THE ASSOCIATION OF THE MENOPAUSE TRANSITION WITH CARDIOMETABOLIC DISEASES AND THEIR RISK FACTORS IN AFRICAN AND OTHER LOW- AND MIDDLE-INCOME COUNTRIES

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A thesis submitted to the Faculty of Health Sciences at the University of the Witwatersrand in fulfilment of the requirements for the Doctor of Philosophy degree

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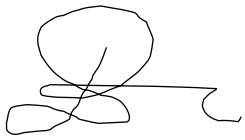
Declaration

I, Raylton Prosper Chikwati, declare that this PhD thesis is the result of my original research work under the supervision of Professor Jaya George, Professor Nigel Crowther, and Dr Nasrin Goolam Mahyhoodeen, and has not been previously submitted for examination at this or any other institution.

I further declare that the research presented here is fully my own and that all sources consulted or referred to in the preparation of this thesis have been duly acknowledged and cited.

Signed:  this day of 21st of June 2023

Name: Raylton Prosper Chikwati

Dedication

To my future self

Research output from PhD

The manuscripts from this PhD are as follows:

1. Chikwati RP, Goolam Mahyoodeen N, Jaff N, Ramsay M, Micklesfield L, Wade A, Agongo G, Asiki G, Choma S, Boua P, George JA, and Crowther NJ. Cardiometabolic Disease Risk Factors in Pre- and Postmenopausal Women from Four Sub-Saharan African Countries; a Cross-Sectional Study (published and awarded Editor's choice by *Maturitas*).
2. Chikwati RP, Chikowore T, Goolam Mahyoodeen N, Jaff N, George JA, and Crowther NJ. Association of Menopause with Cardiometabolic Disease Risk in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis (will be submitted to *Menopause*).
3. Chikwati RP, Goolam Mahyoodeen N, Jaff N, Ramsay M, George JA, and Crowther NJ. The Association of HIV and Menopause with Cardiometabolic Disease Risk Factors in Midlife Women from Kenya and South Africa (will be submitted to *Maturitas*).
4. Chikwati RP, Jaff N, Goolam Mahyoodeen N, George JA, and Crowther NJ Changes in cardiometabolic disease risk factors over the menopausal transition in rural Burkina Faso and Ghana; a longitudinal study (will be submitted to *Journal of Clinical Endocrinology and Metabolism*).

Results from this study were also presented at various scientific conferences in as indicated below:

Oral/poster	**Title of presentation**	**Name of Congress, Location**	**Dates**
Oral	Comparison of cardiometabolic risk factors between pre- and postmenopausal women from diverse sub-Saharan populations	54th SEMDSA Congress, online*.	25-28 March, 2021
Oral	Comparison of cardiometabolic risk factors between pre- and postmenopausal women from diverse sub-Saharan populations	PathRed 2021 Congress, online.	19-22 August, 2021
Oral	Regional variation in cardiometabolic risk factors in pre- and postmenopausal women from four sub-Saharan populations	AfHSG/ H3Africa 18th Consortium Meeting, online.	30 August-4 September, 2021
Oral	Menopause, HIV & cardiometabolic disease risk: a cross-sectional analysis on middle-aged women from Kenya & South Africa	H3Africa 19th Consortium Meeting, Abuja, Nigeria.	29 May-2 June, 2022
Oral	Menopause, HIV & cardiometabolic disease risk: a cross-sectional analysis on middle-aged women from Kenya & South Africa	Wits FHS Research Day 2022, Medical School.	15 September, 2022
Poster	A cross-sectional comparison of cardiometabolic disease risk factors in pre- and postmenopausal women from four sub-Saharan African countries	Wits FHS Research Day 2022, Medical School.	15 September, 2022

*Best oral presentation (Endocrinology/Metabolism), SEMDSA - Society for Endocrinology, Metabolism and Diabetes of South Africa, PathReD - Pathology Research and Development Congress, H3Africa – Human Heredity & Health in Africa, FHS, Faculty of Health Sciences.

Abstract

Background: The changes in risk factors of cardiometabolic disease (CMD) over women's midlife remain understudied in sub-Saharan African (SSA) and other low- and middle-income countries (LMICs). It is unclear whether these changes are driven by the menopause transition (MT) or chronological aging. LMICs including those from SSA have a high population growth of midlife women, and a high prevalence of CMD and human immunodeficiency virus (HIV). The aim of this study was therefore to investigate the MT and its association with CMD risk factor levels in SSA women and women from other LMICs and whether any association is modified by HIV.

Methods: In this study, a meta-analysis of data on menopausal differences in CMD risk from LMICs was performed. Secondly, analysis of cross-sectional data embedded within a multi-centre study in five different sites across four SSA countries namely: South Africa (Dikgale and Soweto), East Africa (Nairobi, Kenya) and West Africa (Nanoro, Burkina Faso, and Navrongo, Ghana) was done. Thirdly, analysis of longitudinal cardiometabolic and sex hormone data from women in Burkina Faso and Ghana was performed, as they passed from pre- to peri- or pre- to post- or early to late postmenopause, at two time points. Lastly, analysis of cross-sectional data from pre- and postmenopausal women in the HIV prevalent regions of Kenya and South Africa was done.

Results: The meta-analysis showed that compared to premenopause, the postmenopausal stage was associated with higher metabolic syndrome (OR=1.18 (95% CI 1.11–1.27)), and higher triglycerides (OR=1.16 (95% CI 1.11–1.21)), elevated blood glucose (OR=1.21 (95% CI 1.15–1.28)), hypertension (OR=1.10 (95% 1.04-1.16)) and higher waist circumference (OR=1.16 (95% CI 1.08–1.25)). However, BMI (OR=1.05 (95% CI 0.96–1.14)), high-density lipoprotein cholesterol (OR=0.71 (95%

CI 0.19–1.22)) and carotid intima media thickness (OR=1.09 (95% CI 0.87–1.36)) did not differ between these groups. In the cross-sectional analyses, combined West African populations demonstrated that postmenopausal women had a larger waist circumference (β = 1.28 (95 % CI: 0.58; 1.98) cm), log subcutaneous fat (β =0.15 (0.10; 0.19)), diastolic (β = 3.04 (1.47; 4.62) mm Hg) and log systolic (β = 0.04 (0.02; 0.06)) blood pressure, log carotid intima media thickness (β = 0.03 (0.01; 0.06)), low-density lipoprotein cholesterol (β = 0.14 (0.04; 0.23) mmol/L) and log triglyceride (β= 0.10 (0.04; 0.16)) levels than premenopausal women. No such differences were observed in the South and East African women. In the longitudinal analyses, the sex hormones estradiol, sex hormone binding globulin, testosterone, and dehydroepiandrosterone decreased significantly but follicle stimulating hormone increased during the MT. Furthermore, androgens mediated minor changes in the levels of CMD risk factors whereas these changes were more strongly influenced by the study site. In the last cross-sectional analysis, age at menopause in women living with HIV was lower than in those living without HIV. However, there were no differences in CMD risk factor levels between menopausal groups and this was not affected by HIV status.

Conclusions: These findings suggest that there is need for a context-specific interventions to lower the risks of CMD in midlife women from SSA populations and other LMICs. Furthermore, this study provides insights into the biology of the menopause in a SSA population by showing minor associations between sex hormones and CMD risk factors but stronger associations with environmental factors. Lastly, HIV does not seem to modulate the menopausal differences on CMD risk factors, but it is associated with a lower age at menopause.

Acknowledgements

This dissertation is more than the sum of three years that I have spent as an international student in South Africa. It comprises all the previous steps I had to take and all the support and guidance that I have received to reach this point. Indeed, the journey has been a long-awaited dream of mine, and am humbled to have been afforded this opportunity to chase down that red gown in a good and nurturing environment. I have equally faced the many challenges of any typical student, surrounded, and battered by troubles, but not demoralised, thrown down, but not broken, and numbed my being home sick. Without such experience I wouldn't have this much sense of accomplishment and sentimental about the many places this journey has taken me.

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Abbreviations

AWI-Gen	Africa Witwatersrand- International Network for the Demographic Evaluation of Populations and Their Health Partnership for Genomics Studies
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CCA	Common Carotid Artery
cIMT	Carotid Intima-Media Thickness
CKD	Chronic Kidney Disease
CHD	Coronary Heart Disease
CMD	Cardiometabolic Disease
CMCS	Chinese Multi-Provincial Cohort Study
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DDR	DNA damage response
DHEAS	Dehydroepiandrosterone Sulphate
DPHRU	Developmental Pathways for Health Research Unit
eGFR	Estimated Glomerular Filtration Rate
E2	Estradiol
FSH	Follicle-Stimulating Hormone
FMP	Final Menstrual Period
GWAS	Genome-wide Association Studies
H3Africa	Human Heredity and Health in Africa
HAART	Highly Active Antiretroviral Therapy
HDL-C	High-Density Lipoprotein Cholesterol

HIV	Human Immunodeficiency Virus
HOMA	Homeostasis Model Assessment
HT	Menopause Hormone Therapy
IQR	Interquartile Range
LDL-C	Low-Density Lipoprotein Cholesterol
LMICs	Lower Middle-Income Countries
MBHS	Michigan Bone Health and Metabolism Study
MT	Menopause Transition
MetS	Metabolic Syndrome
NCDs	Non-Communicable Diseases
NIH	National Institutes of Health
OR	Odds Ratio
ROMO-1	Reactive Oxygen Species Modulator
SAT	Subcutaneous Adipose Tissue
SBP	Systolic Blood Pressure
SD	Standard Deviation
SES	Socio-Economic Status
SHBG	Sex Hormone-Binding Globulin
SWEET	Study of Women Entering and in Endocrine Transition
sVCAM-1	Soluble Vascular Cell Adhesion Molecule 1
STRAW+10	Stages of Reproductive Aging Workshop
SWAN	Study of Women's Health Across the Nation
T2D	Type 2 Diabetes
TLGS	Tehran Lipid and Glucose Study
TNFα	Tumour Necrosis Factor Alpha

VAT	Visceral Adipose Tissue
VIF	Variance Inflation Factor
WC	Waist Circumference
WHO	World Health Organisation
WLWH	Women Living With HIV
WLWOH	Women Living Without HIV

Preface

This PhD thesis is presented in a format guided by the University of the Witwatersrand. As such, the structure of this PhD thesis includes six chapters as follows:

- Chapter 1 gives an introduction and literature review, and main objectives of the study, in a conventional monograph format.
- Chapters 2–5 are the four research studies emanating from the PhD study, presented in the publication model. Chapter 2 is a meta-analysis of data from LMICs to review the relationship of the menopause transition (MT) with CMD risk in these countries. Chapter 3 examines menopausal differences on CMD across SSA regions. Chapter 4 investigates the effects of sex hormone changes on CMD risk factors across different stages of the MT. Chapter 5 investigates the effect of HIV on MT-associated changes in CMD risk factors.
- Chapter 6 integrates a discussion and conclusions from the PhD study.

Candidate's contribution

I worked closely with my supervisors and played an active role in shaping the current thesis from its inception to completion. Here is a list of my inputs into this thesis:

1. **Research design and planning:** I conducted an extensive literature search on the relationship between menopause and cardiometabolic diseases and their risk factors, identifying knowledge gaps and proposing research questions that formed the foundation of this study.
2. **Data collection and analysis:** I was responsible for collecting and analysing data relevant to the research objectives. Specifically, I collected and analysed data for the meta-analysis, which involved developing the literature search strategy and screening the relevant literature and applying relevant statistical methods for analysis. In addition, I accessed the AWI-Gen dataset and collected relevant data for the PhD study. Under the supervision of Prof. Nigel Crowther, I applied several statistical methods relevant to the research objectives. Furthermore, I performed all laboratory measurements to collect sex hormonal data from blood specimens.
3. **Writing and editing:** I actively participated in the writing and editing process of this thesis. Under the guidance of my supervisors, I authored four journal articles relevant to the research objectives. One of the publications earned the Editor's choice for the June Edition of the *Maturitas* Journal. In addition, I contributed to the editing and proofreading of the entire thesis, ensuring coherence, clarity, and adherence to the University's guidelines.
4. **Collaboration and teamwork:** I collaborated with fellow researchers, attended regular research group meetings, presenting my findings, and

received feedback. Under the guidance of my supervisors, I prepared and presented at five scientific conferences (5 oral and 1 poster presentation).

Chapter 1: Introduction and Literature Review

1.1 Introduction

This study investigates the levels of cardiometabolic disease (CMD) and their risk factors during the menopause transition (MT) in midlife sub-Saharan African (SSA) women and other Low- and Middle-Income Countries (LMICs). The Western literature indicates that menopause is associated with an increase in the risk of CMD (1–10) which is driven by changes in sex hormone levels (11–13). However, no longitudinal data exist on increased CMD risk during the MT and its relationship to changes in sex hormone levels and anthropometry in indigenous SSA populations. Such data is important because SSA has a high prevalence of CMD (14), particularly in midlife women however, it is not known whether menopause contributes to their increased risk. Furthermore, the prevalence of HIV in midlife SSA women has been reported to be high, ranging from 20-28% in Eswatini, Lesotho, Botswana and South Africa (15). However, the combined effect of HIV and menopause on CMD risk has not been studied in these populations. Moreover, there is a paucity of global research on HIV, menopause and CMD risk.

1.2 An evolutionary perspective

The word menopause originates from the Greek words *mēnos* and *pausis,* translated month and pause respectively, literally meaning the end of monthly cycles or reproductive life. According to the Darwinian theory of fitness, natural selection does not favour the prolonged lifespan of a species beyond its reproductive years (16). However, humans and some mammals such as whales (17) and chimpanzees (18) have shown a high survival rate after menopause. Therefore, the evolutionary advantage of menopause in these species is indeed a complex puzzle.

Several hypotheses on the selection advantage of menopause in humans have been proposed. These have been summarised into three hypotheses; namely the lifespan-artifact, grandmother and the mate-choice hypotheses (19). The lifespan-artifact hypothesis states that menopause is a by-product of the improved longevity in humans (19). Indeed, the lifespan of modern humans has increased over the years largely due to improved living conditions and healthcare, and is associated with genomic changes such as telomere elongation (19,20). In the last two decades (2000–2019) alone, the World Health Organisation (WHO) estimates that global life expectancy in women has increased by at least 6 years, from 67 years in 2000 to 73 years in 2019 (21).

The grandmother hypothesis postulates that long life allows older women to contribute to the reproductive success of their daughters through sharing the burden of provision and protection for their grandchildren, instead of attempting further reproduction themselves (22,23). The mate-choice hypothesis states that novel population genetic changes occurred in the mating behaviour of humans following the preference for young adult females by adult males which was a selection pressure that diminished fertility in older females (24).

1.3 Historical background

While the term menopause is used frequently in colloquial language, the standardisation of the staging and definition of menopause has been a gradual and evolving process. Various definitions have been proposed through international collaborative efforts from the World Health Organisation (WHO), the Council of Affiliated Menopause Societies (CAMS), the Stages of Reproductive Ageing Workshop (STRAW) and STRAW+10 (25–30). Table 1.1 shows the series of menopause staging definitions by the respective organisations.

1.3.1 STRAW+10

As shown in Table 1.1, the STRAW+10 are the latest recommended criteria for accurate staging of menopause. These criteria are further described in Figure 1.1. According to the STRAW+10 the female reproductive lifecycle is divided into three categories: the reproductive, menopausal transition and postmenopause phases. Further classification showed that five stages (-5 to -1) precede the FMP and two follow the FMP (+1 and +2) (30). Stages -5 to -4 constitute the reproductive stage, -2 to -1 the menopausal transition, and +1 to +2 the postmenopause stage (30).

1.3.1.1 The reproductive stage

The reproductive phase is characterised by regular menstrual cycles, which typically occur between 21 and 35 days. It has been observed that women's fertility peaks in their mid-20s and thereafter gradually decreases (stages -4 and -3a) as the MT begins (30). As shown in Figure 1.1, the late reproductive phase (stage -3) is divided into -3a and -3b. In stage -3a, subtle changes in the menstrual flow and length occur while in -3b, the menses are regular.

1.3.1.2 The menopause transition

The MT is defined as the period between a woman's reproductive years and menopause, typically beginning five to ten years before the FMP (28). In the early MT (stage -2), the menstrual cycles remain regular but the length changes by at least seven days (28). In the late menopausal transition (stage -1), the length of the menstrual cycles become more prolonged, ranging from two or more skips of a normal cycle to at least sixty days or more without a period (28). As shown in Figure 1.1, the follicle-stimulating hormone (FSH) levels increase gradually throughout the MT and into the postmenopausal years.

Table 1.1 Standardisation of menopause staging.

Organisation	Year	Definitions
WHO (25,26)	1980, 1990	Menopause is the permanent cessation of menstrual cycles following the loss of ovarian follicular activity. The premenopause stage is the reproductive period before the menopause. The perimenopause stage is the period before the menopause and at least one year after the menopause. The postmenopause stage is the time frame beginning from the date of FMP, although it can only be confirmed after one-year of amenorrhea. The menopausal transition as a period between a woman's reproductive years and menopause, whose duration varies across individuals, typically beginning 5 to 10 years before the FMP.
CAMS (27)	1999	Adopted the WHO criteria. Climacteric is the period in the transition from a woman's reproductive to their non-reproductive state. This period overlaps the perimenopause, by stretching for a longer variable time before and after perimenopause.
STRAW (28)	2001	Divided the stages of the female reproductive life into seven distinct categories based on the dominant bleeding patterns seen in healthy women undergoing natural menopause.
ReSTAGE (29)	2008	Validated the STRAW criteria on the use of bleeding patterns using longitudinal data from different populations.
STAW+10 (30)	2011	Modified the STRAW criteria to align with the endocrine changes and the length of menstrual cycles.

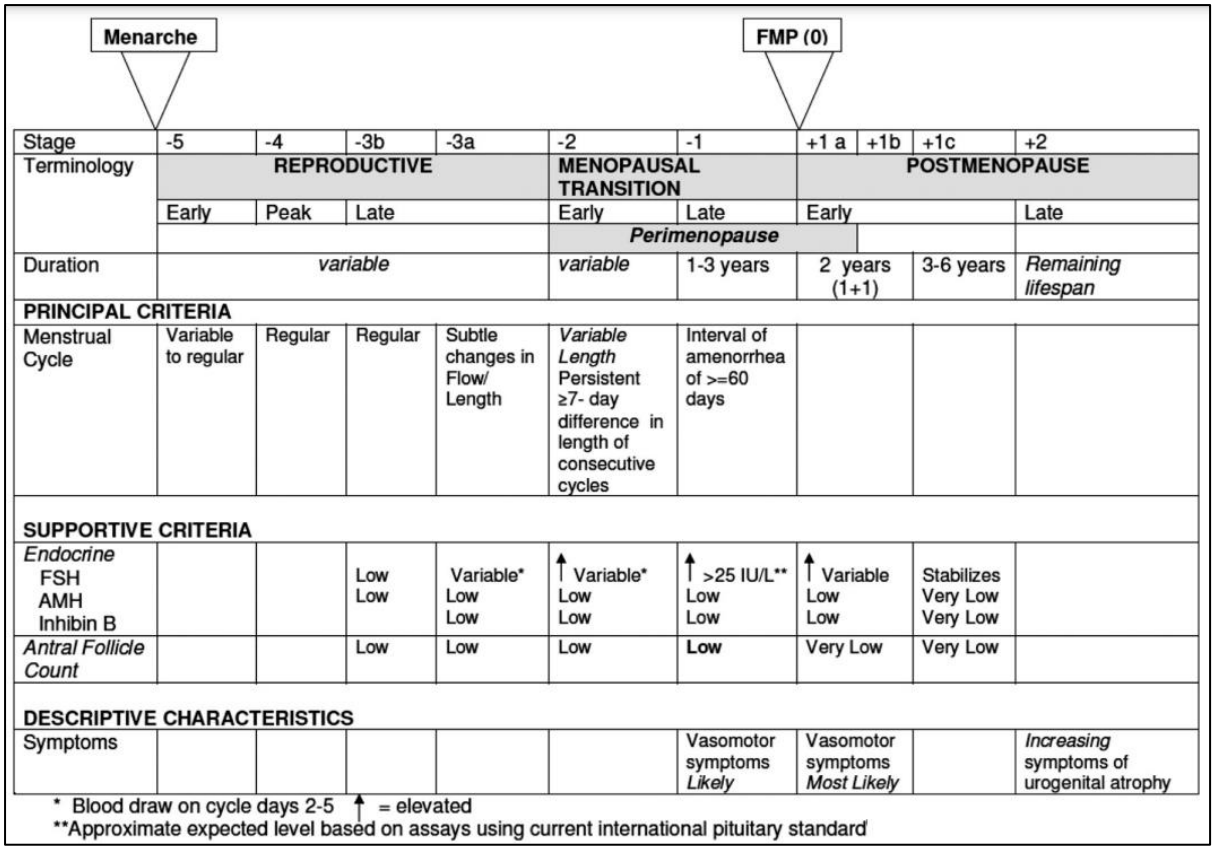

Figure 1.1. Menopause Staging according to STRAW+10 guidelines. Adapted from Soules *et al.,* (28).

1.3.1.2 The Postmenopausal Stage

The postmenopausal stage is divided into early (+1) and late postmenopause (+2). In Figure 1.1, the early postmenopause is subdivided into stages +1a, +1b, and +1c. Stage +1a represents twelve months of amenorrhea and can be used to derive the FMP. Stage +1b is characterised by rapid changes in FSH and estradiol levels. In stage +1c FSH and estradiol reach steady levels that can last between 3 and 6 years, the follows stage +2, late postmenopause.

1.3.2 Utility of the STRAW+10

Since its inception in 2011, the utility of the STRAW+10 criteria in the accurate staging of menopause have been on an upward but slow trend. A few large scale longitudinal studies such as the Penn Ovarian Aging Study (POAS) and the Estrogenic Regulation of Muscle Apoptosis (ERMA) study have since used the STRAW+10 criteria for

menopause staging (10,31). However, some large scale longitudinal studies such as the Atherosclerosis Risk in Communities (ARIC) study from the US and the Montreal Ottawa New Emerging Team (MONET) study from Canada maintained the traditional bleeding criteria that does not require blood FSH measurements (8,9). The STRAW+10 criteria remains the gold standard, and one study from South Africa confirmed its utility in an African population (32). However, in LMICs such as those from SSA, the unavailability of skilled personnel and other resources makes it difficult to implement the STRAW+10 criteria of staging.

1.4 Biology of female sex hormones

The main hormones that regulate the female reproductive system are, the gonadotropin hormone-releasing hormone (GnRH), luteinising hormone (LH), FSH, estrogen, progesterone, inhibin A and B. Other hormones such as, testosterone dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) androstenedione, and sex hormone-binding globulin (SHBG), , are also implicated in the female reproductive axis. Combined, these hormones can be grouped according to their structural similarities as peptides, sex steroids, and glycoproteins, thus the following section discusses the synthesis, and similarities and differences between these hormones.

1.4.1 Peptide hormones

GnRH is a ten-amino acid peptide produced by the hypothalamus and functions as a central regulator of the reproductive system (33). GnRH acts on the pituitary to stimulate the synthesis and secretion of FSH and LH (33).

1.4.2 Sex steroid hormones

The sex steroids, progesterone, DHEA, androstenedione, testosterone, and estrogens are mainly synthesised in the ovary, adrenal gland, skin, and adipose tissue. In the ovaries, steroidogenesis has been described by a two-cell hypothesis model which involves the granulosa and theca cells of maturing follicles (34). Metabolism in these cells is under the regulation of FSH and LH, both of which increase the uptake of cholesterol required for steroidogenesis in these cells (33). FSH exclusively acts on granulosa cells whereas LH acts on both granulosa and theca cells. The theca cells synthesise androgens, whereas the granulosa cells synthesise estrogens (34).

In the ovaries, steroidogenesis begins with a rate limiting step that converts cholesterol to pregnenolone (Figure 1.2) (34). This process is mediated by the cytochrome P450 11A1 enzyme (CYP11A1) (34). Pregnenolone, a 21-carbon (C_{21}) then migrates into the adjacent theca cells to enter the Δ5 pathway (major pathway). Alternatively, low levels of pregnenolone are converted into progesterone before entering the Δ4 pathway (minor pathway) in the theca cells. In the Δ5 pathway, pregnenolone is decarboxylated to 17OH-progesterone, a C_{17} steroid by the cytochrome P450 17A1 (CYP17A1) enzyme (34). In the presence of the cytochrome B5 Type A (CYB5A) protein, CYP17A1 further catalyses 17OH-pregnenolone into DHEA. DHEA is then converted into 5-diol by an aldo-keto reductase enzyme (AKR1C3). In addition, DHEA from the adrenal glands enters this pathway or exits into circulation in its sulphated form, DHEAS. In the Δ4 pathway, progesterone is converted to 17OH-progesterone, then androstenedione, and lastly testosterone (Figure 1.2) (34).

The CYP17A1 enzyme has more affinity for 17OH-pregnenolone than 17OH-progesterone, therefore the contribution of the Δ5 pathway to the production of sex steroids is higher than that of the Δ4 pathway. As a result, DHEA is the main precursor

for sex steroids. As shown in Figure 1.2, the 3ß-hydroxysteroid dehydrogenase type 2 enzymes (HSD3B2) convert metabolites in the ∆5 pathway to their associated hydroxysteroid in the ∆4 pathway. As such, pregnenolone is converted to progesterone, 17OH-pregnenolone to 17OH-progesterone, DHEA to androstenedione and androstenedione (5-diol) into testosterone (34). The conversion of androstenedione to testosterone contributes approximately two-thirds of the testosterone in circulation (33).

Testosterone and androstenedione diffuse into the granulosa cells where they are metabolised into three major estrogens, estrone (E1), estradiol (E2), and estriol (E3) (34). The aromatase CYP19A converts androstenedione to E1 and testosterone to E2, respectively. The predominant and potent ovarian estrogen is E2, thus estrone can be converted into estradiol by the 7ß-hydroxysteroid dehydrogenase type 1 (HSD17B1) enzyme. Estrone can also be sulphated to estrone sulphate by the estrogen sulfotransferase (STS) (34). In addition, either E1 or E2 can be metabolised into E3 which has no known biological function but is produced by the placenta in larger quantities during pregnancy. It is estimated that ovarian E2 and E1 account for approximately 95% and 50% of the circulating E2 and E1 respectively whereas the rest are derived from the periphery (33).

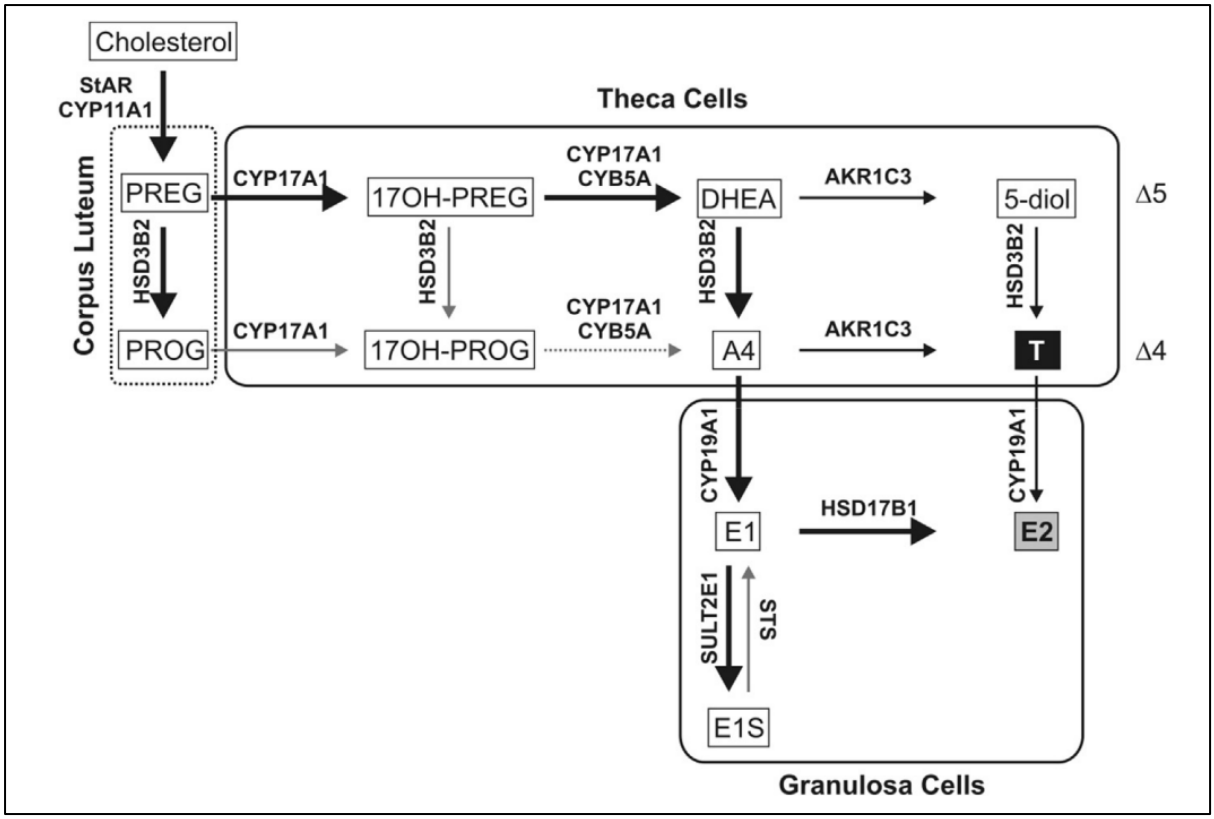

Figure 1.2 Biosynthesis of sex steroids. Grey box: active estrogen; black box: active androgen. Bold arrows show main pathways, and all enzymes are shown for all pathways. Source Schiffer *et al.,* (34).

As previously mentioned, the adipose tissue contributes to the synthesis of some sex steroids. This is facilitated by the enzyme aromatase in adipocytes which converts testosterone to E2 and androstenedione to E1 (33). In addition, the hair follicles in the skin have the HSD3B2 enzymes to convert circulating DHEAS and androstenedione to testosterone (33).

1.4.3 Glycoprotein hormones

The glycoproteins FSH and LH are synthesised by the gonadotrophs located in the anterior pituitary gland (35). Both hormones share a heterodimeric quaternary structure composed of two glycoproteins, the α- and ß-subunits. FSH and LH share a common α-subunit amino acid sequence but different amino acid sequence and oligosaccharides on the ß-subunits, therefore the latter subunit confers differential biological specificity (35). In addition, FSH and LH are synthesised in the same

gonadotropic cells, but their secretion is different. FSH secretion is dependent on the expression of its ß-subunit therefore there is minimal storage of FSH within the gonadotropes. This suggests that the secretion of FSH is constitutive. On the other hand, LH is stored in organelles after its synthesis and only released after an appropriate trigger (increased GnRH pulse frequency), therefore the secretion of LH follows a regulated pathway (33).

Another important glycoprotein is SHBG, which is synthesised in the liver. SHBG has two monomers that form a binding site for sex steroids such as testosterone and E2 (36). In addition, due to their different chemical structures, steroids have different binding affinities to SHBG, thus it has been shown that testosterone and E2 have higher binding affinities to SHBG than androstenedione and E1 (37).

In addition, inhibins are part of the glycoproteins involved in modulating the menstrual cycle by regulating the secretion of FSH from the pituitary gland (38). In females, inhibins are primarily produced by the ovary and corpus luteum. Inhibins have two subunits, the α- and ß-subunits (38). Two isoforms of the ß-subunit exist as βA and βB, thus the nomenclature, inhibin A (αβA) and inhibin B (αβB) (38). Small antral follicles have been shown to predominantly secrete inhibin B, whereas the dominant follicles and the corpus luteum predominantly secrete inhibin A (38). The synthesis of inhibin is regulated by the hypothalamic-pituitary-ovarian (HPO) axis (33). The hypothalamus secretes GnRH, which stimulates the anterior pituitary gland to release FSH and LH. FSH then acts on the ovaries, stimulating the growth and development of ovarian follicles (33). As the ovarian follicles grow, the granulosa cells within them starts producing inhibin. Inhibin selectively suppresses the secretion of FSH from the pituitary gland, forming a negative feedback loop (33). The inhibition of FSH secretion

by inhibin helps regulate the maturation of ovarian follicles and the production of estrogen.

1.5 Physiological functions of female sex hormones

Table 1.2 shows the physiological functions of female sex hormones during the reproductive years. Overall, female sex hormones extend from their traditional role in the maintenance of the menstrual cycle to metabolic functions.

1.6 Measurement of sex hormones

The current methods on the measurement of sex hormones are dominated by radioimmunoassays, direct immunoassays, and mass spectrometry-based assays (39,40). Between these methods, mass spectrometry-based assays have higher sensitivity and lower inter- and intra-assay variability, thus are considered gold standard or comparator methods. However, direct immunoassays are widely used because of their lower cost, ease of use, high throughput, commercial availability, and faster turnaround times. Furthermore, the performance of the newer generation of immunoassays is comparable to those from mass spectrometry-based assays and devoid of some of the pitfalls in the earlier immunoassay generations such as low sensitivity and cross-reactivity (40).

The most common assay method used for sex hormone immunoassays is based on the sandwich principle and detection is achieved by electrochemiluminescence (41). The principle of the method entails the incubation of a sex hormone containing sample with two differently labelled hormone-binding monoclonal antibodies which form a sandwich complex. The sandwich complex is then retained on a solid phase that contains streptavidin-coated microparticles. The microparticles are then magnetically captured on an electrode. The passage of an electric voltage on the electrode then

generates light electrochemically which is finally measured by a photomultiplier, thus the amount of light measured is equivalent to the hormonal levels in the sample (41).

Table 1.2: Physiological functions of female sex hormones

Hormone	Function
Estrogen	• Inhibits lipolysis in adipocytes (42). • Lowers vascular oxidative stress (43). • Modulates blood pressure (44). • Regulates the production of inflammatory cytokines in the liver (45). • Promotes bone health by regulating bone turnover, growth, and maturation (46).
SHBG	• Regulates free estrogen and testosterone concentrations in the circulation by acting as a carrier protein and competitive inhibitor (47). • Effects steroid signalling by binding to cell membrane receptors (48).
Testosterone	• Precursor for estrogens (33). • Regulates body fat composition and muscle mass by mediating carbohydrate, lipid and protein metabolism (49).
DHEAS	• Reservoir for DHEA (50).
DHEA	• Major precursor for androgens and estrogens (50). • Modulates multiple signalling pathways including vascular modelling and immunomodulatory actions (50).
Androstenedione	• Precursor for estrogens (51).
FSH	• Stimulates differentiation of granulosa cells and the production of estrogen (51). • Regulates the homeostasis of body fat and energy (52). • Regulates cholesterol levels by influencing hepatic cholesterol production and endocytosis of (low-density lipoprotein cholesterol (LDL-C) (11,52).
LH	• Stimulates oocyte maturation and rupture of follicular wall during ovulation (51). • Maintenance of the corpus luteum (53).
Progesterone	• Stimulates the midcycle peak in FSH and the coordination of the LH surge (51). • Stimulates the rupture of follicular wall during ovulation (51).
Inhibin A and B	• Suppresses the pituitary production of FSH (33). • Regulates follicular dominance by acting as intraovarian paracrine signalling molecules (38). • Regulates the production of DHEA and androstenedione in the ovaries by paracrine signalling.

SHBG, Sex hormone binding globulin, DHEAS, Dehydroepiandrosterone sulphate, DHEA, Dehydroepiandrosterone, FSH, Follicle Stimulating Hormone, LH, Luteinising Hormone.

1.7 Physiology of reproductive aging

1.7.1 Regular menstrual cycles

A regular menstrual cycle depends on the interactions between the hypothalamus, pituitary, and ovary and the endometrium (Figure 1.3) (53).

As previously mentioned, the hypothalamus secretes GnRH into the pituitary portal system to stimulate the synthesis and secretion of the FSH and LH activating the ovaries to release an egg in preparation for fertilisation (33). At the same time, the ovarian follicles begin to synthesise sex steroid hormones (estradiol and progesterone) and glycoproteins (inhibin A and B). These hormones regulate the menstrual cycle through negative and positive feedback loops (Figure 1.3) (53).

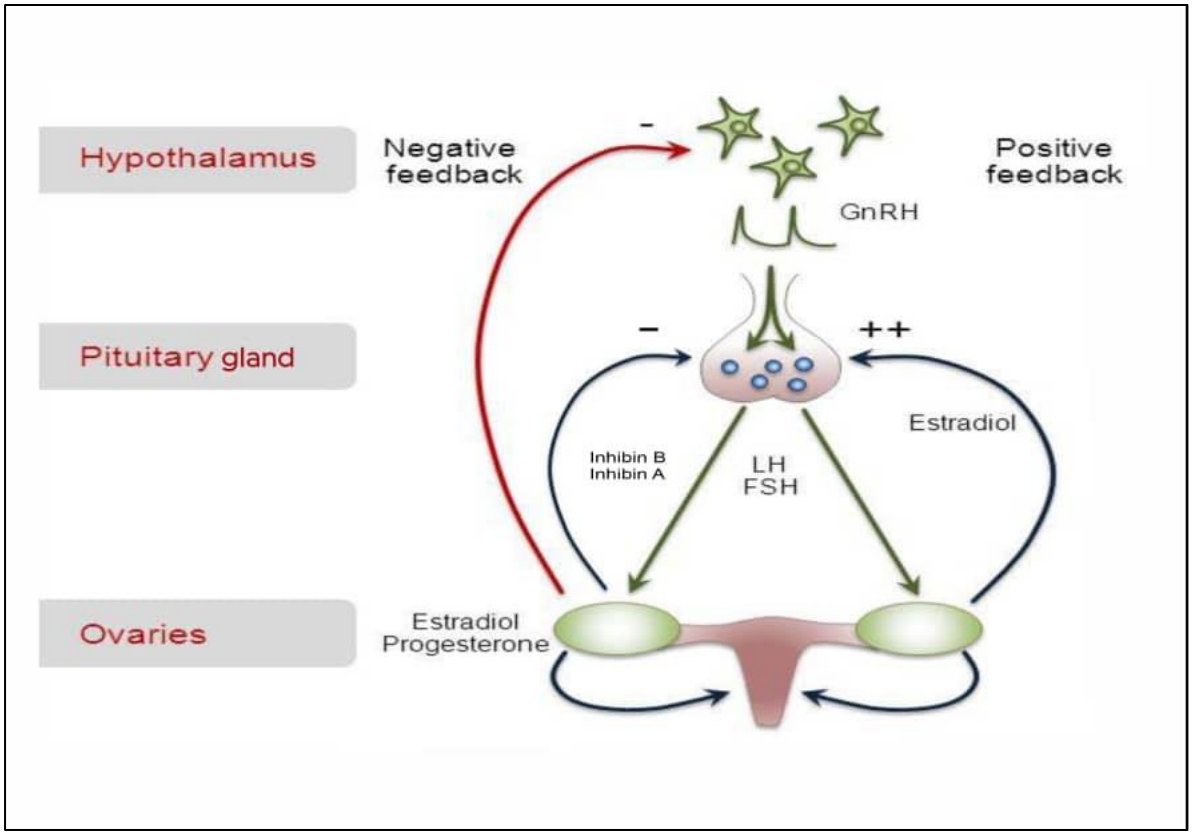

Figure 1.3 The hypothalamus-pituitary-ovarian axis. GnRH, gonadotropin-releasing hormone, LH, luteinising hormone, FSH, follicle stimulating hormone. Adapted from Hall *et al.,*(53).

Each menstrual cycle consists of the luteal and follicular phases, altogether lasting between 25 and 35 days (Figure 1.4). In the late luteal phase, a slower pulsative release of GnRH stimulates a gradual increase in FSH levels. This promotes the growth and maturation of primordial follicles which in turn release estradiol as the follicular phase begins. The increasing estradiol levels restrain any further increase in FSH levels by an inhibiting the release of GnRH from the hypothalamus (Figure 1.4). In addition, the ovarian secretion of the peptide hormones, inhibin A and B act in synergy with estradiol to inhibit the secretion of FSH. In the late follicular phase, peak estradiol levels stimulate the hypothalamus to further increase the pulsative release of GnRH and LH release by the pituitary. LH further stimulates the ovary to produce more estradiol. At this point there is no change in the pulsative release of GnRH. As shown in Figure 1.4, this generates the preovulatory LH surge necessary for ovulation. Once ovulation occurs, the luteal phase begins. At this stage, the raptured follicle now referred to as the corpus luteum assumes a temporary but crucial role in the secretion of progesterone for the maintenance of the endometrium in preparation for implantation (Figure 1.4). Progesterone is also involved in the negative feedback mechanism to reduce the pulsative release of GnRH by the hypothalamus. (33,53).

If pregnancy has not occurred between 10-12 days after ovulation, the corpus luteum disintegrates and two to three days later the shedding of the endometrium lining begins (53). In addition, the loss of a progesterone and estradiol feedback on the hypothalamus is important to achieving adequate levels of FSH that for the next wave of follicular recruitment. This is also associated with declining levels of inhibin A and B, and secretory phase of the endometrium (Figure 1.3). Collectively this forms part of a luteal-follicular phase transition, as follicular recruitment stimulates follicular development in the ovaries (Figure 1.4) (53).

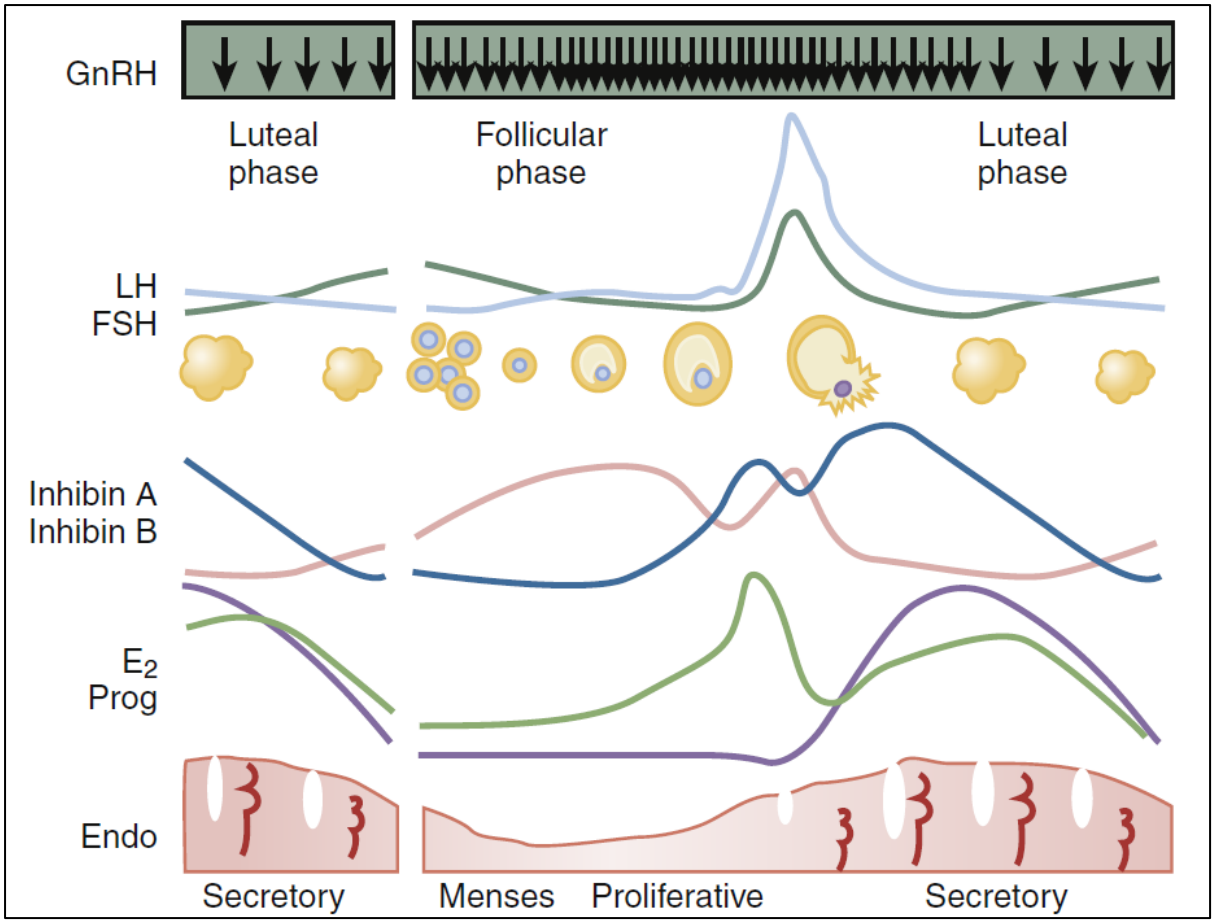

Figure 1.4. The menstrual cycle. GnRH, gonadotropin-releasing hormone, E2-estradiol, Prog-progesterone, LH-luteinising hormone, FSH-follicle stimulating hormone. Source Hall *et al.,* (53).

1.7.2 Reproductive aging

While the bleeding patterns in the younger and older women may be similar, studies have shown that there are underlying hormonal changes associated with reproductive aging in these two groups (54). Evidence shows that there is a linear decline of ovarian reserve and function with increasing age (54). This has been confirmed by studies showing a decreasing number of antral follicles, and lower levels of anti-Mullerian hormone (AMH) and inhibin B associated with aging (55,56). As shown in Figure 1.3, inhibin A and B are a negative feedback modulator for further FSH secretion. Some studies have shown that the decline of inhibin B precedes that of inhibin A, suggesting that inhibin B is the most significant factor in modulating the earliest rise of FSH levels

in reproductive aging (57). This indicates that these elevations in FSH levels may serve as a compensatory mechanism to sustain ovulation in a background of a diminishing primordial follicle pool (58). Figure 1.5 shows the trajectory of increasing FSH and declining estradiol levels relative to the FMP.

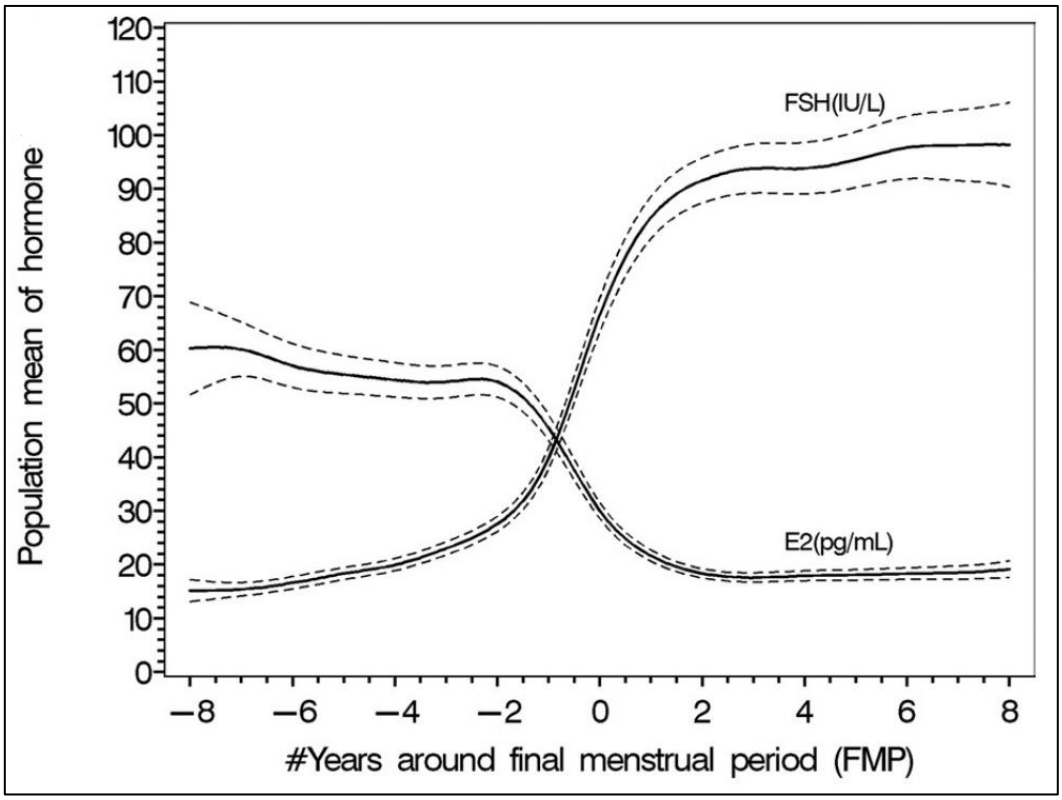

Figure 1.5 FSH and E2 changes relative to the FMP. Data expressed as adjusted means (95% confidence interval for FSH, follicle stimulating hormone, E2, estradiol. Source, Randolf *et al.,* (59).

1.7.3 The menopause transition

As previously discussed, the onset of the MT is characterised by a diminishing pool of primordial follicles and irregular bleeding patterns which are associated with changes in the levels of sex hormones (28). This is supported by data from longitudinal studies in Table 1.3. These studies show that during the MT, FSH and LH levels increases rapidly, at least 6 years before the FMP (59–65). However, during this period, progesterone and estrogen levels decrease (59,61–66). Of the two major estrogens in

circulation, estradiol levels decline more rapidly than those of estrone, suggesting an extra-ovarian source for estrone (65). The declining levels of estradiol at the beginning of the MT are associated with fewer granulosa cells and poor responsiveness of the granulosa cells to gonadotropic hormones such as FSH (33).

Changes in the levels of androgens during the MT are much more complex. Considering that adrenal and ovarian androgens in the general circulation exist in equilibrium, it is probable that for the declining ovarian androgens, a compensatory mechanism to activate the adrenal gland maintains steady androgen levels during the MT (65). In addition, the MT is associated with a decrease in the levels of SHBG (61,66). This leads to an increased androgenicity as measured by the free androgen index (FAI), a ratio between testosterone and SHBG levels. Furthermore, only a few data have explored the association between adrenal androgens and the MT. Some studies have shown that testosterone levels decrease with chronological aging, but this decrease is offset by an increase in the SHBG-unbound testosterone. Therefore, no net change in testosterone levels should be expected during the MT. In addition, estradiol is essential for the synthesis of SHBG in the liver, therefore the declining estradiol levels during the MT are associated with decreased SHBG levels (66,67). Furthermore, Table 1.3 shows that in three studies, the declining levels of dehydroepiandrosterone sulphate (DHEAS) were not associated with the MT, but with chronological aging (66,68) while one study showed that DHEAS levels transiently increased during the MT (69) and in the other, DHEAS levels were stable during the MT (70), therefore data on changes in DHEAS during the MT is inconclusive.

Table 1.3: Hormonal changes during menopause transition; findings from longitudinal studies

Study (reference)	Sample size	Hormonal changes
Michigan Bone Health and Metabolism Study (MBHS) (63,64)	629	Increase in FSH but decrease in E2 levels at least 2 years before the FMP.
Melbourne Women's Midlife Health Project (MWMHP) (66)	172	Decrease in SHBG levels from 4 years before the FMP. SHBG levels were directly associated with decreasing E2 levels. No changes in testosterone levels with time relative to the FMP. Increasing FAI from 4 years pre- FMP, maximum increase, 2 years pre-FMP. Fall in DHEAS levels were not related to the FMP, but age.
Study of Women's Health Across the Nation (SWAN) (59)	1215	Increase in FSH levels 6 years before the FMP. E2 levels decreased 2 years before the FMP and largest drop at the FMP.
Penn Ovarian Aging cohort (60)	105	Increase in FSH and LH but decrease in AMH and inhibin B levels before the FMP. No changes in E2 levels.
Malmo Perimenopausal Project (61)	160	Increase in FSH and LH levels from 5 years before FMP. Largest increase 6-months before the FMP. Declining E2 levels, largest decrease at 6 months pre-FMP. Small decrease in testosterone, androstenedione and SHBG levels around the FMP.
Trevoux *et al.,* (62)	483	E2 levels decreased from 3 years pre-FMP. Progesterone levels decreased from 3 years pre- FMP. Gradual increase of LH and FSH levels before the FMP.
Overlie *et al.,* (65)	59	Increase in FSH and LH but decrease in E2 and E1 levels before FMP. Androstendione and testosterone decreased 3-years pre-FMP.

FSH, follicle stimulating hormone, E2, estradiol, E1, estrone, FMP, final menstrual period, SHBG, sex hormone binding globulin, AMH, Anti-Mullerian hormone, FAI, free androgen index, DHEAS, dehydroepiandrosterone sulphate, LH, luteinising hormone.

1.7.4 The postmenopause stage

The early postmenopause stage is characterised by a diminished pool of ovarian follicles (30). As such, ovarian sex hormonal levels decrease. Table 1.4 shows the findings from longitudinal studies on the sex hormonal changes after the FMP.

As shown in Table 1.4, most studies showed that the levels of FSH, LH, estradiol, estrone, SHBG, androstenedione and DHEAS decreased, while the free androgen index (FAI) increased after at least 2 years post FMP. The exceptions were testosterone levels which did not change in the Melbourne Women's Midlife Health Project study whereas Overlie *et al.,* showed that testosterone levels increased until 2-years post FMP but the levels decreased in the Malmo Perimenopausal Project study (61,65,66). Further along the postmenopausal stage, most sex hormones stabilised to steady levels, except for the second drop in estradiol levels between 6 and 8 years and an increase in FSH levels between 3 and 8 years in the Michigan Bone Health and Metabolism Study (63,64). These hormonal changes that occur after the FMP are associated with further loss of ovarian function. According to the Melbourne Women's Midlife Health Project study, the second drop in estradiol levels was only present in women with BMI <30kgm^2 and associated with a lack of supplementation pool of androstenedione and estrone from the peripheral tissues (64).

Table 1.4: Hormonal changes after the final menstrual point, findings from longitudinal studies

Study (reference)	Hormonal changes
Michigan Bone Health and Metabolism Study (MBHS) (63,64)	FSH levels increase until 1 year post FMP, then a major decrease from 1 to 3 years post FMP, then a steady increase from 3 to 7 years, and last drop at 7 years post FMP. Rapid decline of E2 levels to 2 years post FMP, steady levels between 2- and 6-years post FMP and a further decrease between 6- and 8-years post FMP. Steady state levels maintained at 8 years post FMP.
Melbourne Women's Midlife Health Project (MWMHP) (66)	SHBG levels decreased until 2 years post FMP and thereafter stabilised to 7 years post FMP. No changes in testosterone post FMP. FAI increased until 2 years post FMP. Fall in DHEAS levels were not related to menopause, but age.
Study of Women's Health Across the Nation (SWAN) (59)	Increase in FSH levels 2 years post FMP and stabilisation between 2- and 8-years post FMP. E2 levels further decreased up to 2 years post FMP and stabilised between 2- and 8-years post FMP.
Malmo Perimenopausal Project (61)	Decrease of LH levels until 8 years post FMP. Drop in FSH levels 4 years post FMP. E1:E2 ratio increased. Gradual decrease in testosterone, androstenedione and SHBG levels until 3 years postmenopause where the levels achieved a steady state up to 8 years post FMP.
Trevoux *et al.,* (62)	LH and FSH levels decreased until 1 year post FMP then stabilised between 1- and 7-years post FMP. E2 levels decrease until 4-years post FMP when they reached steady state levels.
Overlie *et al.,* (65)	LH, testosterone, and FSH levels increased until 2 years post the FMP. E2, E1 and SHBG levels gradually decreased in the first 2 years post FMP. Androstenedione and DHEAS decreased 1 year post FMP and gradually increased between 1- and 2-years post FMP.

FSH, follicle stimulating hormone, E2, estradiol, E1, estrone, FMP, final menstrual period, SHBG, sex hormone binding globulin, DHEAS, dehydroepiandrosterone sulphate, LH, luteinising hormne, LH, luteinising hormone.

1.8 Changes in cardiometabolic disease risk during the menopause transition

CMD, defined as a combination of related cardiovascular and metabolic disorders that include hypertension, stroke, angina, chronic kidney disease, and type 2 diabetes mellitus are prevalent causes of morbidity and mortality (71). CMD share common risk factors particularly obesity (72). Several studies have shown that percentage body fat and its distribution are directly associated with CMD morbidity and mortality (73,74).

One well described mechanism for increased CMD risk in obese individuals involves the expansion of the adipose tissue and its associated infiltration by macrophages (75). This interaction between adipocytes and macrophages increases pro-inflammatory cytokines such as plasminogen activation inhibitor 1 (PAI-1) and monocyte chemoattractant protein 1 (MCP-1) while downregulating anti-inflammatory adipokines such as adiponectin (75). PAI-1 inhibits the activation of plasminogen, a protein involved in the dissolution of clots (76). The actions of PAI-1 therefore inhibit fibrinolysis therefore promote a pro-thrombotic state (76). MCP-1 promotes the recruitment of macrophages and monocytes into the arterial cell wall, and therefore is actively involved in atherosclerosis (76).

Furthermore, the adipose tissue has specific regional depots with distinct functions namely subcutaneous adipose tissue (SAT), and the visceral adipose tissue (VAT) (Figure 1.6) (77). The SAT depot is located directly below the skin, and includes the abdominal, gluteofemoral, and leg fat depots (78). As shown in Figure 1.6, the VAT depot is in the abdominal cavity (78). The distribution of fat in these depots plays a significant role towards CMD risk. Increased VAT (central obesity) is directly associated with CMD risk and there is some evidence that abdominal SAT may also contribute, whereas increased SAT in the gluteofemoral and leg region (peripheral obesity) are associated with little or reduced CMD risk (79). The reasons for these

depot-specific differences are not entirely clear, but two theories have emerged. The first is that the VAT depot acts as an endocrine organ which drains its products (cytokines and free fatty acids) into the circulation where they can reach the liver and affect metabolism (80). Recent literature suggests that the additional contribution of free fatty acids from the abdominal SAT depot also adds to the free fatty acids pool in systemic circulation (81). The second hypothesis states that the adipose depots may have different regional gene expression profiles that are linked to the development of glucose intolerance and dyslipidemia (82). For example, gluteofemoral and leg SAT have been shown to express higher levels of adipokines and act as triglyceride reservoirs (83).

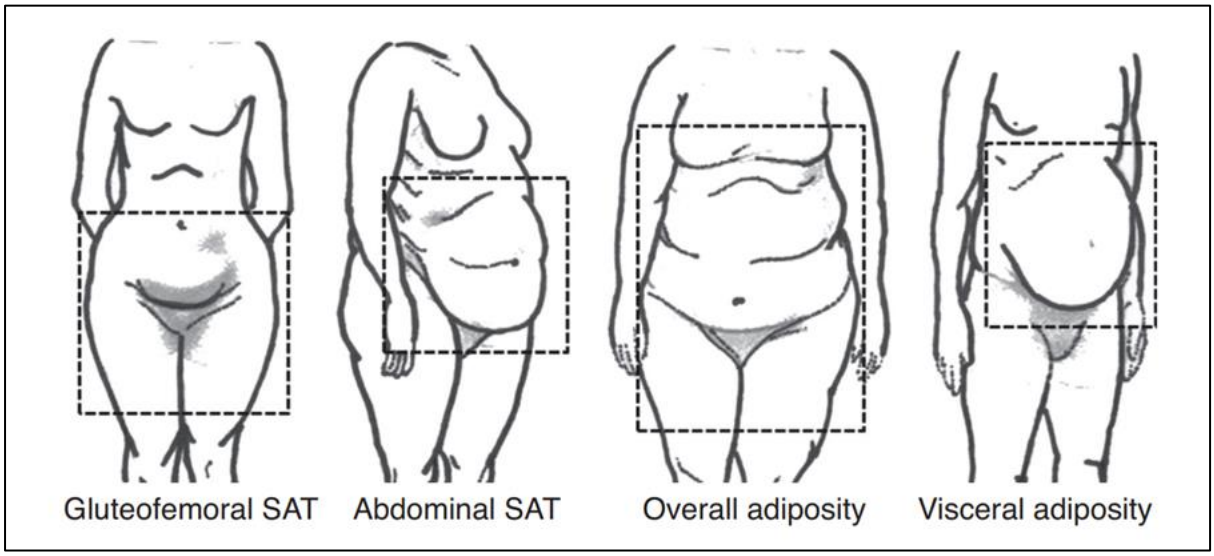

Figure 1.6 Distribution of regional body fat depots. Source Marcadenti *et al.,* (78).

Premenopausal women accumulate fat predominantly in the hips, buttocks, and thighs, giving them a pear shape also referred to as the gluteofemoral or gynoid pattern of adipose tissue distribution (84). Men tend to store fat in the abdominal region, giving them an apple shape also referred to as the android pattern. This has been confirmed by imaging studies which report that premenopausal women exhibit a regional fat distribution that is different from that in age-matched men (85). Specifically, Lemieux

et al., reported that men had higher VAT values than premenopausal women, and women had higher SAT values than age-matched men (85). In a separate study, Kvist *et al.,* showed that in women, no changes in VAT stores occur until a threshold of 30 litres of total adipose tissue stores are reached (86). Furthermore, changes associated with the MT have been associated with the expansion of the VAT depot (84). It has been estimated that the composition of the VAT depot to the total body fat changes from 5–8% in the premenopausal stage to 15–20% after the FMP (87). This shift is associated with a parallel increase in CMD risk that is comparable to that seen in men.

In the following section, evidence of changes on the relationship between CMD risk and MT is discussed, according to the respective timelines of the common longitudinal studies.

1.8.1 Evidence of changes in CMD prevalence and associated risk factors from longitudinal studies

One of the earliest pieces of evidence on changes in CMD prevalence amongst midlife women was from the Framingham Heart study (1). In this study, postmenopausal stage was associated with approximately three-times greater relative risk for cardiovascular (CVD) events than in the premenopausal stage (1). However, this was based on a small sample size of ninety participants with cardiovascular events. Further analysis revealed that only total cholesterol levels increased but not blood pressure, and glucose levels and BMI were not different over the MT (1). More data after over 56 years of follow-up of this cohort showed no differences in the incidence of stroke between men and women during midlife (45–54 years) (88).

In 1990, longitudinal analyses from a study in Denmark reported that menopause was associated with an atherogenic lipid profile (2). Although chronological aging was

related to these changes, the authors showed that the increase in triglycerides, total and LDL-C levels was at its maximum 2-3 months after the FMP (2).

In 1996, the Research Plan for Radiation Effects Research Foundation Adult Health Study (RERF) in Japan reported that levels of total cholesterol increased drastically from three years before FMP to one-year post-FMP, independent of age (3). However, the longitudinal increase in BMI and systolic blood pressure were associated with chronological aging but not menopause (3).

In 1999, Guthrie *et al.,* reported that in a cohort of Australian women; waist circumference and suprailiac skin-fold measurements increased during the MT (89). However, the overall weight gain was only associated with chronological aging but not menopause.

In 2000, the Melbourne Women's Midlife Health Project study reported that declining HDL-C levels were the only CMD risk factor associated with menopause (4). In addition, levels of HDL-C increased around the FMP and declined sharply after the FMP. However, changes to BMI, blood pressure, total cholesterol, triglycerides, and LDL-C levels associated with age.

In 2007, the Michigan site of the SWAN study reported that, only total body fat mass and waist circumference increased but lean mass decreased in the 2-year interval around the FMP (90). Furthermore, in 2009, when all study participants across the seven SWAN study sites were combined, menopause was a risk factor for increased total cholesterol, LDL-C, and apolipoprotein B (7). This atherogenic profile was shown to increase within the 1-year interval around the FMP. In the subsequent reports in 2013 and 2017, further analyses showed that the MT was associated with subclinical atherosclerosis (91,92). The analyses also showed this association was mediated by

increased LDL-C levels during the MT (92). In addition, the MT was a risk factor for increased HDL-C levels and cIMT progression, contrary to the traditional cardioprotective role of HDL-C (93). Further analysis showed that this effect was more pronounced after the FMP. It was then suggested that the changes on the quality of HDL-C over the MT may render it dysfunctional in cardioprotection (93). In 2018, data from the Chicago and Pittsburgh sites of the SWAN study reported accelerated aortic stiffness in women undergoing the MT than women who either remained premenopausal or postmenopausal (94). Other changes in the anthropometric and CMD risk factors, BMI, blood pressure, insulin, and blood glucose levels were associated with increasing age but not the MT (7,95). Moreover, the SWAN study showed that the metabolic syndrome increases during the MT, and is independent of age and other potential confounders (96).

In 2016, the ARIC study in the USA showed that the prevalence of metabolic syndrome increased during the MT but decreased after the FMP, and this was more prominent in African American than Caucasian women (8).

In 2018, Razmjou *et al.,* showed that from the MONET study in Canada, the postmenopausal stage was associated with increased waist circumference, percentage body fat, and low-grade inflammation (9). Both the MT and progression of the postmenopausal stage was associated with increasing levels of inflammatory markers including apolipoprotein B, ferritin, haptoglobulin, and soluble cluster of differentiation 14 (sCD14), whereas interleukin 8, and soluble tumour necrosis factor receptors 1 and 2 decreased. In addition, total cholesterol and HDL-C increased but blood glucose levels decreased during the MT. No changes in BMI were reported (9).

In 2019, the ERMA study showed that the menopause was associated with elevations in total cholesterol, LDL-C, and HDL-C levels. No other changes in any other CMD risk factors were reported (10).

Many of the referenced longitudinal studies show that the MT predisposes midlife women to CMD risk through increased atherogenic dyslipidemia, obesity, metabolic syndrome, and inflammation (1,3,4,7–10,89,90). However, blood pressure, glucose, and insulin levels and BMI were associated with aging but not the MT (1–4,7,9,10,89,95). Furthermore, the relationship between the MT and CVD events is not fully understood. However, data from randomised clinical trials (RCTs) has since shown the potential benefits of menopausal hormone therapy (HT) in reducing atherosclerosis in healthy women early in menopause (97–99). The following section discusses some of the key RCTs on menopause HT and CMD prevalence.

1.8.2 Evidence of changes in CMD prevalence from randomised clinical trials

In the late 1990s, menopause HT was gaining popularity as a preferred strategy for the prevention of CMD in menopausal women (100). However, the supporting evidence at the time was from small observational studies that investigated intermediate end points such as blood lipids and not CMD endpoints (100). As a result, RCTs were designed to critically assess the effectiveness of HT in CMD prevention in postmenopausal women. In 2006, a meta-analysis on 23 RCTs comprised of 39,049 participants reported that there was no effect of HT on coronary heart disease (CHD) prevalence (OR 0.99 [95% CI, 0.88–1.11]) (99). Further analysis showed that HT was effective only in younger women, where the CHD prevalence was lowered by at least 32%, (OR 0.68 [95% CI, 0.48–0.96]). No such effect was reported in older women, 1.03 (95% CI, 0.91–1.16) (99). Similar findings were reported in another meta-analysis that focused on CHD mortality, where in younger women, HT reduced the total

mortality by at least 40% (OR 0.6 [CI, 0.4–0.95]) (101). Whether these differences in CHD morbidity and mortality between younger and older women is attributed to either the MT or aging or a combination of both remains to be answered.

Beyond the discussed meta-analyses, more RCTs have since examined the effects of early menopausal HT on the progression of subclinical atherosclerosis. These include the Kronos Early Estrogen Prevention Study (KEEPS) (2014 publication) (102) and the Early versus Late Intervention Trial with Estradiol (ELITE) (2016 publication) trials (103). The KEEPS assessed changes to the carotid intima media thickness (cIMT) after HT (102). Briefly, the KEEPS recruited women aged 42–58 years and in early menopause. The intervention group received two different formulations, either a low dose of estrogens or transdermal estradiol. Results showed that after 48 months, there were no differences in cIMT levels between the intervention and control groups (102). On the other hand, the ELITE trial enrolled women who were between 6 (early menopausal) and 10 (late postmenopausal) years from their FMP (103). In contrast to the KEEPS, the treatment group in the ELITE received a higher dose of estrogen-containing HT. After 5 years of follow-up, results showed differences in the progression of subclinical atherosclerosis between the treated and control groups. HT was associated with less cIMT progression when therapy was initiated within 6 years of the FMP. However, no differences were observed in the group that initiated therapy 10 years after the FMP (103).

The cumulated data from these RCTs therefore demonstrates that differences in the approach to the administration of HT leads to different outcomes in CHD prevalence. As discussed, the effectiveness of HT in reducing CHD prevalence during the menopause is dependent on early initiation of therapy and the dosage of the HT regimens.

1.8.3 Mechanisms for changes in CMD risk during the menopause transition

In this section, the mechanisms linking the MT to changes in CMD risk are discussed. These include changes in body composition and fat distribution, hormonal levels, age at FMP, environmental, lifestyle and, genetic factors, and the human immunodeficiency virus (HIV).

1.8.3.1 Body composition and fat distribution

Hormonal changes such as the declining of estrogens and increasing FSH levels during the MT have been associated with higher percentage body fat but lower lean (fat-free) mass (5). These changes contribute to central or visceral obesity even in the face of a normal BMI (84). Data from the SWAN study has shown that VAT increases by 8.2% (95% CI: 4.1–12.5) per year, in the 2 years preceding the FMP and by 5.8% (95% CI: 3.7–7.9%) per year, after the FMP (104). The MT was associated with increased VAT and a higher risk of subclinical atherosclerosis (104). The expansion of the VAT depot has also been associated with increased pro-inflammatory adipokines and free fatty acids which in turn increase the risk of insulin resistance, and hypertension (105). However, in some reports such as the MONET study, despite an increase in VAT across the MT, no changes in CMD risk were reported (106).

Data from experimental work on abdominal and femoral adipocytes have provided more insight on how estradiol mediates the expansion of the VAT depot (107). In this work, Rebuffé-Scrive *et al.,* observed that in premenopausal women, femoral adipocytes express a higher lipoprotein lipase (LPL; this enzyme increases free fatty acid uptake into adipocytes) activity than in abdominal and mammary adipocytes. This was suggestive of a preferential fat storage in femoral adipocytes than at the other sites (107). When adipocytes from postmenopausal women were examined, no differences were observed between the adipocytes from the three regions. Therefore,

it was suggested that menopausal changes are associated with lower expression of LPL in femoral adipocytes which lead to the relative expansion of abdominal fat. In addition, Pedersen *et al.,* observed that by adding estradiol to cultured adipocytes, the hormone sensitive lipase (HSL) activity in SAT adipocytes decreased but no changes were seen in VAT adipocytes (108). Hormone sensitive lipase sits within adipocytes and is responsible for the hydrolysis of stored triglycerides. This demonstrates that estradiol levels are necessary for inhibiting HSL in the SAT depot thereby retaining the assimilation of fat in this depot, rather than the VAT.

1.8.3.2 Hormonal changes

As discussed earlier, the MT is associated with increased levels of LDL-C, apolipoprotein B and total cholesterol (3,7,10,59). Song *et al.,* has since demonstrated that FSH downregulates the hepatic LDL-receptor (LDLR) thereby reducing the clearance of LDL-C from the general circulation (11).

Furthermore, the SWAN study demonstrated an association between decreasing estradiol and increasing FSH levels with more risk of subclinical atherosclerosis during the MT (12). In this study, cIMT was associated with changes in estradiol and FSH levels during the MT (12). Women who had lower estradiol levels before and after the FMP had a 43% higher risk of developing subclinical atherosclerosis compared to those who had higher estradiol levels before their FMP (12). In addition, women who experienced a pronounced increase in FSH levels after the FMP had higher cIMT levels than those who experienced a slower increase in FSH levels (12).

It has been reported that in parallel with declining estradiol levels, the ratio of androgens to estrogens increases, leading to a relative androgen excess (13). Moreover, declining E2 levels have been associated with reduced synthesis of SHBG which further increases the free androgen index (FAI) (13,66). However, it is difficult

to ascertain which of the two; relative androgen excess (mostly testosterone) or lower SHBG levels have a stronger contribution towards CMD risk.

Data from the SWAN study also shows that SHBG levels are directly associated with insulin sensitivity (109). The direction of this relationship is unclear. It is possible that insulin suppresses the hepatocyte synthesis of SHBG, thereby SHBG levels could serve as surrogate markers for insulin levels (110). In the Melbourne Women's Midlife Health Project study, SHBG levels were negatively associated with BMI (66).

1.8.3.3 Age at final menstrual period

The onset of menopause is a marker for both reproductive and chronological aging (111). Thus, the age at FMP is important in understanding CMD risk. In a recent meta-analysis involving 321,233 women, premature menopause (<40 years) was associated with CVD (112). The strength of this association was greater than that for premature menopause (40-44 years) and early menopause (45-49 years) (112). Similarly, a longitudinal study involving 3,639 women from the Netherlands showed that the risk for type 2 diabetes was highest in premature menopause, higher in early menopause, and lower in those with late onset menopause (113). Conversely, in a meta-analysis involving 177,131 women from four countries (UK, Japan, Australia and Sweden), premenopausal women who had had a cardiovascular event (CHD or stroke) before the age of 35 years were twice as likely to reach early menopause (<45 years) than their peers who did not experience a CVD event and reached menopause at age 54 years (114). The study also showed that premenopausal women who experienced their first CVD event later (>40 years) had a later onset of menopause (50-51 years). Therefore, the relationship between age at menopause and CMD risk might be bidirectional, with cardiometabolic health potentially affecting the age at menopause or vice-versa.

1.8.3.4 Environmental and lifestyle factors

Environmental and lifestyle-associated factors including dietary patterns, cigarette smoking, smokeless tobacco, alcohol consumption and sociodemographic factors have been associated with age at FMP and CMD risk (115).

Some studies have reported that women who consume more tea, fruits and vegetables, and high protein foods such as meat, seafoods, soybean, and dairy products have a later onset of menopause compared to those that did not (116,117). However, in some studies, the consumption of more vegetables and carbohydrates were associated with an earlier age at menopause (117).

Cigarette smoking is associated with an earlier age at FMP (118). The mechanism involves the estrogenic effects of cigarette smoking (119). From *in vitro* studies, cigarette smoke condensates actively bind to estrogen receptors and induce the cytochrome P450 enzymes 1A1 (CYP1A1) and 1A2 (CYP1A2) to a shunt pathway which increases the levels of a by-product known as 2-hydroxyestrogen thereby creating a net reduction in estradiol levels (119). Other polycyclic aromatic hydrocarbons in cigarette smoke are associated with a cumulative damage on ovarian follicles (120). Furthermore, smokeless tobacco (snuff) has been associated with increased VAT (121). The high nicotine content in snuff have been shown to activating lipolysis in the adipose tissue thereby increasing the levels of free fatty acids in circulation and oxidative stress. In addition, nicotine mediates endothelial dysfunction thereby further increasing CMD risk (122).

The effect of alcohol consumption on the age at FMP has also been studied. In a meta-analysis on six longitudinal studies involving 63,868 women, the relative risk of earlier age at FMP was lower in women who consumed alcohol compared to abstainers

(115). Further analyses revealed that this was only true in women who consumed low to moderate levels of alcohol (up to 16g/day), but not heavy drinkers. However, it is unclear if this association was dependent on the type(s) of alcohol.

There is also evidence showing a relationship between socioeconomic status and age at FMP (123). In these studies, women from lower socioeconomic positions were more likely to experience earlier menopause than their peers with higher socioeconomic status (123). One British study further demonstrated that both childhood and adult socioeconomic status were associated with age at FMP (124). It was also noted that other early life influences including childhood trauma such as parental divorce were associated with earlier menopause (123). In addition, children who were breastfed for more than 7 months had a later menopause than those breastfed for 6 months and less (125). Lastly, higher education, urban residence, and married status have also been associated with later age at menopause (126).

1.8.3.5 Genetic factors

Family studies on mother-daughter pairs, and twins have shown strong associations between genes and age at FMP, ranging from 45 to 87% heritability (127,128). Furthermore, one genome wide association study (GWAS) involving 201,323 women identified 290 loci associated with age at FMP (129). These loci were shown to be involved in a wide range of DNA damage response (DDR) pathways responsible for the detection of DNA damage and signalling pathways to promote DNA repair (130). The loss-of-function in these DDR genes has been associated with ovarian aging (130). From this GWAS, women with the loss-of-function variants on the genes *BRCA2, CHEK2* and *BRCA1* reached menopause between 1.5 and 2.6 years earlier than their non-carrier peers (129). As previously discussed, early FMP is associated

with higher CMD risk so it is conceivable that these variants may also be associated with higher risk, although there are no studies indicating this.

1.8.3.6 HIV

HIV is an independent risk factor for both earlier age at FMP and CMD. In a systematic review on six studies from three countries (USA, Brazil, and France), only two studies compared age at FMP between women living without HIV (WLWOH) and women living with HIV (WLWH) (131). From these studies, one reported that WLWH had a lower median age at menopause (132), but no differences were observed in the other study (133). Outside of this review, one study from Thailand showed that WLWH had lower age at FMP than WLWHOH (134).

In as much as these studies have given some insights on the relationship between HIV and age at FMP, the overall global data on HIV and menopause and CMD risk remains scarce. More so, the HIV prevalence is highest in SSA, yet no studies have investigated the impact of HIV on menopause and CMD risk in this region.

1.9 Menopause research in low-and-middle-income countries (LMICs)

There are few longitudinal studies investigating menopause and CMD risk in LMICs. Cross-sectional studies are more common but are still much less frequent compared to studies conducted in higher income countries. These studies are discussed in the following section.

1.9.1 Longitudinal studies

Current literature only has four longitudinal studies from three LMICs namely Chile, China, and Iran that focused on the relationship between the MT and CMD risk (135–141). In the Barros Luco Study from Chile, there were no associations between the MT and blood pressure, glucose and lipid levels. (135) Furthermore, a sub analysis showed that weight changes were related to aging (137).

In the Zhou *et al.,* study from China, a total of 593 women were recruited to assess changes in serum lipid profiles during the MT (142). After 3 years of follow-up, the transition from pre- to perimenopausal stage was associated with increased levels of total cholesterol and triglycerides, but not HDL-C levels. However, no changes were observed in women who transitioned from the pre- to the postmenopausal stage, therefore suggesting that the MT but not menopause per se is related to the changes in serum lipids (142). In another study from China, the Chinese Multi-Provincial Cohort Study (CMCS), a total of 2104 women were recruited to study age at FMP and prevalence of fatal and non-fatal CVD events for close to 26 years (141). The results showed that early menopause was associated with a high risk of ischemic stroke and death compared with late menopause (141).

In a longitudinal study from Iran, involving 929 women and a follow-up of at least 15-years, menopausal changes in BMI, and serum lipids (139,140,143) were assessed. Menopause associated with BMI, but age at FMP modified this effect. Women with a later age at FMP (>49 years) had decreasing future BMI compared to those with an earlier age at FMP (143). Menopause was associated with increased total cholesterol and LDL-C levels (139). In addition, the risk of CHD in the premenopausal stage was associated with increasing HDL-C whereas in the postmenopausal stage, the risk of CHD was associated with decreasing HDL-C levels (140).

1.9.2 Cross-sectional studies

Few cross-sectional studies from SSA namely South Africa (121,144), Ghana (145), and the Democratic Republic of Congo (146) have investigated the relationship between CMD risk factors and menopausal stage. In one of the South African studies, no differences in anthropometric and CMD variables between pre- and postmenopausal women were observed (147). Furthermore, the levels of testosterone

and DHEA did not differ between the menopausal groups. Lower SHBG levels correlated with increased VAT, waist circumference and BMI, and lower DHEAS levels were associated with increased total fat mass (147). In the other study from South Africa, the postmenopausal stage was associated with higher percentage fat mass, waist circumference and VAT, but less gynoid percentage fat mass (144). In the studies from Ghana and the DRC, the prevalence of metabolic syndrome were higher in post- than in premenopausal women (145,146).

The rest of the cross-sectional studies from LMICs were based in non-SSA countries, including China (148–154), Brazil (155–159), India (160–163), Tunisia (164), Thailand (165,166), Mexico (167), and Bangladesh (168). Most of these studies showed that postmenopausal women had a worse cardiometabolic profile characterised by elevations in both anthropometric and CMD variables when compared to their premenopausal peers. Some of the studies showed that menopause was associated with increased levels of novel oxidative stress biomarkers such as lipoperoxides and remnant lipoprotein cholesterol (TRL-C). Increased levels of oxidative stress have been known to mediate the relationship between menopause and increased CMD risk (167,169). Associations with hormonal changes have also been reported. In one study from China, testosterone levels were directly associated with HDL-C and blood pressure levels, whereas estradiol levels were negatively associated with total cholesterol and blood pressure levels (153). In another study from Brazil that studied WLWH, elevated FSH levels and BMI in postmenopausal women were related to the metabolic syndrome (158). However, some studies showed no differences in the levels of CMD risk factors such as the metabolic syndrome, blood glucose, blood pressure and HDL-C levels between the two menopausal groups after adjusting for age (151,155,165).

1.10 Summary and knowledge gaps

Overall, the MT is associated with increased CMD risk. However, there are no longitudinal data on SSA women exploring the contribution of the MT on these changes in CMD risk. These data are important to expand the knowledge from cross-sectional study designs and provide relevant context in SSA, a region plagued by multi-morbidities that include infectious and non-communicable diseases.

The current evidence from cross-sectional studies on menopause and CMD risk in SSA also has further shortcomings that need to be addressed. Firstly, the selection of premenopausal women in these studies have included a wide age range (from 20 years onwards) (144,145). Secondly, the modest sample sizes: 590 (South Africa) (121), 206 (South Africa) (144), 250 (Ghana) (145), and 200 (DRC) likely reduces statistical power, and could bias the overall interpretation of these studies. Thirdly, there were no data on the effect of HIV across different menopausal groups, despite the high prevalence of HIV in SSA.

1.11 Study aim, hypothesis, and objectives

The aim of this study is to determine whether the MT is associated with increased levels of CMD risk factors in both SSA women and women from other LMICs and whether any association is modified by HIV. The hypothesis that will be investigated is that CMD risk factors are higher in post- than premenopausal women and are associated with changes in sex hormone levels and augmented by HIV. The specific objectives are described below:

1. To perform a systematic review and meta-analysis on the association between menopause and CMD risk factors in studies from LMICs.

2. To examine differences in anthropometric and cardiometabolic variables between pre-menopausal and postmenopausal women in 4 SSA countries – Burkina Faso, Ghana, Kenya, and South Africa – in a cross-sectional analysis. The variables are as follows:

- Fasting serum levels of total cholesterol, triglycerides, LDL-C and HDL-C
- Carotid intima-media thickness (cIMT)
- Insulin resistance and fasting plasma glucose levels
- Systolic and diastolic blood pressure
- Body mass index (BMI)
- Waist and hip circumferences
- Visceral and abdominal subcutaneous fat levels

3. To examine longitudinal changes (over 5 years) in the above variables in menopause-transitioning and postmenopausal women.

4. To establish if a relationship exists between changes in hormone levels (FSH, estradiol, testosterone, DHEAS, SHBG) and changes in cardiometabolic and anthropometric variables in menopause-transitioning and postmenopausal women from Burkina Faso and Ghana.

5. To compare CMD risk factors between pre- and postmenopausal SSA WLWH and WLWOH and to determine whether menopausal differences for these risk factors were modified by Highly Active Antiretroviral Therapy (HAART).

6. To determine whether HIV is associated with earlier age at menopause.

1.12 Conceptual framework

A conceptual framework outlining the proposed relationships between the MT, sex hormones, HIV and HAART, anthropometric and CMD risk factors are shown in Figure 1.7. The figure also highlights the proposed influence of sociodemographic factors over all these relationships. Overall, this conceptual framework acts as a summary and guide for this PhD study. The relationships shown in Figure 1.7 have been fully described in the preceding literature review.

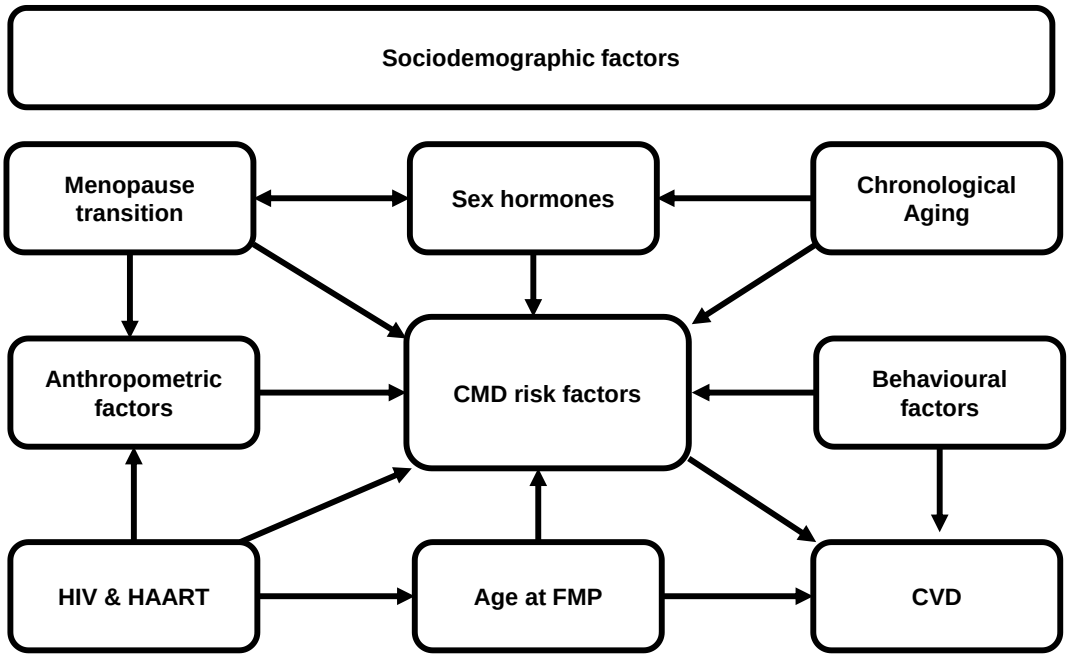

Figure 1.7 Conceptual framework. CMD risk factors- blood pressure, cIMT, SAT, VAT, lipid, glucose and insulin levels, Anthropometric factors-BMI, waist, and hip circumference. Sex hormones- FSH, estradiol, testosterone, DHEAS, SHBG), Sociodemographic factors-study site, highest level of education, socioeconomic status, Behavioural factors-smoking, alcohol consumption, physical activity, diet, CVD-cardiovascular disease.

Chapter 2: Association of Menopause with Cardiometabolic Disease Risk Factors in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis

Chikwati RP, Chikowore T, Goolam Mahyoodeen N, Jaff N, George JA, and Crowther NJ. Association of Menopause with Cardiometabolic Disease Risk in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis (will be submitted to *Menopause*).

2.1 Abstract

Importance: Menopause is an integral part of women's health. Therefore, its impact on cardiometabolic disease risk factors should be assessed. However, to date no study has combined and assessed such studies across LMICs. This would better inform early monitoring and intervention strategies for reducing CMD in mid-life women in these regions.

Objective: To evaluate the association between menopause and CMD risk in LMICs.

Evidence Review: A systematic review with meta-analyses of original articles from PubMed, PubMed Central, Scopus, and ISI Web of Science databases was conducted from conception until January 18, 2023. Observational studies that met the inclusion criteria were included in the analysis. Quality assessment of the articles was done using the Newcastle-Ottawa Scale for cross-sectional and longitudinal studies. The study protocol was registered with the International Prospective Register of Systematic Reviews and adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis

Findings: The initial search identified 7,124 studies; 47 for the systematic review and 16 for the meta-analysis, in accordance with the inclusion criteria. Compared with

premenopausal women, postmenopausal stage was associated with metabolic syndrome OR=1.18 (95% CI 1.11–1.27), high waist-hip ratio OR=1.22 (95% CI 1.12 – 1.32), hypertension OR=1.10 (95% 1.04-1.16), elevated triglycerides OR=1.16 (95% CI 1.11–1.21), elevated plasma glucose OR=1.21 (95% CI 1.15–1.28). These results show an independent association between menopause and CMD risk factors within populations in LMICs.

Conclusions and Relevance: CMD risk factors metabolic syndrome, waist-hip ratio, blood pressure, triglyceride, and blood glucose levels were associated with the postmenopausal stage. This presents an urgent need for public health to focus on early monitoring and interventions targeted at reducing CMD risk and related adverse outcomes in midlife women.

2.2 Introduction

Adverse changes in cardiometabolic health in midlife women are thought to be driven by hormonal changes along the HPO axis during the MT (170,171). In one report nested within the SWAN cohort, it was hypothesised that a relative androgen excess characterised by an increased testosterone:estrogen ratio during the MT could be associated with the redistribution of body fat (172). In particular, the expansion of the abdominal visceral fat depot accompanied by an increase in adipokine secretion and overall chronic inflammation (173), which are associated with increased CMD risk (174).

Several studies show that women from low- and middle-income countries (LMICs) reach menopause at an earlier age than those from high income countries (175). In one meta-analysis involving thirty-six studies across the six continents, the mean (with 95% CIs) age at menopause in LMICs was lower in Africa (48.4 (48.1–48.7)), Latin

America (47.2 (45.9–48.6)), Asia (48.8 (48.1– 49.4)), and the Middle East (47.4 (46.9–47.8)), compared to Australia (51.3 (49.8–52.8)), Europe (50.5 (50.0–51.1)) and the United States (49.1 (48.8–49.4)) (175). Early age at menopause has been linked with increased CMD risk (176), therefore suggesting heightened risk in LMICs. However, there are no data quantifying the association of menopause with CMD in LMICs. The objective of this systematic review and meta-analysis was therefore to evaluate evidence from the literature of links between menopause and CMD risk factors in midlife women living in LMICs.

2.3 Methods

2.3.1 Protocol

This systematic review and meta-analysis were done following the Preferred Reporting Items for Systematic Review (PRISMA) 2020 guidelines and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) with the number CRD42021295401 (177) (Appendix 3).

2.3.2 Search strategy and data sources

The databases: PubMed, PubMed Central, Scopus, and ISI Web of Science were searched for original articles from inception until January 18, 2023. The query terms consisted of the key words related to “premenopause”, “postmenopause”, “cardiometabolic disease risk factors” and “LMICs”. The search strategy is fully detailed in Appendix 5 (Table 1). Furthermore, the website, “Connected Papers” was used to apply snowball sampling to find similar articles. This was applied only on articles that were selected for the systematic review.

2.3.3 Eligibility criteria

Only studies conducted in LMICs as defined by the World Bank list of economies (June 2020) were eligible (178). These studies assessed pre- and postmenopausal women and their associated risk for cardiometabolic disease. The literature search strategy was defined by a combination of the study population (midlife women in LMICs), exposure (menopause), comparison (post- vs premenopause), and outcomes (CMD risk factors). The inclusion criteria comprised: 1) studies that enrolled both pre- and postmenopausal women, 2) studies evaluating differences in cardiometabolic disease risk factors according to the menopausal stage, and 3) studies published in English. Articles were excluded if they were: reviews, editorials, or preliminary reports.

2.3.4 Data extraction

One researcher (the PhD candidate) independently screened all initially identified articles and abstracts using the Rayyan software (179). The number of included and excluded records is mapped in Figure 2.1. Studies deemed to potentially meet inclusion criteria underwent a full-text assessment by two independent reviewers (RPC and NGM). The consensus between two authors satisfied the inclusion criteria. Disagreements were resolved by a third reviewer, NJC (supervisor).

2.3.5 Quality Assessment

Two reviewers, RPC (PhD candidate) and NGM (supervisor), independently used the modified Newcastle-Ottawa Scale (NOS) (180) to assess the methodological quality of selected articles. Briefly, the NOS uses a nine-star system with three key domains: selection of participants (representativeness of sample), comparability (adjustments of age and other potential confounders such as smoking status, BMI etc.) and identification of the outcomes of interest (CMD risk factors). Based on the total score, the risk of bias was assigned into two categories: low risk (7-9) and high risk (0-6).

Only studies with a low risk of bias were included in this study. Any disagreements were referred to a third reviewer, NJC.

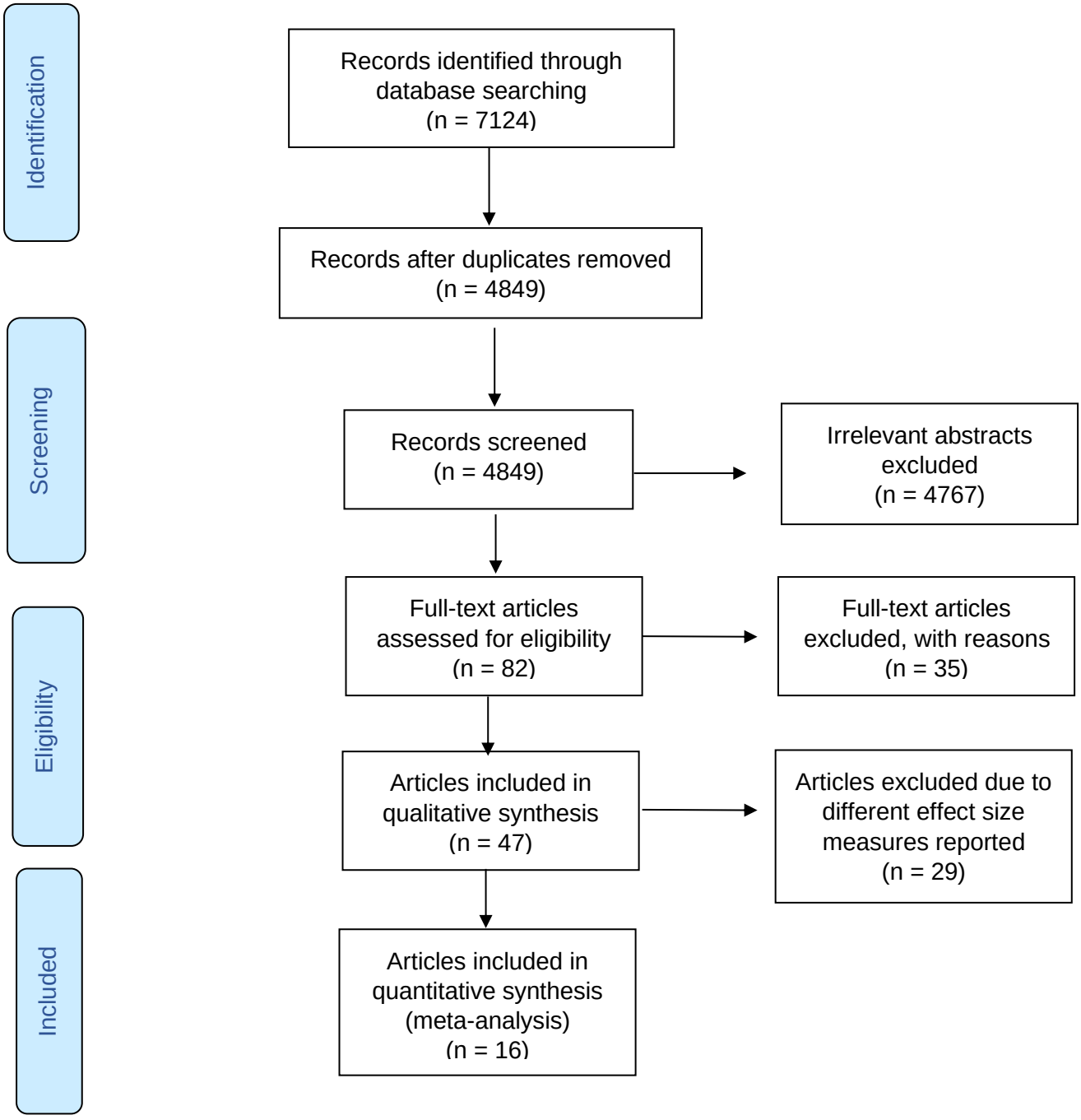

Figure 2.1: PRISMA flow chart of literature screening and selection. PRISMA, preferred reporting items for systematic reviews and meta-analysis (130).

2.3.6 Statistical Analyses

To quantitatively assess the association between menopausal differences and the CMD risk factors metabolic syndrome (MetS), blood pressure, triglycerides, HDL-C, blood glucose, and cIMT levels, general obesity, waist circumference and waist-hip ratio, and type 2 diabetes mellitus, pooled estimates of odds ratios and associated 95% confidence intervals were calculated using the inverse variance fixed-effect model. In the analyses, studies were grouped based on the outcome of interest (CMD risk factor), and the definition of the outcome of interest.

Heterogeneity between studies was assessed using Cochran's Q statistic ($p<0.01$ indicative of heterogeneity) and the I^2 index (values 25%, 50% and 75% suggestive of low, moderate, and high heterogeneity, respectively). Statistical analyses were performed using Stata 16.1 (StataCorp LP, College Station, TX, USA).

2.4 Results

2.4.1 Search results

Figure 2.1 shows the PRISMA flow chart on the screening and selection of the research articles. Briefly, the initial search identified 7,124 abstracts. After removing duplicates, 4,849 titles and abstracts were screened. Of these, 4,767 irrelevant articles were excluded, leaving 82 articles for full-text review. Thirty-three of the 82 articles were excluded in the quality assessment. From the "Connected Papers" website search, 5 articles were identified through snowballing on each of the articles that fit the inclusion criteria. Three of these articles were excluded in the quality assessment. As a result, 47 articles constituted the systematic review. Of these 47 articles, 16 were eligible for the quantitative analysis and 31 were excluded. At this stage, articles were removed due to the following: reporting of a CMD risk factor that was uncommon to other articles (n=3), no combined comparison of pre- and postmenopausal stages on

CMD risk factors (n=1), different definition criterion for MetS (n=1), prevalence studies (n=10), randomised clinical trial (n=1) and studies with different effect measures (n=31).

2.4.2 Study characteristics and populations

Table 2.1 and 2.2 present the characteristics of the forty-five studies included in the systematic review. The studies were from the following countries: China (148,149,185–187,150–153,181–184) (n=14), Brazil (156,159,169,188–191) (n=7), Iran (139,140,143,192–197) (n=9), India (160,162,163) (n=3), Tunisia (198–200) (n=3),Thailand (165,166) (n=2), Mexico (167) (n=1), Democratic Republic of Congo (146) (n=1), Ghana (201) (n=1), South Africa (147) (n=1), Bangladesh (202) (n=1), and Chile (137) (n=1). In total, the studies comprised of 353,589 participants, with sample sizes in each study ranging from 122 to 281,319 participants. Articles were grouped according to each CMD risk factor as shown in Table 2.1.

Table 2.1 CMD risk factors and corresponding articles examined.

CMD risk factor	Number of articles examined
MetS	16
Obesity	14
Blood lipids	12
WC and WHR	11
Blood glucose and insulin levels	11
BP	9
cIMT	2
Others	4

CMD-cardiometabolic disease, MetS-metabolic syndrome, WC-waist circumference, WHR-waist hip ratio, BP-blood pressure, DM-diabetes mellitus, cIMT-carotid intima media thickness, Others-Fat mass, visceral and subcutaneous adipose tissues.

Table 2.2 presents the 47 articles included in the systematic review. In the 16 articles describing the MetS, 10 showed higher MetS in post- than premenopause (150,159,160,181,184,187,194,195,202,203), and 6 showed no differences (165,187,188,190,191,193). In the 14 articles focused on obesity, 3 showed higher

obesity risk in post- than premenopause (143,156,204), and 11 showed no differences (137,147,199,152,156,161,182,185,189,192,198). In the 12 articles on blood lipids, HDL-C was lower in post- than in premenopause in one study (140), but no differences were reported in triglycerides in a separate study (196). Elevated total cholesterol, LDL-C, lipoperoxides, and triglyceride-rich lipoprotein-cholesterol (TLR-C) levels were reported in 9 articles (139,149,151–153,162,167,169,197) in post- compared to premenopausal women. In the 11 articles on WC and WHR, 5 showed that postmenopausal women had higher WC (149,161,182,189,198) and 4 articles showed higher WHR (151,152,161,189), but no menopausal differences were reported on WC in 4 studies (147,156,192,196). In the 11 articles on blood glucose and insulin levels, 3 showed higher glucose levels in post- than premenopause (162,198,199) but 5 showed no difference (149,151,152,182,192), and 2 showed higher insulin in post- than premenopausal women (198,199). In the 9 articles on blood pressure, 6 showed higher blood pressure levels in post- than premenopause (149,153,162,163,182,198) and 3 showed no differences (151,152,192). In the 2 articles describing cIMT, postmenopausal women had higher cIMT levels than their premenopausal counterparts (166,186). In the other articles, menopause was associated with diabetes mellitus (183), 10-year risk of cardiovascular disease (148), and increased body fat mass (161), but no differences in VAT and SAT (121).

In the meta-analysis, fourteen studies from the following countries, China (n=5), Brazil (n=3), Tunisia (n=2), Thailand (n=1), Bangladesh (n=1), and Iran (n=1) constituted a total of 29,361 women. Studies were further categorised according to standard definitions of the risk factors as follows: 1) MetS defined by the National Cholesterol Education Program Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adult Treatment Panel III (NCEP-ATP III criteria) (n=6), 2)

elevated serum triglycerides (≥1.69 mmol/L) (n=4), 3) low HDL-C (<1.29 mmol/L) (n=2), 4) hypertension (SBP ≥140 mmHg, DBP ≥90 mmHg and use of antihypertensives) (n=5) (24–26), 5) hypertension (SBP ≥135 mmHg, DBP ≥85 mmHg and/or use of antihypertensives) (n=3) 6) high waist circumference (≥80 cm) (n=2) and 7) high waist circumference (≥88 cm) (n=3), 8) high WHR (≥0.86) (n=3), and obesity (BMI ≥28kg/m^2) (n=2).

Table 2.2: Studies included in the qualitative analyses.

First Author, Year, Reference	Country	Study Type	Sample Size	Outcome	Main Results
Neto (2010) (188)*	Brazil	Cross-sectional	323	MetS	Influence of age on MetS was prevalent, and attenuated any menopausal differences
Moreira (2020)(191)*	Brazil	Cross-sectional	419	MetS	No association between menopausal stage and MetS
Jesmin (2013) (202)*	Bangladesh	Cross-sectional	1802	MetS	MetS higher in post vs premenopausal women
Belfki (2012)(203) ***	Tunisia	Cross-Sectional	961	MetS	Postmenopausal stage was associated with higher risk of MetS
Jeenduang (2014)(165)*	Thailand	Cross-sectional	361	MetS	No association between menopausal stage and MetS
Ali (2014) (199)*	Tunisia	Cross-sectional	1311	BP, obesity, glucose, and insulin resistance	Only hyperglycemia was associated with postmenopausal stage
Ren (2019) (149)*	China	Cross-sectional	8191	BMI, TGs, glucose, BP, WC	Menopause associated with increased risk of higher BMI, hypertension, TGs, and WC
He (2012) (151)*	China	Cross-sectional	4743	BMI, WHR, lipids, glucose, BP	Elevated total cholesterol, LDL-C, triglycerides, and waist hip ratio were the only risk factors associated with postmenopausal status.
Zhou(2014)(184) *	China	Cross-Sectional	6324	MetS	Postmenopausal status was a risk factor for hypertension
Ali (2016) (198)*	Tunisia	Cross-sectional	242	BMI, WC, BP, glucose, HOMA, lipids	Waist circumference, HOMA, and apo B levels were associated with hypertension in postmenopausal women.
Tehrani (2013)(192)*	Iran	Longitudinal	675	BMI, glucose, lipids, WC, BP	Only LDL-C and total cholesterol were associated with postmenopause
Zhou(2018) (181)*	China	Cross-Sectional	6022	MetS	MetS was higher in postmenopausal women
Chen(2020) (185)*	China	Cross-Sectional	5373	Obesity	Menopause was a risk factor for central and visceral obesity but not general obesity
Donato (2006)(189)*	Brazil	Cross-sectional	358	WC, WHR, BMI	Postmenopausal women had higher WC & WHR than premenopausal women
Ieamtairat (2019) (166)*	Thailand	Cross-sectional	122	cIMT	Menopause was associated with increased cIMT levels

Zhou (2015) (186)*	China	Cross-Sectional	2131	cIMT	Postmenopausal had higher cIMT levels than premenopausal women
Montazeri (2018) (143)	Iran	Longitudinal	929	BMI	Menopause was associated with increasing BMI.
Nazari (2003) (140)	Iran	Longitudinal	3778	HDL-C	HDL-C associated with coronary heart disease in postmenopause
Tehrani (2014) (139)	Iran	Longitudinal	755	Lipids	Dyslipidemia associated with lower AMH levels
Heidari (2010)(193)	Iran	Cross-sectional	1596	MetS	Menopause was only associated with elevated triglycerides.
Maharlouei (2014) (194)	Iran	Cross-sectional	924	MetS	Menopause was associated with higher prevalence of MetS
Ainy (2007) (195)	Iran	Cross-sectional	2182	MetS	Menopause was associated with higher prevalence of MetS
Sarrafzadega (2013) (196)	Iran	Cross-sectional	4146	TGs, WC	Menopause was not associated with a high triglyceride/waist circumference phenotype
Yousefzadeh (2013) (197)	Iran	Cross-sectional	1538	Lipids	LDL-C and total cholesterol levels were higher in post- than in premenopausal women
Wang(183)	China	Longitudinal	281,319	DM	Postmenopausal women had higher risk of developing diabetes
Zhou (2019) (148)	China	Cross-sectional	569	10-year risk of CVD in DM	Menopause was associated with 10-year risk of CVD
Yu (2021) (150)	China	Cross-sectional	1352	MetS	Menopause was associated with higher prevalence of MetS
Feng (2008) (152)	China	Cross-sectional	9097	BMI, WHR, glucose, insulin, lipids, BP	Only WHR, TGs, total cholesterol, HDL-C and LDL-C were higher in post- than in premenopausal women
Wu (1990) (153)	China	Cross-sectional	598	BP, TC, TGs, HDL-C	Postmenopausal women had higher BP and lipid levels
Li (2019) (182)	China	Cross-sectional	3227	BMI, WC, BP, glucose, lipids, TP	Waist circumference, systolic and diastolic blood pressure, triglycerides, ALT, TP, and BUN were risk factors for DM in postmenopausal women.
Strand (2014) (187)	China	Cross-Sectional	440	MetS	Prevalence of MetS was similar in pre- and postmenopausal women
Blümel (2001) (137)	Chile	Longitudinal	271	BMI	BMI independent of menopausal differences

Theodoro (2012)(156)	Brazil	Cross-sectional	617	WC, BMI	Postmenopause was associated with increased general obesity but not abdominal obesity when compared to premenopausal women
Akl (2017)(190)	Brazil	Cross-sectional	273	MetS	No association between menopausal status and metabolic syndrome
Fonseca (2019)(169)	Brazil	Cross-sectional	1916	Lipoprotein subfractions	Menopause was associated with TRL-C levels. Duration since menopause <2 years had the highest association with higher TRL-C and VLDL3-C.
Mendes (2013)(159)	Brazil	Cross-Sectional	551	MetS	Menopause was associated with high blood pressure and elevated glucose levels.
Ghosh (2010)(161)	India	Cross-sectional	245	BMI, WC, WHR, total fat mass, fat free mass	Increased total fat mass, free fat mass, WC, and WHR in post- than premenopausal women
Ghosh (2008)(160)	India	Cross-sectional	200	MetS	MetS was higher in postmenopausal women
Dasgupta (2012)(162)	India	Cross-sectional	316	Lipids, glucose, BP	Postmenopausal stage was associated with elevated glucose, total cholesterol, triglycerides, LDL-C, and BP
Dasgupta (2020)(163)	India	Cross-sectional	1400	BMI, BP	Menopause was associated with higher BMI and BP
Sanchez-Rodriguez (2012) (167)	Mexico	Cross-sectional	374	Oxidative stress	Menopause was associated with oxidative stress as measured by the high lipoperoxide biomarkers
Muchanga (2014) (146)	DRC	Cross-sectional	200	BP	Menopause was associated with prehypertension
Setroame (2020)(201)	Ghana	Cross-sectional	185	MetS	Higher prevalence of metabolic syndrome in post- vs premenopausal women
Jaff (2015) (121)	South Africa	Cross-sectional	702	BMI, WC, visceral fat, subcutaneous fat	No differences in BMI, WC, visceral, and subcutaneous fat between pre- and postmenopausal women.

*Articles used in the quantitative meta-analysis, MetS-metabolic syndrome, BP-blood pressure, BMI-body mass index, TGs-triglycerides, WC-waist circumference, WHR-waist-to-hip ratio, LDL-C-low density lipoprotein-cholesterol, HOMA-homeostatic model assessment for insulin resistance, cIMT-carotid intima media thickness, HDL-C-high density lipoprotein-cholesterol, AMH-anti-mullerian hormone, DM-diabetes mellitus, CVD-cardiovascular disease, TP-total protein, TRL-C- triglyceride-rich lipoprotein-cholesterol, VLDL3-C- very-low-density lipoprotein cholesterol subfraction 3.

2.4.3 Primary outcomes

Figure 2.2 shows the combined effect size estimates in studies that evaluated the differences between CMD risk factors according to menopausal stage. Overall, postmenopausal stage was associated with greater CMD risk as supported by the high prevalence of MetS, elevated blood pressure, triglycerides, fasting blood glucose, waist circumference, and WHR. However, there were no differences in BMI, HDL-C and cIMT levels between pre- and postmenopausal women (Figure 2.2). The individual forest plots for each CMD risk factor are shown in Appendix 5 (Figures 1–11).

Metabolic Syndrome

Six studies involving 5,177 women from Brazil (n=2), Thailand (n=1), Tunisia (n=2), and Bangladesh (n=1) were meta-analysed. Pooled analysis of these studies showed that the risk of MetS was higher in post- than premenopausal women (OR=1.18; 95% CI, 1.11–1.27, P=0.19, and I^2=33.4% (Figure 2.2 and Appendix 5, Figure 1; P is the Chi-squared test p-value showing that the studies were homogenous, and I^2 indicates the level of heterogeneity between the studies).

Blood Pressure

Five studies involving 16,602 women from China (n=3), Tunisia (n=1), and Iran (n=1) showed that when using a definition of hypertension of systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg, postmenopausal women had a higher risk of hypertension compared to their premenopausal peers (OR=1.10; 95% CI 1.04–1.16, P=0.22, and I^2 =29.9%) (Figure 2.2 and Appendix 5, Figure 2). A similar trend was shown in three studies that defined hypertension as SBP ≥130 mmHg and DBP ≥85 mmHg (OR=1.32; 95% CI 1.26–1.38, P<0.001, and I^2

=97.9%) (Figure 2.2 and Appendix 5, Figure 3. The I^2 and P-value for this analysis indicates a highly significant level of heterogeneity between the studies.

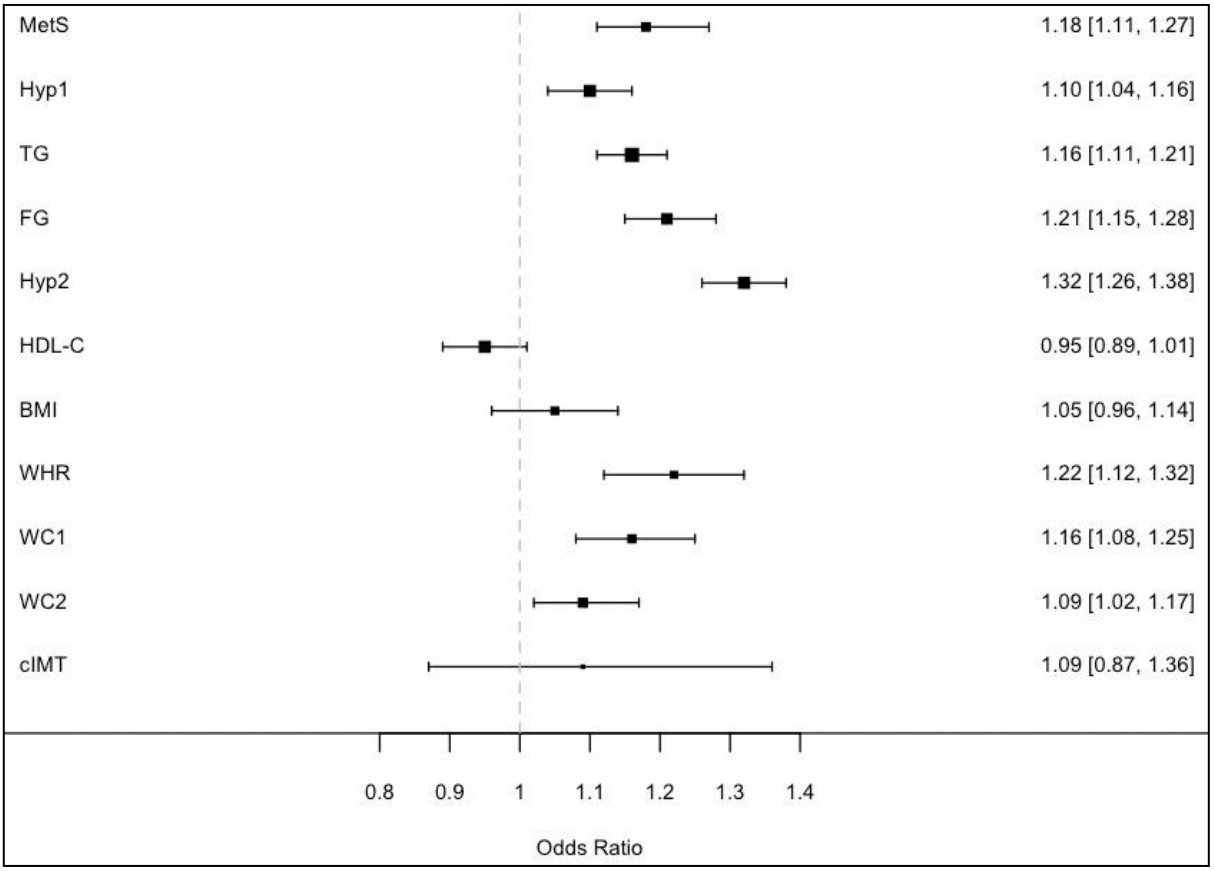

Figure 2.2: Meta-analysis of studies showing differences in cardiometabolic disease risk factors according to menopausal stage. Pooled odds ratios presented as post- vs. premenopausal stage. See Appendix 5, Figures 1-11 for data from each individual study.

MetS, metabolic syndrome: NCEP ATP III definition; Hyp1, high systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg; TG, high triglycerides: ≥1.69 mmol/L; FG, elevated fasting glucose (≥6.1 mmol/L); Hyp2, high systolic BP ≥130 mmHg and diastolic BP ≥85 mmHg; HDL-C, low high density lipoprotein cholesterol: <1.29 mmol/L; BMI, high body mass index: ≥28 kg/m²; WHR, high waist hip ratio: ≥0.85; WC1, high waist circumference: ≥80 cm; WC2, high waist circumference: ≥88 cm; and cIMT, elevated carotid intima media thickness cIMT ≥0.70mm

Triglycerides and HDL-C

Four studies involving 13,465 women from China (n=2), Bangladesh (n=1), and Tunisia (n=1) showed that the risk of elevated triglyceride levels (≥1.69 mmol/L) was higher in post- than in premenopausal women (OR=1.16; 95% CI 1.11–1.21, P<0.001, and I^2 =87.7%) (Figure 2.2 and Appendix 5, Figure 4). However, when low HDL-C levels (<1.29 mmol/L) were compared in two studies with a combined sample size of 4,313 women, no differences were present between pre- and postmenopausal women (OR=0.95; 95% CI 0.89–1.01, P=0.001, and I^2 =91.6%) (Figure 2.2 and appendix 5, Figure 5).

Glucose

Three studies involving 5,274 women from China, Bangladesh and Tunisia showed that the risk of pre-diabetic blood glucose levels (≥6.1 mmol/L) were higher in post- than premenopausal women (OR=1.21; 95% CI 1.15–1.28, P=0.001, and I^2=91.1%) (Figure 2.2 and Appendix 5, Figure 6).

Obesity

Pooled results of two studies from China involving 13,654 women showed that the risk of obesity (BMI ≥28 kg/m²) was similar in pre- and postmenopausal women (OR=1.05; 95% CI 0.96–1.14, P=0.13, and I^2 =56.8%) (Figure 2.2 and Appendix 5, Figure 7).

Waist circumference and waist-to-hip ratio

In two studies from China involving 10,702 women, postmenopausal women had a higher risk of an increased waist circumference (≥80 cm) than their premenopausal peers (OR=1.16; 95% CI 1.08–1.25, P=0.02, and I^2=81.9%) (Figure 2.2 and Appendix 5, Figure 8), A similar trend was observed when three studies from Brazil, Bangladesh

and Tunisia, that defined high waist circumference as ≥88 cm were meta-analysed (OR=1.09; 95% CI 1.02–1.17, P=0.01, and I^2=77.3%) (Figure 2.2 and Appendix 5, Figure 9). Furthermore, pooled analyses from two studies from China and Brazil showed that postmenopausal women had higher risk of increased waist-to-hip ratio (≥0.85) than premenopausal women (OR=1.22; 95% CI 1.12–1.32, P=0.14, and I^2 =54.5%) (Figure 2.2 and Appendix 5, Figure 10).

cIMT

In two studies from China and Thailand involving 2,253 women, there were no differences in the risk of high cIMT levels (≥0.70mm) between post- and premenopausal women (OR=1.09; 95% CI 0.87–1.36 *P*=0.09, and I^2=64.4%) (Figure 2.2 and Appendix 5, Figure 11).

2.5 Discussion

This systematic review and meta-analysis on midlife women from LMICs show that the postmenopausal stage is associated with higher risk of MetS, elevated triglycerides, elevated blood glucose, high blood pressure, and high waist circumference but no differences when obesity, HDL-C and cIMT levels were compared between the two menopausal groups. These observations highlight a disproportionate burden of CMD risk factors in post- compared to premenopausal women in LMICs. Identifying this high-risk population is crucial for public health interventions tailored toward reducing these CMD risk factors.

The current study broadens the understanding of menopause and CMD risk factors by combining studies from LMICs into a large sample size (40,517 participants). These findings are similar to a meta-analysis on MetS which included all studies around the world (205). In their analysis, postmenopausal women were 3.5 times more likely to

develop MetS compared to premenopausal women (205). Furthermore, the higher prevalence of the individual components of MetS in post- than in premenopausal women observed in that study, corroborate these findings.

In longitudinal studies from high-income countries, menopause has been shown to have differential effects on CMD risk. In the SWAN study, MetS, total cholesterol, LDL-C, HDL-C, and apo-B lipoproteins were independently associated with menopause only in the first year after FMP (7,170). The study also showed no influence of menopause on BMI, blood glucose, insulin, triglyceride, and blood pressure levels (7). In the ARIC study, the progression of MetS was rapid during the MT but it decreased after the FMP, which was more prominent in African Americans than Caucasian women (13). In the Melbourne Women's Midlife Health Project, HDL-C levels increased around the first year before FMP but decreased in the first year postmenopause (172). Other changes in blood lipids (triglycerides and LDL-C), BMI and diastolic blood pressure were only related to chronological ageing or one of the traditional risk factors (172). Furthermore, the Radiation Effects Research Foundation (RERF) showed that total serum cholesterol levels increased from three years before FMP to one-year post-FMP whereas increased BMI and systolic blood pressure were associated with chronological ageing but not menopause (3). Guthrie *et al.,* observed that women gained an average of approximately 2.1 kilograms over five years, but these differences were not menopause related. However, the study showed that waist circumference and waist-hip ratio increased with MT (89). There are many possible reasons for these different outcomes across studies, as also observed in the current systematic review, including differences in sample size, ethnicity, and time points at which CMD risk factors were measured. However, it is interesting to note that in these

studies changes in BMI were not related to the menopause but changes in waist and WHR were, and this was also observed in the current meta-analysis.

The differences in CMD risk between pre- and postmenopausal women may relate to hormonal changes during the MT. In the SWAN study, menopause was associated with increasing bioavailable testosterone, and declining estradiol and SHBG levels (170). The changes in testosterone and SHBG were associated with the MetS and its components. However, neither baseline estradiol levels nor its decline during menopausal transition was associated with MetS (13,170). In the age-adjusted analyses, the testosterone: estradiol ratio and FAI increased by approximately 10% from baseline to the five years of follow-up. Supporting evidence from one meta-analysis study showed that women with type 2 diabetes mellitus had higher testosterone but lower SHBG levels than controls (206). It is hypothesised that the association between SHBG and MetS is mediated by the inhibitory effect of insulin on the synthesis of SHBG (207).

This study has some limitations. Firstly, the number of identified articles per each CMD risk factor in these analyses were small; thus, sources of heterogeneity were not further interrogated. Secondly, the studies assessed in the meta-analysis were dominated by large studies from China with none available from sub-Saharan Africa. Thirdly, these analyses were based on observational data and were therefore limited by study design as far as potential unmeasured confounders and direction of associations were concerned. Despite this, the present study provides a comprehensive review of the current literature on LMICs and was guided by a registered protocol.

The results of this systematic review and meta-analysis show that menopause is an independent risk factor for MetS, elevated triglycerides, elevated blood glucose, high blood pressure, and high waist circumference in LMICs. Therefore, it is important to focus on prevention strategies such as lifestyle and behavioural changes to mitigate the development of CMD in midlife women. The feasibility of using therapeutic interventions such as hormone replacement is debatable in under-resourced healthcare systems in LMICs and very few studies have investigated its use in such environments. In a large cross-sectional study across 11 Latin American countries, the Collaborative Group for Research of the Climacteric in Latin America (REDLINC) showed that the current use of menopausal hormonal therapy (MHT) was associated with reduced risk of MetS (208). Furthermore, a study from Brazil showed that the use of MHT was associated with a lower risk for hypertension (209). However, these were cross-sectional studies and the use of MHT in these studies was low (12.5%) (208).

The current study clearly shows that menopause is associated with an increased risk for CMD in LMICs. However, very few studies investigating the relationship of menopause with CMD, and their risk factors are available from sub-Saharan Africa, where the prevalence of obesity and related diseases in midlife women is known to be high. Therefore, the aim of the next study was to examine differences in anthropometric and cardiometabolic variables between premenopausal and postmenopausal women in four sub-Saharan African countries – Burkina Faso, Ghana, Kenya, and South Africa – in a cross-sectional analysis.

Chapter 3: Cardiometabolic Disease Risk Factors in Pre- and Postmenopausal Women from Four Sub-Saharan African Countries; a Cross-Sectional Study

Chikwati RP, Mahyoodeen NG, Jaff NG, Ramsay M, Micklesfield LK, Wade AN, Agongo G, Asiki G, Choma SS, Boua PR, George JA. Cardiometabolic disease risk factors in pre-and postmenopausal women from four sub-Saharan African countries: A cross-sectional study*. Maturitas.* 2023 Jun 1;172:60-8 (Appendix 6).

3.1 Abstract

Objectives: To compare CMD risk factors between pre- and postmenopausal women from four SSA countries.

Study design: This cross-sectional study included 3609 women (1740 premenopausal and 1869 postmenopausal) from sites in Ghana (Navrongo), Burkina Faso (Nanoro), Kenya (Nairobi), and South Africa (Soweto and Dikgale). Demographic, anthropometric and cardiometabolic variables were compared between pre- and postmenopausal women, within and across sites using multivariable regression analyses. The sites represent populations at different stages of the health transition with those in Ghana and Burkina Faso being rural, whilst those in Kenya and South Africa are more urbanised.

Main Outcome Measures: Anthropometric and cardiometabolic variables.

Results: CMD risk factors levels were higher in South (Soweto and Dikgale) and East (Nairobi) Africa than West Africa (Nanoro and Navrongo), irrespective of menopausal stage. Regression models in combined West African populations demonstrated that postmenopausal women had higher waist circumference (ß=1.28 (95% CI: 0.58; 1.98) cm), log subcutaneous fat (ß=0.15 (0.10; 0.19)), diastolic (ß=3.04 (1.47; 4.62) mmHg)

and log systolic (ß=0.04 (0.02; 0.06)) blood pressure, log carotid intima media thickness (ß=0.03 (0.01; 0.06)), low-density lipoprotein cholesterol (ß=0.14 (0.04; 0.23) mmol/L)) and log triglyceride (ß=0.10 (0.04; 0.16)) levels than premenopausal women. No such differences were observed in the South and East African women.

Conclusions: Menopause-related differences in cardiometabolic disease risk factors were prominent in West but not East or South African study sites. These novel findings should inform cardiometabolic disease prevention strategies in midlife women specific to rural and urban and peri-urban locations in sub-Saharan Africa.

3.2 Introduction

SSA is experiencing a health transition (210) characterised by an increased life expectancy that is associated with a growing population of peri- and postmenopausal women (211). This parallels a rising prevalence of cardiometabolic diseases (CMD), including obesity (212,213), hypertension (213), and diabetes mellitus (214) within the SSA region.

Evidence from North American populations demonstrate anthropometric and cardiometabolic differences between pre- and postmenopausal women (170), with CMD risk increasing on the approach of menopause (215). The MT is associated with shifts in body fat distribution, particularly increased deposits of abdominal fat, independent of body mass index (BMI) (170,215). Although the association of CMD risk factors with menopause status has been studied in detail in high income countries, only a small number of such studies have been conducted in SSA. These include cross-sectional investigations in pre- and postmenopausal women from South Africa (121,144), Ghana (145), and the DRC (146). These studies included premenopausal women with a wide age range (from 20 years onwards) (144,145) and modest sample

sizes: South Africa (n=590) (147), South Africa (n=206) (144), Ghana (n=250) (145), and the DRC (n=200) (146).

Therefore, the aim of the current study was to measure and compare CMD risk factors between pre- and postmenopausal women from four SSA countries i.e., South Africa, Burkina Faso, Ghana, and Kenya. Study participants were from the Africa-Wits International Network for the Demographic Evaluation of Populations and Their Health (INDEPTH) Partnership for Genomic studies (AWI-Gen) (216) which allowed this study to perform the first pan-SSA analysis of the relationship between menopause stage and CMD risk factors. Furthermore, the populations within AWI-Gen arose from different demographic strata varying from rural subsistence farmers to more urbanised communities and therefore included SSA populations at different stages of the health transition (217). In addition, regional differences in the prevalence of obesity have been reported in these midlife participants from these four locations (217). Therefore, this study population also allowed us to determine whether any relationship between menopause and CMD risk factors varies across these distinct SSA populations.

3.3 Methods

3.3.1 Study Design and Setting

This cross-sectional population-based analysis of midlife women is embedded within the AWI-Gen study (216) which involves five different sites across four SSA countries namely: South Africa (Dikgale and Soweto), East Africa (Nairobi, Kenya) and West Africa (Nanoro, Burkina Faso, and Navrongo, Ghana). These sites included a mixture of urban (Soweto and Nairobi), peri urban (Dikgale) and rural (Nanoro and Navrongo) environments.

3.3.2 Recruitment

In the main study, women aged between 40 and 60 years were recruited from 2013–2016 at each of the study centres, approximately 1000 participants per site (216). This was performed using random sampling of the general population with some exceptions in Soweto. In Soweto, 700 women were selected from a previous study, the Study of Women Entering and in Endocrine Transition (SWEET) (218) which was a subset of another study, the Birth to Twenty Plus cohort (BT20+) (219). The remaining 300 women were randomly selected from the Soweto community. In total, 5184 women from all five study sites were available for selection. For this PhD study, exclusion criteria were then applied (unknown menopausal stage, perimenopausal stage, <40 and >60 years of age), reducing the sample size to 3609 study participants.

Figure 3.1 shows the locations of the study centres, and the corresponding sample sizes according to menopausal stage.

3.3.3 Ethics

The study was approved at the University of the Witwatersrand by the Human Research Ethics Committee (Medical) (M2010106) (Appendix 1). In addition, local ethics approval was obtained in the respective AWI-Gen study sites. All study participants provided written informed consent.

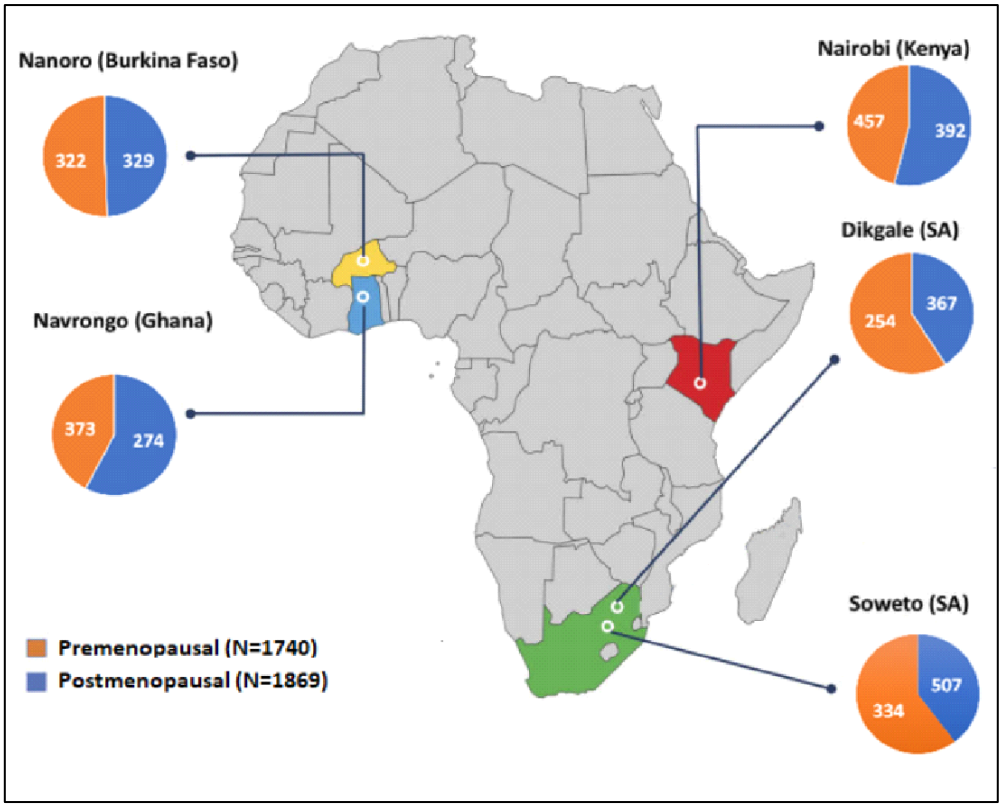

Figure 3.1: Distribution of women according to menopausal stage across the five AWI-Gen Study Centres. Modified from Ramsay *et al.,* (217).

3.3.4 Sociodemographic and Behavioural Variables

Self-reported information on age, physical activity, socioeconomic status, highest level of education, dietary intake, alcohol intake, and smoking were obtained using a questionnaire (Appendix 4). The Global Physical Activity Questionnaire was used to assess moderate-vigorous intensity physical activity (MVPA) (220). The MVPA was derived from time spent on occupational, leisure time and travel-related physical activity. Insufficient physical activity was defined as MVPA <150 min/week (221). Socio-economic status was estimated from a household assets index tool that combines data on household possessions. Ultimately, the total asset score was categorised into either of the five quintiles namely, “poorest”, “poorer”, “poor”, “less poor” and “least poor”. Participants were asked about the highest level of education they had attained. These were categorised into, no formal education, completion of

primary, secondary, or tertiary education. Dietary intake focused on questions regarding consumption of fruits and vegetables, bread, soft drinks (i.e., sugar-sweetened beverages) and fruit juices. Participants were asked to provide the number of days per week in which each of these items were consumed. Smoking and alcohol intake were defined by three categories: “never”, “current”, and “former”.

3.3.5 Menopausal staging

Menopause was staged by asking each woman about her menstrual bleeding patterns. The premenopausal group was then defined as women with regular menstrual cycles, the perimenopausal group were those having irregular menstrual cycles within the past 12 months, whilst the postmenopausal group were those who had had amenorrhea for twelve consecutive months or more. Perimenopausal women were excluded to effectively separate individuals who had experienced the menopause transition and those that had not.

3.3.6 Anthropometric and blood pressure measurements

The recruitment process and collection of anthropometric measurements was done by trained staff. Waist, and hip circumference measurements were taken using a soft tape measure (SECA, Hamburg, Germany). For the measurement of waist circumference, participants were asked to remove their outer clothing to allow for the correct positioning of the tape just above the umbilicus and a reading was taken after normal expiration. Hip circumference measurements were taken around the bulging part of the buttocks. Height was measured using a Harpenden stadiometer (Holtain, Wales) fixed to a wall. The procedure involved asking the participants to remove their shoes and maintain a straight posture against the stadiometer with their head in a horizontal plane. Weight was measured using digital scales (Kendon Medical, South Africa). Before a reading was taken, participants were asked to step onto the scale barefooted,

and space their heels and hang arms at their sides. Body mass index (BMI) was then calculated from weight in kg/height in m^2.

Systolic and diastolic blood pressure were measured on the left arm of a seated participant using a sphygmomanometer (Omron M6, Omron, Kyoto, Japan). Three measurements were taken within two-minute intervals and the last two readings averaged for the final blood pressure level.

3.3.7 SAT, VAT, and cIMT measurements

SAT, VAT, and cIMT measurements of both the right and left layers of the common carotid arteries were measured using a LOGIQ e ultrasound system (GE Healthcare, CT, USA). For each ultrasound scan, study participants were asked to lie down with their face and torso facing up, and a gel applied to their lower abdomen and neck. To measure VAT, an ultrasound probe was positioned between the lower rib (coastal) margin and the high point of the pelvic bone (iliac crest), and the depth set to 15 cm. An ultrasound image was taken, upon which two cursors were used to indicate the abdominal aorta peritoneum and the anterior of the spine. The distance between these two cursors was the VAT thickness. The measurement was repeated, and the two values averaged for the final VAT. Thereafter, SAT measurement was done by rotating the ultrasound probe by 90 degrees and adjusting the depth to 9 cm. After visualising the symmetry of the rectus abdominus muscles and the linea alba (the connective tissue separating the rectus abdominus) an ultrasound image was taken. Again, two cursors on the image were used to identify the locations of the skin and the linea alba. The distance between these two was the SAT thickness. The measurement was repeated, and the two values averaged for the final SAT. To measure the right cIMT, each participant was asked to tilt their head to the left at 45 degrees while lying in the supine position. The exposed neck area was scanned in a transverse manner until the

common carotid artery (CCA) of the posterior of the neck was visualised. A continuous 10mm segment of the CCA posterior wall was identified, and a cursor put on either side of this identified segment (two points were 10 mm apart). The starting point was 10 mm from the bulb of the CCA. The instrument software was then used to measure the cIMT in millimetres. Similarly, the left carotid was measured by asking the participant to turn towards the right, and the process repeated. Ultimately, the cIMT measurements from both the left and right posterior CCA were averaged. To validate the measurement of SAT, VAT and cIMT, each trainee and trainer were asked to perform repeated measurements on 15 volunteers. The coefficient of variation between and within trainees was less than 2%.

3.3.8 Blood tests

All blood tests were performed at the MRC-Wits Developmental Pathways for Health Research Unit (DPHRU) laboratory, Chris Hani Baragwanath Hospital, Soweto. Measurements of fasting serum triglyceride, total cholesterol, HDL-C, and plasma glucose levels were performed on the Randox Plus clinical chemistry analyser (Randox Laboratories Limited, Dublin, UK) through enzymatic colorimetric methods. The measuring ranges for triglycerides, total cholesterol, and HDL-C were 0.13–5.60 mmol/l, 0.30–17.20 mmol/l and 0.05–3.80 mmol/l respectively. The measuring range for plasma glucose was 0.36–35 mmol/l. Fasting insulin levels were assayed on the Immulite 1000 analyser (Siemens, Germany). The assay was based on a chemiluminescent immunoassay. The measuring range for insulin levels was 2–300 µIU/ml.

LDL-C levels were calculated from the Friedewald formula (222). The homeostatic model assessment (HOMA) formula was used to assess insulin resistance (223).

3.3.9 Classification of CMD and risk factors

Diabetes mellitus was confirmed by a fasting plasma glucose ≥7.0 mmol/L (224) and/or use of anti-diabetic agents and/or previous diagnosis by a health care professional. Obesity was defined as BMI ≥30 kg/m² (225), hypertriglyceridaemia as triglycerides ≥1.69 mmol/L (226), and hypercholesterolaemia as cholesterol ≥5.18 mmol/L and/or treatment with anti-lipid agents (226). Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or use of anti-hypertensive agents and/or previous diagnosis by a healthcare professional (227).

3.4 Statistical analyses

All analyses were performed using Stata 16.1 (StataCorp LP, College Station, TX, USA). Continuous variables were presented as means ± standard deviations (SD) or medians and interquartile ranges (IQR) for data with normal or skewed distribution, respectively, and as proportions (%) for categorical variables. Non-parametric continuous dependent variables were log transformed to normality before being analysed using multivariable linear regression analysis. Categorical variables were compared across groups using the chi-squared test. Continuous variables were compared between two groups (e.g., pre- vs postmenopausal) or more (e.g., between study sites) using the appropriate parametric (Student's t-test or one-way ANOVA) or non-parametric (Mann–Whitney U test or Kruskal–Wallis) statistical tests. The level of significance of all statistical analyses was set at $p<0.05$.

Multivariable linear regression was used to compare the relationship of CMD risk factors, namely systolic and diastolic blood pressure, BMI, waist and hip circumference, VAT and SAT, triglycerides, LDL-C, HDL-C, blood glucose, HOMA-IR and average cIMT (dependent variables) with menopausal stage (independent

variable; post- compared to premenopausal group). Additional independent variables were chosen based on scientific plausibility (Table 2, Appendix 5). In the univariate (unadjusted) models, each CMD risk factor was regressed against menopause status. In the adjusted model, age, and other independent variables (Table 2, Appendix) were incorporated into a multivariable model. Multivariable linear regression analyses were performed for each individual study site and for the sites combined, against each CMD risk factor. In addition, a sub-analysis on participants who were not receiving therapy by removing participants who reported use of anti-hypertensive medication or therapies for the treatment of diabetes was performed. Collinearity of variables within each regression model was assessed using the variance inflation factor (VIF): all regression models included independent variables with VIF <3.0. Assumptions of constant variance for the residuals from each regression model were checked by the Breusch-Pagan/Cook-Weisberg test and correction for unequal co-variances was accomplished using robust standard errors.

3.5 Results

3.5.1 Participant characteristics

A total of 3609 women (1740 premenopausal and 1869 postmenopausal) from across the five study sites (Figure 3.2) were included in the statistical analyses. The number of study participants and variables collected are shown in Table 3, Appendix 5. Table 3.1 and Figure 3.2 show that the women at the West African sites had a more favourable cardiometabolic profile, whilst those from South Africa had the worst. Thus, Table 3.1 shows the prevalence of behavioural CMD risk factors and of CMD across all five study sites for all women (i.e., pre-, and postmenopausal combined). Smoking was rare at all sites, whilst alcohol intake was highest at the two West African sites. Insufficient physical activity varied across sites being more prevalent in Soweto.

Obesity, hypertension, diabetes, and dyslipidaemia had the lowest prevalence at the two West African sites and the highest prevalence at one or both South African sites (except for diabetes). Table 3.1 shows that study participants from West and East Africa consumed more vegetables than those from South Africa. In addition, South and East African women consumed more fruits, soft drinks, and bread than their counterparts in West Africa. However, data on alcohol and dietary intake, and cIMT measurements were missing in Soweto (Table 3.1).

Table 3.1: Prevalence of cardiometabolic diseases and risk factors in women at each AWI-Gen study site

Risk factors	All Sites (n=3609)	Nanoro (n= 651)	Navrongo (n=647)	Nairobi (n=849)	Dikgale (n=621)	Soweto (n= 841)	p value
Age in years	49.3±5.8	49.1±5.8	50.1±5.8	48.3±5.5	50.3±6.2	49.3±5.8	0.03
Current Smoking	90 (3)	0 (0.0) *	2 (0.3)	23 (3)	22 (4)	43 (5)	<0.001
Current Alcohol Consumption	865 (31)	389 (60)	344 (53)	50 (6)	82 (13)	..	<0.001
Insufficient MVPA	659 (18)	79 (12)	125 (19)	84 (10)	24 (4)	347 (41)	<0.001
Obesity	1193 (33)	10 (2)	35 (5)	272 (32)	319 (51)	557(66)	<0.001
Hypertension	1221 (34)	76 (12)	146 (22)	250 (29)	290 (47)	459 (55)	<0.001
Diabetes Mellitus	160 (5)	11 (2)	2 (0.3)	67 (8)	36 (7)	44 (6)	<0.001
Hypertriglyceridaemia	388 (11)	19 (3)	27 (4)	93 (11)	84 (14)	165 (20)	<0.001
Hypercholesterolaemia	582 (16)	23 (4)	21 (3)	179 (21)	107 (17)	252 (30)	<0.001
Diet (days consumed per week):							
Vegetables	4.1±2.3	3.2±2.8	4.7±2.0	5.1±1.7	3.1±1.8	..	<0.001
Fruits	2.0±2.2	0.8±1.8	1.0±1.2	3.5±2.4	2.1±1.8	..	<0.001
Soft drinks	0.7±1.9	0.1±0.5	0.1±0.3	0.5±1.1	2.0±3.2	..	<0.001
Fruit Juices	0.5±1.4	0.1±0.2	0.3±0.9	0.2±0.7	1.5±2.4	..	<0.001
Bread	1.9±2.3	0.6±1.4	0.7±1.3	2.2±2.2	4.0±2.4	..	<0.001

Data expressed as mean ± standard deviation or numbers (percentage), MVPA: moderate-vigorous intensity physical activity. *There were no smokers in Nanoro and data on alcohol consumption and diet were missing for Soweto. Significance testing across the sites completed by one-way ANOVA or chi squared test. Definitions were as follows; insufficient MVPA: <150 min/week, obesity: BMI ≥30 kg/m², hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, diabetes mellitus: fasting plasma glucose ≥ 7.0 mmol/L, hypertriglyceridaemia: triglycerides ≥1.69 mmol/L and hypercholesterolaemia: cholesterol ≥ 5.18 mmol/L.

In Figure 3.2, selected CMD risk factors are expressed as continuous variables and presented for all women per study region i.e., West Africa (Navrongo with Nanoro), East Africa (Nairobi) and South Africa (Dikgale with Soweto). Again, these data highlight the tendency for these risk factors to be lowest in the West African region. Similar patterns were observed for the other CMD risk factors i.e., waist circumference, VAT, SAT, and HDL-C (data not shown). When systolic and diastolic blood pressure, BMI, waist circumference, LDL-C, triglycerides, and HDL-C were stratified by menopausal stage (Table 4, Appendix 5), the same patterns shown in Figure 3.2 were observed in both menopausal groups across the three regions.

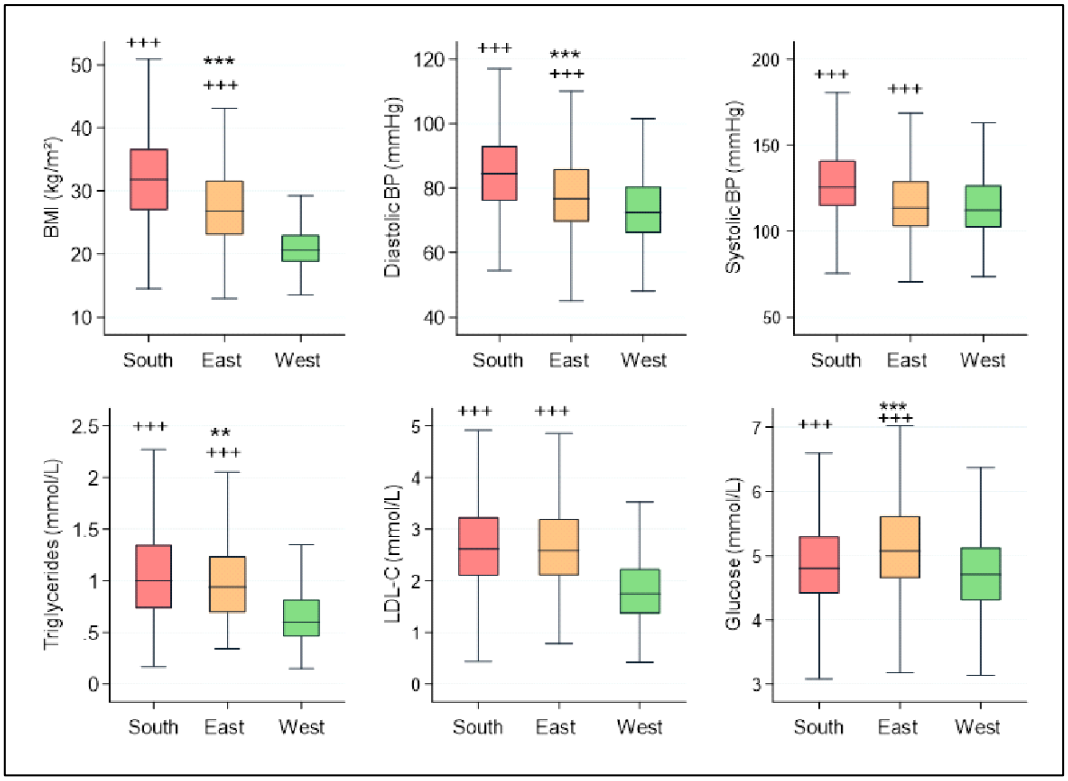

Figure 3.2: CMD risk factor levels across South, East and West Africa.

CMD risk factor levels; BMI (body mass index), diastolic and systolic BP, triglycerides, LDL-C (low density lipoprotein cholesterol), and glucose compared across the three regions by Tukey *post hoc* analysis. *** p<0.001, **p<0.01 vs South Africa. +++ p<0.001 vs West Africa.

3.5.2 Comparison of Anthropometry and CMD Risk Factors across Menopause Groups

When data were combined from all sites and stratified by menopause groups, all anthropometric measures except BMI were higher in post- than in premenopausal women (Table 3.2).

Anthropometric and cardiometabolic risk factors were then compared between menopause groups at the individual sites (Tables 5–9 in Appendix 5). The differences observed in the combined analyses were the same at each of the individual sites except for BMI, VAT, SAT, and hip circumference which were lower in the postmenopausal groups at one or more study sites.

Table 3.2: Comparison of age, anthropometry and cardiometabolic risk factors between menopause groups for all AWI-Gen study sites combined.

Variables	Premenopause	Postmenopause	p value
Age (years)	45.4±4.0	53.0±4.8	<0.001
BMI (kg/m²)	25.2 (10.3)	26.4 (12.1)	0.19
Hip circumference (cm)	101.8±16.0	102.9±17.6	0.05
Waist circumference (cm)	86.5±15.4	89.3±16.3	<0.001
SAT (cm)	1.65 (1.61)	1.86 (1.69)	<0.001
VAT (cm)	4.43 (2.35)	4.55 (2.50)	<0.001
Systolic BP (mmHg)	114.5 (23.5)	123 (29.5)	<0.001
Diastolic BP (mmHg)	77.5±13.0	81.6±13.0	<0.001
Total Cholesterol (mmol/L)	3.71±1.08	4.15±1.23	<0.001
LDL-C (mmol/L)	2.22±0.86	2.51±0.94	<0.001
Triglycerides (mmol/L)	0.74 (0.51)	0.90 (0.60)	<0.001
HDL-C (mmol/L)	1.10 (0.43)	1.13 (0.43)	<0.001
Glucose (mmol/L)	4.75 (0.80)	4.90 (0.95)	<0.001
HOMA-IR	1.04 (1.97)	1.24 (2.11)	<0.001
cIMT average (mm)	0.59 (0.12)	0.65 (0.15)	<0.001

Data expressed as mean±standard deviation or median (interquartile range); BMI (body mass index), VAT (abdominal visceral fat), SAT (abdominal subcutaneous fat); LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), HOMA-IR (homeostatic model for assessment of insulin resistance), cIMT average (average of left and right carotid intima-media thickness).

Thus, BMI was lower in postmenopausal women in the West African sites, Nanoro and Navrongo, compared to their premenopausal counterparts, but was not different between the menopausal groups at any of the other sites. Compared to premenopausal women, postmenopausal women in Dikgale and Nairobi had lower SAT, in Nanoro and Nairobi had lower VAT, and in Dikgale, Nanoro and Navrongo had lower hip circumference.

The cardiometabolic variables (cIMT, blood pressure, lipids, glucose, and HOMA-IR) were higher in post- vs premenopausal women when data from all sites were combined (Table 3.2) and at individual sites (Table 5-9 in Appendix 5). The only exceptions were HOMA-IR, which was only higher in post- vs. premenopausal women

in Nairobi (Table 9 in Appendix 5), HDL-C levels which were only higher in Dikgale, Nanoro and Nairobi (Tables 5, 7 and 9 in Appendix 5) and glucose levels which were only higher in the postmenopausal group in Dikgale, Soweto and Nairobi (Tables 5, 6 and 7 in Appendix 5).

3.5.3 Multivariable Linear Regression Analyses

Due to differences between sites in the levels of CMD risk factors (Table 3.1), univariate and multivariable linear regression models were performed for each risk factor at each site. In the univariate models (Table 3.3), each CMD variable was regressed against menopause status alone (the unadjusted model), whilst in adjusted models age and other independent variables were selected depending on the identity of the dependent variable (Table 2 in Appendix 5). Only significant models are presented in Table 3.3 (Table 10 in Appendix 5 includes all the multivariable regression models for all CMD risk factors at each site). These results clearly show that nearly all the associations between the different CMD risk factors and menopausal status were observed in the West African sites, Nanoro and Navrongo. Thus, at either one or both sites postmenopausal status was positively associated with waist circumference, SAT, diastolic and systolic blood pressure, LDL-C, triglycerides, glucose and cIMT, whilst postmenopausal status was inversely associated with BMI at both Nanoro and Navrongo. In addition to the West African sites, postmenopausal status was associated with elevated triglycerides in Dikgale and inversely associated with systolic blood pressure in Nairobi.

Table 3.3: Multivariate linear regression models showing relationship of menopausal status with CMD risk factors for individual sites.

Dependant Variable	Site	Menopausal stage† (unadjusted)	Menopausal stage† (adjusted)††
Waist Circumference (cm)	Nanoro Navrongo	0.24 (-1.00; 1.48) -0.88 (-2.38; 0.63)	1.54 (0.63; 2.45)** 1.06 (0.07; 2.06)*
Visceral Fat (log; mm)	Nanoro	-0.05 (-0.09; -0.01)*	-0.06 (-0.12; -0.004)*
Subcutaneous Fat (log;mm)	Nanoro Navrongo	0.09 (0.02; 0.16)* 0.05 (-0.03; 0.12)	0.13 (0.05; 0.21)** 0.14 (0.08; 0.20)***
BMI (log; kg/m²)	Nanoro Navrongo	-0.05 (-0.07; -0.03)*** -0.06 (-0.09; -0.04)***	-0.03 (-0.07; -0.004) -0.04 (-0.07; -0.01)*
Diastolic Blood Pressure (mmHg)	Nanoro Navrongo	2.85 (1.34; 4.36)*** 3.04 (1.15; 4.93)**	2.62 (0.549; 4.69)* 3.26 (0.918; 5.61)*
Systolic Blood Pressure (log; mmHg)	Nairobi Navrongo	0.05 (0.03; 0.07)*** 0.06 (0.04; 0.09)***	-0.03 (-0.06; -0.003)* 0.05 (0.02; 0.08)**
LDL-C (mmol/L)	Nanoro Navrongo	0.17 (0.07; 0.26)** 0.18 (0.07; 0.29)**	0.16 (0.01; 0.30)* 0.14 (0.14; 0.28)*
Triglycerides (log; mmol/L)	Dikgale Navrongo	0.27 (0.20; 0.35)*** 0.13 (0.06; 0.21)***	0.20 (0.09; 0.31)*** 0.11 (0.03; 0.20)*
Glucose (Reciprocal, mmol/L)	Navrongo	-0.004 (-0.01; 0.001)	-0.01 (-0.01; -0.002)*
Average cIMT (log;mm)	Navrongo	0.10 (0.07; 0.12)***	0.05 (0.02; 0.08)**

Data is unstandarised ß (95% Confidence Interval); †post- vs pre-menopause; *p<0.05, **p<0.01, ***p<0.001; BMI, body mass index, LDL-C, low-density lipoprotein cholesterol, cIMT average, carotid intima-media thickness average of left and right cIMT; ††all models adjusted for age, physical activity, socioeconomic status, HIV status, level of education, smoking status, alcohol, vegetable, fruit, soft drink, fruit juice, and bread intake; additional adjustments were made for specific variables as shown in Supplemental Table 2.

The data in Table 3.3 demonstrates that associations between menopause stage and CMD risks factors are observed predominantly at the West African sites. We therefore ran multivariable regression models for each CMD risk factor in which the two West African sites were combined, and we also combined the East and South African sites to improve the power of the models. In addition, all five sites were combined to enhance the ability to identify associations that may not have been observable in the

lower-powered regional models; the results are shown in Table 3.4. The adjusted regression models for waist circumference, BMI, SAT, systolic and diastolic blood pressure, glucose, cIMT, LDL-C and triglycerides demonstrate associations with menopause status in West Africa but not in combined sites from East and South Africa. In the models for LDL-C, associations were observed with menopause status in both the regional models, but the association was stronger for West Africa. The models for HOMA-IR demonstrated an association with menopause stage only when all five study sites were combined, whilst for hip circumference, VAT and HDL-C no associations were observed in any of the models.

To determine the effect of anti-hypertensive and diabetic medication on the observed associations removed all participants who reported use of these therapies from the regression models were removed and the above associations between menopausal groups and CMD risk factors were not altered. The number of participants taking lipid-lowering medication was very low (n=20) and therefore associated adjustments to the regression models were not warranted.

Table 3.4: Univariate and multivariate linear regression models showing relationship of menopausal stage with CMD risk factors for all sites combined, for West African sites and for South and East African sites combined.

Dependant variable	Sites included in model	Menopausal stage (unadjusted)* ß (95% CI)	Menopausal stage (adjusted)* ß (95% CI)
Waist Circumference (cm)	All Sites West Africa South and East Africa	2.83 (1.79; 3.86) -0.39 (-1.36; 0.58) 2.51 (1.26; 3.77)	1.10 (0.49; 1.73) 1.28 (0.58; 1.98) 0.92 (-0.09; 1.93)
Hip Circumference (cm)	All Sites West Africa South and East Africa	1.12 (0.02; 2.21) -2.83 (-3.73; -1.93) 0.86 (-0.43; 2.21)	-0.35 (-0.88; 0.19) -0.40 (-1.10; 0.30) -0.17 (-0.97; 0.63)
BMI (log; kg/m²)	All Sites West Africa South and East Africa	0.01 (-0.01; 0.03) -0.06 (-0.08; -0.05) 0.01 (-0.01; 0.03)	-0.02 (-0.04; 0.0002) -0.03 (-0.06; -0.01) 0.0004 (-0.03; 0.03)
VAT (log;mm)	All Sites West Africa South and East Africa	0.03 (0.01; 0.06) 0.0003 (-0.03; 0.03) 0.02 (-0.01; 0.06)	-0.01 (-0.04; 0.02) -0.01 (-0.04; 0.03) -0.02 (-0.06; 0.02)
SAT (log; mm)	All Sites West Africa South and East Africa	0.11 (0.07; 0.15) 0.06 (0.01; 0.11) 0.04 (-0.001; 0.08)	0.07 (0.04; 0.11) 0.15 (0.10; 0.19) 0.03 (-0.01; 0.08)
Systolic Blood Pressure (log; mmHg)	All Sites West Africa South and East Africa	0.07 (0.06; 0.08) 0.06 (0.04; 0.08) 0.07 (0.05; 0.08)	0.01 (-0.01; 0.03) 0.04 (0.02; 0.06) -0.02 (-0.04; 0.01)
Diastolic Blood Pressure (mmHg)	All Sites West Africa South and East Africa	4.09 (3.21; 4.98) 2.44 (1.19; 3.69) 3.98 (2.87; 5.09)	1.44 (0.20; 2.68) 3.04 (1.47; 4.62) 0.06 (-1.79; 1.92)
Glucose (Reciprocal, mmol/L)	All Sites West Africa South and East Africa	-0.01 (-0.01; -0.01) -0.01 (-0.01; -0.001) -0.01 (-0.01; -0.003)	-0.002 (-0.01; 0.001) -0.0060 (-0.01; 0.002) 0.002 (-0.004; 0.007)
Average cIMT (log;mm)	All Sites West Africa South and East Africa	0.09 (0.07; 0.10) 0.09 (0.08; 0.11) 0.09 (0.07; 0.11)	0.01 (-0.003; 0.03) 0.03 (0.01; 0.06) -0.003 (-0.024; 0.02)
LDL-C (mmol/L)	All Sites West Africa South and East Africa	0.31 (0.24; 0.36) 0.16 (0.09; 0.23) 0.29 (0.22; 0.37)	0.13 (0.05; 0.20) 0.14 (0.04; 0.23) 0.10 (-0.01; 0.22)
Triglycerides (log;mmol/L)	All Sites West Africa South and East Africa	0.19 (0.16; 0.22) 0.16 (0.11; 0.21) 0.16 (0.12; 0.20)	0.08 (0.04; 0.12) 0.10 (0.04; 0.16) 0.05 (-0.01; 0.11)
HOMA-IR (log)	All Sites West Africa South and East Africa	0.13 (0.05; 0.21) 0.06 (-0.09; 0.21) 0.13 (0.04; 0.22)	0.16 (0.04; 0.28) 0.18 (-0.02; 0.37) 0.16 (0.001; 0.32)
HDL-C (log; mmol/L)	All Sites West Africa South and East Africa	0.04 (0.02; 0.06) 0.04 (0.01; 0.08) 0.03 (0.002; 0.06)	0.01 (-0.02; 0.04) 0.01 (-0.04; 0.05) 0.01 (-0.03; 0.05)

*Post- vs pre-menopause, West African sites: Navrongo and Nanoro, South African sites: Soweto and Dikgale, East African site: Nairobi, BMI, body mass index, VAT, abdominal visceral fat, SAT, abdominal subcutaneous fat, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness. Adjusted for age, BMI, physical activity, socioeconomic status, HIV status, level of education, smoking status, HOMA-IR, LDL-C, eGFR, study sites, and diet.

3.6 Discussion

This study demonstrates that the CMD risk factor profile of midlife SSA women is more favourable for those women from West Africa compared to those from South and East Africa, irrespective of menopausal stage. When comparing CMD risk factors between menopausal groups, many of these risk factors are higher in postmenopausal West African women compared to their premenopausal counterparts, with differences observed less frequently in the East and South African study populations.

The lower level of CMD risk factors in the West African women may be due to differences in environmental and/or lifestyle related factors (217). Thus, both West African populations were predominantly subsistence farmers whereas the East and South African sites were more urbanised (217). In addition, the West African study participants had a lower dietary intake of bread, soft drinks and fruit than observed at the other study sites. Obesity was also far less prevalent in the West African population.

The current analyses demonstrate that differences in CMD variables between pre- and postmenopausal women were observed more frequently at the West African than the East or South African sites. No other studies have been conducted in SSA comparing CMD risk factors across menopausal groups within different countries. The few studies that have been undertaken were restricted to individual countries. Thus, a study in women of mixed ancestry from South Africa demonstrated higher levels of CMD risk factors in post- compared to premenopausal women; however, the premenopausal group varied in age from 20 to 49 years (median age of 39) and no adjustment for age was made when comparing variables between the menopausal groups (144,145). A second study from South Africa (121), which included data from participants who were

part of the Soweto cohort in the current study, observed no differences in anthropometric and body composition variables between pre- and postmenopausal women after adjusting for confounders. A study from Ghana (West Africa) (145) demonstrated that metabolic syndrome was more prevalent in post- than premenopausal women, but the premenopausal group varied in age from 20 years and upwards (mean age, 34.5 years) and age was shown to be a major determinant of metabolic syndrome. A similar study from the DRC (Central Africa) (146) demonstrated a higher prevalence of metabolic syndrome in post- compared to premenopausal women, which was maintained after adjustment for age.

A major characteristic of the West African sites are much lower levels of CMD risk factors, particularly the lower prevalence of obesity (1.5-5.4%) in comparison to East (32.0%) and South Africa (51.3-66.2%). This may be significant because Tufano *et al.,* showed that cholesterol levels were higher in lean women post menopause compared to lean premenopausal women, but these differences were not observed between obese pre- and postmenopausal groups (228). The authors suggested that in the obese women, body fat was a more important determinant of CMD risk than menopausal status. It should be noted however, that the sample size in this study was low (n=92). In addition, a large cross-sectional study in rural China reported associations between postmenopausal status and elevated glucose, blood pressure, triglycerides, and abdominal obesity amongst women with mean BMI 24.9-25.2 kg/m² (181). Another study from rural China reported that postmenopausal women had elevated levels of total cholesterol, LDL-C and triglycerides than premenopausal women independent of age and other confounders (151). Furthermore, cross-sectional studies from Indian populations with mean BMI ≤25 kg/m² demonstrated that postmenopausal status was positively associated with waist circumference (161) and

mean arterial pressure (229). The data from these studies therefore suggest that differences in CMD risk factors between pre- and postmenopausal women may be greater in lean than obese women and in rural than urban areas. It must be noted that several studies have demonstrated increased levels of metabolic syndrome and its individual components in post- compared to premenopausal women (205); however, none of these studies have examined these differences within study sub-groups based on BMI or other anthropometric measures.

The reason why lower BMI may be associated with greater differences in CMD risk factors between pre- and postmenopausal women is not known. However, it is possible that this may be related to the hormonal changes that occur during menopause. It is well known that estrogen levels (specifically estradiol) decline during the MT (230). As E2 levels fall, circulating C_{19} steroids i.e., testosterone, androstenedione, DHEA and DHEAS become the major substrate for E2 biosynthesis in extra-gonadal tissues (230). Studies have shown that the expression of the enzyme aromatase, which is responsible for the production of E2 in adipose tissue, increases with age and BMI (230). Therefore, it is possible that the lower BMI in West African women could be associated with lower postmenopausal E2 levels when compared to the women from East and South Africa, who have much higher levels of obesity. The lower postmenopausal E_2 levels in the West African women may then lead to enhanced CMD risk, as some studies have shown that the fall in the serum concentrations of estrogens across the MT is associated with higher levels of CMD risk factors (231).

In West Africa, although postmenopausal women have a higher prevalence of CMD risk factors than their premenopausal counterparts, CMD risk factor levels are still

lower than those of both pre- and postmenopausal women in East or South Africa. Thus, in these latter two regions emphasis must be placed on prevention of obesity and related comorbidities in both pre- and postmenopausal women. In West Africa, data from the present study demonstrates that the MT is a critical window for the attenuation of CMD risk, and therefore the potential benefits of MHT become apparent. Data from previous studies suggest that when prescribed in healthy women early in menopause, MHT may help decrease the risk of CMD (97). Unfortunately, data on the use of hormone therapy in SSA women and its relation to CMD risk factors are absent.

The main limitations of this study are its cross-sectional design, limited dietary data, lack of data on sex hormone levels and the fact that populations at the study sites may not be representative of the national population. The time available for collecting data by interviews was restricted and therefore an in-depth analysis of dietary intake using a food frequency questionnaire was not feasible. Therefore, we focused on some key dietary constituents such as fruit and vegetables and high carbohydrate-containing foods and drinks that have been associated with obesity and cardiometabolic diseases i.e., sugar sweetened soft-drinks, fruit juices and bread. Although regional differences were noted in consumption of these dietary items, their inclusion in the regression models did not modify any of the observed associations.

Despite the shortcomings of a cross-sectional design, comparable findings to those observed in the West African populations were found in longitudinal studies where the menopausal transition was associated with increased levels of total cholesterol and LDL-C (10,137), and increased waist circumference (137). One of the largest longitudinal studies of the health consequences of the MT, SWAN, also demonstrated similar findings to those observed in the West African population with regards lipid

levels, body fat distribution and cIMT whereas menopausal increases in blood pressure, glucose and insulin levels were found to be related to chronological aging (232). The data from longitudinal studies therefore demonstrate that the MT is associated with the worsening of cardiometabolic health, which suggests that the data from the South and East African sites are not representative of the current literature and warrant further investigation.

The strengths of the study are the large sample size, the use of multiple sites representing different stages of the health transition across SSA and the measurement of a wide array of appropriate anthropometric, metabolic, demographic, and behavioural data. Furthermore, standardised methodologies were implemented across the study sites and all blood analytes were measured at a central laboratory.

The current study shows that the difference in the levels of CMD risk factors between pre- and postmenopausal women varied across geographical regions within SSA. In the more rural West African populations with a lower prevalence of obesity and other CMD risk factors, there were differences in the levels of CMD risk factors according to menopausal stage, but these differences were largely absent in the more urbanised and more obese South and East African populations. This indicates a need for context specific interventions to lower the risks of CMD in these populations. Thus, public health measures to curb the obesity epidemic in urban SSA populations, and clinical trials to assess the effects of hormone therapy in both urban and rural midlife women on menopause symptomology and CMD are required.

Having compared the CMD variables from four SSA countries in a cross-sectional format, the next chapter will focus on the longitudinal changes on CMD variables in the two West African countries. The two countries selected were those that showed

differences in CMD variables between pre- and postmenopausal women in this cross-sectional analysis. In addition, sex hormone levels at baseline and follow-up were also measured to determine their association with any changes in CMD and associated risk factors during the MT.

Chapter 4: Changes in Cardiometabolic Disease Risk Factors over the Menopausal Transition in Rural Burkina Faso and Ghana: A Longitudinal Study

Chikwati RP, Jaff N, Goolam Mahyoodeen N, George JA, and Crowther NJ. Changes in cardiometabolic disease risk factors over the menopausal transition in rural Burkina Faso and Ghana; a longitudinal study (will be submitted to *Journal of Clinical Endocrinology and Metabolism*).

4.1 Abstract

Context: Menopause is associated with an increased risk of cardiometabolic disease (CMD) which may be driven by changes in sex hormone levels. However, no longitudinal data exist on this relationship in SSA.

Objective: To investigate whether there is an association between changes in sex hormone levels and CMD risk factors (anthropometry, lipids, blood pressure, and glucose) during the menopausal transition in two rural SSA populations.

Design: Longitudinal.

Setting: A population-based study across two rural sites in Ghana (Navrongo) and Burkina Faso (Nanoro).

Participants: Sex hormone and CMD risk factor data were collected from 331 women from Ghana (Navrongo) and Burkina Faso (Nanoro) as they transitioned from pre- to perimenopause (group 1), pre- to postmenopause (group 2), or early to late postmenopause (group 3), or those that remained premenopausal (group 4). Data were collected at baseline and 5–6 years later. Multivariable linear regression was performed to identify the principal explanatory variables associated with changes in CMD risk factors in all three groups.

Main Outcome(s) and Measure(s): Multivariable linear regression was performed to identify the principal explanatory variables associated with changes in CMD risk factors in all three groups.

Results: In groups 1 and 2, levels of E2, SHBG, testosterone (T), and DHEAS decreased significantly whilst FSH levels increased. In group 3 only T and DHEAS levels fell. In group 1, E2 and free testosterone (FT) levels correlated negatively with triglyceride ($p=0.01$) and glucose ($p=0.03$) levels, respectively. In all groups, waist and visceral fat increased more in Ghana than Burkina Faso ($p<0.001$ for all) as did total- ($p=0.03$) and LDL-cholesterol ($p=0.02$) in group 2. In Burkina Faso there was an increase in diastolic blood pressure in groups 2 and 3 ($p<0.001$ for both), and in triglycerides ($p=0.04$) but only in group 3.

Conclusions: In this population of midlife women , changes in CMD risk factors were more strongly linked to the study site than to changes in sex hormone levels. This suggests that behavioural or socio-cultural elements have a substantial influence on CMD risk factors during and after menopause in this socio-demographic.

4.2 Introduction

In the previous systematic review and meta-analysis (Chapter 2), menopause was associated with an increased risk for CMD in LMICs, with some exceptions. These associations have been confirmed in the previous cross-sectional analysis (Chapter 3). However, very few longitudinal studies have been conducted in LMICs (135,141–143) and more are needed to fully understand whether CMD and associated risk factors change across the menopausal transition in these countries. Currently, no such longitudinal data exist in SSA populations. Such data are important in SSA, particularly

in rural populations where cross-sectional menopausal differences in CMD risk factors were more prevalent in these settings than in urban populations (Chapter 3).

The MT has been associated with diminishing activity of ovarian function which leads to a decline in the production of E2, and activation of the hypothalamus to stimulate the pituitary gland to release more FSH and the adrenal gland to release more androgens, particularly DHEA which exists in circulation in its sulphated form, DHEAS (59,66). In addition, the MT is associated with decreased SHBG levels (65,66). E2 is known for its modulation of blood pressure, inhibition of lipolysis in adipocytes, and regulating oxidative stress in the vascular system (42–45). FSH has been associated with regulating the synthesis and uptake of very-low density cholesterol (VLDL-C) and LDL-C by the liver (52). T is a known precursor for E2 and regulates body fat composition by mediating carbohydrate, lipid and protein metabolism (49). SHBG functions as a carrier protein and competitive inhibitor for E2 and T binding to cell membrane for steroid signalling (47,48). Lastly, DHEAS is a precursor for DHEA which in turn is a reservoir for androgens and E2, and is involved in vascular modelling (50). Thus, these hormones have important metabolic functions, and it is important to determine whether they play any role in the development of CMD in under-studied SSA populations in which the prevalence of CMD is increasing in midlife women.

The aim of the current study was to investigate longitudinal changes in anthropometric and CMD variables across different menopausal stages in midlife women from two rural sites in West Africa, Nanoro (Burkina Faso) and Navrongo (Ghana). In addition, the relationship between changes in sex hormone levels E2, FSH, total testosterone (TT), DHEAS and SHBG and changes in cardiometabolic and anthropometric variables in menopause-transitioning and postmenopausal women was investigated.

The hypothesis was that the changes in sex hormones during the menopause transition are associated with changes in CMD risk factors.

4.3 Methods

4.3.1 Study design and setting

This longitudinal study on midlife women is part of the AWI-Gen study in two rural sites in West Africa. As shown in the cross-sectional analysis (Chapter 3), these sites were selected based on their pronounced differences in CMD variables between pre- and postmenopausal women compared to the other sites in East Africa (Kenya) and South Africa (Dikgale and Soweto). The recruitment of study participants was performed using random sampling in the general population of women aged 40–60 years at each of the study centres (216). Data collection comprised two phases: baseline (phase 1, 2012–2016) and the follow-up visit (phase 2, 2019–2022).

Figure 4.1 shows the inclusion/exclusion criteria used in the current PhD study The initial selection comprised of 2133 women (1041 from Nanoro and 1092 from Navrongo). A total of 448 women (40 Nanoro and 408 Navrongo) who lacked menopausal staging data, and 385 women (348 Nanoro and 37 Navrongo) who were perimenopausal were excluded from the current study. In addition, eight women who were living with HIV (4 Nanoro and 4 Navrongo), and 389 women (244 Nanoro and 145 Navrongo) who were below the age of 44 years and 542 women (211 Nanoro and 331) who were aged above 55 years were excluded. The age selection was performed to recruit only a group of premenopausal women (>44 years) who over 5 years would have possibly become postmenopausal and a group of early postmenopausal women (<55 years) who would progress to late menopause at five years. This left 625 women for whom baseline anthropometric and cardiometabolic data had already been collected. Blood serum specimens were retrieved for these participants at the two

points i.e., baseline and follow-up at 5 years, for the measurement of sex hormones, as presented in Figure 4.2.

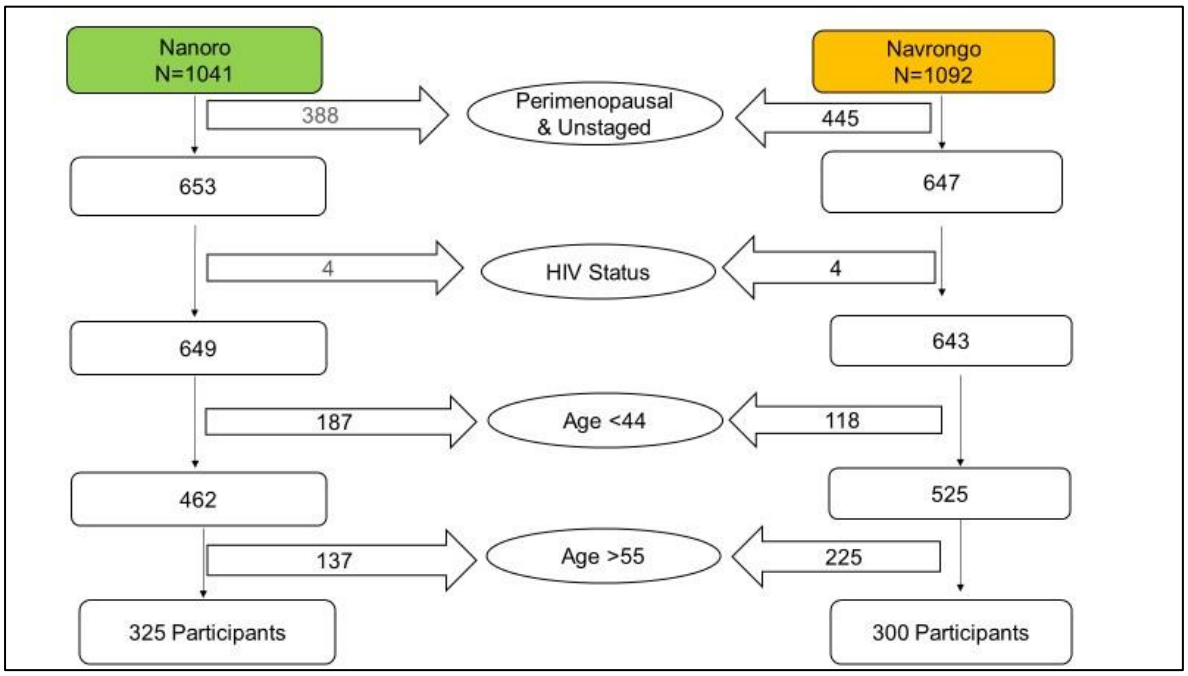

Figure 4.1 Selection of participants at baseline from the two sites, Nanoro and Navrongo.

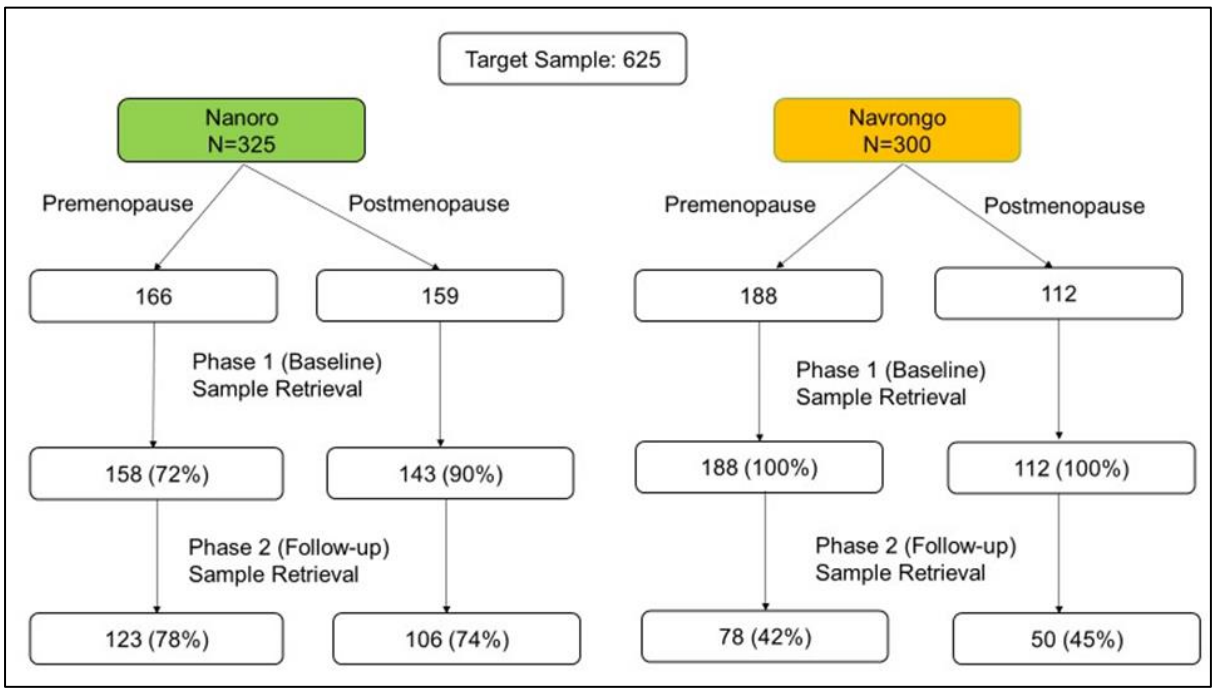

Figure 4.2 Collection of blood specimens at both phase 1 (baseline) and phase 2 (follow-up) visits.

4.3.2 Definitions of menopausal staging

The staging of menopause in the AWI-Gen study was determined by asking participants about their menstrual bleeding patterns. The premenopausal group was defined as women with regular menstrual cycles, the perimenopausal group were those who had had irregular menstrual cycles within the past 12 months, whilst the postmenopausal group were those who had had amenorrhea for twelve consecutive months or more in accordance with the STRAW+10 guidelines (30).

4.3.3 Covariates

From the AWI-Gen dataset, the PhD candidate obtained data on demography, behaviour, and lifestyle, and health history including self-reported information on age, physical activity, socioeconomic status, dietary intake, alcohol intake, and smoking status which had been previously obtained by trained personnel through questionnaires (Appendix 4). Standardised methods had been used to collect measurements such as waist, and hip circumference, standing height, weight, SBP and DBP, SAT, and VAT, and cIMT (22). The SAT, VAT and cIMT measurements had been obtained using ultrasound scans, and validation of these measurements had been done by asking all trained personnel to repeat measurements on 15 volunteers. The reported coefficient of variation between and within trainees was <2 %. Furthermore, fasting blood samples had been taken, and all laboratory measurements performed at the DPHRU laboratory in Soweto. Laboratory measurements included fasting serum triglyceride, total cholesterol, HDL-C, and insulin and fasting plasma glucose. LDL-C levels were calculated using the Friedewald formula (24).

4.3.4 Laboratory methods

For this PhD, the candidate retrieved blood serum specimens from −80°C storage and thawed them once at room temperature before testing on the cobas® 8000 analyser

using Elecsys® assay reagents (Roche Diagnostics, Indianapolis, USA). Serum levels of E2, FSH, TT, SHBG, and DHEAS were measured on the cobas e 601, and albumin on the e 702 modules of the cobas® 8000 analyser. Specimens were paired according to their unique identifications at baseline and the follow-up visit such that each participant's samples were assayed in the same batch. Testing was done according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, USA). The sex hormone measurements were based on either a sandwich or competitive electrochemiluminescence immunoassay (ECLIA). The sandwich ECLIA, applicable for FSH and SHBG includes three steps, recognition, separation, and detection and these are illustrated in Figure 4.3. As shown in Figure 4.3, the specimen is initially incubated with two antibodies, a primary monoclonal antibody labelled with biotin and a secondary monoclonal antibody labelled with a ruthenium complex. The two antibodies form an immunocomplex (sandwich) with the analyte in the specimen. In the second incubation, streptavidin-coated microparticles are added. These microparticles bind to the biotinylated antibody, and the resultant mixture is then transferred to a measuring cell (electrode). In the measuring cell, a magnetic field is applied and the microparticle beads bind to the surface of the measuring cell. A ProCell/ProCell M solution is then introduced to remove the unbound particles and introduce tripropylamine (TPA) which is essential for the electrochemiluminescence (ECL) reaction. The application of voltage on the electrode induces the ruthenium and TPA to their excited states. The TPA radical then serves as a reductant, causing the ruthenium to return to its base state and this is associated with the emission of light because of a chemical reaction (electrochemiluminescence). The emitted light is detected by a photomultiplier and then extrapolated onto a calibration curve to give a reading of the actual concentration of the analyte in the specimen. The competitive

ECLIA, used for measuring E2 and DHEAS, differed from the sandwich ECLIA in the incubation steps where the specimen is incubated with two monoclonal biotinylated antibodies to form an immunocomplex with the analyte in the specimen. In the second incubation, streptavidin-coated microparticles and an analyte derivative labelled with a ruthenium complex competes for the binding sites on the still-vacant biotinylated antibodies to form an antibody-hapten complex. The complex is then bound to the solid phase through the interaction of biotin and streptavidin. After the mixture is aspirated to the measuring cell, the chemiluminescence technology is similar to the one described for the sandwich ECLIA.

The measuring ranges of the sex hormones were as follows: E2, 18.4−11010 pmol/L; FSH, 0.3−200 mIU/ml; DHEAS, 0.003−27.1 µmol/L; TT, 0.087−52.0 nmol/L; SHBG, 0.35−200 nmol/L. The limits of quantitation (LOQ) were as follows: E2, 91.8 pmol/L; FSH, 1.00 mIU/ml; DHEAS, 0,065 µmol/L; TT; 0.416 nmol/L; SHBG, 1.00 nmol/L.

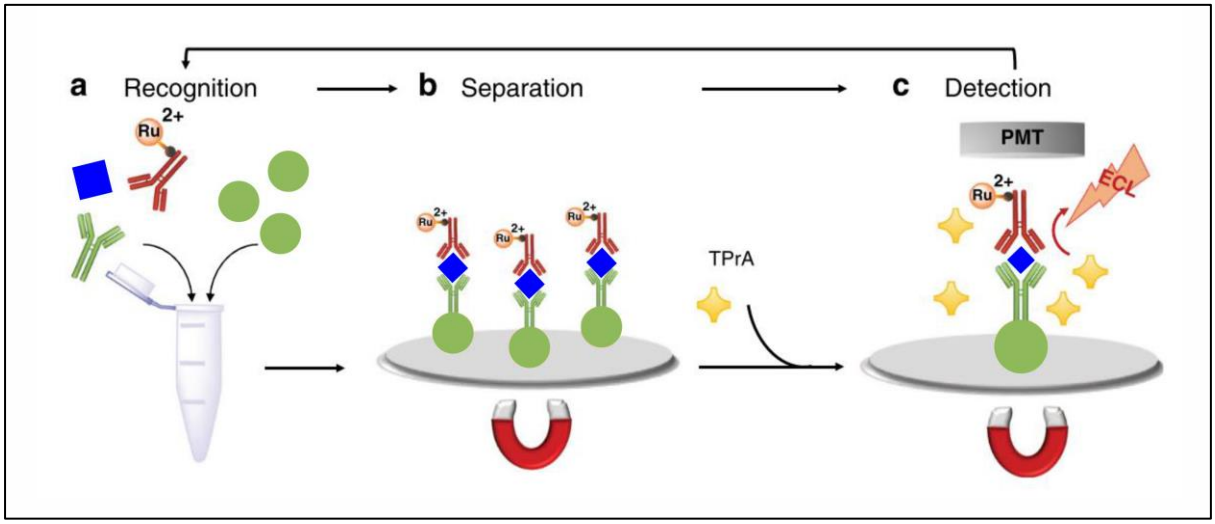

Figure 4.3 Principle of the sandwich electrochemiluminescent immunoassay. Adapted from Zanut *et al.,* (233). **a** Recognition: A biotinylated monoclonal antibody (in green) and a ruthenium labelled monoclonal antibody (in red) are mixed with the analyte (in blue) and streptavidin-coated microparticles (green spheres). **b** Separation: magnetic field is applied to capture microparticles to surface of the cell, and Tri-n-propylamine (TPA) is added**. c** Detection: application of voltage induces an electrochemiluminescence emission, which is captured by the photomultiplier (PMT).

The measurement of serum albumin was based on a colorimetric principle. This involved a chemical reaction between bromocresol green (BCG) and albumin at a low pH (4.1) to form a blue-green albumin-BCG complex. A photometer then measures the colour intensity of the complex which is directly proportional to the concentration of the albumin in the specimen. The measuring range for albumin was 2–60 g/L.

The free androgen index (FAI) was calculated as a percentage of the ratio between TT and SHBG levels, and the testosterone-to-estradiol ratio (TT:E2) was calculated from TT and E2 levels. Furthermore, levels of free testosterone (FT) were calculated using the Vermeulen equation (234,235) as follows:

$FT = (T - N - S + \sqrt{((N + S - T)^2 + 4NT)})\ /2N$

where FT, T, S are free testosterone, TT (nmol/L), and SHBG (nmol/L) concentrations, respectively. *N*=0.5217A+1, where A is the concentration of albumin in g/dL. The latter equation was derived from the association constants of testosterone for albumin (3.6 x 10^4 L/mol) and SHBG (10^9 L/mol) (234,235).

Levels of bioavailable testosterone (BAT) levels were calculated using the Vermeulen equation (234,235):

$BAT = 0.5217A \times FT + FT$

where BAT, A and FT are the bioavailable testosterone (nmol/L), albumin (g/dL) and free testosterone (nmol/L) concentrations, respectively. These units of measurement were later converted into pmol/L for further analyses.

4.3.4 Statistical Analyses

Descriptive statistics for the studied variables at both baseline and follow-up visits were presented as means and standard deviations (SD) or medians and interquartile ranges (IQR) for Gaussian and non-Gaussian distributed variables respectively.

Based on menopausal staging, participants were assigned to one of four possible groups i.e., women who either remained premenopausal or remained postmenopausal, premenopausal women who transitioned to perimenopause, and premenopausal women who transitioned to postmenopause. In each group, levels of E2, FSH, SHBG, DHEAS, TT, FT, FAI, BAT, and albumin levels were compared between baseline and follow-up time points. Comparisons within groups were also made for the cardiometabolic and anthropometric variables i.e., total cholesterol, LDL-C, HDL-C, triglycerides, SBP and DBP, glucose, cIMT, BMI, waist and hip circumference, VAT, and SAT. To identify significant changes in variables within each of the 4 menopausal groups, continuous variables were compared using the parametric (Student's paired t-test) or non-parametric (Wilcoxon matched paired test) statistical tests. Furthermore, absolute changes in variables between the baseline and follow-up time points were calculated and compared between the 4 groups using parametric (one-way ANOVA) and non-parametric (Kruskal-Wallis H) tests. In cases where ANOVA provided a significant F-value and the Kruskal-Wallis H test showed statistical differences between groups, the Tukey and Dunn's *post hoc* tests, respectively, were used to compare paired means. Lastly, multivariable linear regression was performed to identify the principal explanatory variables associated with the absolute changes in anthropometric and CMD variables (dependent variables) within three of the four groups. The exception was the group of women who remained premenopausal for who there was a low sample size. Independent variables (all expressed as absolute change) for these linear models were selected based on scientific plausibility (Table 4.1) and these variables were then included in univariate analyses against the dependent variable using the Spearman's rank correlation test. Only those variables from the univariates that correlated with the dependent variables

at p<0.20 were included in the multiple regression models. This process was chosen due to the limited sample sizes to ensure that models were not over fitted. In addition, the regression models for absolute changes in blood pressure were run with and without all those participants who were pre-diagnosed with hypertension and may therefore be receiving therapy. A similar process was applied to the multiple regression model for absolute changes in glucose and pre-diagnosed diabetic study participants.

Multicollinearity was assessed using the variance inflation factor (VIF) which was set at a threshold of 5.0. However, none of regression models were found to include independent variables with VIF >3. All analyses were performed using Stata 16.1 (StataCorp LP, College Station, TX, USA). The level of significance was set at a two-tailed threshold of p<0.05.

Table 4.1 List of variables included in univariate analyses

Dependent variables (expressed as absolute change)	Independent variables (expressed as absolute change)
Each anthropometric variable	Age, BMI, E2, FSH, TT, FT, BAT, SHBG, DHEAS, FAI, study site
All lipids	Age, BMI, E2, FSH, TT, FT, BAT, SHBG, DHEAS, FAI, waist, hip, VAT, SAT, glucose, study site
Diastolic and systolic BP	Age, E2, FSH, TT, FT, BAT, SHBG, DHEAS, FAI, waist, hip, BMI, VAT, SAT, TC, LDL-C, TGs, glucose, study site
Glucose	Age, E2, FSH, TT, FT, BAT, SHBG, DHEAS, FAI, waist, hip, BMI, VAT, SAT, study site
cIMT	Age, E2, FSH, TT, FT, BAT, SHBG, DHEAS, FAI, waist, hip, BMI, VAT, SAT, DBP, TC, LDL-C, TGs, glucose, study site

BMI-body mass index, E2-estradiol, FSH-follicle stimulating hormone, TT-total testosterone, FT-free testosterone, BAT-bioavailable testosterone, SHBG-sex hormone binding globulin, DHEAS-dehydroepiandrosterone sulphate, FAI-free androgen index, VAT- abdominal visceral adipose tissue, SAT-abdominal subcutaneous adipose tissue, TC-total cholesterol, LDL-C-low-density lipoprotein cholesterol, TGs-triglycerides, cIMT-carotid intima media thickness, BP-blood pressure, study site-Navrongo and Nanoro.

4.4 Results

4.4.1 Participant characteristics

A total of 625 women were recruited at baseline (Figure 4.1). At the follow-up visit (5.4±0.8 years later), 337 had complete data for analyses (Figure 4.2), except for 6 participants who had missing data on menopause staging. In total, 331 (53%) had complete data for the current analyses. The major reasons for the high loss to follow-up were, migration from the study area, death, refusal to participate due to the volume of blood collected, and personal reasons. For the current analyses, the study participants comprised 18 women who had remained premenopausal, 60 who had transitioned from pre- to perimenopause, 108 women who were now postmenopausal at follow-up, and 146 who were postmenopausal from baseline. The incidence of diabetes mellitus and hypertension was 2.1% and 8.7%, respectively.

4.4.2 Changes in sex hormone levels

Tables 4.2 to 4.5 show the levels of sex hormones in the in the four possible groups at the two time-points. In the group that remained premenopausal E2 levels were higher at follow-up, but DHEAS decreased, and no significant differences were observed in the TT, FT, BAT, FAI, FSH and SHBG levels (Table 4.2). Women who transitioned from pre- to perimenopause had significantly lower levels of E2, TT, TT:E2 ratio, SHBG, and DHEAS, while their FSH levels increased, but the levels of FT and BAT which were no different in the follow-up (Table 4.3). In women who were premenopausal at baseline and postmenopausal at follow-up, the levels of sex hormones were all significantly different between the two visits, lower for E2, TT, FT, BAT, FAI, TT:E2 ratio, SHBG, and DHEAS, but higher for FSH (Table 4.4). In Table 4.5, women who were early postmenopausal at baseline and late postmenopausal at

follow-up showed no differences in the levels of E2, TT:E2 ratio and SHBG, but lower FSH, TT, FT,BAT, FAI, and DHEAS from their respective baseline levels.

Table 4.2: Age, sex hormones and albumin levels in women who remained premenopausal (n=18)

	Baseline	Follow-up visit	P value
Age (years)	44.9±1.1	50.6±1.4	<0.001
E2 (pmol/L)	310.7 (175.3–563.1)	552.5 (172.4–1372.0	0.01
FSH (mIU/L)	9.1 (6.9–15.8)	11.5 (5.3–20.2)	0.89
Total Testosterone (pmol/L)	365 (230–540)	410 (210–620)	0.31
Free testosterone (pmol/L)	3.20 (1.31–4.27)	2.81 (1.73–4.61)	0.74
Bioavailable testosterone (pmol/L)	70.1 (32.6–103.4)	61.0 (36.0–105.0)	0.78
FAI	0.38 (0.25–0.52)	0.33 (0.17–0.54)	0.83
TT:E2 ratio	0.99 (0.53–2.73)	0.49 (0.36–1.45)	0.19
SHBG (nmol/L)	102.5±41.1	115.6±44.2	0.25
DHEAS (µmol/L)	1.89±0.83	1.52±0.58	0.02
Albumin (g/L)	42.5±3.6	40.6±2.7	0.06

Data expressed as mean±SD or median (IQR); E2-estradiol, FSH-Follicle Stimulating Hormone, FAI-Free Androgen Index, TT:E2-testosterone:estradiol ratio, SHBG-Sex Hormone Binding Globulin, DHEAS-Dehydroepiandrosterone sulphate.

Table 4.3: Age, sex hormones and albumin levels in women who transitioned from pre- to perimenopause (n=60)

	Baseline	Follow-up visit	P value
Age (years)	45.8±1.5	51.2±1.6	<0.001
E2 (pmol/L)	462.3 (233.6–827.3)	55.4 (37.5–125.7)	<0.001
FSH (mIU/L)	7.4 (5.0–19.4)	50.6 (36.3–70.9)	<0.001
Total Testosterone (pmol/L)	455 (290–735)	370 (210–570)	0.001
Free testosterone (pmol/L)	3.99 (2.15–5.57)	3.06 (1.73–5.21)	0.15
Bioavailable testosterone (pmol/L)	86.8 (50.5–130.4)	72.8 (39.5–122.7)	0.14
FAI	0.46 (0.27–0.71)	0.41 (0.20–0.65)	0.28
TT:E2 ratio	0.90 (0.44–1.92)	5.40 (2.16–9.35)	<0.001
SHBG (nmol/L)	114.1±42.4	93.8± 37.2	<0.001
DHEAS (µmol/L)	2.07±0.87	1.70±0.87	0.03
Albumin (g/L)	41.4±2.9	41.2±3.2	0.42

Data expressed as mean±SD or median (IQR); E2-estradiol, FSH-Follicle Stimulating Hormone, FAI-Free Androgen Index, TT:E2-testosterone:estradiol ratio, SHBG-Sex Hormone Binding Globulin, DHEAS-Dehydroepiandrosterone sulphate.

Table 4.4: Age, sex hormones and albumin levels in women who had become postmenopausal at follow-up (n=108)

	Baseline	Follow-up visit	P value
Age (years)	46.8±2.00	52.3±2.2	<0.001
E2 (pmol/L)	282.8 (90.6–681.2)	37.9 (18.6–64.1)	<0.001
FSH (mIU/L)	13.3 (6.7–35.0)	60.5 (45.8– 78.3)	<0.001
Total Testosterone (pmol/L)	290.0 (100.0–540.0)	185.0 (60.0–370.0)	<0.001
Free testosterone (pmol/L)	2.30 (0.87–4.17)	1.45 (0.50–3.09)	<0.001
Bioavailable testosterone (pmol/L)	48.1 (19.4–88.7)	32.9 (11.3–71.8)	<0.001
FAI	0.27 (0.11–0.48)	0.17 (0.06–0.38)	<0.001
TT:E2 ratio	0.82 (0.54–2.64)	3.31 (1.93–9.13)	<0.001
SHBG (nmol/L)	114.5± 43.6	105.7±34.9	0.01
DHEAS (µmol/L)	1.80±0.80	1.41±0.76	<0.001
Albumin (g/L)	41.2±3.4	41.4±2.9	0.40

Data expressed as mean±SD or median (IQR); E2-estradiol, FSH-Follicle Stimulating Hormone, FAI-Free Androgen Index, TT:E2-testosterone:estradiol ratio, SHBG-Sex Hormone Binding Globulin, DHEAS-Dehydroepiandrosterone sulphate.

Table 4.5: Age, sex hormones and albumin levels in women who were postmenopausal at baseline (n=146)

	Baseline	Follow-up visit	P value
Age (years)	52.5±2.1	57.8±2.1	<0.001
E2 (pmol/L)	39.1 (23.4–52.9)	38.8 (25.1–50.7)	0.82
FSH (mIU/L)	60.4 (43.5–75.5)	59.5 (46.7–75.0)	0.01
Total Testosterone (pmol/L)	300 (160–560)	260 (60–460)	0.03
Free testosterone (pmol/L)	2.36 (1.23–4.14)	1.90 (0.68–3.68)	0.03
Bioavailable testosterone (pmol/L)	52.8 (29.1–97.9)	43.6 (15.2–86.7)	0.02
FAI	0.29 (0.15–0.52)	0.24 (0.08–0.46)	0.03
TT:E2	7.18 (3.68–14.2)	2.83 (6.54–11.9)	0.08
SHBG (nmol/L)	109.4±41.7	110.0±39.1	0.93
DHEAS (µmol/L)	1.58±0.73	1.37±0.72	0.03
Albumin (g/L)	41.8±3.8	41.2±3.2	0.07

Data expressed as mean±SD or median (IQR); E2-estradiol, FSH-Follicle Stimulating Hormone, FAI-Free Androgen Index, TT/E2-testosterone:estradiol ratio, SHBG-Sex Hormone Binding Globulin, DHEAS-Dehydroepiandrosterone sulphate.

4.4.3 Changes in anthropometric and CMD risk factors

Tables 4.6 to 4.9 show the levels of anthropometric and CMD risk factors in the four possible groups at the two time-points. Table 4.6 shows that in women who remained premenopausal, only total cholesterol, triglycerides, cIMT and glucose levels increased in the follow-up. No other changes in anthropometric and CMD risk factors were observed. In Table 4.7, the shift from pre- to perimenopause was associated with

increased anthropometric and cardiometabolic variables, but not DBP and cIMT levels. As presented in Table 4.8, the shift from pre- to postmenopause was associated with increased waist and hip circumference, DBP, VAT, SAT, total cholesterol, LDL-C, HDL-C, triglycerides, glucose, and cIMT levels, but not BMI, and SBP levels. In Table 4.9, women who were postmenopausal at baseline had increased, waist circumference, VAT, blood lipids, glucose and cIMT levels from their respective baseline levels, but no differences on SBP, DBP, BMI, SAT, and hip circumference were observed.

Table 4.6: Age, anthropometric and cardiometabolic variables in the women who remained premenopausal (n=18)

	Baseline	Follow-up visit	P value
Age (years)	44.9±1.1	50.6±1.4	<0.001
Waist circumference (cm)	73.8 (70.2–84.2)	80.3 (71.9–92.0)	0.08
Hip circumference (cm)	92.1 (86.7–94.9)	92.7 (85.4–102.0)	0.63
BMI (kg/m²)	21.7 (20.0–24.2)	21.8 (19.0–25.2)	0.41
SBP (mmHg)	114.0 (103.5–127.0)	116.5 (103.5–131.0)	0.39
DBP (mmHg)	75.5 (66.0–79.0)	72.7 (68.0–82.0)	0.53
VAT (mm)	4.15 (3.16–5.47)	5.24 (4.45–5.74)	0.15
SAT (mm)	1.03 (0.66–1.49)	0.87 (0.64–1.51)	0.67
Total cholesterol (mmol/L)	2.95±1.02	3.80±1.05	0.01
LDL-cholesterol (mmol/L)	1.75 (1.02–2.24)	1.90 (1.52–2.92)	0.12
HDL-cholesterol (mmol/L)	1.07 (0.89–1.31)	1.27 (1.22–1.35)	0.09
Triglycerides (mmol/L)	0.55 (0.47–0.59)	0.78 (0.56–0.84)	0.03
Glucose (mmol/L)	4.68 (4.15–5.11)	5.49 (5.10–5.70)	<0.001
Average cIMT (mm)	0.57 (0.54–0.65)	0.64 (0.61–0.69)	0.01

Data expressed as mean±SD or median (IQR), BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, VAT-abdominal visceral adipose tissue, SAT-abdominal subcutaneous adipose tissue, LDL-low density lipoprotein, HDL-high density lipoprotein, cIMT-carotid intima thickness.

Table 4.7: Age, anthropometric and cardiometabolic variables in the women who progressed from pre- to perimenopause (n=60)

	Baseline	Follow-up visit	P value
Age (years)	45.8±1.5	51.2±1.6	<0.001
Waist circumference (cm)	76.0 (73.0–82.0)	83.0 (75.2–88.3)	<0.001
Hip circumference (cm)	90.0 (87.0–94.2)	93.0 (87.3–99.9)	0.001
BMI (kg/m²)	21.2 (19.9–23.2)	22.6 (20.3–24.4)	0.004
SBP (mmHg)	109.0 (100.3–120.0)	114.8 (103.8–124.5)	0.004
DBP (mmHg)	71.3 (65.5–76.5)	72.8 (67.0–79.3)	0.33
VAT (mm)	3.63 (3.00–4.65)	5.39 (4.90–7.14)	<0.001
SAT (mm)	0.91 (0.56–1.28)	1.13 (0.81–1.63)	<0.001
Total cholesterol (mmol/L)	3.03±0.84	3.64±1.02	<0.001
LDL-cholesterol (mmol/L)	1.63 (1.22–2.10)	1.99 (1.73–2.49)	<0.001
HDL-cholesterol (mmol/L)	1.05 (0.88–1.22)	1.23 (1.03–1.37)	<0.001
Triglycerides (mmol/L)	0.60 (0.44–0.75)	0.77 (0.59–0.94)	<0.001
Glucose (mmol/L)	4.72 (4.23–5.13)	5.32 (5.06–5.70)	<0.001
Average cIMT (mm)	0.61 (0.55–0.68)	0.62 (0.57–0.73)	0.17

Data expressed as mean±SD, median (IQR), BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, VAT-abdominal visceral adipose tissue, SAT-abdominal subcutaneous adipose tissue, LDL-low density lipoprotein, HDL-high density lipoprotein, cIMT-carotid intima media thickness.

Table 4.8: Age, anthropometric and cardiometabolic variables in the women who were premenopausal at baseline and postmenopausal at follow-up

	Baseline	Follow-up visit	P value
Age (years)	46.8±2.0	52.3±2.2	<0.001
Waist circumference (cm)	74.0 (69.3–80.3)	78.2 (71.2–88.3)	<0.001
Hip circumference (cm)	88.0 (82.5–93.0)	89.9 (83.0–97.5)	0.002
BMI (kg/m²)	20.2 (18.5–22.4)	20.4 (18.5–23.1)	0.07
SBP (mmHg)	105.5 (98.5–118.5)	109.0 (98.8–120.3)	0.08
DBP (mmHg)	69.0 (63.0–75.0)	71.3 (65.0–77.3)	0.02
VAT (mm)	4.14 (3.22–5.24)	5.22 (4.56–6.34)	<0.001
SAT (mm)	0.79 (0.54–1.22)	0.86 (0.54–1.31)	0.001
Total cholesterol (mmol/L)	2.99±0.80	3.77±0.90	<0.001
LDL-cholesterol (mmol/L)	1.66 (1.24–2.08)	2.05 (1.64–2.64)	<0.001
HDL-cholesterol (mmol/L)	0.96 (0.81–1.10)	1.19 (0.99–1.42)	<0.001
Triglycerides (mmol/L)	0.57 (0.41–0.78)	0.82 (0.64–1.03)	<0.001
Glucose (mmol/L)	4.79 (4.41–5.22)	5.37 (5.11–5.58)	<0.001
Average cIMT (mm)	0.62 (0.56–0.68)	0.64 (0.58–0.72)	0.01

Data expressed as mean±SD or median (IQR), BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, VAT-abdominal visceral adipose tissue, SAT-abdominal subcutaneous adipose tissue, LDL-low density lipoprotein, HDL-high density lipoprotein, cIMT-carotid intima media thickness.

Table 4.9: Age, anthropometric and cardiometabolic variables in the women who remained postmenopausal (n=146)

	Baseline	Follow-up visit	P value
Age (years)	52.5±2.0	57.7±2.1	<0.001
Waist circumference (cm)	74.9 (70.0–79.9)	78.8 (72.6–84.0)	<0.001
Hip circumference (cm)	87.2 (82.0–91.7)	87.7 (81.5–92.4)	0.27
BMI (kg/m^2)	19.7 (18.0–21.7)	19.6 (17.7–21.3)	0.74
SBP (mmHg)	115.3 (103.0–131.5)	116.8 (106.5–130.0)	0.41
DBP (mmHg)	73.0 (66.5–80.5)	71.8 (64.5–79.5)	0.16
VAT (mm)	3.72 (3.12–4.58)	5.14 (4.60–6.30)	<0.001
SAT (mm)	1.00 (0.72–1.26)	1.01 (0.74–1.47)	0.12
Total cholesterol (mmol/L)	3.37±0.92	3.91±0.86	<0.001
LDL-cholesterol (mmol/L)	1.95 (1.43–2.35)	2.18 (1.67–2.67)	<0.001
HDL-cholesterol (mmol/L)	1.03 (0.88–1.31)	1.25 (1.04–1.46)	<0.001
Triglycerides (mmol/L)	0.72 (0.55–0.93)	0.87 (0.70–1.17)	<0.001
Glucose (mmol/L)	4.79 (4.42–5.20)	5.46 (5.17–5.87)	<0.001
Average cIMT (mm)	0.65 (0.59–0.73)	0.68 (0.61–0.76)	<0.001

Data expressed as mean±SD, median (IQR), BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, VAT-abdominal visceral adipose tissue, SAT-abdominal subcutaneous adipose tissue, LDL-low density lipoprotein, HDL-high density lipoprotein, cIMT-carotid intima media thickness.

4.4.4. Absolute changes in anthropometric variables, CMD risk factors and sex hormone levels across the menopause transition

Table 4.10 shows a comparison of absolute changes in age, anthropometric and CMD risk factors and sex hormone levels in the four possible menopausal groups. In these comparisons, the changes on SAT, BMI, DBP, E2, FSH, TT, TT:E2 ratio and SHBG were different between these four groups. Thus, change in SAT levels were higher in women who transitioned from pre- to perimenopause than those who either remained premenopausal or postmenopausal. The change in BMI was higher in the pre- to perimenopausal transition as compared to women who remained premenopausal. The level of DBP lowering in women who were postmenopausal at baseline was significantly different to the increase in DBP seen in the pre- to postmenopausal women at follow-up. The decline in E2 levels was high in the pre- to perimenopausal group and the pre- to postmenopausal group and no change was seen in the group that was postmenopausal at baseline. The trajectory of FSH showed that the largest

increase was in the pre- to postmenopause and the pre- to perimenopause groups, which were both significantly different to the changes in those women who were initially postmenopausal, and those that remained premenopausal. The decrease in TT levels was highest in the pre- to perimenopausal group followed by the pre- to postmenopausal group which were both significantly different to the increase seen in those who remained premenopausal whilst the small fall seen in the early post- to late postmenopausal group was significantly different to that observed in the pre- to perimenopause group. The rise in the TT:E2 ratio was highest in the pre- to perimenopausal group followed by the pre- to postmenopausal group both of which were significantly different to the decrease seen in the early to late postmenopausal group with the former also being significantly different to the small decrease seen in those that remained premenopausal. The SHBG levels decreased the most in the pre- to perimenopause group followed by the pre- to postmenopause group with the former being significantly different to the increases observed in the group that remained premenopausal and those that were late postmenopausal at follow up. The magnitude of the changes observed on SAT, BMI, and DBP although significant between some of the groups was very small and therefore of little clinical significance, thus no further statistical analyses i.e., ANCOVAs were performed.

Table 4.10 Absolute changes of all variables across the four menopausal groups in the longitudinal study

Variable	Pre- to Premenopausal at follow-up	Pre- to Perimenopause at follow-up	Pre- to Postmenopause at follow-up	Postmenopausal at baseline
Age (years)	5.72±0.46	5.44±0.76	5.46±0.70	5.30±0.76
WC (cm)	4.29±8.10	5.90±6.11	4.47±7.09	3.73±5.74
HipC (cm)	1.14±7.80	2.57±5.45	2.21±6.15	0.87±5.65
VAT (mm)	0.77±1.81	1.93±1.74	1.27±2.04	1.49±1.68
SAT (mm)	-0.08 (-0.32 –0.21)	0.21 (0.01–0.45)* ††	0.06 (-0.08 –0.29)	0.03 (-0.18–0.26)
BMI (kg/m²)	0.17±1.62	0.85±1.98†	0.27±2.14	0.01±1.93
DBP (mmHg)	0.94±8.20	1.04±8.73	1.58±8.37†	-1.68±9.41
SBP (mmHg)	4.27±18.3	4.13±13.4	2.37±13.9	0.33±17.1
T.Chol (mmol/L)	0.91±1.32	0.65±0.71	0.79±0.72	0.59±0.88
LDL-C (mmol/L)	0.04 (-0.08 –0.55)	0.35 (0.12 –0.58)	0.40 (0.08–0.75)	0.25 (-0.11–0.69)
HDL-C (mmol/L)	0.13±0.38	0.16±.23	0.25±0.24	0.20±0.31
TGs (mmol/L)	0.10±0.43	0.16±0.31	0.27±0.26	0.17±0.32
Gluc. (mmol/L)	0.44 (0.63–0.99)	0.27 (0.72–1.08)	0.29 (0.61–1.21)	0.20 (0.67–1.17)
cIMT (mm)	0.05±0.07	0.01±0.09	0.02±0.09	0.03±0.10
E2 (pmol/L)	186.3 (-20.5–880.8)	-355.2 (-771.2 – -160)*** ††† †††	-175.7 (-528.9 – -29.1)*** †††	0.00 (-9.00–9.70)***
FSH (mIU/L)	0.7 (-4.3–4.4)	36.1 (19.4–52.7)*** †††	40.2 (17.5 –62.2)*** †††	-3.0 (-1.5–5.1)
TT (pmol/L)	80.0 (-110.0–170.0)	-90.0 (-300.0–20.0)** †	-40.0 (-220.0–0.0)*	-10.0 (-130.0–60.0)
TT:E2 ratio	-0.16 (-0.82–0.07)	3.62 (1.34–7.97)** †††	2.16 (0.62–5.85) †††	-1.15 (-3.86–2.03)
FT (pmol/L)	0.34 (-0.74–1.18)	-0.37 (-1.70–0.78)	-0.32 (-1.47–0.09)	-0.13 (-1.02 –0.44)
BAT (pmol/L)	1.54 (-23.4–23.9)	-10.2 (-34.3–15.4)	-6.74 (-33.1 –1.48)	-3.05 (-24.2–9.24)
FAI	0.02 (-0.09–0.14)	-0.03 (-0.18–0.11)	-0.04 (-0.17–0.02)	-0.02 (-0.12 –0.05)
SHBG (nmol/L)	13.1±46.8	-20.2±36.6***†††	-8.84±35.0	0.29±27.5
DHEAS (µmol/L)	-0.18 (-0.88–0.06)	-0.24 (-0.61–0.08)	-0.29 (-0.70–-0.03)	-0.19 (-0.44–0.08)
Albumin (g/L)	-1.76±3.61	-0.38±2.96	0.26±3.21	-0.53±3.76

Data expressed as mean±SD and median (IQR), Tukey post hoc test, †p<0.05, ††p<0.01, †††p<0.001 vs post-post; *p<0.05, **p<0.01, ***p<0.001 vs pre-pre; †††p<0.001 vs pre-post; WC-waist circumference, HipC-hip circumference, VAT-visceral adipose tissue, SAT-subcutaneous adipose tissue BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, T.Chol-total cholesterol, LDL-C, low density lipoprotein-cholesterol, HDL-C, high density lipoprotein-cholesterol, TGs-triglycerides, Gluc, glucose, cIMT, carotid intima media thickness, E2, estradiol, FSH, follicle stimulating hormone, TT, total testosterone, FT, free testosterone, BAT, bioavailable testosterone, FAI, free androgen index, SHBG, sex steroid binding globulin, DHEAS, dehydroepiandrosterone sulphate.

4.4.5. Univariate and multivariate analyses

Tables 4.11–4.13 show changes in anthropometric and CMD risk factors (dependent variables) and associations with independent variables from univariate and multivariable linear regression analyses. Table 4.11 shows that in the group that transitioned from pre- to perimenopause, decreasing BAT levels were associated with higher waist circumference, while decreasing E2 levels were associated with higher levels of triglycerides, and increasing FSH levels were associated with lower hip circumference. SHBG levels had near significant (p=0.06) positive associations with changes in VAT. Furthermore, the increased TT:E2 ratio was associated with higher LDL-C, but lower SBP. When hypertensives and diabetics were excluded from the analysis, there was no longer an association between the TT:E2 ratio and SBP. In addition, Table 4.11 shows that the differences between the sites were associated with many anthropometric and CMD risk factors. Thus, participants from Navrongo (Ghana) had higher VAT, LDL-C, and hip circumference changes than their peers in Nanoro (Burkina Faso). Furthermore, during the pre- to perimenopausal transition, the rise in BMI was significantly associated with increased waist and hip circumference, total cholesterol, and LDL-C.

In those women who were premenopausal at baseline and had become postmenopausal at follow up, (shown in Table 4.12), increasing BMI and study site were associated with higher waist and hip circumference. Chronological ageing was associated with increasing VAT, while increasing blood glucose correlated with increased triglycerides, and elevations in BMI with increased blood glucose levels. Increases in FSH levels were associated with near significant increases in total cholesterol (p=0.06) and LDL-C (p=0.07). Furthermore, participants from Navrongo had higher VAT, total cholesterol, and LDL-C, but lower DBP changes than those from

Nanoro. In a sub-analysis where hypertensive and diabetic participants were excluded, the associations between glucose and triglyceride levels, as well as BMI and glucose levels disappeared, but the differences in DBP levels between sites remained significant (data not shown).

Table 4.13 presents the analyses in those women who were postmenopausal at baseline. The univariate analyses showed that rising FAI, SHBG, BMI and study site were associated with increasing waist circumference. Chronological ageing, rising BMI, blood glucose levels and study site correlated with increasing DBP. In the multivariable analyses, both BMI and study site were associated with waist circumference and DBP. Thus, participants in Navrongo had a greater increase in DBP than their counterparts in Nanoro. Furthermore, in a sub-analysis where all participants who reported the use of anti-hypertensive and diabetic medication were excluded, there was no longer an association between BMI and DBP, but study site remained significant (data not shown). In the univariate model, falling BAT levels and study site were associated with increased VAT, and after the adjustment for age and BMI, only study site was associated with VAT. Rising SAT and BMI were associated with total cholesterol, but these differences disappeared in the multivariable analyses. Rising SAT, TT:E2 ratio and study site were associated with LDL-C. In the adjusted model, only changes in TT:E2 ratio had near significant increases in LDL-C ($p=0.09$). Rising SHBG levels were associated with increasing HDL-C. Rising BMI and study site were associated with increased changes in triglycerides and blood glucose, but these were no significant in the multivariable models. Rising waist circumference and SHBG levels correlated with cIMT levels, but not in the adjusted analyses.

Table 4.11: Univariate and multivariable linear regression models showing associations between changes in variables in women who transitioned from pre- to perimenopause

Dependent variable	Independent variable	Univariate model	Multivariable model
Waist circumference (mm)	Age BMI BAT Study site	15.6 (-0.81, 32.1)* 16.1 (8.78, 23.5)*** -0.37 (-0.61, -0.14)** 71.3 (43.7, 98.9)***	0.03 (–9.19, 9.26) 16.4 (11.1, 21.7)*** -0.13 (-0.30, 0.03)* 73.8 (53.4, 94.3)***
Hip circumference (mm)	BMI FSH BAT Study site	13.7 (9.3, 18.3)*** -0.50 (-1.10, 0.03)** -0.25 (-0.48, -0.02)** 60.2 (36.0, 84.3)***	14.8 (10.5, 19.2)*** -0.34 (-0.66, -0.03)** -0.04 (-0.18, 0.09) 60.9 (39.9, 81.9)***
SBP (mmHg)	BMI VAT TT:E2 ratio LDL-C Study site	1.82 (0.33, 3.31)** -1.84 (-3.51, 0.18)** -0.48 (-0.69, -0.26)*** -2.32 (-9.87, 5.24)* -6.67 (-13.1, -0.29)**	1.42 (-0.32, 3.16)* -0.62 (-2.80, 1.56) -0.41 (-0.66, -15)** -0.50 (-8.27, 7.27) -3.18 (-11.1, 4.69)
BMI (kg/m²)	TT:E2 ratio	-0.03 (-0.06, -0.01)**	-0.03 (-0.06, -0.01)**
VAT (mm)	SHBG Study site	0.01 (-0.003, 0.02)* 1.56 (0.68, 2.43)***	0.01 (-0.001, 0.02)* 1.60 (0.73, 2.46)***
SAT (mm, log)	TT:E2 ratio BAT Study site	0.01 (-0.01, 0.04)* 0.001 (-0.004, 0.004)* 0.56 (-0.08, 1.21)**	0.01 (-0.02, 0.04) 0.001 (-0.003, 0.004) 0.49 (-0.23, 1.21)*
Total cholesterol (mmol/L)	BMI SAT TT:E2 ratio Study site	0.10 (0.02, 0.19)** 0.39 (-0.02, 0.81)* 0.01 (-0.002, 0.02)* 0.43 (0.05, 0.80)**	0.15 (0.03, 0.28)** -0.11 (-0.48, 0.25) 0.01 (-0.004, 0.02)* 0.62 (0.11, 1.13)**
LDL-cholesterol (mmol/L)	BMI SAT TT:E2 ratio Study site	0.08 (-0.01, 0.17)* 0.14 (-0.10, 0.39)* 0.01 (0.003, 0.02)** 0.33 (0.03, 0.63)**	0.12 (0.02, 0.23)** -0.29 (-0.62, 0.03)* 0.01 (0.003, 0.02)** 0.51 (0.14, 0.89)**
HDL-cholesterol (mmol/L)	Age E2 TT	-0.04 (-0.13, 0.03)* 5×10^{-5} (-4×10^{-5}, 2×10^{-4})* 1×10^{-4} (-1×10^{-4}, 3×10^{-5})*	-0.03 (-0.12, 0.04) 4×10^{-5} (-4×10^{-5}, 2×10^{-4}) 7×10^{-5} (-2×10^{-4}, 3×10^{-4})
Triglycerides (mmol/L)	Age BMI VAT E2 FSH BAT	0.02 (-0.07, 0.13)* 0.03 (0.007, 0.07)** -0.04 (-0.09, 0.006)* -1×10^{-4} (-2×10^{-4} , 2×10^{-6})** 0.002 (-0.001, 0.01)* -0.001 (-0.002, -1×10^{-5})**	0.07 (-0.03, 0.17)* 0.03 (-0.001, 0.07)** -0.03 (-0.09, 0.02)* -1×10^{-4} (-2×10^{-5} ,4×10^{-5})** 0.002 (-0.002, 0.01) -0.001 (-0.001, 3×10^{-4})
Glucose (mmol/L)	FT VAT	-0.04 (-0.13, 0.04)* 0.04 (-0.09, 0.18)*	-0.04 (-0.14, 0.05)* 0.04 (-0.09, 0.18)

Data is unstandarised ß (95% Confidence Interval); *p<0.20, **p<0.05, ***p<0.001; Study site- Navrongo vs. Nanoro.

Table 4.12: Univariate and multivariable linear regression models showing associations between changes in variables in women who were premenopausal at baseline and postmenopausal at follow-up

Dependent variable	Independent variable	Univariate model	Multivariable model
Waist circumference (mm)	BMI	10.6 (3.46, 17.8)**	13.7 (6.84, 20.6)***
	SHBG	-0.37 (-0.79, 0.04)*	-0.14 (-0.42, 0.13)
	DHEAS	-21.2 (-40.4, -2.05)**	-2.29 (-15.3, 10.7)
	Study site	93.2 (66.2, 120.2)***	101.8 (74.3, 129.2)***
Hip circumference (mm)	BMI	7.55 (1.72, 13.3)***	12.4 (7.62, 17.3)***
	SHBG	-0.39 (-0.71, -0.07)**	-0.10 (-0.30, 0.09)
	DHEAS	-20.0 (-35.9, -4.09)**	-1.82 (-10.6, 6.96)
	TT:E2 ratio	1.82 (-0.17, 3.82)*	0.57 (-0.89, 2.04)
	Study site	82.7 (60.3, 105.1)***	84.5 (62.5, 106.7)***
DBP (mmHg)	Age	-2.08 (-3.99, -0.17)**	0.38 (-1.55, 2.33)
	BMI	0.94 (0.15, 1.73)**	0.59 (-.46, 1.65)
	VAT	-1.38 (-2.07, -0.69)***	-0.40 (-1.35, .53)
	SAT	-4.27 (-7.48, -1.06)**	0.48 (-4.16, 5.14)
	LDL-C	-1.91 (-4.74, 0.90)*	-1.18 (-3.60, 1.22)
	TGs	3.25 (-3.86, 10.3)*	1.57 (-5.11, 8.27)
	Study site	-10.2 (-13.4, -7.06)**	-9.58 (-14.8, -4.31)***
VAT (mm)	Age	-0.29 (-0.77, 0.18)*	-0.81 (-1.32, -0.30)**
	BMI	0.17 (-0.07, 0.42)*	0.16 (-0.12, 0.46)
	FSH	0.01 (-0.004, 0.02)*	0.01 (-0.003, 0.02)*
	DHEAS	-0.27 (-0.88, 0.33)*	0.19 (-0.32, 0.71)
	Study site	1.97 (1.00, 2.93)***	2.34 (1.23, 3.46)***
Total cholesterol (mmol/L)	FSH	0.01 (-4×10^{-4}, 0.01)*	0.004 (-3×10^{-4}, 0.001)*
	FAI	0.59 (-0.11, 1.30)*	0.51 (-0.21, 1.23)*
	SAT	-0.10 (-0.80, 0.60)*	-0.15 (-1.10, 0.79)
	Study site	0.33 (0.002, 0.66)**	0.39 (.02, 0.76)**
LDL-cholesterol (mmol/L)	SAT	-0.03 (-0.62, 0.55)*	-0.08 (-0.86, 0.68)
	TT:E2 ratio	0.01 (-0.004, 0.04)*	-0.002 (-0.03, 0.02)
	FSH	0.003 (-4×10^{-4},0.01)**	0.004 (-4×10^{-4}, 0.01)*
	FAI	0.62 (0.07, 1.16)**	0.45 (-0.25, 1.15)*
	SHBG	-0.004 (-0.01,-0.001)**	-0.003 (-0.01, 0.001)*
	Study site	0.34 (0.07, 0.61)**	0.34 (0.04, 0.63)**
HDL-cholesterol (mmol/L)	TT:E2 ratio	-0.01 (-0.01, 0.001)*	-0.01 (-0.01, 0.002)*
	SHBG	0.001 (-0.001, 0.002)*	0.001 (-0.001,0.002)
Triglycerides (mmol/L)	Glucose	0.08 (0.02, 0.14) **	0.07 (-2×10^{-4}, 0.13)**
	FSH	0.001 (-0.001, 0.003)*	0.001 (-0.001, 0.003)*
	TT:E2 ratio	0.01 (-0.002, 0.011)*	0.002 (-0.01, 0.01)
Glucose (mmol/L)	BMI	0.06 (0.003, 0.11)**	0.06 (3×10^{-4}, 0.13)*
	SAT	0.18 (-0.11, 0.47)*	0.12 (-0.14, 0.37)
Average cIMT (mm)	HipC	-2×10^{-4} (-5×10^{-4}, 5×10^{-5})*	-3×10^{-4} (-5×10^{-4}, 1×10^{-4})
	Study site	-0.02 (-0.05, 0.01)*	-0.01 (-0.05, 0.03)

Data is unstandarised ß (95% Confidence Interval); *p<0.20, **p<0.05, ***p<0.001; Study site-Navrongo vs. Nanoro.

Table 4.13: Univariate and multivariable linear regression models showing associations between changes in variables in women who were postmenopausal at baseline

Dependent variable	Independent variable	Univariate model	Multivariable model
Waist circumference (mm)	Age	19.4 (6.9, 31.8)*	-1.1 (-10.2, 8.0)
	BMI	7.4 (2.3, 12.4)**	12.2 (7.2, 17.2)***
	FAI	51.3 (14.4, 88.4)**	18.9 (-15.5, 53.4)
	E2	-0.08 (-0.16, -0.004)	-0.02 (-.07, 0.03)
	SHBG	-0.55 (-0.90, -0.19)**	-0.16 (-0.42, 0.10)
	Study site	77.1 (59.9, 94.1)***	86.1 (66.1, 106.1)***
DBP (mmHg)	Age	-2.5 (-4.4, -0.5)*	1.2 (-0.8, 3.1)
	BMI	2.0 (0.65, 3.24)**	1.52 (0.22, 2.82)**
	E2	-3×10^{-4} (-0.01, 0.01)*	-0.01 (-0.02, 0.01)
	Glucose	2.08 (-0.12, 4.29)*	0.64 (-1.49, 2.79)
	Study site	-8.7 (-12.2, -5.3)***	-8.33 (-12.2, -4.44)***
VAT (mm)	Age	0.29 (-0.05, 0.64)*	0.14 (-0.27, 0.55)
	BMI	0.07 (-0.08, 0.23)*	0.13 (-0.07, 0.34)*
	BAT	0.01 (-0.001, 0.01)*	0.004 (-0.004, 0.01)
	Study site	1.08 (0.41, 1.70)**	1.00 (0.07, 1.93)**
Total cholesterol (mmol/L)	BMI	0.05 (-0.03, 0.14)*	0.05 (-0.09, 0.20)
	SAT	0.62 (0.20, 1.04)***	0.40 (-0.26, 1.07)
	Study site	0.31 (-0.04, 0.67)*	0.26 (-0.26, 0.78)
LDL-cholesterol (mmol/L)	BMI	0.05 (-0.01, 0.12)*	0.04 (-0.08, 0.16)
	SAT	0.45 (0.12, 0.77)**	0.26 (-0.28, 0.81)
	TT:E2 ratio	0.01 (0.004, 0.03)**	0.01 (-0.002, 0.03)*
	Study site	0.20 (-0.05, 0.46)*	0.20 (-0.23, 0.64)
HDL-cholesterol (mmol/L)	BMI	0.02 (-0.004, 0.04)*	0.01 (-0.01, 0.04)
	SHBG	-0.002 (-0.01, -4×10^{-4})**	-0.002 (-0.01, -0.01)**
	SAT	0.13 (-0.01, 0.27)*	0.05 (-0.09, 0.21)
Triglycerides (mmol/L)	Age	-0.02 (-0.09, 0.04)*	0.01 (-0.01,0.09)
	BMI	0.02 (-0.01, 0.05)*	0.02 (-0.01, 0.05)
	Site	-0.10 (-0.23, 0.01)*	-0.09 (-0.24, 0.05)*
Glucose (mmol/L)	Age	-0.06 (-0.23, 0.09)*	-0.01 (-0.20, 0.18)
	BMI	0.08 (0.02, 0.14)**	0.08 (0.01, 0.15)**
	SHBG	-0.002 (-0.01, 0.002)*	-0.002 (-0.01, 0.003)
	Site	-0.14 (-0.41, 0.11)*	-0.06 (-0.37, 0.24)
Average cIMT (mm)	WC	-3×10^{-4} (-6×10^{-4}, -8×10^{-5})**	-4×10^{-4} (-8×10^{-4}, 9×10^{-6})
	TT	2×10^{-5} (-8×10^{-5}, 1×10^{-4})*	2×10^{-5} (-1×10^{-4}, 1×10^{-4})
	SHBG	0.001 (-9×10^{-5}, 0.002)*	0.001 (-4×10^{-4}, 0.002)
	Site	-0.02 (-0.07, 0.03)*	0.01 (-0.06, 0.08)

Data is unstandarised ß (95% Confidence Interval) Univariate analyses; *p<0.20, **p<0.05, ***p<0.001; Study site-Navrongo vs. Nanoro.

4.5 Discussion

The current study investigated the association between the MT and CMD risk by exploring longitudinal changes in the MT and in postmenopausal women from rural West Africa. This study shows that the MT is characterised by changes in sex hormones, anthropometric measures and CMD risk factors. Furthermore, the data suggest that different environmental and/or lifestyle factors may also contribute to the development of CMD risk factors in the studied populations.

This is the first longitudinal study to be conducted in SSA women showing that the sex hormones E2, SHBG, testosterone, and DHEAS decreased significantly across the MT while FSH increased. The present findings on these changes are consistent with those reported in longitudinal cohorts from high income countries including the Michigan Bone Health and Metabolism Study, the Melbourne Women's Midlife Health Project, the SWAN, the POAS, and the Malmo Perimenopausal Project (59,61–65). These studies have shown that E2 (59,61,62,64), SHBG (61,66) and TT (61,65) levels decreased, while FSH levels increased during the MT (59–63). In one study, DHEAS levels were shown to decrease in the MT, but these changes were only associated with chronological ageing (66). However, in contrast to the present findings, Burger *et al.,* reported no differences in the levels of TT during the MT despite an increased FAI (66).

The present findings in the group of women who were postmenopausal at baseline show there were no changes in E2, TT:E2 ratio and SHBG levels but decreasing levels of TT and DHEAS in the 5-year follow-up period; FSH levels also decreased but by such small levels that although statistically significant, appear to be of no clinical significance. Previously, longitudinal studies from high income countries have characterised the early postmenopausal stage using serial measurements of sex

hormones (59,61–64,66). These studies showed that FSH levels significantly increase between 1 and 2 years after the FMP, then plateau from year 2–8 years after the FMP (59,62,63). Levels of E2 have been reported to further decline up to 2 years post-FMP, then reach steady levels between year 2–6 years after the FMP (59,61,62,64). In one study, TT levels were shown to decrease until 3 years after the FMP (61) as shown in the present results, while in another study, no changes in testosterone after the FMP were observed despite an increasing FAI until 2 years post FMP (66). As shown in some studies, the current data found that SHBG levels decreased until 2–3 years after the FMP and thereafter stabilised to 7 years after the FMP (61,65,66). The current study shows that DHEAS levels further decreased after the FMP, and this has been associated with chronological aging (61,65). These studies demonstrate that despite the lack of serial sex hormone measurements, the current data in the postmenopausal period tend to agree with those reported in the literature from high income countries.

The current data confirm those from studies showing that the MT is associated with decreased levels of sex steroids and increased gonadotrophins (59–65). The biological mechanism has been associated with the diminishing activity of the granulosa and theca cells of the ovaries which typically synthesise inhibins and androgens, respectively (34). Reduced synthesis of inhibins in turn stimulates the release of FSH and restrains further synthesis of ovarian androgens, androstenedione and DHEA (33,38). Thus, the downstream precursors, including E2, decrease.

In the current study, the increase in the levels of total cholesterol, LDL-C and triglycerides during the MT has been confirmed by others (2,3,7,10,139,142). In contrast to other reports on decreasing HDL-C during the MT (2,4), the current study shows that HDL-C levels increased across the MT and during the postmenopausal years. Similar findings have been reported arguing that the cardioprotective role of

HDL-C may be diminished after the MT (10,93,236,237). It has been hypothesised that since the conventional methods of HDL-C measurement only report the amount of cholesterol on the HDL particles, this might not fully capture the possible composition and conformational changes to the HDL subclass profile which may be related to CMD risk (236).

This study shows that blood glucose levels increased from their respective baseline levels across the MT and further into the postmenopausal years. This is in agreement with some studies (7,164) but not all (1,9,135).

In this study, the anthropometric variables, waist, and hip circumference, VAT, SAT increased across the MT, however changes in BMI were only observed in the group that transitioned from pre- to perimenopause. The present findings agree with others on the increase of waist circumference (9,89,90) and no changes in BMI (9) during the MT. However, some studies have reported increasing BMI and body weight during the MT (89,143).

The SBP and DBP levels did not change from pre- to postmenopausal stage. However, SBP levels increased in the transition from pre- to perimenopause while DBP decreased in the postmenopause. In the literature, some reports show no association between menopause and BP (1,7,135), and others show an indirect association (9).

The current findings on the changes in CMD risk factors across the MT largely agree with other longitudinal studies. However, it must be noted that there are some discrepancies within the literature that may be related to measurements occurring at differing time points, variation in sample sizes, misclassification of menopausal stages and differences between ethnic groups.

In the multivariable regression analyses, the results have demonstrated that the 'relative androgen excess' as hypothesised by one report from the SWAN study influenced the changes in anthropometric and CMD risk factors in the pre- to perimenopausal transition (13,238) but not in the direction one would have expected. Thus, the present results show that falling BAT and FT were associated with increased waist and hip circumference and blood glucose levels. Furthermore, the rising TT:E2 ratio was associated with falling SBP and BMI. The 'relative androgen excess' hypothesis highlights the fact that despite the levels of total testosterone levels remaining constant or decreasing during the MT, the more rapid decline of E2 creates a more androgenic sex hormone profile that may contribute to unfavourable cardiometabolic changes (238). However, this is not supported by the current study and requires further investigation.

The current study showed that in the pre- to postmenopausal transition, no effect of sex steroid changes on the changes in CMD risk factors were observed. Interestingly rising FSH levels demonstrated near significant positive associations with rising total cholesterol and LDL-C. Previous studies have also shown effects of FSH on cholesterol metabolism (11,239).

In the women who moved from early to late postmenopause there were also few associations of hormone levels with CMD risk factors with increasing SHBG being associated with decreasing HDL-C levels.

The small effect of sex hormonal changes on the CMD risk factors is further emphasised by the data shown in Table 4.10. These analyses show that the much larger changes observed in hormone levels in the pre- to perimenopausal and pre- to postmenopausal groups compared to those seen in women who remained pre- or

postmenopausal do not translate into significant differences in the changes in CMD risk factor variables observed across these study groups.

Noticeable effects of the two study sites were observed in all three of the study groups with increases in anthropometric measures being greater in Navrongo than Nanoro whilst increases in DBP was greater in Nanoro. These associations were very significant and suggest that changes in menopause stage may be strongly affected by environmental factors.

The major strengths of the present study were its longitudinal design, the use of standardised protocols for data collection and centralised laboratory measurements, and collection several anthropometric and cardiometabolic variables, and the comprehensive list of sex hormones that include the physiologically active hormones such as the FT and BAT. The main limitations were the small sample size particularly the subgroups of women who remained premenopausal. Furthermore, there was a high loss to follow-up, mainly due to refusal to participate, outmigration from study area, lost contact, death, and others were too ill to participate. In addition, the immunoassay methods used for the measurement of the sex steroids have limitations on sensitivity and chemical interference and therefore might lack the accuracy that the LC/MS offers on the measurement of E2 and testosterone levels which are known to be low in menopausal women. Lastly, the study participants were from a rural population and may not be representative of the national population. Further studies are warranted to assess the reproducibility of the reported data.

The present study has demonstrated that during the MT, the effects of sex hormone changes on CMD risk factors are small. The androgens were more strongly associated with changes in CMD risk factors than E2. Study site differences were noted in the

magnitude of changes in blood pressure and anthropometry suggesting that environmental factors are important influences on CMD risk factors. Many of these results agree with those from other high-income countries and therefore more research is needed to confirm these findings in more SSA studies given the high prevalence of CMD in the region.

The previous studies have concentrated on the assessment of CMD risk factors across the MT in the context of SSA. This region has the world's highest prevalence of HIV infection, which is prominent even in midlife, but the effects of HIV on CMD risk factors during the MT have not been studied. Therefore, the purpose of the next study was to analyse these associations in midlife women from Kenya and South Africa.

Chapter 5: The Association of HIV and Menopause with Cardiometabolic Disease Risk in Midlife Women from sub-Saharan Africa

Chikwati RP, Goolam Mahyoodeen N, Jaff N, Ramsay M, George JA, and Crowther NJ. The Association of HIV and Menopause with Cardiometabolic Disease Risk Factors in Midlife Women from Kenya and South Africa (will be submitted to *Maturitas*).

5.1 Abstract

Background: Menopause and HIV are both associated with CMD. In SSA, the health transition, access to effective HAART and improved life expectancy is mirrored by a growing population of midlife WLWH. It is therefore important to examine differences in CMD risk factor levels between pre- and postmenopausal SSA women in relation to HIV and HAART.

Objectives: To compare CMD risk factors between pre- and postmenopausal SSA WLWH and WLWOH and to determine whether menopausal differences for these risk factors were modified by HIV and therapy.

Study design: The analysis included 1665 WLWOH (733 pre- and 932 postmenopausal) and 414 WLWH (211 pre- and 203 postmenopausal) between the ages of 40–60 years from the Africa Wits-INDEPTH partnership for Genomics studies (AWI-Gen) at sites in Kenya (Nairobi), and South Africa (Soweto and Dikgale).

Main outcome measures: Demographic, anthropometric and cardiometabolic variables were compared between the WLWH and WLWOH and between pre- and postmenopausal women according to HIV status using multivariable linear regression analyses.

Results: The mean age and HIV prevalence in the study population were 49.2±5.9 years and 19.9% respectively. Age at menopause was lower in WLWH compared to WLWOH (48.1±5.1 *vs* 50.1±4.7years, p<0.001) after adjusting for confounding variables.

Compared to the WLWOH, WLWH on HAART had lower BMI, hip circumference, systolic and diastolic blood pressure, and cIMT, but elevated triglycerides and insulin resistance after adjusting for confounding variables. In addition, WLWH who were HAART naïve had lower total cholesterol, HDL-cholesterol, and abdominal visceral fat, but higher triglycerides compared to WLWOH.

When comparing CMD risk factors between pre- and postmenopausal women in those with or without HIV infection, there were no differences between menopausal groups regardless of HIV status.

Conclusion: HIV is an independent risk factor for earlier onset of menopause. Therefore improved clinical care should focus on reducing cardiometabolic disease risk in midlife women living with HIV.

5.2 Introduction

The eastern and southern regions of SSA remain disproportionately affected by HIV, accounting for approximately 55% of the global prevalence (15). The prevalence of HIV in midlife SSA women has been reported to be high, approaching 20% in South Africa (218,240). Moreover, the high prevalence of obesity in midlife SSA women has been associated with metabolic disorders such as diabetes, hypertension, and the metabolic syndrome (241). Combined with a growing population of menopausal women within SSA, it is apparent that the aetiology and burden of CMD in the region is complex. However, the combined effect of HIV, menopause and obesity has not

been studied amongst midlife SSA women. Furthermore, there have been no data on CMD risk change in menopausal SSA women and little data on HIV and CMD risk have been reported. The few SSA studies focusing on the effects of HIV and its progression with or without HAART have reported an increased risk for CMD. In a recent meta-analysis on SSA studies, HIV was associated with higher triglycerides but lower HDL-C, systolic and diastolic blood pressure levels, while HAART exposure was associated with higher LDL-C and HDL-C levels (242). Lastly, HIV has been associated with earlier age at menopause in non-SSA studies which is linked to higher CMD risk (243). Thus, it is important to examine the association of HIV and CMD risk at different menopausal stages. This study therefore aimed to investigate whether differences in CMD risk factors between pre- and postmenopausal midlife women from SSA were modified by HIV and HAART. The study also aimed to compare age at menopause between midlife WLWH and WLWOH.

5.3 Methods

5.3.1 Study Population

This population based cross-sectional study is part of the AWI-Gen study, in which midlife women from three study sites in the two countries of Kenya (Nairobi) and South Africa (Dikgale and Soweto) were selected. The three sites were selected from six possible AWI-Gen sites based on their high prevalence of HIV.

The recruitment of AWI-Gen study participants has been previously detailed (Chapter 3). In summary, over 12 000 unrelated participants aged 40–60 years including both men and women were randomly selected from the general population from six study sites based in the four countries of Kenya, South Africa (3 sites), Ghana, and Burkina Faso. For the present study, all men (n=5405), all women from Ghana (n=1086) and Burkina Faso (n=1032), where HIV infection was nearly completely absent,

perimenopausal women (n=421), and women with missing menopausal staging data (n=1170) were excluded.

5.3.2 Study Variables

Questionnaires were used to gather information on menopause stage, demographics (socioeconomic status and level of education) and behaviour (dietary intake, alcohol consumption, and smoking) (Appendix 4).

A voluntary HIV rapid antibody test (Alere Determine HIV-1/2; Alere San Diego Inc, San Diego, CA) was offered to all study participants. WLWH were asked if they were receiving HAART or not. Menopausal stage was defined by asking each woman about her menstrual bleeding patterns. Premenopausal women had regular menstrual cycles, while the postmenopausal women had experienced amenorrhea for at least twelve consecutive months, or more. Age at final menstrual period (FMP) was calculated as the difference between the self-reported age at recruitment and years since last menses.

The methodologies on the collection of anthropometric and CMD risk factor measurements by trained staff have been previously described (Chapter 3). The study variables collected include height, weight, hip, and waist circumference, SAT VAT, SBP and DBP, cIMT, and total cholesterol, HDL-C, triglyceride, glucose, and insulin levels. LDL-C levels were calculated using the Friedewald equation (222). Insulin resistance was estimated using the HOMA-IR (223). All laboratory tests were performed in the same laboratory at Baragwanath Academic Hospital and have been previously detailed (Chapter 3).

5.3.3 Statistical Analyses

Parametric and non-parametric continuous data were presented as means ± SD or medians and IQR, respectively. Non-parametric continuous dependent variables were

log transformed to normality before being analysed using multivariable linear regression analyses. Categorical data were presented as frequency or percentage. Comparisons of categorical data between groups were performed using either the Chi-squared or Fisher exact test, as appropriate. Continuous data were compared between groups using the appropriate parametric (Student's unpaired t-test or one-way ANOVA) or non-parametric (Mann–Whitney U test or Kruskal–Wallis ANOVA) statistical tests.

Postmenopausal participants were grouped into quartiles of the self-reported years since the FMP, and an ANOVA was used to compare the calculated age at FMP in each quartile. Pearson univariate correlation analysis was used to determine the relationship between the age at FMP and the years since FMP. Multivariable linear regression with age at FMP as the dependent variable was performed to investigate if differences in the age at FMP between WLWH and WLWOH were maintained after adjusting for possible confounders i.e., BMI, physical activity, socioeconomic status, level of education, smoking status, and HAART status.

Multivariable linear regression was also used to compare the relationship of anthropometric and CMD risk factors, namely SBP, DBP, BMI, waist and hip circumference, VAT and SAT, triglycerides, LDL-C, HDL-C, glucose, HOMA-IR and average cIMT (dependent variables) with HIV status (independent variable; WLWOH, WLWH-HAART naive, and WLWH receiving HAART). In the adjusted model, age, and other independent variables (Appendix 5, Table 12) were incorporated into a multivariable model. In addition, participants who were diabetic and reported the use of therapies for the treatment of diabetes mellitus (n=137) or hypertension (n=217) were excluded from the analyses.

Lastly, participants were divided into two groups, WLWOH and WLWH. In each of these groups, multivariable linear regression analyses were performed to compare anthropometric and CMD risk factors (dependent variables) between post- and premenopausal groups (used as an independent variable) while adjusting for confounders. For each regression model, collinearity of variables was assessed using VIF and in all models the VIFs were found to be <3. All analyses were performed using Stata 16.1 (StataCorp LP, College Station, TX, USA). Statistical significance was set at a two-tailed $p<0.05$.

5.4 Results

5.4.1 Participant characteristics

A total of 1665 WLWOH (733 pre- and 932 postmenopausal) and 414 WLWH (211 pre- and 203 postmenopausal) were recruited from two study sites in South Africa (Dikgale and Soweto) and one in Kenya (Nairobi). The HIV prevalence at these sites was 22.2%, 20.6% and 17.5% respectively. In total, 79% of the WLWH were on HAART and 21% were not on HAART. Table 5.1 shows that WLWH were younger, consumed more alcohol, and were at a lower socio-economic status than WLWOH.

5.4.2 HIV, anthropometry and CMD risk

Compared to WLWOH, WLWH had a lower BMI, hip and waist circumference, SAT, VAT, SBP and DBP, total cholesterol, LDL-C, glucose, and cIMT than WLWOH (Table 5.1). In addition, WLWH had lower BMI, hip circumference, systolic and diastolic blood pressure, cIMT, but elevated triglycerides and insulin resistance after adjusting for confounding variables (Table 5.2). In a sub-analysis where WLWH were divided into those on and not on HAART, similar associations on anthropometry, blood pressure, triglycerides, insulin resistance and cIMT were shown to be largely driven by HAART after the adjustment for possible confounding variables (Table 5.3). Furthermore,

WLWH who were HAART naïve had lower total cholesterol and HDL-C, but higher triglycerides compared to WLWOH.

Table 5.1: Baseline characteristics of study participants according to HIV status.

Variables	WLWOH (n=1665)	WLWH (n=414)	P value
Age (years)	49.6±5.8	47.4±5.6	<0.001
Alcohol consumption			
Never	816 (73.0)	159 (58.2)	<0.001
Current	99 (8.9)	31 (11.4)	
Former	203 (18.2)	83 (30.4)	
Smoking			
Never	1515 (91.2)	369 (89.1)	0.13
Current	69 (4.2)	16 (3.9)	
Former	203 (18.6)	29 (7.0)	
Socioeconomic status			
Poorest	238 (15.1)	75 (18.8)	0.002
Poorer	462 (29.3)	135 (33.8)	
Poor	296 (18.7)	70 (17.5)	
Less Poor	292 (18.5)	77 (19.2)	
Least Poor	291 (18.4)	42 (10.5)	
Level of education			
None	121 (7.8)	25 (6.4)	0.52
Primary	889 (57.6)	241 (61.5)	
Secondary	512 (33.2)	122 (3.1)	
Tertiary	22 (1.4)	4 (1.0)	
MPA (min/week)			
Insufficient (<150)	305 (18.3)	74 (17.9)	0.83
Sufficient (>150)	1358 (81.7)	340 (82.1)	

Data expressed as mean±standard deviation and frequency (percentage).

Table 5.2: Comparison of anthropometric and CMD risk factors according to HIV status after adjustment for possible confounding variables

Variables	WLWOH (n= 1484)	WLWH (n=268)
BMI (kg/m²)	30.8±7.3	26.7±6.9***
Hip circumference (cm)	110.9±15.3	102.1±15.2*
Waist circumference (cm)	94.9±15.1	87.1±14.1
SAT (cm)	2.50±1.10	2.12±1.09
VAT (cm)	5.30±2.02	4.67±1.88
Systolic BP (mmHg)	121.5 (109.5–136.5)	116.3 (102.5–130)***
Diastolic BP (mmHg)	83.1±13.5	77.7±13.2***
Total Cholesterol (mmol/L)	4.38±1.09	4.19±1.17
LDL-C (mmol/L)	2.69±0.87	2.46±0.88
Triglycerides (mmol/L)	0.95 (0.70–1.29)	1.00 (0.70–1.28) ***
HDL-C (mmol/L)	1.18 (1.00–1.40)	1.14 (0.94–1.44)
Glucose (mmol/L)	4.88 (4.50–5.40)	4.85 (4.50–5.31)
HOMA-IR	1.29 (0.62–2.44)	1.36 (0.66–2.37)**
cIMT average (mm)	0.60 (0.53–0.66)	0.56 (0.50–0.62)**

Data expressed as mean ± SD or median interquartile range (IQR).WLWOH, women living without HIV, WHLH, women living with HIV, *p<0.05 vs WLWOH, **p<0.01 vs WLWOH, ***p<0.001 vs WLWOH.

Table 5.3: Comparison of age, anthropometric and CMD risk factors according to HIV and HAART status

Variables	WLWOH (n=1484)	WLWH HAART+ (n=212)	WLWH HAART- (n=56)
Age (years)	49.1± 5.7	47.3±5.4 ***	47.5±6.0
BMI (kg/m²)	30.8±7.3	25.8±6.2 ***	30.2±8.3 *
Hip circumference (cm)	110.9±15.3	99.8±13.4*	110.8±18.5
Waist circumference (cm)	94.6±15.1	86.0±13.6 *	91.1±15.3
SAT (cm)	2.50±1.09	2.00±1.01	2.60±1.25
VAT (cm)	5.30±2.02	4.66±1.86	4.70±1.97
Systolic BP (mmHg)	122 (27)	114 (25) ***	131 (28)
Diastolic BP (mmHg)	83±14	75±12 ***	87±13
Total Cholesterol (mmol/L)	4.38±1.09	4.23±1.20	4.03±1.00 *
LDL-C (mmol/L)	2.69±0.87	2.46±0.89	2.44±0.85
Triglycerides (mmol/L)	0.95 (0.52)	0.96 (0.62) **	1.10 (0.34) *
HDL-C (mmol/L)	1.18 (0.38)	1.17 (0.49)	1.10 (0.31) **
Glucose (mmol/L)	4.88 (0.90)	4.85 (0.85)	4.90 (0.60)
HOMA-IR	1.29 (1.83)	1.39 (1.84) **	1.19 (1.68)
cIMT average (mm)	0.60 (0.13)	0.56 (0.12) *	0.58 (0.12)

Data expressed as mean ± SD or median interquartile range (IQR). WLWOH, women living without HIV, WHLH, women living with HIV, HAART+, on therapy, HAART-, not on therapy. *p<0.05 vs WLWOH, **p<0.01 vs WLWOH, ***p<0.001 vs WLWOH.

5.4.3 HIV and Menopausal Stage and CMD risk

When comparing CMD risk factors between pre- and postmenopausal women in WLWH or WLWOH, the risk factor levels did not differ significantly between the menopausal groups after adjusting for multiple confounders and this was irrespective of HIV status (Table 5.4).

5.4.4 Age at FMP across quartiles of years after FMP

When age at FMP was compared between WLWH and WLWOH, it was observed that WLWH reached menopause at an earlier age than their uninfected counterparts (46.8±5.2 *vs* 48.4±5.6 years, $p<0.001$). Due to these self-reported low ages for FMP, further analyses were undertaken. Thus, Pearson correlation analysis showed an inverse relationship between the calculated age at FMP and the reported years since menopause ($r =-0.59$, $p<0.001$, $n=962$), suggestive of an underestimation of age at FMP with increasing years after menopause. This was confirmed when women were divided into four groups based on years since calculated FMP i.e., ≤3 years, >3 to ≤6 years, >6 to ≤9 years and >9 years, and age at FMP was compared between WLWH and WLWOH in each of those 4 groups. The age at FMP was always found to be lower in the WLWH group but was only significant in the group within ≤3 years of FMP and in the combined group (Table 5.5). In addition, the calculated age at FMP fell in both groups the further the women reported that they were from FMP in years (Table 5.5). The significant difference in age at FMP between WLWH and WLWOH was maintained for all study participants and for those who reported that their FMP was within 3 years of their interview in a multivariable linear regression model for FMP which was adjusted for BMI, physical activity, socioeconomic status, education, smoking, and study site (Table 5.6). Irrespective of HIV status, sub-analysis showed differences in the age at FMP across the different sites, Nairobi, 47.2±4.7; Dikgale

50.1±4.7 and Soweto; 51.6±4.4. The differences were significant between Nairobi and Dikgale, $p<0.001$; Nairobi and Soweto, $p<0.001$; but not different between the South African sites Dikgale and Soweto, $p=0.05$.

Table 5.4: Univariate and multivariate linear regression models showing relationship of menopausal status with CMD risk factors for all sites combined.

Dependant Variable	HIV Status	Menopausal status (unadjusted)*	Menopausal status (adjusted)*
Waist Circumference (cm)	WLWH	0.71 (-2.76–4.20)	0.22 (-2.33–2.78)
	WLWOH	2.47 (0.88–4.05)	0.84 (-0.22–1.90)
Hip Circumference (cm)	WLWH	-0.47 (-4.23–3.28)	-0.86 (-2.69–.96)
	WLWOH	0.76 (-0.84–2.37)	-0.39 (-1.26–0.48)
VAT (cm)	WLWH	-0.07 (-0.53–.38)	0.03 (-0.52–0.44)
	WLWOH	0.10 (-0.11–0.31)	-0.06 (-0.23–0.19)
SAT (cm)	WLWH	0.04 (-0.22–0.31)	0.05 (-0.15–0.26)
	WLWOH	0.08 (-0.03–0.19)	-0.07 (-0.18–0.04)
BMI (kg/m^2)	WLWH	-0.18 (-1.89–1.52)	0.07 (-2.06–2.21)
	WLWOH	0.36 (-0.40–1.12)	-0.15 (-1.13–0.81)
Diastolic Blood Pressure (mmHg)	WLWH	3.23 (1.63–6.45)	-1.16 (-4.84–2.52)
	WLWOH	4.25 (2.84–5.67)	0.37 (-1.67–2.42)
Systolic Blood Pressure (log;mmHg)	WLWH	0.03 (-0.002–.08)	-0.03 (-0.08–0.01)
	WLWOH	0.06 (0.05–0.08)	-0.01 (-0.03–0.01)
LDL-C (mmol/L)	WLWH	0.39 (0.18–0.61)	0.22 (-0.04–0.48)
	WLWOH	0.27 (0.18–0.36)	0.10 (-0.02–0.23)
Triglycerides (log;mmol/L)	WLWH	0.17 (0.05–0.28)	0.04 (-0.11–0.18)
	WLWOH	0.15 (0.10–0.20)	0.06 (0.01–0.13)
HDL-C (log;mmol/L)	WLWH	0.06 (-0.02–0.15)	0.01 (-0.10–0.11)
	WLWOH	0.03 (0.001–0.06)	-0.01 (-0.05–0.03)
Glucose (mmol/L)	WLWH	0.22 (0.07–0.37)	0.18 (-0.01–0.39)
	WLWOH	0.07 (0.003–0.14)	-0.04 (-0.13–0.04)
HOMA-IR (log)	WLWH	0.15 (-0.09–0.40)	0.23 (-0.08–0.55)
	WLWOH	0.07 (-0.03–0.19)	0.03 (-0.12–0.19)
Average cIMT (log; mm)	WLWH	0.09 (0.04–0.14)	0.01 (-0.03–0.06)
	WLWOH	0.07 (0.05–0.09)	-0.01 (-0.03–0.02)

Data is unstandarised ß (95 Confidence Interval); *post- vs pre-menopause. WLWH, women living with HIV, WLWOH, women living without HIV.

Table 5.5: Age at FMP by quartiles of years after FMP

	WLWH		WLWOH		P Value
Years after FMP	Age at FMP,y	n	Age at FMP,y	n	
≤3 y	48.1±5.1	98	50.9±4.7	428	<0.001
>3 to ≤6y	47.8±4.7	33	48.5±4.5	137	0.38
>6 to ≤9y	45.1±4.2	17	45.5±4.8	78	0.71
>9y	41.4±3.6	24	42.5±4.3	147	0.27
Combined	46.8±5.2	172	48.4±5.6	790	0.001

Data expressed as mean ± SD, and sample size n. WLWH, women living with HIV, WLWOH, women living without HIV.

Table 5.6: Relationship of HIV status and age at final menstrual period

Dependent variable	Independent variable	
Age at FMP	HIV status† (unadjusted)	HIV status† (adjusted)
If Years after FMP ≤3 y	-2.7 (-3.8– -1.6)***	-2.2 (-3.3– -1.0)***
In all postmenopausal women	-1.1 (-2.0– -0.2)***	-1.6 (-2.4– -0.8)***

Data is unstandarised ß (95% confidence interval); †HIV status (WLWH vs. WLWOH), *p<0.05, **p<0.01, ***p<0.001. Adjusted for BMI, physical activity, socioeconomic status, level of education, smoking status, study sites, Nairobi, Soweto and Dikgale.

5.5 Discussion

The current study compared CMD risk factors between midlife SSA WLWH and WLWOH and further investigated whether the differences in these risk factors between pre- and postmenopausal women were modified by HIV. The results showed that HIV and HAART were associated with increased insulin resistance and triglyceride levels but lower BMI, cIMT and blood pressure. Furthermore, the age at menopause was lower in WLWH than WLWOH. However, the levels of CMD risk factors were similar in pre- and postmenopausal women regardless of HIV status.

The association between HAART and lower BMI has been reported in a previous meta-analysis on SSA studies. (242) . At the time of this study, participants were receiving the efavirenz-tenofovir-lamivudine combination therapy as per the respective national guidelines (244,245). The pathophysiology for lower BMI in HIV and HAART

is complex and partially understood. However, the most common hypothesis applicable to this study is that the combination of lamivudine and efavirenz induces mitochondrial toxicity in adipocytes leading to oxidative stress and subsequent apoptosis and loss of subcutaneous fat mass (246,247).

The link between HAART and insulin resistance and higher triglyceride levels has been confirmed in previous studies (248,249). The mechanisms behind these associations have been thought to be driven by the effect of HAART on body fat redistribution and impairment of adipocyte function (246). It is thought that anti-retroviral agents block lipoprotein lipase activity in adipocytes reducing triglyceride uptake leading to higher serum levels of triglycerides (250). Moreover, HAART can induce insulin resistance by direct effects on insulin signalling or indirectly by causing lipodystrophy (246,249).

The current study also shows that HIV but not HAART was associated with lower total and HDL-C levels. Other studies that have shown similar associations include Granfeld *et al.,* (251), Nguemaïm *et al.,* (252), and Riddler *et al.,* (253). It has been suggested that the decline of HDL-C begins in the early stages of HIV infection and precedes that of increasing triglycerides levels. Furthermore, Riddler *et al.,* demonstrated that the initiation of HAART significantly improved total cholesterol levels but the changes on HDL-C were minimal (253). The negative effector (nef) peptide, an HIV accessory protein has been shown to inhibit the expression of the ATP-binding cassette transporter A1 (ABCA1) which is responsible for the efflux of cholesterol (254). The inhibition of the ABCA1 thereby retains cholesterol within macrophages, leading to lower levels of total and HDL-C in the circulation.

The current study shows an association between lower systolic and diastolic blood pressure and HAART even after excluding participants on anti-hypertensive and anti-diabetic medication. Previous investigations on the relationship between blood pressure and HIV, or HAART have been inconclusive, with some reporting direct associations (255–258), some reporting inverse associations (242,259,260), and others reporting no associations (261,262). For the present study, the mechanism behind HAART, lower BP and WLWH is unclear. Some studies have suggested that the autonomic dysfunction of the nervous system in chronic HIV infection affects the modulation of heart rate (263,264). In addition, WLWH on HAART may have greater contact with healthcare services compared to either individuals not on HAART or their uninfected counterparts and thus receiving earlier therapy for any newly diagnosed comorbid diseases (260).

Consistent with the present findings, lower cIMT levels in HAART have also been reported by Fourie *et al.,* (265). In that study, the endothelial activation biomarkers; intercellular adhesion molecule 1 (ICAM) and vascular cell adhesion molecule 1 (VCAM) were higher in HAART naive than in HAART receiving people living with HIV (PLWH) (265). This suggests that HAART may be responsible for a reduction in the HIV-induced endothelial activation and consequently lower the risk for atherosclerosis. However, other studies from the SSA countries Uganda (266), South Africa (267), Botswana (268), and Ethiopia (269) showed no associations between cIMT and HIV. In addition, a meta-analysis study showed that there were no differences in cIMT levels between PLWH or uninfected counterparts from pooled cohort studies, while differences were observed in the pooled cross-sectional studies (270). Together, there may be several reasons for these inconsistencies. Firstly, most of these studies had relatively smaller sample sizes: ranging from 306 to 548 study participants (266–269).

Secondly, the selection of study participants was not uniform, for example in this study included both pre- and postmenopausal women, while other studies recruited mostly premenopausal women (266), who otherwise carry a lower CMD risk. In another study, the selection of the WLWOH control group was based on a relatively unhealthy referral group from another clinic so the CMD risk differences with PLWH were likely to be minimal (269). Thirdly, the use of different classes of HAART and duration of use could be an additional source of heterogeneity across studies, as some specific HAART regimens and their prolonged use have been linked with greater CMD risk than others. Finally, the cross-sectional nature of these studies (including the current study) makes it difficult to control for all residual confounding.

The present study shows that the level of CMD risk factors was similar in both pre- and postmenopausal women regardless of HIV status. No similar studies have compared CMD risk factors between pre- and postmenopausal women and then compared these differences between WLWH and WLWOH. However, there are studies that have focused on just WLWH. Thus, in the REPRIEVE study, only waist circumference and haemoglobin levels were higher in post- than in premenopausal WLWH (271). In a Women's Interagency HIV Study (WIHS) sub-analysis, blood pressure, BMI, and diabetes were higher in post- than premenopausal women (272). However, age was not adjusted for in this study. Overall, data on CMD risk during the MT in WLWH remain scarce and therefore warrants further investigation.

In the current study, midlife WLWH from South Africa and Kenya experienced menopause earlier (48.1±5.1 years) than their uninfected counterparts (50.9±4.7 years). Similar findings have been reported in studies outside SSA including Thailand, where WLWH had an earlier mean age at menopause (47.3±5.1 years) than their uninfected counterparts (49.5±3.6 years) (134), and in the United States, where the

median age at menopause in WLWH was 46 (IQR: 39-49) years compared to 47 (IQR:44.5-48) years in the uninfected group (273). However, one study from Brazil showed no differences in age at menopause between WLWH and WLWOH; 48.9±7.4 years and 51.0±8.7 years respectively (274). In the present study, the reason for a lower age at menopause in WLWH is unclear. However, other studies have reported that severe immunosuppression (CD4+<200 cells/mm^3), longer duration of HIV infection, and co-infection with hepatitis C may be associated with earlier age of menopause in WLWH (273,275,276). Furthermore, one study from Denmark showed that AMH levels were lower in WLWH than in uninfected premenopausal counterparts (277). Since AMH is a measure of ovarian reserve, these findings suggest physiological differences that predispose WLWH to an early age at menopause.

The present study showed that women from Kenya had an earlier age at FMP than those from South Africa. Geographical differences in FMP have been reported in a previous meta-analysis where the mean age at menopause (with 95% confidence interval) was lower in Africa (48.4 (48.1–48.7)), Latin America (47.2 (45.9–48.6)), Asia (48.8 (48.1– 49.4)), and the Middle East (47.4 (46.9–47.8)), than in Australia (51.3 (49.8–52.8)), Europe (50.5 (50.0–51.1)) and the United States (49.1 (48.8–49.4)) (175). Furthermore, the study showed that higher level of education and occupation were associated with later age at menopause, while smoking was associated with earlier age at menopause. It is therefore possible that socio-demographic differences between the South African and Kenyan populations may explain the different FMPs observed in these groups.

The current study had its limitations and strengths. Firstly, this study was cross-sectional; therefore, could only measure associations and could not account for all confounders. Secondly, data on the duration of HIV infection and HAART use, and

measurements on viral load and CD4 counts were unavailable. Lastly, the sample size was small particularly when comparing variables across HIV groups. There is therefore a need to repeat these analyses in a larger population. Despite these limitations, the current study is the largest cross-sectional analysis of HIV, menopause and CMD in SSA. In addition, many study variables were measured, and all biochemical assays were conducted in a single laboratory to ensure standardisation.

The current study reports that HIV and HAART are independent risk factors for higher triglyceride levels, insulin resistance, and earlier onset of menopause but lower blood pressure and cIMT levels were observed in WLWH receiving HAART+. Although there were no differences in CMD risk factors between menopausal groups regardless of HIV status, the monitoring of CMD risk in this population, given their high BMI levels is important for curtailing morbidity and mortality.

Chapter 6: Discussion and Conclusions

This PhD study included a systematic review with meta-analysis, and three population-based cross-sectional and longitudinal analyses with an overall aim of investigating whether the MT is associated with increased levels of CMD risk factors in both SSA women and women from other LMICs and whether any association is modified by HIV. Table 6.1 summarises the main objectives and findings of this study.

In Chapter 2, the systematic review and meta-analysis shows that menopause is associated with increased CMD risk in LMICs, with some exceptions. These data are important in informing public health strategies to reduce the development of CMD in midlife women. However, the feasibility of using therapeutic interventions such as hormone replacement is debatable in already over-burdened healthcare systems in LMICs and very few studies have investigated its use in these environments. Furthermore, the data from the longitudinal study described in Chapter 4 suggests that estrogens may have very little effect on CMD risk factors during the MT.

In Chapter 3, differences in anthropometric and cardiometabolic variables between pre- and postmenopausal women in four sub-Saharan African countries, Burkina Faso, Ghana, Kenya, and South Africa were examined – in a cross-sectional analysis. The results show that CMD risk factors were higher in post- than pre-menopausal women only at the West African study sites (Burkina Faso and Ghana), which are far more rural than the study sites in East (Kenya) and South Africa. In addition, obesity and related cardiometabolic diseases were more prevalent in the East and South African than the West African sites. This identifies the premenopausal stage as a critical stage for early intervention of CMD risk in West Africa, whereas in the more urban East and South Africa areas interventions focusing on obesity are necessary

across all stages of the menopause. Thus, this study suggests that a ‘one-size-fits-all’ non-communicable disease intervention policy for menopausal women is not possible across SSA and must be tailored to each community.

In Chapter 4, longitudinal changes on sex hormones, anthropometric and CMD variables in the two West African countries, Burkina Faso, and Ghana were examined. The results showed that sex hormone levels, anthropometric and CMD variables changed rapidly in the perimenopausal transition and in women who became postmenopausal in the follow-up, but the changes were minimal in women who remained premenopausal and those that were postmenopausal at baseline. Further analyses showed that the associations between the changes in sex hormones and CMD variables were few and of a smaller magnitude. These changes were more associated with androgens than estrogens. Furthermore, the longitudinal changes in anthropometric measures except for DBP, were greater in Navrongo than Nanoro across all the three menopausal groups. This suggests strong influences from environmental, lifestyle or socio-demographic factors. It is therefore important to further assess such factors to help mitigate the increase of CMD in menopause in these populations. This study once again suggests that interventions for CMD in midlife woman in different regions of Africa may require more tailored methodologies.

In Chapter 5, the study examined whether differences in CMD risk factors between pre- and postmenopausal midlife women from urban Kenya and South Africa were modified by HIV and HAART. The findings show that the level of CMD risk factors was similar in both pre- and postmenopausal women regardless of HIV status. The study also demonstrated that the age at menopause was lower in WLWH than in those free of infection. Furthermore, the study showed that HIV was associated with lower total cholesterol and HDL-C while HAART was associated with higher triglyceride levels,

insulin resistance but lower cIMT and blood pressure. These findings highlight the complex interaction between HIV, its therapy and prevalent CMD in SSA populations.

Table 6.1 Integrated findings

Objective	Main findings
To perform a systematic review and meta-analysis on the association between menopause and CMD risk factors in studies from LMICs.	Postmenopausal women had a higher risk of MetS, and elevated triglycerides, blood glucose, BP, and WC than premenopausal women. No differences in obesity, HDL-C and cIMT levels were noted in most studies
To examine differences in anthropometric and cardiometabolic variables between pre- and postmenopausal women in 4 SSA countries	CMD risk factors were higher in post- than premenopausal women only at the rural West African sites than the urban study sites in East and South Africa.
To examine longitudinal changes in the menopause-transitioning and postmenopausal women in two SSA countries.	E2, SHBG, testosterone, and DHEAS levels decreased while FSH increased during the MT. CMD and anthropometric measures increased during the MT and postmenopause.
To establish if a relationship exists between changes in sex hormone levels and changes in cardiometabolic and anthropometric variables in menopause-transitioning and postmenopausal women from Burkina Faso and Ghana.	Associations between changes in sex hormone levels and cardiometabolic and anthropometric variables were few and their overall effect small. These changes were more associated with androgens than estrogens.
To compare CMD risk factors between pre- and postmenopausal SSA WLWH and WLWOH and to determine whether menopausal differences for these risk factors were modified by HAART.	HIV did not affect CMD risk factors in pre- and postmenopausal women. HAART was associated with higher triglyceride and higher insulin resistance but lower cIMT and BP levels.
To determine whether HIV is associated with earlier age at menopause.	WLWH had an earlier age at menopause than WLWOH.

CMD-cardiometabolic disease, MetS-metabolic syndrome, WC-waist circumference, WLWH-women living with HIV, WLWOH- women living without HIV, HAART-highly active antiretroviral therapy, cIMT-carotid intima media thickness.

6.1 Limitations and strengths

The limitations and strengths of the current study have been covered with greater detail in the preceding Chapters 2–5. However, other limitations and strengths of the study should be further explored.

Firstly, the advantage of conducting meta-analysis studies such as the one from this PhD (Chapter 2) is that it provides high level evidence by combining data from multiple sources and has strong statistical power. However, as a limitation of this analyses, we did not assess for publication bias. As such, there is a chance that the reported pooled estimates may over- or underestimate the true effect of menopause as a risk factor for CMD levels.

Secondly, as shown in Chapter 3, the present study was the first multi-centre study in SSA to investigate differences in CMD risk factors between pre- and postmenopausal women. However, these analyses may not be representative of the national populations of the respective study sites.

Thirdly, conducting a longitudinal study (Chapter 4) was advantageous in that it allowed for a follow up on individuals at different time points across the MT. However, the study had a high rate of attrition (45%), and the statistical power was reduced particularly in the analyses where individuals were partitioned into the three menopausal groups. In addition, the selection of only the participants from the two countries (Burkina Faso and Ghana) may not be transferable to the Kenyan and South African populations, which were more urban and had the highest prevalence of CMD risk factors. Furthermore, financial resources limited measurements on sex hormones in participants from Kenya and South Africa, therefore these were excluded. One more

limitation was that immunoassays used for the sex hormone assays may be less accurate than measurements from mass spectrometry.

Fourthly, in Chapter 5, the investigations on the age at menopause were based on self-reported data. As has been shown by the analyses, this was associated with recall bias, particularly in the women who experienced their last periods beyond 3 years from participating in the study. Furthermore, the interaction terms to capture the effect of a combination of variables such as alcohol, socioeconomic status, and level of education could have been included in the analyses. However, these were excluded because of lack of significance in each of the regression models.

6.2 Future directions

Having demonstrated that menopause is a risk factor for increased CMD, there is therefore a need for future studies to consider promoting intervention strategies that could lower CMD in midlife women. These should include lifestyle changes such as diet and physical exercise before or during the menopause transition. Furthermore, studies that assess the efficacy of these interventions could be important for improving awareness and possibly informing implementation policies. Hormone replacement should also be investigated not only for its effects on CMD risk but also on menopause symptoms.

There is also a need to conduct more longitudinal studies with longer follow-up periods in SSA populations. These studies would better assess the long-term effects of menopause on CMD risk factor levels, and the development of CMD. As suggested by the cross-sectional analyses in Chapter 3 and 4, menopause affects each population differently, therefore future menopause studies could benefit from looking at sub-groups based on BMI or socio-demographic factors.

The longitudinal study was hampered by small sample sizes and therefore future studies should recruit larger numbers of study participants and focus on the effects of FSH on lipids and the role of androgens in modulating CMD risk factors.

6.3 Conclusions

The current study has shown that menopause is a risk factor for CMD in LMICs and SSA populations. These associations are complex, and environmental factors had strong influences on the differential associations across the study sites. Furthermore, this study provides insights into the biology of the menopause in a SSA population by showing that androgens had a stronger effect on the levels of CMD risk factors than estrogens. Many of these results agree with those from other high-income countries and therefore indicate that more research is needed to confirm these findings in SSA given a high prevalence of CMD in the region. Furthermore, interventions such as behavioural modification may be effective strategies as shown by the strong influences of environmental factors.

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Appendices

Appendix 1: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Postgraduate Affairs

TO: Mr RP Chikwati and Professor M Ramsay
School of Pathology
Department of Chemical Pathology
Medical School
University

E-mail: rayltonc@gmail.com

CC: Supervisor: P{rofessor NJ Crowther
<Nigel.Crowther@nhls.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2021/03/01

REF: R14/49

PROTOCOL NO: **M2010106** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Body fat distribution, sex hormone levels and cardiometabolic disease risk factors during the menopause transition in African women*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Mr RP Chikwati and Professor M Ramsay

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M2010106

NAME: (Principal Investigator)	Mr RP Chikwati and Professor M Ramsay
DEPARTMENT:	School of Pathology Department of Chemical Pathology Medical School University
PROJECT TITLE:	*Body fat distribution, sex hormone levels and cardiometabolic disease risk factors during the menopause transition in African women*
DATE CONSIDERED:	2020/10/30
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M170880 and M180535
SUPERVISOR:	P{rofessor NJ Crowther
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/03/01

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

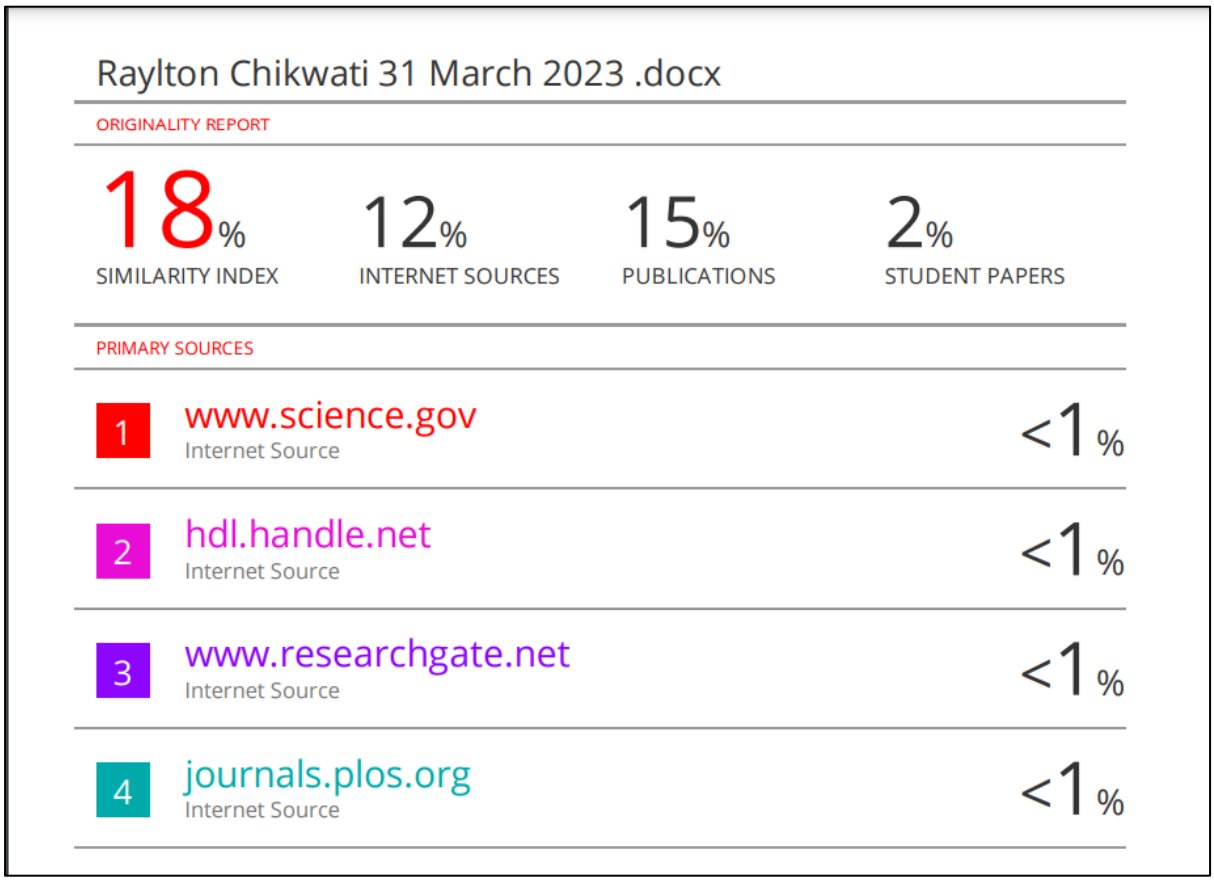
To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **«Missing mail merge field»** and therefore reports and re-certification will be due in the month of **«Missing mail merge field»** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

M. Ramsay — 1 March 2021

Signature of Principal Investigator — Date

Appendix 2: Turnitin report



Raylton Chikwati 31 March 2023 .docx

ORIGINALITY REPORT

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Appendix 3: PROSPERO protocol

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

UNIVERSITY of York
Centre for Reviews and Dissemination

Systematic review

A list of fields that can be edited in an update can be found here

1. * Review title.

Give the title of the review in English

Menopause and Cardiometabolic Disease Risk in Low- and Middle-Income Countries, a Systematic Review

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

English

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

01/12/2021

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

30/06/2022

5. * Stage of review at time of this submission.

This field uses answers to initial screening questions. It cannot be edited until after registration.

Tick the boxes to show which review tasks have been started and which have been completed.

Update this field each time any amendments are made to a published record.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

6. * Named contact.

The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.

Raylton Chikwati

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Mr Chikwati

7. * Named contact email.

Give the electronic email address of the named contact.

rayltonc@gmail.com

8. Named contact address

Give the full institutional/organisational postal address for the named contact.

7 York Road, Parktown

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

+27731675184

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be

completed as 'None' if the review is not affiliated to any organisation.

Department of Chemical Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Sydney Brenner Institute for Molecular Bioscience (SBIMB), University of Witwatersrand, Johannesburg, South Africa

Organisation web address:

https://www.wits.ac.za/pathology/divisions/chemical-pathology/

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country now MUST be entered for each person, unless you are amending a published record.**

Mr Raylton Chikwati. University of the Witwatersrand, Johannesburg
Dr Nasrin Goolam Mahyoodeen. Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Dr Nicole G Jaff. Department of Chemical Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
Professor Jaya A George. Department of Chemical Pathology, National Health Laboratory Service, Johannesburg, South Africa.
Professor Nigel J Crowther. Department of Chemical Pathology, National Health Laboratory Service, Johannesburg, South Africa.

12. * Funding sources/sponsors.

Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

RPC received a bursary from Shimadzu South Africa.

Grant number(s)

State the funder, grant or award number and the date of award

13. * Conflicts of interest.

List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are

not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.

State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

What is currently known about the association between menopause and cardiometabolic disease risk in Low- and Middle-Income Countries (LMICs).

16. * Searches.

State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

Without any restrictions on publication dates or language, we searched the databases; PubMed, PMC, Scopus, Scopus, and ISI Web of Science, for original articles until November 20, 2021. Only original and published article were sought.

17. URL to search strategy.

Upload a file with your search strategy, or an example of a search strategy for a specific database, (including the keywords) in pdf or word format. In doing so you are consenting to the file being made publicly accessible. Or provide a URL or link to the strategy. Do NOT provide links to your search **results**.

https://www.crd.york.ac.uk/PROSPEROFILES/295401_STRATEGY_20211130.pdf

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Cardiometabolic disease (CMD) is a constellation of cardiovascular and metabolic derangements including hypertension, dyslipidaemia, obesity, diabetes, stroke, angina, and kidney disease. These diseases individually and collectively increase the risk and prevalence for CMD morbidity and mortality.

19. * Participants/population.

Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

Inclusion: Participants from Low- and Middle-Income Countries

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

Not applicable

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Post- vs Premenopausal women

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.

We will include original articles on menopause and cardiometabolic disease risk in LMICs. Review articles will be excluded.

23. Context.

Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

Research from low- and middle-income countries only will be included.

24. * Main outcome(s).

Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

Review literature on menopause and cardiometabolic disease risk in Low- and Middle- Income Countries (LMICs) using the electronic databases PubMed, PMC, Scopus, and Web of Science.

Measures of effect

Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

Associations between menopause and cardiometabolic risk factors (p-values), beta coefficients and odds ratios.

25. * Additional outcome(s).

List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review

None

Measures of effect

Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.

26. * Data extraction (selection and coding).

Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.

Study selection

Study will apply the eligibility criteria and select studies to include in the systematic review based on the title and abstracts of identified articles. Articles that meet the selection criteria will be selected and shared between all five authors using a web software: Ryaan (https://www.rayyan.ai/). Any disagreements will be resolved through discussion with all authors.

Data extraction

The selected papers that meet the inclusion criteria will be fully reviewed. The data for the systematic review extracted will be summarised on an extraction table in Microsoft Office Excel software. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline will be followed throughout the review.

The extraction table will include information about author name, country, year of publication, sample size, study design, study setting, selection of participants, mean age, definition of menopause, cardiometabolic risk factors and key conclusion(s).

Retrieved articles will be assessed for inclusion using their title and abstract and then a full-text review of articles will be done by all authors before inclusion in the final review. Any disagreements will be resolved through a discussion.

27. * Risk of bias (quality) assessment.

State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.

For cross-sectional and cohort studies, the risk of bias will be assessed and based on the Newcastle-Ottawa Scale (NOS). The NOS uses a nine-star system which uses three pre-defined domains; selection of

participants (representativeness of sample), comparability (adjustments of age and other potential confounders such as smoking status, BMI etc) and identification of outcomes of interest. The scoring system on the NOS assigns a maximum of four points for selection, two points for comparability, and three points for outcome. Nine points on the NOS reflects the highest study quality. A threshold score of ? 5 will define adequate quality for inclusion in this review. Two authors will assess the quality of the studies to be included in the review and a consensus reached with the other three co-authors.

28. * Strategy for data synthesis.

Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.

Due to the many cardiometabolic disease risk factors (outcomes), we will conduct a systematic review instead of a meta-analysis.

29. * Analysis of subgroups or subsets.

State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.

Not applicable

30. * Type and method of review.

Select the type of review, review method and health area from the lists below.

Type of review

Cost effectiveness

No

Diagnostic

No

Epidemiologic

No

Individual patient data (IPD) meta-analysis

No

Intervention

No

Living systematic review

No

Meta-analysis

No

Methodology

No

Narrative synthesis
No

Network meta-analysis
No

Pre-clinical
No

Prevention
No

Prognostic
No

Prospective meta-analysis (PMA)
No

Review of reviews
No

Service delivery
No

Synthesis of qualitative studies
No

Systematic review
Yes

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
Yes

Care of the elderly
No

Child health

No

Complementary therapies
No

COVID-19
No

Crime and justice
No

Dental
No

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
Yes

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity
No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing

No

Obstetrics and gynaecology
No

Oral health
No

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
Yes

Rehabilitation
No

Respiratory disorders
No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.

English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.

South Africa

33. Other registration details.

Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format)

Not applicable

Add web link to the published protocol.

Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Do you intend to publish the review on completion?

Yes

Give brief details of plans for communicating review findings.?

On completion, the systematic review will be published in a peer-review journal and will be part of a PhD thesis at the University of the Witwatersrand, Johannesburg, South Africa.

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords help PROSPERO users find your review (keywords do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

Systematic review; Premenopause; Postmenopause; Cardiometabolic risk factors; Low- and Middle-Income Countries.

37. Details of any existing review of the same topic by the same authors.

If you are registering an update of an existing review give details of the earlier versions and include a full bibliographic reference, if available.

Not applicable

38. * Current review status.

Update review status when the review is completed and when it is published.New registrations must be ongoing so this field is not editable for initial submission.

Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any other information relevant to the registration of this review.

Not applicable

40. Details of final report/publication(s) or preprints if available.

Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format.

Give the link to the published review or preprint.

Appendix 4: AWI-Gen questionnaire

AWI-Gen H3Africa

Genomic and Environmental Risk Factors for Cardiometabolic Disease in Africans

AWI-Gen Study Number: |__|__|__|__||__|	**Unique Site Identifier :** |__|__|__|__||__|__|__|

BARCODE STICKER
Please stick the barcode sticker here

1. GENERAL INFORMATION		
1.1	Data collection date	| d | d | | m | m | m | | y | y | y | y |
1.2	Interviewer code	_____
1.3	Start time	| h | h |:| m | m | ***[Based on a 24 hour clock eg. 15:30]***

2. DEMOGRAPHIC INFORMATION		
2.1	Date of birth known? ***[If no, skip to Q.2.3]***	☐ Yes ☐ No
2.2	Date of Birth ***[eg. 27 SEP 1957]***	| d | d | | m | m | m | | y | y | y | y |
2.3	Approximate year of birth	| **1** | **5** | | **J** | **U** | **N** | | y | y | y | y |
2.4	Gender	☐ Female ☐ Male ***[please tick the appropriate box]***
2.5	Country	South Africa
2.6	Home language ***[please tick the appropriate box]***	☐ Afrikaans ☐ English ☐ isiNdebele ☐ isiXhosa ☐ isiZulu ☐ Sesotho ☐ Sepedi ☐ Setswana ☐ siSwati ☐ Tshivenda ☐ Xitsonga ☐ Shona ☐ Unknown

		☐ Other (specify)_____
2.7	Ethnic/tribal affiliation ***[please tick the appropriate box]***	☐ Zulu ☐ Xhosa ☐ Ndebele ☐ Sotho ☐Venda ☐ Tsonga ☐ Tswana ☐ BaPedi ☐ Shona ☐ Unknown ☐ Swati ☐ Other (specify)_____

2.8. Family Ethnicity

This question relates to the ethnicity and home languages of your parents and grandparents.

Please tick the applicable columns

	Father		Paternal Grandfather		Paternal Grandmother		Mother		Maternal Grandfather		Maternal Grandmother	
Ethnicity/Language	E	L	E	L	E	L	E	L	E	L	E	L
Afrikaans	■		■		■		■		■		■	
English												
	■		■		■		■		■		■	
Ndebele/isiNdebele												
Sotho/Sesotho												
BaPedi/Sepedi												
Swati/siSwati												
Tsonga/Xitsonga												
Tswana/Setswana												
Venda/Tshivenda												
Xhosa/isiXhosa												
Shona/Shona												
Zulu/isiZulu												
Unknown												

Other												

If other, please specify

Father __________ PG\father __________ PG\mother __________

Ethnicity Language Ethnicity Language Ethnicity Language

Mother __________ MG\father __________ MG\mother __________

Ethnicity Language Ethnicity Language Ethnicity Language

3. FAMILY COMPOSITION		
3.1	Do you have any siblings* with which you share at least one parent? ****half siblings and those that have passed away are included***	☐ Yes ☐ No ***[If no, skip to Q.3.4]***
3.2	How many brothers do you have?	|__|__|
3.3	How many sisters do you have?	|__|__|
3.4	Do you have any biological children?	☐ Yes ☐ No ***[If no, skip to the next section- Phenotypic Data Collection]***
3.5	How many biological sons do you have?	|__|__|
3.6	How many biological daughters do you have?	|__|__|

PHENOTYPIC COLLECTION DATA

4. PREGNANCY **[If male, please skip to the next section - Marital Status]**		
4.1	Are you pregnant?	☐ Yes ☐ No
4.2	How many pregnancies have you had? ***[If none, please skip to Q.4.4]***	|__|__|
4.3	How many live births have you had?	|__|__|

4.4	Do you have regular (28-35 day) periods?	☐ Yes ☐ No ☐ NA
4.5	Date of last period	|__|__| |__|__|__|__|

5. MARITAL STATUS		
5.1	Marital Status ***[please tick the appropriate box]***	☐ Married ☐ Living together ☐ Never married or co-habited ☐ Divorced with a living partner ☐ Separated with a living partner ☐ Partner deceased

6. EDUCATION		
6.1	Highest level of Education ***[please tick the appropriate box]*** ***[If "no formal education" please skip to Q.7]***	☐ No formal education ☐ Primary ☐ Secondary ☐ Tertiary* ****tertiary education includes qualifications such as certificates, diplomas or degrees***
6.2	Total number of successfully completed years at highest level of education	|__|__| **years**

7. EMPLOYMENT		
7.1	Employment	☐ Self employed ☐ Formal full-time employment by someone else ☐ Part-time employment by someone else ☐ Informal employment (dependent on availability of work) ☐ Unemployed

8. HOUSEHOLD ATTRIBUTES

8.1. How many people besides you are in your household? **|__|__| people**

This applies to people who sleep in separate buildings on the property but share their main meal in the same house as you
If none, please skip to Q.8.3.

8.2.	**Name**	**Sex**	**Age (years)**	**Relationship to you (eg. father, husband, daughter, etc.)**
	1.	☐ Male ☐ Female	|__|__|__|	
	2.	☐ Male ☐ Female	|__|__|__|	
	3.	☐ Male ☐ Female	|__|__|__|	
	4.	☐ Male ☐ Female	|__|__|__|	
	5.	☐ Male ☐ Female	|__|__|__|	
	6.	☐ Male ☐ Female	|__|__|__|	
	7.	☐ Male ☐ Female	|__|__|__|	
	8.	☐ Male ☐ Female	|__|__|__|	
	9.	☐ Male ☐ Female	|__|__|__|	
	10.	☐ Male ☐ Female	|__|__|__|	
8.2.	**Name**	**Sex**	**Age (years)**	**Relationship to you (eg. father, husband, daughter, etc.)**
	12.	☐ Male ☐ Female	|__|__|__|	

8.3	How many rooms are there in the house and outside structures used by household members?	**|__|__| rooms**
8.4	How many rooms are used for sleeping in?	**|__|__| rooms**

8.5	**Which of the following items in working order, do you have in your household at the present time?**			
1	Electricity	☐ Yes	☐ No	☐ Don't know
2	Solar energy	☐ Yes	☐ No	☐ Don't know
3	Power generator	☐ Yes	☐ No	☐ Don't know
4	Alternative power source	☐ Yes	☐ No	☐ Don't know
5	Television	☐ Yes	☐ No	☐ Don't know
6	Radio	☐ Yes	☐ No	☐ Don't know
7	Motor vehicle	☐ Yes	☐ No	☐ Don't know
8	Motorcycle	☐ Yes	☐ No	☐ Don't know
9	Bicycle	☐ Yes	☐ No	☐ Don't know
10	Refrigerator	☐ Yes	☐ No	☐ Don't know
11	Washing machine	☐ Yes	☐ No	☐ Don't know
12	Sewing machine	☐ Yes	☐ No	☐ Don't know
13	Telephone	☐ Yes	☐ No	☐ Don't know
14	Mobile phone	☐ Yes	☐ No	☐ Don't know
15	Microwave	☐ Yes	☐ No	☐ Don't know
16	DVD player	☐ Yes	☐ No	☐ Don't know
17	Satellite TV or DSTV	☐ Yes	☐ No	☐ Don't know
18	Computer or laptop	☐ Yes	☐ No	☐ Don't know
19	Internet by computer	☐ Yes	☐ No	☐ Don't know
20	Internet by mobile phone	☐ Yes	☐ No	☐ Don't know
21	Electric or gas stove	☐ Yes	☐ No	☐ Don't know
22	Toilet facilities	☐ Yes	☐ No	☐ Don't know
23	Cattle	☐ Yes	☐ No	☐ Don't know
24	Other livestock *	☐ Yes	☐ No	☐ Don't know
25	Poultry **	☐ Yes	☐ No	☐ Don't know

8.5	**Which of the following items in working order, do you have in your household at the present time?**			
26	Tractor	☐ Yes	☐ No	☐ Don't know
27	Plough	☐ Yes	☐ No	☐ Don't know

*** other livestock includes donkeys, goats, sheep and pigs**

**** poultry includes ducks, chickens, geese and other fowl**

9. SUBSTANCE USE

9.1. Tobacco use		
9.1.1	Have you ever smoked any tobacco products such as cigarettes, cigars or pipes? ***[If no, skip to Q.9.1.8]***	☐ Yes ☐ No
9.1.2	Do you *currently smoke any tobacco products, such as cigarettes, cigars or pipes? ****We are asking if they smoke when they have the opportunity to do so.*** ***[If no, skip to Q.9.1.6]*** ***[If yes, please do not answer Q.9.1.7]***	☐ Yes ☐ No
9.1.3	What do you smoke? ***[please tick the appropriate boxes]***	☐ Cigarettes ☐ Pipe ☐ Hand rolled ☐ Cigars ☐ E-cigarettes
9.1.4	How often do you smoke tobacco products?	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 days per month ☐ Less than once per month
9.1.5	On the days that you smoke, how many tobacco products do you smoke?	|__|__|__|
9.1.6	How old were you when you first started smoking?	|__|__| years old
9.1.7	When did you stop smoking completely?	|y|y|y|y|

9.1.8	Have you ever used any smokeless tobacco such as snuff, snus, betel with tobacco or chewing tobacco? ***[If no, skip to Q.9.2]***	☐ Yes ☐ No
9.1.9	Do you use snuff? ***[If no, skip to Q.9.1.13]***	☐ Yes ☐ No
9.1.10	How do you take snuff?	☐ Through your nose ☐ Through your mouth/on your lip
9.1.11	How often do you use snuff?	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 days per month ☐ Less than once per month
9.1.12	On the days that you use snuff, how many times a day do you use it?	☐ Once a day ☐ Twice a day ☐ Three times a day ☐ More than three times a day
9.1.13	Do you use chewing tobacco? ***[If no, skip to Q.9.2]***	☐ Yes ☐ No
9.1.14	How often do you use chewing tobacco?	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 days per month ☐ Less than once per month
9.1.15	On the days that you use chewing tobacco, how many times a day do you use it?	☐ Once a day ☐ Twice a day ☐ Three times a day ☐ More than three times a day

9.2. Alcohol use		
9.2.1	Have you ever consumed an alcoholic drink such as beer, wine, spirits, fermented cider, thothotho, or traditional beer? ***[If "no" or "don't know", skip to Q.9.3]***	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.2	Do you currently (in the last 30 days) consume any alcoholic drink such as beer, wine, spirits, fermented cider, thothotho, or traditional beer? ***[If no, skip to Q.9.2.10]***	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.3	How often do you have at least one alcoholic drink?	☐ Daily ☐ 5-6 days per week ☐ 1-4 days per week ☐ 1-3 days per month ☐ Less than once per month
9.2.4	On the days that you drink alcoholic drinks, how many alcoholic drinks do you have? USE CARDS TO SHOW STANDARD DRINKS	|__|__|
9.2.5	Have you ever felt that you should cut down on your drinking?	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.6	Have people annoyed you by criticising your drinking?	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.7	Have you ever felt bad or guilty about your drinking?	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.8	Have you ever had an alcoholic drink first thing in the morning to steady your nerves or get rid of a hangover?	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

9.2.9	In the past year, did you ever take 6 or more alcoholic drinks in a single morning, afternoon, or night? I understand that you may share drinks and that some drinks have different sizes, but please do your best to answer. USE CARDS TO SHOW STANDARD DRINKS.	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer
9.2.10	What type of alcoholic beverage do you, or did you, usually drink?	☐ Beer ☐ Wine ☐ Spirits ☐ Home brew ☐ Other (specify) _____

9.3. Drug use		
9.3.1	Do you, or have you ever taken marijuana, methamphetamines, cocaine or any other drugs (dagga, nyaope, tik, glue, rocks, crack, buttons, mandrax, acid)?	☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

10. GENERAL HEALTH

10.1. Please indicate whether you have, or have had, any of the following illnesses

[Please tick the appropriate boxes] [If male, please proceed to question 10.1.3] [If female, please skip question 10.1.3]

10.1.1	Breast cancer ***[for females only]***	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.1.2	Cervical cancer ***[for females only]***	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.1.3	Prostate cancer ***[for males only]***	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.1.4	Other cancers	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.1.5	Asthma or reactive air diseases	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.1.6	Have you ever received treatment prescribed by a doctor to treat any of the above illnesses? ***[If no, skip to Q.10.1.9]***	☐ Yes	☐ No	☐ Don't know	
10.1.7	Are you currently on treatment prescribed by a doctor to treat any of the above illnesses? ***[If no, skip to Q.10.1.9]***	☐ Yes	☐ No	☐ Don't know	
10.1.8	What medication has been prescribed? Please list names if possible.	_____			

10.1.9	Are you currently taking any herbal or traditional remedy for any of the above illnesses?	☐ Yes ☐ No ☐ Don't know

10.2. Please indicate if your <u>mother</u> has, or has had, any of the following illnesses
[Please tick the appropriate boxes]

10.2.1	Weight problem/obesity	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.2	High blood pressure	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.3	High cholesterol	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.4	Breast cancer	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.5	Cervical cancer	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.6	Other cancers	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.2.7	Asthma or reactive air diseases	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer

10.3. Please indicate if your <u>father</u> has, or has had any of the following illnesses

[Please tick the appropriate boxes]

10.3.1	Weight problem/obesity	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.3.2	High blood pressure	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.3.3	High cholesterol	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.3.4	Prostate cancer	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.3.5	Other cancers	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10.3.6	Asthma or reactive air diseases	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer

10.4. Diet ***The following questions ask about what you eat and drink.***		
10.4.1	In a typical week, on how many days do you eat fruit?	|__| days
10.4.2	How many servings of fruit do you eat on a typical day? USE CARDS TO SHOW STANDARD SERVINGS	|__|__| servings
10.4.3	In a typical week, on how many days do you eat vegetables?	|__| days
10.4.4	How many servings of vegetables do you eat on a typical day? USE CARDS TO SHOW STANDARD SERVINGS	|__|__| servings
10.4.5	How many meals per week do you buy from a *vendor or takeaway or restaurant? By meal, I mean breakfast, lunch or dinner. ****A vendor is any person or place that sells food***	|__|__| meals
10.4.6	In a typical week, on how many days do you eat bread bought from a shop?	|__| days
10.4.7	How many slices of bread bought from a shop, do you eat on a typical day? USE CARDS TO SHOW STANDARD SLICES	|__|__| slices
10.4.8	How many cans, bottles, or cups of sugary cold drinks (eg. Coke, Pepsi, Fanta, Sprite, etc.), do you drink in a week?	|__|__| drinks
10.4.9	How many cans or bottles or cups of fruit juice do you drink in a week? (eg. Liquifruit, Tropicana, Oros, etc.)	|__|__| drinks
10.4.10	Has a doctor, nurse, or other healthcare worker ever told you to change your diet (eg.To eat less sugar)?	☐ Yes ☐ No ☐ Don't know
10.4.11	Has a doctor, nurse, or other healthcare worker ever advised you to lose weight?	☐ Yes ☐ No ☐ Don't know

10.5. Exposure to Pesticides		
10.5.1	Do you work with insecticides or pesticides? ***[If no, please skip to Q.10.5.3.]***	☐ Yes ☐ No ☐ Don't know
10.5.2	How long have you been working with insecticides or pesticides?	|__|__| years
10.5.3	Do you live close to a farm or region where insecticides or pesticides are used? ***[If no, please skip to Q.11]***	☐ Yes ☐ No ☐ Don't know
10.5.4	Do you know what type of pesticides or insecticides are used, either by you, or within your area? ***[If not yes, please skip to Q.11]***	☐ Yes ☐ No ☐ Don't know
10.5.5	Please list them if possible :	

11. INFECTION HISTORY

11.1. Malaria		
1	Have you ever had Malaria? ***[If no, skip to Q.11.2]***	☐ Yes ☐ No ☐ Don't know
2	Have you had malaria fever in the last month? ***[If no, skip to Q.11.2]***	☐ Yes ☐ No ☐ Not sure
3	Have you travelled to an area with a *high incidence of malaria, in the last 2 months? ****Some areas with high incidence of malaria include; Angola, Burkina Faso, Cameroon, Cote d'Ivoire, DRC, Ghana, GuineaBissau, Liberia, Malawi, Sierra Leone, Togo, Coastal or Western Kenya, Uganda and Zambia***	☐ Yes ☐ No ☐ Not sure

11.2. TB		
11.2.1	Have you ever been told by a doctor, nurse or other healthcare worker that you have TB? ***[If no, skip to Q.11.2.7]***	☐ Yes ☐ No ☐ Don't know
11.2.2	Have you been newly-diagnosed with TB in the last 12 months?	☐ Yes ☐ No ☐ Don't know
11.2.3	When was it diagnosed?	|y|y|y|y|
11.2.4	Have you ever received treatment for TB prescribed by a doctor, nurse, or other healthcare worker? ***[If no, skip to Q.11.2.7]***	☐ Yes ☐ No ☐ Don't know
11.2.5	Are you currently receiving treatment for TB prescribed by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
11.2.6	Have you ever been counselled by a doctor, nurse or other healthcare worker, on how you can avoid passing TB onto others ?	☐ Yes ☐ No ☐ Don't know
11.2.7	Are you currently taking any herbal or traditional remedy for TB?	☐ Yes ☐ No ☐ Don't know

12. CARDIOMETABOLIC RISK FACTORS

12.1. Diabetes		
12.1.1	Has a doctor, nurse, or other healthcare worker ever measured your blood or urine for diabetes (sugar in the blood)?	☐ Yes ☐ No ☐ Don't know
12.1.2	Have you ever been told by a doctor or healthcare worker, that you have diabetes or high blood sugar (outside of pregnancy)? ***[The brackets apply only to females]*** ***[If "no" or "don't know", skip to Q. 12.1.7]***	☐ Yes ☐ No ☐ Don't know
12.1.3	Have you been newly diagnosed with diabetes in the last 12 months?	☐ Yes ☐ No
12.1.4	Have you ever received treatment for diabetes prescribed by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.1.5	Are you currently receiving treatment for diabetes prescribed by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.1.6	Are you doing anything to treat your diabetes (sugar in the blood)? *Note: Insulin injections and pills can't be taken at the same time!*	☐ Insulin injection ☐ Pills (that you swallow) ☐ Special Diet ☐ Weight Loss ☐ Other (specify) _____
12.1.7	Are you currently taking any herbal or traditional remedy for diabetes (sugar in the blood)?	☐ Yes ☐ No ☐ Don't know
12.1.8	Do you have a family history of diabetes? ***[If "no" or "don't know", skip to Q.12.2]***	☐ Yes ☐ No ☐ Don't know

12.1.9. Which of your family members have, or have had diabetes (sugar in the blood)

	Mother	☐ Yes	☐ No	☐ Don't know
	Father	☐ Yes	☐ No	☐ Don't know
	Brother 1	☐ Yes	☐ No	☐ Don't know
	Brother 2	☐ Yes	☐ No	☐ Don't know
	Brother 3	☐ Yes	☐ No	☐ Don't know
	Brother 4	☐ Yes	☐ No	☐ Don't know
	Sister 1	☐ Yes	☐ No	☐ Don't know
	Sister 2	☐ Yes	☐ No	☐ Don't know
	Sister 3	☐ Yes	☐ No	☐ Don't know
	Sister 4	☐ Yes	☐ No	☐ Don't know
	Son 1	☐ Yes	☐ No	☐ Don't know
	Son 2	☐ Yes	☐ No	☐ Don't know
	Son 3	☐ Yes	☐ No	☐ Don't know
	Son4	☐ Yes	☐ No	☐ Don't know
	Daughter 1	☐ Yes	☐ No	☐ Don't know
	Daughter 2	☐ Yes	☐ No	☐ Don't know
	Daughter 3	☐ Yes	☐ No	☐ Don't know
	Daughter 4	☐ Yes	☐ No	☐ Don't know
	Other, specify :	☐ Yes	☐ No	☐ Don't know

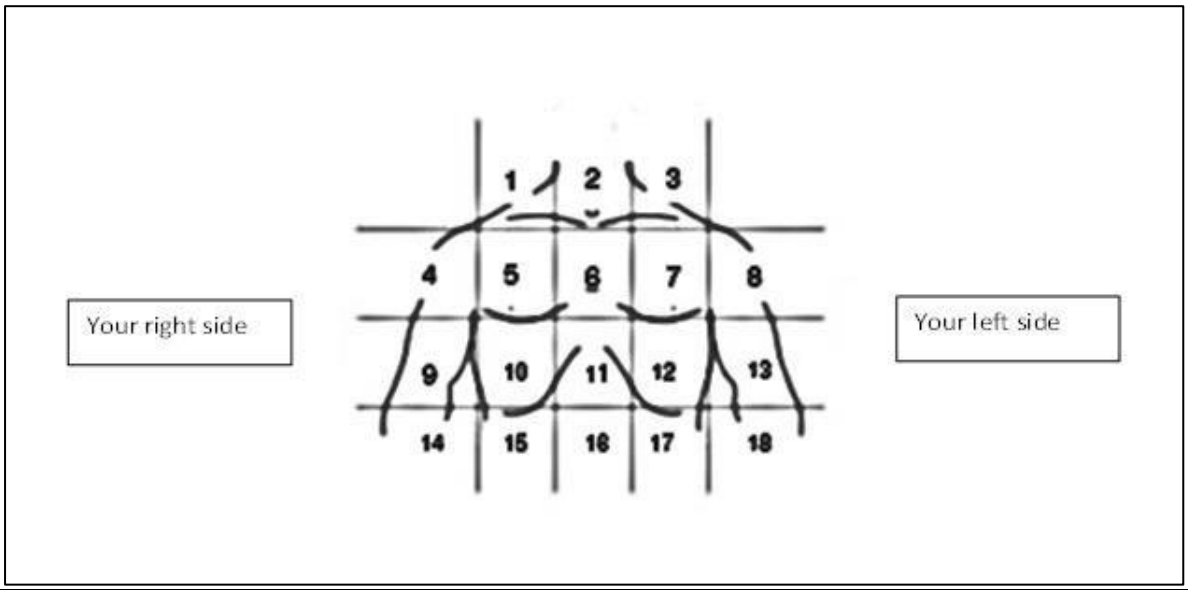
12.2. Stroke* ***Jones, W.J., Willams, L. S. and Meschia, J. F. (2001) Validating the Questionnaire for Verifying Stroke-Free Status (QVSFS) by neurological history and examination. Stroke, 32(10): 22232-6. Please note that the word, "physician" has been replaced with the phrase, "doctor, nurse or other healthcare worker" to accommodate the AWI-Gen study.***		
12.2.1	Have you ever been told by a doctor, nurse, or other healthcare worker that you have had a stroke? ***[If "no" or "don't know", skip to Q. 12.2.3]***	☐ Yes ☐ No ☐ Don't know
12.2.2	When was it first diagnosed? ***[Please indicate in which year your stroke was diagnosed]***	|_y_|_y_|_y_|_y_|
12.2.3	Have you ever been told by a doctor, nurse, or other healthcare worker that you have had a ministroke, or transient ischemic attack (TIA)?	☐ Yes ☐ No ☐ Don't know
12.2.4	Have you ever had sudden painless weakness on one side of your body?	☐ Yes ☐ No ☐ Don't know
12.2.5	Have you ever had sudden numbness or a dead feeling on one side of your body?	☐ Yes ☐ No ☐ Don't know
12.2.6	Have you ever had sudden painless loss of vision in one or both eyes?	☐ Yes ☐ No ☐ Don't know
12.2.7	Have you ever suddenly lost one half of your vision?	☐ Yes ☐ No ☐ Don't know
12.2.8	Have you ever suddenly lost the ability to understand what people are saying?	☐ Yes ☐ No ☐ Don't know
12.2.9	Have you ever suddenly lost the ability to express yourself verbally, or in writing?	☐ Yes ☐ No ☐ Don't know

12.3. Hypertension		
12.3.1	Has a doctor, nurse, or other healthcare worker ever measured your blood pressure?	☐ Yes ☐ No ☐ Don't know
12.3.2	Have you ever been told by a doctor, nurse, or other healthcare worker that you have hypertension (high blood pressure)? ***[If "no" or "don't know", skip to Q. 12.3.7]***	☐ Yes ☐ No ☐ Don't know
12.3.3	Have you been newly diagnosed with hypertension in the last 12 months?	☐ Yes ☐ No ☐ Don't know
12.3.4	Have you ever received treatment for hypertension prescribed by a doctor, nurse or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.3.5	Are you currently on treatment for hypertension prescribed by a doctor, nurse or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.3.6	What medicine are you taking for this? Please list if possible.	_____
12.3.7	Are you currently taking any herbal or traditional remedy for hypertension?	☐ Yes ☐ No ☐ Don't know

12.4. Angina*		
Angina is characterised as an intense chest pain or discomfort. This pain may occur in the shoulders, arms, neck, back and jaw as well. It may also feel like indigestion. It is considered to be the main symptom of Coronary Heart Disease (CHD).		
12.4.1	Have you ever been told by a doctor, nurse, or other healthcare worker that you have angina (chest pain due to heart disease)? ***[If "no" or "don't know", skip to Q. 12.4.5]***	☐ Yes ☐ No ☐ Don't know
12.4.2	Have you ever received treatment for chest pain due to heart disease prescribed by a doctor, nurse or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.4.3	Are you currently taking any medication for angina prescribed by a doctor or other healthcare worker for this?	☐ Yes ☐ No ☐ Don't know
12.4.4	What medicine are you taking for this? Please list if possible.	_____
12.4.5	Are you currently taking any herbal or traditional remedy for angina?	☐ Yes ☐ No ☐ Don't know
12.4.6	During the last 12 months, have you experienced any pain or discomfort in your chest, or pain going to the left arm or neck, when you **walk uphill or hurry**?	☐ Yes ☐ No ☐ Don't know
12.4.7	During the last 12 months, have you experienced any pain or discomfort in your chest, or pain going to the left arm or neck, when you **walk at an ordinary pace on level ground**? ***[If no to both 12.4.6 and 12.4.7, please skip to Q. 12.5]***	☐ Yes ☐ No ☐ Don't know
12.4.8	What do you do if you get the pain or discomfort when you are walking?	☐ Stop or slow down ☐ Rest for a while then carry on ☐ Carry on after taking a pain relief medicine that dissolves in your mouth (a nitro spray or tablet) ☐ Carry on walking
12.4.9	Is the pain or discomfort relieved if you stand still?	☐ Yes ☐ No ☐ Don't know

12.4.10. Will you show me where you usually experience the pain or discomfort?

Please circle the numbers in the box in the area of the body shown by the participant



12.5. Heart Attack		
12.5.1	Have you ever been told by a doctor, nurse, or other healthcare worker that you have had a heart attack? ***[If "no" or "don't know", skip to Q. 12.5.3]***	☐ Yes ☐ No ☐ Don't know
12.5.2	Did you ever receive medical treatment for your heart attack?	☐ Yes ☐ No ☐ Don't know
12.5.3	Are you currently taking any herbal or traditional remedy for your heart attack?	☐ Yes ☐ No ☐ Don't know

12.6. Congestive Heart Failure		
12.6.1	Have you ever been told by a doctor, nurse, or other healthcare worker that you have had heart failure? ***[If "no" or "don't know", skip to Q. 12.6.5]***	☐ Yes ☐ No ☐ Don't know
12.6.2	Have you ever received medical treatment for heart failure prescribed by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.6.3	Are you currently on treatment for heart failure prescribed by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.6.4	What medicine are you taking for this? Please list if possible.	_____
12.6.5	Are you currently taking any herbal or traditional remedy for heart failure?	☐ Yes ☐ No ☐ Don't know

12.7. High Cholesterol		
12.7.1	Has a doctor, nurse or other healthcare worker ever measured your cholesterol?	☐ Yes ☐ No ☐ Don't know
12.7.2	Have you ever been told by your doctor or other healthcare worker told you that you have high cholesterol? ***[If "no" or "don't know", skip to Q. 12.7.5]***	☐ Yes ☐ No ☐ Don't know
12.7.3	Have you ever been treated for high cholesterol by a doctor, nurse, or other healthcare worker?	☐ Yes ☐ No ☐ Don't know
12.7.4	Are you currently using any of the following to treat your high cholesterol, as prescribed by a doctor, nurse, or other healthcare worker? ***[Please tick the appropriate boxes, more than one may be selected]***	☐ Special diet ☐ Weight loss ☐ Medicine ☐ Other
12.7.5	Are you currently taking any herbal or traditional remedy for high cholesterol?	☐ Yes ☐ No ☐ Don't know

13. THYROID DISEASE* ***This includes underactive thyroid, over active thyroid, thyroid nodule enlargement and thyroid cancer***		
13.1	Has a doctor ever told you that you have thyroid disease? ***[If no, skip to Q. 13.5]***	☐ Yes ☐ No ☐ Don't know
13.2	Do you know what type of thyroid disease you were diagnosed with? If yes, please specify	☐ Yes ☐ No Specify _____
13.3	Have you ever been treated for it? ***[If no, skip to Q. 13.5]***	☐ Yes ☐ No ☐ Don't know
13.4	What treatment did you use?	☐ Thyroid hormone ☐ Surgery ☐ Radioactive iodine ☐ Antithyroid drugs
13.5	Do either of your parents have, or have they had, thyroid disease? If yes, please specify. ***[If yes please indicate which parent eg.mother, father or both]***	☐ Yes ☐ No Specify _____

14. KIDNEY DISEASE ***The following questions are aligned with the H3Africa Kidney Disease Study***		
14.1	Has a doctor ever told you that you have kidney disease? ***[If no, skip to Q. 14.3]***	☐ Yes ☐ No ☐ Don't know
14.2	Do you know what type of kidney disease? If yes, please specify.	☐ Yes ☐ No Specify_____
14.3	Has a doctor ever told you that your kidneys have low function?	☐ Yes ☐ No ☐ Don't know
14.4	Has anyone in your family either had kidney disease, or died from it? ***[If no, skip to Q. 15]***	☐ Yes ☐ No ☐ Don't know
14.5	If yes, who? ***["Other" refers to any other blood relatives eg sister, aunt etc]***	☐ Mother ☐ Father ☐ Other (specify)_____
14.6	Do you know what kind of kidney disease she or he had? If yes, specify.	☐ No ☐ Yes Specify_____

15. PHYSICAL ACTIVITY

The following questions are about the time you spend doing different types of physical activities. This includes activities you do at home, at work, travelling from place to place and during your spare time. Work can be paid or unpaid. You are requested to answer the questions even if you don't consider yourself an active person.

15.1.1	How many days do you work per week?	|__| days
15.1.2	Do you work over the weekend?	☐ Yes ☐ No

15.2. Occupation-related Physical Activity (paid or unpaid work)

The following questions have been aligned with the validated GPAQ for Physical Activity. Respondents should consider their activity during a usual week. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. In the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate. PLEASE USE THE SHOWCARDS FOR PHYSICAL ACTIVITY TO ANSWER THESE QUESTIONS

15.2.1	Does your work involve mostly sitting or standing still, or walking for very short periods (less than 10 minutes)? ***[If yes, skip to Q. 15.2.8]***	☐ Yes ☐ No
15.2.2	Does your work involve **vigorous** activities (heavy lifting, digging, manual labour or construction) for at least 10 minutes at a time? ***[If no, skip to Q.15.2.5]***	☐ Yes ☐ No
15.2.3	In a usual week, how many days are spent doing **vigorous** activities as part of your work?	|__| days
15.2.4	On a usual day of **vigorous** work, how many hours are spent doing these activities?	|__|__| hrs |__|__| mins
15.2.5	Does your work involve **moderate-intensity** activities (brisk walking or carrying light loads) for at least 10 minutes at a time? ***[If no, skip to Q.15.3]***	☐ Yes ☐ No
15.2.6	In a usual week, how many days are spent doing **moderate intensity** activities at work?	|__| days
15.2.7	On a usual work day, how many hours are spent doing **moderate-intensity** activities?	|__|__| hrs |__|__| mins
15.2.8	How long is your usual work day? ***[Please indicate how many hours you work per day on average]***	|__|__| hrs |__|__| mins

15.3. Travel-related physical activity

The following questions have been aligned with the validated GPAQ for Physical Activity. Respondents should consider their activity during a usual week. These questions exclude the physical activities at work that you have already mentioned. These questions are about the usual way you travel to and from places. For example, to work, for shopping, to market, to place of worship.

PLEASE USE THE SHOWCARDS FOR PHYSICAL ACTIVITY TO ANSWER THESE QUESTIONS

15.3.1	Do you walk or use a bicycle (for at least 10 minutes at a time) to get to and from places? ***[If no, please skip to Q.15.4.]***	☐ Yes ☐ No
15.3.2	In a usual week, how many days do you walk or cycle for at least 10 minutes to get to and from places?	|__| days
15.3.3	On a usual day, how many hours do you spend walking or cycling for travel?	|__|__|hrs |__|__| mins

15.4. Non-work related and leisure time Physical Activity

The following questions have been aligned with the validated GPAQ for Physical Activity. Respondents should consider their activity during a usual week. The next questions, exclude the work and transport activities that you have already mentioned, they are about sports, fitness and recreational activities (leisure). PLEASE USE THE SHOWCARDS FOR PHYSICAL ACTIVITY TO ANSWER THESE QUESTIONS

15.4.1	In your spare time, do you engage in any **vigorous** or **moderateintensity** physical activities lasting more than 10 minutes at a time? ***[If no, skip to Q. 15.5]***	☐ Yes ☐ No
15.4.2	In your spare time do you do any **vigorous** activities like running, strenuous sport or exercise for at least 10 minutes at a time? ***[If no, skip to Q.15.4.5]***	☐ Yes ☐ No
15.4.3	In a usual week, how many days do you engage in **vigorous** activities as part of your leisure time?	|__| days
15.4.4	In a normal day, how many leisure hours are spent doing **vigorous** activities?	|__|__| hrs |__|__| mins
15.4.5	In your spare time, do you engage in any **moderately intense** physical activities like walking or swimming for at least 10 minutes at a time? ***[If no, skip to Q.15.5]***	☐ Yes ☐ No
15.4.6	In a normal week, how many days are spent engaging in **moderately intense** physical activity as part of your leisure time?	|__| days
15.4.7	How many leisure hours are spent doing **moderate-intensity** activities in a normal day?	|__|__| hrs |__|__| mins

15.5. Sitting/Resting Activity ***The first question has been aligned with the validated GPAQ for Physical Activity. Respondents should consider their activity during a usual week. A usual day is defined as a weekday.***		
15.5.1	Over the past 7 days, how many hours did you spend sitting or reclining on a usual day (excluding sleep)? This may include time sitting at a desk, visiting friends, reading, or sitting down to watch television during working hours and leisure or spare time.	|__|__| hrs |__|__|mins
The following questions provide a better understanding of sedentary behaviour.		
15.5.2	How many hours per day do you spend **sitting**, while you are at work?	|__|__| hrs |__|__|mins
15.5.3	How many hours do you spend sitting **watching TV** per day, during the **week**?	|__|__| hrs |__|__|mins
15.5.4	How many hours per day, do you spend **watching TV** during the **weekend**?	|__|__| hrs |__|__|mins
15.5.5	How many hours per day, are spent sitting while using a **computer** outside of your normal working hours during the **week**?	|__|__| hrs |__|__|mins
15.5.6	How many hours per day are spent using a **computer** during the **weekend**?	|__|__| hrs |__|__|mins
15.5.7	How many hours per day do you spend sitting (eg. in car, bus, train) while **travelling** from place to place during the **week**?	|__|__| hrs |__|__|mins
15.5.8	How many hours per day are spent sitting while **travelling** from place to place (eg. in car, bus, train) during the **weekend**?	|__|__| hrs |__|__|mins
15.5.9	How many hours per day do you spend sitting while **socialising** during the **week**?	|__|__| hrs |__|__|mins
15.5.10	How many hours per day do you spend sitting while **socialising** over the **weekend**?	|__|__| hrs |__|__|mins
15.5.11	How many hours per day do you spend sitting while **relaxing** during the **week**?	|__|__| hrs |__|__|mins
15.5.12	How many hours per day do you spend sitting while **relaxing** during the **weekend**?	|__|__| hrs |__|__|mins

15.5.13	How many hours per day are spent sitting while at church during the **week**?	|__|__| hrs |__|__|mins
15.5.14	How many hours per day are spent sitting while at church during the **weekend** ?	|__|__| hrs |__|__|mins

16. SLEEP

The following questions relate to how much time is spent asleep per day

[Based on a 24 hour clock eg.15:30]

16.1	What time do you go to sleep during the week?	|h|h|:|m|m|
16.2	What time do you wake up during the week?	|h|h|:|m|m|
16.3	What time do you go to sleep during the weekend?	|h|h|:|m|m|
16.4	What time do you wake up during the weekend?	|h|h|:|m|m|

17.	Time at completion of questionnnaire	|h|h|:|m|m|	***[Based on a 24 hour clock eg.15:30]***

AWI-Gen H3Africa

Genomic and Environmental Risk Factors for Cardiometabolic Disease in Africans

Please place barcode sticker here	**Unique Site Identifier :** |__|__|__|__||__|__|__|

SAMPLE COLLECTION DATA

1. ANTHROPOMETRIC MEASUREMENTS		
1.1	Standing height	|__|__|__|__| mm
1.2	Weight	|__|__|__|.|__| kg
1.3	Waist circumference	|__|__|__|__| mm
1.4	Hip circumference	|__|__|__|__| mm

2. BLOOD PRESSURE		
2.1	Systolic 1	|__|__|__| mmHg
2.2	Systolic 2	|__|__|__| mmHg
2.3	Systolic 3	|__|__|__| mmHg
2.4	Diastolic 1	|__|__|__| mmHg
2.5	Diastolic 2	|__|__|__| mmHg
2.6	Diastolic 3	|__|__|__| mmHg
2.7	Time blood pressure taken in the first instance ***[Based on a 24 hour clock eg.15:30]***	| h | h |:| m | m |

3. PULSE		
3.1	Pulse 1	|__|__|__| beats/min
3.2	Pulse 2	|__|__|__| beats/min
3.3	Pulse 3	|__|__|__| beats/min
3.4	Person performing measurements	_____
3.5	Notes or comments related to measurements	

4. ULTRASOUND MEASUREMENTS		
4.1	Ultrasound	☐ Yes ☐ No
4.2	Visceral (medial) fat	|__|__|.|__|__| cm
4.3	Subcutaneous (transverse) fat	|__|__|.|__|__| cm
4.4	Ultrasound operator name	_____
4.5	Date ultrasound taken ***[eg. 27 SEP 1957]***	| d | d | | m | m | m | | y | y | y | y |
4.6	cIMT	☐ Yes ☐ No
4.7	Minimum cIMT on the right	|__|.|__|__| mm
4.8	Maximum cIMT on the right	|__|.|__|__| mm
4.9	Mean cIMT on the right	|__|.|__|__| mm
4.10	Minimum cIMT on the left	|__|.|__|__| mm
4.11	Maximum cIMT on the left	|__|.|__|__| mm
4.12	Mean cIMT on the left	|__|.|__|__| mm

4.13	Notes or comments related to ultrasound measurements	

AWI-Gen H3Africa

Genomic and Environmental Risk Factors for Cardiometabolic Disease in Africans

Please place barcode sticker here	**Unique Site Identifier:**|__|__|__|__||__|__|__|

1. BLOOD COLLECTION

1.1	Fasting blood? ***[If YES, skip to Q1.3]***	☐ Yes ☐ No
1.2	At what time did you last eat? ***[Based on a 24 hour clock eg.15:30]***	| h | h |:| m | m |
1.3	Fasting confirmed	☐ Yes ☐ No

1.4. Please complete the following table

4ml or 6ml Tubes	Number		Reason	Notes
Red	**2**	☐ Yes ☐ No	**lipid**	
Purple	**2**	☐ Yes ☐ No	**DNA extraction**	
Grey	**1**	☐ Yes ☐ No	**glucose**	

1.5	Phlebotomist Name	_____
1.6	Date blood taken ***[eg. 27 SEP 1957]***	| d | d | | m | m | m | | y | y | y | y |
1.7	Time blood taken	| h | h |:| m | m |

2. HIV		
2.1	Have you ever been tested for HIV? ***[If "no" or "don't know", skip to Q 2.5]***	☐ Yes ☐ Don't know ☐ No ☐ Refuse to answer
2.2	Do you know your status? ***[If "no" or "don't know", skip to Q.2.5.]***	☐ Yes ☐ Don't know ☐ No ☐ Refuse to answer
2.3	Have you ever tested HIV positive? ***[If "no" or "don't know", skip to Q.2.5.]***	☐ Yes ☐ Don't know ☐ No ☐ Refuse to answer

2.4	Do you use medication prescribed by a doctor, nurse, or healthcare worker to treat it?	☐ Yes ☐ Don't know ☐ No ☐ Refuse to answer
2.5	Are you currently taking any herbal or traditional remedy for HIV?	☐ Yes ☐ Don't know ☐ No ☐ Refuse to answer
2.6	Do you agree to have your blood sample tested for HIV? ***[If no, please mark Q. 2.6 as NA]***	☐ Yes ☐ No
2.6	Result	☐ Positive ☐ Negative ☐ NA

3. Test Results

Test	Result	Notes
Fasting Plasma Glucose		
Fasting Insulin		
HbA1c		
HDL		
LDL		
Triglycerides		
Total Cholesterol		

4. Urine Collection		
4.1	Urine sample taken	☐ Yes ☐ No

AWI-Gen H3Africa

Genomic and Environmental Risk Factors for Cardiometabolic Disease in Africans

Please place barcode sticker here	**Unique Site Identifier :** |__|__|__|__||__|__|__|

Appendix 5: Supplementary data

Table 1: Search Strategy

No.	Query Terms	PubMed	PMC	Scopus	Web of Science
#1 Menopause status	{Postmenopause} OR {Postmenopaus*} OR {Post-menopaus*} OR {After menopause} OR {Post menopaus*} OR {ovarian failure} OR {ovarian insufficiency} OR {Premenopaus*} OR {Pre menopaus*} OR {Before menopause} OR {Climacteric} OR {climacteric} OR {Menopause} OR {Menopause}	113,695	252,625	227,277	143,163
#2 Cardiometabolic disease	({Hypertension} OR {Blood Pressure} OR {Body Mass Index} OR {Obesity} OR {Hypercholesterol*} OR {Waist Circumference} OR {Waist-Hip Ratio} OR {Hip Circumference} OR {Intra-Abdominal Fat} OR {Intra-Abdominal Fat} OR {Abdominal Visceral Fat} OR {Subcutaneous Fat} OR {Abdominal subcutaneous Fat} OR {Body fat*} OR {Hyperlipid*} OR {Diabetes Mellitus} OR {HOMA} OR {Insulin*} OR {Glomerular Filtration Rate} OR {Chronic Kidney Disease} OR {Carotid Intima-Media Thickness} OR {Metabolic Syndrome} OR {cardiovascular disease} OR {coronary heart disease} OR {coronary artery disease} OR {cerebrovascular disease} OR {stroke} OR {cardiometabolic} OR {hypertriglycerid*} OR {hypertriglycerid*} OR {glucose*} OR {triglycerides} OR {cholesterol} OR {atherosclerosis} OR {adipos*} OR {metabolic syndrome} OR {vascular dysfunction} OR {vasoconstriction} OR {vasoconstriction} OR {visceral fat} OR {visceral adipose} OR {subcutaneous fat} OR {subcutaneous fat}) AND (LIMIT-TO (LANGUAGE , "English"))	5,480,343	2,837,193	3,667,176	3,337,457

#3 Low-middle income countries	({Afghanistan} OR {Albania} OR {Algeria} OR {american samoa} OR {angola} OR {antigua and barbuda} OR {Argentina} OR {Armenia} OR {aruba} OR {Azerbaijan} OR {Bahrain} OR {Bangladesh} OR {Barbados} OR {republic of Belarus} OR {belize} OR {benin} OR {Bhutan} OR {Bolivia} OR {bosnia and Herzegovina} OR {Botswana } OR {brazil} OR {Bulgaria} OR {burkina faso} OR {Burundi} OR {cabo verde} OR {Cambodia} OR {Cameroon} OR {central african republic} OR {chad} OR {chile} OR {china} OR {Colombia} OR {comoros} OR {congo} OR {DRC} OR {costa rica} OR {cote d'ivoire} OR {Croatia} OR {cuba} OR {cyprus} OR {czech republic} OR {Djibouti} OR {Dominican*} OR {Ecuador} OR {Egypt} OR {el Salvador} OR {equatorial guinea} OR {Eritrea} OR {Estonia} OR {Swaziland} OR {Ethiopia} OR {fiji} OR {gabon} OR {gambia} OR {Georgia} OR {ghana} OR {Gibraltar} OR {Greece} OR {grenada} OR {guam} OR {Guatemala} OR {guinea} OR {guinea Bissau} OR {Guyana} OR {Haiti} OR {Honduras} OR {hungary} OR {india} OR {Indonesia} OR {iran} OR {Iraq} OR {Jamaica} OR {Jordan} OR {Kazakhstan} OR {kenya} OR {democratic people's republic of korea} OR {republic of korea} OR {Kosovo} OR {Kyrgyzstan} OR {laos} OR {Latvia} OR {Lebanon} OR {Lesotho} OR {Liberia} OR {Libya} OR {Lithuania} OR {macau} OR {republic of north Macedonia} OR {Madagascar} OR {Malawi} OR {Malaysia} OR {indian ocean islands} OR {mali} OR {malta} OR {Micronesia} OR {palau} OR {Mauritania} OR {Mauritius} OR {Mexico} OR {Moldova} OR {Mongolia} OR {Montenegro} OR {Morocco} OR {Mozambique} OR {Myanmar} OR {Namibia} OR {Nepal} OR {netherlands Antilles} OR {Nicaragua} OR {niger} OR {Nigeria} OR {oman} OR {Pakistan} OR {Panama} OR {papua new guinea} OR {Paraguay} OR {peru} OR {Philippines} OR {Poland} OR {Portugal} OR {puerto rico} OR {Romania} OR {Russia} OR {Rwanda} OR {samoa} OR {sao tome and principe} OR {saudi arabia} OR {Senegal} OR {Serbia} OR {Seychelles} OR {sierra leone} OR {Slovakia} OR {Slovenia} OR	1,201,860	1,750,030	5,666,077	3,956,987

	{Melanesia} OR {Somalia} OR {south Africa} OR {south sudan} OR {sri lanka} OR {saint kitts and nevis} OR {saint lucia} OR {saint vincent and the grenadines} OR {sudan} OR {suriname} OR {Syria} OR {Tajikistan} OR {Tanzania} OR {Thailand} OR {timor leste} OR {togo} OR {tonga} OR {trinidad and Tobago} OR {Tunisia} OR {turkey} OR {Turkmenistan} OR {Uganda} OR {Ukraine} OR {Uruguay} OR {Uzbekistan} OR {Vanuatu} OR {Venezuela} OR {Vietnam} OR {middle east} OR {yemen} OR {Yugoslavia} OR {Zambia} OR {Zimbabwe} OR {africa south of the sahara} OR {africa, central} OR {africa, northern} OR {africa, southern} OR {africa, eastern} OR {africa, western OR west indies} OR {indian ocean islands} OR {caribbean region} OR {central America} OR {latin America} OR {south America} OR {asia, central} OR {asia, northern} OR {asia, southeastern} OR {asia, western} OR {europe, eastern} OR {developing countries}) AND (LIMIT-TO (LANGUAGE , "English"))				
#5	#1 AND #2 AND #3 AND #4	1177	1876	2095	1976

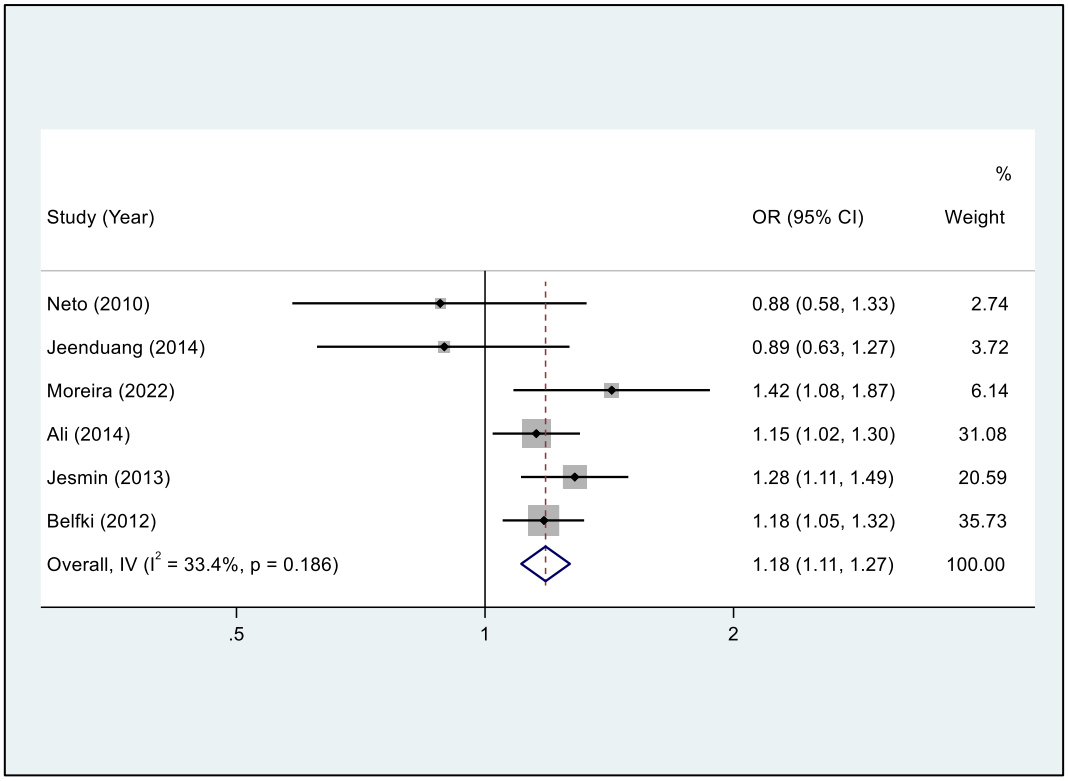


Figure 1: Post- vs premenopausal stage and metabolic syndrome. Adjusted for age, BMI, smoking, alcohol intake, physical activity, and education. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size. Metabolic syndrome as defined by the National Cholesterol Education Program (NCEP) Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (NCEP ATP III).

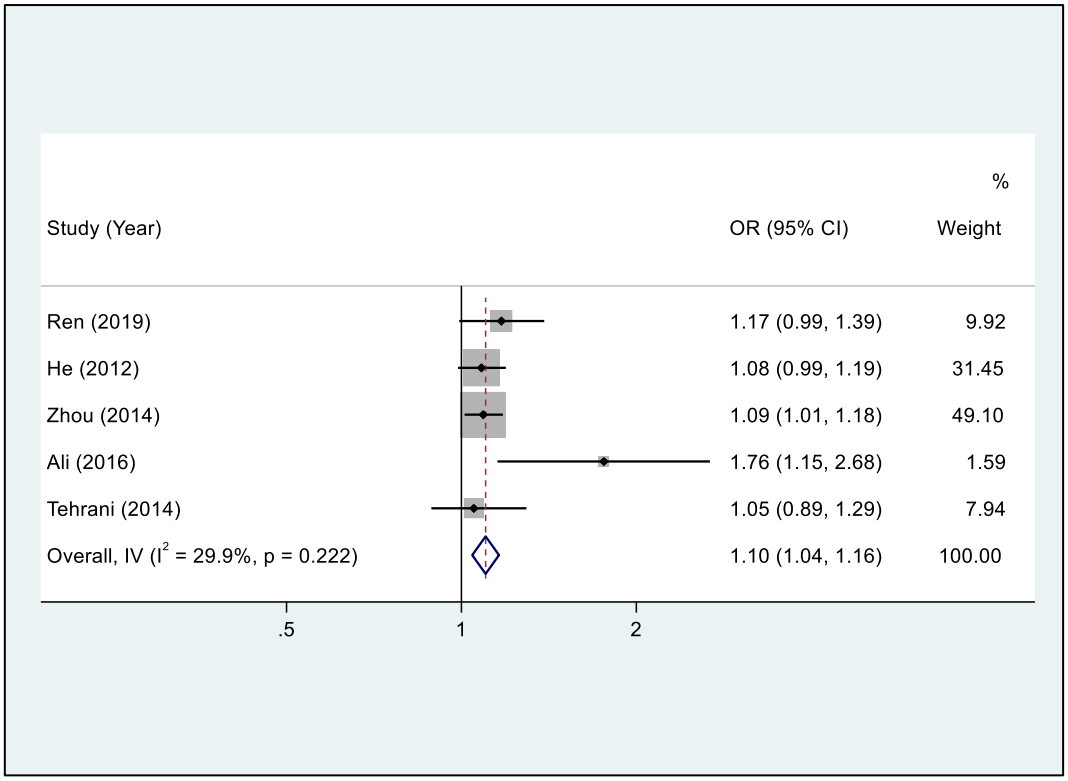


Figure 2: Post- vs premenopausal stage and hypertension. Adjusted for age, BMI, smoking, education, income, marital status, physical activity, hypercholesterolemia, high triglycerides, lipid lowering and blood pressure lowering therapy. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size. Hypertension defined as SBP ≥140 mmHg, DBP ≥90 mmHg and/or HTN medications.

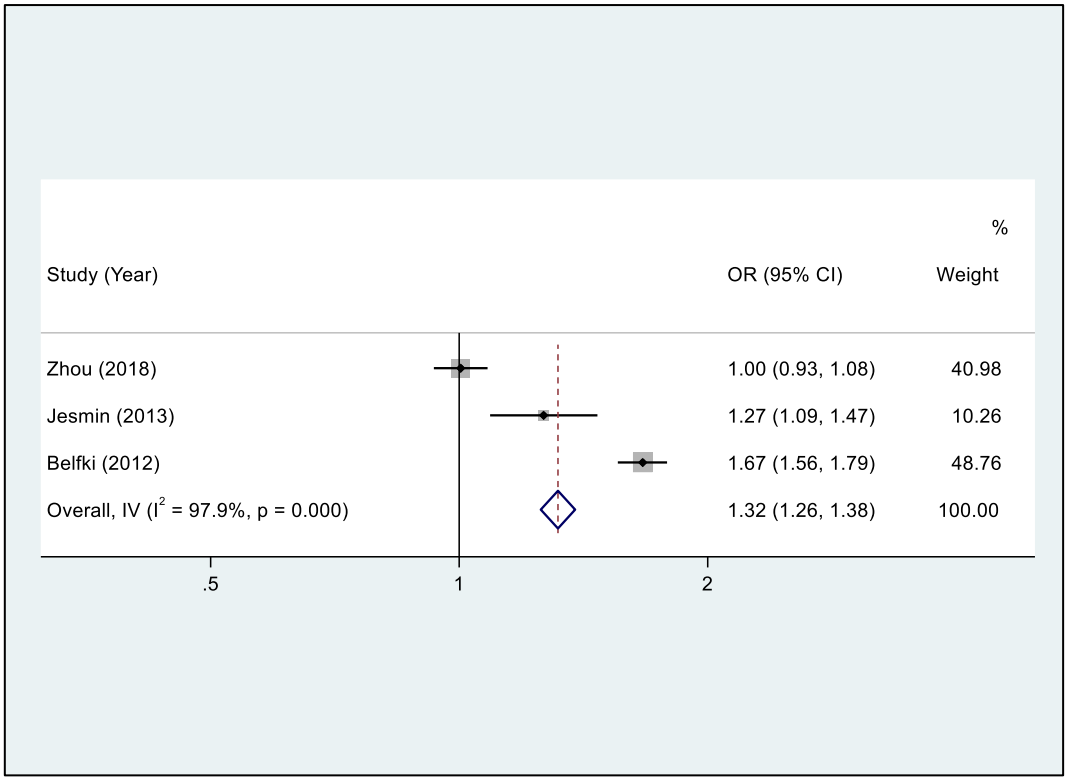


Figure 3: Post- vs premenopausal stage and hypertension. Adjusted for age, BMI, smoking, education, income, marital status, physical activity, hypercholesterolemia, high triglycerides, lipid lowering and blood pressure lowering therapy. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.Hypertension defined as SBP ≥130 mmHg, DBP ≥85 mmHg and/or HTN medications.

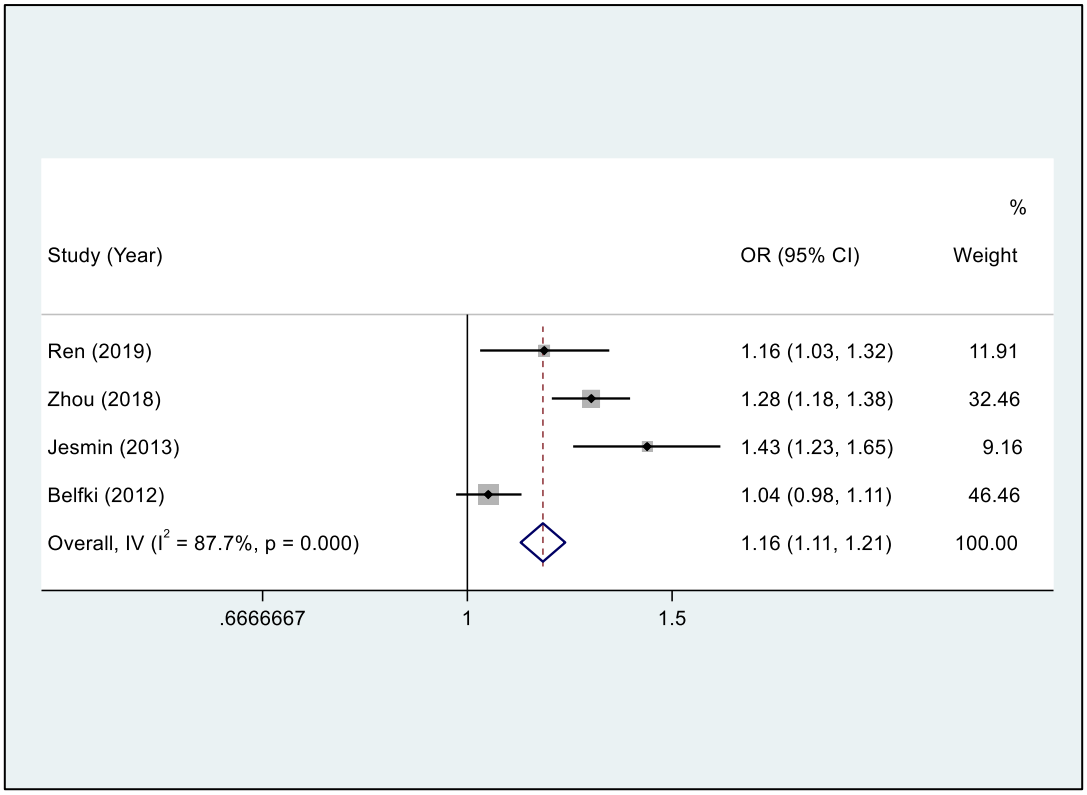


Figure 4: Post- vs premenopausal stage and high triglycerides. adjusted for age, body mass index, income, education, physical activity, ethnicity, diet score, smoking, alcohol, and sleep duration. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size. High triglycerides were defined as serum levels ≥1.69 mmol/L.

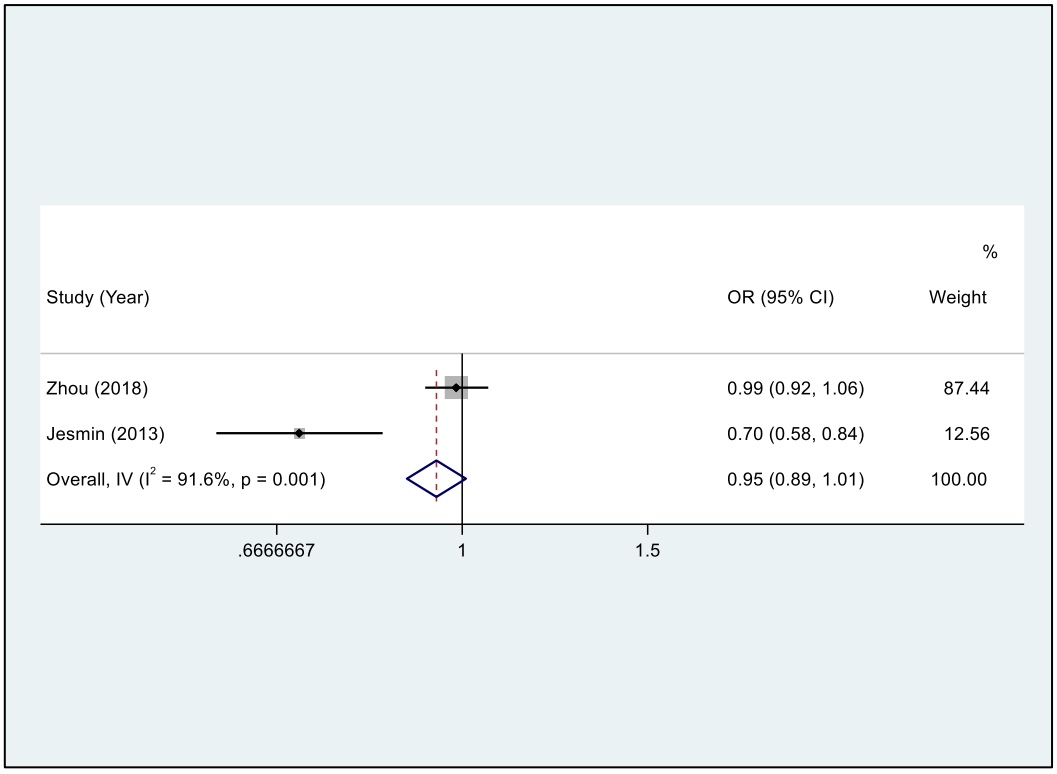


Figure 5: Post- vs premenopausal stage and low HDL-C. Adjusted for age, body mass index, income, education, physical activity, ethnicity, diet score, smoking, alcohol, and sleep duration. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size. Low HDL-C were defined as HDL-C levels <1.29 mmol/L.

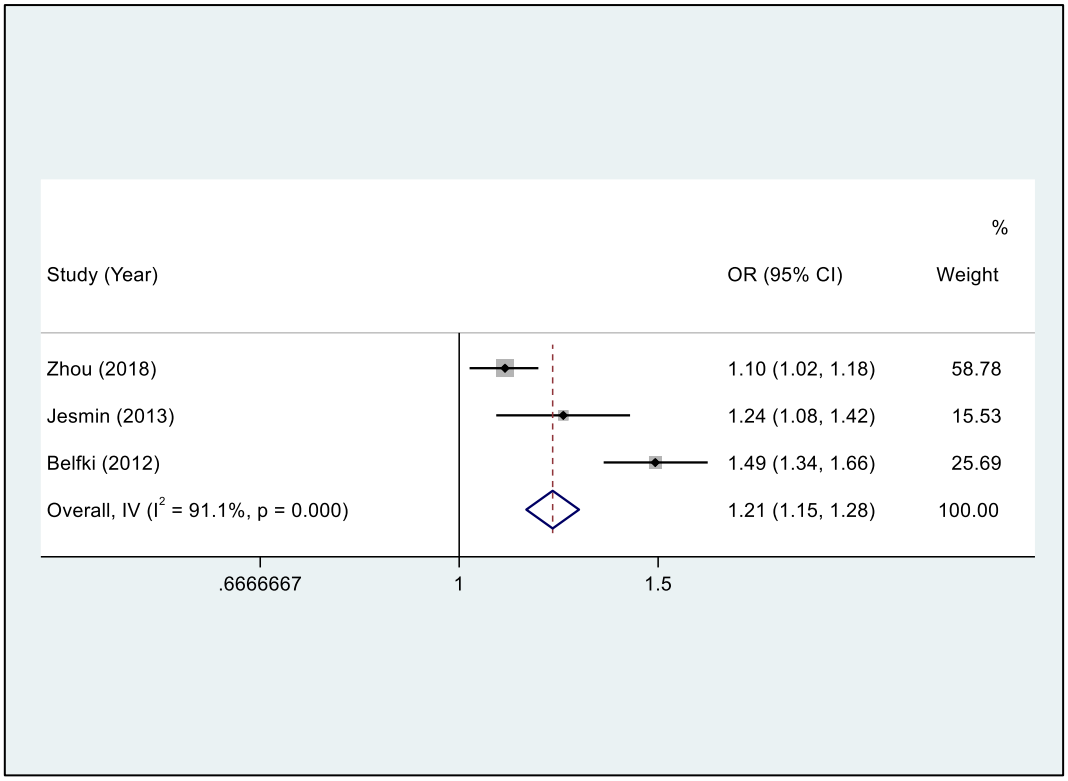


Figure 6: Post- vs premenopausal stage and elevated plasma glucose (≥6.1 mmol/L). Adjusted for age, body mass index, annual income, education status, physical activity, ethnicity, diet score, smoking status, drinking status, and sleep duration. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.

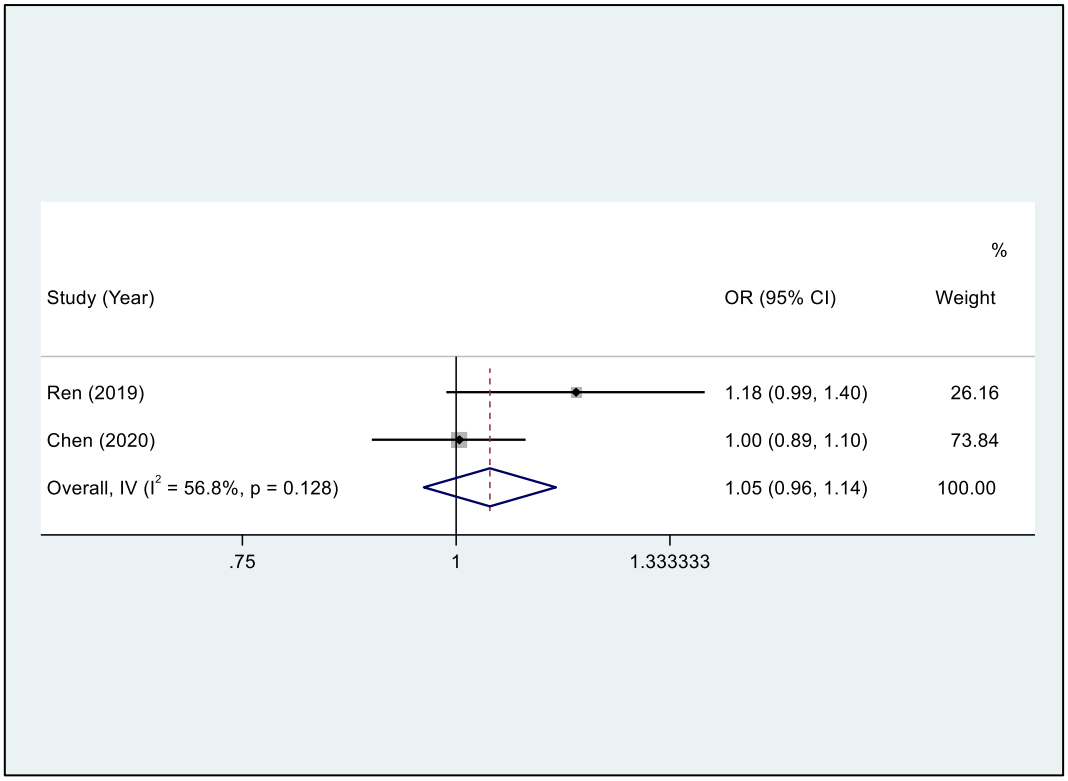


Figure 7: Post- vs premenopausal stage and obesity (BMI ≥28 kg/m²). Adjusted for age, smoking, alcohol, menarche, history of DM, education, income, physical activity, and marital status. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.

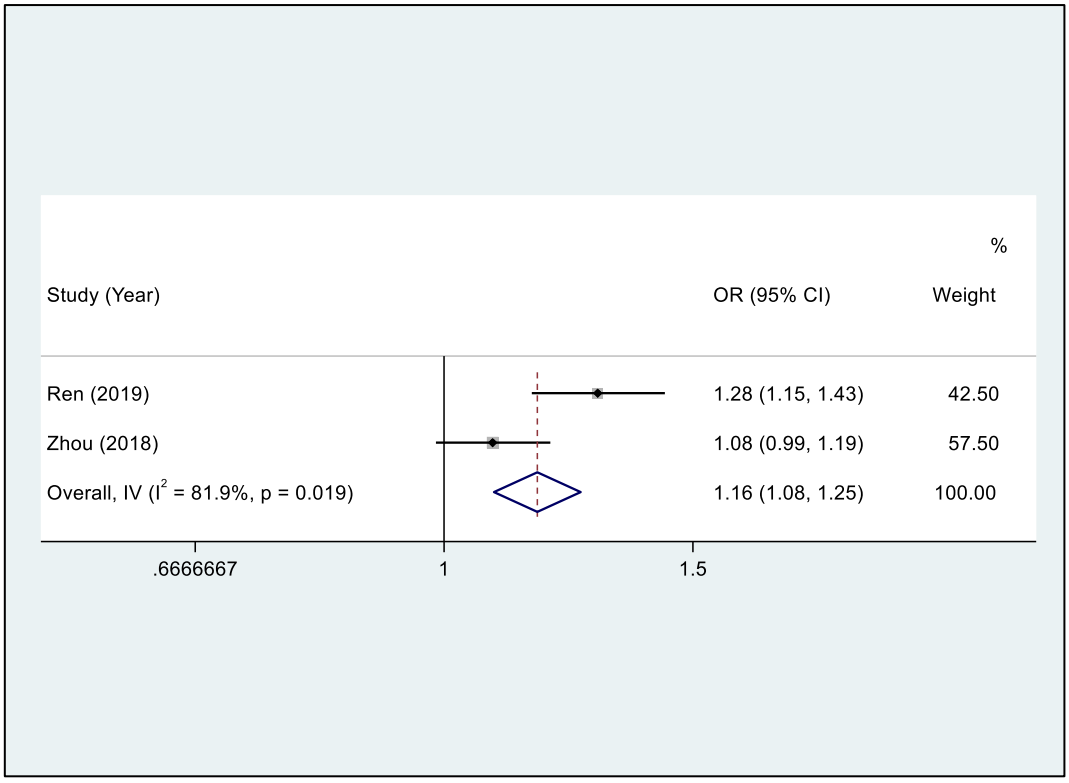


Figure 8: Post- vs premenopausal stage and high waist circumference. Adjusted for age, parity, hormonal contraceptive use, physical activity, alcohol, and smoking. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size. High waist circumference was defined as ≥80 cm.

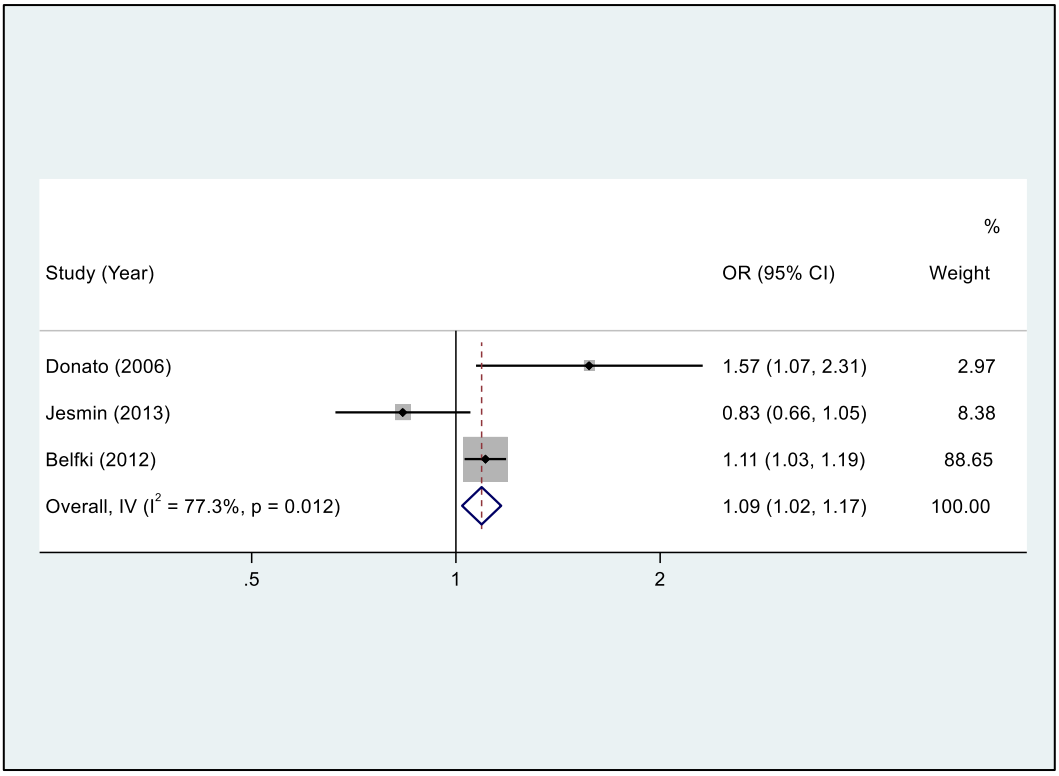


Figure 9: Post- vs premenopausal stage and high waist circumference (≥88 cm). Adjusted for age, education, income, parity, hormonal contraceptive use, physical activity, alcohol consumption, and smoking. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.

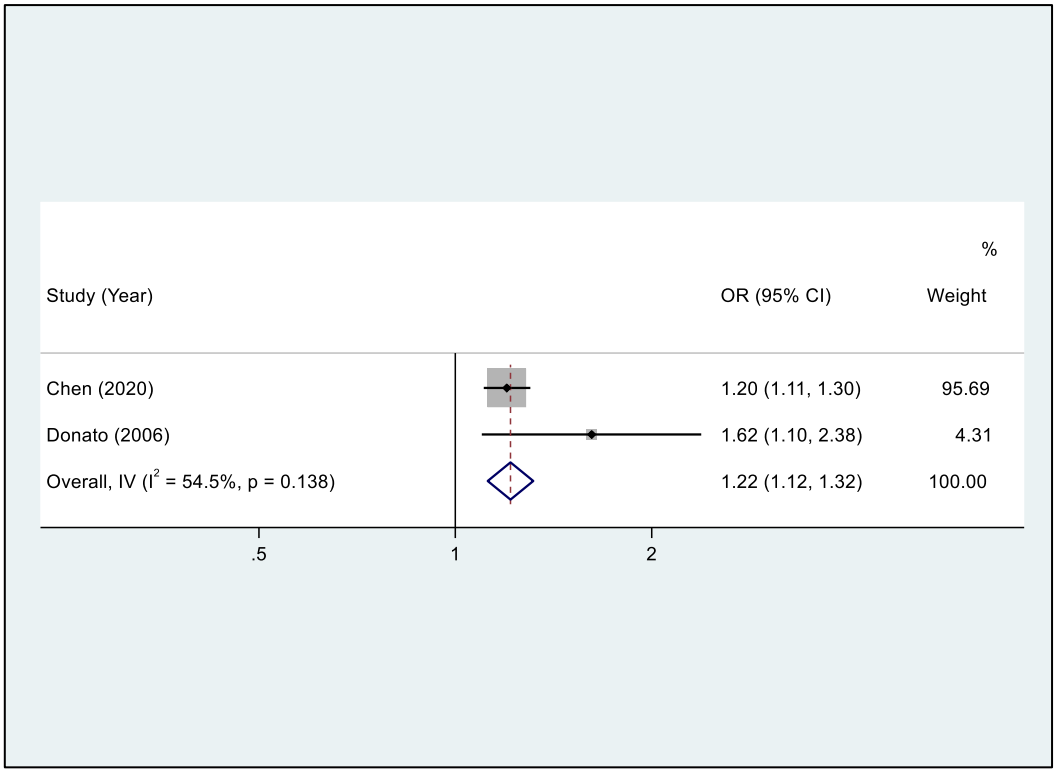


Figure 10: Post- vs premenopausal stage and high waist-hip ratio (≥0.85). Adjusted for age, education, income, parity, hormonal contraceptive use, physical activity, alcohol consumption, and smoking. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.

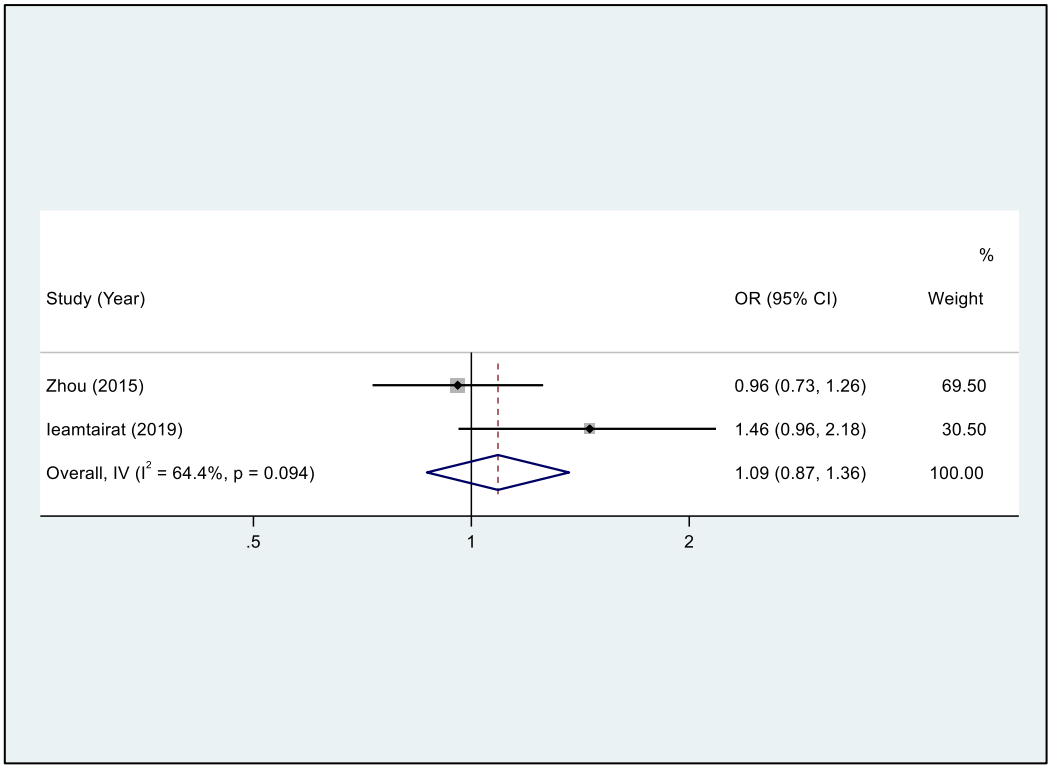


Figure 11: Post- vs premenopausal stage and high cIMT (cIMT ≥0.70mm). Adjusted for age, BMI, hypertension, dyslipidemia, diabetes mellitus, and triglyceride. Horizontal lines represent 95% confidence intervals, boxes show the study-specific weight, diamond represents combined effect size.

Table 2: Covariates used in multivariable linear regression models for each CMD risk factor.

Dependent Variable (CMD risk factor)	Independent Variable
Waist Circumference Hip Circumference Visceral Fat Subcutaneous Fat	Age, BMI, MVPA, SES, HIV status, education, smoking status, alcohol status, vegetables, fruits, soft drinks, fruit juices, bread, and study sites
BMI	Age, MVPA, SES, HIV status, education, smoking status, alcohol status, vegetables, fruits, soft drinks, fruit juices, bread, and study sites
Diastolic Blood Pressure Systolic Blood Pressure	Age, BMI, MVPA, SES, eGFR, LDL-C, HOMA-IR, HIV status, education, smoking status, alcohol status, vegetables, fruits, soft drinks, fruit juices, bread, and study sites
LDL-C Triglycerides HDL-C Glucose	Age, BMI, MVPA, SES, HOMA-IR, eGFR, HIV status, education, alcohol status, smoking status, vegetables, fruits, soft drinks, fruit juices, bread, and study sites
HOMA-IR	Age, BMI, SES, MVPA, HIV status, education, smoking status, alcohol status, vegetables, fruits, soft drinks, fruit juices, bread, and study sites
Average cIMT	Age, BMI, MVPA, LDL-C, SES, HIV status, smoking status, alcohol status, glucose, hypertension, education, vegetables, fruits, soft drinks, fruit juices bread, and study sites

LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment for insulin resistance, HIV status, human immunodeficiency virus status, cIMT:carotid media intima thickness, BMI: body mass index, MVPA: moderate-vigorous physical activity, SES: Socioeconomic status five quintiles; poorest, poorer, poor, less poor and least poor, study sites: Navrongo, Nanoro, Dikgale, Soweto and Nairobi, (Nanoro used as reference).

Table 3: Study Participants and Variables According to Menopausal Status

Variables	Premenopausal	Postmenopausal	Total
Age (years)	1740	1869	3609
BMI (kg/m²)	1736	1866	3602
Hip Circumference (cm)	1736	1863	3599
Waist Circumference (cm)	1737	1862	3599
SAT (mm)	1704	1821	3525
VAT (mm)	1701	1808	3509
Systolic BP (mmHg)	1735	1863	3598
Diastolic BP (mmHg)	1738	1862	3600
LDL-C (mmol/L)	1689	1796	3485
Triglycerides (mmol/L)	1707	1817	3524
HDL-C (mmol/L)	1706	1819	3525
Glucose (mmol/L)	1694	1804	3498
HOMA	1694	1804	3498
eGFR (ml/min/1.73m²)	1740	1869	3609
cIMT average (mm)	1337	1299	2636

Data expressed as number of study participants available for each variable; BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure, HOMA, homeostatic model for assessment of insulin resistance, eGFR, estimated glomerular filtration rate, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 4: Regional differences in anthropometric and cardiometabolic risk factors in women by menopausal status across the AWI-Gen study centres

Variable	Premenopausal Women				Postmenopausal Women			
	West Africa	East Africa	South Africa	P value	West Africa	East Africa	South Africa	P value
Age (years)	47.5±5.5	46.6±4.9	48.0±5.6	<0.001	51.2±5.61	51.5± 5.6	51.1±5.8	<0.001
Systolic BP (mmHg)	115.5 (24.5)	114.5 (25.0)	119.5 (27.5)	<0.001	119.0 (29.5)	120.8 (29.5)	122.5 (27.5)	<0.001
Diastolic BP (mmHg)	76.9±12.8	78.0±13.2	80.6±13.8	<0.001	79.2±13.4	80.6±13.8	82.2±13.7	<0.001
BMI (kg/m²)	23.1 (9.4)	25.6 (10.2)	27.5 (11.6)	<0.001	23.6 (10.5)	26.4 (11.5)	27.8 (12.4)	<0.001
Waist Circumference (cm)	83.8±14.8	87.5±15.3	90.1±16.2	<0.001	85.8±15.7	89.3±15.9	90.8±16.3	<0.001
LDL-C (mmol/L)	2.14±0.81	2.33±0.88	2.40±0.91	<0.001	2.31±0.93	2.52±0.92	2.51±0.91	<0.001
Triglycerides (mmol/L)	0.71 (0.49)	0.79 (0·54)	0.84 (0.61)	<0.001	0.79 (0.59)	0.90 (0.59)	0.90 (0.59)	<0.001
HDL-C (mmol/L)	1.10 (0.36)	1.12 (0.43)	1.12 (0.43)	<0.001	1.10 (0.42)	1.14 (0.44)	1.14 (0.43)	<0.001
HOMA-IR	0.97 (1·97)	1.10 (2·0)	1.18 (1.90)	<0.001	1·09 (2.23)	1·22 (2.04)	1·26 (2.00)	<0.001

Data expressed as mean±standard deviation or median (interquartile range), BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, Systolic BP,systolic blood pressure, Diastolic BP, diastolic blood pressure, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol and HOMA-IR (homeostatic model assessment insulin resistance index). Significance testing performed using Kruskal-Wallis (non-parametric data) or one-way ANOVA (parametric data) tests, comparing women of the same menopausal stage between the three regions.

Table 5: Comparison of age, anthropometry and cardiometabolic risk factors between pre- and postmenopausal women in Dikgale

Variables	Premenopause	Postmenopause	p value
Age	45.0±4.0	54.1±4.4	<0.001
Systolic BP (mmHg)	118.5 (19)	125.5 (28.5)	<0.001
Diastolic BP (mmHg)	80.2±12.5	83.2±13.6	0.008
Height (m)	1.60±0.1	1.58±0.1	0.004
Weight (kg)	80.2± 21.0	76.1±20.4	0.02
BMI (kg/m^2)	30.5 (10.7)	29.6 (10.0)	0.14
Hip Circumference (cm)	110.7±15.1	108.1±16.2	0.04
Waist Circumference (cm)	93.8±16.7	94.4±16.3	0.65
VAT (mm)	6.65 (3.42)	6.78 (3.40)	0.56
SAT (mm)	2.38 (1.47)	2.13 (1.30)	0.01
Total Cholesterol (mmol/L)	3.88±1.02	4.36±1.18	<0.001
LDL-C (mmol/L)	2.38±0.83	2.65±0.92	<0.001
Triglycerides (mmol/L)	0.84 (0.47)	1.06 (0.73)	<0.001
HDL-C (mmol/L)	1.12±0.32	1.19±0.35	0.02
Glucose (mmol/L)	4.78 (0.78)	4.93 (1.07)	0.002
HOMA-IR	1.35 (1.40)	1.69 (2.06)	0.06
cIMT average (mm)	0.58 (0.12)	0.64 (0.14)	<0.001

Data expressed as mean±standard deviation or median (interquartile range); Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 6: Comparison of age, anthropometry and cardiometabolic risk factors between pre- and postmenopausal women in Soweto

Variables	Premenopause	Postmenopause	p value
Age	44.7±3.6	52.3±5.0	<0.001
Systolic BP (mmHg)	125 (23)	132 (28)	<0.001
Diastolic BP (mmHg)	86.7±12.8	89.4±12.3	<0.001
Height (m)	1.59±0.06	1.58±0.06	0.02
Weight (kg)	83.6±18.8	82.7±19.4	0.49
BMI (kg/m²)	32.9 (8.8)	32.8 (9.1)	0.95
Hip Circumference (cm)	118.2±15.8	118.3±14.8	0.90
Waist Circumference (cm)	98.0±14.7	99.6±14.2	0.13
Visceral Fat (mm)	4.80 (2.39)	4.69 (2.37)	0.81
Subcutaneous Fat (mm)	3.11(1.44)	3.14(1.29)	0.66
Total Cholesterol (mmol/L)	4.29±0.98	4.69±1.10	<0.001
LDL-C (mmol/L)	2.58±0.87	2.91±0.91	<0.001
Triglycerides (mmol/L)	0.91 (0.60)	1.10 (0.64)	<0.001
HDL-C (mmol/L)	1.20 (0.46)	1.20 (0.40)	0.90
Glucose (mmol/L)	4.70 (0.73)	4.80 (0.8)	<0.001
HOMA-IR	1.29 (1.70)	1.38 (1.83)	0.54

Data expressed as mean±standard deviation or median (interquartile range); Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance.

Table 7: Comparison of age, anthropometry and cardiometabolic risk factors between pre- and postmenopausal women in Nanoro

Variables	Premenopause	Postmenopause	p value
Age	44.9±3.5	53.2±4.6	<0.001
Systolic BP (mmHg)	104 (16.5)	112 (25)	<0.001
Diastolic BP (mmHg)	69.5±9.3	72.3±10.3	<0.001
Height (m)	1.62±0.1	1.61±0.1	0.003
Weight (kg)	55.2±9.6	51.7±8.9	<0.001
BMI (kg/m^2)	20.3 (3.7)	19.3 (3.6)	<0.001
Hip Circumference (cm)	90.1±7.5	87.5±7.8	<0.001
Waist Circumference (cm)	75.7±7.8	76.0±8.3	0.71
Visceral Fat (mm)	4.33 (1.58)	4.13 (1.44)	0.02
Subcutaneous Fat (mm)	0.85(0.62)	0.95(0.56)	0.02
Total Cholesterol (mmol/L)	3.00±0.79	3.34±0.86	<0.001
LDL-C (mmol/L)	1.68±0.62	1.85±0.63	0.001
Triglycerides (mmol/L)	0.61 (0.33)	0.73 (0.40)	<0.001
HDL-C (mmol/L)	0.98 (0.34)	1.07 (0.42)	<0.001
Glucose (mmol/L)	4.85 (0.73)	4.91 (0.93)	0.51
HOMA-IR	0.83 (2.53)	0.88 (2.29)	0.95
cIMT average (mm)	0.60 (0.12)	0.65(0.15)	<0.001

Data expressed as mean±standard deviation or median (interquartile range); Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 8: Comparison of age, anthropometry and cardiometabolic risk factors between pre- and postmenopausal women in Navrongo

Variables	Premenopause	Postmenopause	p value
Age	47.3±4.8	54.1±4.6	<0.001
Systolic BP (mmHg)	115 (22)	123 (28)	<0.001
Diastolic BP (mmHg)	75.5±11.4	78.6±13.0	0.002
Height (m)	1.59±0.1	1.57±0.1	<0.001
Weight (kg)	57.9±10.4	53.2±11.5	<0.001
BMI (kg/m²)	22.3 (4.4)	20.7 (4.4)	<0.001
Hip Circumference (cm)	90.8±9.7	87.8±9.5	<0.001
Waist Circumference (cm)	77.2±7.2	76.3±8.3	0.25
Visceral Fat (mm)	3.29 (1.38)	3.36 (1.25)	0.46
Subcutaneous Fat (mm)	0.98 (0.73)	1.03 (0.70)	0.22
Total Cholesterol (mmol/L)	3.14±0.85	3.30±0.98	0.02
LDL-C (mmol/L)	1.83±0.66	2.02±0.73	0.001
Triglycerides (mmol/L)	0.52 (0.27)	0.59 (0.37)	<0.001
HDL-C (mmol/L)	1.07 (0.41)	1.08 (0.43)	0.89
Glucose (mmol/L)	4.53 (0.71)	4.58 (0.77)	0.11
HOMA-IR	0.62 (1.29)	0.68 (1.19)	0.29
cIMT average (mm)	0.63 (0.12)	0.7 (0.14)	<0.001

Data expressed as mean±standard deviation or median (interquartile range); Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 9: Comparison of age, anthropometry and cardiometabolic risk factors between pre- and postmenopausal women in Nairobi

Variables	Premenopause	Postmenopause	p value
Age	45.0±3.4	52.0±5.0	<0.001
Systolic BP (mmHg)	111 (23.5)	117 (28.5)	<0.001
Diastolic BP (mmHg)	76.6±12.5	80.2±13.6	<0.001
Height (m)	1.59±0.06	1.57±0.06	<0.001
Weight (kg)	69.8±15.6	68.5±14.8	0.22
BMI (kg/m^2)	26.6 (8.4)	26.9 (8.7)	0.54
Hip Circumference (cm)	102.3±11.7	102.0±12.2	0.76
Waist Circumference (cm)	89.1±14.0	91.7±13.9	0.01
Visceral Fat (mm)	4.60 (2.42)	4.68 (2.24)	0.98
Subcutaneous Fat (mm)	1.96 (0.99)	1.96 (0.89)	0.88
Total Cholesterol (mmol/L)	4.21±1.00	4.60±1.15	<0.001
LDL-C (mmol/L)	2.56±0.82	2.83±0.90	<0.001
Triglycerides (mmol/L)	0.90 (0.57)	0.98 (0.53)	0.004
HDL-C (mmol/L)	1.16 (0.45)	1.17 (0.45)	0.19
Glucose (mmol/L)	4.98 (0.93)	5.20 (0.93)	<0.001
HOMA-IR	1.09 (1.90)	1.44 (2.34)	0.02
cIMT average (mm)	0.56 (0.12)	0.61 (0.15)	<0.001

Data expressed as mean±standard deviation or median (interquartile range); Systolic BP, systolic blood pressure, Diastolic BP, diastolic blood pressure BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 10: Univariate and multivariate linear regression models showing relationship of menopausal status with CMD risk factors for individual sites

Dependant Variable	Site	Menopausal stage (unadjusted)*	Menopausal stage (adjusted)*
Waist Circumference (cm)	Dikgale	0.62 (-2.02; 3.25)	0.52 (-1.12; 2.17)
	Nairobi	2.59 (0.702; 4.48)	1.18 (-0.11; 2.47)
	Nanoro	0.24 (-1.00; 1.48)	1.54 (0.63; 2.45)
	Navrongo	-0.88 (-2.38; 0.63)	1.06 (0.07; 2.06)
	Soweto	1.53 (-0.46; 3.53)	-0.08 (-3.28; 3.12)
Hip Circumference	Dikgale	-2.68 (-5.22; -0.16)	-0.03 (-1.47; 1.41)
	Nairobi	-0.25 (-1.86; 1.37)	-0.27 (-1.21; 0.67)
	Nanoro	-2.40 (-3.51; -1.28)	-0.60 (-1.36; 0.16)
	Navrongo	-3.19 (-4.61; -1.76)	-0.45 (-1.518; 0.63)
	Soweto	0.14 (-1.92; 2.19)	-0.44 (-1.80; 0.93)
Visceral Fat (log; mm)	Dikgale	0.02 (-0.04; .074)	0.003 (-0.06; 0.06)
	Nairobi	-0.003 (-0.05; 0.05)	-0.03 (-0.08; 0.03)
	Nanoro	-0.05 (-0.09; -0.01)	-0.06 (-0.12; -0.004)
	Navrongo	0.02 (-0.03; 0.06)	0.03 (-0.02; 0.08)
	Soweto	0.01 (-0.05; 0.07)	-0.001 (-0.08; 0.08)
Subcutaneous Fat (log;mm)	Dikgale	-0.09 (-0.17; 0.0001)	-0.0004 (-0.09; 0.08)
	Nairobi	0.09 (-0.04; 0.07)	0.04 (-0.01; 0.09)
	Nanoro	0.09 (0.02; 0.16)	0.13 (0.05; 0.21)
	Navrongo	0.05 (-0.03; 0.12)	0.14 (0.08; 0.20)
	Soweto	0.04 (-0.02; 0.09)	-0.03 (-0.11; 0.04)
BMI (log; kg/m²)	Dikgale	-0.03 (-0.08; 0.01)	-0.002 (-0.06; 0.05)
	Nairobi	0.01 (-0.02; 0.04)	0.004 (-0.03; 0.04)
	Nanoro	-0.05 (-0.07; -0.03)	-0.03 (-0.07; -0.004)
	Navrongo	-0.06 (-0.09; -0.04)	-0.04 (-0.07; -0.01)
	Soweto	-0.001 (-0.03; 0.03)	-0.002 (-0.05; 0.05)
Diastolic Blood Pressure (mmHg)	Dikgale	2.88 (0.77; 5.00)	1.10 (-2.22; 4.43)
	Nairobi	3.61 (1.85; 5.38)	-0.95 (-3.21; 1.30)
	Nanoro	2.85 (1.34; 4.36)	2.62 (0.55; 4.69)
	Navrongo	3.04 (1.15; 4.93)	3.26 (0.92; 5.61)
	Soweto	2.62 (0.88; 4.36)	0.69 (-1.93; 3.30)
Systolic Blood Pressure (log; mmHg)	Dikgale	0.06 (0.04; 0.09)	0.02 (-0.02; 0.05)
	Nairobi	0.05 (0.03; 0.07)	-0.03 (-0.06; -0.003)
	Nanoro	0.07 (0.05; 0.09)	0.02 (-0.001; 0.06)
	Navrongo	0.06 (0.04; 0.09)	0.05 (0.02; 0.08)
	Soweto	0.06 (0.03; 0.08)	-0.003 (-0.04; 0.03)
LDL-C (mmol/L)	Dikgale	0.30 (0.15; 0.43)	0.16 (-0.03; 0.36)
	Nairobi	0.27 (0.15; 0.38)	0.09 (-0.06; 0.23)
	Nanoro	0.17 (0.07; 0.26)	0.16 (0.01; 0.30)
	Navrongo	0.18 (0.07; 0.29)	0.14 (0.14; 0.28)
	Soweto	0.34 (0.21; 0.47)	0.15 (-0.03; 0.33)
Triglycerides (log; mmol/L)	Dikgale	0.27 (0.20; 0.35)	0.20 (0.09; 0.31)
	Nairobi	0.09 (0.03; 0.15)	-0.03 (-0.10; 0.05)
	Nanoro	0.16 (0.10; 0.22)	0.09 (-0.01; 0.18)
	Navrongo	0.13 (0.06; 0.21)	0.11 (0.03; 0.20)
	Soweto	0.13 (0.07; 0.20)	0.003 (-0.09; 0.101)
Glucose (Reciprocal, mmol/L)	Dikgale	-0.01 (-0.02; -0.002)	0.004 (-0.01; 0.01)
	Nairobi	-0.01 (-0.01; -0.004)	0.002 (-0.01; 0.01)
	Nanoro	-0.003 (-0.01; 0.002)	-0.003 (-0.01; 0.004)
	Navrongo	-0.004 (-0.01; 0.001)	-0.01 (-0.01; -0.002)
	Soweto	-0.01 (-0.01; -0.003)	0.01 (-0.0004; 0.01)
Average cIMT (log;mm)	Dikgale	0.10 (0.07; 0.12)	-0.01 (-0.04; 0.03)
	Nairobi	0.07 (0.05; 0.10)	-0.0001 (-0.03; 0.03)

	Nanoro Navrongo	0.10 (0.08; 0.12) 0.10 (0.07; 0.12)	0.01 (-0.03; 0.04) 0.05 (0.02; 0.08)
HDL-C (mmol/L)	Dikgale Nairobi Nanoro Navrongo Soweto	0.06 (0.003; 0.11) 0.03 (-0.01; 0.08) 0.11 (0.06; 0.16) -0.02 (-0.07; 0.03) 0.01 (-0.03; 0.05)	0.04 (-0.03; 0.11) -0.01 (-0.07; 0.05) 0.03 (-0.04; 0.11) -0.03 (-0.09; 0.04) -0.003 (-0.06; 0.06)
HOMA-IR (log)	Dikgale Nairobi Nanoro Navrongo Soweto	0.11 (-0.05; 0.27) 0.18 (0.02; 0.34) -0.01 (-0.24; 0.21) 0.08 (-0.13; 0.28) 0.05 (-0.09; 0.20)	0.12 (-0.12; 0.37) 0.14 (-0.06; 0.34) 0.14 (-0.19; 0.47) 0.15 (-0.09; 0.38) -0.07 (-0.28; 0.14)

Data is unstandardised ß (95% confidence interval); *post- vs premenopause. BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT.

Table 11: Reference intervals for sex hormone and albumin levels

	Median	5-95th percentiles
E2 (pmol/L)		
Follicular phase	196	45.4–854
Ovulation phase	462	151–1461
Luteal phase	370	81.9–1251
Postmenopause	<18.4	< 18.4–505
FSH (mIU/L)		
Follicular phase	6.9	3.5–12.5
Ovulation phase	12.3	4.7–21.5
Luteal phase	3.6	1.7–7.7
Postmenopause	67.0	25.8–134.8
Total Testosterone (pmol/L)		
20–49 years	941	290–1670
≥ 50 years	563	101–1420
Free testosterone (pmol/L)		
Premenopausal	–	2.8–31.9
Postmenopausal	–	2.1–23.2
Bioavailable testosterone (pmol/L)		
Premenopausal	–	66.0–79.0
Postmenopausal	–	55.0–66.0
FAI		
20–50 years	–	0.18–7.07
≥ 50 years	–	0.10–4.95
SHBG (nmol/L)		
(20-49 years)	67.8	32.4–128
(≥ 50 years)	62.4	27.1–128
DHEAS (µmol/L)		
45–54	3.28	0.96–6.95
55–64	2.08	0.51–5.56
Albumin (g/L)	44.3	39.7–49.4

E2, estradiol, FSH, follicle stimulating hormone, FAI, free androgen index, SHBG, sex-hormone binding globulin, DHEAS, dehydroepiandrosterone sulphate.

Table 12: Covariates used in multivariable linear regression models for each CMD risk factor.

Dependent Variable (CMD risk factor)	Independent Variable
Waist Circumference Hip Circumference Visceral Fat Subcutaneous Fat	Age, HIV status, menopausal status, BMI, MVPA, SES, Education, Smoking status, Study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week.
BMI	Age, HIV status, menopausal status, MVPA, SES, Education, Smoking status, Study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week
Diastolic Blood Pressure Systolic Blood Pressure	Age, HIV status, menopausal status, BMI, MVPA, SES, eGFR, LDL-C, HOMA-IR, Education, Smoking status, Study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week
LDL-C Triglycerides HDL-C Glucose	Age, HIV status, menopausal status, BMI, MVPA, SES, HOMA-IR, eGFR, Education, Smoking status, Study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week
HOMA-IR	Age, HIV status, menopausal status, BMI, SES, MVPA, Education, Smoking status, Study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week
Average cIMT	Age, HIV status, menopausal status, BMI, MVPA, LDL-C, SES, smoking status, alcohol status, glucose, hypertension, education, study sites, fruits and vegetables, bread, soft drinks, and fruit juices per week

LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment for insulin resistance, ART status, cIMT:carotid media intima thickness, BMI: body mass index, MVPA: moderate-vigorous physical activity, SES: Socioeconomic status five quintiles; poorest, poorer, poor, less poor and least poor, study sites: Dikgale, Soweto and Nairobi, (Dikgale used as reference), HIV status: WLWOH, WLWOH on HAART, WLWOH HAART naïve (WLWOH used as reference), menopausal status: pre- and postmenopausal (premenopause used as reference).

Table 13: Comparison of HIV status and years since menopause quartiles by multivariable linear regression

	HIV status† (unadjusted)	**HIV status† (adjusted)**
Years after FMP	**ß (95 % C.I)**	**ß (95 % C.I)**
≤3 y	–2.7 (–3.8; –1.6)***	–2.2 (<–3.3; –1.0)***
>3 to ≤6y	–0.8 (–2.5; 1.0)	–0.8 (–2.6; 1.1)
>6 to ≤9y	–0.5 (–2.7; 1.7)	–0.4 (–3.2; 2.3)
>9y	–1.0 (–2.6; 0.6)	–1.4 (–2.7; –0.01)*
Combined	–1.5 (–2.4; –0.7)***	–1.6 (–2.3; –0.7)***

Data is unstandarised ß (95% Confidence Interval); † HIV status (WLWH vs WLWOH), *p<0.05, **p<0.01, ***p<0.001 Adjusted for BMI, physical activity, socioeconomic status, level of education, smoking status, study sites, Nairobi, Soweto and Dikgale (Dikgale as reference).

Appendix 6: Publication

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Original article

Cardiometabolic disease risk factors in pre- and postmenopausal women from four sub-Saharan African countries: A cross-sectional study

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ABSTRACT

Objective: To compare the risk factors for cardiometabolic disease between pre- and postmenopausal women from four sub-Saharan African countries.
Study design: This cross-sectional study included 3609 women (1740 premenopausal and 1869 postmenopausal) from sites in Ghana (Navrongo), Burkina Faso (Nanoro), Kenya (Nairobi), and South Africa (Soweto and Dikgale). Demographic, anthropometric and cardiometabolic variables were compared between pre- and postmenopausal women, within and across sites using multivariable regression analyses. The sites represent populations at different stages of the health transition, with those in Ghana and Burkina Faso being rural, whilst those in Kenya and South Africa are more urbanised.
Main outcome measures: Anthropometric and cardiometabolic variables.
Results: The prevalence rates of risk factors for cardiometabolic disease were higher in South (Soweto and Dikgale) and East (Nairobi) Africa than in West Africa (Nanoro and Navrongo), irrespective of menopausal status. Regression models in combined West African populations demonstrated that postmenopausal women had a larger waist circumference (β = 1.28 (95 % CI: 0.58; 1.98) cm), log subcutaneous fat (β =0.15 (0.10; 0.19)), diastolic (β = 3.04 (1.47; 4.62) mm Hg) and log systolic (β = 0.04 (0.02; 0.06)) blood pressure, log carotid intima media thickness (β = 0.03 (0.01; 0.06)), low-density lipoprotein cholesterol (β = 0.14 (0.04; 0.23) mmol/L) and log triglyceride (β= 0.10 (0.04; 0.16)) levels than premenopausal women. No such differences were observed in the South and East African women.
Conclusions: Menopause-related differences in risk factors for cardiometabolic disease were prominent in West but not East or South African study sites. These novel findings should inform cardiometabolic disease prevention strategies in midlife women specific to rural and urban and peri-urban locations in sub-Saharan Africa.

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1. Introduction

Sub-Saharan Africa (SSA) is experiencing a health transition [1] characterised by an increased life expectancy that is associated with a growing population of peri- and postmenopausal women [2]. This parallels a rising prevalence of cardiometabolic diseases (CMDs), including obesity [3,4], hypertension [4], and diabetes mellitus [5] within the SSA region.

Data from a number of studies have demonstrated anthropometric and cardiometabolic differences between pre- and postmenopausal women, with CMD risk increasing on the approach of menopause [6–8]. The menopause transition is associated with shifts in body fat distribution, particularly increased deposits of abdominal fat, independent of body mass index (BMI) [6]. Although the association of CMD risk factors with menopause stage has been studied in detail in high income countries, only a small number of such studies have been conducted in SSA. These include cross-sectional investigations in pre- and postmenopausal women from South Africa [9,10], Ghana [11], and the Democratic Republic of Congo (DRC) [12]. These studies included premenopausal women with a wide age range (from 20 years onwards) [10,11] and modest sample sizes: 590 (South Africa) [9], 206 (South Africa) [10], 250 (Ghana) [11], and 200 (DRC) [12].

Therefore, the aim of the current study was to measure and compare CMD risk factors between pre- and postmenopausal women from four SSA countries i.e., Ghana, Burkina Faso, Kenya, and South Africa. Study participants were from the Africa-Wits International Network for the Demographic Evaluation of Populations and Their Health (INDEPTH) Partnership for Genomic studies (AWI-Gen) [13] which allowed us to perform the first pan-SSA analyses of the relationship between menopause stage and CMD risk factors. Furthermore, the populations within AWI-Gen arose from different demographic strata varying from rural subsistence farmers to more urbanised communities and therefore included SSA populations at different stages of the health transition [14]. This allowed us to determine if any differences observed in CMD risk factors between pre- and postmenopausal women were similar across SSA populations with varying health and demographic profiles. Our hypothesis was that the same biological processes in all populations drive the increase in CMD risk that is observed during the menopause transition and therefore the prevalence of CMD risk factors would be higher in post- than premenopausal women at all the study sites irrespective of differences in sociodemographic factors.

2. Methods

2.1. Study design and setting

This cross-sectional population-based study on midlife women is a sub-study of the AWI-Gen study [13] from five different sites across four African countries namely: South Africa (Dikgale and Soweto), East Africa (Nairobi, Kenya) and West Africa (Nanoro, Burkina Faso, and Navrongo, Ghana) (Fig. 1). These sites included a mixture of urban (Soweto and Nairobi), peri urban (Dikgale) and rural (Nanoro and Navrongo) environments. In the main study, recruitment was performed using random sampling in the general population of women aged 40–60 years at each of the study centres [13]. The recruitment target for each site was at least 1000 women, thus 5184 women were recruited for this study.

Using a questionnaire, data on demography, behaviour, and health history were collected from all study participants. Menopause was staged by asking each woman about her menstrual bleeding patterns. The premenopausal group was then defined as women with regular menstrual cycles, the perimenopausal group was those having irregular menstrual cycles within the past 12 months, whilst the postmenopausal group was those who had had amenorrhea for twelve consecutive months or more. A total of 1575 women had unknown menopausal stage, were perimenopausal, on hormonal therapy or were <40 and >60 years of age and were excluded resulting in 3609 women being included in the final analyses. Perimenopausal women were excluded to

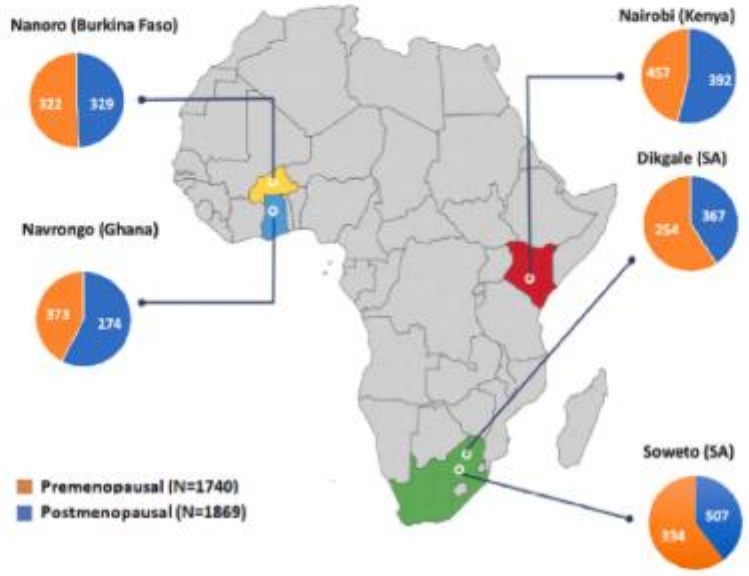

Fig. 1. Distribution of women according to menopausal stage across the five AWI-Gen Study Centres. Adapted from Ali et al. [13].

effectively separate individuals who had experienced the menopause transition and those that had not.

The recruitment process and collection of anthropometric measurements by trained staff has been detailed previously [14]. Waist, and hip circumference measurements were taken using a tape measure (SECA, Hamburg, Germany). Height was measured using a Harpenden digital stadiometer (Holtain, Wales) fixed to a wall. Weight was measured using digital Physician Large Dial 200 kg capacity scales (Kendon Medical, South Africa) whilst abdominal subcutaneous (SAT), visceral fat (VAT) and carotid intima media thickness (cIMT) of both the right and left layers of the common carotid arteries were measured using a LOGIQ e ultrasound system (GE Healthcare, CT, USA). All ultrasound readings were taken by trained field workers and to validate the measurement of SAT, VAT and cIMT, each of them was asked to perform repeated measurements on 15 volunteers. The coefficient of variation between and within trainees was <2 %. Self-reported information on age, physical activity, socioeconomic status, dietary intake, alcohol intake, and smoking were also obtained. Moderate-vigorous intensity physical activity (MVPA) was assessed in minutes per week using the Global Physical Activity Questionnaire (GPAQ) [15]. The MVPA was calculated from the accumulation of occupation, travel-related and leisure time physical activity. Socio-economic status was estimated from household assets and categorised into either of the five quintiles namely, "poorest", "poorer", "poor", "less poor" and "least poor". Dietary intake focused on questions regarding consumption of fruits and vegetables, bread, soft drinks (i.e., sugar-sweetened beverages) and fruit juices. Participants were requested to provide the number of days per week in which each of these items were consumed. Smoking and alcohol intake were defined by three categories: "never", "current", and "former".

Systolic blood pressure and diastolic blood pressure were measured using a digital sphygmomanometer (Omron M6, Omron, Kyoto, Japan). Three readings were taken within two-minute intervals and the last two readings averaged for the final blood pressure level. Measurements of serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), fasting glucose and creatinine levels were performed on the Randox Plus clinical chemistry analyser (Randox Laboratories Limited, Dublin, UK) through colorimetric methods. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation [16]. For the assessment of kidney function, estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation without the African American ethnicity correction formula [17].

Body mass index (BMI) was calculated from weight in kg/height in m^2. The homeostatic model assessment insulin resistance index (HOMA-IR) was calculated using the formula fasting insulin (μIU/L) × fasting glucose (mmol/L) / 22.5 [18]. Diabetes mellitus was defined as a fasting plasma glucose ≥7.0 mmol/L [19], and/or use of anti-diabetic agents and/or previous diagnosis by a health care professional. Obesity was defined as BMI ≥30 kg/m^2 [20], hypertriglyceridemia as triglycerides ≥1.69 mmol/L [21], and hypercholesterolaemia as cholesterol ≥5.18 mmol/L and/or treatment with anti-lipid agents [21]. Insufficient physical activity was defined as MVPA <150 min/week [22], and hypertension as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of anti-hypertensive agents and/or previous diagnosis by a healthcare professional [23].

2.2. Statistical analyses

All statistical analyses were performed using Stata version 16.1 (StataCorp LP, College Station, TX, USA). Continuous variables were presented as means ± standard deviations (SD) or medians and interquartile ranges (IQR) for data with normal or skewed distribution, respectively, and as proportions (%) for categorical variables. Non-parametric continuous dependent variables were log transformed to normality before being analysed using multivariable linear regression analysis. Categorical variables were compared across groups using the chi-squared test. Continuous variables were compared between two groups (e.g., pre- vs postmenopausal) or more (e.g., between study sites) using the appropriate parametric (Student's *t*-test or one-way ANOVA) or non-parametric (Mann–Whitney *U* test or Kruskal–Wallis) statistical tests. The level of significance of all statistical analyses was set at $p < 0.05$.

Multivariable linear regression was used to compare the relationship of CMD risk factors, namely systolic and diastolic blood pressure, BMI, waist and hip circumference, VAT and SAT, triglycerides, LDL-C, HDL-C, blood glucose, HOMA-IR and average cIMT (dependent variables) with menopausal stage (independent variable; post- compared to premenopausal group). Additional independent variables were chosen based on scientific plausibility (Supplemental Table 1). In the univariate (unadjusted) models, each CMD risk factor was regressed against menopause stage. In the adjusted model, age, and other independent variables (Supplemental Table 1) were incorporated into a multivariable model. Multivariable linear regression analyses were performed for each individual study site and for the sites combined, against each CMD risk factor. In addition, we performed a sub-analysis on participants who were not receiving therapy by removing participants who reported use of anti-hypertensive medication or therapies for the treatment of diabetes.

Unstandardised β coefficients (with 95 % CIs) were used in all the regression models. In cases where the dependent variable was log-transformed to obtain a normal distribution, the unstandardised β coefficient represents the fractional increase in the dependent variable for every 1 unit increase in the independent variable. Thus, a β coefficient of 0.10 signifies a 10 % increase. Collinearity of variables within each regression model was assessed using the variance inflation factor (VIF): all regression models included independent variables with VIF <3. Assumptions of constant variance for the residuals from each regression model were checked by the Breusch-Pagan/Cook-Weisberg test and correction for unequal co-variances was accomplished using robust standard errors.

3. Results

3.1. Participant characteristics

A total of 3609 women (1740 premenopausal and 1869 postmenopausal) were recruited across the five study sites (Fig. 1). The number of study participants and variables collected are shown in Supplemental Table 2. Table 1 and Fig. 2 show that the women at the West African sites had a more favourable cardiometabolic profile, whilst those from South Africa had the worst. Thus, Table 1 shows the prevalence of behavioural CMD risk factors and of CMDs across all five study sites for all women (i.e., pre-, and postmenopausal combined). Smoking was rare at all sites, whilst alcohol intake was highest at the two West African sites. Insufficient physical activity varied across sites being more prevalent in Soweto. Obesity, hypertension, diabetes, and dyslipidaemia had the lowest prevalence at the two West African sites and the highest prevalence at one or both South African sites (except for diabetes). Table 1 shows that study participants from West and East Africa consumed more vegetables than those from South Africa. In addition, South and East African women consumed more fruits, soft drinks, and bread than their counterparts in West Africa. However, data on alcohol and dietary intake, and cIMT measurements were missing in Soweto (Table 1).

In Fig. 2, selected CMD risk factors are expressed as continuous variables and presented for all women per study region i.e., West Africa (Navrongo with Nanoro), East Africa (Nairobi) and South Africa (Dikgale with Soweto). Again, these data highlight the tendency for these risk factors to be lowest in the West African region. Similar patterns were observed for the other CMD risk factors i.e., waist circumference, VAT, SAT, and HDL-C (data not shown). When systolic and diastolic blood pressure, BMI, waist circumference, LDL-C, and triglycerides were

Table 1
Prevalence of cardiometabolic diseases and risk factors in women at each AWI-Gen study site.

Risk factors	All sites (n = 3609)	Nanoro, West Africa (n = 651)	Navrongo, West Africa (n = 647)	Nairobi, East Africa (n = 849)	Dikgale, South Africa (n = 621)	Soweto, South Africa (n = 841)	p value
Age in years	49.3 ± 5.8	49.1 ± 5.8	50.1 ± 5.8	48.3 ± 5.5	50.3 ± 6.2	49.3 ± 5.8	0.03
Current smoking	90 (3)	0 (0.0)[a]	2 (0.3)	23 (3)	22 (4)	43 (5)	<0.001
Current alcohol consumption	865 (31)	389 (60)	344 (53)	50 (6)	82 (13)	…	<0.001
Insufficient MVPA	659 (18)	79 (12)	125 (19)	84 (10)	24 (4)	347 (41)	<0.001
Obesity	1193 (33)	10 (2)	35 (5)	272 (32)	319 (51)	557(66)	<0.001
Hypertension	1221 (34)	76 (12)	146 (22)	250 (29)	290 (47)	459 (55)	<0.001
Diabetes mellitus	160 (5)	11 (2)	2 (0.3)	67 (8)	36 (7)	44 (6)	<0.001
Hypertriglyceridemia	388 (11)	19 (3)	27 (4)	93 (11)	84 (14)	165 (20)	<0.001
Hypercholesterolaemia	582 (16)	23 (4)	21 (3)	179 (21)	107 (17)	252 (30)	<0.001
Diet (days consumed per week)							
Vegetables	4.1 ± 2.3	3.2 ± 2.8	4.7 ± 2.0	5.1 ± 1.7	3.1 ± 1.8	…	<0.001
Fruits	2.0 ± 2.2	0.8 ± 1.8	1.0 ± 1.2	3.5 ± 2.4	2.1 ± 1.8	…	<0.001
Soft drinks	0.7 ± 1.9	0.1 ± 0.5	0.1 ± 0.3	0.5 ± 1.1	2.0 ± 3.2	…	<0.001
Fruit juices	0.5 ± 1.4	0.1 ± 0.2	0.3 ± 0.9	0.2 ± 0.7	1.5 ± 2.4	…	<0.001
Bread	1.9 ± 2.3	0.6 ± 1.4	0.7 ± 1.3	2.2 ± 2.2	4.0 ± 2.4	…	<0.001

Data presented as mean ± standard deviation or numbers (percentage), MVPA: moderate-vigorous intensity physical activity. Significance testing across the sites completed by one-way ANOVA or chi squared test. Definitions were as follows; insufficient MVPA: <150 min/week, obesity: BMI ≥30 kg/m^2, hypertension: systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, diabetes mellitus: fasting plasma glucose ≥ 7.0 mmol/L, hypertriglyceridemia: triglycerides ≥1.69 mmol/L and hypercholesterolaemia: cholesterol ≥ 5.18 mmol/L.

[a] There were no smokers in Nanoro and data on alcohol consumption and diet were missing for Soweto.

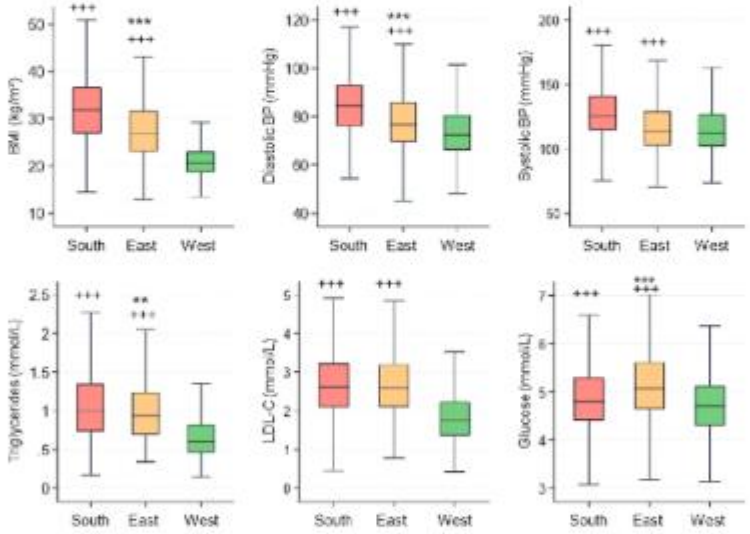

Fig. 2. CMD risk factor levels across South, East and West Africa.
CMD risk factor levels (BMI (body mass index), diastolic and systolic BP (blood pressure), triglycerides, LDL-C (low density lipoprotein cholesterol) and glucose) compared across the three regions by Tukey post hoc analysis. ***p < 0.001, **p < 0.01 vs South Africa. $^{+++}$p < 0.001 vs West Africa.

stratified by menopausal stage (Supplemental Table 3), the same patterns shown in Fig. 2 were observed in both menopausal groups across the three regions.

3.2. Comparison of anthropometry and CMD risk factors across menopause groups

When data were combined from all sites and stratified by menopause stage, all anthropometric measures except BMI were higher in post- than in premenopausal women (Table 2).

Anthropometric and cardiometabolic risk factors were then compared between menopause groups at the individual sites (Supplemental Tables 4–8). The differences observed in the combined analyses were the same at each of the individual sites except for height, weight, BMI, VAT, SAT, and hip circumference which were lower in the postmenopausal groups at one or more study sites. The BMI was lower in postmenopausal women in the West African sites, Nanoro and Navrongo, compared to their premenopausal counterparts, but was not different between the menopausal groups at any of the other sites. Compared to premenopausal women, postmenopausal women in

Table 2
Comparison of age, anthropometry and cardiometabolic risk factors between menopause groups for all AWI-Gen study sites combined.

Variables	Premenopause	Postmenopause	p value
Age (years)	45.4 ± 4.0	53.0 ± 4.8	<0.001
BMI (kg/m^2)	25.2 (10.3)	26.4 (12.1)	0.19
Hip circumference (cm)	101.8 ± 16.0	102.9 ± 17.6	0.05
Waist circumference (cm)	86.5 ± 15.4	89.3 ± 16.3	<0.001
SAT (cm)	1.65 (1.61)	1.86 (1.69)	<0.001
VAT (cm)	4.43 (2.35)	4.55 (2.50)	<0.001
Systolic BP (mm Hg)	114.5 (23.5)	123 (29.5)	<0.001
Diastolic BP (mm Hg)	77.5 ± 13.0	81.6 ± 13.0	<0.001
Total cholesterol (mmol/L)	3.71 ± 1.08	4.15 ± 1.23	<0.001
LDL-C (mmol/L)	2.22 ± 0.86	2.51 ± 0.94	<0.001
Triglycerides (mmol/L)	0.74 (0.51)	0.90 (0.60)	<0.001
HDL-C (mmol/L)	1.10 (0.43)	1.13 (0.43)	<0.001
Glucose (mmol/L)	4.75 (0.80)	4.90 (0.95)	<0.001
HOMA-IR	1.04 (1.97)	1.24 (2.11)	<0.001
cIMT average (mm)	0.59 (0.12)	0.65 (0.15)	<0.001

Data presented as mean ± standard deviation or median (interquartile range); BMI (body mass index), VAT (abdominal visceral fat), SAT (abdominal subcutaneous fat); LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), HOMA-IR (homeostatic model for assessment of insulin resistance), cIMT average (average of left and right carotid intima-media thickness).

Dikgale and Nairobi had lower SAT, in Nanoro and Nairobi had lower VAT, and in Dikgale, Nanoro and Navrongo had lower hip circumference.

The cardiometabolic variables (cIMT, blood pressure, lipids, glucose, and HOMA-IR) were higher in post- vs premenopausal women when data from all sites were combined (Table 2) and at individual sites (Supplemental Tables 4–8). The only exceptions were HOMA-IR, which was only higher in post- vs. premenopausal women in Nairobi (Supplemental Table 8), HDL-C levels which were only higher in Dikgale, Nanoro and Nairobi (Supplemental Tables 5, 6 and 8) and glucose levels which were only higher in the postmenopausal group in Dikgale, Soweto and Nairobi (Supplemental Tables 4, 5 and 8).

3.3. Multivariable linear regression analyses

Due to differences between sites in the levels of CMD risk factors (Table 1), univariate and multivariable linear regression models were performed for each risk factor at each site. In the univariate models (Table 3), each CMD variable was regressed against menopause stage alone (the unadjusted model), whilst in adjusted models age and other independent variables were selected depending on the identity of the dependent variable (Supplemental Table 1). Only significant models are presented in Table 3. Supplemental Table 9 includes all the multivariable regression models for all CMD risk factors at each site. These results clearly show that nearly all the associations between the different CMD risk factors and menopausal stage were observed in the West African sites, Nanoro and Navrongo. Thus, at either one or both sites postmenopausal stage was positively associated with waist circumference, SAT, diastolic and systolic blood pressure, LDL-C, triglycerides, glucose and cIMT, whilst postmenopausal stage was inversely associated with BMI at both Nanoro and Navrongo. In addition to the West African sites, postmenopausal stage was associated with elevated triglycerides in Dikgale and inversely associated with systolic blood pressure in Nairobi.

The data in Table 3 demonstrates that associations between menopause stage and CMD risks factors are observed predominantly at the individual West African sites. We therefore ran multivariable regression models for each CMD risk factor in which the two West African sites were combined, and we also combined the East and South African sites. We hoped that these combinations would reveal new significant associations that could not be observed at the individual sites (as shown in Table 3) due to a lack of power. There was only one East African site i.e., Nairobi, and therefore it was decided to combine it with the South African sites. These three sites also share some similarities particularly the high prevalence of CMDs (Table 1) and as shown in Table 3, very few associations of menopausal stage with CMD risk factors. In addition, we combined all five sites to enhance the ability to identify associations that may not have been observable in the lower-powered regional models; the results are shown in Table 4. The adjusted regression models for waist circumference, BMI, SAT, systolic and diastolic blood pressure, glucose, cIMT, LDL-C and triglycerides demonstrate associations with menopause stage in West Africa but not in combined sites from East and South Africa. In the models for LDL-C, associations were observed with menopause stage in both the regional models, but the association was stronger for West Africa. The models for HOMA-IR demonstrated an association with menopause stage only when all five study sites were combined, whilst for hip circumference, VAT and HDL-C no associations were observed in any of the models.

Table 3
Multivariate linear regression models showing relationship of menopausal stage with CMD risk factors for individual sites.

Dependent variable	Site	Menopausal stage[a] (unadjusted)	Menopausal stage[a] (adjusted)[b]
Waist circumference (cm)	Nanoro	0.24 (−1.00; 1.48)	1.54 (0.63; 2.45)**
	Navrongo	−0.88 (−2.38; 0.63)	1.06 (0.07; 2.06)*
Visceral fat (log; mm)	Nanoro	−0.05 (−0.09; −0.01)*	−0.06 (−0.12; −0.004)*
Subcutaneous fat (log; mm)	Nanoro	0.09 (0.02; 0.16)*	0.13 (0.05; 0.21)**
	Navrongo	0.05 (−0.03; 0.12)	0.14 (0.08; 0.20)***
BMI (log; kg/m^2)	Nanoro	−0.05 (−0.07; −0.03)***	−0.03 (−0.07; −0.004)
	Navrongo	−0.06 (−0.09; −0.04)***	−0.04 (−0.07; −0.01)*
Diastolic blood pressure (mm Hg)	Nanoro	2.85 (1.34; 4.36)***	2.62 (0.549; 4.69)*
	Navrongo	3.04 (1.15; 4.93)**	3.26 (0.918; 5.61)*
Systolic blood pressure (log; mm Hg)	Nairobi	0.05 (0.03; 0.07)***	−0.03 (−0.06; −0.003)*
	Navrongo	0.06 (0.04; 0.09)***	0.05 (0.02; 0.08)**
LDL-C (mmol/L)	Nanoro	0.17 (0.07; 0.26)**	0.16 (0.01; 0.30)*
	Navrongo	0.18 (0.07; 0.29)**	0.14 (0.14; 0.28)*
Triglycerides (log; mmol/L)	Dikgale	0.27 (0.20; 0.35)***	0.20 (0.09; 0.31)***
	Navrongo	0.13 (0.06; 0.21)***	0.11 (0.03; 0.20)*
Glucose (reciprocal, mmol/L)	Navrongo	−0.004 (−0.01; 0.001)	−0.01 (−0.01; −0.002)*
Average cIMT (log; mm)	Navrongo	0.10 (0.07; 0.12)***	0.05 (0.02; 0.08)**

Data is unstandardised β (95 % confidence interval); additional adjustments were made for specific variables as shown in Supplemental Table 1.

[a] Post- vs pre-menopause; BMI, body mass index, LDL-C, low-density lipoprotein cholesterol, cIMT average, carotid intima-media thickness average of left and right cIMT.

[b] All models adjusted for age, physical activity, socioeconomic status, HIV status, level of education, smoking status, alcohol, vegetable, fruit, soft drink, fruit juice, and bread intake.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

To determine the effect of anti-hypertensive and diabetic medication on the observed associations we removed all participants who reported use of these therapies from the regression models and the above associations between menopausal groups and CMD risk factors were not altered. The number of participants taking lipid-lowering medication was very low (n = 20) and therefore associated adjustments to the regression models were not warranted.

4. Discussion

This study demonstrates that the CMD risk factor profile of midlife African women is more favourable for those women from West Africa compared to those from South and East Africa, irrespective of

Table 4
Univariate and multivariate linear regression models showing relationship of menopausal stage with CMD risk factors for all sites combined, for West African sites and for South and East African sites combined.

Dependent variable	Sites included in model	Menopausal stage[a] (unadjusted)	Menopausal stage[a] (adjusted)[b]
Waist circumference (cm)	All Sites	2.83 (1.79; 3.86)***	1.10 (0.49; 1.73)***
	West Africa	−0.39 (−1.36; 0.58)	1.28 (0.58; 1.98)***
	South and East Africa	2.51 (1.26; 3.77)***	0.92 (−0.09; 1.93)
Hip circumference (cm)	All Sites	1.12 (0.02; 2.21)	−0.35 (−0.88; 0.19)
	West Africa	−2.83 (−3.73; −1.93)***	−0.40 (−1.10; 0.30)
	South and East Africa	0.86 (−0.43; 2.21)	−0.17 (−0.97; 0.63)
BMI (log; kg/m^2)	All Sites	0.01 (−0.01; 0.03)	−0.02 (−0.04; 0.0002)
	West Africa	−0.06 (−0.08; −0.05)***	−0.03 (−0.06; −0.01)**
	South and East Africa	0.01 (−0.01; 0.03)	0.0004 (−0.03; 0.03)
VAT (log; mm)	All Sites	0.03 (0.01; 0.06)*	−0.01 (−0.04; 0.02)
	West Africa	0.0003 (−0.03; 0.03)	−0.01 (−0.04; 0.03)
	South and East Africa	0.02 (−0.01; 0.06)	−0.02 (−0.06; 0.02)
SAT (log; cm)	All Sites	0.11 (0.07; 0.15)***	0.07 (0.04; 0.11)***
	West Africa	0.06 (0.01; 0.11)*	0.15 (0.10; 0.19)***
	South and East Africa	0.04 (−0.001; 0.08)	0.03 (−0.01; 0.08)
Systolic blood pressure (log; mm Hg)	All Sites	0.07 (0.06; 0.08)***	0.01 (−0.01; 0.03)
	West Africa	0.06 (0.04; 0.08)***	0.04 (0.02; 0.06)***
	South and East Africa	0.07 (0.05; 0.08)***	−0.02 (−0.04; 0.01)
Diastolic blood pressure (mm Hg)	All Sites	4.09 (3.21; 4.98)***	1.44 (0.20; 2.68)*
	West Africa	2.44 (1.19; 3.69)***	3.04 (1.47; 4.62)***
	South and East Africa	3.98 (2.87; 5.09)***	0.06 (−1.79; 1.92)
Glucose (reciprocal, mmol/L)	All Sites	−0.01 (−0.01; −0.01)***	−0.002 (−0.01; 0.001)
	West Africa	−0.01 (−0.01; −0.001)**	−0.0060 (−0.01; −0.002)*
	South and East Africa	−0.01 (−0.01; −0.003)**	0.002 (−0.004; 0.007)
Average cIMT (log; mm)	All Sites	0.09 (0.07; 0.10)***	0.01 (−0.003; 0.03)
	West Africa	0.09 (0.08; 0.11)***	0.03 (0.01; 0.06)*
	South and East Africa	0.09 (0.07; 0.11)***	−0.003 (−0.024; 0.02)
LDL-C (mmol/L)	All Sites	0.31 (0.24; 0.36)***	0.13 (0.05; 0.20)***
	West Africa	0.16 (0.09; 0.23)***	0.14 (0.04; 0.23)*
	South and East Africa	0.29 (0.22; 0.37)***	0.10 (−0.01; 0.22)
Triglycerides (log; mmol/L)	All Sites	0.19 (0.16; 0.22)***	0.08 (0.04; 0.12)***
	West Africa	0.16 (0.11; 0.21)***	0.10 (0.04; 0.16)**
	South and East Africa	0.16 (0.12; 0.20)***	0.05 (−0.01; 0.11)
HOMA-IR (log)	All Sites	0.13 (0.05; 0.21)**	0.16 (0.04; 0.28)*
	West Africa	0.06 (−0.09; 0.21)	0.18 (−0.02; 0.37)
	South and East Africa	0.13 (0.04; 0.22)**	0.16 (0.001; 0.32)
HDL-C (log; mmol/L)	All Sites	0.04 (0.02; 0.06)***	0.01 (−0.02; 0.04)
	West Africa	0.04 (0.01; 0.08)**	0.01 (−0.04; 0.05)
	South and East Africa	0.03 (0.002; 0.06)*	0.01 (−0.03; 0.05)

Data is unstandardised β (95 % confidence interval); West African sites: Navrongo and Nanoro, South African sites: Soweto and Dikgale, East African site: Nairobi, all sites: Navrongo, Nanoro, Soweto, Dikgale and Nairobi. BMI, body mass index, VAT, abdominal visceral fat and SAT, abdominal subcutaneous fat, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol, HOMA-IR, homeostatic model for assessment of insulin resistance, cIMT average, carotid intima-media thickness average of left and right cIMT; additional adjustments were made for specific variables as shown in Supplemental Table 1.

[a] Post- vs pre-menopause.

[b] All models adjusted for age, physical activity, socioeconomic status, HIV status, level of education, smoking status, study sites, alcohol, vegetable, fruit, soft drink, fruit juice and bread intake.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

menopausal stage. When comparing CMD risk factors between menopausal groups, many of these risk factors are higher in postmenopausal West African women compared to their premenopausal counterparts, with differences observed less frequently in the East and South African study populations.

In the current study, the staging of menopause was determined by asking participants about their menstrual bleeding patterns. The use of self-reported bleeding patterns is considered to be the most appropriate technique for the staging of menopause [24,25] and is universally used. A study conducted in Soweto, South Africa confirmed the accuracy of the staging in an African population using both estradiol and follicular stimulating hormone (FSH) serum level trends as supportive criteria across menopause stages determined from self-reported bleeding patterns [9,26].

The lower level of CMD risk factors in the West African women may be due to differences in environmental and/or lifestyle related factors [13,14]. Thus, both West African populations were predominantly subsistence farmers whereas the East and South African sites were more urbanised [14]. In addition, the West African study participants had a lower dietary intake of bread, soft drinks and fruit than observed at the other study sites and obesity was far less prevalent in the West African population.

The current analyses demonstrate that differences in CMD variables between pre- and postmenopausal women were observed more frequently at the West African than the East or South African sites. No other studies have been conducted in SSA comparing CMD risk factors across menopausal groups within different countries. The few studies that have been undertaken were restricted to individual countries. Thus, a study in women of mixed ancestry from South Africa demonstrated higher levels of CMD risk factors in post- compared to premenopausal women; however, the premenopausal group varied in age from 20 to 49 years (median age of 39) and no adjustment for age was made when comparing variables between the menopausal groups [10]. A second study from South Africa [9], which included data from participants who were part of the Soweto cohort in the current study, observed no differences in anthropometric and body composition variables between pre- and postmenopausal women after adjusting for confounders. A study from Ghana (West Africa) [11] demonstrated that metabolic syndrome was more prevalent in post- than premenopausal women, but

the premenopausal group varied in age from 20 years and upwards (mean age, 34.5 years) and age was shown to be a major determinant of metabolic syndrome. A similar study from the DRC (Central Africa) [12] demonstrated a higher prevalence of metabolic syndrome in post- compared to premenopausal women, which was maintained after adjustment for age.

A major characteristic of the West African sites are much lower levels of CMD risk factors, particularly the lower prevalence of obesity (1.5–5.4 %) in comparison to East (32.0 %) and South Africa (51.3–66.2 %). This may be significant because Tufano et al. showed that cholesterol levels were higher in lean women post menopause compared to lean premenopausal women, but these differences were not observed between obese pre- and postmenopausal groups [27]. The authors suggested that in the obese women, body fat was a more important determinant of CMD risk than menopausal stage. It should be noted however, that the sample size in this study was low (n = 92). In addition, a large cross-sectional study in rural China reported associations between postmenopausal stage and elevated glucose, blood pressure, triglycerides, and abdominal obesity amongst women with mean BMI 24.9–25.2 kg/m^2 [28]. Furthermore, cross-sectional studies from Indian populations with mean BMI ≤25 kg/m^2 demonstrated that postmenopausal stage was positively associated with waist circumference [29] and mean arterial pressure [30]. The data from these studies therefore suggest that differences in CMD risk factors between pre- and postmenopausal women may be greater in lean than obese women. It must be noted that several studies have demonstrated increased levels of metabolic syndrome and its individual components in post- compared to premenopausal women [31]; however, none of these studies have examined these differences within study sub-groups based on BMI or other anthropometric measures.

Another difference between the West and the East and South African populations is that those in West Africa are residing in a more rural location. This may explain the lower prevalence of obesity in the West African sites, but it is also possible that rural residence could influence the relationship between menopausal stage and CMD risk factors independent of its association with lower BMI. Thus, the studies described above that showed an increase in CMD risk factors in post- compared to premenopausal women in populations with low BMI were all conducted at rural sites [28–30]. However, a study conducted in a large rural population (N = 4743) in China demonstrated a higher prevalence of CMD risk factors in post- than premenopausal women, but with a prevalence of obesity at close to 22 %, which is far higher than that observed in the West African populations [32]. In addition, a study from Poland comparing rural to urban postmenopausal women, demonstrated a higher prevalence of metabolic syndrome in the rural (70 %) than the urban group (22 %) [33]. Furthermore, two recent studies from Mexico [34] and China [35] have demonstrated that age at menopause is lower in rural than urban women. Earlier age at menopause is known to be associated with a higher risk of cardiovascular disease [36]. The study from China also showed that rural women had a higher prevalence of menopausal symptoms and that these were associated with a higher risk of non-communicable diseases [35]. These studies suggest than rural residence may be associated with a higher risk of CMDs in postmenopausal women, but longitudinal studies are required to determine whether the menopause transition is differentially associated with CMD risk in rural and urban populations.

The reason why lower BMI or a rural environment may be associated with greater differences in CMD risk factors between pre- and postmenopausal women is not known. However, regarding low BMI, it is possible that this may be related to the hormonal changes that occur during menopause. It is well known that estrogen levels (specifically estradiol) decline during the menopause transition [37]. As estradiol (E_2) levels fall, circulating C_{19} steroids i.e., testosterone, androstenedione, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) become the major substrate for E_2 biosynthesis in extra-gonadal tissues [38]. Studies have shown that the expression of the enzyme aromatase, which is responsible for the production of E_2 in adipose tissue, increases with age and BMI [38]. Therefore, it is possible that the lower BMI in West African women could be associated with lower postmenopausal E_2 levels when compared to the women from East and South Africa, who have much higher levels of obesity. The lower postmenopausal E_2 levels in the West African females may then lead to enhanced CMD risk, as studies have shown that the fall in the serum concentrations of estrogens across the menopause transition is associated with higher levels of CMD risk factors [38].

In West Africa, although postmenopausal women have a higher prevalence of CMD risk factors than their premenopausal counterparts, CMD risk factor levels are still lower than those of both pre- and postmenopausal women in East or South Africa. Thus, in these latter two regions emphasis must be placed on prevention of obesity and related comorbidities in both pre- and postmenopausal women. In West Africa, our data demonstrate that the menopause transition is a critical window for the attenuation of CMD risk, and therefore the potential benefits of menopausal hormone therapy (MHT) become apparent. Data from previous studies suggest that when prescribed in healthy women early in menopause, MHT may help decrease the risk of CMD [7]. Unfortunately, data on the use of hormone therapy in sub-Saharan African women and its relation to CMD risk factors are absent.

4.1. Strengths and limitations

The main limitations of this study are its cross-sectional design, limited dietary data, lack of data on sex hormone levels and the fact that populations at the study sites may not be representative of the national population. The time available for collecting data by interviews was restricted and therefore an in-depth analysis of dietary intake using a food frequency questionnaire was not feasible. Therefore, we focused on some key dietary constituents such as fruit and vegetables and high carbohydrate-containing foods and drinks that have been associated with obesity and cardiometabolic diseases i.e., sugar sweetened soft-drinks, fruit juices and bread. Although regional differences were noted in consumption of these dietary items, their inclusion in the regression models did not modify any of the observed associations. A further limitation of this study was that food and alcohol intake were not recorded at the Soweto site and cIMT data were also not available.

Despite the shortcomings of a cross-sectional design, comparable findings to those observed in the West African populations were found in longitudinal studies where the menopausal transition was associated with increased levels of total cholesterol and LDL-cholesterol [39,40], and increased waist circumference [40]. One of the largest longitudinal studies of the health consequences of the menopause transition, SWAN, also demonstrated similar findings to those observed in the West African population with regards lipid levels, body fat distribution and cIMT whereas menopausal increases in blood pressure, glucose and insulin levels were found to be related to chronological aging [40]. The data from longitudinal studies therefore demonstrate that the menopause transition is associated with the worsening of cardiometabolic health, which suggests that the data from the South and East African sites are not representative of the current literature and warrant further investigation.

The strengths of the study are the large sample size, the use of multiple sites representing different stages of the health transition across SSA and the measurement of a wide array of appropriate anthropometric, metabolic, demographic, and behavioural data. Furthermore, standardised methodologies were implemented across the study sites and all blood analytes were measured at a central laboratory.

5. Conclusions

The current study shows that the difference in the levels of CMD risk factors between pre- and postmenopausal women varied across geographical regions within SSA. In the more rural West African

populations with a lower prevalence of obesity and other CMD risk factors, there were differences in the levels of CMD risk factors according to menopausal stage, but these differences were largely absent in the more urbanised and more obese South and East African populations. This indicates a need for context specific interventions to lower the risks of CMD in these populations. Thus, public health measures to curb the obesity epidemic in urban SSA populations, and clinical trials to assess the effects of hormone therapy in both urban and rural midlife women on menopause symptomology and CMD and cancer risk, are required.

Contributors

Raylton P. Chikwati contributed to statistical analyses, drafting, and revision of the manuscript.

Nasrin Goolam Mahyoodeen contributed to interpretation and revision of the manuscript.

Nicole G. Jaff contributed to the acquisition and interpretation of data and revision of the manuscript.

Michele Ramsay contributed to the conception and design of the study, interpretation of data and critical revision of the manuscript.

Lisa K. Micklesfield, Alisha N. Wade, Godfred Agongo, Gershim Asiki, Solomon S. R. Choma and Palwende R. Boua contributed to the acquisition and interpretation of data and revision of the manuscript.

Jaya A. George contributed to the conception and design of the study, interpretation of data and critical revision of the manuscript.

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Ethical approval

Ethics approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand for the AWI-Gen study (M121029 and M170880) and for the current sub-study (M2010106). In addition, local ethics approval was obtained in the respective AWI-Gen study sites. All study participants provided written informed consent.

Provenance and peer review

This article was not commissioned and was externally peer reviewed.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. The data have been submitted to the European Genome-Phenome Archive (EGA), accession number EGA00001002482 [41]. The Human Heredity and Health in Africa (H3Africa) Data and Biospecimen Access Committee (DBAC) will review requests for the AWI-Gen phenotype dataset. Related documents including study protocol and statistical analysis plan will be available upon request from the corresponding author.

Declaration of competing interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.maturitas.2023.04.005.

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