development of diabetes mellitus, is related to unfavorable levels of established cardiovascular risk factors such as dyslipidemia and hypertension, promotes the development of atherosclerosis and contributes to an increase in risk of symptomatic cardiovascular events (7, 8).

Several measures of adiposity are available to assess various overall and central body fat phenotypes. Thus, for general obesity, body mass index (BMI) is the most common measure while total body fat can be measured using dual-energy X-ray absorptiometry (DXA). Central obesity is often assessed using waist circumference (WC), with specific abdominal fat depots i.e., visceral (VAT) and subcutaneous (SCAT) adipose tissue assessed using imaging techniques such as magnetic resonance imaging (MRI), computerized tomography (CT) and B-mode ultrasonography (9, 10). Peripheral or lower body obesity, particularly fat tissue in the gluteofemoral region, is commonly measured using hip circumference (HC) whilst gluteofemoral fat can be specifically measured using the imaging methodologies described above. These adiposity phenotypes have been suggested to exert different effects on the development

of CVD risk factors and atherosclerosis (11). Body fat distribution has been shown to be different in black Africans compared to their white counterparts (12, 13).

The relationship between obesity and atherosclerosis can be investigated by the use of carotid intima-media thickness (CIMT) of the common carotid artery (CCA). Extensive research has shown that common CIMT, assessed using B-mode ultrasonography, is a marker of the presence of atherosclerosis locally and elsewhere in the arterial system, and, as such, is an intermediate stage for the development of CVDs (14-16). However, most of the research examining the relationship of adiposity with atherosclerosis, assessed using common CIMT has been conducted in Whites (17), Asians (18-20) and African–Americans (21) from high income countries, but little information is available for black African populations from SSA.

In the present study, we used data from the Africa-Wits-INDEPTH [International Network for the Demographic Evaluation of Populations and Their Health] Partnership for Genomic Studies (AWI-Gen) project (22) to determine the relationship of various adiposity phenotypes with CIMT in four SSA countries – Kenya, Ghana, Burkina Faso and South Africa.

Methods

Study design, setting and participants
The AWI–Gen study is embedded within the NIH–funded Human Heredity and Health in Africa (H3Africa) Consortium. AWI–Gen is a population–based longitudinal study conducted in six sites in four SSA countries with baseline data collection occurring between 2013 and 2016 as described previously (23, 24). The study was conducted at five Health and Socio–Demographic Surveillance Sites (HDSS) under the INDEPTH Network and the MRC/Wits Developmental Pathways to Health Research Unit (DPHRU) in Soweto, South Africa (25). The HDSS sites in South Africa were the cohort from Bushbuck Ridge in Mpumalanga (referred to as Agincourt HDSS in this publication) (26) and Dikgale HDSS (27). The other sites were in East Africa: African Population and Health Research Center HDSS, Nairobi, Kenya (28) and two rural sites in West Africa: Nanoro HDSS, Burkina Faso (29) and the Navrongo HDSS, Ghana (30). These countries are located in three sub-regional African blocks and may be representative of the social, geographical and genetic diversity of SSA. Study participants were adult women and men aged 40–60 years living in the various sites. Details of the sampling methods and recruitment strategies used by the various sites have been described in a previous publication (24).

Ethical considerations

The AWI-Gen study received ethics approval from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg, South Africa (Ethics approval identification number: M121029, renewed in 2017 with number: M170880). Additional ethics approvals were obtained from the national and institutional ethics boards/committees of the University of Limpopo, Nanoro HDSS, Burkina Faso; Nairobi HDSS, Kenya and Navrongo HDSS, Ghana. Written informed consent was obtained from all participants prior to recruitment.

Data availability

The datasets generated and/or analyzed during the current study will be made publicly available in the European Genome–phenome Archive under the set of projects related to the Human Heredity and Health in Africa (H3Africa) Consortium. Details concerning access to data and DNA can be found in the document titled H3Africa Data and Biospecimen Access Committee Guidelines, available in the consortium documents section of the H3Africa website (www.h3africa.com).

Data collection

The data collected included socio–demographic determinants of health, behavioral risk factors, and metabolic risk factors of CVDs, and the variables relevant to this paper are described briefly below.

Outcome variable: Common CIMT

The outcome variable was the mean CIMT thickness in micrometers (μm) of the far walls of both the left and right CCA. Images were taken with a linear-array 12L-RS transducer using GE Healthcare B-mode LOGIQ e ultrasound machine (GE, Healthcare, CT, USA). To measure the right CCA, the participant was asked to lie down in a supine position with a pillow underneath the neck for slight extension, head turned towards the left at a 45-degree angle and gel applied to the exposed neck area. Using the two sternocleidomastoid muscles as landmarks, the exposed area was scanned along the longitudinal plane until the CCA was found. The operator then identified a continuous one-centimeter segment (10mm) of the CCA far wall after which the image was frozen. The operator then placed a cursor between two points (10mm apart) on this identified segment of the far wall with the proximal starting point one centimeter from the bulb of the CCA. The inbuilt software in the ultrasound equipment then automatically detected the intima-lumen and the media-adventitia interfaced and calculated the minimum, maximum and mean common CIMT in mm to two decimal places. To measure the left carotid, the participant's head was turned to the opposite side, and the process was repeated (24). We measured one site or angle of the CCA instead of multiple carotid sites or angles, because

it was easier to measure and was equally reliable at enabling its widespread use at all study sites and aligned to real life setting measurements (31-33). To ensure reproducibility and reduce CIMT measurement variability, masked repeated measurements of the 15 volunteers were conducted by each trainee and the lead trainer. The coefficient of variation between and within trainees was calculated and maintained at <2%. Subsequently, the same settings and calibrations of the ultrasound equipment were used at each site for data collection.

Exposure variables: Adiposity phenotypes

The different adiposity phenotypes included in this analysis were body mass index, hip circumference, waist circumference, visceral adipose tissue and subcutaneous adipose tissue. BMI: Standing height to the nearest 0.1mm and weight to the nearest 0.1kg of each participant were measured without shoes and in light clothes using a Harpenden stadiometer (Holtain, Crymych, Wales) fixed to the wall and a digital calibrated weighing scale (SECA, Hamburg, Germany) respectively. The BMI was subsequently calculated as weight in kg/height in m^2.

Waist circumference (WC) and hip circumference (HC)

WC was measured using a stretch-resistant tape measure (SECA, Hamburg, Germany). Participants were asked to wear only light or tight-fitting clothing, with the outer clothing removed to enable the tape to be positioned correctly. The participant breathed normally and stood straight with arms slightly abducted when the tape was placed horizontally around the narrowest part of the torso, about halfway between the iliac crest and the lowest rib. Measurements of the WC were taken at the end of a normal expiration without the compression of the tape. The WC was recorded to the nearest 0.1cm. The HC was measured by placing the tape around the most protruding part of the buttocks, ensuring that the zero mark was to the participant's side. The HC was measured to the nearest 0.1cm.

Visceral (VAT) and subcutaneous (SCAT) adipose tissues

These were measured using a B-mode. LOGIQ e ultrasound machine (GE, Healthcare, CT, USA) with a 2.5MHz 3C-RS curved array transducer. A depth of 15cm and 9cm were used for VAT (medial) and SCAT measurements, respectively. For both measurements, participants were in the supine position, gel applied to the lower abdomen and the probe positioned with minimal compression on the midline at a level midway between the lower costal margin and the iliac crest with appropriate adjustments in the gain settings. The xiphi-sternum and umbilicus were used as a guide for accurate positioning. For VAT imaging, the transducer was held horizontal and the spine visualised in the

horizontal position with the vertebra in the centre of the image. The participant was then asked to breathe quietly, and the measurement was taken at the end of the exhalation. To calculate the amount of VAT, the paused ultrasound image was brought up onto the screen. The first cursor was placed anterior to the spine (on the fat pad if visible), and the second cursor on the thin peritoneal layer beneath the anterior rectus abdominal muscles. Care was taken to ensure that the measurement was perpendicular to the surface of the lumbar vertebra and taken between the peritoneum and the spine where there is a clear space between the vertebra and the aorta. The measurement was repeated by producing a second image, and the results recorded in cm to two decimal places. An immediate quality check was done to ensure that the spine, abdominal aorta and rectus abdominal muscle were visualized on the image (34, 35).

For the measurement of SCAT (transverse), the ultrasound probe was rotated through 90 degrees and the depth setting adjusted to 9cm. The rectus abdominus muscles were visualized, with care taken to ensure that both muscles were symmetrical in the image, and that the linea alba was centrally located, the gain adjusted accordingly, and the image captured. To calculate the SCAT measurement, the distance between the skin and the outer edge of the linea alba was measured on the screen, as described above. The measurement was then taken from a second image, and the results recorded similarly, in cm to two decimal places. The reliability of these ultrasound measurements in estimating adiposity has previously been validated in a black South African population (35).

Covariates
Selected covariates were those that contribute to the development of CVDs and can confound the association between adiposity phenotypes and CIMT. These included age, level of education (highest level of education obtained), household socio-economic status (estimated by calculations based on household assets, according to the practice implemented by the Demographic and Health Surveys (DHS) Program) (24), alcohol consumption, smoking and physical inactivity assessed by measuring moderate-to-vigorous physical activity (MVPA). Physical inactivity was defined as MVPA < 150 mins/week. Other covariates included systolic blood pressure, glucose, total cholesterol, HDL-C, LDL-C and HIV infection and ART use. Post–menopausal status in women was defined as having no period within the past 12 months. Details of how these variables were measured have been reported previously (24).

Data analysis
All data analyses were conducted with STATA version 14.1 (College Station, Texas, USA). We computed an average of the mean right and left far wall common CIMT thickness in micrometers (µm) as the main outcome variable. Continuous data were normally distributed and were summarized as means with standard deviations (±SD) and categorical data presented as absolute counts with corresponding percentages (%). Differences in mean (±SD) levels of the various adiposity phenotypes between women and men within the sites were determined using Student t-tests. We determined the association between each adiposity phenotype and CIMT using an inverse-variance weighted fixed-effect individual participant data meta-analysis (IPD-MA) using the ***"ipdmetan"*** package in STATA. This approach offered standardisation of analyses across study sites while taking into account potential clustering and heterogeneity of the different study populations (36). We were thus able to compare the magnitude of the effect of each adiposity phenotype on CIMT across the various study sites. In these analyses, we obtained a test of the overall effect of each adiposity measure on CIMT in the total sample. We also obtained between-study variance as a percentage of the total variance between study populations (I^2), giving us an idea of the extent of heterogeneity. Forest plots are plotted to display the effect measure and the percentage weight of the various study populations.

Where heterogeneity is significant as demonstrated by a higher % I^2, subgroup analyses were conducted for each site. In these sub-group analyses, the association of adiposity phenotypes with CIMT in each separate site was assessed using adjusted multiple linear regression analyses. These analyses were done in multiple sequential models. In the first step, we initially determined the independent association between each adiposity phenotype and CIMT. We then adjusted for covariates in a sequential manner. In Model 1, we adjusted for age, level of education, and household SES; Model 2 included variables from Model 1 plus additional adjustments for smoking, alcohol consumption, and MVPA. Model 3 was based on Model 2 plus each of the other body adiposity phenotypes. Adiposity phenotypes that had a variance inflation factor (VIF) >5 were dropped from Model 3. Thus, BMI, WC and HC were strongly correlated and were therefore not included in Model 3, whereas VAT and SCAT were included. Model 4 included Model 3 plus systolic blood pressure, glucose, HDL-C, LDL-C, total cholesterol and HIV infection. In women, menopausal status was also adjusted for in Model 4.

Measures of associations are reported as standardised beta (β) coefficients denoting differences in mean CIMT in µm caused by a unit increase in the adiposity phenotypes. Statistical significance was set at two– sided, $p < 0.05$.

Results

Data were available for 10,863 participants, but 1,341 had no CIMT data which included all women from Soweto. In addition, 512 participants had missing data from the other variables. Therefore, we conducted complete case analyses of 9,010 participants from six sites in four countries.

Descriptive data
The basic characteristics of the AWI–Gen study participants stratified by study site and sex are presented in Table 1. For the combined data from all sites, women had a mean age of 50.1±6 years compared to 49.8±9 years for men. In all sites, men had a higher mean household SES compared to women and similar observations were made for educational attainment (Table 1). Men showed a higher prevalence of both current smoking and alcohol consumption than women at all sites. Physical activity levels varied across sites, but women were more likely to be physically active. The prevalence of obesity was higher in women than men at all sites except Nanoro, Burkina Faso. Both women and men from Navrongo had the highest mean common CIMT followed by Nanoro, Dikgale, Agincourt and Soweto (men only) with the lowest levels in both sexes being observed in Nairobi (Table 1).

The mean levels of the various adiposity phenotypes by site and gender are summarized in Table 2. Gender differences (p < 0.001 for all) were noted for all adiposity measures at all sites, with the exception of VAT and SCAT at Nanoro.

Table 1: Basic characteristics of AWI-Gen study participants from six sites stratified by sex

Characteristics	All sites (N=9010)	Nanoro (n=2074)	Navrongo (n=1729)	Agincourt (n=1248)	Dikgale (n=1142)	Soweto (n=904)	Nairobi (n=1913)
Women (n (%))	**4536 (50.3)**	**1033 (49.8)**	**935 (54.1)**	**738 (59.1)**	**788 (69.0)**	**-**	**1042 (54.5)**
Age (years)	50.1±5.77	49.79±5.65	51.53±5.74	50.29±5.69	50.53±5.95	-	48.28±5.29
Formal education	2221 (51.5)	70 (6.84)	235 (22.2)	253 (66.1)	723 (90.9)	-	940 (89.4)
Household SES	9.61±3.80	10.74±3.39	9.01±4.52	6.86±2.89	10.07±3.06	-	10.79±3.01
Current alcohol	1383 (32.0)	618 (59.9)	581 (54.7)	135 (18.3)	100 (12.6)	-	294 (28.2)
Current Smoking	176 (3.89)	2 (0.19)	31 (3.32)	9 (1.22)	54 (6.86)	-	80 (7.68)
Obesity	1084 (23.9)	12 (1.16)	36 (3.85)	302 (41.3)	402 (51.0)	-	332 (31.9)
Low MVPA	545 (12.6)	136 (13.2)	199 (18.7)	85 (22.2)	27(3.44)	-	95 (9.12)
Height (m)	1.59±0.07	1.62±0.06	1.58±0.07	1.61±0.07	1.59±0.07	-	1.59±0.06
Weight (kg)	64.25±17.81	53.20±9.42	55.58±10.71	75.67±18.81	77.76±20.76	-	68.48±15.45
Systolic blood pressure (mmHg)	121.7±22.7	111.2±17.5	123.3±23.0	134.5±23.4	126.7±21.1	-	117.8±21.8
Diastolic blood pressure (mmHg)	77.5±12.9	71.1±9.81	77.1±12.6	80.6±13.2	82.5±12.9	-	78.3±13.2
Glucose (mmol/l)	5.09±1.75	5.00±0.91	4.59±0.70	4.94±1.39	5.28±2.46	-	5.59±2.36
LDL-C (mmol/l)	2.27±0.98	1.93±0.81	1.71±0.72	2.34±0.94	2.64±1.02	-	2.86±0.91
HDL-C (mmol/l)	1.15±0.38	1.05±0.35	1.12±0.34	1.19±0.35	1.18±0.35	-	1.26±0.44
HIV+	612 (13.5)	4 (0.39)	6 (0.64)	260 (35.2)	171 (21.7)	-	171 (16.4)
Post-menopausal	1481 (40.1)	352 (34.8)	262 (39.4)	83 (31.8)	376 (52.7)	-	409 (39.1)
Average common CIMT (μm)	636.3±113.9	648.3±112.1	692.2±119.3	609.8±88.9	635.7±111.8	-	593.6±104.9

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Table 1: Basic characteristics of AWI-Gen study participants from six sites stratified by sex (continued)

Characteristics	All sites (N=9010)	Nanoro (n=2074)	Navrongo (n=1729)	Agincourt (n=1248)	Dikgale (n=1142)	Soweto (n=904)	Nairobi (n=1913)
Men (n (%))	4474 (49.7)	1041 (50.2)	794 (45.9)	510 (40.9)	354 (31.0)	904 (100)	871 (45.5)
Age (years)	49.8±5.89	49.80±6.00	50.51±5.71	50.25±5.96	50.04±6.01	49.35±5.99	48.78±5.60
Formal education	2959 (67.3)	280 (27.3)	342 (37.9)	252 (77.8)	332 (94.1)	919 (99.1)	834 (96.1)
Household SES	10.62±4.31	11.85±4.18	10.12±4.87	6.52±2.62	9.52±3.38	12.11±3.12	11.59±3.51
Current alcohol	2696 (61.3)	691 (67.3)	702 (77.8)	135 (41.7)	216 (61.2)	660 (71.2)	292 (33.6)
Current Smoking	1521 (34.6)	142 (13.8)	384 (42.5)	81 (25.1)	222 (63.1)	490 (53.0)	202 (23.3)
Obesity	299 (6.69)	23 (2.21)	9 (1.13)	64 (12.6)	10 (2.82)	150 (16.6)	43 (4.94)
Low MVPA	716 (16.3)	255 (24.8)	90 (10.0)	69 (21.3)	13 (3.7)	254 (27.4)	34 (4.0)
Height (m)	1.71±0.07	1.73±0.07	1.67±0.08	1.72±0.07	1.69±0.06	1.71±0.06	1.69±0.07
Weight (kg)	65.91±14.31	65.22±11.98	58.70±9.69	72.59±17.00	62.18±12.62	73.09±17.48	65.58±11.89
Systolic blood pressure (mmHg)	125.4±20.5	120.1±17.8	124.4±20.0	132.2±21.5	124.6±20.4	131.1±21.1	122.4±20.2
Diastolic blood pressure (mmHg)	79.9±13.3	76.0±10.8	76.6±12.9	79.9±12.9	78.4±12.0	89.3±13.2	78.1±2.4
Glucose (mmol/l)	5.05±1.51	5.11±1.51	4.49±0.77	5.06±1.93	4.97±1.71	5.29±1.56	5.24±1.46
LDL-C (mmol/l)	2.36±0.99	2.49±1.01	1.70±0.76	2.29±0.94	2.35±1.01	2.37±0.92	2.87±0.95
HDL-C (mmol/l)	1.22±0.44	1.19±0.38	1.18±0.42	1.21±0.44	1.24±0.47	1.21±0.46	1.28±0.50
HIV+	496 (11.1)	5 (0.48)	7 (0.88)	168 (32.9)	72 (20.3)	179 (19.8)	65 (7.46)
Average common CIMT (μm)	637.9±120	679.9±121	688.1±120	608.2±97	635.7±119	616.9±112	581.9±101

Obesity defined as BMI ≥30kg/m^2 and data presented as absolute count and percentages = n (%) or as mean ± SD (standard deviation); SES, socio-economic status; MVPA, moderate-to-vigorous physical activity and low is defined as MVPA<150mins/week; CIMT, carotid intima-media thickness; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol and HIV, human immunodeficiency virus

Table 2: Mean distribution of the various adiposity measures among men and women by study site

Adiposity phenotype	All sites (N=9010)	Nanoro (n=2074)	Navrongo (n=1729)	Agincourt (n=1248)	Dikgale (n=1142)	Soweto (n=904)	Nairobi (n=1913)
Women							
BMI (kg/m^2)	25.6±6.99	20.2±3.13	22.1±3.79	29.2±6.75	30.8±7.98	–	27.6±6.08
WC (cm)	85.5±15.2	75.8±8.06	76.3±9.25	94.6±14.9	94.1±16.6	–	90.6±14.3
HC (cm)	98.2±14.4	88.6±7.57	89.1±9.43	105±12.9	109±15.5	–	102±12.1
VAT (cm)	4.94±2.00	4.38±1.16	3.51±1.08	5.74±2.19	6.83±2.25	–	4.77±1.61
SCAT (cm)	1.72±1.14	0.99±0.52	1.13±0.54	2.58±1.74	2.29±1.04	–	1.94±0.71
Men							
BMI (kg/m^2)	22.6±4.56	21.6±3.58	20.9±3.25	24.1±5.33	21.7±3.95	24.9±5.65	22.8±3.89
WC (cm)	82.4±12.5	81.4±9.83	73.2±7.19	87.5±13.5	80.3±11.3	88.9±14.8	83.4±10.7
HC (cm)	91.9±10.6	90.9±9.04	84.1±7.94	95.9±10.2	89.5±8.98	97.9±11.1	93.2±9.65
VAT (cm)	5.33±1.94	4.43±1.32	4.21±1.19	6.47±2.08	6.15±1.87	6.58±2.07	5.21±1.71
SCAT (cm)	1.16±0.89	0.95±0.49	0.77±0.37	1.71±1.79	0.94±0.49	1.55±0.92	1.14±0.54

Data expressed as mean ±SD; BMI, body mass index; WC, waist circumference; HC, hip circumference; VAT, visceral adipose tissue and SCAT, subcutaneous adipose tissue.

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Relationship between adiposity phenotypes and CIMT

Figures 1–5 present the forest plots showing the site-specific and pooled IPD-MA of the association of each adiposity phenotype with CIMT. There was an overall positive effect of BMI on CIMT in the pooled analyses and in all sites, but the effect in Navrongo was not statistically significant (Figure 1). The IPD-MA for WC (Figure 2), HC (Figure 3) and SCAT (Figure 5) also gave significant positive pooled effects. In Nanoro, VAT had an inverse association with CIMT while the pooled effect was positive (Figure 4). The pooled IPD-MA demonstrated that BMI had the highest effect measure followed by SCAT, VAT, WC and HC. These analyses demonstrated between-site heterogeneity which shows that the fixed-effect assumption (that the effect is the same at each site) is incorrect.

Figure 1: Forest plot displaying an inverse-variance weighted fixed-effect individual participant data meta-analysis of the effect of BMI (kg/m^2) on common carotid intima-media thickness (μm); the effect size (beta, β) and 95% CIs are presented by the symbol and the bars respectively; the big diamond represents the overall effect of BMI in the poled data and the grey squares represent the % weight of each study site.

Sites		Effect (95% CI)	% Weight
Nanoro		1.21 (0.70, 1.72)	10.87
Navrongo		1.11 (0.48, 1.75)	7.09
Nairobi		0.74 (0.39, 1.09)	23.08
Agincourt		0.99 (0.67, 1.32)	26.20
Digkale		1.71 (1.31, 2.11)	17.86
Soweto		2.15 (1.71, 2.58)	14.89
Overall (I-squared = 84.4%)		1.27 (1.10, 1.44)	100.00

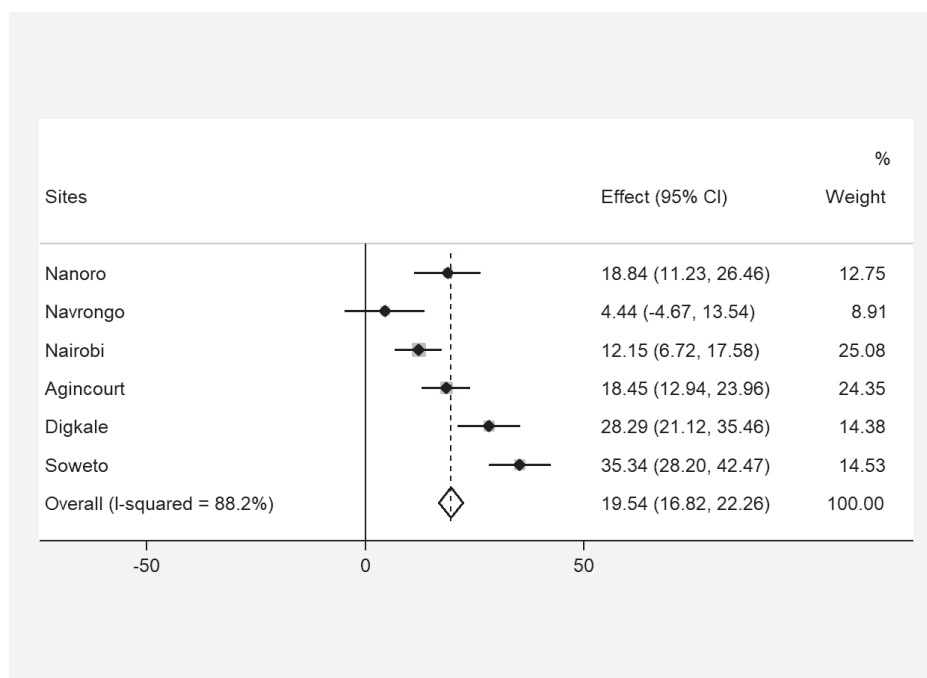**Figure 2**: Forest plot displaying an inverse-variance weighted fixed-effect individual participant data meta-analysis of the effect waist circumference (cm) on common carotid intima-media thickness in μm; the effect size (beta, β) and 95% CIs are presented by the symbol and the bars respectively; the big diamond represents the overall effect of waist circumference in the poled data and the grey squares represent the % weight of each study site.
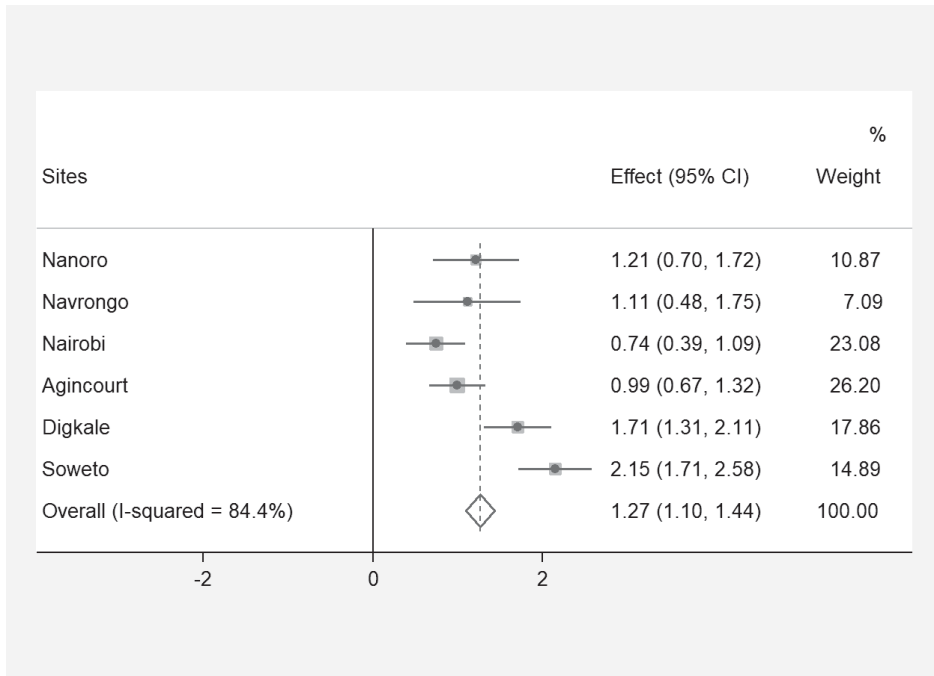
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Figure 3: Forest plot displaying an inverse-variance weighted fixed-effect individual participant data meta-analysis of the effect hip circumference (cm) on common carotid intima-media thickness in μm; the effect size (beta, β) and 95% CIs are presented by the symbol and the bars respectively; the big diamond represents the overall effect of hip circumference in the poled data and the grey squares represent the % weight of each study site.
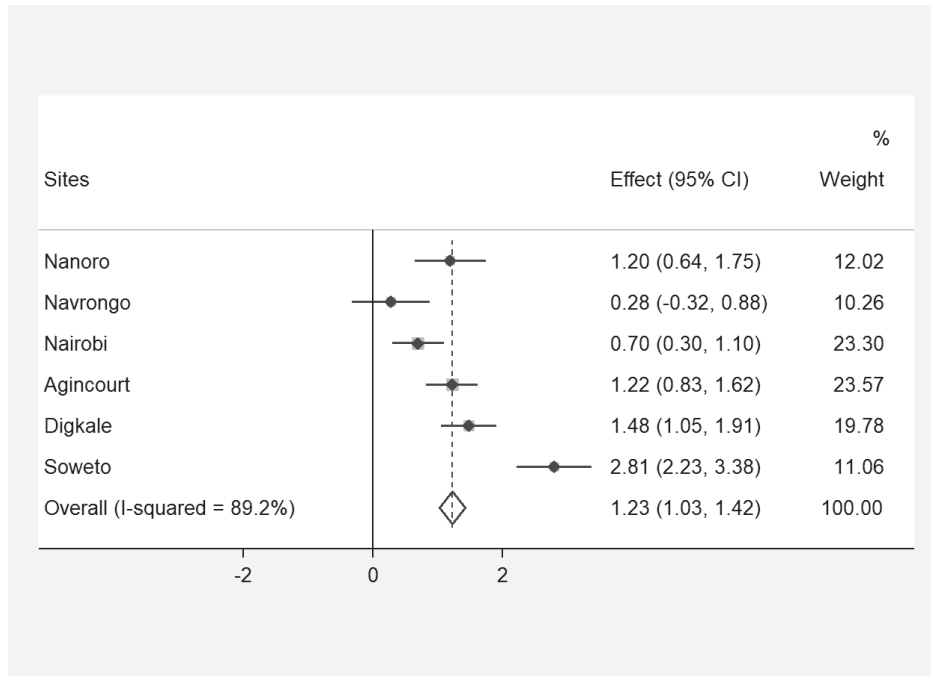
Figure 4: Forest plot displaying an inverse-variance weighted fixed-effect individual participant data meta-analysis of the effect visceral adipose tissue (cm) on common carotid intima-media thickness in μm; the effect size (beta, β) and 95% CIs are presented by the symbol and the bars respectively; the big diamond represents the overall effect of visceral adipose tissue in the pooled data and the grey squares represent the % weight of each study site.
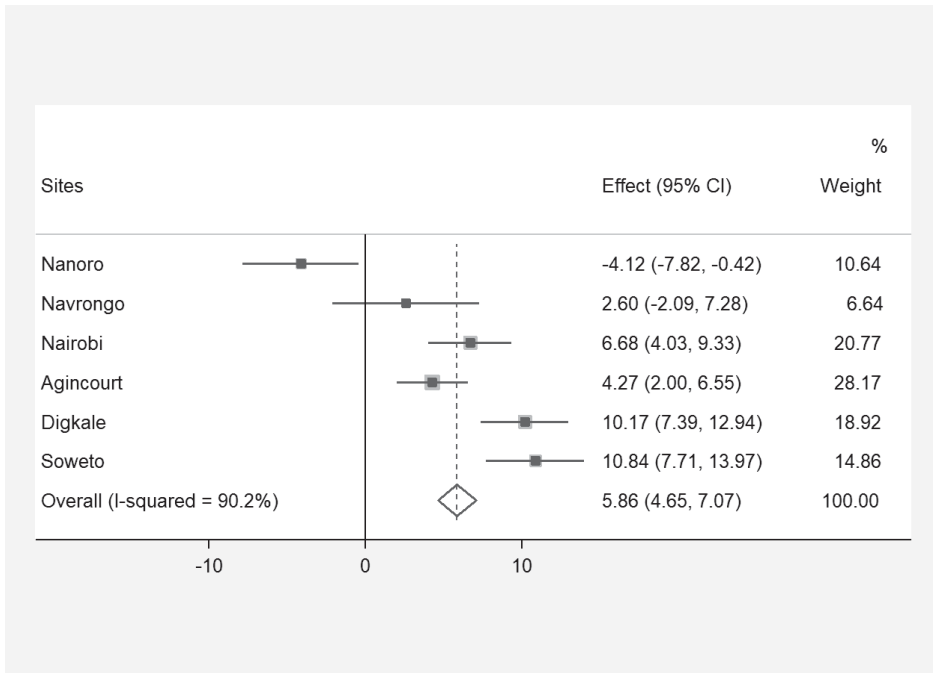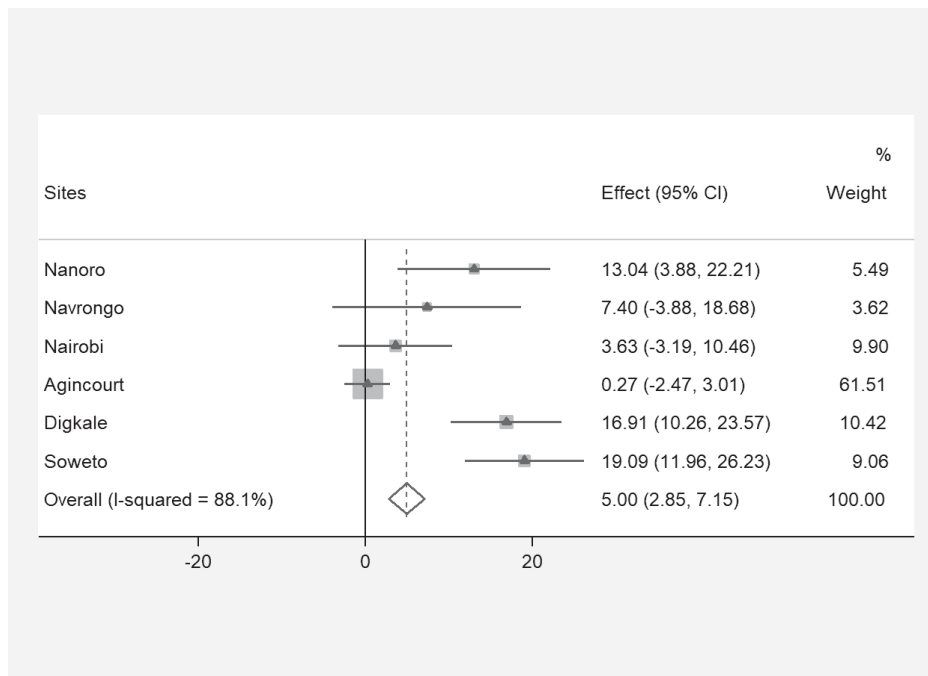
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Figure 5: Forest plot displaying an inverse-variance weighted fixed-effect individual participant data meta-analysis of the effect subcutaneous adipose tissue (cm) on common carotid intima-media thickness in μm; the effect size (beta, β) and 95% CIs are presented by the symbol and the bars respectively; the big diamond represents the overall effect of subcutaneous adipose tissue in the pooled data and the grey squares represent the % weight of each study site.

The variability in the effect-size estimates is therefore due to between-site differences rather than sampling variation. We therefore conducted separate analyses for women and men in each site and reported these results in supplementary Tables S1 and S2. The summary measures of heterogeneity and the test of overall effect are presented in supplementary Table S3.

The strength of the associations between each adiposity measures and CIMT stratified by sex varied across the sites, however the direction of the significant associations were the same for all the adiposity phenotypes except VAT and SCAT. VAT was negatively associated with CIMT at Nanoro in both women and men and negatively associated in men at the Navrongo site while SCAT was inversely associated with CIMT among women at all sites. Adjusting for covariates in the models that included all women or all men did tend to affect the strength of the associations, but it did not change the direction of the relationship or render the association significant or non-significant when compared to the initial unadjusted model. The strength of association of each

anthropometry measure with CIMT was higher in men than in women at all the study sites (supplementary Tables S1 and S2).

Discussion

In this study, we examined the association between various adiposity phenotypes and common CIMT in African populations from Burkina Faso, Ghana, Kenya and South Africa. The selected age group of 40–60 years has been identified by World Health Organization to be at risk of "premature death" from CVDs (5), indicating significant public health implications of the current findings. We standardized our estimates to enable us to compare the magnitude of the effect of the various adiposity phenotypes on CIMT by sex and the various study sites. We observed that all adiposity phenotypes were positively associated with an increase in CIMT in the pooled analyses. The associations were stronger in men than in women except for SCAT where the association was stronger in women than in men. We further observed that, after adjusting for possible confounding variables, BMI had the strongest association with CIMT followed by VAT, SCAT, WC and HC.

Our finding that various adiposity phenotypes were associated with greater CIMT in both women and men expands the evidence on this matter since it has been previously reported in European, American, African– American and Asian populations (17-19, 37, 38). In our study, BMI showed a stronger association with CIMT compared to the other measures of adiposity. This is contrary to many studies which have shown that central adiposity, particularly VAT, is a stronger determinant of CVD risk than BMI (11, 39). However, none of these studies included data collected from African populations resident in sub–Saharan Africa. A study from Malawi previously reported a stronger association of general obesity with hyperglycemia and elevated blood pressure over that of central obesity (40). In support of the results produced in our investigation, a study conducted on HIV-positive participants from Uganda demonstrated that BMI, but not waist circumference, correlated positively with CIMT (41). The attenuated effect of VAT and markers of central adiposity on CIMT in sub–Saharan African populations may be related to the lower level of VAT observed in these populations when compared to other ethnic groups (12, 13, 42, 43).

We observed that there was a positive effect of BMI on CIMT in the two West African sites which had the lowest levels of BMI but the highest CIMT levels. Biologically, obesity is known to be associated with CVD risk and our finding supports this observation. However, we noticed the magnitude of the effect of BMI on atherosclerosis was smaller in these two sites compared to the other sites that had higher obesity levels. The high CIMT in West Africa must therefore

be due to other variables that were not captured in this study as many factors are known to influence CIMT other than BMI.

The association between various measures of body adiposity and CIMT may be mediated by other CVD risk factors such as high serum cholesterol and blood pressure levels. However, after adjusting for these risk factors in multivariable regression models, the association between adiposity phenotype and CIMT was attenuated, but still remained significant. Thus, other factors must be mediating the effect of adiposity on CIMT and previous studies suggest that these may include inflammation, insulin resistance and endothelial dysfunction (44).

In our study, SCAT was positively associated with CIMT in men but inversely associated with CIMT in women, and these gender–specific associations were consistently observed at each study site. The inverse association of SCAT with CIMT observed in women is supported by other studies that have observed that SCAT may be protective against several diseases including atherosclerosis. Narumi et al (2009), who assessed atherosclerosis using the calcium score of the whole aorta, demonstrated an inverse association between SCAT and atherosclerosis in an apparently healthy Japanese population (45). Bouchi et al (2015) further observed a protective effect of SCAT on CIMT among Japanese type 2 diabetes patients (46). A study by Glesby et al (2018) in a group of HIV-positive American females showed that higher SCAT levels were associated with a lower risk of carotid artery plaques (47). The protective effect of SCAT may be due to the expression of a less pro-inflammatory adipokine profile compared to visceral fat (39, 48) and/or the ability of SCAT to act as a buffer for lipid flux thus reducing triglyceride deposition in visceral or ectopic fat depots (49). Women had higher mean SCAT levels compared to men and this may contribute to the greater protective effect observed in this gender.

In addition, abdominal SCAT is composed of two different compartments termed superficial and deep SCAT, with women having lower levels of deep but higher levels of superficial SCAT than men (50). Furthermore, a study has shown that deep SCAT is positively associated with the Framingham risk score in multivariable regression models in men but not women (51). It is uncertain whether these sex differences in abdominal SCAT compartments and their differential association with cardiovascular risk may explain the opposing effects of SCAT on CIMT observed in men and women. Levels of deep and superficial SCAT were not measured in this study and further analysis of these SCAT compartments and their association with CIMT is required in African populations. Also, the higher 95% CIs for the coefficients for SCAT and the high standard deviations for the SCAT mean values may imply some level of measurement imprecision, further affecting the outcome.

We observed that the association of each adiposity phenotype (with the exception of SCAT) with CIMT was stronger in men than women. This is supported by a study from Taiwan which showed that BMI was more strongly associated with CIMT in men than women (52). Studies have shown that sex differences exist in adipocyte function and secretory profiles (53), but whether such differences explain the differential effects of obesity on CIMT in men and women is not known. It is also possible that this sex difference may be due to differences in the component causes for increased CIMT between men and women.

At most of the study sites, VAT was positively associated with CIMT, but in men in Navrongo and both women and men in Nanoro, West Africa, these associations were in the opposite direction. The reason for this is not known. However, VAT levels at these sites were the lowest of all the sites, whilst CIMT was the highest. In addition, the majority of the participants from West Africa were subsistence farmers, whilst this was not the case in the other regions. It is therefore possible that biological, behavioural and socio-demographic differences across the sites that have not been captured in the current study may explain these variations in the association of VAT with CIMT, and require further investigation. However, it must also be noted that some of the site differences may be due to random error or differences in the population selection at the various sites.

Strengths and limitations
This study contains data on CIMT and adiposity phenotypes from a large population of black African subjects from three sub-continental regions, that is, East, West and South Africa and, to the best of our knowledge, is the largest study conducted on populations residing in SSA. Measurements of exposure and outcome variables and covariates were harmonized across the six study sites. This minimized variability and enabled us to make valid comparisons by sex and site across the study populations. Most large population-based studies use crude markers of adiposity, however, in this study we used ultrasound to assess VAT and SCAT levels. Due to the broad range of adiposity measures used in our analyses, we were able to investigate the contribution of general, central and peripheral adiposity to atherosclerosis in the study population. Furthermore, we adjusted for a wide range of confounding variables including socio–economic status, lifestyle factors and other cardiovascular risk factors, to minimize the effect of residual confounding.

There are some limitations to our study. Due to the cross–sectional study design, it is not possible to infer causality. Even though we included a wide range of possible confounding variables, our observed associations may still be affected by persistent residual confounding due to unmeasured variables that may better explain reported associations. Since most of the risk factors were

self–reported, there is the risk of recall bias and selective response leading to some biased estimates. The gold–standard methods for measurement of VAT and SCAT include CT and MRI imaging, whereas we used B–mode ultrasound measures. However, this method was the only practical way of measuring VAT, SCAT and CIMT in a large population–based study across multiple sites. Despite significant efforts instituted to reduce inter-sonographer measurement variation, we fully acknowledge that there may be some level of error obtained in the measurements in the field.

Conclusions

The results from this study suggest that the increasing prevalence of obesity within SSA will likely result in a surge in atherosclerotic cardio- and cerebrovascular events, especially in males, unless addressed through primary prevention of obesity in both rural and urban communities across Africa. The inverse association between VAT and CIMT in West Africa (Burkina Faso and Ghana) requires further investigation.

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Competing Interests
The authors have no competing interest to declare.

Author Contribution
EAN, MLB, KKG and NJC conceived the paper. EAN, ARO, GA, CCS, LKM, FM, PRB, SFM, ANW, ARO, CK, SAN, SMT, HT, MR and NJC contributed to data collection and data quality control in the H3Africa AWI–Gen study. EAN conducted the analyses and wrote the first draft with contributions from NJC, MLB and KKG. All authors critically reviewed and approved the final manuscript prior to submission.

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Table S1: Association of adiposity phenotypes with common CIMT in women aged 40–60 years from SSA stratified by site

	Nanoro, Burkina Faso Standardised β (95%CI)	Navrongo, Ghana Standardised β (95%CI)	Agincourt, South Africa Standardised β (95%CI)	Dikgale, South Africa Standardised β (95%CI)	Soweto, South Africa	Nairobi, Kenya Standardised β (95%CI)	Combined population Standardised β (95%CI)
BMI (SD=6.9kg/m²)							
Crude model	0.59 (–1.43, 2.63)	–1.17 (–3.25, 0.92)	1.77 (0.89, 2.65)	2.53 (1.54, 3.52)	–	1.34 (0.30, 2.38)	0.91 (0.41, 1.41)
Model 1	1.94 (–0.13, 4.02)	1.06 (–1.12, 3.25)	1.49 (0.63, 2.36)	2.46 (1.50, 3.41)	–	1.04 (–0.01, 2.09)	0.85 (0.35, 1.36)
Model 2	1.93 (–0.15, 4.02)	1.09 (–1.13, 3.32)	1.52 (0.66, 2.37)	2.45 (1.49, 3.41)	–	1.04 (–0.01, 2.09)	0.96 (0.45, 1.46)
Model 3	5.56 (2.76, 8.36)	0.37 (–2.59, 3.34)	1.69 (0.37, 3.03)	1.97 (0.15, 3.79)	–	1.80 (0.28, 3.33)	2.68 (1.89, 3.47)
Model 4	4.97 (2.13, 7.81)	–0.32 (–3.25, 2.60)	1.54 (0.19, 2.89)	2.08 (0.23, 3.93)	–	1.74 (0.09, 3.41)	2.30 (1.49, 3.11)
WC (SD=15.2cm)							
Crude model	0.68 (–0.12, 1.48)	0.51 (–0.33, 1.35)	0.84 (0.48, 1.21)	1.44 (0.98, 1.89)	–	0.75 (0.31, 1.21)	0.46 (0.24, 0.68)
Model 1	0.33 (0.45, 1.12)	0.98 (0.13, 1.84)	0.53 (0.17, 0.89)	1.33 (0.88, 1.78)	–	0.36 (–0.08, 0.80)	0.21 (–0.01, 0.43)
Model 2	0.36 (–0.42, 1.16)	1.04 (0.17, 1.90)	0.54 (0.18, 0.90)	1.31 (0.86, 1.76)	–	0.35 (–0.08, 0.80)	0.25 (0.03, 0.48)
Model 3	1.17 (0.16, 2.18)	2.30 (0.76, 3.85)	0.57 (–0.01, 1.14)	0.81 (0.08, 1.55)	–	0.74 (0.07, 1.41)	0.74 (0.40, 1.08)
Model 4	1.11 (0.06, 2.17)	1.18 (–0.07, 2.42)	0.48 (–0.16, 1.13)	0.97 (0.12, 1.82)	–	0.69 (–0.03, 1.41)	0.59 (0.23, 0.95)

Table S1: Association of adiposity phenotypes with common CIMT in women aged 40–60 years from SSA stratified by site (continued)

	Nanoro, Burkina Faso Standardised β (95%CI)	Navrongo, Ghana Standardised β (95%CI)	Agincourt, South Africa Standardised β (95%CI)	Dikgale, South Africa Standardised β (95%CI)	Soweto, South Africa	Nairobi, Kenya Standardised β (95%CI)	Combined population Standardised β (95%CI)
HC (SD=14.4cm)							
Crude model	-0.07 (-0.91, 0.77)	-0.79 (-1.59, 0.02)	0.78 (0.35, 1.22)	1.05 (0.50, 1.60)	-	0.58 (0.07, 1.10)	0.10 (-0.15, 0.35)
Model 1	0.43 (-0.39, 1.25)	-0.06 (-0.85, 0.73)	0.74 (0.32, 1.17)	1.22 (0.73, 1.72)	-	0.44 (-0.06, 0.93)	0.16 (-0.09, 0.41)
Model 2	0.42 (-0.40, 1.25)	-0.05 (-0.86, 0.76)	0.76 (0.34, 1.18)	1.21 (0.71, 1.72)	-	0.43 (-0.06, 0.93)	0.21 (-0.04, 0.46)
Model 3	0.92 (-0.87, 0.93)	-0.44 (-1.36, 0.49)	0.73 (0.16, 1.31)	0.62 (-0.14, 1.38)	-	0.79 (0.10, 1.48)	0.55 (0.19, 0.91)
Model 4	1.01 (-0.03, 2.05)	-0.41 (-1.32, 0.50)	0.77 (0.14, 1.40)	0.52 (-0.31, 1.35)	-	0.91 (0.16, 1.66)	0.44 (0.08, 0.79)
VAT (SD=23.9cm)							
Crude model	-6.58 (-12.4, -0.75)	5.26 (-2.14, 12.7)	4.49 (1.72, 7.26)	9.87 (6.52, 13.2)	-	7.62 (3.55, 11.7)	1.71 (0.06, 3.36)
Model 1	-7.47 (-12.9, -1.99)	6.78 (-0.38, 13.9)	4.42 (1.83, 7.02)	8.26 (5.18, 11.3)	-	6.16 (2.33, 9.99)	1.02 (-0.58, 2.62)
Model 2	-7.56 (-13.1, -2.06)	6.55 (-0.56, 13.7)	4.48 (1.86, 7.11)	8.27 (5.17, 11.4)	-	6.23 (2.40, 10.1)	1.18 (-0.42, 2.78)
Model 3	-7.57 (-13.1, -2.05)	7.74 (-0.34, 15.8)	4.45 (1.76, 7.13)	7.04 (2.79, 11.3)	-	8.00 (3.94, 12.07)	1.82 (0.19, 3.44)
Model 4	-7.59 (-13.2, -2.01)	5.34 (-2.66, 13.3)	3.02 (0.19, 5.85)	7.01 (2.78, 11.2)	-	5.72 (1.51, 9.94)	2.08 (0.37, 1.65)
SCAT (SD=7.5cm)							
Crude model	4.61 (-15.6, 10.4)	-2.38 (-16.4, 11.6)	-0.49 (-3.49, 2.95)	10.4 (2.86, 17.9)	-	-2.39 (-11.0, 6.25)	-2.82 (-5.59, -0.05)
Model 1	-2.59 (-5.19, 3.83)	-2.67 (-11.3, 16.7)	-2.51 (-5.85, -0.84)	13.7 (6.92, 20.55)	-	-3.69 (-12.1, 4.74)	-4.49 (-7.25, -1.74)
Model 2	-2.49 (-15.5, 10.5)	-2.32 (-11.9, 16.6)	-2.52 (-5.90, 0.86)	13.7 (6.86, 20.5)	-	-3.71 (-12.2, 4.77)	-4.13 (-6.91, -1.35)
Model 3	-25.6 (-33.3, 2.07)	-17.6 (-37.7, 2.40)	-3.19 (-8.05, 1.67)	-2.61 (-14.6, 9.35)	-	-21.8 (-34.3, -9.33)	-4.42 (-7.27, -1.57)
Model 4	-6.58 (-20, 7.29)	-12.8 (-29.3, 3.73)	-0.09 (-3.99, 3.80)	-1.25 (-10.6, 8.13)	-	-15.4 (-24.5, -6.32)	-3.94 (-7.03, -0.84)

Model 1: age, educational status and household SES; **Model 2:** Model 1 + alcohol, smoking and physical activity and **Model 3:** model 2 + other adiposity measures except those that were collinear. **Model 4:** Model 3 + glucose, HDL-C, LDL-C, SBP and HIV

Table S2: Association of adiposity phenotypes with CIMT in men aged 40–60 years from SSA stratified by site

	Nanoro, Burkina Faso	Navrongo, Ghana	Agincourt, South Africa	Dikgale, South Africa	Soweto, South Africa	Nairobi, Kenya	Combined population
	Standardised β (95%CI)	Standardised β (95%CI)	Standardised β (95%CI)	Standardised β (95%CI)	Standardised β (95%CI)	Standardised β (95%CI)	Standardised β (95%CI)
BMI (SD=4.6kg/m²)							
Crude model	3.15 (1.04, 5.25)	-0.39 (-3.10, 2.32)	4.79 (2.90, 6.69)	9.78 (6.24, 13.31)	6.86 (5.48, 8.24)	4.94 (3.17, 6.72)	4.33 (3.51, 5.15)
Model 1	6.54 (4.46, 8.63)	2.64 (-0.30, 5.59)	4.59 (2.82, 6.35)	9.24 (5.88, 12.59)	6.06 (4.79, 7.33)	4.65 (2.94, 6.37)	4.29 (3.50, 5.09)
Model 2	6.17 (4.05, 8.29)	2.82 (-0.19, 5.83)	4.79 (2.95, 6.63)	9.18 (5.79, 12.56)	6.30 (4.99, 7.61)	4.64 (2.89, 6.38)	4.49 (3.68, 5.31)
Model 3	6.62 (3.19, 10.1)	2.06 (-1.24, 5.38)	3.22 (6.15, 6.28)	9.92 (4.17, 17.68)	6.01 (2.83, 9.09)	5.54 (2.34, 8.74)	5.44 (4.26, 6.61)
Model 4	6.60 (3.32, 9.88)	2.05 (-0.98, 5.09)	3.41 (0.91, 5.91)	8.69 (3.38, 14.00)	7.16 (4.92, 9.41)	4.87 (2.03, 7.71)	4.86 (3.64, 6.09)
WC (SD=12.5cm)							
Crude model	1.38 (0.60, 2.16)	0.99 (-0.27, 2.25)	2.13 (1.41, 2.86)	4.10 (2.81, 5.39)	2.71 (2.19, 3.22)	1.71 (1.08, 2.35)	1.61 (1.31, 1.90)
Model 1	1.98 (1.23, 2.73)	1.47 (0.22, 2.72)	1.80 (1.09, 2.52)	3.52 (2.31, 4.73)	2.18 (1.70, 2.67)	1.48 (0.89, 2.08)	1.30 (1.01, 1.59)
Model 2	1.87 (1.12, 2.61)	1.54 (0.28, 2.81)	1.86 (1.13, 2.59)	3.57 (2.35, 4.79)	2.26 (1.77, 2.76)	1.46 (0.86, 2.06)	1.37 (1.07, 1.67)
Model 3	2.34 (1.26, 3.42)	1.57 (0.05, 3.10)	2.08 (1.06, 3.11)	4.72 (2.07, 7.38)	2.33 (1.61, 3.06)	1.27 (0.29, 2.24)	1.62 (1.19, 2.04)
Model 4	2.16 (0.92, 3.39)	1.64 (0.11, 2.79)	1.61 (0.43, 2.79)	3.56 (1.76, 5.36)	2.53 (1.65, 3.41)	1.27 (0.15, 2.39)	1.38 (0.91, 1.84)
HC (SD=10.6cm)							
Crude model	0.88 (0.01, 1.74)	-0.29 (-1.31, 0.73)	2.41 (1.46, 3.36)	3.46 (1.96, 4.95)	3.35 (2.69, 4.02)	1.23 (0.31, 2.16)	1.26 (0.88, 1.63)
Model 1	1.85 (0.90, 2.81)	0.86 (-0.19, 1.92)	2.19 (1.31, 3.09)	3.29 (1.86, 4.73)	2.91 (2.29, 3.52)	1.17 (0.18, 2.15)	1.22 (0.85, 1.59)
Model 2	1.70 (0.74, 2.67)	0.94 (-0.12, 2.01)	2.25 (1.35, 3.14)	3.22 (1.80, 4.65)	3.01 (2.38, 3.64)	1.12 (0.14, 2.12)	1.27 (0.89, 1.65)
Model 3	1.43 (0.34, 2.52)	0.72 (-0.36, 1.80)	2.16 (1.13, 3.19)	2.53 (0.87, 4.18)	2.70 (1.89, 3.51)	0.52 (-0.73, 1.77)	0.97 (0.49, 1.45)
Model 4	1.55 (0.40, 2.70)	0.56 (-0.54, 1.67)	1.62 (0.42, 2.81)	1.89 (0.07, 3.72)	2.71 (1.75, 3.67)	0.36 (-0.94, 1.67)	0.75 (0.25, 1.24)

Table S2: Association of adiposity phenotypes with CIMT in men aged 40–60 years from SSA stratified by site (continued)

	Nanoro, Burkina Faso Standardised β (95%CI)	Navrongo, Ghana Standardised β (95%CI)	Agincourt, South Africa Standardised β (95%CI)	Dikgale, South Africa Standardised β (95%CI)	Soweto, South Africa Standardised β (95%CI)	Nairobi, Kenya Standardised β (95%CI)	Combined population Standardised β (95%CI)
VAT (SD=24.6cm)							
Crude model	−0.61(−6.69, 5.48)	−3.19(−10.4, 4.01)	3.06 (−1.79, 7.91)	14.7(7.37, 21.9)	13.3 (9.41, 17.2)	8.09(4.32, 11.9)	4.72 (2.73, 6.71)
Model 1	−3.10(−8.59, 2.39)	−1.48 (−8.23, 5.27)	3.69 (−0.97, 8.36)	14.7 (7.99, 21.4)	10.6 (6.87, 14.3)	7.26 (3.68, 10.8)	3.99 (2.09, 5.90)
Model 2	−2.15(−7.61, 3.31)	−1.77(−8.52, 4.99)	3.59 (−1.05, 8.24)	14.4 (7.81, 21.0)	10.9 (7.16, 14.8)	7.34(3.77, 10.9)	4.09 (2.19, 5.98)
Model 3	−5.21(−11.1, 0.59)	−3.40 (−105, 3.69)	5.15(0.62, 9.67)	12.9 (5.71, 20.2)	9.19(5.04, 13.3)	6.30(2.53, 10.1)	3.09 (1.12, 5.07)
Model 4	−4.93 (−11.1, 1.26)	−4.76 (−11.8, 2.28)	1.01 (−4.13, 5.93)	11.0 (3.89, 18.2)	6.83 (2.66, 10.9)	4.16 (0.09, 8.22)	3.98 (0.16, 4.07)
SCAT (SD=5.9cm)							
Crude model	25.1(10.4, 40.5)	19.7(0.26, 39.1)	4.85(−0.67, 10.4)	45.8(19.3, 72.4)	24.8(15.9, 33.7)	20.1(7.34, 32.9)	8.90 (4.59, 13.2)
Model 1	26.8(12.1, 41.6)	16.8(−2.42, 35.9)	3.83(−1.25, 8.90)	37.6(12.2, 63.0)	19.1(10.8, 27.5)	17.7(4.85, 30.5)	6.29 (2.34, 10.2)
Model 2	26.8 (12.1, 41.6)	18.2 (−0.79, 37.3)	3.64 (−1.41, 8.69)	35.9 (11.2, 60.6)	19.3 (10.6, 28.0)	17.7 (4.82, 30.6)	6.46 (2.47, 10.5)
Model 3	31.2(15.9, 46.6)	21.7 (1.92, 41.6)	10.6(3.76, 17.5)	12.4 (−14.9, 39.6)	11.5(2.29, 20.6)	11.9 (−1.71, 25.4)	9.78 (5.08, 14.5)
Model 4	14.8 (−3.80, 33.4)	10.9 (−10.2, 32.1)	7.66 (−0.45, 15.8)	0.54 (−26.6, 27.7)	9.70 (0.96, 18.4)	8.89 (−5.71, 23.5)	6.24 (1.14, 11.3)

Model 1: age, educational status and household SES; **Model 2:** Model 1 + alcohol, smoking and physical activity and **Model 3:** model 2 + other adiposity measures except those that were collinear. **Model 4:** Model 3 + glucose, HDL-C, LDL-C, SBP and HIV

Table S3: Output of meta-analysis pooling of main effect estimates of adiposity phenotype on CIMT using the fixed-effect inverse-variance model with heterogeneity measures

Sites	BMI in kg/m	WC in cm	HC in cm	VAT in cm	SCAT in cm
Nanoro	18.8 (11.3, 26.5)	1.21 (0.70, 1.72)	1.19 (0.64, 1.75)	-4.12 (-7.82, -0.42)	13.0 (3.88, 22.2)
Navrongo	4.44 (-4.67, 13.55)	1.11 (0.48, 1.75)	0.28 (-0.32, 0.88)	2.59 (-2.09, 7.29)	7.40 (-3.88, 18.6)
Nairobi	12.1 (6.72, 17.6)	0.74 (0.39, 1.09)	0.69 (0.30, 1.09)	6.68 (4.03, 9.33)	3.63 (-3.19, 10.5)
Agincourt	18.5 (12.9, 23.9)	0.99 (0.67, 1.32)	1.22 (0.83, 1.62)	4.27 (1.99, 6.55)	0.27 (-2.47, 3.01)
Dikgale	28.3 (21.1, 35.5)	1.71 (1.31, 2.11)	1.48 (1.05, 1.92)	10.2 (7.39, 12.9)	16.9 (10.3, 23.6)
Soweto	35.4 (28.2, 42.5)	2.15 (1.71, 2.58)	2.81 (2.23, 3.39)	10.8 (7.71, 13.9)	19.1 (11.9, 26.2)
Overall effect	19.5 (16.8, 22.3)	1.27 (1.09, 1.44)	1.23 (1.04, 1.42)	5.86 (4.65, 7.07)	5.00 (2.85, 7.15)
Test of overall effect	z = 14.09, p<0.001	z = 14.78 p<0.001	z = 12.49 p<0.001	z = 9.512 p<0.001	z = 4.564 p<0.001
Heterogeneity measures					
I^2	88.2%	84.4%	89.2%	90.2%	88.1%
Modified H^2	7.48	5.42	8.25	9.19	7.41
Tau^2	88.7	0.25	0.49	21.7	75.6

BMI, body mass index; WC, waist circumference; HC, hip circumference; VAT, visceral adipose tissue and SCAT, subcutaneous adipose tissue; I^2 = between-study variance (tau²) as a percentage of total variance; Modified H^2 = ratio of tau² to typical within-study variance

chapter 3

Chapter 4

Race-ethnic differences in the association between classical cardiovascular risk factors and common carotid intima-media thickness: an individual participant data meta-analysis

Nonterah EA, Crowther NJ, Klipstein-Grobusch K, Oduro AR, Kavousi M, Agongo G, Anderson TJ, Asiki G, Boua PR, Choma SSR, Couper DJ, Engström G, de Graaf J, Kauhanen J, Lonn EM, Mathiessen EB, Micklesfield LK, Okazaki S, Polak JF, Rundek T, Salonen JT, Tollman SM, Tuomainen T-P, Grobbee DE, Ramsay M, and Bots ML, for the H3Africa AWI-Gen and USE-IMT collaborative study group.

Abstract

Background: The major risk factors for atherosclerotic cardiovascular disease (CVD) differ by race-ethnicity, but have largely been defined using populations of European ancestry. Despite the rising prevalence of CVD in Africa there is little related data from African populations. Therefore, we compared the association of established cardiovascular risk factors with carotid-intima media thickness (CIMT), a subclinical marker of atherosclerosis, between African, African American, Asian, European and Hispanic populations.

Methods: Cross sectional analyses of 34,025 men and women drawn from 15 cohorts in Africa, Asia, Europe, and North America was undertaken. Classical cardiovascular risk factors were assessed and CIMT measured using B-mode ultrasound. Ethnic differences in the association of established cardiovascular risk factors with CIMT were determined using a two-stage individual participant data meta-analysis (IPD-MA) with beta coefficients expressed as a percentage using the White population as the reference group.

Results: CIMT adjusted for risk factors was the greatest among African Americans followed by Asian, European, and Hispanic populations with Africans having the lowest mean CIMT. In all race-ethnic groups, men had higher CIMT levels compared to women. Age, sex, body mass index (BMI) and systolic blood pressure (SBP) had a significant positive association with CIMT in all ethnicities at varying magnitudes. When compared to European populations, the association of age, sex and SBP with CIMT was weaker in all ethnicities. Smoking (beta coefficient [95% CI]: 0.39 [0.09, 0.70]), BMI (0.05 [0.01, 0.08]) and glucose (0.13 [0.06, 0.19]) had the strongest positive association with CIMT in the Asian population when compared to all other ethnic groups. High density lipoprotein-cholesterol (HDL-C) had significant protective effects in African American (-0.31 [-0.42, -0.21]) and African (-0.26 [-0.31, -0.19]) populations only.

Conclusion: The strength of association between established cardiovascular risk factors and CIMT differed across the race-ethnic groups and may be due to lifestyle risk factors and genetics. These differences have implications for race-ethnic specific primary prevention strategies and also give insights into the differential contribution of risk factors to the aetiology of CVD. The greatest burden of subclinical atherosclerosis in African Americans warrants further investigations.

Key words: Race; ethnicity; carotid intima-media thickness; atherosclerosis; cardiovascular disease risk; individual participant data meta-analysis

Introduction

Cardiovascular disease (CVD) is the primary cause of deaths globally (1, 2). Differences in the prevalence of the classical risk factors between ethnic groups have been reported to account for the differences in CVD burden (3). Previous studies examining ethnic differences in CVD events have been largely conducted outside of the African region mostly in migrant populations residing in high income countries (4-6).

The INTERHEART and PURE studies (7, 8) are the only studies that have included Africans residing in Africa. These studies demonstrated that the risk of myocardial infarction due to smoking, BMI, blood pressure and lipids did not differ by geographical location or ethnicity. The INTERHEART study included 578 Africans drawn from Mozambique, Nigeria, South *Africa*, Sudan, and Uganda while the PURE study included study participants from Zimbabwe and South Africa. The paucity of data from African countries is problematic as the prevalence of CVDs has been reported to be increasing across the continent (2, 8). In terms of primary prevention it is important to identify risk factors and the strength of their association with cardiovascular events or measures of atherosclerosis, and whether differences exist between ethnic groups for these associations.

Carotid intima-media thickness (CIMT) is a proxy for subclinical atherosclerosis, and is related to CVD risk (9). With regard to African-ancestry participants, most studies on CIMT have been conducted in African American populations (4, 6) with one in Africans residing in Africa (10). The recent USE-IMT analyses of ethnic differences in the association between cardiovascular risk factors and CIMT used African Americans as a proxy for endemic African populations (6).

The current study uses individual participant data meta-analyses (IPD-MA) utilizing data from the USE-IMT project (11) combined with the large pan-African Africa-Wits-INDEPTH Genomic (AWI-Gen) study (12), a Human Hereditary and Health in Africa (H3Africa) Collaborative Center, to investigate the association of classical cardiovascular risk factors with CIMT with the inclusion of a large pan-African cohort. In this study we determined the differences in the association of classical cardiovascular risk factors with CIMT among Africans residing in Africa and African American, Asian, Hispanic and European populations.

Methods

Study population

Africans participants were recruited for H3Africa AWI-Gen study, a population-based cross-sectional study investigating genomic and environmental risks factors for cardiometabolic diseases across six African sites including in Burkina Faso and Ghana (West Africa); Kenya (East Africa) and South Africa (12). The six cohorts were drawn from five Health and Socio-Demographic Surveillance Sites (HDSS) of the International Network for Demographic Evaluation of Populations and their Health (INDEPTH) and adults of the same age from Soweto, South Africa. We included all 6 cohorts from the AWI-Gen study composed of adults aged 40-60 years old. The USE-IMT project is an individual participant data meta-analysis (IPD-MA) as described in detail elsewhere (11). The USE-IMT comprised of adult men and women recruited for 18 cohorts from various countries in Europe, Asia and North America.

In order to adequately compare the USE-IMT cohorts with the AWI-Gen cohorts, we applied an age restriction. We excluded 9 cohorts from the 18 cohorts contributing to USE-IMT based on participant's age less than 40 years or greater than 60 years. Further exclusions were based on missing data for biomarkers: glucose, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). The final dataset included in this IPD-MA consisted of 34,025 adults aged 40-60 years drawn from 15 cohorts in 11 countries.

The following cohorts contributed the recruited participants of European ancestry: FATE, Firefighters and Their Endothelium Study in Canada (13); ARIC, Atherosclerosis Risk in Communities study (14); MESA, Multi-Ethnic Study of Atherosclerosis (15) and NOMAS, Northern Manhattan (16) Studies all from the USA; KIHD, Kuopio Ischemic Heart Disease Risk Factor Study in Finland (17); Malmö, Malmö Diet and Cancer Study in Sweden (18); Tromsø Study in Norway (19) and the NBS, Nijmegen Biomedical Study in the Netherlands (20). African Americans and Hispanics were recruited from the ARIC, MESA and NOMAS studies in USA while participants from the Asian ethnic group were recruited from the OSACA2, Osaka Follow-up Study for Carotid Atherosclerosis study in Japan (21) and migrant Asians (Chinese) from the NBS, ARIC and MESA studies from the USA (supplementary Table S1). Analyses were limited to participants with data on carotid intima-media thickness (CIMT) and the following established cardiovascular risk factors: age, sex, smoking, alcohol, physical activity, body mass index, blood pressure, fasting glucose, LDL-C and HDL-C.

Race and ethnicity were self-identified by the participants of the various cohorts. Most cohorts adopted separate questions about ethnicity (Hispanic/

Latino ethnicity) and race (white/black) since these are distinct concepts (22). In addition, some studies collected information on country of origin among all races and language spoken at home to help classify multi-racial individuals who may only identify with a single group (23). We used the sociocultural context of race and ethnicity variables to assign sufficient attribution to explanatory biological factors (and based on the literature), and reported on the relationships among pre-specified race-ethnic groups according to recent recommendations for use of race and ethnicity in research (24). White race-ethnic group refers to participant of European ancestry while black race-ethnic group are participants of African ancestry classified as Africans residing in Africa and African Americans referring to Africans in the diaspora.

Data harmonization
Several steps were taken to harmonize all extracted data for the current analyses. Data included were cross-sectional baseline data from the various cohorts thus offsetting differences in study designs. We obtained the crude individual level data (for each cohort) on all variables giving us the opportunity to standardize units of measurements of the variables. Weight was measured in kilogram in all cohorts while height was measured in cm in the USE-IMT and mm in the AWI-Gen study and was converted to meters. Subsequent body mass index (BMI) was calculated as weight divided by height in meters squared. We converted all serum biochemical marker data (glucose, LDL-C and HDL-C) into mmol/L. Blood pressure was measured as systolic and diastolic pressures in mmHg. Alcohol intake in the USE-IMT was reported as the number of beverages consumed per week while in the AWI-Gen study it was reported as never consumed; current non-problematic consumer; current problematic consumer; former consumer (12). We thus recoded the merged variable into two mutually exclusive variables as 0 "never consumed" and 1 "ever consumed" (i.e. reporting consumption of at least one beverage per week or current non-problematic consumer; current problematic consumer or former consumer). Smoking was coded as 0, never smoked and 1, current or former smoker. Physical activity was measured differently within the various study cohorts. Some studies used questionnaires to assess leisure activity and moderate-to-vigorous physical activity (MVPA) in some detail, whilst others asked whether subjects engaged in any form of physical activity, the type and duration per day or week. Those who reported not engaging in any form of physical activity or lower than 150mins of MVPA per week were coded as 0 (no form of physical activity) and those who reported ≥150mins of MVPA per week or engaging in activities that require physical exertion (e.g. walking, cycling) or some form of physical activity for at least 4 hours per week or several times per week were categorized as 1 (some form of physical activity).

Common carotid artery intima-media thickness

Images of CIMT obtained varied across cohorts and from two to six. Some cohorts measured one angle and others multiple angles. The outcome variable, CIMT was thus computed as an average of all available angles (from the number of angles; from the far wall; and from one or both sides of the neck) of the common CIMT measurements from each included cohort. All common CIMT values were used in the analysis, including values larger than 1.5mm, which are suggestive of plaque. The same protocol was used for the six cohorts in the H3Africa AWI-Gen study limiting variability in measurements while different protocols were used by the various USE-IMT cohorts. As absolute differences in common CIMT levels between AWI-Gen and the USE-IMT cohorts could arise from differences in methodology employed in obtaining measurements, we harmonized the mean common CIMT by computing cohort-specific standard z-scores using the formula, $z = (x–\mu)/\sigma$, where x is the crude participant CIMT, μ is the populations mean CIMT, and σ is the population standard deviation.

Statistical analyses

Participants were stratified by race-ethnic background and by cohorts and their characteristics were summarized using counts and frequencies for categorical data and means with standard deviation (±SD) for normally distributed continuous data. We used mixed effect multilevel (ML) linear regression analyses to compute and compare mean (with 95% confidence intervals) CIMT between the different race-ethnicities while adjusting for the classical CVD risk factors. We further compared mean differences between African Americans, West, East and South Africans. We also conducted similar sub-group analyses for populations of Asian and European ancestry. Adjustments were done in order to determine whether any variations in ethnic differences were due to differential patterning of CVD risk factors by ethnicity. Due to significant sex-ethnicity interactions in the combined sample, we presented mean differences in CIMT for the combined sample and for women and men separately.

To determine the association between the established cardiovascular risk factors and common CIMT z-score, we conducted a two-staged IPD-MA. In the first stage, we conducted a mixed-effect multilevel regression with a random-effects approach for each original cohort. We adjusted for all the established CVD risk factors separately for each ethnic group. For the AWI-Gen cohorts, we also adjusted for HIV as its prevalence was high in East and South African cohorts and it is established to contribute to the risk of CVD (25). In the second stage, a fixed-effect inverse-variance pooled IPD-MA approach was used to determine ethnic differences in CVD risk factors associated with common CIMT z-score. The meta-analysis pooling of the main effects on mean CIMT of each risk factor enabled us to obtain the associations of each risk factor with CIMT

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across the ethnic groups. The IPD was conducted for each established classical CVD risk factor and in each analysis adjustments were made for the other established classical CVD risk factors. We further conducted pooled analyses for men and women separately due to sex*ethnicity interactions. For Africans in the AWI-Gen cohorts we tested for the significance of an HIV*sex interaction. This is because the epidemiology of HIV in SSA differs between women and men. There was no sex*HIV interaction, but sex-ethnicity interaction terms were significant. Due to similarities in the effect sizes of the risk factors with raw mean CIMT and CIMT z-scores, we reported the associated differences in CIMT in mm.

To further quantify the differences in the association of each risk factor with common CIMT according to ethnicity, we conducted a mixed-effect ML regression with a random-effects approach for each original cohort. We then extended this model by adding the interaction terms for ethnicity with each CVD risk factor. Beta coefficients (with 95% confidence intervals) for each risk factor in each ethnic group were obtained with populations of European ancestry as the reference group in all analyses. From the obtained effect measure (beta value) for each race-ethnic group we converted the beta value to a percentage, again using the European race-ethnic group as a reference and for whom the beta coefficient was set at 100%. This enabled us to observe the difference in the magnitude of the association of the risk factors with CIMT for each ethnic group compared to the European race-ethnic group. The established classical cardiovascular risk factors that were adjusted for in all the inferential analyses were: age, sex, smoking, alcohol intake, body mass index (BMI), SBP, glucose, LDL-C and HDL-C. We used LDL-C (more atherogenic lipoprotein) in preference to total cholesterol due the universal use of LDL-C in treatment guidelines for CVD prevention (26-28). We further analysed whether LDL-C/HDL-C ratio was more important than LDL-C and HDL-C in predicting CIMT. We first included all three markers in the same model and checked for significant collinearity using the variance inflation factor. We then compared the variance (adjusted R^2) of the model that included both HDL-C and LDL-C with that of the model that included the ratio only. A two-sided *p<0.05* is considered statistically significant in all inferential analyses. All statistical analyses were conducted in STATA v14.2 (StataCorp LP, Texas USA).

Ethical approval
The AWI-Gen study received ethics approval from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa (initial approval number: M121029 and renewed approval number: M170880) and cohort specific ethical approvals were also obtained. The USE-IMT study received ethics approval from the institutional review committee of the University Medical Centre Utrecht, Utrecht, the Netherlands. The individual U.S. studies received

ethical approval from their respective ethics committees or institutional review boards. All participants within each individual study provided informed consent prior to recruitment. Approval for use of the data included in these analyses was provided by the principal investigators of the H3Africa AWI-Gen and USE-IMT through a signed data transfer agreement.

Data availability and access
Data for these analyses are made up of baseline data from the USE-IMT initiative and the H3Africa AWI-Gen studies. AWI-Gen data can be accessed from the European Genome-phenome Archive (www.ega459archive.org/datasets/EGAD00001006425). The phenotype dataset accession IDs is EGAD00010001996. The procedure to obtain access to the USE-IMT data goes through contacting the principal investigator (M.L.B.). The PI will then contact the PIs of the individual cohorts to ask for access through cohort specific existing procedures.

Results

Basic characteristics of cohorts by race-ethnic groups
The study consisted of 15 cohorts drawn from Africa, Asia, Europe and North America. Baseline characteristics of the included cohorts are presented in supplementary Table S1 while baseline characteristics of participants by ethnicity are presented in Table 1. A total of 34,025 individuals with a mean±SD age of 52±5 years and crude CIMT of 0.69±0.14 mm were studied. Hispanics were older (54±5years) than other ethnic groups with Africans having the lowest mean age (50±6years). An approximate equal number of women and men were included in this meta-analysis. More men were smokers and current alcohol consumers than women across all ethnic groups. Africans (30%) and African Americans (29%) had the highest smoking rates with Asians (15%) having the lowest rates. African Americans and Hispanics had the highest mean BMI while Africans and Asians reported the lowest levels. African women had higher mean BMI than their male counterparts while the opposite was observed in all other race-ethnic groups. Africans generally had lower levels of fasting glucose, HDL-C and LDL-C compared to all other ethnicities. African Americans had the highest glucose and HDL levels while Europeans had the highest LDL-C levels (Table 1).

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Table 1: Baseline characteristics of USE-IMT and AWI-Gen collaborative study participants according to race-ethnic groups

Factors	Asian	African	African American	European	Hispanic	All
Combined population						
N	573	9428	4182	18817	980	34025
Age, years	53±5	50±6	52±5	53±5	54±5	52±5
Smoking. %	15.3	29.5	28.9	26.6	19.3	27.2
Alcohol, %	88.7	93.3	38.8	95.3	70.5	78.3
Physical activity, %	83.1	94.9	88.9	91.3	74.2	90.3
HIV+ART, %	-	12.2	-	-	-	-
BMI, kg/m²	24.2±3.3	24.1±1.07	29.7±4.56	26.7±4.5	29.6±5.3	26.4±5.7
SBP, mmHg	123±20	124±22	127±20	125±19	123±20	125±20
DBP, mmHg	77±12	79±13	79±12	77±12	75±11	78±12
Glucose, mmol/l	5.52±1.73	5.06±1.62	6.21±2.88	5.37±1.47	5.4±2.1	5.39±1.8
HDL, mmol/l	1.33±0.72	1.18±0.41	1.40±0.44	1.35±0.41	1.21±0.33	1.30±0.43
LDL, mmol/l	3.03±0.75	2.30±0.99	3.41±1.08	3.80±1.12	3.2±0.86	3.28±0.14
Common CIMT in mm	0.72±0.22	0.64±0.12	0.70±0.15	0.69±0.15	0.68±0.12	0.68±0.14
Women						
N (%)	282 (49)	4745 (50)	2548 (61)	8947 (48)	534 (54)	17055 (50)
Age, years	53 ±5	50 ±6	52 ±5	53 ±5	54 ±5	52 ±5
Smoking,%	6.2	3.9	24.8	26.2	16.7	19.1
Alcohol,%	87.9	29.8	93.0	94.0	64.0	75.0
Physical activity,%	81.9	91.4	94.7	91.7	71.8	91.2
HIV+ART,%	-	13.2	-	-	-	-
BMI, kg/m²	23.7 ±3.3	25.5 ±6.9	30.8 ±6.4	26.1 ±5.1	30.3 ±5.9	26.7 ±6.0

Factors	Asian	African	African American	European	Hispanic	All
Combined population						
SBP, mmHg	121±22	122±23	127±21	123±20	122±20	123±21
DBP, mmHg	77 ±11	78 ±13	77 ±11	74 ±12	73 ±11	76 ±12
Glucose, mmol/l	5.33 ±1.56	5.08 ±1.74	6.28 ±3.10	5.29 ±1.47	5.30 ±2.02	5.41 ±1.92
HDL, mmol/l	1.45 ±0.40	1.15 ±0.38	1.49 ±0.45	1.53 ±0.42	1.31 ±0.35	1.44 ±0.45
LDL, mmol/l	3.03±0.74	2.27 ±0.98	3.42 ±1.09	3.75 ±1.16	3.15±0.86	3.28 ±1.23
Common CIMT in mm	0.68 ±0.20	0.64 ±0.12	0.69 ±0.16	0.66 ±0.13	0.66 ±0.11	0.66 ±0.13
Men						
N (%)	291 (51)	4683 (50)	1634 (39)	9870 (52)	446 (46)	16924 (50)
Age, years	53 ±5	50 ±6	52 ±5	53 ±5	53 ±5	52 ±5
Smoking,%	23.9	54.3	36.7	27.0	22.4	35.3
Alcohol,%	83.6	45.8	93.7	96.5	78.3	81.6
Physical activity,%	84.5	86.4	95.2	91.0	77.1	89.5
HIV+ART, %	–	11.0	–	–	–	–
BMI, kg/m^2	24.7 ±3.1	22.6 ±4.6	27.9 ±4.7	27.2 ±3.8	28.7 ±4.5	26.2 ±4.5
SBP, mmHg	125 ±19	126 ±21	128 ±20	127 ±19	123 ±19	127 ±19
DBP, mmHg	80 ±11	80 ±13	82 ±12	80 ±12	77 ±11	80 ±12
Glucose, mmol/l	5.70 ±1.86	5.03 ±1.50	6.11 ±2.50	5.43 ±1.47	5.62 ±2.17	5.43 ±1.69
HDL, mmol/l	1.23 ±0.33	1.21 ±0.44	1.27 ±0.41	1.20 ±0.33	1.09 ±0.28	1.23 ±0.37
LDL, mmol/l	3.13 ±0.75	2.34 ±0.99	3.41 ±1.07	3.86 ±1.08	3.15 ±0.86	3.40 ±1.20
Common CIMT in mm	0.76 ±0.23	0.64 ±0.12	0.73 ±0.15	0.72 ±0.15	0.69±0.13	0.71 ±0.15

Data presented as percentages (%) or mean ± standard deviation (SD); ART, antiretroviral therapy; BMI, body mass index; CIMT, carotid intima-media thickness; DBP, diastolic blood pressure; HDL, high density lipoprotein cholesterol; HIV, human immune deficiency virus and LDL, low density lipoprotein cholesterol

Mean CIMT levels across race-ethnic groups

The adjusted CIMT levels for each race-ethnic group as total, men and women separately are presented in Figure 1. In the combined populations, age, sex, smoking, alcohol, physical activity, glucose, HDL-C and LDL-C were adjusted sequentially (model 1, unadjusted; model 2, adjusted for age, sex and sex*ethnicity interaction; and model 3, model 2 plus the classical cardiovascular risk factors) to see the differential effect of behavioral and metabolic risk factors on mean common CIMT levels across the ethnicities. The results of these sequential models are presented in supplementary Table S2. The adjusted mean CIMT was highest among African Americans followed by Asian, White, and Hispanic populations with Africans having the lowest mean CIMT. There was a significant sex and race-ethnicity interaction, *p=0.001*(supplementary Table S3) hence we conducted separate analyses for women and men.

	African	Hispanic	White	Asian	AA
■ Total	0.67	0.69	0.7	0.7	0.75
■ Men	0.69	0.7	0.71	0.72	0.76
■ Women	0.66	0.67	0.68	0.65	0.72
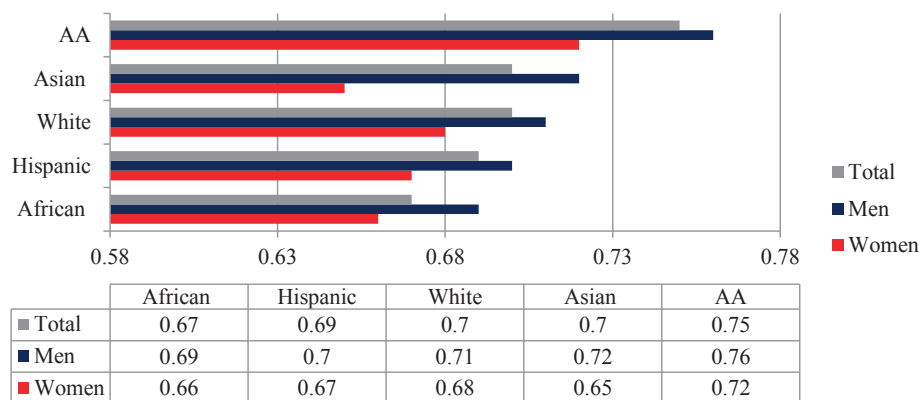
Figure 1: Adjusted means of common CIMT in mm (with 95% CIs) across the race-ethnic groups in the USE-IMT & AWI-Gen collaborative study's combined sample. Values adjusted for age, sex (in the total sample), smoking, alcohol consumption, physical activity, systolic blood pressure, body mass index, high density lipoprotein cholesterol and low density lipoprotein cholesterol; AA, African American; white, European race-ethnic group

Men from all the ethnic groups had higher adjusted CIMT compared to women. African American women had the highest adjusted mean CIMT levels followed by African, European and Hispanic women with Asian women recording the lowest adjusted CIMT levels. A similar trend was observed in men except that Asian men had the second highest CIMT level. We further performed a sub-group analysis among African-ancestry participants by comparing mean CIMT levels between African Americans and East (Kenya), West (Ghana and Burkina Faso) and South Africans. In these analyses we observed that African Americans had the highest mean CIMT followed by West, South and East Africans (see Table 2).

Table 2: Adjusted mean levels of carotid intima-media thickness (with 95% CIs) in participants with African ancestry including African Americans and East, West, and South Africans

	African American (n=4182)	West African (N=4090)	East African (N=1940)	South African (N=3398)	P interaction
Combined sample					
Model 1	0.78 (0.74, 0.82)	0.68 (0.66, 0.69)	0.59 (0.57, 0.61)	0.62 (0.61, 0.64)	–
Model 2	0.78 (0.74, 0.81)	0.68 (0.66, 0.69)	0.60 (0.58, 0.62)	0.62 (0.61, 0.63)	<0.001
Model 3	0.75 (0.72, 0.77)	0.69 (0.67, 0.70)	0.60 (0.57, 0.62)	0.61 (0.59, 0.62)	<0.001
Women					
Model 1	0.76 (0.72, 0.81)	0.67 (0.65, 0.69)	0.60 (0.56, 0.63)	0.62 (0.60, 0.65)	–
Model 2	0.75 (0.71, 0.79)	0.67 (0.65, 0.69)	0.61 (0.58, 0.64)	0.62 (0.60, 0.64)	–
Model 3	0.72 (0.68, 0.75)	0.68 (0.66, 0.70)	0.60 (0.58, 0.63)	0.61 (0.59, 0.62)	–
Men					
Model 1	0.79 (0.75, 0.83)	0.69 (0.67, 0.70)	0.58 (0.57, 0.60)	0.62 (0.61, 0.63)	–
Model 2	0.78 (0.75, 0.82)	0.68 (0.67, 0.69)	0.59 (0.57, 0.61)	0.62 (0.61, 0.63)	–
Model 3	0.76 (0.73, 0.78)	0.69 (0.67, 0.72)	0.59 (0.56, 0.62)	0.61 (0.59, 0.63)	–

Model 1 is crude unadjusted; model 2 is adjusted for age, sex and sex*ethnicity interaction (sex stratified analyses were adjusted for age only) and model 3 is model 2 with adjustment for smoking, alcohol, physical activity, SBP, BMI, glucose, HDL and LDL. $P_{interaction}$, p-value for ethnicity*sex multiplicative interaction term

We also conducted sub-group analyses comparing adjusted mean CIMT levels of Chinese American with Japanese subjects (these two populations constitute the 'Asian' race-ethnic group in the present study) as well as European Americans with Europeans resident in Europe (these two populations constitute the 'European' race-ethnic group in the present study). In these analyses we observed that the CIMT level of Japanese subjects (0.72 (0.57, 0.83) mm) was not significantly different to that of Chinese Americans (0.73 (0.67, 0.79) mm). The CIMT for European subjects resident in Europe (0.74 (0.74, 0.76) mm) was not significantly different from that of European Americans (0.73 (070, 0.78) mm).

Race-ethnic differences in association of CVD risk factors with common CIMT
Results of the IPD-MAs to determine whether there were ethnic differences in the association of classical CVD risk factors with CIMT are presented as forest plots for the combined sample (Figure 2), women (Figure 3) and men (Figure 4). The data is also presented for the combined sample in Table 3 in which the beta coefficients for the associations with CIMT are expressed as percentages using the European race-ethnic group as a reference with a set value of 100%. The measure of heterogeneity obtained from the IPD-MA is

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presented in supplementary Table S4. In the pooled IPD-MA for each risk factor with adjustment for other factors, we noticed that age, sex, BMI and SBP were significantly associated with CIMT in all ethnicities and at varying magnitudes (Table 3). The association between age and CIMT was significantly smaller in Africans (88%), Hispanics (63%) and Asians (50%) but the same for African Americans when compared to European race-ethnic group.

Table 3: Relations of the established CVD risk factors in ethnic groups for the outcome common carotid intima-media thickness

	European		Asian		African		African American		Hispanic	
	β (95%CIs)		β (95%CIs)	%	β (95%CIs)	%	β (95%CIs)	%	β (95%CIs)	%
Age	0.08 (0.07, 0.09)	ref	0.04 (0.03, 0.09)	50	0.07 (0.06, 0.07)	88	0.08 (0.07, 0.09)	100	0.05 (0.04, 0.08)	63
Men vs. women	0.46 (0.42, 0.50)	ref	0.37 (0.13, 0.61)	80	0.09 (0.03,0.15)	20	0.36 (0.26, 0.46)	78	0.31 (0.16, 0.46)	67
Smoking	0.25 (0.21, 0.30)	ref	0.39 (0.09, 0.70)	140	0.24 (0.19, 0.29)	96	0.12 (0.03, 0.21)	48	0.13 (−0.06, 0.31)	52
Alcohol	−0.05 (−0.14, 0.04)	ref	0.06 (−0.22, 0.33)	120	−0.09 (−0.16, −0.04)	180	−0.20 (−0.38, −0.03)	400	−0.03 (−0.16, 0.15)	60
Physical activity	−0.39 (−0.47, −0.33)	ref	0.29 (0.02, 0.55)	74	−0.18 (−0.26, −0.11)	46	−0.13 (−0.33, 0.07)	33	0.14 (−0.03, 0.31)	36
BMI (kg/m^2)	0.02 (0.02, 0.03)	ref	0.05 (0.01, 0.08)	250	0.02 (0.01, 0.02)	100	0.02 (0.01, 0.03)	100	0.04 (0.02, 0.05)	200
SBP (mmHg)	0.17 (0.16, 0.19)	ref	0.13 (0.08, 0.20)	78	0.06 (0.05, 0.08)	35	0.13 (0.11, 0.15)	76	0.09 (0.05, 0.13)	53
Glucose (mmol/l)	0.02 (0.01, 0.03)	ref	0.13 (0.06, 0.19)	650	−0.01 (−0.03, 0.02)	50	0.04 (0.02, 0.05)	167	0.06 (0.02, 0.09)	300
HDL (mmol/l)	−0.07 (−0.12, −0.02)	ref	0.05 (−0.30, 0.39)	71	−0.26 (−0.31, −0.19)	371	−0.31 (−0.42, −0.21)	443	0.09 (−0.14, 0.33)	128
LDL (mmol/l)	0.16 (0.15, 0.18)	ref	0.01 (−0.14, 0.16)	6	0.06 (0.03, 0.08)	38	0.13 (0.08, 0.16)	81	0.14 (0.06, 0.22)	89

Data presented as β (beta) coefficients (differences in CIMT in mm); beta coefficients also expressed as a percentage of that observed in the European race-ethnic group; ref (100%), reference group with 100% set as the reference value

For all race-ethnic groups, SBP and men had lower beta coefficients compared to European race-ethnic group. Smoking was significantly associated with CIMT in all groups except for the Hispanic population, being strongest in the Asian group (140%). Alcohol intake was inversely associated with common CIMT in all groups except Asians but was significant only in Africans (180%) and African Americans (400%). Physical activity was inversely associated with CIMT in Africans and European but positively associated with CIMT in Asians. Body mass index was significantly associated with CIMT in all population, being strongest in the Asian group (250%).

The beta coefficients for the association between glucose and CIMT were highest in Asians (650%), and were non-significant only in the African population. The association of HDL-C with CIMT was negative and significant in European, African and African-American populations whilst LDL-C was positively associated with common CIMT in all groups except Asians, being strongest in the European population. When analyzing whether the LDL-C/HDL-C ratio better predicted CIMT when compared to LDL and HDL, we noticed significant collinearity when LDL-C, HDL-C and LDL-C/HDL-C were included in the same model. We therefore split these models into two with HDL-C and LDL-C in one model and LDL-C/HDL-C in the second. A comparison of the adjusted R^2 indicated that LDL-C/HDL-C ratio was no more predictive of CIMT than HDL-C and LDL-C (supplementary Table S5). In sex stratified analyses, similar associations of age, BMI and SBP with CIMT were observed for women and men except for SBP where Asian women (Figure 3) and Hispanic men displayed a marginal association (Figure 4).
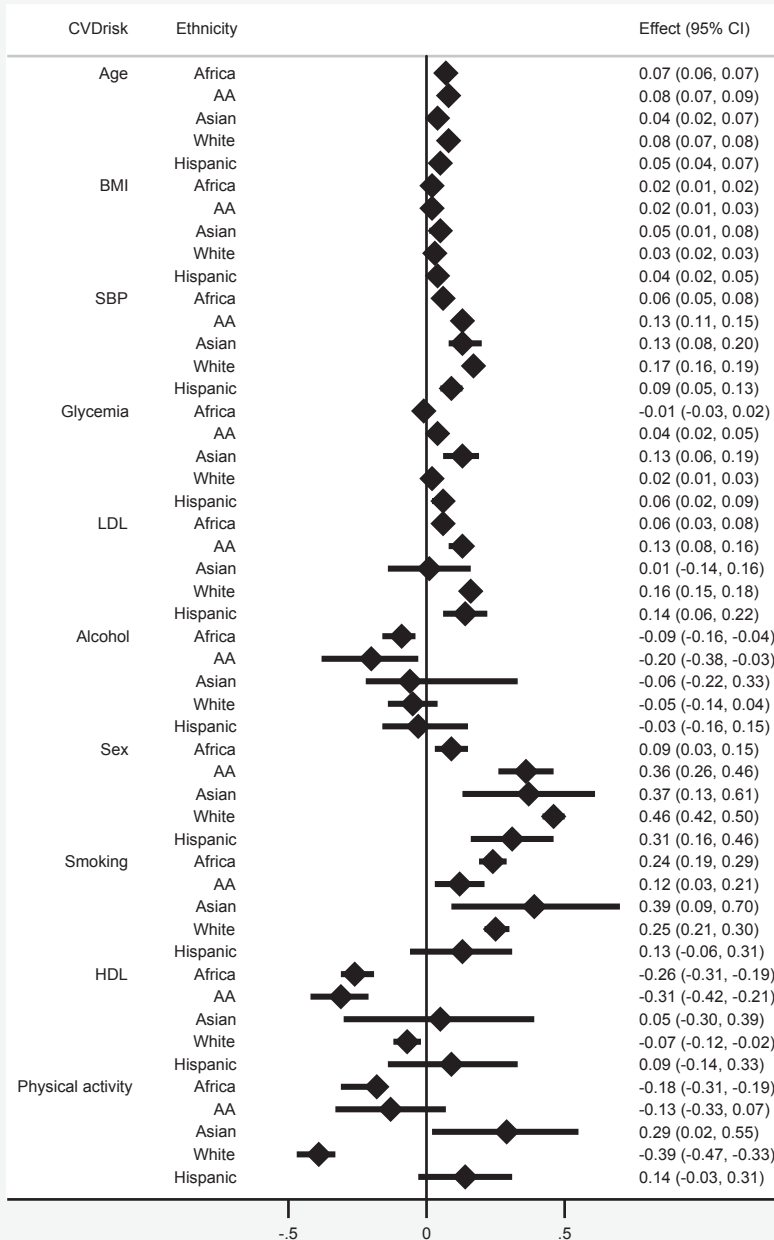
CVDrisk	Ethnicity		Effect (95% CI)
Age	Africa		0.07 (0.06, 0.07)
	AA		0.08 (0.07, 0.09)
	Asian		0.04 (0.02, 0.07)
	White		0.08 (0.07, 0.08)
	Hispanic		0.05 (0.04, 0.07)
BMI	Africa		0.02 (0.01, 0.02)
	AA		0.02 (0.01, 0.03)
	Asian		0.05 (0.01, 0.08)
	White		0.03 (0.02, 0.03)
	Hispanic		0.04 (0.02, 0.05)
SBP	Africa		0.06 (0.05, 0.08)
	AA		0.13 (0.11, 0.15)
	Asian		0.13 (0.08, 0.20)
	White		0.17 (0.16, 0.19)
	Hispanic		0.09 (0.05, 0.13)
Glycemia	Africa		-0.01 (-0.03, 0.02)
	AA		0.04 (0.02, 0.05)
	Asian		0.13 (0.06, 0.19)
	White		0.02 (0.01, 0.03)
	Hispanic		0.06 (0.02, 0.09)
LDL	Africa		0.06 (0.03, 0.08)
	AA		0.13 (0.08, 0.16)
	Asian		0.01 (-0.14, 0.16)
	White		0.16 (0.15, 0.18)
	Hispanic		0.14 (0.06, 0.22)
Alcohol	Africa		-0.09 (-0.16, -0.04)
	AA		-0.20 (-0.38, -0.03)
	Asian		-0.06 (-0.22, 0.33)
	White		-0.05 (-0.14, 0.04)
	Hispanic		-0.03 (-0.16, 0.15)
Sex	Africa		0.09 (0.03, 0.15)
	AA		0.36 (0.26, 0.46)
	Asian		0.37 (0.13, 0.61)
	White		0.46 (0.42, 0.50)
	Hispanic		0.31 (0.16, 0.46)
Smoking	Africa		0.24 (0.19, 0.29)
	AA		0.12 (0.03, 0.21)
	Asian		0.39 (0.09, 0.70)
	White		0.25 (0.21, 0.30)
	Hispanic		0.13 (-0.06, 0.31)
HDL	Africa		-0.26 (-0.31, -0.19)
	AA		-0.31 (-0.42, -0.21)
	Asian		0.05 (-0.30, 0.39)
	White		-0.07 (-0.12, -0.02)
	Hispanic		0.09 (-0.14, 0.33)
Physical activity	Africa		-0.18 (-0.31, -0.19)
	AA		-0.13 (-0.33, 0.07)
	Asian		0.29 (0.02, 0.55)
	White		-0.39 (-0.47, -0.33)
	Hispanic		0.14 (-0.03, 0.31)

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Figure 2: A forest plot of individual participant data meta-analysis of ethnic differences in the adjusted association between classical cardiovascular risk factors and common carotid intima-media thickness for the combined population; AA, African American; white, European race-ethnic group; sex, men vs. women; plots illustrate the beta coefficients with 95% confidence intervals.
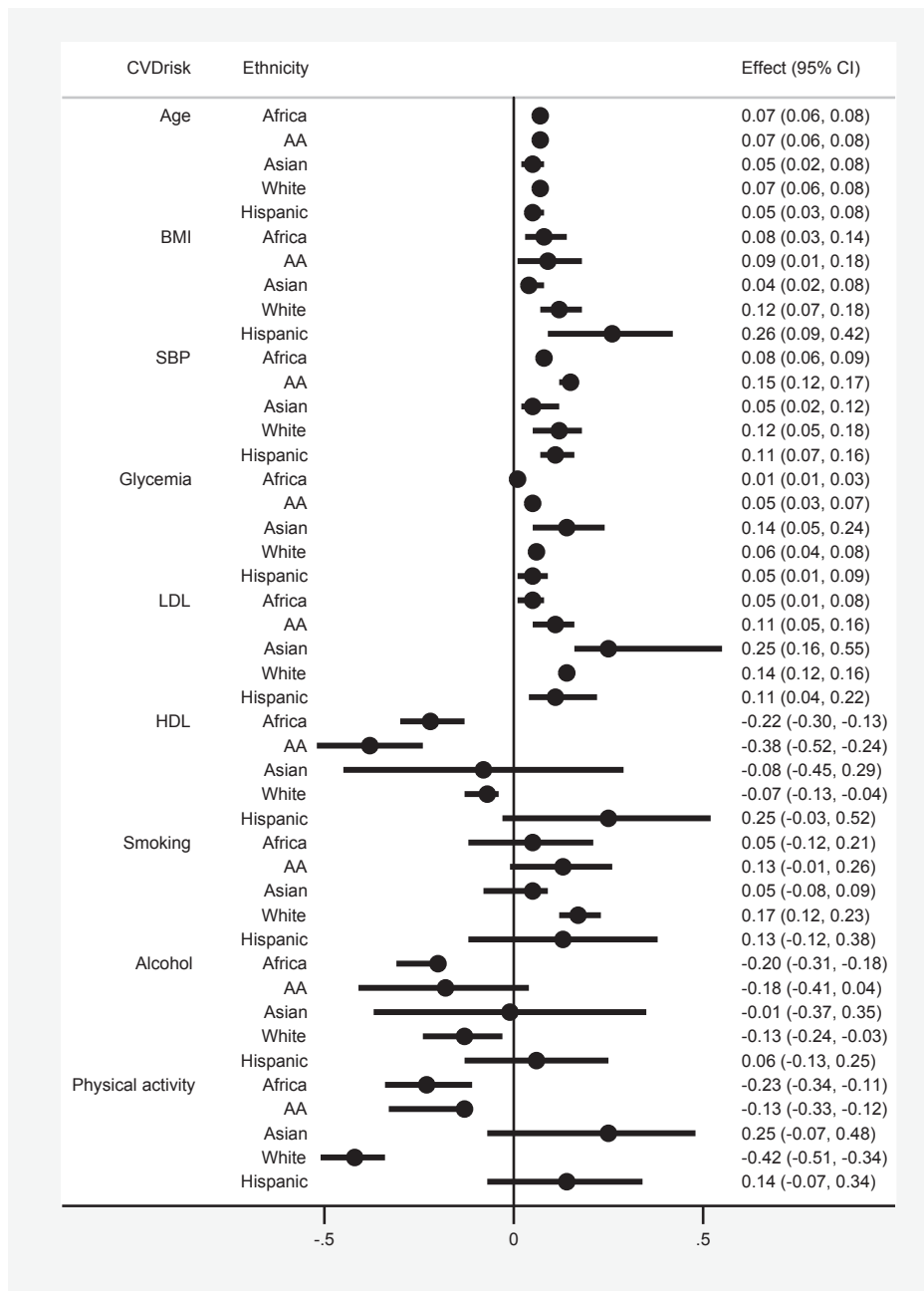
Figure 3: A forest plot of individual participant data meta-analysis of ethnic differences in the adjusted association between classical cardiovascular risk factors and common carotid intima-media thickness in women; AA, African American; white, European race-ethnic group; plots illustrate the beta coefficients with 95% confidence intervals.
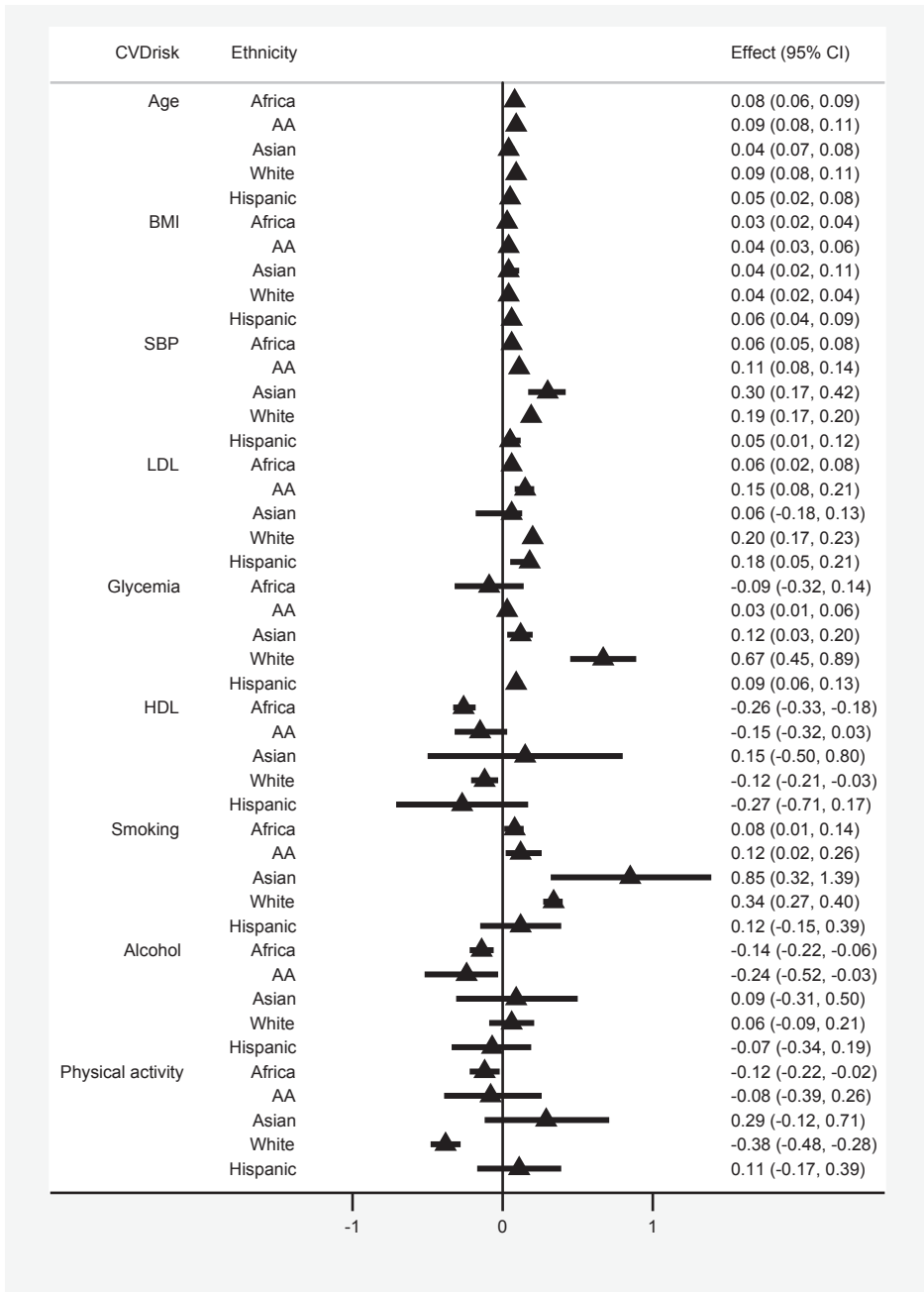
CVDrisk	Ethnicity		Effect (95% CI)
Age	Africa		0.08 (0.06, 0.09)
	AA		0.09 (0.08, 0.11)
	Asian		0.04 (0.07, 0.08)
	White		0.09 (0.08, 0.11)
	Hispanic		0.05 (0.02, 0.08)
BMI	Africa		0.03 (0.02, 0.04)
	AA		0.04 (0.03, 0.06)
	Asian		0.04 (0.02, 0.11)
	White		0.04 (0.02, 0.04)
	Hispanic		0.06 (0.04, 0.09)
SBP	Africa		0.06 (0.05, 0.08)
	AA		0.11 (0.08, 0.14)
	Asian		0.30 (0.17, 0.42)
	White		0.19 (0.17, 0.20)
	Hispanic		0.05 (0.01, 0.12)
LDL	Africa		0.06 (0.02, 0.08)
	AA		0.15 (0.08, 0.21)
	Asian		0.06 (-0.18, 0.13)
	White		0.20 (0.17, 0.23)
	Hispanic		0.18 (0.05, 0.21)
Glycemia	Africa		-0.09 (-0.32, 0.14)
	AA		0.03 (0.01, 0.06)
	Asian		0.12 (0.03, 0.20)
	White		0.67 (0.45, 0.89)
	Hispanic		0.09 (0.06, 0.13)
HDL	Africa		-0.26 (-0.33, -0.18)
	AA		-0.15 (-0.32, 0.03)
	Asian		0.15 (-0.50, 0.80)
	White		-0.12 (-0.21, -0.03)
	Hispanic		-0.27 (-0.71, 0.17)
Smoking	Africa		0.08 (0.01, 0.14)
	AA		0.12 (0.02, 0.26)
	Asian		0.85 (0.32, 1.39)
	White		0.34 (0.27, 0.40)
	Hispanic		0.12 (-0.15, 0.39)
Alcohol	Africa		-0.14 (-0.22, -0.06)
	AA		-0.24 (-0.52, -0.03)
	Asian		0.09 (-0.31, 0.50)
	White		0.06 (-0.09, 0.21)
	Hispanic		-0.07 (-0.34, 0.19)
Physical activity	Africa		-0.12 (-0.22, -0.02)
	AA		-0.08 (-0.39, 0.26)
	Asian		0.29 (-0.12, 0.71)
	White		-0.38 (-0.48, -0.28)
	Hispanic		0.11 (-0.17, 0.39)

-1   0   1

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Figure 4: A forest plot of individual participant data meta-analysis of ethnic differences in the adjusted association between classical cardiovascular risk factors and common carotid intima-media thickness for men; AA, African American; white, European race-ethnic group; plots illustrate the beta coefficients with 95% confidence intervals.

Discussion

Our study involving a large cohort of adults from different race-ethnic groups shows that Africans, African Americans, Asians, Europeans and Hispanics differ markedly in the levels of CIMT and associated CVD risk factors. African Americans had significantly higher mean CIMT levels compared to all the other ethnic groups whilst among Africans, West Africans had higher levels of CIMT than East and South Africans. In the pooled individual participant data meta-analyses and adjusted multilevel analyses, age, sex, BMI, and SBP were significantly associated with CIMT in all race-ethnic groups but with the strength of the associations varying considerably. Compared to European populations, populations of African ancestry (African and African Americans) had higher strength of association between alcohol, HDL-C and CIMT while all other race-ethnicities had a lower strength of association between LDL-C and CIMT.

It has been observed that race-ethnic differences in carotid artery thickness, diameter and stiffness are due to genetic factors as well as anatomical differences in the carotid artery (29) which may explain the higher CIMT in African American race-ethnic group in our study. African Americans also have disproportionately narrower internal and larger external carotid arteries compared to Asians, Europeans and Hispanics (29). This increases turbulent blood flow around the bifurcation resulting in high shear stress and exposure to risk factors enhances the thickening of the adjoining common carotid artery (29). African Americans are an admixed group with considerably variations in the relative proportions of African, European and other race-ethnic group ancestry between individuals. However, majority of African Americans are known to have largely originated from Yoruba, Bantu and Mandeka speaking individuals of West African origin, as well as from Central Africa, who were forcibly moved to North America as part of the slave trade (30, 31). The thicker CIMT in African Americans may therefore be due to their ancestry with West Africans who had thicker CIMT compared to the other African populations in the AWI-Gen study. In addition, the higher burden of established CVD risk factors among the African American compared to the African populations may be a possible contributor to the greater CIMT observed among the former group.

The independent association of age with increased atherosclerosis in all ethnic groups is consistent with published literature (6, 10). The common carotid artery is highly susceptible to the effects of aging with resultant thickening of the intima-media and increased vascular stiffness (32). Increasing life expectancy is therefore likely to result in higher CVD related morbidity and mortalities. The consistent finding of age effects on CIMT across all ethnic groups suggests that screening and management of risk factors should be targeted at older

individuals in all populations. The positive association of common CIMT levels with smoking in all ethnicities has been previously reported (7, 8, 10) as has the association of systolic blood pressure with high CIMT (6). High blood pressure is reported to cause detrimental effects on the vascular tree by inducing higher pressure overload causing arterial hypertrophy or hyperplasia (33).

The inverse association of alcohol intake with CIMT in Africans and African Americans could be attributed to genetic factors and the types of alcohol consumed. Allele frequencies of polymorphic loci in the gene that encodes one of the alcohol dehydrogenase isoforms (ADH1B) differ between populations of African and European ancestry and confer different levels of cardio-protection in both race-ethnicities. Thus, the alcohol-metabolizing ADH1B*3 functional allele, found almost exclusively in populations with African ancestry is associated with a higher conversion rate of ethanol to acetaldehyde (34, 35). This should not be regarded as a public health advocacy for the consumption of alcohol as a means of reducing lipid levels. A randomized cross-over feeding trial among men of African ancestry showed that alcohol improves lipid profiles and reduces atherosclerosis-related inflammatory markers in plasma (36). Furthermore, sorghum-based beers are widely consumed in African populations and the high phenolic content of these drinks has been found to reduce the levels of leukocyte adhesion molecules and inflammatory biomarkers (10). Contrary to our findings the ARIC study observed that African Americans who are current drinkers were more likely to develop coronary heart disease than Europeans (37). These inconsistent findings further highlight the complex association of alcohol with CVDs and calls for more in-depth longitudinal, gene-alcohol interaction and genome wide association studies (GWAS) to provide further insight into the relationship of alcohol intake with atherosclerotic diseases in African populations.

Africans showed the weakest positive association between LDL-C, and one of the strongest negative associations between HDL-C and CIMT compared to the other race-ethnic groups. Previous studies had demonstrated that African populations have lower lipid levels (especially LDL-C and isolated HDL-C) and this may have resulted in a weaker association with CVD morbidity and mortality compared to other race-ethnic groups (38, 39). Low LDL-C and weak association of LDL with CIMT may be a driver of the lower level of atherosclerosis in African populations but the effect of HDL-C on CVD risk is uncertain. Lifestyle differences between various race-ethnic groups may explain some of the observed differences in both the risk factors for CVD and the CIMT measurements.

The raw CIMT levels were higher in men compared to women for all cohorts except those from Africa, where CIMT was similar across genders. Gender

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differences in CIMT may be related to the relative exposure to CVD risk factors (40) and when CIMT values were adjusted for these risk factors their levels were found to be higher in men than women within the African race-ethnic group and in all the other groups.

Our study has demonstrated that race-ethnic variations exist in the association of classical cardiovascular risk factors with CIMT. These observed differences may result from a complex gene-environment and other environmental factors and highlight the importance of further research to elucidate the mechanisms involved in the differential association of CVD risk factors with sub-clinical atherosclerosis across different population groups.

Strengths and limitations: In these IPD-MAs, we pooled data from 34,025 adults between the ages of 40 and 60 years from 15 cohorts drawn from Africa, Asia, Europe and North America. This is the first study to compare the association of CVD risk factors with CIMT in a large population of Africans residing in Eastern, Western and Southern Africa with that in other large cohorts drawn from different ethnic groups. The addition of the African population to the meta-analysis considerably expands our understanding of race-ethnicity and atherosclerotic cardiovascular disease. Using an IPD approach makes this study robust since we have been able to make direct comparison across the various ethnic groups. Harmonization of the outcome measure and the variables used in these analyses limited bias. The use of adjusted multilevel mixed-effect analyses to determine the mean differences in CIMT makes our results reliable and comparable across race-ethnic groups. We also acknowledge that due to the relatively small number of Asian and Hispanic subjects included in this study, our results may not be generalizable to these ethnicities as a whole. Furthermore, the Hispanic population and a large majority of the Asian population were resident in the USA and therefore any data should not be extrapolated to such race-ethnic groups resident outside of the USA.

Adjustment of the mean CIMT levels for the effect of established CVD risk factor enabled us to determine whether observed variations in mean CIMT across race-ethnic groups were due to differential patterning of CVD risk factors. We were also able to account for across and within ethnic group differences conferred by the different cohorts included in the IPD while preserving the original design and composition of the cohort. The H3Africa AWI-Gen study was conducted recently (2013 to 2015) while studies in the USE-IMT cohorts were mainly conducted between 1990 and 2000. This however should not affect the observed relationships since evidence from the Framingham study demonstrates significant relations that were observed in the baseline cohort 73 years ago still exist (41). We do acknowledge that residual confounding from

some unmeasured factors may contribute to some of the observed ethnic differences.

A limitation of any meta-analysis is that analyses can only be performed on variables that are common to all the studies. In the present meta-analysis, dietary intake data was not available across all the studies and this is a limitation since previous investigations have shown associations between nutrient intake and CIMT. Thus, a recent meta-analysis of clinical trials focusing on the reduction of CIMT demonstrated that dietary interventions significantly slowed CIMT progression (42). In addition, we did not have medication use for all the studies.

A recent review by Mitchell et al (2021) reported that over time changes in technology have improved image resolutions of CIMT measurements (43). This may therefore affect cross cohort comparisons due to differences in the ultrasound technologies used. We believe however that the use of z-scores may offer a measure of standardization to overcome this problem and that the observed associations of CIMT with CVD risk factors will be minimally affected.

Conclusion

The differences in the magnitude of the associations of CVD risk factors with CIMT has implications for ethnic specific primary prevention strategies and also offer insights into ethnic-specific mechanisms involved in the aetiology of CVD. The current study does suggest that high CIMT levels in African Americans could be the result of genetics, with alleles predisposing to higher CIMT levels possibly originating in West Africa. However, it cannot be excluded that lifestyle factors, such as diet, may also contribute to these differences in CIMT levels and that gene-environment interactions may also be involved.

Author contribution

Acknowledgements

Disclosures

None

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Supplementary material

Table S1: Baseline characteristic of the cohorts in the USE-IMT and AWI-Gen collaborative study

Cohorts	Country	Women	Men	Age, years	CIMT, mm	Smoking %	Alcohol %	PA %	BMI, kg/m²	SBP, mm Hg	DBP, mm Hg	Glucose, mmol/l	LDL, mmol/l	HDL, mmol/l
ARIC[14]	USA	6920	5355	52 ±5	0.66 ±0.14	27	75	98	27.6 ±5.3	120±18	74±11	5.96 ±2.14	3.53 ±1.02	1.34 ±0.44
AWI-Gen[12]	SSA	4745	4683	50 ±6	0.64 ±0.12	29	38	89	24.1 ±6.1	124±22	79±13	5.06 ±1.62	2.30 ±0.99	1.18 ±0.41
	SA*	758	515	51 ±6	0.61 ±0.09	20	21	83	27.1 ±6.7	134±23	80±13	4.99 ±1.63	2.33 ±0.93	1.20 ±0.39
	SA†	804	356	51 ±6	0.64 ±0.12	31	28	79	30.0 ±8.2	126±21	81±13	5.18 ±2.26	2.54 ±1.03	1.19 ±0.40
	SA‡	–	965	49 ±6	0.62 ±0.12	69	51	75	24.9 ±5.7	132±21	89±13	5.29 ±1.54	2.37 ±0.92	1.21 ±0.46
	Kenya	1056	884	49 ±5	0.59 ±0.11	28	19	89	25.4 ±5.8	120±21	78±13	5.43 ±1.99	2.86 ±0.93	1.26 ±0.47
	BF	1038	1043	50 ±6	0.66 ±0.12	13	64	84	20.9 ±3.5	116±18	74±11	5.05 ±1.24	2.20 ±0.95	1.12 ±0.37
	Ghana	1089	920	51 ±6	0.69 ±0.13	31	65	89	21.6 ±3.6	124±22	78±13	4.54 ±0.81	1.72 ±0.74	1.14 ±0.38
FATE[13]	Canada	1	1112	49 ±5	0.71 ±0.16	14	100	–	28.8 ±3.6	127±16	82±10	5.34 ±1.01	3.38 ±0.83	1.24 ±0.28
KIHD[17]	Finland	–	1055	50 ±5	0.76 ±0.16	42	100	80	26.5 ±3.5	132±16	88±10	4.72 ±1.16	3.93 ±0.97	1.29 ±0.29

Table S1: Baseline characteristic of the cohorts in the USE–IMT and AWI–Gen collaborative study (continued)

Cohorts	Country	Women	Men	Age, years	CIMT, mm	Smoking %	Alcohol %	PA %	BMI, kg/m²	SBP, mm Hg	DBP, mm Hg	Glucose, mmol/l	LDL, mmol/l	HDL, mmol/l
Malmö[18]	Sweden	1920	1331	54 ±4	0.74 ±0.14	25	100	79	25.4 ±3.8	137±18	86±9.5	5.08 ±1.23	4.06 ±0.98	1.39 ±0.37
MESA[15]	USA	1631	1433	53 ±5	0.68 ±0.14	18	72	85	28.8 ±5.8	119±18	72±10	5.20 ±1.66	3.08 ±0.83	1.29 ±0.37
NBS[20]	NLD	327	66	56 ±3	0.79 ±0.09	21	90	40	26.2 ±4.0	124±14	77±10	5.04 ±0.81	3.77 ±0.93	1.47 ±0.39
NOMAS[16]	USA	210	144	57 ±2	0.69 ±0.08	20	69	47	28.7 ±5.2	133±19	82±11	5.37 ±2.17	3.22 ±0.86	1.21 ±0.35
OSACA2[21]	Japan	84	114	55 ±5	0.84 ±0.13	31	100	98	23.9 ±3.3	135±20	83±11	5.88 ±1.48	3.52 ±0.66	1.43 ±0.40
Tromsø[19]	Norway	1235	1459	55 ±5	0.74 ±0.13	36	79	69	26.1 ±3.7	139±19	83±12	4.84 ±1.13	4.84 ±1.23	1.52 ±0.43
Combined	–	17073	16952	52 ±5	0.69 ±0.14	27	78	90	26.4 ±5.3	125±20	78±12	5.41 ±1.81	3.34 ±1.22	1.33 ±0.43

Data presented as mean ±standard deviation (SD) or %; ARIC, Atherosclerosis Risk in Communities study; AWI–Gen, African–Wits–INDEPTH Genomic study; BF, Burkina Faso; BMI, body mass index in kg/m²; CIMT, carotid intima–media thickness; FATE, Firefighters and Their Endothelium Study; LDL–C, low–density lipoprotein cholesterol; HDL–C, high–density lipoprotein cholesterol; KIHD, Kuopio Ischemic Heart Disease Risk Factor Study; Malmö, Malmö Diet and Cancer Study; MESA, Multi-Ethnic Study of Atherosclerosis; NBS, Nijmegen Biomedical Study; NLD, Netherlands; NOMAS, Northern Manhattan Study; OSACA2, Osaka Follow-up Study for Carotid Atherosclerosis 2; PA, Physical activity; *SA, South Africa –Agincourt; †SA, South Africa – Dikgale; ‡SA, South Africa – Soweto; USA, United States of America; USE–IMT, USE Intima–Media Thickness collaboration.

Table S2: Adjusted mean common carotid intima-media thickness (with 95% CIs) for the various ethnicities in the USE-IMT AWI-Gen collaborative study and stratified by women and men

	Asian (n=572)	African (n=9428)	African American (n=4182)	European (n=18817)	Hispanic (n=980)	$P_{interaction}$ (ethnicity*sex)
Combined sample						
Model 1	0.72 (0.68, 0.76)	0.64 (0.53, 0.75)	0.78 (0.74, 0.82)	0.73 (0.69, 0.77)	0.73 (0.69, 0.77)	–
Model 2	0.72 (0.68, 0.76)	0.66 (0.55, 0.77)	0.78 (0.74, 0.81)	0.71 (0.68, 0.75)	0.72 (0.68, 0.75)	< 0.0001
Model 3	0.70 (0.67, 0.73)	0.67 (0.59, 0.76)	0.75 (0.72, 0.77)	0.70 (0.67, 0.73)	0.69 (0.66, 0.72)	0.001
Women						
Model 1	0.69 (0.65, 0.74)	0.64 (0.53, 0.75)	0.76 (0.72, 0.81)	0.70 (0.66, 0.75)	0.72 (0.67, 0.76)	–
Model 2	0.68 (0.64, 0.72)	0.66 (0.55, 0.76)	0.75 (0.71, 0.79)	0.69 (0.65, 0.73)	0.70 (0.66, 0.74)	–
Model 3	0.65 (0.62, 0.69)	0.66 (0.58, 0.75)	0.72 (0.68, 0.75)	0.68 (0.63 0.70)	0.67 (0.64, 0.71)	–
Men						
Model 1	0.74 (0.70, 0.78)	0.64 (0.53, 0.75)	0.79 (0.75, 0.83)	0.75 (0.71, 0.79)	0.74 (0.70, 0.78)	–
Model 2	0.73 (0.69, 0.77)	0.66 (0.55, 0.77)	0.78 (0.75, 0.82)	0.73 (0.70, 0.77)	0.73 (0.69, 0.77)	–
Model 3	0.72 (0.69, 0.76)	0.69 (0.61, 0.77)	0.76 (0.73, 0.78)	0.71 (0.68, 0.74)	0.70 (0.67, 0.74)	–

Model 1 is crude unadjusted; model 2 is adjusted for age, sex and sex*ethnicity interaction (sex stratified analyses were adjusted for only age) and model 3, model 1 + smoking, alcohol, physical activity, SBP, BMI, glucose, HDL-C and LDL-C; BMI, body mass index, HDL-C, high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol and SP, systolic blood pressure

Table S3: Multiplicative interaction terms between race–ethnicity and sex

Multiplicative interaction terms	Coefficient (95%CI)	SE	x² (p-value)
Ethnicity x Sex for whole sample			
African x Women	-0.59 (-1.77, 0.58)	0.599	1204.88 (<0.00001)
African x Men	-0.58 (-1.75, 0.59)	0.599	
African American x Women	0.60 (0.53, 0.66)	0.032	
African American x Men	0.99 (0.92, 1.07)	0.037	
Asian x Women	-0.16 (-0.34, 0.03)	0.094	
Asian x Men	0.64 (0.42, 0.87)	0.113	
European x Women	Ref (0)	Ref (0)	
European x Men	0.69 (0.50, 0.87)	0.094	
Hispanic x Women	0.17 (0.03, 0.31)	0.070	
Hispanic x Men	0.43 (0.28, 0.57)	0.073	
HIV x Sex for Africans only			
HIV negative x Women	Ref (0)	Ref (0)	60.41 (0.169)
HIV negative x Men	0.048 (-0.003, 0.101)		
HIV+ART+ x Women	-0.213 (-0.111, 0.314)		
HIV+ART+ x Men	-0.324 (-0.212, 0.436)		

ART, antiretroviral therapy

Table S4: Individual Participant Data-Meta-analysis on ethnic differences of the association between classical CVD risk factors and carotid intima-media thickness in the combined sample, women and men

Factors	African β (95%CI)	African American β (95%CI)	Asian β (95%CI)	European β (95%CI)	Hispanic β (95%CI)	I^2, P-value
Combined population						
Age	0.07 (0.06, 0.07)	0.08 (0.07, 0.09)	0.04 (0.02, 0.07)	0.08 (0.07, 0.08)	0.05 (0.04, 0.07)	68%; p=0.014
Men vs. women	0.09 (0.03, 0.15)	0.36 (0.26, 0.46)	0.37 (0.13, 0.61)	0.46 (0.42, 0.50)	0.31 (0.16, 0.46)	97%; p<0.001
Smoking	0.24 (0.19, 0.29)	0.12 (0.03, 0.21)	0.72 (0.29, 1.16)	0.25 (0.21, 0.30)	0.13 (-0.06, 0.31)	96%; p<0.001
Alcohol	-0.09 (-0.16, -0.04)	-0.20 (-0.38, -0.03)	0.06 (-0.22, 0.33)	-0.05 (-0.14, 0.04)	-0.03 (-0.16, 0.15)	92%; p<0.001
Physical activity	-0.18 (-0.26, -0.11)	-0.13 (-0.33, 0.07)	0.29 (0.02, 0.55)	-0.39 (-0.47, -0.33)	0.14 (-0.13, 0.31)	93%; p<0.001
BMI (kg/m^2)	0.02 (0.01, 0.02)	0.02 (0.01, 0.03)	0.05 (0.01, 0.08)	0.02 (0.02, 0.03)	0.04 (0.02, 0.05)	89%; p<0.001
SBP (mmHg)	0.06 (0.05, 0.08)	0.13 (0.11, 0.15)	0.13 (0.08, 0.20)	0.17 (0.16, 0.19)	0.09 (0.05, 0.13)	98%; p<0.001
Glucose (mmol/l)	-0.01 (-0.03, 0.02)	0.04 (0.02, 0.05)	0.13 (0.06, 0.19)	0.02 (0.01, 0.03)	0.06 (0.02, 0.09)	89%; p<0.001
HDL-C (mmol/l)	-0.26 (-0.31, -0.19)	-0.31 (-0.42, -0.21)	0.05 (-0.30, 0.39)	-0.07 (-0.12, -0.02)	0.09 (-0.14, 0.33)	88%; p<0.001
LDL-C (mmol/l)	0.06 (0.03, 0.08)	0.13 (0.08, 0.16)	0.01 (-0.14, 0.16)	0.16 (0.15, 0.18)	0.14 (0.06, 0.22)	97%; p<0.001
Women						
Age	0.07 (0.06, 0.08)	0.07 (0.06, 0.08)	0.05 (0.02, 0.08)	0.07 (0.06, 0.08)	0.05 (0.03, 0.08)	0.0%; p=0.451
Smoking	0.05 (-0.12, 0.21)	0.13 (-0.01, 0.26)	0.05 (-0.08, 0.09)	0.17 (0.12, 0.23)	0.13 (-0.12, 0.38)	5%; p=0.066
Alcohol	-0.20 (-0.31, -0.18)	-0.18 (-0.41, 0.04)	-0.01 (-0.37, 0.35)	-0.13 (-0.24, -0.03)	0.06 (-0.13, 0.25)	88%; p<0.001
Physical activity	-0.23 (-0.34, -0.12)	-0.14 (-0.40, 0.11)	0.25 (-0.07, 0.56)	-0.42 (-0.51, -0.34)	0.14 (-0.07, 0.34)	89%; p<0.001
BMI (kg/m^2)	0.08 (0.03, 0.14)	0.09 (-0.01, 0.18)	0.04 (0.02, 0.08)	0.12 (0.07, 0.18)	0.26 (0.09, 0.42)	85%; p<0.001
SBP (mmHg)	0.08 (0.06, 0.09)	0.15 (0.12, 0.17)	0.05 (-0.02, 0.12)	0.12 (0.05, 0.18)	0.11 (0.07, 0.16)	95%; p<0.001
Glucose (mmol/l)	0.01 (-0.01, 0.03)	0.05 (0.03, 0.07)	0.14 (0.05, 0.24)	0.06 (0.04, 0.08)	0.05 (0.01, 0.09)	78%; p=0.001
HDL-C (mmol/l)	-0.22 (-0.30, -0.13)	-0.38 (-0.52, -0.24)	-0.08 (-0.45, 0.29)	-0.07 (-0.13, -0.04)	0.25 (-0.03, 0.52)	85%; p<0.001
LDL-C (mmol/l)	0.05 (0.01, 0.08)	0.11 (0.05, 0.16)	0.25 (0.16, 0.55)	0.14 (0.12, 0.16)	0.11 (0.04, 0.22)	93%; p<0.001

Table S4: Individual Participant Data-Meta-analysis on ethnic differences of the association between classical CVD risk factors and carotid intima-media thickness in the combined sample, women and men (continued)

Factors	African β (95%CI)	African American β (95%CI)	Asian β (95%CI)	European β (95%CI)	Hispanic β (95%CI)	I², P-value
Men						
Age	0.08 (0.07, 0.09)	0.09 (0.08, 0.11)	0.04 (0.07, 0.08)	0.09 (0.08, 0.11)	0.05 (0.02, 0.08)	66%; p=0.018
Smoking	0.08 (0.01, 0.14)	0.12 (-0.02, 0.26)	0.85 (0.32, 1.39)	0.34 (0.27, 0.40)	0.12 (-0.15, 0.39)	77%; p=0.002
Alcohol	-0.14 (-0.22, -0.06)	-0.24 (-0.52, 0.03)	0.09 (-0.31, 0.50)	0.06 (-0.09, 0.21)	-0.07 (-0.34, 0.19)	82%; p<0.001
Physical activity	-0.12 (-0.22, -0.02)	-0.08 (-0.39, 0.26)	0.29 (-0.12, 0.71)	-0.38 (-0.48, -0.28)	0.11 (-0.17, 0.39)	84%; p<0.001
BMI (kg/m²)	0.03 (0.02, 0.04)	0.04 (0.03, 0.06)	0.04 (-0.02, 0.11)	0.04 (0.02, 0.04)	0.06 (0.04, 0.09)	55%; p=0.062
SBP (mmHg)	0.06 (0.05, 0.08)	0.11 (0.08, 0.14)	0.30 (0.17, 0.42)	0.19 (0.17, 0.20)	0.05 (-0.01, 0.12)	97%; p<0.001
Glucose (mmol/l)	-0.09 (-0.32, 0.14)	0.03 (0.01, 0.06)	0.12 (0.03, 0.20)	0.67 (0.45, 0.89)	0.09 (0.06, 0.13)	82%; p<0.001
HDL-C (mmol/l)	-0.26 (-0.33, -0.18)	-0.15 (-0.32, 0.03)	0.15 (-0.50, 0.80)	-0.12 (-0.21, -0.03)	-0.27 (-0.71, 0.17)	35%; p=0.186
LDL-C (mmol/l)	0.06 (0.02, 0.09)	0.15 (0.08, 0.21)	0.06 (-0.18, 0.31)	0.20 (0.17, 0.23)	0.18 (0.05, 0.31)	95%; p<0.001

I², is the between-study variance as a percentage of total variance; BMI, body mass index, CI, confidence interval; HDL-C, high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol and SP, systolic blood pressure

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Table S5: Comparing the variance explained by modeling the association of LDL, HDL and LDL/HDL ratio with common carotid intima-media thickness

	Variance inflation factor for model 1	R^2 for model 2	R^2 for model 3	Comment
African ancestry	7.32	0.18	0.18	No change in R^2
African American	8.29	0.17	0.17	No change in R^2
Asians	12.6	0.26	0.26	No change in R^2
European ancestry	8.41	0.16	0.16	No change in R^2
Hispanic	10.04	0.24	0.24	No change in R^2

Model 1 included the following independent variables: age, sex, smoking, physical activity, alcohol, BMI, SBP, Glucose, HDL-C, LDL-C and LDL-C/HDL-C ratio; Model 2 included age, sex, smoking, physical activity, alcohol, BMI, SBP, Glucose, LDL-C and HDL-C and Model 3 included age, sex, smoking, physical activity, alcohol, BMI, SBP, Glucose, LDL-C/HDL-C ratio

Part 2:

CIMT and cardiovascular disease risk

Chapter 5

Poor cardiovascular health is associated with subclinical atherosclerosis in apparently healthy sub-Saharan African populations: an H3Africa AWI-Gen study

Nonterah EA, Crowther NJ, Oduro A, Agongo G, Micklesfield LK, Boua PR, Choma SSR, Mohamed SF, Sorgho H, Tollman SM, Norris SA, Raal FJ, Grobbee DE, Ramsay M, Bots ML, Klipstein-Grobusch K; as part of the H3Africa AWI-Gen study.

Abstract

Background: The cardiovascular health index (CVHI) introduced by the American Heart Association is a valid, accessible, simple, and translatable metric for monitoring cardiovascular health in a population. Components of the CVHI include the following seven cardiovascular risk factors (often captured as life's simple 7): smoking, dietary intake, physical activity, body mass index, blood pressure, glucose, and total cholesterol. We sought to expand the evidence for its utility to under-studied populations in sub-Saharan Africa, by determining its association with common carotid intima-media thickness (CIMT).

Methods: We conducted a cross-sectional study involving 9011 participants drawn from Burkina Faso, Ghana, Kenya, and South Africa. We assessed established classical cardiovascular risk factors and measured carotid intima-media thickness of the left and right common carotid arteries using B-mode ultrasonography. Adjusted multilevel mixed-effect linear regression was used to determine the association of CVHI with common CIMT. In the combined population, an individual participant data meta-analyses random-effect was used to conduct pooled comparative sub-group analyses for differences between countries, sex, and socio-economic status.

Results: The mean age of the study population was 51 ± 7 years and 51% were women, with a mean common CIMT of 637 ± 117 µm and CVHI score of 10.3 ± 2.0. Inverse associations were found between CVHI and common CIMT (β-coefficients [95% confidence interval]: Burkina Faso, $- 6.51$ [$- 9.83, - 3.20$] µm; Ghana, $- 5.42$ [$- 8.90, - 1.95$]; Kenya, $- 6.58$ [$- 9.05, - 4.10$]; and South Africa, $- 7.85$ [$- 9.65, - 6.05$]). Inverse relations were observed for women ($- 4.44$ [$- 6.23, - 2.65$]) and men ($- 6.27$ [$- 7.91, - 4.64$]) in the pooled sample. Smoking ($p < 0.001$), physical activity ($p < 0.001$), and hyperglycemia ($p < 0.001$) were related to CIMT in women only, while blood pressure and obesity were related to CIMT in both women and men ($p < 0.001$).

Conclusion: This large pan-African population study demonstrates that CVHI is a strong marker of subclinical atherosclerosis, measured by common CIMT and importantly demonstrates that primary prevention of atherosclerotic cardiovascular disease in this understudied population should target physical activity, smoking, obesity, hypertension, and hyperglycemia.

Keywords: Cardiovascular health index, Carotid intima-media thickness, Subclinical atherosclerosis, Sub-Saharan Africa, Primary prevention, Cardiovascular diseases, Screening, Understudied populations

Background

Cardiovascular disease (CVD) is a leading cause of global deaths (1, 2) raising huge public health concerns. Sub-Saharan Africa (SSA) is experiencing a rising burden of non-communicable diseases including CVD (3), alongside a reduction in the prevalence of infectious diseases (4). Increasing urbanization, changing lifestyles and population ageing is probably accounting for the surge in the prevalence of CVD in SSA (3, 4). This presents a challenge to the health systems of African countries and requires a pragmatic approach to the prevention of CVD. Where risk equations such as Framingham (5) and Pooled Cohort Equations (PCE) (6) have served as valuable tools in assessing the risk of CVD globally, these have inherent limitations especially when applied to an African population (7). The American Heart Association and the American Stroke Association (AHA/ASA) developed the Cardiovascular Health Index (CVHI) as a new public health metric for assessing and monitoring cardiovascular health (8-11). The components of the CVHI often referred to as life's simple seven include smoking, dietary intake, physical activity, body mass index (BMI), blood pressure, glucose and total cholesterol.

Due to the lack of data on incident CVD events, carotid-intima media thickness (CIMT) is often used as an alternate marker of CVD risk. Increased CIMT has been shown to be related to several risk factors for CVD and CVD risk globally (12, 13) including SSA populations (14).

The CVHI metric is proven to be related to CIMT, a marker of subclinical atherosclerosis (15), and coronary artery calcification (16) in both Caucasian and Asian populations (17). In Africa, the association of CVHI with subclinical atherosclerosis has only been reported in a relatively small study of HIV-positive (n=105) and non-infected (n=100) individuals (18) in Uganda and recently in a rural setting in Limpopo, South Africa (19). Expanding the evidence to a larger pan-African population would help to clarify whether the CVHI can be used as a simple screening tool for cardiovascular health within the existing health systems of resource-constrained SSA countries. Therefore, we set out to assess the association between cardiovascular health, using CVHI, and subclinical atherosclerosis in a large understudied African population living in SSA.

Methods

Study design and population
The H3Africa Africa-Wits-INDEPTH (www.indepthnetwork.org) partnership for genomic studies (AWI-Gen) project is a population-based cross-sectional study. Participants included older adult women and men aged 40-60 years drawn from six sites in four SSA countries (20, 21).

Five of the sites were INDEPTH-Network (International Network for the Demographic Evaluation of Populations and Their Health) Health and Demographic Surveillance Study (HDSS) sites namely Navrongo HDSS in Ghana, Nanoro HDSS in Burkina Faso, Nairobi urban HDSS in Kenya and Agincourt and DIMAMO HDSS's in South Africa. The sixth site was the South African MRC/ University of the Witwatersrand Developmental Pathways to Health Research Unit (DPHRU) in Soweto, South Africa. Participants were randomly sampled from existing sampling frames in the various recruitment sites. Those who reported history of cardiovascular diseases (myocardial infarction, stroke) and chronic kidney disease were excluded from the analyses. Pregnant women, closely related individuals (first degree relatives) and those residents in the communities for less than 10 years were excluded.

Independent variable: Cardiovascular health index (CVHI)
The components of the CVHI as defined by AHA/ASA include blood pressure, fasting glucose, total cholesterol, body mass index, physical activity, diet, and smoking (8). Each CVHI component was given a point score of 0, 1, or 2 to represent poor, intermediate and ideal health respectively. Details of how the categories were defined are presented in Additional file 1: Table S1. The AHA recommended cutoffs are consistent with recommendations from the World Health Organization (22, 23) and other organizations (24). These cutoffs have recently been used in a study in rural South Africa (19). An overall CVHI score was computed as the sum of all 7 CVHI components, ranging from 0 to 14. We stratified participants into 3 mutually exclusive categories based on overall CVHI score: Ideal cardiovascular health (iCVH) defined as 12-14 points, intermediate cardiovascular health (8-11 points), and poor cardiovascular health (0-7 points) (25). Details of how the various components of CVHI were measured and defined in the AWI-Gen study have been published elsewhere (21).

Briefly, the history of smoking tobacco products was self-reported and defined as never smoked, previous smoker and current smoker. Physical activity was measured as the level of moderate-to-vigorous physical activity (MVPA) from self-reported data on occupation, travel-related and leisure time physical activity. Diet was measured as the self-reported daily number of servings

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of fruits and vegetables. Weight was measured to the nearest 0.1 kg using a digital 200 kg capacity scale (Kendon Medical, South Africa). Standing height to the nearest millimeter was measured using a Harpenden digital stadiometer (Holtain, Wales). The BMI was then calculated as weight divided by height squared in kg/m^2. Blood pressure was measured from the left arm of the participant, seated with the arm at the level of the heart and having rested 5-10 minutes. Three readings were taken with the first value discarded and an average of the second and third computed as blood pressure. Venous blood was taken with the participant having fasted overnight and this sample was used to measure glucose and total cholesterol using colorimetric assays in a Randox Plus clinical chemistry analyser (Crumlin, UK). The coefficient of variation of the laboratory measurements for glucose was 2.3% and 1.5% for total cholesterol. All analyses were performed in a centralized laboratory at the DPHRU in Soweto according to good laboratory practice, and with external monitoring for quality control.

Dependent variable: Common carotid atherosclerosis

Carotid intima-media thickness was used as a marker of sub-clinical atherosclerosis. A carotid B-mode ultrasound scan (GE Healthcare, USA) with a 12L-RS straight probe was used to measure the CIMT of the right and left common carotid artery (CCA). Details of the measurement procedure used in this population are described elsewhere (4, 21). Briefly the right and left intima-media thickness of the common carotid arteries (CCA) were measured at a single angle of 45 degree tilt with the participant lying supine and neck supported with a pillow. A mean of the right and left common CIMT (in micrometers) was computed as the outcome variable.

Potential confounders and effect modifiers

Age was self-reported or calculated from reported date of birth while sex was reported at the time of recruitment. Household socio-economic status (SES) was determined based on household assets and quintiles calculated according to the practice implemented by the Demographic and Health Surveys (DHS) Program (http://www.dhsprogram.com/topics/wealth-index/Wealth-Index-Construction.cfm). A principal component analysis of the listed household assets is conducted and predicting factor scores generated from these analyses. The predicted factor scores are then categorized into quintiles for each of the participating sites separately (21). Educational status was by asking the number years spent in school. Educational status was ascertained by asking the highest level of education (no formal education, primary, secondary or tertiary education). This was subsequently categorized as no formal education and yes formal education (primary, secondary and tertiary education). This took into consideration the number of years in each country at the different levels of

school. HIV infection was self-reported in Burkina Faso and Ghana and testing was not offered due to the very low prevalence. Kenya and South Africa used locally available, government-approved rapid-test to conduct voluntary HIV rapid test. Antiretroviral therapy (ART) use was self-reported across all sites.

Statistical analyses

All data analyses were conducted using STATA version 14.2 (Statacorp, College Station, TX, USA). We used descriptive and inferential statistics to summarize our data. Using Epanechnikov kernel function in STATA, density curves were plotted to determine the distribution of the CVHI score and common CIMT for the various countries represented in the AWI-Gen study (Additional file 2: Figs S1 and S2). Continuous variables are reported as mean and standard deviation (±SD) due to approximate normal distribution and categorical variables as sample number with respective percentages. The differences in prevalence of overall cardiovascular health between women and men were examined using Pearson's Chi squared test.

As the participants were resident in four different countries, we used multilevel mixed-effects generalized linear models to determine the associations of CVHI score with common CIMT. A variance co-variance method was used to obtain robust standard errors. Four progressively adjusted models were conducted as follows: model 1 was adjusted for age and sex; model 2 was adjusted for age, sex, level of education and household socio-economic status; in model 3, we adjusted for all the model 2 variables in addition to HIV and ART use. The fixed effects portion of the model was considered to vary according to country and hence country was specified as the random effect part of the mixed effect model. Separate models were fitted for the combined populations, women and men and countries.

The country specific models were fitted sequentially following the pattern explained above using adjusted linear regression models. Multilevel mixed-effects generalized linear models were conducted for South Africa which contained three different sites (site as the random effect). In the combined population, an individual participant data (IPD) meta-analyses was conducted. Pooled comparative sub-group analyses included comparison by country, sex and household SES. Our sub-group analyses included household SES since it has previously been linked to poor health outcomes (26) and atherosclerosis (27). Forest plots were subsequently plotted and weighted by sample size to avoid biased estimates (and for ease of comparison) and by default sample size instead of study weights appear on the plots. In addition line plots of the predicted linear association between CVHI score and CIMT were drawn and presented in supplementary material (Additional file 2: Figs S3 and S4).

To determine the discriminatory power of each CVHI component metric to predict common CIMT, we generated dummy variables for the 3 levels of each CVHI metric using 'poor' as the reference. We then conducted adjusted multilevel mixed effects linear regression analyses of the dummy variables, with adjustment for age, sex, educational status and household SES in the pooled sample and for women and men separately. A two-tailed $p<0.05$ was considered statistically significant in all inferential analyses.

Results

After excluding participants (7.5%) with missing data, 9011 adults from Burkina Faso, Ghana, Kenya and South Africa were included in these analyses. Basic characteristics are presented in Table 1. Fifty one percent of the study population was women and the average age was 51±7 years. Physical activity levels were high (higher mean moderate-to-vigorous physical activity - minutes/week) in the populations from the two rural sites of West Africa. The average fruit and vegetable intake in the combined AWI-Gen population was 3±2 servings per day. Mean BMI, systolic and diastolic blood pressure were highest in South Africa and Kenya and lowest in Ghana and Burkina Faso (Table 1).

Table 1: Basic characteristics of the AWI-Gen participants per research site

Characteristics	Total population N = 9011	Burkina Faso n=2074	Ghana n=1729	Kenya n=1913	South Africa n=3295
Women	4948 (50.8)	1035 (49.6)	936 (54.1)	1067 (54.1)	1910 (48.2)
Age in years	50.9±6.9	49.8±5.9	51.1±5.8	48.8±5.8	52.5±8.0
Formal education in yes/no	6675 (63.8)	353 (17.0)	594 (29.6)	1795 (92.4)	3933 (88.5)
*Household SES	10.1±4.1	11.3±3.9	9.50±4.6	11.1±3.3	9.1±4.0
Household SES in quintiles					
Q1 (Poorest)	336 (16.2)	372 (18.5)	235 (12.1)	605 (13.4)	1548 (14.7)
Q2 (Poorer)	404 (19.4)	360 (17.9)	432 (22.3)	1100 (24.4)	2296 (21.8)
Q3 (Poor)	405 (19.5)	387 (19.2)	450 (23.2)	655 (14.6)	1897 (18.0)
Q4 (Less poor)	386 (18.6)	473 (23.5)	396 (20.4)	949 (21.1)	2204 (20.9)
Q5 (Least poor)	546 (26.3)	421 (20.9)	429 (22.1)	1194 (26.5)	2590 (24.6)
MVPA in minutes/weeks	1573±1550	2077±1590	2036±1707	1749±1732	1074±1189
Servings of fruits & vegetables/day	2.9±2.0	1.8±0.9	3.4±1.5	4.11±2.5	2.23±1.7
BMI in kg/m^2	25.0±6.8	20.9±3.4	21.60±3.6	25.40±5.8	28.22±7.6
SBP in mmHg	124.6±21.8	115.6±18.2	123.9±21.6	120.0±21.2	130.9±21.7
DBP in mmHg	79.7±3.7	73.5±10.6	76.9±12.7	78.4±12.9	84.29±14.2
Glucose in mg/dL	91.3±29.4	90.9±22.5	81.8±13.21	97.8±36.1	92.5±33.5
Total cholesterol in mg/dL	148±43.7	132±36.9	125±35.8	169±41.9	159±43.2
CVHI (0-14)	10.4±2.0	10.8±1.5	11.4±1.6	10.7±1.9	9.3±2.0
Common CIMT in μm	637±117	664±118	690±119	588±104	620±106

Values are an (%) or mean ±SD (standard deviation); CVHI = cardiovascular health index; CIMT = carotid intima-media thickness; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; SES = socio-economic status; Q1 to Q5 = wealth quintiles; MVPA = moderate-to-vigorous physical activity in minutes per week. Formal education is defined as having attained primary or secondary or tertiary education; *Household SES presented as a mean ±SD of household assets.

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The prevalence of each of the 3 levels of cardiovascular health (poor, intermediate and ideal) for the total sample, the total sample stratified by sex, and the various countries is presented in Figure 1. In the combined sample iCVH was 31.9% with women (38.2%) having a higher prevalence than men (25.6%). Rural sites in Ghana (50.6%) and Burkina Faso (44.3%) had the highest prevalence of iCVH whilst the more urban sites in Kenya (37.4%) and South Africa (11.3%) had the lowest. The prevalence of the poor, intermediate and ideal levels of each of the 7 CVHI components, according to sex and country, are presented in Table 2. The majority of participants were in the ideal category for total cholesterol, fasting glucose, physical activity, BMI and blood pressure with unhealthy diet and smoking placing the majority of participants in the poor category. Burkina Faso and Ghana had more participants in the ideal category for most CVHI components than South Africa and Kenya, except diet and physical activity where Burkina Faso had the least ideal levels compared to other countries.
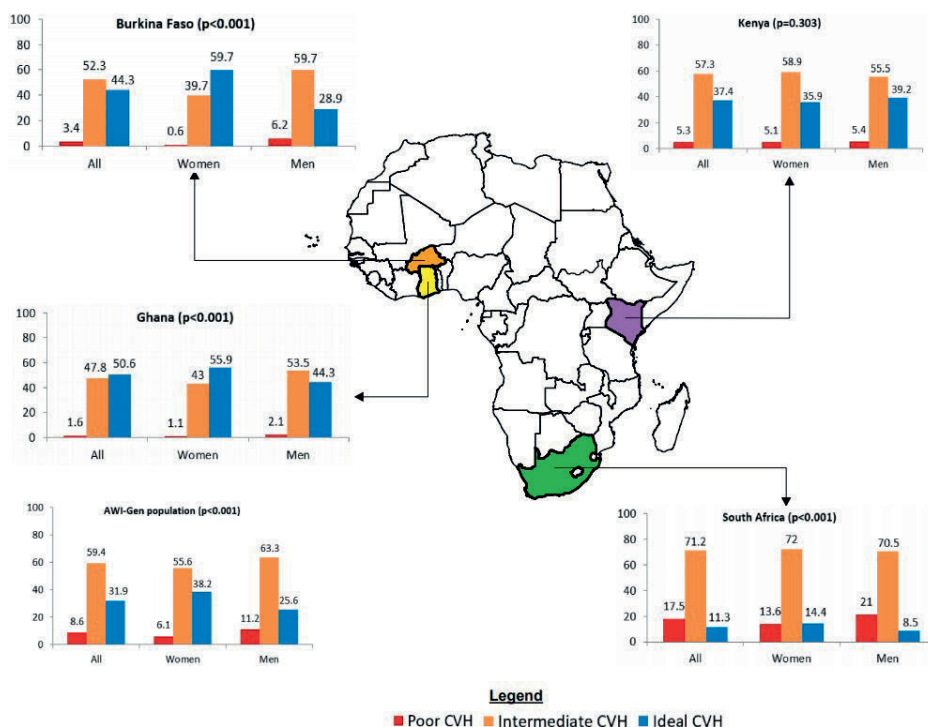

Figure 1: Map of Africa showing the prevalence of levels of cardiovascular health (CVH) for the various countries of the AWI-Gen study and according to sex, AWI-Gen population is the combined participant analysis. *P*-values denote differences between women and men.

Table 2: The distribution of the poor, intermediate, and ideal levels of cardiovascular health for each of the 7 CVHI metrics in the AWI-Gen sample stratified by sex and country

CVHI metrics	Levels	Combined sample	Women	Men	Burkina Faso	Ghana	Kenya	South Africa
Smoking	Poor	984 (10.9)	103 (2.3)	881 (19.7)	124 (6.0)	190 (11.0)	309 (16.2)	361 (11.0)
	Intermediate	1600 (17.8)	73 (1.6)	1527 (34.1)	142 (6.8)	355 (20.5)	230 (12.0)	873 (26.5)
	Ideal	6427 (71.3)	4361 (96.1)	2066 (46.2)	1808 (87.2)	1184 (68.5)	1374 (71.8)	2061 (62.5)
Physical activity	Poor	1355 (15.0)	603 (13.3)	752 (16.8)	402 (19.4)	257 (14.9)	130 (6.8)	566 (17.2)
	Intermediate	442 (4.9)	227 (5.0)	215 (4.8)	63 (3.0)	86 (5.0)	129 (6.7)	164 (5.0)
	Ideal	7214 (80.1)	3707 (81.7)	3507 (78.4)	1609 (77.6)	1386 (80.2)	1654 (86.5)	2565 (77.8)
Healthy diet score	Poor	4266 (56.3)	2359 (56.2)	1907 (56.5)	1324 (82.4)	518 (30.0)	578 (30.2)	1846 (79.4)
	Intermediate	1946 (25.7)	1070 (25.5)	876 (26.0)	271 (16.9)	778 (45.0)	620 (32.4)	277 (11.9)
	Ideal	1361 (18.0)	770 (18.3)	591 (17.5)	12 (0.7)	433 (25.0)	715 (37.4)	201 (8.6)
Body mass index	Poor	1393 (15.5)	1092 (24.1)	301 (6.7)	36 (1.7)	45 (2.6)	375 (19.6)	937 (28.4)
	Intermediate	1673 (18.6)	914 (20.2)	759 (17.0)	177 (8.5)	168 (9.7)	494 (25.8)	834 (25.3)
	Ideal	5943 (66.0)	2529 (55.8)	3414 (76.3)	1861 (89.7)	1516 (87.7)	1043 (54.6)	1523 (46.2)
Blood pressure	Poor	1211 (13.4)	548 (12.1)	663 (14.8)	123 (5.9)	210 (12.1)	216 (11.3)	662 (20.1)
	Intermediate	2204 (24.5)	1006 (22.2)	1198 (26.8)	331 (16.0)	380 (22.0)	445 (23.3)	1048 (31.8)
	Ideal	5596 (62.1)	2983 (65.7)	2613 (58.4)	1620 (78.1)	1139 (65.9)	1252 (65.4)	1585 (48.1)
Fasting glucose	Poor	513 (5.7)	281 (6.2)	232 (5.2)	77 (3.7)	70 (4.0)	127 (6.6)	239 (7.3)
	Intermediate	1203 (13.4)	569 (12.5)	634 (14.2)	305 (14.7)	113 (6.5)	354 (18.5)	431 (13.1)
	Ideal	7295 (81.0)	3687 (81.3)	3608 (80.6)	1692 (81.6)	1546 (89.4)	1432 (74.9)	2625 (79.7)
Total cholesterol	Poor	350 (3.9)	185 (4.1)	165 (3.7)	44 (2.1)	10 (0.6)	107 (5.6)	189 (5.7)
	Intermediate	805 (8.9)	425 (9.4)	382 (8.5)	97 (4.7)	44 (2.5)	282 (14.6)	382 (11.6)
	Ideal	7856 (87.2)	3927 (86.6)	3929 (87.8)	1933 (93.2)	1675 (96.9)	1524 (79.7)	2724 (82.7)

Data presented as n (%); Levels of CVH = cardiovascular health which is defined as poor (CVHI score of 0-7), intermediate (CVHI score of 8-11) and optimum (CVHI score of 12-14)

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There were inverse linear associations between CIMT and CVHI in our combined population (r = −0.064; p<0.001) and for Burkina Faso (r = −0.126; p<0.001), Ghana (r = −0.085; p<0.001), Kenya (r = −0.178; p<0.001) and South Africa (r = −0.199; p<0.001) and these are presented as line plots in Additional file 2: Figs S3 and S4. In the adjusted multilevel mixed-effects generalized linear regression model for the combined population a unit increase in the CVHI was associated with −5.54 [−6.75, −4.32] µm decrease in common CIMT (Figure 2). The risk of atherosclerosis (high common CIMT) was progressively lower with increasing CVHI in each country, sex and household SES category in both the combined sample (Figure 2) and the sex stratified analyses (Figures 3 and 4). A similar inverse association was observed in women (−4.44 [−6.23, −2.65]) and in men (−6.27 [−7.98, −4.64]) (Figures 3 and 4, respectively).
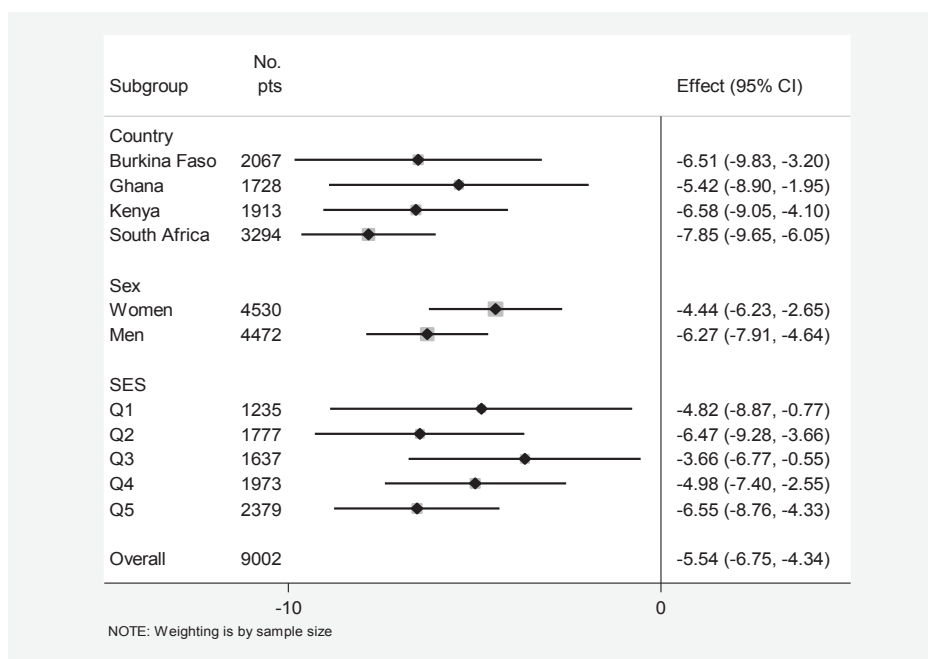

Figure 2: Adjusted* association of cardiovascular health index (CVHI) score with common carotid intima-media thickness (CIMT in µm) in the combined AWI-Gen population stratified by country, sex and socio-economic status (SES) subgroups. Effect is represented by β-coefficients and is a change in mean CIMT (in µm) with each unit increase in the CVHI score (0-14). Wealth quintile defined as Q1 = poorest, Q2 = poorer, Q3 = poor, Q4 less poor and Q5 = least poor. *Adjusted for age, educational status, household SES, HIV+ART, self-reported history of stroke, heart attack, congestive heart failure and chronic kidney disease.
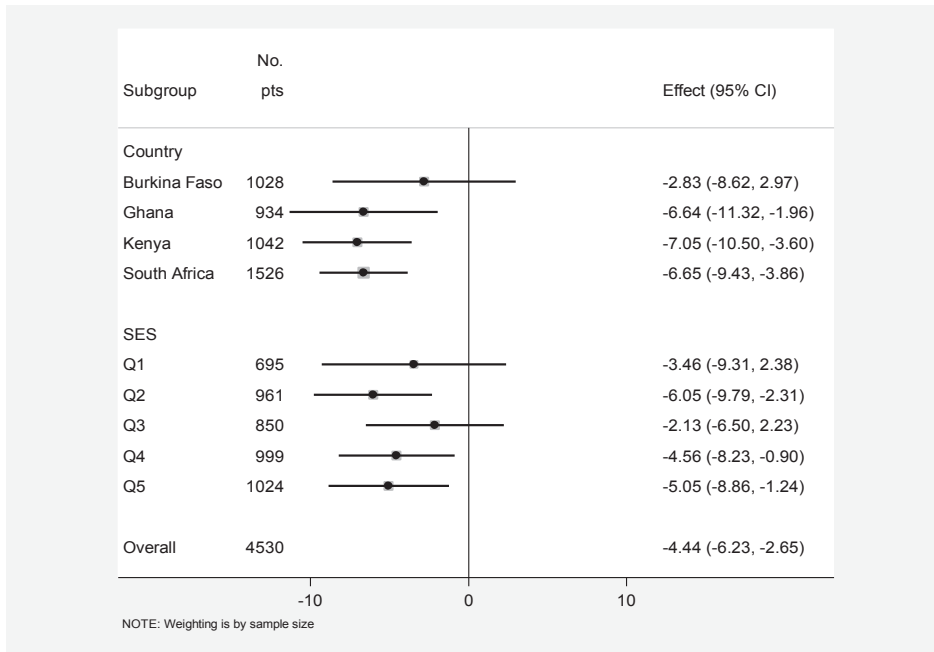
Figure 3: Adjusted* association of cardiovascular health index (CVHI) score with common carotid intima-media thickness (CIMT in μm) in women stratified by country and socio-economic status (SES). Effect is represented by β-coefficients and is a change in mean CIMT (in μm) with each unit increase in the CVHI score (0-14). Wealth quintile defined as Q1 = poorest, Q2 = poorer, Q3 = poor, Q4 less poor and Q5 = least poor. *Adjusted for age, educational status, household SES, HIV+ART, self-reported history of stroke, heart attack, congestive heart failure and chronic kidney disease.
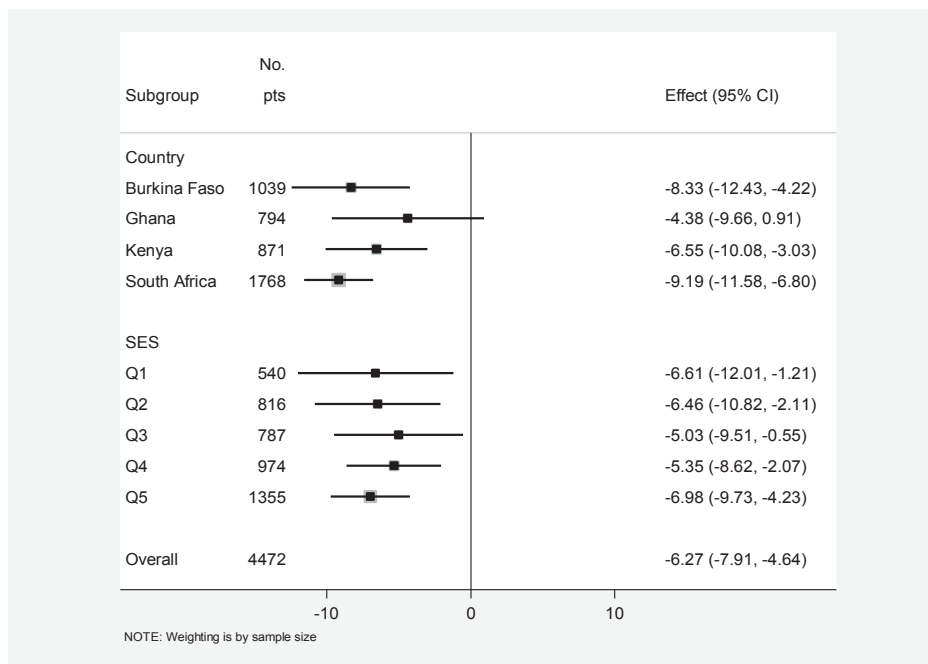
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Figure 4: Adjusted* association of cardiovascular health index (CVHI) score with common carotid intima-media thickness (CIMT in μm) in men stratified by country and socio-economic status (SES). Effect is represented by β-coefficients and is a change in mean CIMT (in μm) with each unit increase in the CVHI score (0-14). Wealth quintile defined as Q1 = poorest, Q2 = poorer, Q3 = poor, Q4 less poor and Q5 = least poor. *Adjusted for age, educational status, household SES, HIV+ART, self-reported history of stroke, heart attack, congestive heart failure and chronic kidney disease.

Sequential adjustments of the regression models are presented in Table 3 and demonstrate minimal effects of adjustment on the β-coefficients. All models demonstrated a significant inverse association between the CVHI and common CIMT, except in women from Burkina Faso and men from Ghana, where the associations were again negative but with the upper 95% CI just overlapping zero. In adjusted analyses looking at the contribution of the individual components of CVHI to common CIMT, we observed that blood pressure, BMI and hyperglycemia were associated with common CIMT in the combined population, whilst smoking and physical activity were associated with common CIMT in women only (see Table 4).

Table 3: Association of CVHI score with common CIMT (µm) in women and men in the combined AWI-Gen sample and by countries

	All countries	Burkina Faso	Ghana	Kenya	South Africa
Combined sample	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Model 1	−7.39 [−8.49, −6.27]	−6.48 [−9.80, −3.16]	−5.42 [−8.89, −1.94]	−6.79 [−9.24, −4.35]	−8.31 [−10.1, −6.52]
Model 2	−7.15 [−7.89, −6.41]	−6.43 [−9.76, −3.09]	−5.46 [−8.94, −1.97]	−6.66 [−9.12, −4.19]	−7.66 [−9.47, −5.85]
Model 3	−7.22 [−8.04, −6.39]	−6.51 [−9.83, −3.19]	−5.42 [−8.91, −1.94]	−6.57 [−9.05, −4.10]	−7.85 [−9.65, −6.05]
Model 4	−7.11 [−7.74, −6.48]	−6.42 [−9.75, −3.09]	−5.51 [−8.99, −2.02]	−6.63 [−9.10, −4.16]	−7.49 [−9.31, −5.68]
Women					
Model 1	−5.98 [−7.44, −4.52]	−2.05 [−7.73, 3.62]	−6.04 [−10.60, −1.46]	−6.81 [−10.2, −3.43]	−6.89 [−9.69, −4.10]
Model 2	−5.85 [−7.00, −4.71]	−2.18 [−7.98, 3.62]	−6.59 [−11.20, −1.96]	−7.05 [−10.50, −3.64]	−6.62 [−9.39, −3.86]
Model 3	−4.87 [−5.69, −4.05]	−2.83 [−8.63, 2.97]	−6.64 [−11.30, −1.96]	−7.05 [−10.50, −3.59]	−6.65 [−9.44, −3.86]
Model 4	−5.93 [−7.02, −4.85]	−2.29 [−8.10, 3.53]	−6.79 [−11.40, −2.15]	−7.16 [−10.60, −3.71]	−6.52 [−9.34, −3.69]
Men					
Model 1	−7.83 [−9.79, −5.86]	−8.62 [−12.7, −4.52]	−4.69 [−10.1, 0.66]	−6.66 [−10.20, −3.15]	−9.36 [−11.70, −7.01]
Model 2	−7.42 [−9.21, −5.63]	−8.25 [−12.4, −4.14]	−4.56 [−9.91, 0.79]	−6.51 [−10.00, −2.99]	−8.84 [−11.20, −6.44]
Model 3	−5.68 [−6.57, −4.80]	−8.33 [−12.4, −4.22]	−4.38 [−9.67, 0.92]	−6.55 [−10.10, −3.02]	−9.19 [−11.60, −6.80]
Model 4	−7.38 [−9.11, −5.66]	−8.18 [−12.3, −4.07]	−4.47 [−9.81, 0.87]	−6.42 [−9.93, −2.91]	−8.71 [−11.10, −6.32]

Effect measured as β-coefficient (change in mean CIMT in µm per unit change in CVHI score); Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, education status, household SES and site; Model 3 adjusted for self-reported history of stroke, heart attack, congestive heart failure and chronic kidney disease in addition to model 2; Model 4 was adjusted for model 2 plus chronic kidney disease and HIV plus antiretroviral therapy (ART) use; Model 1 for women and men were adjusted for only age. CVHI = cardiovascular health index; CIMT = carotid intima-media thickness.

Table 4: Adjusted association of components of the CVHI metric with common CIMT in the AWI-Gen population stratified by sex

CVHI metrics	Levels	All countries		Women		Men	
		β [95%CI]	p-value	β [95%CI]	p-value	β [95%CI]	p-value
Smoking	Poor	ref	ref	Ref	ref	ref	ref
	Intermediate	-1.01 [-13.7,11.7]	0.083	-11.4 [-23.9, 1.15]	<0.001	1.41 [-12.9, 15.7]	0.658
	Ideal	3.28 [-0.09, 7.35]		-7.04 [-10.1, -3.98]		3.83 [-16.7, 9.04]	
Physical activity	Poor	ref	ref	Ref	ref	ref	ref
	Intermediate	-17.5 [-34.9, -0.08]	0.078	-19.8 [-34.0, -5.59]	<0.001	-10.8 [-34.2, 12.6]	0.365
	Ideal	-12.8 [-24.8, -0,78]		-9.28 [-14.1, -4.51]		-12.63 [-30.4, 5.14]	
Diet	Poor	ref	ref	Ref	ref	ref	ref
	Intermediate	1.92 [-4.24, 8.09]	0.169	5.68 [-1.91, 13.3]	0.059	-2.47 [-13.3, 8.33]	0.654
	Ideal	6.75 [-0.24,13.7]		7.55 [-1.08, 16.2]		6.11 [0.32, 11.9]	
Body mass index	Poor	ref	ref	Ref	ref	ref	ref
	Intermediate	-4.06 [-17.4, -2.96]	<0.001	-13.2 [-25.5, -0.94]	<0.001	2.57 [-20.4, 25.6]	<0.001
	Ideal	-28.2 [-44.2, -12.2]		-33.6 [-50.6, -16.6]		-32.7 [-55.1, -10.4]	

Table 4: Adjusted association of components of the CVHI metric with common CIMT in the AWI-Gen population stratified by sex (continued)

CVHI metrics	Levels	All countries β [95%CI]	p-value	Women β [95%CI]	p-value	Men β [95%CI]	p-value
Hypertension	Poor	ref	ref	ref	ref	ref	ref
	Intermediate	-17.7 [-26.0, -9.28]	<0.001	-7.30 [-20.4, -5.75]	<0.001	-23.8 [-27.0, -20.6]	<0.001
	Ideal	-31.4 [-42.4, -20.3]		-20.2 [-39.1, -1.28]		-28.9 [-38.1, -19.7]	
Fasting glucose	Poor	ref	ref	ref	ref	ref	ref
	Intermediate	-18.2 [-35.8, -0.68]	<0.001	-23.3 [-36.8, -9.86]	<0.001	-8.79 [-38.7, 21.1]	0.377
	Ideal	-14.2 [-32.8, -4.42]		-21.8 [-35.2, -8.27]		-0.57 [-29.1, 27.9]	
Total cholesterol	Poor	ref	ref	ref	ref	ref	ref
	Intermediate	-1.89 [-28.3, 24.5]	0.958	5.45 [-15.1, 25.9]	0.542	-15.8 [-43.3, 11.6]	0.265
	Ideal	-0.58 [-28.6, 27.5]		10.53 [-11.2, 32.2]		-17.8 [-41.9, 6.21]	

Adjusted for age, sex, educational status and socio-economic status adjusted for in the combined population while age, educational status and socio-economic status adjusted for in the sex stratified analyses. Effect is represented by β-coefficients and is a change in mean CIMT (in μm) with each unit increase in the CVHI components. CVHI = Cardiovascular heath index

Discussion

Results from this large population-based study of middle-aged men and women from four countries in sub-Saharan Africa highlight relatively high levels of ideal cardiovascular health. We have also shown a significant inverse linear association between increasing CVH score and common CIMT independent of age, educational status, household socio-economic status, HIV infection and ART use and prior history of cardiovascular or chronic kidney diseases. To our knowledge this study is the largest to demonstrate an association between the CVHI metric and subclinical atherosclerosis in an apparently healthy adult population from multiple countries in SSA.

The prevalence of ideal cardiovascular health (iCVH) was 31.9% in the combined AWI-Gen population and this was higher than the 6.8% reported in the multi-ethnic study of atherosclerosis (MESA) conducted among Caucasians, Chinese, African Americans and Hispanics in the United States (25). Similarly, data from the Behavioral Risk Factor Surveillance System study, which used information obtained telephonically from nearly 1.4 million subjects in the United States reported a much lower prevalence of iCVH (5.1%) in 2009 than reported in the current study (28). The iCVH levels among our participants from Ghana was 50.9% and higher than reported by the Research on Obesity and Diabetes among African Migrants (RODAM) study conducted among Ghanaians residing in rural (25.7%) and urban (7.5%) areas and their counterparts in the diaspora (<5%) (29). Further to this a systematic review by Peng et al, 2018 showed that participants from Africa were likely to have higher proportions of ideal CVH compared to North and South Americans, Asians, Europeans and the Oceania's (30). The RODAM study, however, used the comprehensive five dietary components (as described in the original CVHI) to define healthy diet whereas only two components were available for use in the AWI-Gen study. This may therefore result in over classifying AWI-Gen participants into the ideal diet category. This notwithstanding, the broad range of iCVH levels observed in the various settings may be a reflection of the level of ongoing epidemiological transition from communicable to non-communicable diseases. Thus, the iCVH levels were much higher in rural communities in Ghana and Burkina Faso and lower in the more urban settings of Kenya and South Africa and lowest in Europe and the USA.

We observed an inverse association of the iCVH with common CIMT levels independent of age, educational status, socio-economic status, HIV infection and ART use. Similar to our findings, several other studies conducted in Caucasian, African-American and Asian populations have observed increasing CVHI score is associated with lower risk of subclinical atherosclerosis (15, 17, 31-

35). It has been reported that a proportion of residual phenotypic variance is due to the additive effects of genes (36). Although, we did not adjust for the effect of genes in this paper, the Emory Twin Study did report that shared genetic and other familial factors do not confound the association of CVHI with CIMT (15). Our results did not show any significant disparities in the association between CVHI and CIMT across different levels of socioeconomic wealth quintiles as has been previously reported in a multi-ethnic population, including African Americans, in the United States (25). This may be due to the fact that participants in our population did not show significant difference in wealth distribution.

The rising burden of CVD morbidity and mortality in SSA has been reported to occur in younger age groups (1, 37). This, coupled with high levels of unawareness of risk factors and challenges with secondary prevention, calls for the evaluation of simple tools that can be used in routine screening for subjects with poor CVH in primary prevention efforts. The significant independent association of CIMT with the CVHI demonstrated in this study makes a strong case for the utility of the CVHI metric in the routine screening of CVD risk in the broader SSA population irrespective of SES status.

We observed in the combined sample that BMI, blood pressure and hyperglycaemia were the CVHI metrics most strongly associated with common CIMT. The former 2 components were also associated with common CIMT in both women and men whilst physical activity and smoking related with common CIMT in women but not men. This could be due to the fact that women were less likely to smoke compared to men and equally had healthier physical activity levels than men. These findings have implications for prioritizing particular CVHI components for the primary prevention of atherosclerotic cardiovascular diseases in SSA. Although total cholesterol did not correlate with CVHI, cholesterol levels in Africans remain lower than in the Western World. However with urbanisation cholesterol levels are increasing and thus may well contribute in the near future, to atherosclerotic cardiovascular disease in SSA (38).

Our study does have some limitations. Reliance on self-reported smoking, physical activity, HIV infection status and ART use may result in recall and social desirability biases. Diet was ascertained as self-reported servings of fruits and vegetables per day instead of derived from five components (fruits and vegetables per day; portions of fish a week; servings of fibre-rich whole grains per day; sodium per day and sugar-sweetened beverages per week) as recommended by AHA, which could have resulted in misclassification of certain participants in terms of their dietary intake. In addition, the cut points used for classifying each of the components of the CVHI metric were largely based

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on those used in populations of European ancestry. This brings into question whether these cut points are appropriate for use in African populations. However, the cut points used in the CVHI for defining cholesterol, blood pressure, glucose and BMI levels are used globally for the categorisation of these disease markers, and are recognised cut points in the clinical guidelines of many African nations; their use within these countries has not been widely questioned. Other cut points have been challenged for their use within African populations and include those for HDL and triglycerides (39), HbA1c (40) and waist circumference (41), but none of these factors are used within the CVHI metric.

Another possible shortcoming of the use of the CVHI in African populations is the high prevalence of underweight which is classified in the ideal category for BMI but is associated with increased cardiovascular risk. However, a large meta-analysis has shown that low BMI is more strongly associated with respiratory disease than CVD mortality, but it must be noted that this review included few studies from Africa (42). Future studies confirming the applicability of the CVHI criteria to African populations, especially those for BMI, would be important. In the current study it was essential to use the existing CVHI criteria so that we were able to compare our data to those from studies conducted in both African and non-African populations. Despite these limitations, the greatest strength of this work is that it used a large cohort of African individuals from 4 different SSA countries and a standardized data collection protocol, with laboratory assays performed at a single laboratory. In addition, a large number of possible confounding or modulatory variables were measured for use in the multiple regression models. Furthermore, this is the largest African study to use the CVHI to assess cardiovascular health and its association with common CIMT and provides important information on the utility of this metric for screening for individuals at high risk for CVD in resource-poor environments.

Conclusion

The CVHI score has a significant inverse, independent association with common CIMT in older adults across four sub-Saharan African countries. This makes a case for its use as a simple tool to monitor cardiovascular health at a population and clinical level in SSA. The current study demonstrates that primary prevention of atherosclerotic CVDs in SSA should focus on reducing smoking and obesity, improved control of hypertension and hyperglycemia and higher levels of physical activity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-021-01909-6.

Additional file 1: Table S1. Scoring and definition of components of Cardiovascular Health Index

Additional file 2: Figure S1. Kernel density of mean distribution number of ideal cardiovascular health metrics (0–14) by AWI-Gen study countries. **Figure S2.** Kernel density of mean CIMT distribution in μm by AWI-Gen study countries. **Figure S3.** The linear association between CVH score and CIMT in the combined AWI-Gen population. **Figure S4.** The linear association between CVH score and CIMT in the combined four participating countries of the AWI-Gen study.

Abbreviations
AHA/ASA: American Heart Association and the American Stroke Association; AWI-Gen: African Wits-INDEPTH Genomic studies; BMI: Body mass index; CVD: Cardiovascular disease; CIMT: Carotid-Intima media thickness; CVHI: Cardiovascular Health Index; HDSS: Health and Socio-demographic Surveillance System; SSA: Sub-Saharan Africa

Authors' contributions
EAN, NJC, MLB, and KKG conceived the study and designed the analysis plan. EAN conducted the literature search, analyzed the data, and wrote the first draft. MLB, NJC, and KKG contributed to revising the text and writing the paper. EAN, NJC, ARO, GA, LKM, SFM, SSRC, PRB, HS, SMT, SAN, DEG, FJR, MLB, MR, and KKG critically reviewed the paper. MR is overall principal investigator; ARO, SMT, and SAN are site principal investigators of the AWI-Gen study and together with sites investigators EAN, NJC, ARO, GA, LKM, SFM, PRB, HS, SMT, and SAN acquired data and had data oversight. All authors reviewed and approved the manuscript before submitting for publication.

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Funding

Availability of data and materials

The datasets generated and/or analyzed during the current study will be made publicly available in the European Genome-phenome Archive under the set of projects related to the Human Heredity and Health in Africa (H3Africa) Consortium. Details concerning access to data and DNA can be found in the document titled H3Africa Data and Biospecimen Access Committee Guidelines, available in the consortium documents section of the H3Africa website (www. h3africa.com).

Ethics approval and consent to participate

This study received ethical approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Wits) (protocol number M121029) and was renewed in 2017 (protocol number M170880). Additional approvals were obtained from the local ethics review boards in research site prior to commencing any participant-related activities.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Additional file 1

Table S1: Scoring and definition of components of Cardiovascular Health Index

CVHI metric	Levels	Score	Definition
Smoking*	Poor	0	Current
	Intermediate	1	Former
	Ideal	2	Never
Physical activity	Poor	0	No physical activity
	Intermediate	1	1-149mins/week of MVPA[†]
	Ideal	2	≥150mins/week of MVPA[†]
Healthy diet‡ score	Poor	0	< 2 servings per day
	Intermediate	1	2 to 4 servings per day
	Ideal	2	≥ 5 servings per day
Body mass index	Poor	0	≥30 kg/m^2
	Intermediate	1	25 to 29.99 kg/m^2
	Ideal	2	<25 kg/m^2
Blood pressure	Poor	0	SBP ≥140 or DBP ≥90 mm Hg
	Intermediate	1	SBP 120–139 or DBP 80–89 mm Hg or treated with antihypertensive to normal level
	Ideal	2	SBP<120 or DBP<80 mm Hg, without antihypertensive medication
Fasting glucose	Poor	0	≥126 mg/dL
	Intermediate	1	100 to 125 mg/dL or treated with anti-diabetes to normal level
	Ideal	2	<100 mg/dL, without anti-diabetes medication
Total cholesterol	Poor	0	≥240 mg/dL
	Intermediate	1	200 to 239 mg/dL or treated to <200 mg/dL
	Ideal	2	<200 mg/dL, without lipid lowering medication

*Self-reported smoking status was reported as never smoked, previous smoker and current smoker; there was no data on duration of smoking; †MVPA indicates moderate-to-vigorous physical activity; ‡Healthy diet score modified based on available data in the AWI-Gen study on the number of servings of fruits and vegetable per day.

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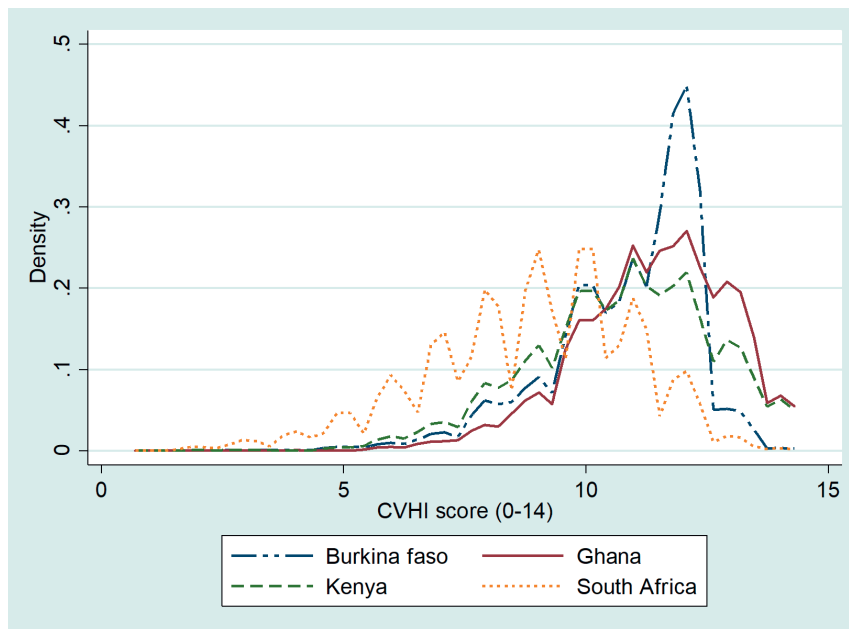
Additional file 2

Figure S1: Kernel density of mean distribution number of ideal cardiovascular health metrics (0-14) by AWI-Gen study countries
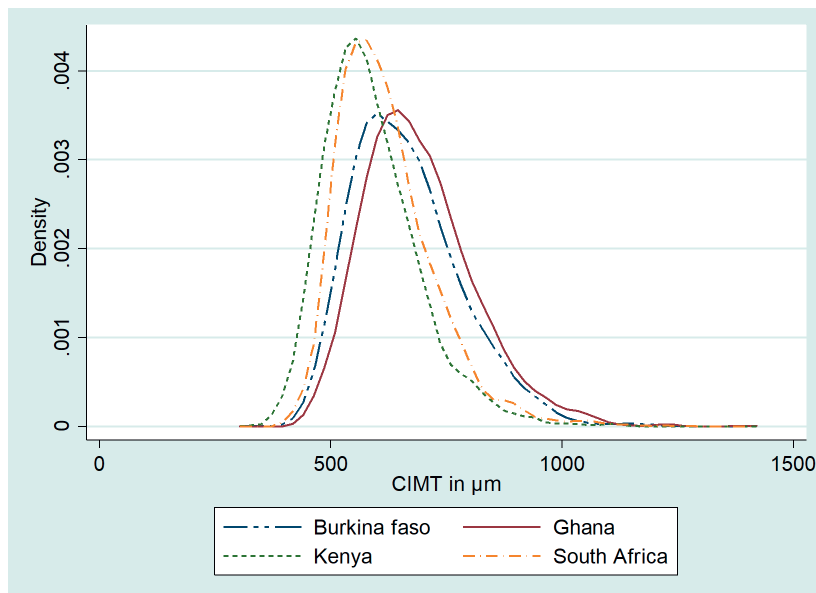

Figure S2: Kernel density of mean CIMT distribution in μm by AWI-Gen study countries
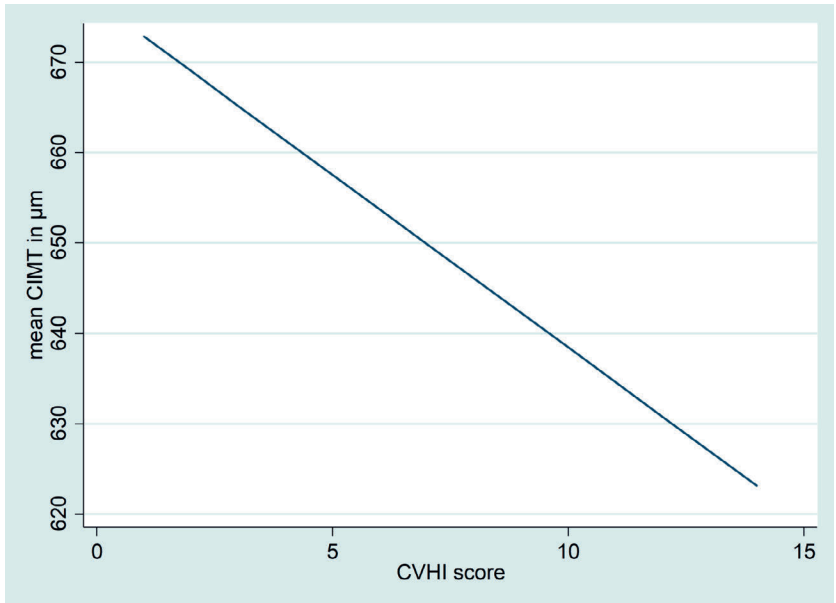
Figure S3: The linear association between CVH score and CIMT in the combined AWI-Gen population
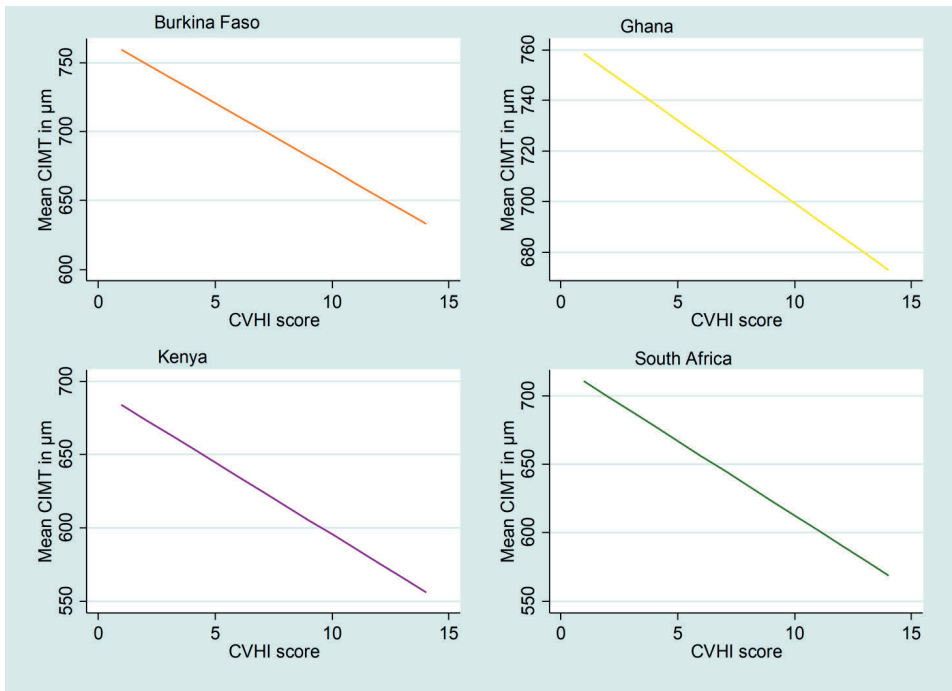
Figure S4: The linear association between CVH score and CIMT in the combined four participating countries of the AWI-Gen study

Chapter 6

Carotid atherosclerosis, microalbuminuria and predicted 10-year atherosclerotic cardiovascular disease risk in sub-Saharan Africa: an H3Africa AWI-Gen study

Nonterah EA, Boateng D, Crowther NJ, Klipstein-Grobusch K, Oduro AR, Agongo G, Mohamed SF, Boua PR, Choma SSR, Norris SA, Tollman SM, Bots ML, Ramsay M and Grobbee DE, for the H3Africa AWI-Gen study.

Abstract

Importance: Carotid atherosclerosis and microalbuminuria predict atherosclerotic cardiovascular disease (ASCVD) but are understudied in sub-Saharan African (SSA).

Objective: We determined the association of these factors with 10-year ASCVD risk in middle-aged Africans.

Design, settings and participants: A cross sectional analyses of baseline data from the African-Wits-INDEPTH genomic study (AWI-Gen). Apparently healthy women and men aged 40-60 years drawn from surveillance sites from Burkina Faso, Ghana, Kenya and South Africa. Additional recruitment was from urban Soweto in Johannesburg, South Africa.

Main outcome and measures: Hypotheses for the analyses formulated after data collection. The main measures were classical cardiovascular risk factors, carotid atherosclerosis assessed using carotid intima-media thickness (CIMT) using B-mode ultrasound and microalbuminuria measured using spot urine albumin (SUA) and urine albumin-creatinine-ratio (uACR). The main outcome being high 10-year ASCVD risk defined as the 2018 Pooled Cohort Equations score ≥7.5. Associations were determined using multivariable logistic regression analysis.

Findings: We studied 9011 participants with an average age of 50 ± 6 years. High CIMT, SUA and uACR were each associated with older age, and high prevalence of both diabetes and hypertension (all associations p<0.01). Smokers were likely to have higher SUA and uACR. Carotid atherosclerosis was common in Burkina Faso and Ghana while microalbuminuria was common in Kenya and South African. The SUA was associated with higher odds of carotid atherosclerosis compared to uACR. Common CIMT, SUA and uACR were strongly associated with 10-year ASCVD risk. CIMT had a higher odds ratio (95% confidence interval) for 10-year ASCVD risk in both women (1.95 [1.78, 2.14]) and men (1.73 [1.55, 1.93]) compared to SUA (1.27 [1.12, 1.43] and 1.40 [1.26, 1.55], respectively) and uACR (1.30 [1.10, 1.54] and 1.30 [1.15, 1.46], respectively).

Conclusion and relevance: The presence of microalbuminuria measured by spot urine albumin may indicate risk of subclinical carotid atherosclerosis and high 10-year CVD risk in middle-aged individuals from SSA. Due to limited regional variation in our findings, SUA may be used to assess CVD risk in both sexes across many African populations. These data must be confirmed in longitudinal studies of cardiovascular events.

Keywords: Carotid atherosclerosis; Microalbuminuria; Pooled Cohort Equation; Atherosclerotic cardiovascular disease risk; sub-Saharan Africa

Introduction

Cardiovascular disease (CVD) is a leading cause of death and disability worldwide (1) with low- and middle-income countries, including the sub-Saharan Africa (SSA) region, currently experiencing 80% of these CVD-related global deaths (2). Most of the CVD-related deaths in SSA occur in younger age groups when compared to the rest of the world (3) posing both health and economic concerns as these individuals represent the more economically productive sectors of society.

Established classical cardiovascular risk factors are major contributors to CVD in many populations. However, these factors alone do not explain the total risk for CVD and several novel factors such as markers of inflammation, atherosclerosis and microalbuminuria have been reported to contribute to CVD risk (4, 5). Common carotid intima-media thickness (CIMT) assessed by B-mode ultrasound is a marker of carotid atherosclerosis which often occurs early in life as a low grade sustained inflammatory process (6, 7) and progresses with age and is augmented by the presence of CVD risk factors. It is an established major precursor to the development of CVD later in life and has the ability to predict future cardiovascular events in the general population (5, 8).

Microalbuminuria has been reported to be an independent predictor of future cardiovascular events such as congestive heart failure, stroke and myocardial infarction (9, 10) especially in high risk groups such as subjects with hypertension or diabetes. However, few studies in high income countries have reported the association of microalbuminuria with CVD in the general population.

Despite being increasingly recognized as risk factors for cardiovascular events, there are few studies in African populations examining the association of microalbuminuria and carotid atherosclerosis with CVD. This is partly due to the difficulty in assessing cardiovascular risk in Africa as a result of the absence of data on clinical events. The PURE and INTERHEART studies are the only studies to have investigated the association of classical risk factors with CVD end points using African populations (11, 12). These studies were limited by their small sample sizes and in the case of the PURE study by selection bias, and neither study examined novel CVD risk factors. Thus, population level data on the association of CVD risk factors with clinical endpoints among individuals free of cardiovascular disease is still largely absent in SSA. This has led to the use of equations to assess the risk of CVD events in SSA populations. A commonly used prediction score is the 2018 revised Pooled Cohort Equation (PCE) which has a high level of accuracy for 10-year ASCVD risk prediction (13). The PCE is

also known to perform very well in ethnic minority groups and included data from a large population of African American subjects (13).

It is important to conduct studies on the association of both classical and novel risk factors with CVD risk because the prevalence of these diseases is increasing across Africa (3) and therefore cost effective methods for assessing the presence of CVD are necessary. Thus, it is important to determine if an easily measured index such as microalbuminuria is associated with carotid atherosclerosis and whether it can be used as an indicator of CVD risk. Furthermore, comparing the ability of CIMT and microalbuminuria to predict CVD will offer new insights on factors that can be used to identify individuals at a high risk of CVD in population level screening programmes. This will also contribute baseline information as a benchmark for future research. Therefore, the aim of this study was to measure the association of microalbuminuria with CIMT and the association of both these variables with predicted 10-year ASCVD risk (using the PCE) in a large population of middle-aged adults drawn from Burkina Faso, Ghana, Kenya and South Africa. A secondary aim was to compare two commonly used methods for assessing microalbuminuria i.e. spot urine albumin (SUA) and urine albumin-creatinine ratio (uACR) for their association with CIMT and 10-year ASCVD risk.

Methods

Study design and population

We conducted a population-based cross-sectional study using the Human Hereditary and Health Africa (H3A) African-Wits-INDEPTH Genomic Study (AWI-Gen) cohort (14, 15). Data were collected from adults residing in health and socio-demographic surveillance sites (HDSS) located in Nanoro (Burkina Faso), Navrongo (Ghana), Nairobi (Kenya) and DIMAMO and Agincourt in South Africa (16). In addition, data was collected from the BT20+ (Birth to Twenty Plus) cohort study run by the Developmental Pathways to Health Research Unit (DPHRU) in Soweto, South Africa (16).

Study participants were adults aged 40-60 years residing in the various study sites and women included were not pregnant at the time of data collection. Details of the recruitment and data collection procedures for AWI-Gen have been published (16). Ineligible participants included those who self-reported a previous diagnosis of CVD (based on an affirmative response to the questions, "Has a doctor or other health professional ever told you that you had coronary heart disease, angina (also called angina pectoris), heart attack (also called myocardial infarction), or stroke?". Participants were also ineligible if their

measured CIMT was greater than 1.5mm, as this was consistent with plaque and not atherosclerosis and so not relevant for this study (17).

Data collection

A structured questionnaire for obtaining information on socio-demographic variables, clinical history and behavioral risk factors was administered by trained researchers. Physical measurements included weight and height for computing body mass index (BMI), waist and hip circumference and blood pressure. Blood samples were used to assess fasting levels of glucose, lipids and insulin while urine samples were used to assess albumin and creatinine. Data collection tools were standardized across sites with centralized training of research coordinators and research officers to minimize variability in the measurements. Community engagement, broad consent as well as individual informed consent were obtained prior to carrying out study procedures. Details of data collection instruments and study procedure have been published elsewhere (16).

Predicted 10-year atherosclerotic cardiovascular disease (ASCVD) risk

Estimated 10-year ASCVD risk was computed using the revised 2018 PCE (13). This risk equation estimates the 10-year absolute risk of ASCVD, defined as death due to coronary heart disease or nonfatal myocardial infarction or fatal or nonfatal stroke over a 10-year period in people free of established CVD. The PCE permits the derivation of sex- and race-specific estimates of the 10-year risk for ASCVD for adults aged 40–79 years (which falls within the age category used by the AWI-Gen study of 40-60 years). Variables in the PCE include age, sex, black race, tobacco smoking, total and high-density lipoprotein cholesterol, treated or untreated systolic blood pressure and diabetes status. A PCE score ≤7.5% classifies an individual participant at a "high ASCVD risk" while a score above 7.5 is low risk (18).

Age and sex were self-reported at the time of data collection. Smoking was self-reported as cigarette use in the twelve months prior to data collection. Blood pressure was measured with the participant seated and with the arm resting on a chair at the level of the heart. An appropriate sized cuff was then applied and three readings taken with the first discarded while an average of the last two was used as the blood pressure reading. Fasting blood samples were taken for the measurement of fasting glucose and lipid fractions. These measurements were performed in a single laboratory using standardized procedures. The coefficient of variation of the glucose and lipid measurements ranged between 0.2% and 1.5%. Diabetes status was defined as fasting glucose levels ≥7mmol/l and/or known diabetic on medication at the time of data collection.

Carotid atherosclerosis

Common carotid intima-media thickness (CIMT)
A LOGIQ e B-mode ultrasound system (GE Healthcare, Chicago, IL, USA) with a 12L-RS straight transducer was used to measure right and left intima-media thickness of the common carotid arteries (CCA). The IMT was measured within a 10mm segment of the CCA starting from 1cm away from the bulb. Measurements were taken of minimum, maximum and average distance in millimeters to two decimal places. Centralized training of selected staff (including nurses, clinicians and researchers) from study sites minimized measurement variability and reduced errors. Common CIMT ≥ 0.90mm was used as the cut-point for diagnosing carotid atherosclerosis (19-22).

Microalbuminuria

Spot urine albuminuria (SUA)
Midstream urine samples (10–15 ml voided into a sterile collection pot) were collected in accordance with the procedures followed by the World Health Organization (WHO) (16). Samples were centrifuged at 1500–2000 x g for 5 minutes and the supernatant aliquoted into cryovials for storage at -80°C until analysis. Albumin concentrations in urine samples were determined using a turbidimetric assay on a Cobas 501 autoanalyser (Roche Diagnostics, Manheim, Germany). The measuring range was 3–400 mg/l for albumin, and the coefficient of variation was less than 2.7%. The use of spot urine as opposed to 24-hour urine collection or albumin-creatinine-ratio is previously validated in the general population (23, 24). Microalbuminuria was defined as spot urine albumin concentration ≥20mg/L (or 20-400mg/L within the confines of the measuring range of the study population) (25).

Urine albumin-creatinine ratio (uACR):
Urinary albumin concentration was measured with immunoturbidimetry as described above and urine creatinine was measured by an IDMS-traceable Jaffe method on the Cobas 501 instrument (Roche Diagnostics, Mannheim, Germany). The measuring range for the urine creatinine assay was 3.75– 550 mmol/l with a coefficient of variation of 2.8%. Urine ACR of more than 3 mg/mmol was defined as albuminuria (25, 26).

Statistical analyses
Characteristics of the AWI-Gen participants are described using counts (n) and proportions (%) for categorical data and means ± standard deviations for the continuous data (all were normally distributed). The results were stratified by CIMT and microalbuminuria cut points. Differences between these groups were

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determined using Pearson's Chi squared test for categorical data and Student t-test for continuous data.

To determine the association between microalbuminuria and CIMT mixed-effect logistic regression analysis was performed using country as a random effect. This was done for the combined sample and for women and men. Adjustments were made for classical CVD risk factors (age, sex except in the sex stratified analyses, smoking, BMI, systolic blood pressure, total cholesterol, HDL). Associations are reported as odds ratios with 95% confidence intervals (CI) and were presented as forest plots.

The association of carotid atherosclerosis and microalbuminuria with 10-year atherosclerotic cardiovascular disease (ASCVD) risk was determined using adjusted logistic regression analyses. Due to differences in their units of measurements, we first generated sex and country specific z-scores for common CIMT, spot urine and urine albumin-creatinine-ratio thus allowing direct comparison of the odds ratios. The z-scores were calculated using the following equation: $(x-\mu)/\sigma$ where x is the raw, individual value, μ is the population mean, and σ is the population standard deviation. As per the recommendations of the American Heart Association (AHA), a high 10-year ASCVD risk is defined as a PCE score ≥7.5%. We therefore generated a binary form of PCE and applied adjusted logistic regression analyses to determine the relationship of CIMT, SAU and uACR with high 10-year CVD risk. Adjustments were made in a sequential manner using the following variables sex (except in sex stratified analyses), educational level, household socio-economic status, body mass index (BMI), and participating country (except in the country specific analyses). The factors used for adjustment of the regression models were socio-demographic factors, which have been demonstrated to be associated with CVD, and BMI which is a well-recognized risk factor for CVD. The Akaike/Bayesian Information Criteria (AIC/BIC) were used to compare the models and the model with the lowest value was considered a better model. This was further confirmed using the Hosmer-Lemeshow (H-L) goodness of fit test. Specifically the test determines whether the observed odds ratios (with 95%CI) match the expected odds ratios in the population and population sub-groups (countries and sex specific subgroups) and a *p>0.05* was considered a good fit.

For the country level comparisons of the strength of association of CIMT, SUA and uACR z-scores with high 10-year CVD risk across, we further conducted an inverse variance (IV) logistic regression meta-analysis. The best fitted model (fully adjusted model i.e. model 3) was used for these country and sex comparative analyses. The adjusted odds ratios are presented as forest plots with the within group variance measured using I-squared (I^2) with a high

score and p<0.05 indicating high within group variance. Heterogeneity was measured to assess differences between study groups with a p>0.05 indicating low heterogeneity between groups. The statistical significance for all inferential statistics was set at a two-tailed *p<0.05*. All analyses were conducted using STATA v14.2 (StataCorp, College Station, TX, USA).

Ethical clearance
The AWI-Gen study received ethics approval from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa (ethics approval identification number: M121029, renewed in 2017 with number: M170880). Ethics approvals were also obtained from relevant ethics bodies in the participating sites and written informed consent obtained from all participants prior to recruitment.

Data availability
The AWI-Gen data used in the current study can be accessed from the European Genome-phenome Archive (www.ega459archive.org/datasets/ EGAD00001006425) with phenotype dataset accession ID of EGA00001002482.

Results

Study population basic characteristics
Presented in Table 1 is a comparison of the characteristics of the 9010 study participants according to carotid atherosclerosis and microalbuminuria status. Higher levels of education were associated with lower levels of atherosclerosis but higher levels of microalbuminuria while socio-economic status did not differ. Ghana and Burkina Faso had higher levels of carotid atherosclerosis than those from Kenya and South Africa. Kenya and South Africa had higher levels of microalbuminuria than Burkina Faso and Ghana. Participants with carotid atherosclerosis (high CIMT) and microalbuminuria (high SUA or high uACR) were more likely to be diabetic or hypertensive. The percentage of participants with high ASCVD risk (PCE ≥7.5) was significantly higher in the subject groups with high CIMT, SUA and uACR levels (Table 1).

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Table 1: Distribution of socio-demographic and cardiometabolic variables by carotid atherosclerosis and microalbuminuria

Variables	Carotid atherosclerosis			Spot urine albuminuria			Albumin-creatinine-ratio		
	Normal CIMT	High CIMT*	p-value	Normal SUA	High SUA†	p-value	Normal ACR	High ACR‡	p-value
Age in years	50 ± 6	55 ± 5	<0.001	50 ± 6	51 ± 6	0.007	50 ± 6	51 ± 6	<0.001
BMI, kg/m2	24.1±6.1	24.3±6.2	0.616	24.8±6.0	24.8±6.1	0.947	24.8±6.4	25.1±6.7	0.244
Household SES:									
Q1	1185 (13.8)	32 (12.2)	0.466	368 (14.5)	130 (11.6)	0.182	394 (14.1)	84 (11.9)	0.516
Q2	1695 (19.7)	53 (20.2)		531 (20.9)	255 (22.8)		590 (21.1)	154 (21.8)	
Q3	1597 (18.4)	40 (15.3)		455 (17.9)	200 (17.9)		482 (17.3)	135 (19.1)	
Q4	1889 (22.0)	57 (21.8)		541 (21.2)	235 (21.0)		598 (21.4)	150 (21.2)	
Q5	2259 (26.3)	80 (30.5)		652 (25.6)	297 (26.6)		731 (26.2)	184 (26.0)	
Highest level of education:									
No formal education	3335 (38.8)	146 (55.9)	<0.001	909 (35.7)	358 (32.1)	0.029	995 (35.6)	216 (30.6)	0.033
Primary	2493 (29.0)	61 (23.4)		793 (31.2)	387 (34.7)		875 (31.3)	245 (34.7)	
Secondary	2449 (28.5)	46 (17.6)		740 (29.1)	339 (30.4)		811 (29.0)	224 (31.7)	
Tertiary	319 (3.7)	8 (3.1)		104 (4.1)	33 (3.0)		113 (4.0)	22 (3.1)	
Country:									
Burkina Faso	1962 (22.8)	82 (31.3)	<0.001	583 (22.9)	175 (15.7)	<0.001	631 (22.6)	100 (14.1)	<0.001
Ghana	1632 (19.0)	91 (34.7)		234 (9.2)	151 (13.5)		270 (9.7)	96 (13.6)	
Kenya	1886 (21.9)	22 (8.4)		678 (26.5)	272 (24.4)		738 (26.4)	165 (23.3)	
South Africa	3131 (36.4)	67 (25.6)		1056 (41.4)	519 (46.5)		1158 (41.4)	346 (48.9)	
Components of PCE:									
Smoking	1541 (17.9)	39 (14.9)	0.210	407 (16.0)	210 (18.8)	0.035	456 (16.3)	138 (19.5)	0.042
SBP in mmHg	123 ± 22	133 ± 25	<0.001	125 ± 22	134 ± 27	<0.001	125 ± 22	134 ± 27	<0.001

Table 1: Distribution of socio-demographic and cardiometabolic variables by carotid atherosclerosis and microalbuminuria (continued)

Variables	Carotid atherosclerosis			Spot urine albuminuria			Albumin-creatinine-ratio		
	Normal CIMT	High CIMT*	p-value	Normal SUA	High SUA†	p-value	Normal ACR	High ACR‡	p-value
Hypertension on medication	946 (11.0)	46 (17.6)	0.001	356 (14.0)	213 (19.1)	<0.001	398 (14.2)	137 (19.4)	0.001
Diabetes	531 (6.2)	29 (11.1)	<0.001	171 (6.7)	128 (11.5)	<0.001	181 (6.5)	99 (13.9)	<0.001
Total cholesterol in mg/dl	147.3 ± 43.2	145.4 ± 46.5	0.489	151.6 ± 43.0	154.0 ± 44.8	0.114	151.3 ± 42.9	155.5 ± 45.6	0.025
HDL-C in mg/dl	46.0 ± 16.1	43.5 ± 14.2	0.013	46.4 ± 16.4	47.8 ± 19.4	0.021	46.6 ± 16.9	47.3 ± 18.8	0.320
10-year CVD risk ³ 7.5	912 (10.6)	69 (26.3)	<0.001	319 (12.5)	262 (23.5)	<0.001	345 (12.3)	195 (27.6)	<0.001

Data presented as mean ± standard deviation (SD) or number and percentage (%); uACR, urine albumin-creatinine-ratio; BMI, body mass index; CIMT, common carotid intima-media thickness; CVD, cardiovascular disease; HDL, High density lipoprotein; LDL, Low density lipoprotein; PCE, Pooled Cohort Equation; SBP, Systolic blood pressure; SUA, Spot urine albuminuria and SES, socio-economic status in wealth quintile Q1–Q5; *High CIMT defined as common CIMT ≥ 0.9mm; †Spot urine albuminuria defined as spot urine albumin concentration ≥ 20mg/L and ‡uACR > 3 mg/mmol.

Association of microalbuminuria with carotid intima-media thickness

The associations of microalbuminuria with CIMT in the combined sample and in women and men are presented in Figure 1. In the combined population high SUA displayed a significant odds ratio for subclinical atherosclerosis (adjusted odds ratio 1.77, 95% confidence interval [1.04, 3.01]) in the combined population but this was not significant in women and men. The uACR was not associated with a higher risk for elevated CIMT in women and men or in the combined population (see Figure 1). There was significant within country variance in the measured SUA and uACR in the combined population and in men with minimal variance in women. There was no heterogeneity between women and men (p=0.895). The country stratified analyses are presented in supplementary Figure S1.
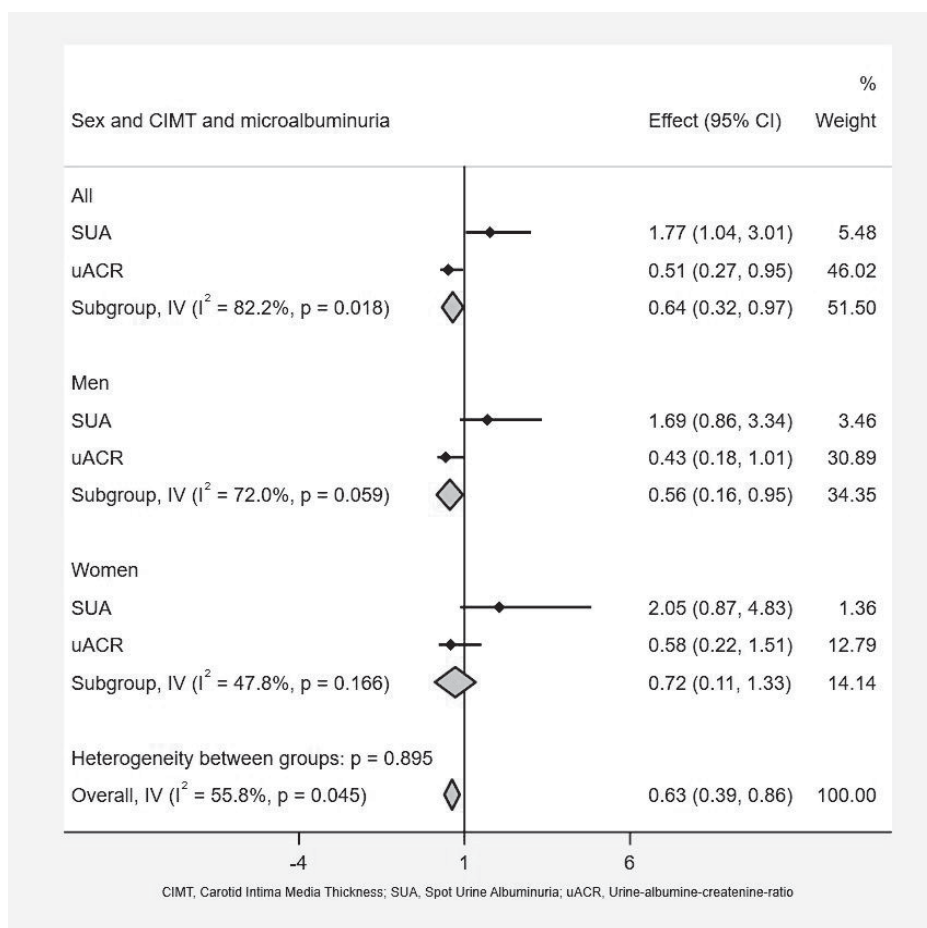

Sex and CIMT and microalbuminuria	Effect (95% CI)	% Weight
All		
SUA	1.77 (1.04, 3.01)	5.48
uACR	0.51 (0.27, 0.95)	46.02
Subgroup, IV (I^2 = 82.2%, p = 0.018)	0.64 (0.32, 0.97)	51.50
Men		
SUA	1.69 (0.86, 3.34)	3.46
uACR	0.43 (0.18, 1.01)	30.89
Subgroup, IV (I^2 = 72.0%, p = 0.059)	0.56 (0.16, 0.95)	34.35
Women		
SUA	2.05 (0.87, 4.83)	1.36
uACR	0.58 (0.22, 1.51)	12.79
Subgroup, IV (I^2 = 47.8%, p = 0.166)	0.72 (0.11, 1.33)	14.14
Heterogeneity between groups: p = 0.895		
Overall, IV (I^2 = 55.8%, p = 0.045)	0.63 (0.39, 0.86)	100.00

CIMT, Carotid Intima Media Thickness; SUA, Spot Urine Albuminuria; uACR, Urine-albumine-createnine-ratio

Figure 1: Association of microalbuminuria with CIMT in the combined study populations and in women and men; IV, inverse variance

Association of carotid atherosclerosis and microalbuminuria with high 10-year ASCVD risk

Presented in Table 2 (combined population) is the association of carotid atherosclerosis or microalbuminuria with high 10-year ASCVD risk, stratified by sex. These associations were determined through sequential model building. In the combined population common CIMT and both measures of microalbuminuria were associated with 10-year ASCVD risk in all the models (for women and men). Thus, in model 3 for the combined population, common CIMT had higher odds of 10-year ASCVD risk (odds ratio 1.85, 95% confidence interval [1.75, 1.99]) compared to SUA (1.34 [1.24, 1.45]) and uACR (1.30 [1.18, 1.43]). This observed pattern was similar in women and men (see Table 2). The fully adjusted models (model 3) had lower AIC/BIC values indicating that they fitted the data better. This was further confirmed by the Hosmer-Lemeshow (H-L) test which showed p-values greater than 0.05 for carotid atherosclerosis and microalbuminuria in the combined population and for women and men (Table 2).

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Table 2: Association of CIMT and microalbuminuria with high ASCVD 10-year risk in combined study populations and in women and men

Models	Common CIMT z-score			SUA z-score			uACR z-score		
	OR [95% CIs]	p-value	AIC/BIC	OR [95% CIs]	p-value	AIC/BIC	OR [95% CIs]	p-value	AIC/BIC
All*									
Model 1	1.55 [1.46, 1.65]	<0.001	5973.58	1.29 [1.20, 1.39]	<0.001	3163.38	1.27 (1.16, 1.39)	<0.001	2983.43
Model 2	1.93 [1.80, 2.07]	<0.001	5264.00	1.32 [1.22, 1.42]	<0.001	2908.98	1.28 (1.16, 1.40)	<0.001	2752.09
Model 3	1.85 [1.73, 1.99]	<0.001	5196.41	1.34 [1.24, 1.45]	<0.001	2809.06	1.30 (1.18, 1.43)	<0.001	2653.46
H-L Chi² (p-value)	7.63 (0.470)			6.46 (0.578)			6.83 (0.555)		
Women‡									
Model 1	1.62 [1.50, 1.75]	<0.001	3596.79	1.24 [1.11, 1.38]	<0.001	1929.75	1.22 [1.06, 1.42]	0.006	1797.27
Model 2	2.01 [1.83, 2.20]	<0.001	3214.92	1.21 [1.08, 1.36]	0.001	1811.38	1.23 [1.05, 1.44]	0.010	1689.03
Model 3	1.95 [1.78, 2.14]	<0.001	3157.65	1.27 [1.12, 1.43]	<0.001	1750.11	1.30 [1.10, 1.54]	0.002	1624.27
H-L Chi² (p-value)	5.72 (0.678)			11.84 (0.159)			10.70 (0.219)		
Men‡									
Model 1	1.52 [1.38, 1.68]	<0.001	2245.76	1.43 [1.30, 1.57]	<0.001	1153.68	1.34 [1.19, 1.52]	<0.001	1124.66
Model 2	1.83 [1.64, 2.03]	<0.001	2058.06	1.40 [1.27, 1.55]	<0.001	1107.17	1.30 [1.52, 1.47]	<0.001	1076.01
Model 3	1.73 [1.55, 1.93]	<0.001	2052.30	1.40 [1.26, 1.55]	<0.001	1079.40	1.30 [1.15, 1.46]	<0.001	1049.15
H-L Chi² (p-value)	13.35 (0.100)			8.55 (0.382)			8.66 (0.372)		

* Model 1 unadjusted model; model 2, adjusted for sex, education, country; model 3, adjustments as for model 2 + household socio-economic status, BMI.
‡Model 1 unadjusted model; model 2, adjusted for education, country; model 3, adjustments as for model 2 + household socio-economic status, BMI.
OR, odds ratio; BMI, body mass index; CIMT, carotid intima-media thickness; uACR, urine albumin-creatinine-ratio; SUA, spot urine albuminuria and H-L Chi² test is the Hosmer-Lemeshow test of goodness of fit for the model

The fully adjusted model was thus used in meta-analyses to compare associations of carotid atherosclerosis and microalbuminuria with 10-year ASCVD risk for the various countries stratified by sex (Figures 2 and 3). These analyses show that across all countries microalbuminuria and carotid atherosclerosis were significantly associated with 10-year ASCVD risk in women and men. However the magnitude of these associations differs between the countries and for women and men. In most instances, the strength of association of CIMT with ASCVD risk was higher than for SUA and uACR with few exceptions. Men from Ghana had higher odds of ASCVD risk from SUA than CIMT and uACR. In women from Kenya uACR presented with a higher odds of ASCVD risk than CIMT and SUA. The within country variance for women was higher for measured SUA (I^2 = 74.6%, p=0.008) with the other measures not showing any significant variance across the countries. Among men there was no significant within country variance in the measures for carotid atherosclerosis and microalbuminuria. The heterogeneity between the z-scores for SUA, uACR and CIMT was significant in women (p<0.001) and men (p=0.002).
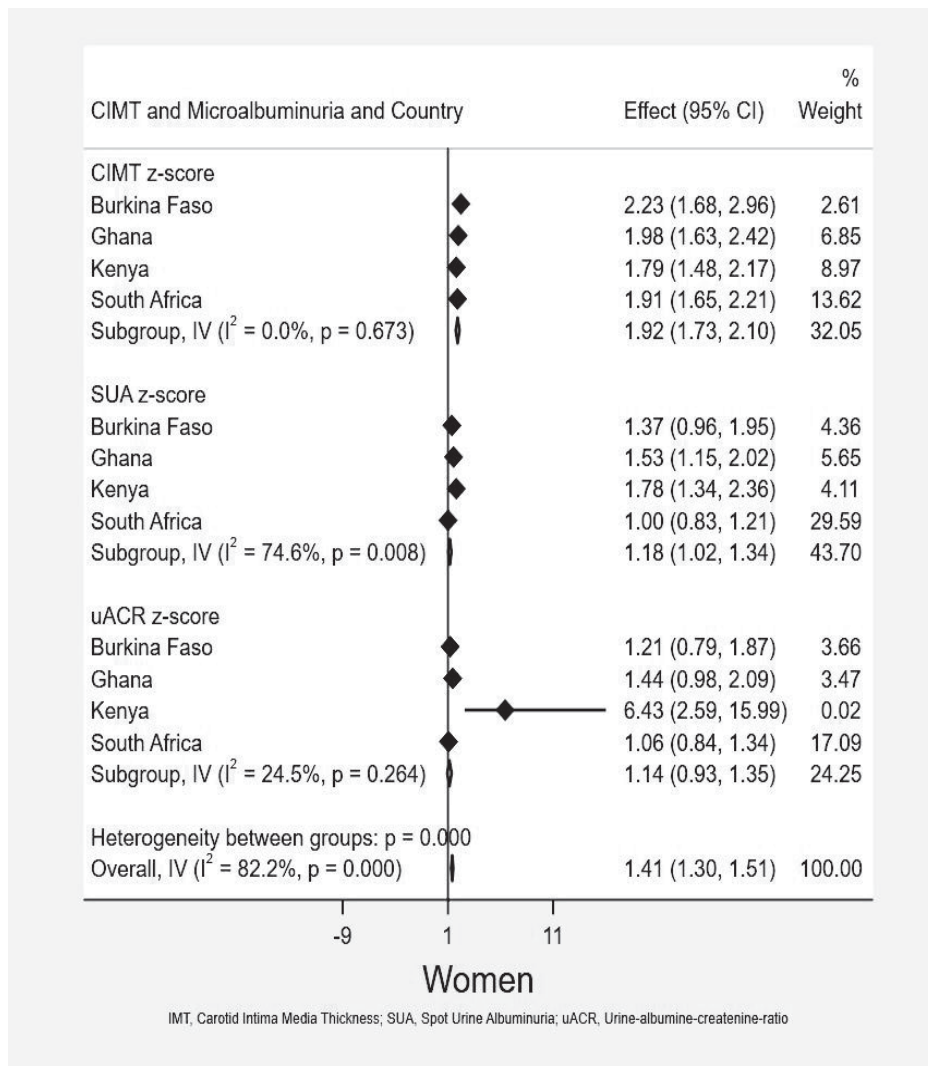
Figure 2: Meta-analyses of the comparative association of carotid atherosclerosis (CIMT) and microalbuminuria (SUA and uACR) with 10-year ASCVD risk in women from the four countries. CIMT, carotid intima-media thickness; IV, inverse variance; SUA, spot urine albuminuria and uACR, urine albumin-creatinine-ratio
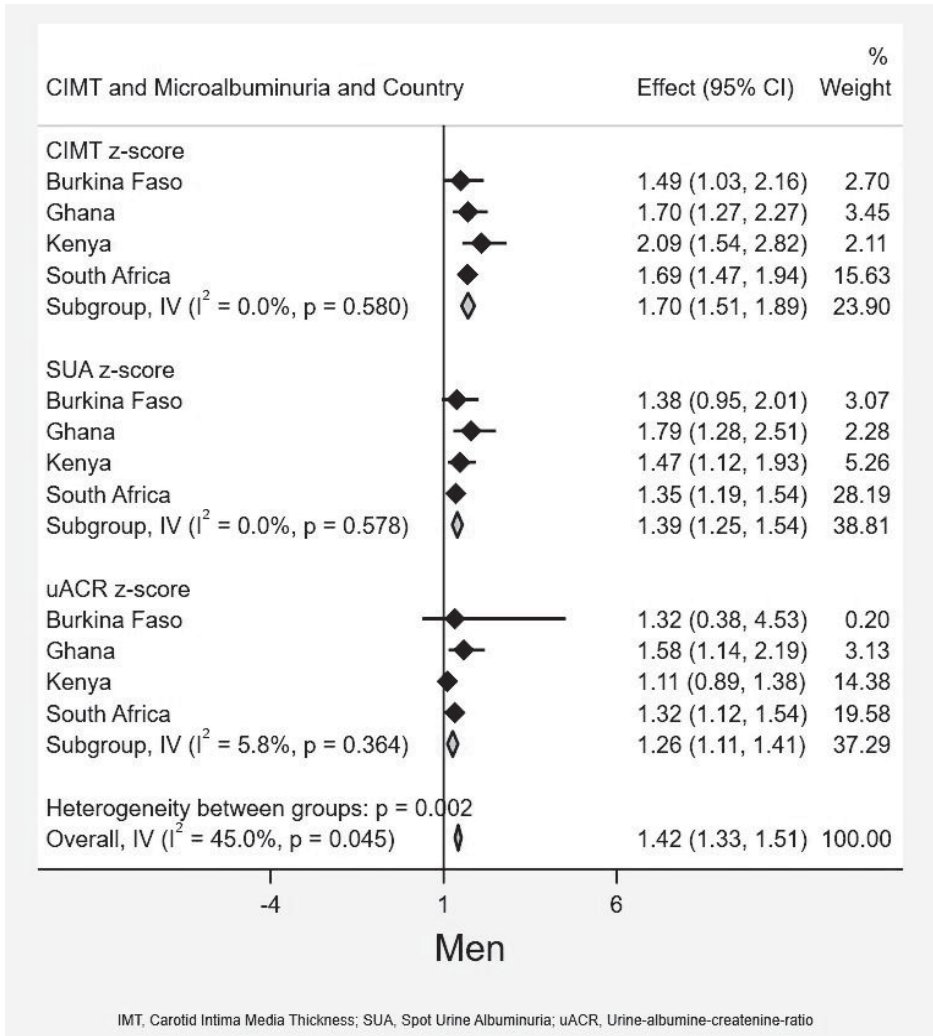
CIMT and Microalbuminuria and Country	Effect (95% CI)	% Weight
CIMT z-score		
Burkina Faso	1.49 (1.03, 2.16)	2.70
Ghana	1.70 (1.27, 2.27)	3.45
Kenya	2.09 (1.54, 2.82)	2.11
South Africa	1.69 (1.47, 1.94)	15.63
Subgroup, IV (I^2 = 0.0%, p = 0.580)	1.70 (1.51, 1.89)	23.90
SUA z-score		
Burkina Faso	1.38 (0.95, 2.01)	3.07
Ghana	1.79 (1.28, 2.51)	2.28
Kenya	1.47 (1.12, 1.93)	5.26
South Africa	1.35 (1.19, 1.54)	28.19
Subgroup, IV (I^2 = 0.0%, p = 0.578)	1.39 (1.25, 1.54)	38.81
uACR z-score		
Burkina Faso	1.32 (0.38, 4.53)	0.20
Ghana	1.58 (1.14, 2.19)	3.13
Kenya	1.11 (0.89, 1.38)	14.38
South Africa	1.32 (1.12, 1.54)	19.58
Subgroup, IV (I^2 = 5.8%, p = 0.364)	1.26 (1.11, 1.41)	37.29
Heterogeneity between groups: p = 0.002		
Overall, IV (I^2 = 45.0%, p = 0.045)	1.42 (1.33, 1.51)	100.00

-4   1   6

Men

IMT, Carotid Intima Media Thickness; SUA, Spot Urine Albuminuria; uACR, Urine-albumine-createnine-ratio

Figure 3: Meta-analyses of the comparative association of carotid atherosclerosis (CIMT) and microalbuminuria (SUA and uACR) with 10-year ASCVD risk in men from the four countries. CIMT, carotid intima-media thickness; IV, inverse variance; SUA, spot urine albuminuria and uACR, urine albumin-creatinine-ratio

chapter 6

Discussion

This study determined the association between microalbuminuria (measured by SUA and uACR) and carotid atherosclerosis (as represented by CIMT) and their association with high 10-year ASCVD risk in Burkina Faso, Ghana, Kenya and South Africa. The present study demonstrated that microalbuminuria is associated with CIMT independent of the traditional CVD risk factors. We also demonstrated that carotid atherosclerosis and microalbuminuria are associated with high 10-year ASCVD risk in middle-aged adults.

We showed differences in the prevalence of carotid atherosclerosis and microalbuminuria between the various countries. Notably, participants from Kenya and South Africa reported high levels of microalbuminuria compared to those from Burkina Faso and Ghana while the opposite was observed for common CIMT. The CIMT results are consistent with a previous study involving this study population (27). The possible reason for the higher prevalence of microalbuminuria in Kenya and South Africa may be due to the higher prevalence of cardiometabolic diseases and HIV infection. The higher prevalence of alcohol use in Burkina Faso and Ghana may result in chronic liver disease and hence low circulatory serum albumin and creatinine levels. Using albumin-creatinine-ratio with a similar cut off to that used in the current study to define microalbuminuria, two studies involving HIV-exposed and HIV-naïve individuals conducted in rural South Africa and Hawaii observed HIV as an important predictor of microalbuminuria (28, 29). The pathophysiological basis for this observation involves HIV-nephropathy and HIV-associated immune complex kidney disease leading to increased leakiness of nephrons to molecules such as creatinine and albumin (30, 31).

We observed that independent of the classical CVD risk factors, SUA had a stronger association than uACR with CIMT. Some studies have reported an association between microalbuminuria (measured with urine albumin and ACR) and subclinical atherosclerosis independent of traditional CVD risk factors (32-34). The underlying mechanisms for the observed association remain unclear. However, it has been reported that vascular endothelial damage may cause atherosclerosis and albuminuria (10, 35). Endothelial dysfunction has been shown to increase vascular leakiness in target vessels. If structural changes occur in the endothelium of the glomerular capillaries there may be increased permeability in the glomerular barrier leading to increased excretion of albumin into the urine (36). The non-significant association of uACR with CIMT warrants further investigation

Carotid atherosclerosis and microalbuminuria were associated with high 10-year ASCVD risk in women and men. Large epidemiological studies such as the Kuopio Ischaemic Heart Disease Risk Factor Study, Atherosclerosis Risk in Communities (ARIC) and the Cardiovascular Health Study have observed that increased CIMT was associated with a higher incidence of myocardial infarction and stroke independent of classical CVD risk factors (21, 37-39). The Rotterdam and USE-IMT studies recently reported a significant association of CIMT with CVD and related risk factors (40, 41). While CIMT and microalbuminuria are strong independent predictors of cardiovascular disease, microalbuminuria is more apparent in individuals with hypertension and diabetes. Our data demonstrates that diabetes, use of anti-hypertensive medication and systolic blood pressure, which are each components of the PCE, are all higher in subjects with microalbuminuria as defined by either SUA or uACR. This is in line with previous studies that reported microalbuminuria was strongly associated with CVD in subjects with hypertension or diabetes (42). This was supported by an observational study that demonstrated that microalbuminuria occurs in approximately 5-15% of the general elderly population, in 11-17% of hypertensive individuals, and approximately 20-30% of individuals with type 2 diabetes (42). Among diabetics, biochemical alterations to the extracellular matrix may lead to glomerulosclerosis and premature atherosclerosis as well as insulin resistance leading to endothelial structural and functional damage which occurs relatively late in the atherothrombotic disease course and is strongly associated with advanced age (43).

Strengths and limitations: Our study presents with certain limitations such as the cross sectional design which prevents us from establishing causality. Social desirability and recall bias may also arise from the self-reported responses to certain questions such as educational status. The absence of clinical event data also affects the observed associations and hence the results must be interpreted with caution. These notwithstanding, there are a number of strengths of this study: the use of z-scores maximizes statistical power of the analyses and limits the non-centrality parameter of the statistic. We are thus able to compare the magnitude of the association of carotid atherosclerosis and microalbuminuria with 10-year CVD risk. The updated 2018 PCE equation has an improved accuracy among all races and sex subgroups (13). The use of a centralized laboratory to measure the biochemical measures and centralized training for the measurement of CIMT minimizes data variability.

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Conclusions

The presence of spot urine microalbuminuria may indicate risk of subclinical atherosclerosis and high 10-year ASCVD risk in middle-aged individuals from SSA. These data suggest that spot urine measures of albumin may be used within population studies to assess CVD risk in both genders and in the majority of countries included in this study. However, these data must be confirmed in longitudinal studies of cardiovascular events in African populations.

Disclosure
The authors have no conflict of interest to declare

Author contribution
EAN, NJC, KKG, ARO, MLB and DEG contributed to conception of the study. EAN, NJC, KKG and MLB designed the analyses plan. EAN and DB conducted the analyses and EAN drafted the manuscript with contributions from DB, NJC, MLB, KKG, ARO and DEG. EAN, ARO, NJC and MR are investigators of the H3Africa AWI-Gen study and contributed to data collection and quality control. All authors critically reviewed and approved the final version of the manuscript for submission.

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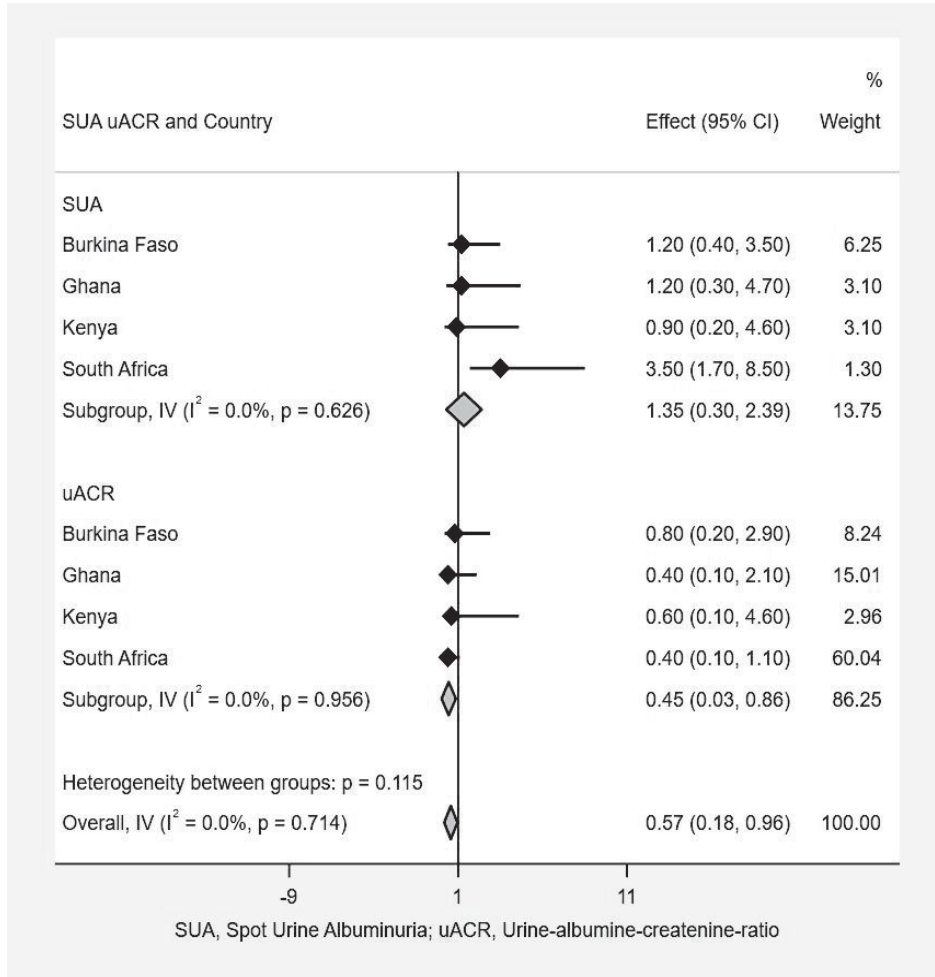
Supplementary material

Figure S1: Association of microalbuminuria (spot urine albuminuria and urine creatinine-albumin-ratio) with carotid intima-media thickness by the study countries; IV, inverse variance; SUA, spot urine albuminuria; uACR, urine albumin-creatinine-ration

Chapter 7

Discussion

Contextualizing the evidence for CVD control

In the past decade several global efforts have been initiated towards the prevention and management of non-communicable diseases (NCDs) which include cardiovascular disease (CVD). For the first time, NCDs were included in the Sustainable Development Goals (SDGs) agenda 2030 that was adopted in 2015 by the United Nations (1). Of the 17 SDGs, SDG3 focuses on health and commits governments to "ensure healthy lives and promote wellbeing for all ages". Specifically target 3.4 seeks to "reduce by one third premature mortality from NCDs through prevention and treatment, and promote mental health and wellbeing", which builds on the World Health Organization (WHO) "25x25" NCD target to reduce NCD mortality by 25% by the year 2025 (2-4). SDG target 3.5 focuses on the prevention and treatment of substance abuse, including harmful use of alcohol while 3.a seeks to strengthen the implementation of the WHO convention on tobacco control (1). Prevention of NCDs offers enormous gains since the major risk factors are also linked to many of the other SDGs such as poverty, nutrition, gender equality, energy, urbanization and climate.

Despite these efforts the burden of CVD continues to grow especially in sub-Saharan African countries. The lack of context specific research on the important risk factors for primary prevention may be a major contributor to the shortfalls in the global efforts. Investigating the burden and risk factors of carotid atherosclerosis among a population of Eastern, Western and southern Africans residing in sub-Saharan Africa (SSA), the H3Africa AWI-Gen study shows differences and similarities in risk of atherosclerotic CVD between these sub-regional blocks. A further comparison between African, African American, Asian, European and Hispanic populations highlights important race-ethnic similarities and differences in risk factors of carotid atherosclerosis. To our knowledge this is by far the largest pan-African study to investigate these differences using harmonized epidemiological data. This is also the first time, data from SSA populations are included in a pooled analyses for a race-ethnicity comparison of risk factors of carotid atherosclerosis.

In the current study, carotid intima-media thickness (CIMT) was used as a measure for atherosclerotic cardiovascular disease. We observed that the classical cardiovascular risk factors such as age, male gender, smoking, body mass index (BMI), systolic blood pressure, and low density lipoprotein (LDL) were associated with increased CIMT. High density lipoprotein (HDL), alcohol intake and physical activity were inversely associated with CIMT (5). We further highlight that in addition to BMI other adiposity measures, i.e. waist (WC) and hip circumference (HC), visceral (VAT) and subcutaneous adipose tissue (SCAT) were associated with CIMT. Specifically, BMI had the greatest positive association followed by VAT, WC and HC whilst SCAT had an inverse

association with CIMT. For the study populations in Burkina Faso and Ghana an inverse association of VAT with CIMT was observed (6). An unexpected finding was the inverse association of HIV with CIMT in this sub-Saharan population. In comparison with other populations, Africans and African Americans had a lower effect of LDL on CIMT compared to Europeans. The protective effect of HDL and alcohol on carotid atherosclerosis was higher in people of African ancestry (African and African Americans) compared to Europeans (7). The thesis further established that a simple screening tool such as the Cardiovascular Health Index proposed by the American Heart Association (AHA) is a strong predictor of carotid atherosclerosis in African populations (8). Finally, the thesis demonstrated that measures of microalbuminuria correlated positively with carotid atherosclerosis and highlights the strong magnitude of the association of carotid atherosclerosis with high 10-year CVD risk (9).

Our observed association of the classical CVD risk factors with CIMT is paralleled by reports from the INTERHEART (10) and the PURE (11) studies which identified these factors as major contributors to myocardial infarction and stroke. The USE-IMT collaboration, also using CIMT, further established these risk factors in African American, Asian, European and Hispanic populations (12, 13). Consistency in the higher risk of CVD conferred by age and sex (men than women) has important implications for mapping out targeted CVD screening programs and management.

The differing burden of classical cardiovascular risk factors within different populations across Africa is possibly driven by the level of epidemiological transition present in each population, and has been highlighted in this thesis and elsewhere. Thus, we observed that lower risk factor burden was observed in rural areas of Nanoro in Burkina Faso and Navrongo in Ghana when compared to peri-urban Dikgale and Agincourt in South Africa and to more urban areas such as Nairobi in Kenya and Soweto in South Africa (14). It has been established that the stage of epidemiological transition is a major contributor to differences in CVD risk in various populations (15, 16). It is also important to note that within the same country the burden of CVD risk factors may differ, particularly between rural and urban populations. This was observed in South Africa where peri-urban Agincourt and Dikgale had a lower burden of risk factors compared to urban Soweto (17, 18). In addition to variability in the geographical distribution of traditional CVD risk factors, variability in the association of novel risk factors with subclinical CVD highlight the need for more in-depth studies across the African continent (9).

Sex related differences in CVD morbidity and risk factors are reported in the literature. African women, just like African American women (19) were reported

to have a higher prevalence of obesity compared to their male counterparts (6) and this is also reflected in other studies from Africa and other low- and middle-income countries (LMIC) (20-22). This thesis has also made similar observations where obesity is more common in women than in men. Several studies have reported a poor perception of the threat of overweight and obesity to health among African overweight/obese women (23-25). Further to this the social desirability for a larger body size among women has been reported in some African communities (26, 27). This context has implications in crafting policies to control obesity among women in African populations.

The contribution of infectious diseases such as HIV, tuberculosis and malaria to the pathophysiology of CVDs in SSA has been described in the literature (28-30). It has been widely reported in Europe and North America that HIV is associated with an increased rate of preclinical atherosclerosis and CVD risk and events. This was largely due to the chronic inflammatory states and immune activation leading to atherosclerosis (31, 32). The use of antiretroviral therapy (ART) thus controls the inflammatory process leading to reduced viraemia and atherosclerosis process (5). For African populations, however, we and others have observed lower prevalence of diabetes and hypertension among people living with HIV (PLWHIV) (33-35), favourable CVD risk profile among HIV positive compared to HIV negative people (35), and an inverse association of HIV with atherosclerosis (5). In addition, a recent study from rural Uganda observed that the progression of carotid atherosclerosis was no different between HIV positive compared to HIV negative people (29). The reason for the reduced levels of CVD risk factors in PLWHIV in SSA is not fully understood, however, one possible explanation is greater healthcare utilization among PLWHIV as well as heightened screening and management of CVD-related co-morbidities in this population, but this requires further investigation. There is however an emerging trend of increased risk of obesity associated with the use of newer anti-retroviral agents such as dolutegravir (36). This observation should serve as a basis for further studies on drug discovery and mapping out of strategies for early detection and management of CVD risk factors with behavioural change interventions.

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Drawing on the infrastructure put in place to develop an efficient HIV care cascade, attempts should be made to use this system for the screening and management of CVD risk factors (37). This we believe will ensure a sustainable way of managing CVD within existing systems without necessarily investing in or establishing new systems. This is advantageous in an era where healthcare funding for CVD care in SSA is already inadequate with communicable diseases taking a much higher priority in healthcare expenditure.

Differences observed in the current study in the association of CVD risk factors with CIMT across race-ethnic groups may be linked to genetic factors. Thus, this thesis and other literature highlight the negative association of alcohol with CIMT, healthy lipid profiles (5) and reduced atherosclerosis-related inflammatory markers among Africans and African Americans may result from specific gene variants in these populations (38-40). In this thesis, African Americans and West Africans had the highest CIMT levels (7) when compared to the other African populations. This is interesting because African American ancestral populations are thought to originate from west and central Africa (41, 42). Thus, it is possible that distinct gene polymorphisms related to high CIMT in the West African population may partially contribute to high CIMT levels in African American subjects. Obviously, this hypothesis must be tested with in depth comparative gene association studies in both populations.

Control of CVD – maximizing efforts at a minimal cost
The current thesis offers evidence on contextual disparities and similarities in CVD risk among African, African American, Asian, European and Hispanic populations (7). It further highlights key priority CVD risk factors (hypertension and obesity) in Eastern, Western and Southern SSA based on the magnitude of the observed associations of these factors to CIMT, and their high prevalence. The region is unprepared for the growing burden of coronary artery disease and cerebrovascular events, as there is evidence of insufficient health care infrastructure and resources as well as serious deficiencies in the numbers of cardiac specialists to combat CVDs in this region (43, 44). Appropriate interventions should therefore be context specific due to the low budgetary allocations to NCDs with prevailing communicable and maternal and child health related morbidities being prioritised.

Considering the numerous challenges and scarce resources in implementing interventions to control CVDs, extrapolating interventions from other settings to Africa may not be appropriate. To overcome these resource constraints, it is important to formulate strategies based on context specific evidenced based research. Following identification of the important risk factors a multisectoral approach to enhance both treatment and prevention is vital taking into account sociocultural factors, infrastructure levels and financial limitations.

Given the relatively short time frame to meet the current global sustainable development goals and other set goals, efforts will have to include both population-based strategies as well as strengthening health service access and quality for the delivery of primary and secondary prevention as well as acute management.

Due to low national budgetary allocations for the management of CVD (43), pragmatic health service solutions through the use of existing health infrastructure will be a major boost to chronic disease identification and care. Health system strengthening through the use of current primary health care modules in various SSA countries is an important step. For instance, existing health care workers and trained community health volunteers could be further trained to promptly screen and identify individuals at a higher risk of CVD. They could also be trained to offer counseling aimed at risk factor reduction. In some African settings it has been demonstrated that nurses and health volunteers at the primary healthcare level have been a useful and effective means of offering basic treatment and monitoring of hypertension and diabetes (37). Valuable lessons can also be learnt from the WHO HEARTS Package which supports Ministries of Health to strengthen CVD management in primary health care settings (45). HEARTS can easily be adopted at the primary healthcare level to target identified CVD risk factors and implement evidence-based strategies for managing CVD.

The major financial commitments to these low budget solutions will include provision of screening tools such as weight scales, blood pressure machines, and point-of-care glucometers and lipid measuring devices. Effective sustained and continuous training of primary healthcare workers as well as health volunteers on the generated protocols may require minimal funding to achieve desired results. These blended pragmatic steps are sure to assist in risk factor reduction, early detection and improve adherence to the treatment of CVD. The above points are supported propositions in the SDGs. The SDG 3.8 echoes the need to achieve universal health coverage, including financial risk protection, access to high quality health-care services and access to safe, effective and affordable essential medicines and vaccines for all (1). This is further supported by 3.b which is aimed at supporting the research and development of vaccines and medicines for communicable and non-communicable diseases that primarily affect LMIC countries, and to provide access to affordable essential medicines and vaccines (1).

This PhD thesis is based on the largest study from SSA to date with harmonized training of researchers regarding data collection methodologies and centralized analysis of the serum biomarkers of CVD risk. The coefficient of variation for most of the anthropometric, ultrasound and serum biochemical measures was maintained at less than 2% thus measurement variability in most of the study parameters was minimized. The study also included populations from across SSA (Eastern, Western and Southern SSA) within countries at different levels of the epidemiological transition thus providing the study with a broad numeric range for each of the socio-demographic and cardiometabolic variables

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and highlighting the heterogeneous nature of African populations. This is an important feature of the study and demonstrates that a 'one size fits all' approach to the development of population-based screening and interventions for CVD may not be appropriate. Potential limitations of the study include the observed association of HIV with CIMT in West Africa, which may be biased due to the self-reported assessment of HIV in Burkina Faso and Ghana. However, we believe the extent of the bias is not sufficient to affect the results due to the very low prevalence of HIV infection in the affected West African sites, which is supported by data from the literature (5). The use of CIMT as a proxy marker for CVD may influence the observations made and hence interpretation of the results. However, we believe the evidence from the literature on the association of CIMT with CVD (46) strongly supports our use of this marker.

Future research

Finally, further research in the form of longitudinal studies to establish key risk factors associated with clinical cardiovascular events are much needed. For instance, the role of visceral adiposity in CVD risk in West African populations needs further validation. Longitudinal research will serve as a good avenue to establish Afrocentric reference values for several of the metabolic risk factors. With several large population-based studies such as the H3Africa AWI-Gen study transitioning into longitudinal cohorts, it may be prudent for researchers to prioritize the collection of clinical event data. The reason for the higher CIMT in West African compared to Eastern and Southern African population may be explained by unmeasured risk factors. Further research may therefore be needed to understand this observation. It will also be useful for a future individual participant data meta-analysis to look at the contribution of HIV and ART use to CVD risk among PLWHIV in sub-Saharan Africa. Exposure adversity (such as poor nutrition) in the early life stages has been shown to result in increased risks for obesity, diabetes and hypertension in later life (47–49). This may partly explain the higher burden of CVD risk among young people in Africa compared to other developed nations. It would therefore be important if future studies could focus on the effect of adverse exposure to infectious agents and malnutrition as predictors of early onset atherosclerosis and cardiovascular risk factors.

Conclusion

Classical cardiovascular risk factors are associated with atherosclerotic cardiovascular diseases in African populations. The emerging trend of increasing obesity in African populations has the potential of increasing the risk of myocardial infarctions and cerebrovascular events. Compared to other race-ethnicities, African population could benefit from interventions

that promote healthy diet (plant source protein) and healthy lipid profiles as a measure to control CVD. Carotid atherosclerosis (carotid intima-media thickness) and microalbuminuria (spot urine albuminuria and urine albumin-creatinine-ratio) are associated with atherosclerotic cardiovascular diseases, hence simple spot urine albuminuria should give a heightened suspicion for onset of CVD. Therefore, simple screening methods such as the use of urine dipstick can safely be used at the primary healthcare setup to identify at risk individuals. The association of cardiovascular health index with atherosclerosis offers an important message in mapping out measures to control the classical cardiovascular risk factors. Particularly the control of smoking, obesity, hypertension and improving physical activities levels are essential in controlling CVD in African populations. Population level strategies could include banning of smoking especially in public places and creating smoking zones. Similarly legal frameworks could adopt reductions in the content of salt in bread and sugar in beverages or imposition of taxes on companies to reduce the content of salt and sugar in their products. There is evidence of the effectiveness of this in high and middle-income countries (50, 51). A review across seven sub-Saharan African countries showed willingness of the countries to adopt Sugar Sweetened Beverage (SSB) tax as a means to control NCDs at a population level (52). Promoting health screening through the adoption of annual days for the screening of risk factors for CVD may be useful avenues to identify undiagnosed cases of hypertension, diabetes or dyslipidaemia. However, such programs will need to be tailored to the populations under investigation, as the current study does highlight the heterogeneous socio-demographic and cardiometabolic features of countries across SSA.

Finally, a blended approach of population level policies such as taxation, ban on smoking indoors and health system approaches such as implementing WHO HEARTS package in existing primary health care systems will make a huge impact in the control of CVDs and associated risk factors.

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Key messages

- It is important to expand the scope of research on the association of carotid intima-media thickness with cardiovascular disease (CVD) in African populations through the use of longitudinal studies and the collection of data on clinical events.
- The classical cardiovascular risk factors are important risk factors in African populations.
- The relative importance of one risk factor over the other based on the magnitude of its effect on atherosclerotic cardiovascular disease is an important consideration in adopting control measures in different populations.
- The use of existing infrastructure for HIV screening and treatment within primary healthcare systems in sub-Saharan Africa is one unique approach to preventing CVD by controlling risk factor burden. These systems will serve as a good foundation for screening for CVD risk factors and treatment monitoring at the primary healthcare level
- Further to this other primary healthcare set-ups such as community health and planning services (CHPS) in Ghana, which are meant for maternal and child health care services could incorporate the screening of CVD risk factors into their routine services within the community.

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Chapter 8

Summary

Summary

The burden of atherosclerotic cardiovascular diseases in sub-Saharan Africa (SSA) is rising steadily and global efforts to reduce cardiovascular disease morbidity and mortality require the use of context specific interventions. The evidence guiding formulations of policies require pragmatic research taking note of local context that influences etiological relationships of risk factors with cardiovascular disease (CVD).

A general background to the study was discussed with **chapter 1** providing an in-depth discussion on the global burden of CVD and the rising burden in the SSA region. We observed the rising burden in SSA to be largely due to improving life expectancies and the rise in CVD risk factors. The chapter also discussed the use of carotid-intima media thickness (CIMT) as a marker for cardiovascular disease.

Part 1 of the thesis assessed the risk factors for increased CIMT in both SSA and other populations.

In **chapter 2** we determined the association of the classical cardiovascular risk factors and HIV with CIMT. The 8872 adults (50.8% men) had a mean age of 50±6 years with age- and sex-adjusted mean (±SE) CIMT of 640±23µm. Participants from Ghana and Burkina Faso had higher CIMT compared with other sites. Age (β = 6.77, 95%CI [6.34, 7.19] µm), body mass index (17.6 [12.5, 22.8] µm), systolic blood pressure (7.52 [6.21, 8.83] µm), low-density lipoprotein cholesterol (5.08 [2.10, 8.06] µm) and male gender (10.3 [4.75, 15.9] µm) were associated with higher CIMT. Smoking was associated with higher CIMT in men. High-density lipoprotein cholesterol (-12.2 [-17.9, -6.41] µm) and alcohol consumption (−13.5 [-19.1, -7.91] µm) were inversely associated with CIMT. HIV was not significantly associated with CIMT (26.80 (-41.40, 95.01) µm; p=0.441). Given the rising prevalence of cardiovascular disease risk factors in SSA, atherosclerotic diseases may become a major pan-African epidemic unless preventive measures are taken particularly for the prevention of hypertension, obesity, and smoking. HIV-specific studies are needed to fully understand the association between HIV and CIMT in SSA.

In response to the rising prevalence of obesity in SSA, **chapter 3** determined the association between various adiposity phenotypes and CIMT in 9010 middle aged adults from SSA. Men had higher levels of visceral fat (VAT) than women while women had higher BMI, waist (WC) and hip circumference (HC) and subcutaneous fat (SCAT) than men at all sites except Burkina Faso. In the pooled analyses, BMI (β−value [95% CIs]: 19.5 [16.8, 22.3] µm) showed the strongest

relationship with CIMT followed by VAT (5.86 [4.65, 7.07] µm), SCAT (5.00 [2.85, 7.15] µm), WC (1.27 [1.09, 1.44] µm) and HC (1.23 [1.04, 1.42] µm). Stronger associations were observed in men than in women. We established that obesity within SSA will likely result in higher levels of atherosclerosis and promote the occurrence of cardio- and cerebrovascular events, especially in males.

Chapter 4 examined race-ethnic differences in the association of classical cardiovascular risk factors with CIMT. This individual participant data meta-analysis included data from 34,025 individuals from African, African American, Asian, European and Hispanic populations. In all race-ethnic groups, men had higher CIMT levels compared to women. Age, sex, body mass index (BMI) and systolic blood pressure (SBP) had a significant positive association with CIMT in all ethnicities at varying magnitudes. When compared to European populations, the association of age, sex and SBP with CIMT was weaker in all ethnicities. Smoking (beta coefficient [95% CI]: 0.39mm [0.09, 0.70]), BMI (0.05mm [0.01, 0.08]) and glucose (0.13mm [0.06, 0.19]) had the strongest positive association with CIMT in the Asian population when compared to all other ethnic groups. High density lipoprotein-cholesterol (HDL-C) had significant protective effects in African American (-0.31mm [-0.42, -0.21]) and African (-0.26mm [-0.31, -0.19]) populations only. Differences in distribution of lifestyle risk factors as well as genetics may contribute to the differences in magnitude of atherosclerosis and CVD. These differences in the association of CVD risk factors with CIMT have implications for race-ethnic-specific primary prevention strategies and also give insights into the differential contribution of risk factors to the aetiology of CVD across populations. The greatest burden of subclinical atherosclerosis in African Americans warrants further investigation.

Part 2 has two chapters with the first chapter examining the association of microalbuminuria with CIMT and the association of microalbuminuria and CIMT with high 10-year CVD risk while the second investigated the association of cardiovascular health index with common CIMT. In **chapter 5** we sought to expand the evidence on the utility of the cardiovascular health index (CVHI) introduced by the American Heart Association as a tool for screening CVD. We therefore determined its association with CIMT in under-studied populations in sub-Saharan Africa. The CVHI is a valid, accessible, simple, and translatable metric for monitoring cardiovascular health and incorporates seven cardiovascular risk factors (often termed 'life's simple 7'): smoking, dietary intake, physical activity, body mass index, blood pressure, glucose, and total cholesterol. We observed inverse associations between CVHI and common CIMT (β-coefficients [95% confidence interval]: Burkina Faso, - 6.51 [- 9.83, - 3.20] µm; Ghana, - 5.42 [- 8.90, - 1.95] µm; Kenya, - 6.58 [- 9.05, - 4.10] µm; and South Africa, - 7.85 [- 9.65, - 6.05] µm). Inverse relations were observed for women

(– 4.44 [– 6.23, – 2.65] µm) and men (– 6.27 [– 7.91, – 4.64] µm) in the pooled sample. Smoking (p < 0.001), physical activity (p < 0.001), and hyperglycemia (p < 0.001) were related to CIMT in women only, while blood pressure and obesity were related to CIMT in both women and men (p < 0.001). We demonstrated that primary prevention of atherosclerotic cardiovascular disease in this understudied population should target physical activity, smoking, obesity, hypertension, and hyperglycemia.

Chapter 6 discussed the association of microalbuminuria with CIMT and of both these variables with 10-year predicted ASCVD risk in 9011 participants. High CIMT, SUA and uACR were each associated with older age and high prevalence of both diabetes and hypertension (all associations p<0.01). The SUA was associated with higher odds of carotid atherosclerosis compared to uACR. Common CIMT, SUA and uACR were strongly associated with 10-year ASCVD risk. The CIMT had a higher odds ratio (95% confidence interval) for 10-year ASCVD risk in both women (1.95 [1.78, 2.14]) and men (1.73 [1.55, 1.93]) compared to SUA (1.27 [1.12, 1.43] and 1.40 [1.26, 1.55], respectively) and uACR (1.30 [1.10, 1.54] and 1.30 [1.15, 1.46], respectively). This data demonstrates that microalbuminuria is associated with subclinical atherosclerosis and high 10-year ASCVD risk in middle-aged individuals from SSA and suggests that urine albumin can be used as a marker of CVD risk in population screening studies.

Appendices

Samenvatting

De ziektelast van atherosclerotische hart- en vaatziekten (HVZ) in sub-Sahara Afrika stijgt gestaag en wereldwijde inspanningen om de morbiditeit en mortaliteit van HVZ terug te dringen vragen om het gebruik van context specifieke interventies. Beleidsvorming moet worden gebaseerd op pragmatisch onderzoek dat rekening houdt met lokale context die de etiologische relaties tussen risicofactoren en HVZ beïnvloedt.

In het begin van dit proefschrift werd een algemene achtergrond van het onderzoek besproken, met in **hoofdstuk 1** een diepgaande discussie over de wereldwijde ziektelast van HVZ en de toenemende ziektelast in de SSA-regio. We hebben geobserveerd dat de toenemende ziektelast in SSA vooral veroorzaakt wordt door de verbetering van de levensverwachting en een toename in risicofactoren voor HVZ: hier is sprake van een epidemiologisch transitie. In dit hoofdstuk werd ook het gebruik van de intima-mediadikte (vaatwanddikte) van de A. carotis (CIMT) als marker voor HVZ besproken.

In deel 1 van het proefschrift werden de risicofactoren voor verhoogde CIMT in zowel SSA als andere populaties onderzocht.

In **hoofdstuk 2** hebben we de associatie tussen klassieke HVZ risicofactoren en HIV met CIMT vastgesteld. De 8872 volwassenen (50.8% man) hadden een gemiddelde leeftijd van 50±6 jaar met een voor leeftijd en geslacht gecorrigeerd gemiddelde CIMT van 640±23μm. Deelnemers uit Ghana en Burkina Faso hadden een hogere CIMT vergeleken met andere locaties. Leeftijd (β = 6.77, 95%BI [6.34, 7.19] μm), body mass index (17.6 [12.5, 22.8] μm), systolische bloeddruk (7.52 [6.21, 8.83] μm), LDL-cholesterol (5.08 [2.10, 8.06] μm) en mannelijk geslacht (10.3 [4.75, 15.9] μm) waren geassocieerd met een hogere CIMT. Roken was geassocieerd met een hogere CIMT in mannen. HDL-cholesterol (-12.2 [-17.9, -6.41] μm) en alcohol consumptie (−13.5 [-19.1, -7.91] μm) waren omgekeerd geassocieerd met CIMT. HIV was niet significant geassocieerd met CIMT (26.80 (−41.40, 95.01) μm; p=0.441). Door de stijgende prevalentie van HVZ risicofactoren in SSA kunnen atherosclerotische ziekten mogelijk een grote pan-Afrikaanse epidemie worden, tenzij er preventieve maatregelen worden genomen specifiek gericht op het voorkomen van hypertensie, obesitas en roken. HIV-specifieke studies zijn nodig om meer inzicht te krijgen in het verband tussen HIV en CIMT in SSA.

Als reactie op de toenemende prevalentie van obesitas in SSA hebben we in **hoofdstuk 3** het verband onderzocht tussen verschillende adipositas fenotypes en CIMT in 9010 volwassenen van middelbare leeftijd uit SSA. Mannen hadden

hogere niveaus van visceraal vet (VAT) dan vrouwen. Vrouwen hadden daarentegen een hogere BMI, middel- en heupomtrek, en subcutaan vet (SCAT) dan mannen in elke regio behalve Burkina Faso. In de gepoolde analyse liet BMI (beta coëfficiënt [95% BIs]: 19.5 [16.8, 22.3] μm) de sterkste associatie zien met CIMT gevolgd door VAT (5.86 [4.65, 7.07] μm), SCAT (5.00 [2.85, 7.15] μm), middelomtrek (1.27 [1.09, 1.44] μm) en heupomtrek (1.23 [1.04, 1.42] μm). Voor mannen waren de associaties sterker dan voor vrouwen. We hebben vastgesteld dat obesitas in SSA waarschijnlijk leidt tot een toename van atherosclerose en het optreden van HVZ bevordert, met name bij mannen.

In **Hoofdstuk 4** onderzochten we raciaal-etnische verschillen in de associatie tussen klassieke HVZ risicofactoren en CIMT. Deze meta-analyse van individuele deelnemersgegevens bevatte data van 34,025 individuen van Afrikaanse, Afrikaans-Amerikaanse, Aziatische, Europeaanse en Latijns-Amerikaanse populaties. In alle raciaal-etnische groepen hadden mannen hogere CIMT waarden dan vrouwen. Leeftijd, geslacht, BMI, en systolische bloeddruk toonden een significant positieve associatie met CIMT in alle etniciteiten in verschillende groottes. Vergeleken met Europese populaties waren de associaties voor leeftijd, geslacht en systolische bloeddruk met CIMT zwakker in andere etnische groepen. Roken (beta coëfficiënt [95% BI]: 0.39mm [0.09, 0.70]), BMI (0.05mm [0.01, 0.08]) en glucose (0.13mm [0.06, 0.19]) hadden de sterkste positieve associatie met CIMT in Aziatische populaties vergeleken met andere raciaal-etnische groepen. HDL-cholesterol had een significante beschermende effect exclusief in Afrikaans-Amerikaanse (-0.31mm [-0.42, -0.21]) en Afrikaanse (-0.26mm [-0.31, -0.19]) populaties. Deze verschillen in de associatie van HVZ risicofactoren met CIMT hebben gevolgen voor specifieke raciaal-etnische preventiestrategieën en geven inzicht in de verschillende invloed van risicofactoren op de etiologie van HVZ over populaties. De grote ziektelast van subklinische atherosclerose bij Afro-Amerikanen vraagt om nader onderzoek.

Deel 2 heeft twee hoofdstukken waarvan in gaat op de associatie van microalbuminurie met CIMT en de associatie van microalbuminurie en CIMT met een hoog 10-jaars HVZ risico. In het tweede hoofdstuk wordt de relatie van de cardiovascular health index met CIMT onderzocht. In **hoofdstuk 5** probeerden we meer bewijs te vinden voor het nut van de cardiovascular health index (CVHI) van de American Heart Association als hulpmiddel voor het screenen van HVZ. Daarvoor hebben we de associatie van de CVHI met CIMT onderzocht in onderbestudeerde populaties in SSA. De CVHI is een valide, toegankelijk, simpel en vertaalbaar meetinstrument voor het monitoren van cardiovasculaire gezondheid en bevat zeven HVZ risicofactoren (vaak 'life's simple 7' genoemd): roken, voedingsinname, lichaamsbeweging, BMI, bloeddruk, glucose en totaalcholesterol. We observeerden een omgekeerd verband tussen CVHI en

CIMT (β-coëfficiënten [95% betrouwbaarheidsinterval]: Burkina Faso, - 6.51 [- 9.83, - 3.20] μm; Ghana, - 5.42 [- 8.90, - 1.95] μm; Kenia, - 6.58 [- 9.05, - 4.10] μm; en Zuid-Afrika, - 7.85 [- 9.65, - 6.05] μm). Omgekeerde associaties werden zowel bij vrouwen (- 4.44 [- 6.23, - 2.65] μm) als mannen (- 6.27 [- 7.91, - 4.64] μm) gezien in de gepoolde steekproef. Roken (p < 0.001), lichaamsbeweging (p < 0.001), en hyperglykemie (p < 0.001) waren alleen bij vrouwen gerelateerd aan CIMT, terwijl bloeddruk en obesitas geassocieerd waren met CIMT bij zowel mannen als vrouwen (p < 0.001). We hebben aangetoond dat primaire preventie van atherosclerotische HVZ in deze onderbelichte populatie gericht moet zijn op lichaamsbeweging, roken, obesitas, hypertensie en hyperglykemie.

In **hoofdstuk 6** bekeken we de associatie van microalbuminurie met CIMT en van beide variabelen met het 10-jaars HVZ risico bij 9011 deelnemers. Hoge CIMT, serum urinezuur (SUA) en urine albumine/creatinine ratio (uACR) waren elk geassocieerd met hogere leeftijd en hoge prevalentie van zowel diabetes als hypertensie (alle associaties p<0.01). SUA was geassocieerd met een hoger risico op halsslagader atherosclerose vergeleken met uACR. Algemene CIMT, SUA en uACR waren sterk geassocieerd met 10-jaars HVZ risico. CIMT had een hogere odds ratio (95% betrouwbaarheidsinterval) voor 10-jaars HVZ risico in zowel vrouwen (1.95 [1.78, 2.14]) als mannen (1.73 [1.55, 1.93]) vergeleken met SUA (1.27 [1.12, 1.43] en 1.40 [1.26, 1.55], respectievelijk) en uACR (1.30 [1.10, 1.54] en 1.30 [1.15, 1.46], respectievelijk). Dit toont aan dat microalbuminurie geassocieerd is met subklinische atherosclerose en hoog 10-jaars HVZ risico in personen van middelbare leeftijd uit SSA. Ook suggereren deze uitkomsten dat urine albumine gebruikt kan worden als marker voor HVZ risico in bevolkingsonderzoeken.

appendices

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Contributing authors (alphabetical order)

Agongo, G.
Navrongo Health Research Centre, Ghana Health Service, Navrongo, Ghana

Alberts, M.
Department of Pathology and Medical Sciences, DIMAMO Health and Demographic Surveillance System, University of Limpopo, South Africa

Ali, S.
Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Anderson, T.J.
Department of Cardiac Sciences and Libin Cardiovascular Institute of Alberta, University of Calgary, Calgary, Alberta, Canada

Asiki, G.
African Population and Health Research Centre (APHRC), Nairobi, Kenya

Boateng, D.
Julius Global Health, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

Bots, M.L.
Julius Global Health, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

Boua, P. R.
Clinical Research Unit of Nanoro, Institut de Recherché en Sciences de la Santé, Nanoro, Burkina Faso

Choma, S.S.R.
Department of Pathology and Medical Sciences, DIMAMO Health and Demographic Surveillance System, University of Limpopo, South Africa

Couper, D.J.
Collaborative Studies Coordinating Center, Department of Biostatistics, University of North Carolina at Chapel Hill, USA

Crowther, N.J.
Department of Chemical Pathology, National Health Laboratory Service, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Debpuur, C.
Navrongo Health Research Centre, Ghana Health Service, Navrongo, Ghana

Engström, G.
Department of Clinical Sciences in Malmö, Lund University, Skåne University Hospital, Malmö, Sweden

de Graaf, J.
Department of General Internal Medicine, Division of Vascular Medicine, Nijmegen University Medical Centre, the Netherlands

Grobbee D.E.
Julius Global Health, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

Hazelhurst, S.
Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Kahn, K.
South African Medical Research Council/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Kauhanen, J.
The Institute of Public Health and clinical Nutrition, School of Medicine, Faculty of Health Sciences, University of Eastern Finland (UEF), Helsinki, Finland

Klipstein-Grobusch, K.
Julius Global Health, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

Kyobutungi, C.
African Population and Health Research Centre (APHRC), Nairobi, Kenya

Lonn, E. M.
Department of Medicine, Division of Cardiology and Population Health Research Institute, McMaster University, Hamilton, Ontario, Canada

Mashinya, F
Department of Pathology and Medical Sciences, DIMAMO Health and Demographic Surveillance System, University of Limpopo, South Africa

Mathiessen, E. B.
Brain and Circulation Research Group, Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway

Micklesfield, L. K.
South African Medical Research Council/Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

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Mohammed, S. F.
African Population and Health Research Centre (APHRC), Nairobi, Kenya

Mukomana, F.
Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Nakanabo-Diallo, S.
Clinical Research Unit of Nanoro, Institut de Recherché en Sciences de la Santé, Nanoro, Burkina Faso

Norris, S. A.
South African Medical Research Council/Developmental Pathways for Health Research Unit (DPHRU), Department of Paediatrics, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Oduro, A. R.
Navrongo Health Research Centre, Ghana Health Service, Navrongo, Ghana

Okazaki, S.
Stroke Center, Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan

Polak, J. F.
Department of Radiology, Tufts Medical Center, Boston, Massachusetts, USA

Raal, F. J.
Carbohydrate and Lipid Metabolism Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Ramsay, M.
Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Rundek, T.
University of Miami Miller School of Medicine, Miami, Florida, USA

Salonen, J. T.
MAS-Metabolic Analytical Services Oy, Helsinki, Finland

Sankoh O.
INDEPTH-Network, Accra, Ghana

Soo, C.
Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Sorgho, H.
Clinical Research Unit of Nanoro, Institut de Recherché en Sciences de la Santé, Nanoro, Burkina Faso

Tinto, H.
Clinical Research Unit of Nanoro, Institut de Recherché en Sciences de la Santé, Nanoro, Burkina Faso

Tollman, S. M.
South African Medical Research Council/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Tuomainen, T-P.
The Institute of Public Health and clinical Nutrition, School of Medicine, Faculty of Health Sciences, University of Eastern Finland (UEF), Helsinki, Finland

Wade, A. N.
South African Medical Research Council/Wits Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

About the author

Engelbert A. Nonterah was born on 10th February, 1984 in Navrongo, Ghana. He attended Notre Dame Minor Seminary School and proceeded to the School of Medicine and Health Sciences of the University for Development Studies where he studied BSc Human Biology. He then proceeded to the University of Ghana Medical School at Korle Bu, Accra where he carried out his clinical studies and graduated with a Bachelors in Medicine and Surgery (MBChB). After compulsory internship in the Eastern Regional Hospital where he undertook rotations in Paediatrics, Obstetrics and Gynecology and Surgery he proceeded to the University of Ghana Hospital in Legon, Accra, Ghana for a rotation in Internal Medicine.

Engelbert joined the Ghana Health Service and was stationed at the Navrongo Health Research Centre (NHRC) where he worked with the H3Africa AWI-Gen study. Engelbert proceeded to pursue Masters in Epidemiology (Epidemiology and Biostatistics) at the University of the Witwatersrand in Johannesburg where He worked as a research intern at the Sydney Brenner Institute of Molecular Bioscience and conducted quality control on the AWI-Gen ultrasound data.

Engelbert cultivated much interest in cardiovascular research and developed a proposal with Prof Nigel J. Crowther and Kerstin Klipstein-Grobusch which later became the focus of His PhD thesis. During the course of the PhD thesis, Engelbert supervised master's students from the University of Groningen in the Netherlands, University of the Witwatersrand in Johannesburg, South Africa and Georgetown University, Washington DC, USA. Engelbert maintained a strong presence in clinical work at the War Memorial Hospital, Navrongo, Ghana.

Engelbert held a research fellows position at the MRC Lifecource Epidemiology Unit, University of Southampton, UK from April, 2018 to December, 2020. During the course of the PhD he led, still leads, and works with several research projects in the NHRC: I. Improved nutrition preconception, during pregnancy and post-delivery (INPreP) – Phase I (April, 2018 – March, 2021; £350,000); II. Saving Brains from malnutrition (August, 2019 – March, 2020; $50,000); III. Adolescents experiences during COVID-19 pandemic - GhanaTec19 (August, 2020 – March, 2021; £41,000); IV. INPreP Phase II - Improving nutrition among pregnant and lactating mothers through provision of bio-fortified food crops (Aril, 2021 – March, 2022; £100,000); v. Phase I and II African-Wits-INDEPTH genomic (AWI-Gen) study (2013 – 2022) and VI. COVID-19 vaccine trials (2021)

He is currently licensed by Ghana Medical and Dental Council as medical practitioner in Ghana in good standing. He is a member of the Public Health Association of Ghana (PHAG) and South Africa (PHASA) as well a member of the Ghana Medical Association (GMA) and the African Chapter of the Development Origins of Health and Disease (DOHaD) society.

Engelbert's hobbies include reading, traveling, playing basket ball and rendering community service.

List of publications

Published (order of publication)

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appendices

Submitted manuscripts

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Nonterah EA, Welaga P, Chatio ST, Kehoe SH, Ofosu W, Ward KA, Godfrey KM, Newell M-L and Oduro AR as members of the INPreP study group. Children born during the hunger season are at a higher risk of severe acute malnutrition: findings from a Guinea Sahelian ecological zone in northern Ghana

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Dalaba M*, **Nonterah EA***, Chatio ST, Adoctor JK, Dambayi E, Nonterah EW, Stephen Azalia S, Ayi-Bisah D, Erzse A, Watson D, Hardy-Johnson P, Kehoe SH, Tugendhaft A, Ward K, Debpuur C, Oduro AR, Ofosu W, Danis M , and Barker M on behalf of the INPreP study group. Engaging community members in priority setting for nutrition interventions in rural northern Ghana

Adoctor JK, Chatio ST, **Nonterah EA**, Kehoe SH, Dalaba MA, Dambayi E, Nonterah EW, Debpuur C, Oduro AR, Ofosu W, Barker M, Ward K. Perceived infant feeding practices and suitable local foods to improve health in the first 1000days of life in rural Kassena-Nankana Districts of Northern Ghana

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