AN INVESTIGATION OF THE METABOLIC, HORMONAL AND ANTHROPOMETRIC CHARACTERISTICS OF THE MENOPAUSAL TRANSITION IN BLACK URBAN SOUTH AFRICAN WOMEN

by

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

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Declaration

I, Nicole Gusti Jaff, declare that the thesis entitled: An investigation of the metabolic, hormonal and anthropometric characteristics of the menopausal transition in black urban South African women submitted for the degree of Doctor of Philosophy to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg is the result of my own original work.

All references made to the work of others and any assistance received has been fully acknowledged.

No part of this work has been submitted for degree or examination purposes to any other university or institution.

Signed: this 17nd day of November 2015

Name: Nicole Gusti Jaff

Dedication

This thesis is dedicated with all my love and gratitude to my husband Nicholas Robert Jaff for his steadfast love, endless support, patience and unswerving belief in me even in those dark days when I had none in myself and completing this PhD thesis seemed impossible.

Publications from PhD thesis and funding of research

In the process of completing this PhD thesis, the research was reported and published in a series of scientific publications in peer-reviewed journals. Results from this study were also presented in the form of posters and oral presentations at various medical and scientific meetings and conferences shown below. Publications 1 and 2 are included in the appendices.

Peer-reviewed publications:

1. Jaff NG, Snyman T, Norris SA, Crowther NJ. 2014. Staging reproductive aging using Stages of Reproductive Aging Workshop + 10 in black urban African women in the Study of Women Entering and in Endocrine Transition. *Menopause* 21:1225-1233
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3. Jaff NG, Norris SA, Snyman T, Toman, M, Raal, FJ, Crowther NJ. Reproductive aging and associated hormonal changes are related to metabolic syndrome and cardiovascular disease risk factors in menopausal African women. *Human Reproduction* (under review)

PhD student's contribution to the publications:

The student was responsible for study design, questionnaire development and piloting, and project management (including supervision of data collection and training of fieldworkers), data management (including data cleaning and coding), data analysis and writing of the manuscripts. Co-authors provided guidance on the conceptualization of the manuscripts, methodology, and statistical analysis, editing manuscripts and/or reviewing drafts. Contributions have been noted where applicable. All co-authors are in agreement that the

scientific papers that they have co-authored may be presented in the results section of this PhD thesis (see Appendices; Page 185).

Conference Presentations:

1. North American Menopause Society (NAMS) 24th Annual Meeting October 9-12, 2013, Dallas, TX. Friday Concurrent Session #2. Jaff NG, Snyman T, Norris SA, Crowther NJ. 'Staging reproductive aging using STRAW+10 in urban African women in the Study of Women Entering and in Endocrine Transition (SWEET)': Oral presentation
2. North American Menopause Society (NAMS) 25th Annual Meeting October 15-18, 2014, Washington, DC. Jaff NG, Snyman T, Toman, M, Norris SA, Crowther NJ. 'Menopausal transition, body adiposity, lean mass and hormonal levels in black urban African women in the Study of Women Entering and in Endocrine Transition (SWEET)': Poster presentation
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4. 12th South African Menopause Society (SAMS) Congress 27-28 February 2015, Johannesburg, South Africa. Jaff NG. 'Menopause in Black South Africans': Plenary session: Friday 27th February.
5. Society for Endocrinology, Metabolism and Diabetes in South Africa (SEMDSA) Congress 17-19 April 2015, Bloemfontein, South Africa. 'Changes in body composition in menopausal, black urban African women in the Study of Women Entering and in Endocrine Transition (SWEET)': Jaff NG, Snyman T, Toman, M, Norris SA, Crowther NJ. Oral presentation (Crowther, NJ).

6. Pfizer Africa & Middle East (AfME) Women's Health Summit 6 September 2014, Irene, South Africa. 'An overview of the Study of Women Entering and in Endocrine Transition (SWEET): Menopause in black South African women': Lecture
7. Faculty of Health Sciences Research Day 2014, Johannesburg South Africa 'Body fat distribution across the menopause transition in a population of obese urban African women in the Study of Women Entering and in Endocrine Transition (SWEET)' September, 2014: Oral presentation

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Abstract

Background and Objectives: The Study of Women Entering and in Endocrine Transition (SWEET) was developed to examine differences in metabolic, hormonal, and anthropometric parameters in black urban South African women at different stages of the menopause transition (MT). Little data are available on accurate staging of the menopausal transition for sub-Saharan African women. There is a plethora of data on this and related subjects in Western women, but little available research on changes in body composition or risk of metabolic syndrome (MetS) in the MT in midlife black South African women, although obesity is prevalent in this group, and there is a high instance of both diabetes and hypertension. The prevalence of HIV infection is also high in these women but it is not known whether this may affect the symptoms and conditions of the MT, contribute to changes in body composition or increased risk of MetS and cardiovascular disease (CVD). No prior study in sub-Saharan Africa has used the Stages of Reproductive Aging Workshop + 10 (STRAW + 10) criteria to stage reproductive aging or assessed their reliability in classifying ovarian status. The MT is closely associated with changes in body composition including lower bone mineral density, decreased lean muscle mass, increased body mass index (BMI) and adiposity, particularly increased central adiposity. Abdominal obesity is a key risk factor for MetS. This, and the subsequent risk of CVD appear to increase as women transition into menopause. It is unclear if this is due to reproductive or chronological aging, or both combined.

Aims: (1) To assess the usefulness of the STRAW + 10 criteria in staging ovarian aging in black South African women. (2) To determine whether there are differences in body adiposity, lean muscle mass, and bone mineral density (BMD) across reproductive groups and ascertain the main correlates of these variables. (3) To determine in this population, if the risk of MetS and the levels of its components and related metabolic factors, differ between women at different stages of the MT and to explore the possible determinants. (4)

To investigate whether the high prevalence of HIV infection in these women affects the age at menopause, menopausal symptoms, body composition, and metabolic variables in midlife black South African women.

Methods: Participants in this cross-sectional study were 702 black urban African women aged 40 to 60 years. The stages of reproductive aging were categorized using STRAW + 10 criteria. The Menopause Rating Scale was used to measure the prevalence of menopausal symptoms including vasomotor symptoms. Study-specific questionnaires were used to obtain relevant demographic and lifestyle data. Blood levels of follicle stimulating hormone (FSH), estradiol (E2), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone (T) and sex hormone blinding globulin (SHBG), insulin, lipids, glucose, leptin and adiponectin were measured. Simple measures of body anthropometry (weight, height, waist and hip circumference) were obtained. Body composition was measured using dual-energy X-ray absorptiometry (DXA) and ultrasonography. Human immunodeficiency virus (HIV) status was assessed using a point-of-care method. Metabolic syndrome and diabetes were diagnosed using internationally recognized criteria.

Results: Reported age at final menstrual period (FMP) was higher in subjects interviewed within 4 years of FMP (49.0±3.80) than in subjects interviewed ≥10 years after FMP (42.0±4.06; $p<0.0005$). Human immunodeficiency virus (HIV) status had no effect on menopause symptoms. A BMI ≥35 kg/m^2 was associated with severe vasomotor symptoms. Estradiol ($p<0.0005$), SHBG ($p<0.0005$) and DHEAS ($p=0007$) were significantly lower in post- than premenopausal groups, whilst FSH was higher ($p<0.0005$). Whole body lean mass ($p=0.002$) and BMD ($p<0.0005$) were significantly lower in postmenopausal compared to premenopausal groups. Multivariable linear regression models and ANCOVA demonstrated that the lower lean mass was related to the high postmenopausal FSH levels, whilst the lower BMD was partially explained by the low postmenopausal E2 levels. Use of antiretroviral therapy (ART) correlated negatively with total fat mass ($\beta=-2.92$, $p=0.008$) and

total bone mineral content (BMC; β=-78.8, p=0.003). The MetS was highly prevalent (49.6%). Levels of total cholesterol (p<0.0005), LDL (p<0.0005), triglyceride (p=0.01), systolic (p<0.0005) and diastolic (p<0.05) blood pressure were all significantly higher in post-menopausal compared to pre-menopausal groups whilst there was a trend for glucose levels (p=0.05) and MetS prevalence (p=0.05) to also be higher. Multiple regression analyses and ANCOVA showed that the higher levels of cholesterol and LDL were related to higher FSH concentrations whilst elevation in systolic blood pressure was linked to lower estradiol levels. The higher postmenopausal glucose and diastolic blood pressure levels and risk of MetS were related to chronological aging. Adiponectin was strongly correlated with all components of the MetS except for blood pressure.

Conclusions: Reporting of age at FMP is unreliable in subjects interviewed ≥ 4 years after the event. The STRAW+10 criteria are accurate in staging reproductive aging, as confirmed by the significant association of FSH and estradiol levels with menopausal transition stage. These guidelines may be appropriate for use in resource-limited settings in the absence of biomarkers. The MT in these women is characterized by lower whole body lean mass and BMD in post- compared to premenopausal subjects but there are negligible differences in fat mass. Lower lean mass and BMD were associated with higher FSH and lower E2 serum levels, respectively. Lower fat mass and BMC were associated with ART use. The lipid profile was more atherogenic and blood pressure was higher in the post- than the premenopausal women. These differences were related to the higher FSH (LDL and total cholesterol) and lower E2 (diastolic blood pressure) levels in the postmenopausal women. These data suggest that the hormonal changes characterizing the menopause may play a role in the etiology of cardiometabolic disease and in the body composition changes that are observed in the MT. The above conclusions should be addressed in longitudinal studies. The terminology of STRAW+10 needs to be simplified and the questions contextualized, and contraceptive use should be specifically addressed in questions on bleeding patterns. In addition there are implications for the use of behavioral interventions

in lowering cardio-metabolic risk factors and hence morbidity and mortality in these women. Further research is needed to examine health risks associated with snuff use, and the long-term effects of HIV-infection and different ART regimens. Additional studies should address the poor understanding of menopausal health consequences in this population with appropriate education programs

Acknowledgements:

When I embarked upon this PhD over six years ago, I believed I was well equipped to achieve it; I was an older student with few of the distractions that might hinder younger research fellows. My children were grown, my husband was extremely supportive, I had returned in my forties to university and achieved two degrees, become a registered healthcare practitioner, sat and passed two board exams, written several books, a monthly column on menopause for a national women's magazine and had a busy counseling practice. I thought I had some conception of what a PhD would entail. I knew it would be rigorous, time consuming and hard work, but I thought it would be a relatively easy process and that it would be accomplished within 3-4 years at most. Blissfully ignorant as I was then, I was mistaken. I have run the gamut of almost every emotion from rage, frustration and despair, to happiness, delight and gratitude. Completing this PhD has been one of the most difficult and challenging experiences of my life. It has also been one of the most fulfilling. Six years later, I know that I would do it all over again, albeit with more humility and caution, greater patience and humor, but with absolute certainty that this was a life-enhancing decision.

I could not have achieved this PhD thesis without enormous help and support from my supervisors. My primary supervisor, associate Professor Nigel Crowther was an extraordinary mentor, and I am profoundly grateful for the unstinting help he has given me. He was always available; he read every draft timeously and with rigorous and meticulous attention to detail. He taught me how to submit papers to scientific journals, and how to create abstracts for submission to congresses. His critiques were insightful and relevant. He was a tough and demanding audience when I was learning how to present my oral abstracts at conferences. He alleviated my anxiety but never allowed me to lose my sense of humor. His witty and ironic perceptions and commentary on academic life were, and are, one of my great delights.

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Definitions of terms and abbreviations

AMH	Anti-Müllerian hormone
ART	Antiretroviral therapy
BFD	Body fat distribution
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BT20	Birth to twenty
CAC	Coronary artery calcium
CAMS	Council of the Affiliated Menopause Societies
CEE	Conjugated equine estrogen
CIMT	Carotid intima-media thickness
CVD	Cardiovascular disease
DHEA	Dyhdroepiandrosterone
DHEAS	Dyhdroepiandrosterone sulfate
DSMB	Data Safety Monitoring Board
E2	Estradiol
ELITE	Early Versus Late Intervention Trial With Estradiol
FAI	Free androgen index
FDA	Food and Drug Administration
FMP	Final menstrual period
FSH	Follicle-stimulating hormone
HDL-C	High-density lipoprotein cholesterol
HERS	Heart and Estrogen/Progestin Replacement Study
HIV/AIDS	Human immunodeficiency virus infection and acquired immune deficiency syndrome
HOMA	Homeostasis Model Assessment
HRT	Hormone replacement therapy
HS	High school

IMS	International Menopause Society
KEEPS	Kronos Early Estrogen Prevention Study
LDL-C	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
LMICs	Low or middle-income countries
MHT	Menopausal hormone therapy
MetS	Metabolic syndrome
MPA	Medroxyprogesterone acetate
MRS	Menopause Rating Scale
MT	Menopause transition
MWMHP	Melbourne Women's Midlife Health Project
NAMS	North American Menopause Society
NCDs	Non-communicable diseases
P4	Progesterone
PEPI	Postmenopausal Estrogen/Progestin Interventions Study
ReSTAGE	Collaboration of four cohorts to identify MT markers and validate STRAW recommendations
SAT	Subcutaneous adipose tissue
SHBG	Sex hormone-binding globulin
SMWHS	Seattle Midlife Women's Health Study
STRAW	Stages of Reproductive Aging Workshop
STRAW + 10	Stages of Reproductive Aging Workshop + 10
SWAN	Study of Women's Health Across the Nation
SWEET	Study of Women Entering and in Endocrine Transition
T	Testosterone
TREMIN	The TREMIN Research Program on Women's Health
VAT	Visceral adipose tissue
VMS	Vasomotor symptoms
WC	Waist circumference
WHI	Women's Health Initiative

Preface

The past 15 years of clinical practice as a Menopause Counselor, studying and researching the topic of menopause and wellness, have shown me that women, with access to either private or public healthcare, have scant knowledge of the health risks that appear to be associated with the menopause transition. Of paramount interest and concern to me were mid-life black South African women, who have very little access to gynaecologists and health-care professionals, and almost no knowledge of menopause. In addition, the cryptic manner in which the menopause transition appears to be frequently addressed, and lack of information from public health care providers in many black communities, adds to the opaque nature of the subject. I examined the available literature and confirmed that this was a very neglected area of research, with a paucity of literature addressing the physiological, psychological, psychosocial aspects of the menopause transition in black mid-life women.

My PhD appears to be the first study of its kind in sub-Saharan Africa, and the results, comparing similarities and differences between black postmenopausal South African women with those worldwide, were explored. The size of the cohort may allow the data gathered to be generalizable to other communities of urban black women in sub-Saharan Africa. Since this research examines the menopause transition and the related hormonal, metabolic and anthropometric changes that are related to it, it was named: The Study of Women Entering and in Endocrine Transition (SWEET).

Access to the mothers and caregivers of the children in the Birth to Twenty study in Soweto, whose database is situated at the Developmental Pathways for Health Research Unit (DPHRU) at Chris Hani Baragwanath Hospital, provided a discreet, homogenous group of midlife women, who were not research naïve, and who understood the benefits of research. They were also acquainted with some of the research team members and were familiar with data collection processes. The data collection for SWEET was completed within two years.

Most of these women had never had an annual medical check-up or gynaecological examination, although they had heard about menopause. However, not many understood its implications or the process of reproductive aging. Very few of these women had ever had the blood assays that act as biomarkers for both metabolic disease and menopausal stage, or dual-energy X-ray absorptiometry (DXA) and ultrasound, to measure body composition. They were therefore eager to participate in the study and to be informed of these results, which would allow them to know more about their health status. It was agreed that if their results were not in the normal range, they would be referred to a primary health care clinic, medical practitioner or specialist for further testing and treatment.

The PhD thesis is by publication and includes three manuscripts. The articles have either been published or have been submitted to peer-reviewed, accredited medical journals. The PhD thesis is in six chapters.

Chapter 1 and Chapter 2 consist of the literature review, contextual background and relevance of the research, and a description of the study cohort and methods.

Chapters 3-5 include the three research studies, presented as scientific publications that were undertaken for this PhD project. Paper 1 is a cross-sectional study investigating the usefulness and validity of staging ovarian aging in black South African women. Paper 2 examines differences in body composition across groups of black sub-Saharan African women at different stages of the menopause transition, and the possible relationship between these and an altered hormonal milieu. Paper 3 explores whether reproductive aging and the associated hormonal changes are related to a greater risk of metabolic syndrome and associated CVD risk factors in this population.

Chapter 6 discusses the findings of these three studies. It contains a conclusion and suggestions for further research in this field

Chapter 1. Literature Review

1 Literature Review

This study examines the metabolic, hormonal and anthropometric parameters across the menopausal transition stages in black urban South African women. The literature review in this chapter provides the background for the research undertaken, highlighting the results and recommendations arising from related studies in this research area.

1.1 Background

The World Health Organization estimates that by 2030, 1.2 billion women will be 50 or over. This almost triples the number of women who were in that age bracket in 1990 (1). It is estimated that by the late 2020's, 76% of postmenopausal women will be living in developing countries (2). Growing numbers of women can expect to live for several decades after menopause; however, it is suggested that women living in poor socio-economic conditions may have their final menstrual period (FMP) at a younger age (3), while a later menopause may be associated with greater longevity (4). There is a great deal of data documenting metabolic syndrome as a risk factor for cardiometabolic disease in postmenopausal women in Western and developed countries but very little data on this subject is available in emerging nations. The Heart of Soweto study (5) showed that cardiovascular disease (CVD) is becoming more prevalent in black urban populations. The study population contained more women than men and the mean age was 53 years, which suggests that many of these women were menopausal, but unfortunately the subjects were not tested to determine menopausal status (5). The Soweto researchers discuss the prevalence of hypertension and type 2 diabetes, either one of which may suggest the presence of metabolic syndrome. Some morbidity in black postmenopausal women in South African women may be due to HIV/AIDS or tuberculosis (6). However, HIV positive women may be at greater risk for metabolic disease (7), and symptoms and conditions of the menopause transition (MT) in midlife women may be affected if they are HIV positive (8). However, if, as the literature

shows, women during the MT and in postmenopause show a greater tendency to develop metabolic syndrome (9), a better understanding of possible health risks may help these women to effect lifestyle changes which could increase longevity.

1.2 An historical perspective of the MT

1.2.1 Reproductive aging is recognized (350 BC - 6th century AD)

The idea that the MT is associated with risk of disease is at least 200 hundred years old as shown in in Figure 1.1, though the cessation of menses has been mentioned throughout the ages. As early as 350 BC, Aristotle, in his seminal work, The History of Animals, wrote: 'In the human species, the male is generative, at the longest, up to seventy years, and the female up to fifty; but such extended periods are rare. As a rule, the male is generative up to the age of sixty-five, and to the age of forty-five the female is capable of conception' (10). Utian explains that even as early as the sixth century, the Byzantine physician, Aëtius of Amida, had described the relationship between menopause and age (Figure 1.1) (11).

1.2.2 Menopause and ill-health (17th century – 19th century)

However, it was towards the end of the 18th century and at the beginning of the 19th century, that several medical tracts, written by doctors in both France and England addressed this subject of menopause and disease in depth. In 1777, John Leake described several problems experienced by menopausal women that seemed connected to the cessation of menses, including as he termed them, 'hysteric disorders' (12). John Burns (1814), made a connection between increased risk of breast cancer and the end of the menses (13). In 1816, a French physician, Charles Pierre Louis De Gardanne wrote a book called Avis Aux Femmes Qui Entrent Dans L'âge Critique (Advice to Women Entering the Critical Age) where he used the term 'menéspausie' (14), which was modified in the second

edition (1821) to ‘menopause’ (15). It seems that the term is Greek in derivation; *mēn* – month and *pausis* – halt (Figure 1.1) (16).

Many 19th century writers believed the onset of menopause and the end of fertility caused ill health in women. In the second edition of Gardanne’s book, now called: De la ménopause ou de l’âge critique des femmes (On Menopause or the Critical Age of Women), he wrote that the diseases that affected women during this critical age were very frequent (15). Laurent Cerise (1845), describes numerous problems affecting mid-life women: ‘Douleurs de tête, vertiges, hallucinations…météorisme, … palpitations, abattement, agitation, graves hémorragies, … insomnie opiniâtre, rêves, cauchemars, inappétence, … chaleur, frissons…’ (Headaches, dizziness, hallucinations…bloating, palpitations, depression, agitation, severe bleeding,… continuous insomnia, dreams, nightmares, loss of appetite, … heat, chills…) (See Figure 1.1) (17).

1.2.3 The medicalization of menopause (19th century – 20th century)

The trend to pathologize the menopause, and to view it as a time of ill-health and increased morbidity in women, continued into the mid 20th century (18), culminating in a book called Feminine Forever by Dr. Robert Wilson (19). The views expressed in this work; that estrogen would protect women from what he described as an estrogen deficiency disease (20), reinforced the perspective of menopause in the medical profession of that time (Figure 1.1).

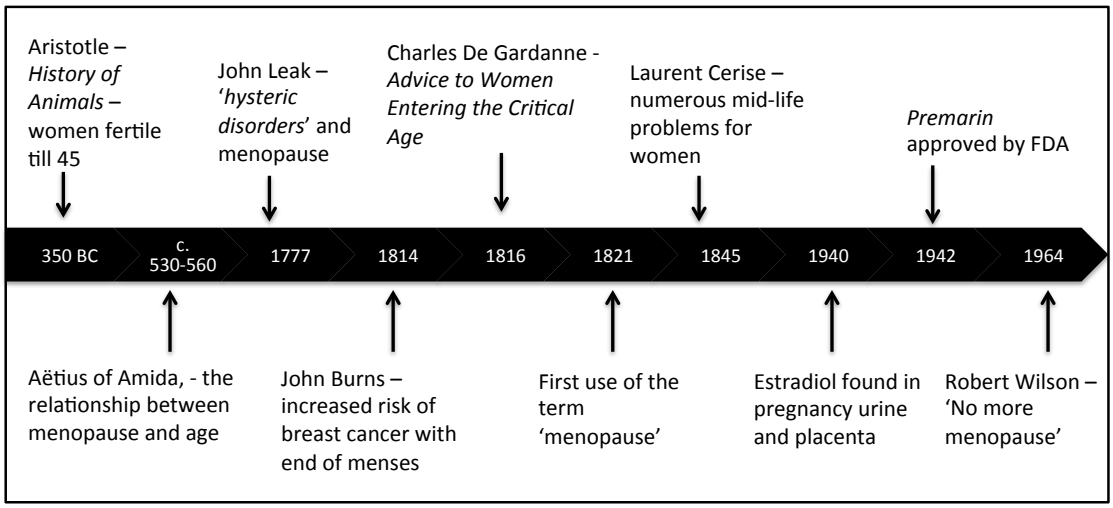

Figure 1.1 An Historical Perspective of Menopause: Adapted from 'Vasomotor symptoms in menopause'; Pinkerton et al (21)

Towards the end of the 19th century, three independent trials, where doctors attempted to counteract the perceived effects of ovarian aging by administering different forms of ovarian tissue from cows, had been conducted (22). However, studies began in earnest at the beginning of the 20th century. By 1930, Butenandt and his colleagues had worked out the structure of estrogens (estrone and estriol), which had been extracted from the urine of pregnant women (23, 24), although it was not until 1940 that estradiol (E2) as shown in Figure 1.1, was found in pregnancy urine and the placenta (11). In 1942, conjugated equine estrogens (CEE), brand name Premarin (Figure 1.1), were approved by the Food and Drug Administration (FDA) of America and by the 1970's, Premarin had become the most prescribed medication in the USA to alleviate menopausal symptoms (25).

The discovery in the mid 1970's, as shown in Figure 1.2, that the use of unopposed, conjugated estrogen therapy was causing a steep increase in endometrial carcinoma (26), caused a temporary decline in the use of Premarin (25). However, when research showed that the addition of progestogens to the estrogen regimen reduced the risk of endometrial hyperplasia (27), wide use of estrogen therapy resumed (25). In 1986 the FDA approved the

use of estrogens as useful in combating osteoporosis, and this, together with the fact that doctors believed that hormone therapy (HT) was effective in combating CVD entrenched its use. In 1995, a combination drug called Prempro (Fig. 1.2) combining Premarin and Provera was introduced (28) By the end of the 20th century, the use of HT was firmly established, and approximately 15 million women in the USA were using it (Fig. 1.2) (29).

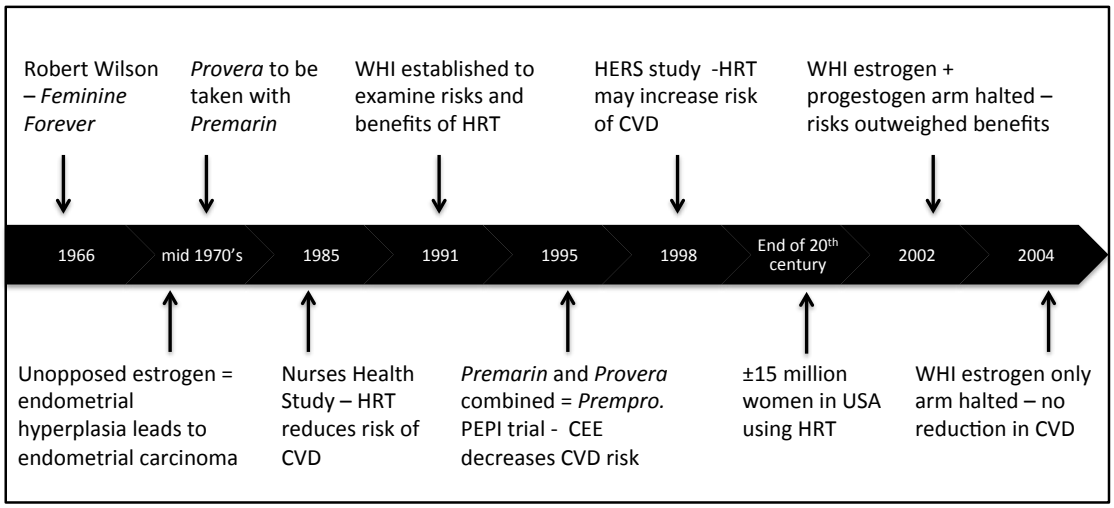

Figure 1.2 An historical perspective of menopause (cont.) (21)

1.2.4 Menopause, HT and heart disease (late 20th century)

The historical perspective and belief that menopause was a time of ill-health, informed much of the hypotheses in research on menopausal women and heart disease in the late 20th century. As shown above, researchers and clinicians believed that although menopause increased the risk of CVD, this could be attenuated by judicious use of estrogen. Researchers used qualitative, observational and anecdotal research to confirm their hypothesis that women, albeit later in life, were at greater risk for CVD than men. Studies about CVD risk focused mainly on men, and these findings were then extrapolated to women (30). However, even when studies focused on women, data seemed to indicate that doctors were correct in their assumptions about the beneficial effects of HT. In 1985, data from the Nurses' Health Study suggested that the use of HT reduced the risk of CVD among

postmenopausal women, since estrogen appeared to have a protective effect on lipids (Figure 1.2) (31). Clinicians generally adhered to this belief, despite data from the Framingham Heart Study, which was published in the same issue of the New England Journal of Medicine, and showed a 50 percent increased risk of CVD and a greater than two fold risk of stroke in women using HT (32).

The practice of prescribing HT to reduce the risk of CVD seemed justified in 1995, when results from the Postmenopausal Estrogen/Progestogen Interventions (PEPI) trial found that unopposed estrogen, in this case, CEE, increased levels of high-density lipoprotein cholesterol (HDL-C) (33) However, in the Heart and Estrogen/Progestogen Replacement Study (HERS) (Fig. 1.2), where researchers assessed the possible benefits of HT on lowering risk of heart disease, results showed that after four years of using estrogen alone or estrogen plus progestogen, the overall risk of heart disease was not reduced. Furthermore there was a three-fold increased risk of stroke, an increased risk of gallbladder disease and an increase of CVD in the early years. The HERS researchers felt that HT should not be recommended for secondary prevention of CVD (34), and the possibility that HT use might increase the risk of CVD in older, postmenopausal women was raised (35).

1.2.5 The WHI - a changing perspective (late 20th century – early 21st century)

In 1991, a very large longitudinal, randomized controlled clinical trial called the Women's Health Initiative (WHI) sponsored by the National Institutes of Health (NIH), National Heart, Lung, and the Blood Institute (NHLBI) was established to investigate the overall risks and benefits of HT for a period of 8.5 years (Figure 1.2). Although the study was due to continue until 2005, a WHI Data Safety Monitoring Board (DSMB) halted the estrogen plus progestogen arm after 5.2 years in 2002. The board found that there was a greater risk of stroke, an increased risk of CVD and breast cancer and that these risks outweighed the benefits. A regimen of CEE plus MPA as a primary prevention for CVD was deemed to be

unsafe (36). Although some believed that these data might be due to the progestogen component, the NIH halted the estrogen-only arm in 2004, since there appeared to be an increased risk of stroke, no reduced risk of CVD, or overall benefit. Nevertheless, since the fracture risk was lowered with the estrogen alone treatment, and there was a possibility of a lower risk of breast cancer in this group, further research was required (Figure 1.2) (37).

In the 10 years since the WHI halted, and although there has been debate concerning the apparent discrepancy between those results and those of previous data, the WHI study changed the way in which HT had been prescribed for several generations; prescription rates dropped precipitously (29). Following the recommendations of certain menopause and endocrine societies, clinicians re-evaluated the prescription of HT. Recommendations were that HT should be prescribed for alleviation of menopause symptoms, including vasomotor symptoms (VMS) and vulvovaginal atrophy, and in certain cases, to decrease fracture risk. (38). The WHI study is ongoing and provides a wealth of information about postmenopausal women's health. By 2013, 573 studies had been published concerning various data collected in the WHI and the WHI extension study, of which 63 focused on CVD (30, 39).

1.2.6 A new conundrum – the timing hypothesis and CVD risk

There was ongoing debate after the WHI, and research into the hypothesis that HT was effective in reducing the risk of CVD across the MT continued. The timing hypothesis, which describes a critical window or time period, when estrogen therapy may have a beneficial effect (40), arose from dissatisfaction with the WHI study design and data showing that older women might be at greater risk for CVD as a possible result of lowered levels of endogenous estrogen (41). Furthermore, the results of the estrogen-only arm of the WHI suggested that in 50 to 59 year old women, exogenous estrogen did lower the risk of CVD and the benefits outweighed the risks in this sample (40).

The focus for proving the timing hypothesis was now on two studies. The first of these was the Kronos Early Estrogen Prevention Study (KEEPS), which hypothesized that HT given within three years of menopause would reduce the progression of atherosclerosis. However, there was no significant difference between the treatment and placebo groups in terms of atherosclerosis, although there was a small non-significant trend in terms of less build–up of coronary artery calcium (CAC) in those women using HT (42).

The second trial that specifically tested the timing hypothesis in healthy women either less than 6 years or more than 10 years since menopause, was the Early versus Late Intervention Trial with Estradiol (ELITE). This trial was designed to measure the progression of carotid intima-media thickness (CIMT) every six months for three years, and after an extension of five years, to measure lesions and calcium in the coronary arteries (43). Results from this trial have shown that this regimen of 1mg of oral micronized estradiol daily with 10 days of vaginal progesterone gel lowered CVD risk factors, when used in younger menopausal women near onset of menopause (44). However, there are some problematic areas in determining the timing hypothesis. These include the low rate of CVD among young, healthy menopausal women and the fact that the long-term effects of this regimen still need to be examined in a sufficiently powered cohort study (45, 46).

As discussed earlier, clinicians believed, based mainly on observational studies, that there was an association between the MT and increased risk of CVD, and therefore encouraged the practice of treating what was described as an ‘estrogen deficiency disease’ with HT to improve arterial health. However, the question still remains as to whether chronological or reproductive aging is responsible for increased CVD risk in women across the MT.

1.3 The biology of reproductive aging

1.3.1 The menstrual cycle

Changes in the menstrual cycle are an important marker of reproductive aging. Menopause is the result of ovarian aging, so an understanding of the normal menstrual cycle and subsequent hormonal changes caused by reproductive aging, is essential. In utero, a woman's two ovaries contain up to two million follicles but by the time she reaches puberty only about 500 000 remain. Atresia, begins in utero and continues throughout a woman's reproductive life, with the loss becoming more rapid over the years. Ovulation accounts for the loss of approximately 500 follicles over a life span. Reproductive and chronological aging cause changes across the MT, and the decrease in follicles intensifies as a woman ages, although chronological age per se cannot accurately predict the time of the MT (47). Approximately 3,000 follicles remain by the time a woman reaches menopause (30).

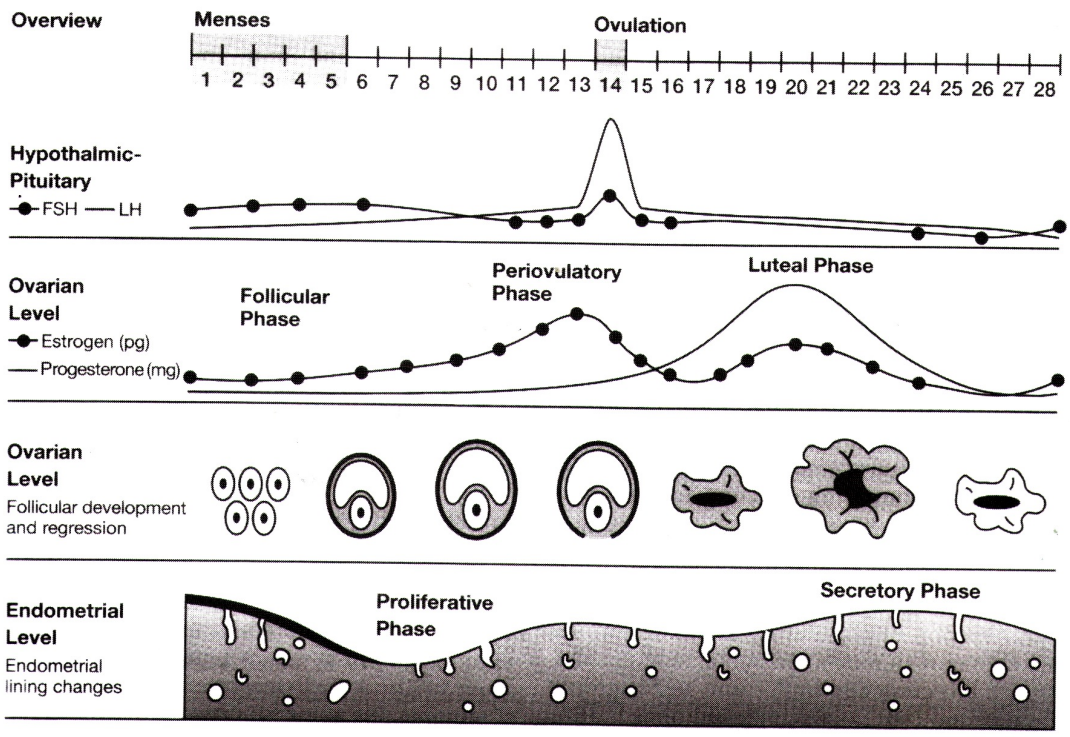

Figure 1.3 The menstrual cycle (Source: Menopause Practice: A Clinicians Guide (4th ed.)) (48)

At the beginning of the menstrual cycle, as shown in Figure 1.6, serum levels of estrogen and progesterone (P4) are low, causing, through a negative-feedback loop, the hypothalamic-pituitary ovarian axis, to increase FSH. As a result, approximately 1000 follicles begin to mature, increasing estrogen production, which thickens the endometrium. This is known as the follicular phase and by the end of this phase, one of the follicles (dominant), matures faster than the others, which begin to degenerate, having supported the dominant follicle thus far. The dominant follicle continues to mature, increasing estrogen production. This is the beginning of the periovulatory phase. Raised levels of estrogen cause the hypothalamus, through the agency of positive feedback, to reduce the production of FSH and to trigger a steep increase in the secretion of luteinizing hormone (LH) leading to the luteal phase. This surge of LH ensures that the dominant follicle continues to mature and then rupture. The ruptured follicle is now known as the corpus luteum and begins to produce estrogen and increasing amounts of P4, helping to stabilize the endometrium, preparing it for the fertilized egg. If there is no pregnancy, the corpus luteum deteriorates causing falling levels of estrogen and P4. The endometrium is thus no longer sustained and the menses, caused by the degeneration of the endometrium begin (30, 49).

1.3.2 Sex steroid and protein hormones, and the MT

In this study, and generally in research investigating the MT, specific sex steroid hormones are examined, in particular; estrogen (with E2 being the principal estrogen examined in this study), FSH and androgens, specifically testosterone, bioavailable testosterone, sex hormone-binding globulin (SHBG), dyhdroepiandrosterone (DHEA), dyhdroepiandrosterone sulfate (DHEAS) and the free androgen index (FAI). As described earlier, ovarian aging will cause hormone levels to fluctuate prior to reaching a plateau in late menopause (50). The literature shows that levels of FSH will rise across the MT while levels of E2 will fall (Figure 1.7) (51). It has been shown that some androgenic hormones, specifically DHEAS, fall

across the MT (52) as do levels of SHBG (53). It appears that testosterone levels do not fall across the MT (54), while the fall in DHEAS may be as a result of chronological aging (54, 55).

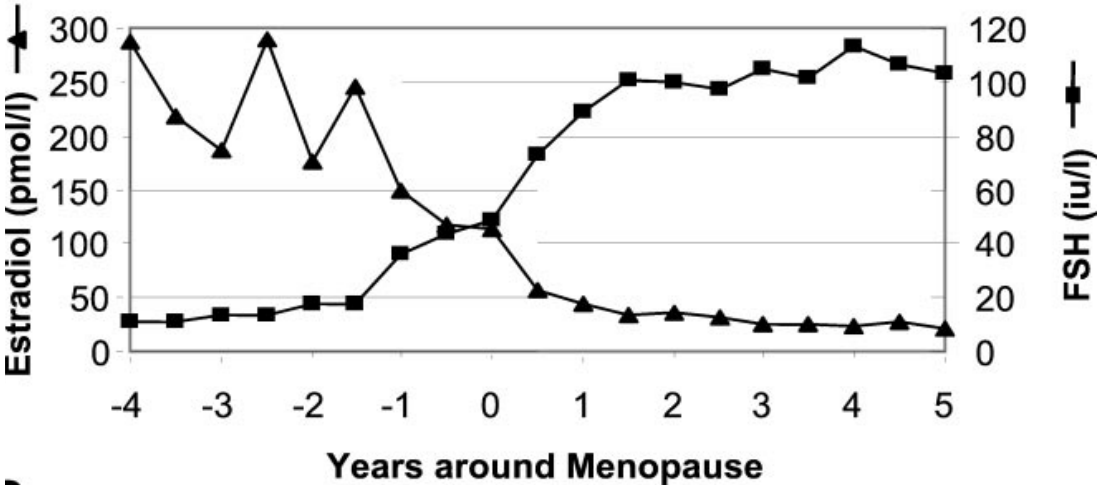

Figure 1.4 Levels of FSH and E2 shown across the MT (Source: Burger et al) (50)

1.3.3 The process of reproductive aging

The menstrual cycle functions, unless disturbed by compromising circumstances or by pregnancy (30), until the late reproductive stage (56). Since atresia causes an increased decline in antral follicles, fertility begins to decline and as a result of a decrease in levels of the gonadal peptide, inhibin B, these aging follicles do not respond as they did previously to FSH. When the negative feedback that has regulated the relationship between inhibin B and FSH changes, greater levels of FSH are secreted in an attempt to recruit and stimulate follicle growth (Figure 1.4.2). This may cause fluctuations in estrogen as well as FSH levels, leading to a shorter follicular phase, changes in normal menstrual cycles, and erratic ovulation or anovulatory cycles (30, 47).

The stages of reproductive aging as described in STRAW+10 (Figure 1.5) (56), show how ovarian aging causes changes both in levels of FSH, E2 (Figure 1.7), and bleeding patterns. In late reproductive stage -3b, regular menses continue and there is no change in FSH

levels in the follicular phase, but fertility begins to decrease due to the falling antral follicle count and declining levels of AMH. In late reproductive stage -3a, changes in bleeding patterns may become apparent, cycle length shortens (57, 58), FSH levels become more erratic and start to rise. In early menopausal transition stage -2, persistent changes of at least 7 days in the duration of successive menstrual cycles are seen and serum FSH levels increase though they are still erratic. In the late menopausal transition stage -1, amenorrhea is experienced for periods of 60 days or more, greater variations in cycle duration and steep hormonal fluctuations occur and FSH levels rise and fall, the latter especially when associated with high levels of E2 (47, 53). Stage -1 may last between 1 to 3 years and it is during this time that VMS and other menopause related symptoms start to become apparent (Figure 1.8). The FSH levels continue to rise in the early postmenopausal stage, +1a, +1b, with levels of E2 dropping until to two to five years after menopause (Figure 1.7) (51, 59). Stage +1a signifies that there have been no menses for twelve months. This stage happens in conjunction with the ending of the perimenopause. This period includes the first year after the final menstrual period (FMP) (Figure 1.5). Hormonal fluctuations seen in stages +1a and +1b, are usually accompanied by VMS and other symptoms. It appears that these hormones start to settle in stage +1c, which lasts between 3 to 6 years. Late post menopause +2 occurs when hormonal changes have stabilized, and is often when the symptom of vaginal atrophy becomes very common (38).

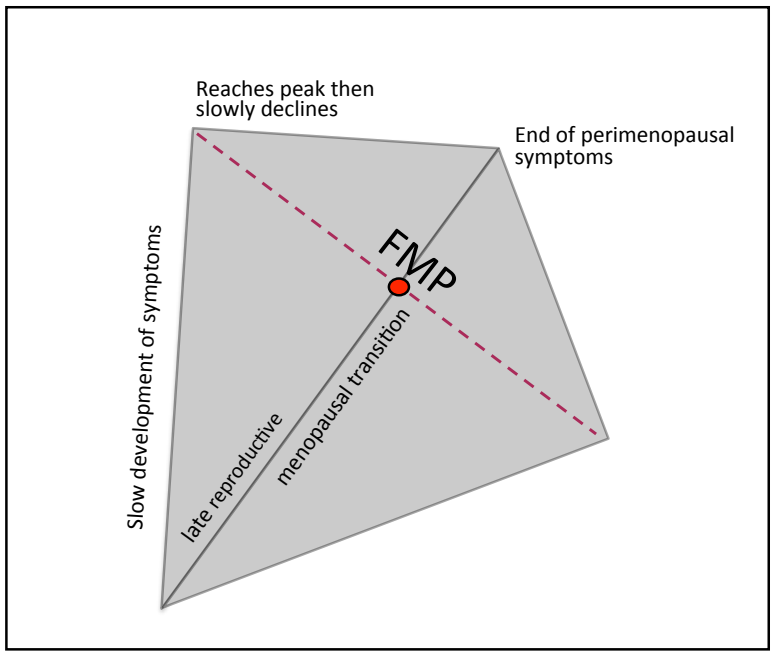

Figure 1.5 Development of symptoms in the MT (30)

1.3.4 Age at FMP

Changes in the menstrual cycle are a defining characteristic of the transition into menopause (56) and are a sign of reproductive aging. Earlier age at FMP has been shown to affect both morbidity and mortality (60), specifically in surgical menopause (bilateral oophorectomy), or chemical menopause, (ovaries cease functioning prematurely due to chemical or radiation treatments), or with a premature menopause caused by primary ovarian insufficiency, where the ovaries cease to function, either temporarily or permanently (48). Median age for natural menopause is around 52 years. Premature menopause is defined as happening in women younger than 40, while early menopause includes women aged 40 to 45 years (61). In a comprehensive editorial, Crawford described several factors related to healthy aging that may also relate to age at menopause (62). Factors considered include genetics where studies have shown that daughters whose mothers had an earlier natural menopause are more likely to experience an earlier menopause relative to the average experience (63). Socioeconomic and life style factors; unemployment, low educational levels and cigarette

smoking (64), and childhood lifestyle also play a role (65). Several studies have confirmed these findings, suggesting that childhood deprivation has an effect on ovarian aging (66, 67). Lawlor et al (68) have suggested that there is a strong association between unfavorable economic circumstances occurring throughout childhood and into adulthood, and a younger age at FMP.

An issue that has been widely discussed amongst those researching the MT is the difficulty in accurately ascertaining age at FMP when it is self-reported. Several studies have shown that recalling this occurrence many years after it had happened is not always accurate (69). A large prospective study generally found that answers, although given on different occasions, were similar (70). However, another very large prospective study found that the type of menopause experienced, whether surgical or natural, might affect accuracy of reporting. Research found that women who had had a natural menopause, reported the date within a year or two of the event, but those women who had had a surgical menopause reported the date with greater accuracy (71). This was also shown in a population-based screening project (72). Several studies agreed that age at menopause becomes less accurate as the number of years since the event increase, and that women who experienced an earlier age at menopause have a better recall than those who were older at onset (72, 73). However, there are other difficulties attendant on accurate recall when reporting age at FMP. The transition into menopause is often very gradual and lengthy, with extreme cycle variability (74), making it harder, in retrospect, to recall an exact date, which is why data from longitudinal studies, using follow-up interviews, may improve accuracy (75). It has been argued that menstrual calendars may be useful in reliably assessing this date, but this method may not be useful in a population with a very low educational level, that does not generally have easy access to primary healthcare clinics or annual medical check-ups. Data from a large multi-ethnic study showed poor concordance between an annual report and menstrual calendar (76), which may relate to differences in understanding of the menstrual cycle changes in the participants as opposed to the researchers (77). As explained, there is

extreme menstrual cycle variability during the late reproductive stage, which may be difficult to identify and describe, so validated questions that clearly relate to the MT stages as described in STRAW+ 10 (56) may be more useful.

1.3.5 Menopause symptoms and the MT

As described earlier, STRAW+10 suggest that two kinds of menopausal symptoms, VMS and vaginal atrophy may be used as supportive criteria in staging menopause. However ovarian aging and the accompanying hormonal changes usually mark the onset of a wide range of different menopausal symptoms (Table 1.3).

Identifying severity and prevalence of menopausal symptoms is important in research, especially in the case of VMS (78). Several studies have found that severe VMS may increase a woman's risk of both CVD and related metabolic variables (79-81). In addition, the genitourinary problems experienced by many women in menopause may seriously affect their quality of life (48), as do many of the symptoms related to mood and cognition (82).

Table 1.1 Symptoms of the Menopause Transition(30)

Symptoms	Symptom Domain
Vasomotor symptoms -hot flushes/flashes -night sweats -body temperature disturbance	Somatic
Heart palpitations	Somatic
Breast changes -tenderness -changes in density	Somatic
Weight gain -android fat deposits	Somatic
Genitourinary symptoms -vaginal atrophy -vaginitis -dyspareunia -changes in sexual functioning -loss of libido -hypoactive sexual disorder -changes in urinary function -urinary and stress incontinence -urinary tract infections	Urogenital-sexual
Sleep disturbance -insomnia -altered sleep patterns -early wakefulness -difficulty falling asleep	Somatic
Mood swings -anger -depression -generalized and free floating anxiety	Psychological
Memory issues -forgetfulness -confusion -impaired concentration	Psychological
Changes in skin and hair -thinning hair -increased facial hair -dry eyes -bleeding gums	Somatic
Joint pain/muscle aches	Somatic
Low grade fatigue	Psychological

1.4 Tools to assess menopausal symptoms

1.4.1 Kupperman Index

There are several instruments that may be used to assess menopausal symptoms. Initially, research into the MT used the Kupperman Index (sometimes called the Blatt-Kupperman or Blatt Index), which was formulated in 1952 and modified in 1953 (83, 84). In line with the clinical understanding of the time and based on their clinical experience, Kupperman and his colleagues developed an index of 11 menopausal symptoms in order to identify and treat what they termed 'the menopause syndrome'. Their intention was to compare the different medications that were being used to treat it. They recommended that three discrete entities should be considered; 'psychotherapy, sedation, and hormonal therapy' (85). This index, which was scored from 0 to 3, depending on prevalence and severity of the 11 symptoms listed the following symptoms: hot flushes, paraesthesia, insomnia, nervousness, melancholia, vertigo, weakness, arthralgia or myalgia, headache, palpitations, and formication. Scores in the 11 categories were added and the total separated into categories: 0 = none, 5 = mild, 10= moderate or ≥ 15 = severe. In 1959, a refinement of the Kupperman Index introduced weighting in selected categories. The weighted categories were divided into three: mild = 15-20, moderate = 20 -35 and severe = ≥ 35 (85). In 1998, a comprehensive critical review by Alder, described the shortcomings and disadvantages of the Kupperman Index (86), and suggested the need for a different tool. Several reasons were given: psychometric standards had advanced, and the index was not psychometrically sound due to bias in the assessment. The list of symptoms excluded vaginal atrophy and decreased libido, which are both commonly expressed menopausal symptoms. The former is a defining symptom of postmenopause and is related to declining levels of estrogen (56), while the latter is one of a group of symptoms that appears to be specifically related to menopause (87). In addition, the terms used in the index were not clearly defined, no mathematical justification was offered for the symptom weightings used, and the index was neither statistically valid nor reliable (86). However, this index was the main tool used to

identify menopause symptoms for approximately 40 years, and is still the preferred tool in some research studies (88).

1.4.2 Greene Climacteric Scale

In 1976, Greene, who became the first researcher to use factor analysis to assess menopause symptoms, proposed a new menopause scale (89). The symptoms were divided into three categories, vasomotor, somatic and psychological. Greene suggested that the MT was not a static event and proposed that changes in symptoms, and the association between different menopausal stages could be measured using this scale. The scale could be used to evaluate treatments, in cross-cultural comparisons, and epidemiological research into menopause and the MT. Greene suggested that VMS were a key factor of the MT and should be placed in an independent category. The scale was amended in 1998 and described as a: 'a standard measure of core climacteric symptoms'. Seven factorial studies, which were in strong agreement as to which symptoms should be included and the specific category to which they belonged, were compared (90). However, there were still some questions concerning the scale. Greene did not include the symptom of vaginal dryness, or that of breast pain (mastalgia) in either the original or the amended scale (91). Nevertheless, the scale is extensively used in women worldwide, and is considered valid, reliable and culturally appropriate (92, 93). Researchers have found the Greene Climacteric Scale easy to work with (48).

1.4.3 The Menopause Rating Scale (MRS)

The Menopause Rating Scale is a popular tool for measuring prevalence and severity of menopausal symptoms. This health related quality of life scale was originally developed in Germany in 1992 by researchers who wanted an instrument to measure menopausal symptoms in aging women and assess how these affected their quality of life (83). This scale was physician administered, but in 1996 a modified version (MRS II) enabled women

to perform it themselves (94). The MRS comprises 11 symptoms, within three different domains (psychological, physiological and urogenital), which are easily scored using values of 0-4 (95). Strengths of the MRS include its ability to assess quality of life (96) and its reliability (97). In addition, it is cross-culturally applicable, has been internationally validated and has been translated into nine different languages (98). Difficulty in scoring has been found in some population groups due to lower education levels and inability to understand the values of the scale, but this disadvantage can be addresses if a member of the research team administers the scale (99). Studies in the following population groups have used the MRS, and found it to be reliable and valid in describing severity and prevalence of symptoms: Latin America (100, 101), Oman (102), Nigeria (103), Malaysia (99) and Sri Lanka (104).

1.5 Reproductive Aging

1.5.1 Staging Menopause

Prior to 2001, there was no specific structure defining the stages of the MT and the system of staging menopause developed gradually. Research in the mid 70's, and focused mainly on HT regimens and their outcomes (105). In 1976, the First International Congress on the menopause was convened in France (16) to examine issues concerning HT use and plan future research (26).

1.5.2 Defining the terminology of menopause stages

In 1981 the World Health Organization convened a group of experts to comprehensively debate issues relating to the menopause (106). It was agreed that there was a lack of consensus in medical research regarding the definitions used to describe the various stages of reproductive aging and clear recommendations were made round the terminology relating to specific stages. Topics included changes in the level of hormones related to the MT and

the need for a culturally appropriate tool to measure menopause symptoms. Issues around age at FMP in different cultural and socioeconomic groups and the different cultural experiences of menopause were discussed. It became evident that the WHO should play a role in addressing these issues, providing direction for further research (106).

Table 1.2 Definitions describing the Menopause; WHO Scientific Group on Research on the Menopause in the 1990s (107)

Natural Menopause	The permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Natural Menopause is recognized to have occurred after 12 consecutive months of amenorrhea for which there is no other pathological or physiological cause. Menopause occurs with the final menstrual period (FMP) which is known with certainty only in retrospect a year or more after the event. An adequate independent biological marker for the event does not exist
Perimenopause	The term perimenopause should include the time immediately prior to the menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and the first year after menopause.
Menopause Transition	The term menopausal transition should be reserved for the time before the final menstrual period when variability in the menstrual cycle is usually increased.
Premenopause	The term premenopause is often used ambiguously, either to refer to the 1 or 2 years immediately before the menopause or to refer to the whole of the reproductive period prior to the menopause. The group recommended that the term be used consistently in the latter sense to encompass the entire reproductive period up to the FMP
Induced Menopause	The term induced menopause is defined as the cessation of menstruation which follows either surgical removal of both ovaries (with or without hysterectomy) or iatrogenic ablation of ovarian function (e.g. by chemotherapy or radiation).
Simple Hysterectomy	Where at least one ovary is conserved is used to define a distinct group of women in whom ovarian function may persist for a variable period after surgery
Postmenopause	The term postmenopause is defined as dating from the final menstrual period, regardless of whether the menopause was induced or spontaneous
Premature menopause	Ideally, premature menopause should be defined as menopause that occurs at an age less than two standard deviations below the mean estimated for the reference population. In practice, in the absence of reliable estimates of the distribution of age at natural menopause in populations in developing countries, the age of 40 years is frequently used as an arbitrary cut-off point, below which menopause is said to be premature.

Subsequent to this meeting, a WHO scientific group met in 1996 to evaluate the progress made on recommendations from the 1981 meeting, and to discuss emerging research issues. As mentioned earlier, the group noted that by 2030, 76% of menopausal women would be living in developing countries (1), but there was a paucity of research on the health risks associated with menopause in these women, and therefore there was a need for more data, to enable the departments in these countries to understand the potential morbidity and mortality risks of aging women (2).

Menopause stage definitions that had been proposed by the scientific group of 1981 were augmented and modified as shown in Table 1.1 Changes in bleeding patterns were discussed, including changes in the length of bleeding and time between bleeds . The issue of age at FMP was explored and it appeared that women in low resource counties were found to have an earlier age at menopause than those in high resource countries (108). The roles of sex steroid hormones, proteins and androgens in the MT were evaluated. Symptoms of the menopause transition were discussed (2). The recommendations suggested a need for more precise definitions than those adopted at the 1981 meeting. The research conducted should be culturally appropriate; and attention should be given to the possibility that ethnicity may affect perception and reporting of VMS. The effects of chronological and/or reproductive aging on health in mid-life women should be noted. A possible association between VMS, CVD and osteoporosis risk was identified, as well as a need for more sensitive and standardized hormone assays. Future research should examine whether changes in hormone levels during perimenopause might be predictive of future health risks (2).

The recommendation, from the WHO scientific group, that standardized definitions should be used in menopause research, was reinforced in 1999, when the Council of the Affiliated Menopause Societies (CAMS) was given the task of defining menopause related terminology to prevent confusion and allow a common understanding in global menopause studies as shown in Figure 1.3 (109). The International Menopause Society (IMS) board approved this terminology and recommended that these definitions should be used internationally (109) . The focus of all these groups described above was primarily on the terminology related to the MT, and not specifically on the staging of reproductive aging.

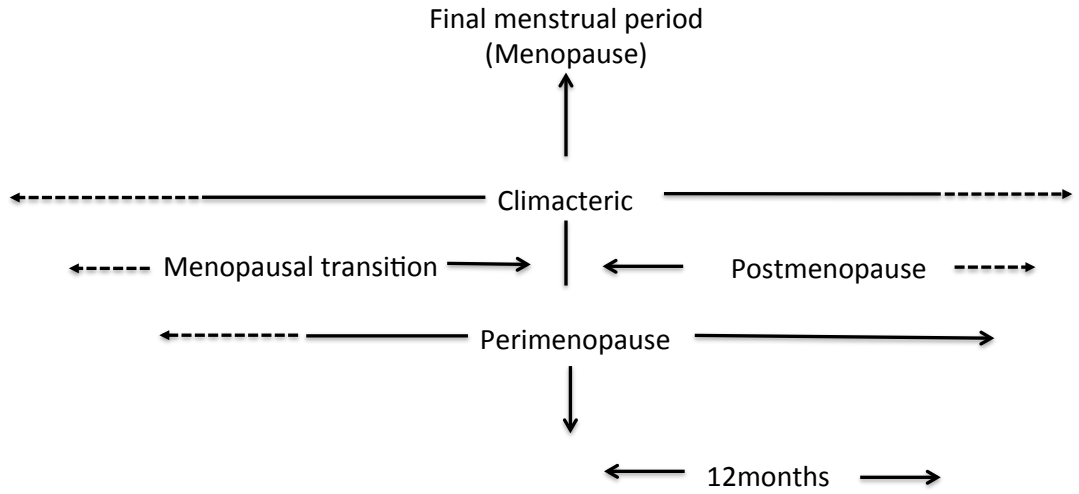

Figure 1.6 Utian 1999 terminology definitions: Relationship between different time periods surrounding the menopause modified from the WHO 96238 report (109)

1.5.3 Cohort studies and the Staging of Reproductive Aging Workshop (STRAW)

Throughout the 1990's, several cohort studies were set up to examine the natural transition into menopause, and many of these looked at ways of staging the MT. These included the very large multi-ethnic cohort Study of Women's Health Across the Nation (SWAN), formed in 1994 (110), and the Seattle Midlife Women's Health Study (SMWHS) begun in 1990 (111). Accurate staging of reproductive aging is fundamental in understanding many aspects of the MT, including symptomatology (112). In July 2001, 27 experts in the field convened the Staging of Reproductive Aging Workshop (STRAW) in Park City, Utah to create an accurate staging system (113). The contribution of STRAW to the field of menopause research cannot be overestimated and the criteria adopted by STRAW were considered the gold standard for staging the MT (114).

The aims of STRAW were to create an appropriate and practical staging tool, incorporating standardized terminology that would be useful in both clinical and academic research to assess and understand the trajectory of women's reproductive aging; specifically the length and time period of the transition towards menopause.

The experts present determined that a staging tool should abide by specific, objective criteria. Tests used should be consistent, not expensive and easily obtainable. Prospective categorization of women should be allowed for appropriate classification (113). The criteria discussed were: menstrual cycles (the high levels of variability, and effective ways to confirm a woman's menstrual history), biochemical and endocrinological measures, fertility and ovarian ultrasound to determine antral follicle count. Due to fluctuations in hormone changes during the MT, FSH was considered the only assay that could be reliably measured in a clinical environment as a biomarker for the staging system.

It was recommended by STRAW that changes in bleeding patterns should be one of the factors used to stage reproductive aging; STRAW used the definition of a 'variable cycle length, more than seven days different from normal', and based this on data from three important studies (115). These were the Melbourne Women's Midlife Health Project (MWMHP), the SMWHS and the long-running TREMIN Research Program on Women's Health (116). These studies described five measures, marking the beginning of the MT (115). Lisabeth et al analysed data from TREMIN and concluded that a cycle of more than 60 days was probably the best indicator of late MT (117). The bleeding criteria suggested by the MWMHP were that the start of the MT was marked by 'irregularity' meaning that for at least 10 menstrual cycles, a woman would have more than two menstrual cycles that were not in a span of 21 to 35 days (74). The SMWHS described variations in cycle using two terms 'irregularity' and 'skipping'. The former meant that there was a greater variation of 6 days between ongoing menstrual cycles and the latter that there was no bleeding at all for more than 58 days between cycles. Specific changes in the menstrual cycle encompassing bleeding flow and the duration of cycles, followed by irregularity as defined above and then skipping prior to the FMP were described (58).

It was agreed that menopause symptoms are subjective and their definition may vary between socioeconomic groups, cultures and ethnicity (113). The group revised the

menopause terminology adopted by WHO in 1996, attempted to find more precise definitions and, as shown in Figure 1.4, described reproductive aging using seven stages: -5, -4, -3 (reproductive stage: early, peak and late); -2, -1(menopausal transition: early and late); +1,+2 (postmenopause: early and late). As shown in Figure 1.4 menopause was described as the point following the FMP, after 12 months of consecutive amenorrhea. The menopause transition is the time defined by rising FSH levels and variations in the menstrual cycle. This stage ends with the FMP. Postmenopause comprises two stages – early and late. The length of the stages were described as variable for reproductive and menopause transition stages, and early post menopause as being five years after the FMP, with a sub-stage (a) being 12 months after the FMP and a second sub-stage (b) being 4 years after the FMP. Late postmenopause was described as five years after the FMP to the end of a woman's life. Perimenopause, was described as beginning in early menopausal transition and ending a year after the FMP. STRAW recommended that the term climacteric no longer be used in scientific menopause literature (113).

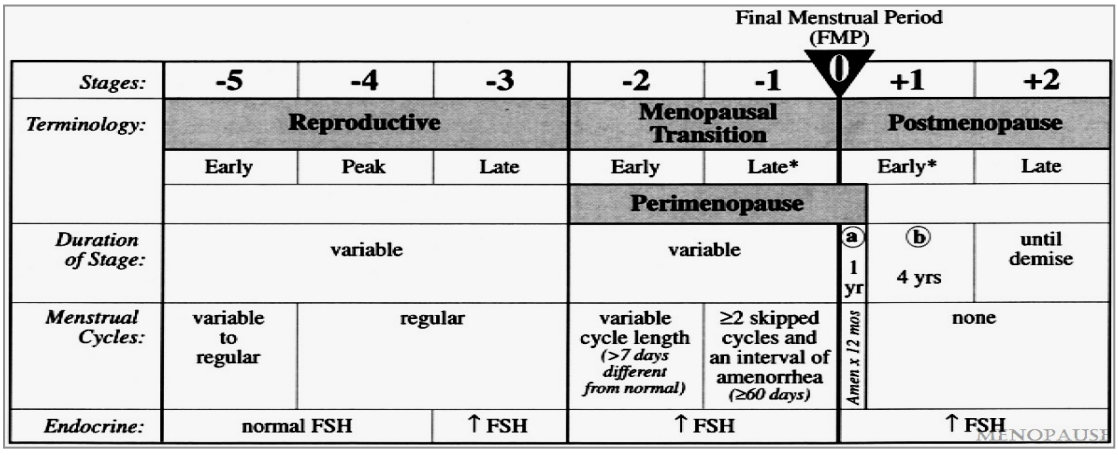

Figure 1.7 Stages of Reproductive Aging Workshop (STRAW) – seven stages of STRAW (Soules et al) (113)

1.5.4 Staging the MT using bleeding criteria.

Subsequent to STRAW, investigators began examining the bleeding criteria recommended by STRAW in relation to different stages of the MT. The problem was that the bleeding

criteria adopted by STRAW were not empirically assessed and had not been validated (118). The ReSTAGE Collaboration was thus constituted to evaluate the consistency and validity of those bleeding criteria, and to examine whether these could be applied to women in different cohorts, both in research and clinical studies. Researchers involved in four cohort studies participated in ReSTAGE. The studies were SWAN, TREMIN, MWMHP and SMWHS. ReSTAGE analyzed prospective data based on information from menstrual calendars from the cohorts (118). Harlow et al focused on whether suggested bleeding patterns defined by STRAW marked the start of the late menopausal transition stage and found that the STRAW recommendations were essentially valid. The results were robust and confirmed that 60 days of amenorrhea marked the start of the late menopausal transition. The STRAW stages needed to be further clarified, and an agreement reached as to which measures of menstrual bleeding would define the start of the MT (118). Serum FSH data from SWAN and MWMHP were analyzed and showed that levels of FSH were closely related to changes in bleeding patterns across the MT (119).

This close association was confirmed in data that showed that increased levels of FSH were related to bleeding changes associated with the start of the early menopausal transition stage (115). When comparing measures, both inter- and intra-, to facilitate prediction of a woman's nearness to the FMP, the ReSTAGE investigators found that women with natural menopause usually had changes in menstrual function as a sign of early transition, described by the bleeding criteria proposed by STRAW. The best measure to describe early menopausal transition appears to be a difference greater than six days in length of menstrual cycle, which seems to happen sooner than in the other suggested markers (115).

The collaborators from ReSTAGE evaluated whether the measures of bleeding patterns, endocrinology and symptomatology recommended by STRAW were appropriate in determining stages in reproductive aging, and confirmed they were valid. ReSTAGE suggested that measures for defining both the early and late menopausal transition stages

should be refined. This included a simple bleeding pattern measure of amenorrhea for 60 days to define the start of late menopausal transition, including an FSH level of ≥ 40 IU/L to forecast the time of the FMP more easily, and to use a measure of an ongoing difference of 7 days or more in cycle duration as the marker for the start of the early menopausal transition stage (114).

The work of the ReSTAGE collaboration set the stage for the modification of the original STRAW criteria. It became clear that basic measures of bleeding criteria were useful in describing the MT, and that a simple questionnaire could be developed so that women's answers could help define at which stage of reproductive aging they found themselves (115).

1.5.5 STRAW +10

In the 10 years following STRAW, clinicians and researchers in the field of menopause and reproductive aging acquired a wider understanding of hypothalamic-pituitary and ovarian function during the MT. This encouraged experts to update the STRAW recommendations. In October 2011, a workshop: ' STRAW + 10: Addressing the Unfinished Agenda of Staging Reproductive Aging' was held in Washington, DC. The aim of the two-day workshop was to assess the STRAW criteria for staging menopause, based on the latest data (56). In addition, presentations examined the relationship between chronic disease and the difficulty of staging the MT in those women who are infected with the human immunodeficiency virus (HIV), or have cancer. On September 22, 2011, after careful appraisal of all STRAW stages, general agreement on a staging system was reached (Table 1.2). These modifications were presented at the annual meeting of the North American Menopause Society (NAMS) (56). STRAW+10 retained the measures for an appropriate and practical staging tool suggested by STRAW and added some modifications (Figure 1.5).

Table 1.3 Consensus of criteria for an ideal staging system (56)

Rely primarily on objective data	STRAW
Use widely available, reliable, noninvasive, and inexpensive tests	STRAW
Allow for prospective classification of women	STRAW
Permit unambiguous classification of women into a unique stage	STRAW
Retain the same widely accepted nomenclature	STRAW+10
Consider menstrual cycle criteria to remain the most important criteria given the continuing lack of international standardization of biomarker assays as well as their cost and/or invasiveness, particularly in the context of resource poor countries	STRAW+10
Consider biomarker criteria as supportive criteria given the lack of assay standardization (supportive criteria are to be used only as necessary and should not be interpreted as required for diagnosis)	STRAW+10
Use criteria that are independent of age, symptoms, and pathology (because no universal menopausal syndrome has been established across ethnic groups, two key symptoms are incorporated only as descriptive additional information that may support other criteria in assessing stage)	STRAW+10

STRAW+10 suggested that recommendations from ReSTAGE, which used more accurate and less complicated definitions of menstrual cycle measures should be adopted for early and late menopausal transition stages (56).

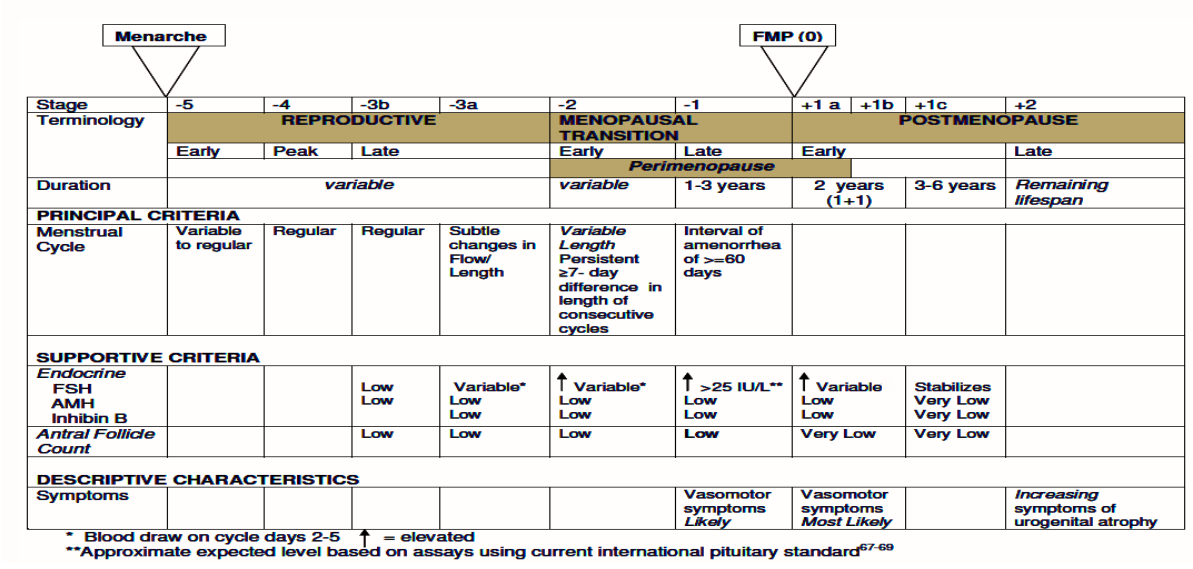

Figure 1.8 STRAW + 10 stages of reproductive aging diagram (Source: Harlow et al) (56)

Bleeding patterns (menstrual cycles) remain the most important criteria for determining the stage of the MT because there is no standardization of biomarker assays (56, 115, 118), although recently more accurate, standardized testing of estrogen has been recommended (120). In addition, low- or middle-income countries may not have the resources for the wide use of blood assays in assessing specific stages in the reproductive cycle (121). In 2001, STRAW recommended that follicle-stimulating hormone (FSH) level be used as a biomarker in the late transition stage. Given the improved understanding of endocrine changes involved in ovarian aging (51), STRAW + 10 suggested that FSH and E2, specifically their concentrations before and after the FMP, should be used as supportive criteria to verify the stage of the MT. It was decided that an FSH serum blood level of >25 IU/L could generally define the late menopausal transition. Data also suggest that FSH levels will continue to increase until approximately two years post FMP, while E2 levels will decline (51). Thereafter these levels will become stable (57). STRAW +10 acknowledged that although no set of menopausal symptoms had been specified worldwide due to different ethnicities, VMS and vaginal atrophy might be used to support other measures to determine the stage of the MT. Since bleeding criteria are used to stage reproductive aging, women who have had hysterectomies cannot be staged using STRAW+10, although in these cases, FSH levels, at least three months post surgery, may be used as a measure of MT. Important areas for ongoing research included the establishment of a standardized assay of anti-Müllerian hormone (AMH); the definition of more precise bleeding patterns in the late reproductive stages; examining changes in sex steroid hormones in early and late post menopausal stages and staging the MT in the HIV-positive women. It was shown that STRAW+10 is a staging tool that may be applied generally to women, irrespective of body mass index (BMI), age, lifestyle, or demography (56).

1.6 Cultural aspects of menopause

Among problems found when researching reproductive aging in different populations, are the cultural and ethnic differences within diverse groups of women. A wide-ranging body of literature examines the problems inherent in attempting to describe the menopause experience as uniform amongst women (122). Issues that may play a role, include socioeconomic status, cultural mores and ethnicity (123). However, in order to carry out cross-cultural comparative research, there needs to be a standardized tool which takes cognizance of menopausal symptoms that may be specific to different cultures and ethnicities (123). The literature shows that menopause symptoms, specifically VMS and their severity and prevalence, are often described and/or perceived differently by women in various population groups in spite of the fact that VMS are widely present (124). It has been suggested that these variances are informed by several different factors, such as lifestyle and cultural norms, and these can also affect the experience of menopause (125). Evidently the cultural imperative is a very important factor in understanding a woman's experience of her menopause (126). A powerful review by Melby et al, suggests that a biocultural approach, which examines differences in both biological and cultural variables and how they affect reproductive aging in women of different population groups, is vital when conducting cross-cultural comparisons (127). However other research suggests that while there are universal aspects to symptom reporting between different population groups, the way in which prevalence and severity are explained are associated with varying cultural imperatives (128). Another problematic area is the difference in menopause perceptions between women from high and low-resource countries and the fact that women have a diverse understanding of menopause, possibly reflected by the way in which their own culture views the transition into menopause (87). It may be important to provide women with culturally specific information on menopause (129). Another important aspect to consider when researching menopause across different cultures, is heterogeneity within each cultural group, since socioeconomic variables may play a part in how women experience the MT in

different ways (130). Given this wide range of variables, methodology in cross-cultural research determining menopause symptoms needs to be very carefully designed and controlled (131). A very large multi-ethnic study suggested that when gathering information about the menopause from women of different cultures, control factors, including socioeconomic status, lifestyle and education, need to be taken into account (122).

Researchers in the field concur that both open and close-ended questions are useful in obtaining information about reproductive aging in different population groups. In addition it has been suggested that more attention be paid to a thorough analysis of the cultural mores of a population group, and in addition, there should be a clear distinction between etic and emic data, whereby data reflecting the areas of interest of the researcher and data reflecting that of the participants, should be considered. Although emic data is more complex because the researcher needs to thoroughly understand the cultural mores of their research subjects, the addition of open-ended questions to close-ended question may go some way to solving this problem (132). As described earlier, biomarkers may also be used as supportive criteria in assessing menopause stage through self-reported bleeding patterns (56). Unfortunately many low-income countries do not have the resources to perform these assays.

1.7 Menopause, morbidity and mortality

1.7.1 Menopause and the metabolic syndrome

A constellation of symptoms described as the metabolic syndrome (MetS) may increase the risk of CVD. The currently accepted definition for this syndrome is the one contained in the Harmonizing statement (133), where a number of experts from different medical organizations agreed that if there was a finding of three or more out of the five criteria described in the statement, which exceeded the stated cut points, a diagnosis of metabolic syndrome may be given. These five diagnostic criteria are: an increased waist circumference, raised triglyceride levels (≥150 mg/dL [1.7 mmol/L]) lowered levels of HDL-C

(<50 mg/dL [1.3 mmol/L] females, and <40 mg/dL [1.0 mmol/L] males), hypertension (systolic ≥130 and/or diastolic ≥85 mm Hg and raised fasting glucose (≥100 mg/dL[≥ 5.6mmol/L]). These are the criteria that will be used in this study. The consensus of the Harmonizing group was that waist circumference should be gender and population specific (133). Thus, the waist cut points for Caucasian males and females were; ≥ 94cm and ≥ 80cm respectively; ≥ 90cm for Asian males and ≥ 80cm for Asian females, and ≥94cm and ≥80cm for sub-Saharan males and females respectively. However, studies on mid-life black African women suggest that a higher waist circumference cut point of between 91.5cm (7) or 92cm (134) may be more appropriate than the ≥80cm cut point that was recommended in the Harmonizing statement.

A wide body of literature has shown that menopausal women have risk factors related to the MetS, and these are discussed in the sections below (Table 4). However, whereas several studies suggested that the transition into natural menopause appeared to increase the risk of MetS (135-138), other data showed that aging attenuates this association, (139, 140). Data from the Study of Women's Health Across the Nation (SWAN) showed that 13.7% of their cohort of 949 women had MetS at the time of final menstrual period (FMP) but suggested that this risk seemed to lessen after FMP (110, 141). Some research has suggested that the combination of age and the MT may explain an increased risk of MetS in postmenopause (142), while other studies found that menopause is not associated with an increased risk of heart disease (143), and even when body composition changes accompany the MT in women with a normal BMI, these changes are not indicative of CVD risk (144). Two comprehensive reviews (9, 145) examined the relationship between increased risk of MetS and the MT and found no obvious association. It appears that several factors, either individually or combined, might be associated with the MT and might increase the risk of cardiometabolic disease in mid life women, but these risk factors may have existed before the transition into menopause (9), so the data remain conflicting.

Table 1.4 Overview of important risk factors related to cardiometabolic disease in menopausal women

Risk factors for cardiometabolic disease in menopause	Research hypotheses
Age and/or MT increase MetS risk	-reproductive or chronological aging or a combination of both increases MetS risk
Changes in body composition	-MT closely related to changes in body composition -increased central adiposity associated with NCDs -changes in abdominal obesity related to changing hormonal milieu across MT -changes in abdominal deposition of VAT and SAT during MT related to either reproductive and/or chronological aging -sarcopenia associated with both chronological and reproductive aging
Obesity	-increased obesity associated with MT -obesity associated with metabolic disease
Sex steroid hormones, FSH, SHBG, DHEAS	-decreased E2 associated with hypertension and dyslipidemia -lower estrogen levels associated with increased body fat and decreased lean mass (sarcobesity) may increase metabolic disease risk -changing androgenic milieu associated with increased abdominal obesity - increasing androgenic milieu and exposure time to higher androgen levels may increase CVD risk -declining SHBG levels related to increased insulin levels and higher BMI increasing MetS risk -DHEAS associated with increased CVD mortality possibly due to lower anti-inflammatory effects
Adipokine levels	-adiponectin and leptin associated with metabolic variables that may increase CVD risk -negative correlation betwen adiponectin and body adiposity, atherogenic lipid levels and BP -positive correlation between adiponectin with insulin sensitivity -leptin levels associated with appetite control, body mass and energy expenditure. -leptin may be significantly related to insulin resistance and MetS independent of its BMI relationship
Age at FMP	-earlier age at FMP may increase risk of CVD and stroke -family history may attenuate CVD risk -HT prescribed for early menopause may attenuate risk
VMS	-VMS associated with increased TC, LDL-C, triglycerides, impaired fasting glucose, increased HOMA index, increased carotid intima media thickness, hypertension
Lifestyle factors	-association between smoking and younger age at FMP - VMS more prevalent and severe in smokers - Smoking associated with increased visceral fat and impaired insulin sensitivity -smokers in the MT at greater risk for atherosclerosis - nicotine may increase insulin resistance

1.7.2 Changes in Body composition across MT and risk of cardiometabolic disease

The literature shows that the MT is closely associated with changes in body composition (146), including lower bone mineral density (BMD) (147), an increase in obesity (148, 149), changes in body fat distribution (BFD) (150), particularly increased central adiposity (151), and a decrease in lean muscle mass (146, 152). The relationship of the MT with changes in abdominal obesity is complex. Several studies have found that increased central adiposity is a result of the changing hormonal milieu; increased FSH levels (146), decreased levels of E2 (153), and changes in androgen levels (154), and there is a strong association with reproductive aging (155), particularly for visceral adipose tissue (VAT) (151, 156). Further research suggests that changes in the abdominal deposition of VAT and subcutaneous adipose tissue (SAT) during the MT are related to chronological aging (157, 158), although the metabolic effects of VAT and SAT have been shown to be different (159-161). However, other data suggest that both aging and hormone changes may explain the changes in body adiposity during the MT (51, 162). As detailed above, abdominal obesity, which can be assessed by measuring waist circumference, is one of the principal risk factors for cardiometabolic disease, including diabetes and CVD (163). Central adiposity and its strong association with non-communicable diseases (NCDs) has been widely described in Western literature (164, 165) Although an increase in abdominal adiposity does not appear to be related to the MT, but rather to chronological aging, longitudinal research has shown that physical activity in mid-life women attenuates weight gain and these women have lower waist circumference which may lower their risk for metabolic disease (166). Thus, studies in menopausal women have shown that physical activity lowers risk of cardiometabolic disease, including diabetes and hypertension (167-169)

Studies show that a decrease in lean muscle mass may be associated with both chronological and reproductive aging (146). A comprehensive review by Messier et al (170) examines the role of ovarian aging in loss of muscle mass, explaining that although declining estrogen levels may contribute to a decrease in lean mass, the role sex steroid hormones

play in this process is complex and difficult to determine. Studies have shown that sarcopenia, defined as decreasing lean mass, is associated with an increased risk for metabolic disease (171-173). Furthermore, the combination of increased obesity and loss of lean mass in post menopausal women, described as sarcobesity, has been shown to be an additional risk for NCDs (171).

As discussed earlier, decreased BMD is another change in body composition that takes place across the MT and has been examined in a large longitudinal study (174). Finkelstein et al show that bone density decreases rapidly during the late menopause transition stage and continues to do so in the years following FMP (175). Moreover, research showed that this loss of BMD increases for about 1.5 years after FMP and then this decline slowed (176). Studies have found that weight is inversely related to the speed at which BMD decreases (175) and that women who were not obese had a greater rate of bone resorption (176). Further research confirms this relationship (177, 178). However an extremely large prospective population based study found that obese postmenopausal women were not protected from fracture risk (179). An association between lean mass and BMD has also been reported (180, 181).

1.7.3 Sex steroid hormones, SHBG and FSH and the risk of cardiometabolic disease

The possible link between changing levels of sex steroid hormones across the MT and an increasing risk of cardiometabolic disease has been extensively studied (9). Chu et al hypothesized that rising levels of FSH were associated with unhealthy lipid levels in premenopausal women, which suggested that when postmenopausal levels of FSH become progressively higher, these may be a correlation with an increased risk of CVD (182). Rising levels of FSH over time have been shown to be associated with sarcopenia (146), which, as described earlier, is associated with an increased risk of metabolic diseases.

A comprehensive review (183) found that declining levels of estrogen were associated with increased risk of CVD. Svendson et al suggested that lower levels of estrogen lead to an increase in body fat and a decrease in lean mass, contributing to sarcobesity, which in turn increases risk of metabolic disease (184). In addition, research showed that decreasing levels of estrogen are related to an increased risk of hypertension (185) and lipid changes leading to dyslipidemia (145).

A large body of research has examined the association between the MT, androgens and increased risk of metabolic syndrome (186). One of the main risk factors for metabolic syndrome is abdominal obesity, and research has shown that as the MT progresses, the changing androgenic milieu is related to increased abdominal obesity which in turn may be responsible for increased CVD risk (187). Research has suggested that this increasing androgenic milieu and the time span that a woman is exposed to higher androgen levels may increase her risk for CVD (188). Although levels of testosterone remain relatively stable throughout the MT (54), declining levels of estrogen create what researchers describe as 'relative androgen excess' (189), which increases the risk of metabolic syndrome in women transitioning through menopause, as described in the SWAN study (134, 180).

Levels of the glycoprotein SHBG appear to decrease across the MT due to declining levels of estradiol, and this occurs concurrently with an increase in the FAI (54). Declining SHBG levels are related to increased levels of androgens (190) and insulin (187, 191), and higher BMI (192). In addition, these decreased SHBG levels which affect adipokines unfavorably, seem to increase the risk of metabolic syndrome in midlife women (193). This effect has been seen in other data which suggested that lower levels of SHBG were associated with increased VAT and impaired glucose tolerance (194). In a very large multiethnic, longitudinal study, a very significant association between declining levels of SHBG, increased bioavailable androgens and a higher prevalence of metabolic syndrome was seen (195). The association of lowered SHBG and decreased insulin sensitivity, and its role as an

independent risk factor for cardiometabolic disease was shown in a large cross-sectional study of Australian women (196). Studies have noted the association of altered lipid profiles and raised blood pressure with low levels of SHBG, specifically, decreased serum HDL-C, increased total cholesterol and raised diastolic blood pressure (197). Lower SHBG levels and increased FAI were noted in participants from the Women's Health Study, who had CVD and who did not use HT. However, this relationship was attenuated after adjustment for BMI and other CVD risk factors (190).

The androgenic sex steroid hormone, DHEAS may decrease the risk for CVD. Thus, data from the Women's Ischemia Syndrome Evaluation (WISE) demonstrated that lower levels of DHEAS were associated with higher levels of CVD mortality (198). The mechanisms by which DHEAS may affect CVD risk is not known, however studies have shown that this hormone has anti-inflammatory effects (199) and may enhance endothelial function (200)

Not all studies show that cardiometabolic disease levels increase across the menopause or that changes in hormone levels during the MT are related to cardiometabolic disease risk factors. Thus, in a large cross- sectional study, Worsley et al (201) showed that neither androgen nor estrogen levels appeared to be associated with a changing lipid profile, while findings from a longitudinal cohort study examined several risk factors for metabolic syndrome and found no association between the menopause transition and increased CVD risk (143).

1.7.4 Adipokines, and risk of cardiometabolic disease

Research has shown that adipose-derived hormones, adiponectin and leptin are associated with metabolic variables that may increase the risk of CVD (202). Adiponectin levels correlate negatively with body adiposity, atherogenic lipid levels and blood pressure but positively with insulin sensitivity (203), while leptin is associated with appetite control, body

mass and energy expenditure (204). Some studies have also found that leptin is significantly related to insulin resistance and MetS independently of its relationship with BMI (205, 206).

The role of sex steroid hormones and their relationship to changing adipokine levels and metabolism, as they alter during reproductive aging, has been explored (204). Studies have shown that when women in MT are not obese there appeared to be very little risk of lowered adiponectin levels and associated insulin resistance, while the MT transition did not appear to affect leptin levels. However during the MT, overweight and obese women are at risk for adverse changes in adiponectin levels (207). It appears that estrogen helps to stabilize adiponectin levels but in the MT, when estrogen levels start to fall, this protective effect is removed (208). Leptin levels were associated with severity and prevalence of VMS in menopausal women but interestingly no association was found between leptin levels and estradiol. However some research has hypothesized that, in obese women, aromatized estrogen from adipose tissue may play a role in increased leptin levels (204, 209). As explained earlier, the relationship between lower levels of SHBG and increased adiposity in midlife women has been shown (210). Data have shown that lower levels of SHBG and its relationship to decreased adiponectin levels lead to a greater risk of metabolic disease (193). This may occur because SHBG appears to play a role in controlling adipokine levels (211).

1.7.5 Age at Final Menstrual Period and cardiometabolic risk

Associated risks for metabolic disease during the MT has been widely examined, however, an important factor associated with risk for metabolic syndrome is the age at which menopause occurs. In fact, it has been suggested that any changes in menstrual cycle length, versus a regular cycle may increase risk for metabolic disease (212). The concern that earlier age at FMP may increase risk of cardiometabolic disease has been well described (213). In a prospective study, data from an extremely large cohort showed that

mortality was decreased by two years in women with a later menopause, and there was a decreased risk for both CVD and stroke risk (214, 215). A large multi-ethnic study found that when established heart disease risk factors were excluded, earlier age at FMP, was associated with both stroke and CVD, although familial CVD history attenuated this (216). The effects of premature and early menopause have been well described and include increased mortality and CVD risk (4). However, these effects, which appear to be caused by a decline in estrogen and androgen levels, may depend on the specific cause of the premature or early menopause, since ovaries, post menopause, may still produce small levels of estrogens and androgens (217), and, as described above, a changing hormone milieu may have diverse risk effects. Although there is no Level 1 evidence to support the following, and where it is not contraindicated, recommendations are that women with premature or early menopause, should be prescribed HT (either estrogen alone or estrogen and progestogen therapy), until the age of natural menopause, since this may help to lessen the risks of morbidity (61, 218).

1.7.6 Vasomotor symptoms and cardiometabolic disease risk

A wide body of research focusing on VMS, a defining symptom of the MT, has shown that these are significantly associated with cardiometabolic disease risk (219-221). As explained earlier, cultural perceptions may affect how VMS are reported (123). Nevertheless, it is clear that most women, as a result of ovarian aging experience VMS, irrespective of their cultural background (128, 158). Postmenopausal women experience higher levels of VMS than those in the late reproductive stage, though reporting of severity and prevalence may differ depending on race and ethnicity (122). Declining levels of estrogen are strongly related to presence of VMS (132, 221), as are increased levels of FSH (222). It has been shown that menopausal symptoms occur across different cultural groups, and it is highly probable that these are related to the changing hormonal environment of the MT (223). However, it is important to remember that levels of estrogen or factors that impact on estrogen levels, may

be affected by race and ethnicity, as well as demographics, lifestyle habits and cultural mores (158). In addition to fluctuating and declining levels of estrogen, there are other factors that may affect severity and prevalence of VMS (224). These include environmental and demographic variables such as climate, socioeconomic status and level of education (126), cultural aspects (225), psychosocial stressors (226) lifestyle factors, including smoking (227, 228), and BMI (229, 230).

Metabolic variables, such as obesity, increased levels of total cholesterol, LDL-C and triglycerides are also associated with VMS (221). The relationship between VMS and an altered lipid profile that was found in the very large population-based study of nearly 6,000 women (221), was also seen in a very large multiethnic cohort study (220). Data from the above cohorts also found an association between VMS and additional symptoms of the metabolic syndrome, impaired fasting glucose and an increased HOMA index. In addition to those metabolic risk markers, an increased carotid intima media thickness was found in both overweight and obese women, who consistently reported VMS over a two week period (80). Obesity, has been shown to be associated with a greater prevalence of VMS (231). The hypothesis is that the ability of adipose tissue to retain body heat results in a complex thermoregulatory process to maintain homeostasis (232). Hypertension has also been reported in association with VMS (221).

It should be noted there are conflicting data as to the association between VMS and increased risk of CVD (233). It has been suggested that researchers should distinguish between hot flushes and night sweats rather than combining them in the broader descriptive term, VMS. This distinction is necessary because it has been found that healthier women who only experience hot flushes and not night sweats have a lower risk for heart disease (234). This finding is similar to data from the Women's Health Initiative Observational Study (WHI-OS), but here, researchers suggested that the timing of the onset of VMS predicted greater risk of CVD in those women who experienced VMS later in menopause (235).

Moreover, results from a population-based study showed that women who experienced night sweats had a lower risk of death over 20 years (236). Other research has disputed the findings that women with VMS have altered lipid profiles, and showed no difference in lipid levels between women who did or did not experience hot flushes, regardless of severity (237), although endogenous estrogen concentrations were comparable in both groups. However, a previous study found that estrogen levels in women without VMS are much higher (221). This variation in findings suggests a need for further research to better understand the usefulness of VMS, or hot flushes or night sweats respectively, as an important marker for future risk of cardiometabolic disease in menopausal women.

1.7.7 Obesity and black South African women

A wide body of research has shown that obesity in postmenopausal women is associated with risk factors for metabolic disease (202, 238), in particular type 2 diabetes and dyslipidemia (239) . It has also been found that these risks are specifically associated with abdominal obesity (240), which has been shown to correlate positively with insulin resistance (145). For the purpose of this study, the WHO definition of obesity, namely levels of BMI ≥30kg/m^2 is used (241).

Since obesity is a risk factor for MetS, and given the high prevalence of obesity amongst midlife black South African women, it is not surprising that there is also a high incidence of metabolic disease in this group (242). This high prevalence of obesity in black South African women aged 45-55, is shown in Figure 1.9, but BMI starts to fall in this group after the age of 65, however since the data in the South African National Health and Nutrition Examination Survey (SANHANES) is cross-sectional, the fall in BMI may be due to a period rather than a cohort effect.

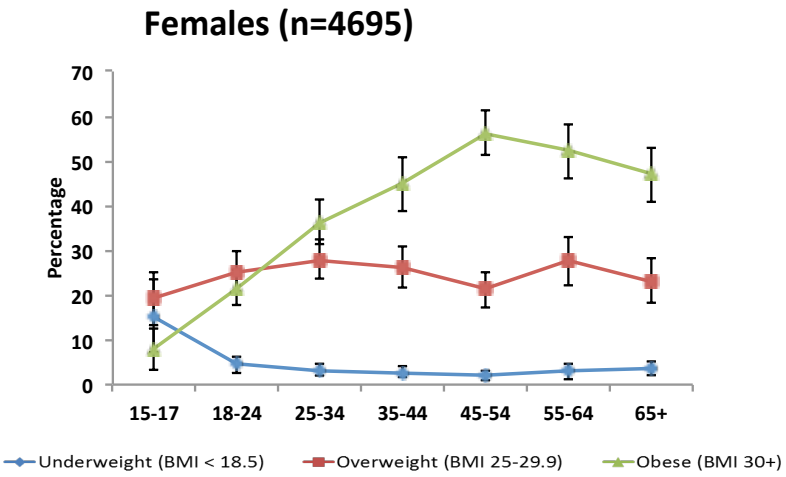

Figure 1.9 Prevalence of underweight, overweight and obesity by sex and age, SA 2012: (Source: SANHANES-1, Shisana, O et al) (6)

A high prevalence of obesity in black midlife urban women was also shown in the Heart of Soweto study, a large study examining the risk of heart disease in Soweto residents (5). In a dedicated screening study, "Heart Awareness Days", of black adults in Soweto, Johannesburg, Tibzarwa et al (243) examined the association between the increased risk of cardiometabolic disease, and, amongst other risk factors, the prevalence of obesity in a black urban population. They found that obesity was more prevalent in mid-life women (55%) than in mid-life men (23%) (Figure 1.10). Likewise, Puoane et al (244), found that obesity was more prevalent in black urban South African women than those living in rural areas and became greater with age. This was also true of abdominal obesity. This high rate of obesity and abdominal obesity was also described in an earlier study of middle-aged women from the BT20 cohort that were used as the source of subjects for SWEET. Data from this study, demonstrated that elevated levels of abdominal obesity in conjunction with low levels of HDL primarily drove the high rate of MetS, with the prevalence of obesity being 50.1% and MetS, 42.1% (7).

A recent South African National Health and Nutrition Examination Survey (SANHANES-1) investigating the ongoing increase in certain NCDs; hypertension, diabetes and heart disease, described an increase of obesity, particularly in older females (6). As shown

earlier, obesity in black women is associated with a variety of metabolic risk factors (245) and both age and higher levels of obesity were associated with increased risk of diabetes in black women (246). In the screening study described above over a third of the participants were hypertensive (243).

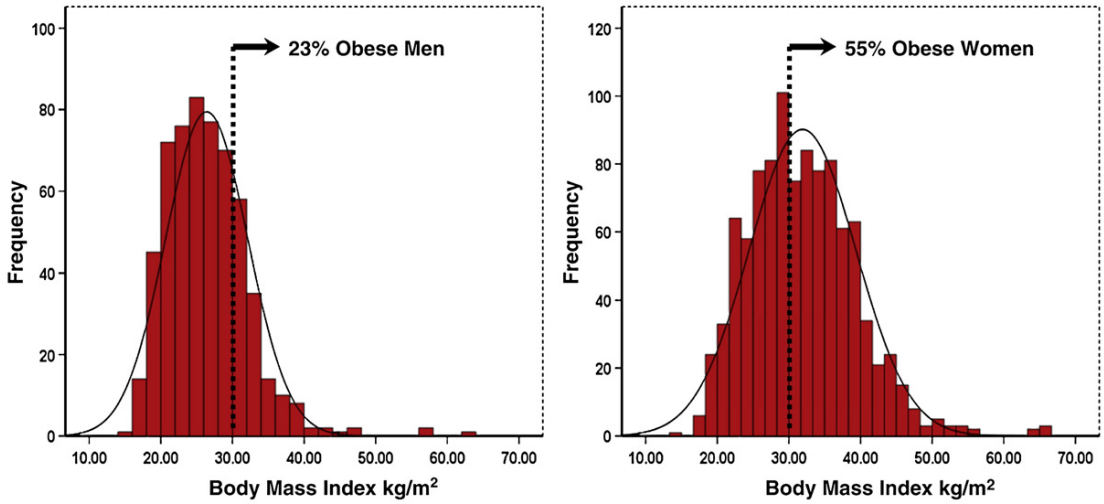

Figure 1.10 Frequency distribution of body mass index in male and female subjects in a black urban South African cohort from Soweto. (Source: K. Tibazarwa et al) (243)

1.7.8 Smoking, smokeless tobacco use and morbidity

Earlier age at FMP and its related risk for metabolic disease has been described. Data have shown there is an association between current smoking and a younger age at FMP (247). Prevalence and severity of VMS have been shown to be a risk factor for metabolic syndrome and it has been shown that women who smoke have worse VMS (127, 227). In addition smokers in the MT appeared to be at greater risk for atherosclerosis (248). Smoking is associated with increased visceral fat and impaired insulin sensitivity (249), and nicotine has been shown to increase insulin resistance (250). Smoking is also a significant risk factor for osteoporosis and cumulative lower bone density in postmenopausal women (251). Although, smoking is not widespread amongst black South African women, use of smokeless tobacco or snuff is prevalent among these midlife women (252, 253). Research

has shown that intensive use of snuff (snus) is significantly related to metabolic syndrome (254). Given the association between nicotine and insulin resistance, it is important to note that levels of nicotine, in the brand of snuff preferred by black South Africans are substantially higher than in brands sold in Europe (255), and hypothetically this may further impact both the risk of insulin resistance and lower bone density in black menopausal South African women.

1.8 HIV and the menopause transition

There is a high prevalence of HIV infection in South Africa. It is estimated that in 2012, 6.4 million people (12.2%) in South Africa were HIV-positive (256). As shown in Figure 1.11, mean HIV infection amongst midlife women aged 40 to 59 years was 18.0%. Due, possibly to the increased use of antiretroviral treatment (ART), the prevalence of HIV-infected older women was higher in 2012 than in 2008 (256). This may have implications for future research in examining whether HIV infection has any effect on reproductive aging and related cardio-metabolic morbidity and mortality.

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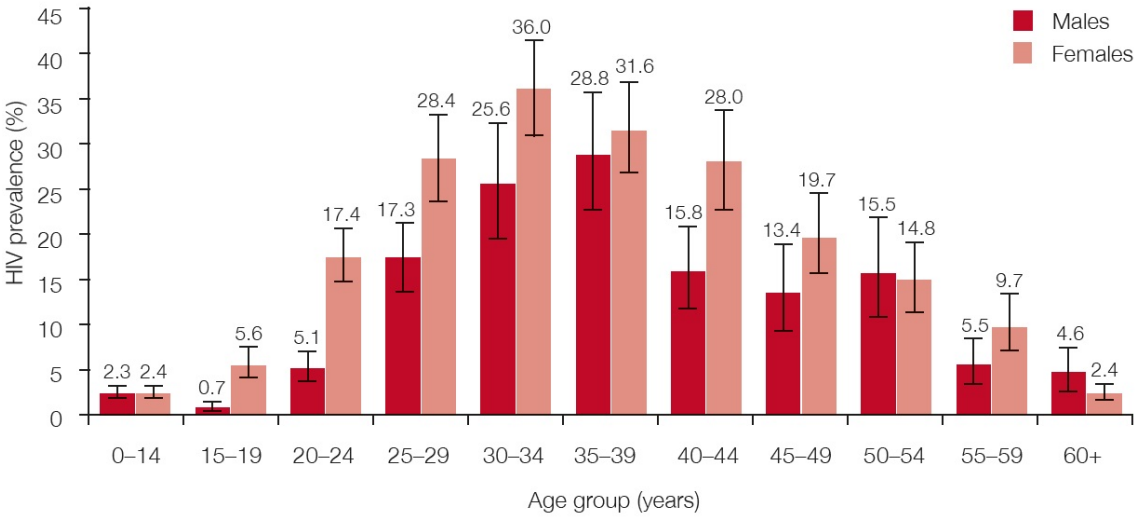

Figure 1.11 HIV prevalence, by sex and age, South Africa 2012 (Source: O. Shisana et al) (256)

There is conflicting research about the effects of HIV on women in MT. Some studies have shown that age at menopause is earlier (47.3 years) in HIV-positive women, and that they experience more symptoms (257). However, other research showed no difference in age at menopause between HIV-positive and HIV-negative women, with 50 being the median age at FMP, which is near to the median age of natural menopause in the USA. However, results from this study did demonstrate that HIV-positive women had more VMS (258). In a very large cohort, researchers found that median age at FMP was comparable between HIV-positive and HIV-negative women (259).

One of the issues confronting researchers studying the effects of HIV infection on reproductive aging, is that in addition to the association between the MT and increased metabolic disease (9), there are independent metabolic effects related to HIV infection and ART (260, 261). Studies have shown that HIV-positive women have greater lipoatrophy than HIV-negative women and increased VAT (262), which increases risk of metabolic disease. Hadigan et al (263) have examined these cardiometabolic risk factors and showed that HIV-positive adults with lipodystrophy have lower levels of HDL-C and higher levels of LDL-C and triglycerides, in addition to impaired fasting glucose and increased diastolic blood pressure. They also showed that in HIV-positive women, fat distribution, as a result of lipoatrophy, increased their risk of CVD (263). In addition, data have shown that ART has a metabolic effect leading to dyslipidemia and decreased insulin sensitivity (264). In a cross-sectional study of HIV-positive and HIV-negative women, HIV-infection was related to dyslipidemia, and ART use was associated with a more atherogenic lipid profile (265).

Body composition changes in HIV-positive women have been explored and some studies found that HIV infected women who had peripheral lipoatrophy did not have increased VAT, but that varying ARTs have different effects on VAT (266). A comprehensive review of South African adults saw no difference in VAT in women using ART, compared with women in the control group, and suggested that increased central adiposity is a result of treating the HIV

infection, but is not related to any class of antiretroviral medication per se. Thus the increased abdominal adiposity seen in HIV-positive women receiving ART, is similar to the fat gain experienced in HIV negative women (267).

Research investigating increased risk for CVD in HIV-positive menopausal women have shown that the MT (135), chronological aging (139), and HIV-infection (260) are all independent risk factors. Comprehensive reviews have shown that HIV-positive women appear to be at greater risk for certain CVD risk factors, including insulin resistance, higher LDL-cholesterol levels and triglyceride levels, lower HDL cholesterol levels (8, 260). In addition, a combination of HIV infection and ART may increase the risk of CVD in HIV-positive women, due to the presence of the factors mentioned above and increased levels of inflammatory markers (262). More research is needed to better understand the effects of HIV infection and ART in combination with reproductive aging, in groups of HIV-positive and HIV-negative women, where the MT has been accurately staged (268).

Studies have shown that women transitioning through menopause have lower BMD (269), and other data have found that HIV-positive women have decreased BMD, which in these HIV infected women, is associated with reduced muscle mass, decreased testosterone levels and menstrual cycle irregularities (270). Therefore low BMD in HIV-positive women may be related to a combination of menopausal status and the effects of HIV infection. However, research that examined BMD in HIV-positive and HIV-negative premenopausal urban black South African women did not find BMD was lower in the HIV-positive group (271). The effect of HIV infection and subsequent treatment on declining BMD has also been examined, suggesting that low bone density in HIV-positive women is related to several different variables, including the effects of being underweight, cigarette smoking and low vitamin D levels (272).

1.9 Menopause research in sub-Saharan Africa

A comprehensive search of the literature using MEDLINE (1970-2015) suggests that there is a paucity of research about menopause in sub-Saharan Africa compared to Western, Asian, and Latin American countries. Although there is research on cardiometabolic disease risk (5, 243, 246) in sub-Saharan African populations, there is almost nothing specifically related to women in MT and CVD risk or the deleterious effect of increasing obesity or changes in body fat distribution in midlife women from this population. A Ghanaian study (273) described body fat distribution changes but only with reference to menopause symptoms, and not CVD. A systematic review of VMS around the world, further highlights this lack, since only two African papers, from Nigeria and Ghana, were included in this review, and, in one of these, the definition of menopause status was not even described (124). There are several studies examining the risk of metabolic disease and changes in body composition in African women in Ghana (274), Nigeria (275-277) and the Congo (138), but generally the cohorts were small, menopause status was self-reported and not accurately staged, nor confirmed with biomarkers. Studies based in Nigeria (103, 278), Tanzania (279), Ghana (273) and Zimbabwe (280) examined African women's experience of the menopause and menopausal symptoms. VMS were widely experienced and joint pain was particularly common (277, 281, 282). Some research investigated hormone levels during the MT, and, as expected, found that levels of FSH were higher and E2 levels lower in postmenopausal women (283, 284). Age at FMP was also explored in Ghanaian (285), Kenyan (286), Nigerian (287) and South African (280) women. Data from these four studies showed an approximate mean and median age of 48.04 and 48 years respectively which were significantly lower than age at FMP in some Western studies, where age at FMP in a multiethnic American study was 51,4 years (64), and 54 years in a very large European cohort (288). The problem of accurately determining menopause stage in these groups of women remains.

1.10 Summary and research gaps

There is a significant body of research investigating cardiometabolic disease and associated risk factors in South African women. Metabolic syndrome (7, 243), obesity (242, 245, 289), diabetes (246, 290) and heart disease (5), have all been examined. However, the MT was not staged in these studies of midlife women, so it is difficult to determine whether chronological or reproductive aging, or both, may be risk factors for cardiometabolic disease in this population. Accurate staging of the MT is important in understanding this complex association. However, there appear to be no studies in sub-Saharan Africa using a validated staging tool. Most of the studies, described in this African population, rely on self- reporting to establish whether women are pre- or postmenopausal (12 months of amenorrhea), and no validated staging method has been used. It can therefore be seen that there is a lack of research on the MT in sub-Saharan African females, particularly in relation to cardiometabolic diseases and body composition. The following list highlights some of these knowledge gaps:

- Changing sex steroid hormone levels across the MT have been shown to be associated with an increase in the risks of MetS and CVD (9, 145, 182, 183, 188, 189); however, such studies have not been performed in African females during the MT.

- Obesity and a changing hormonal milieu may increase the severity and prevalence of VMS (155, 221, 229, 291), but VMS, which have been found to be a risk factor for metabolic syndrome (79, 219, 220), have not been assessed in African females.

- Body composition changes during the MT may predispose women to greater risk of MetS (9, 155, 292) but there are little data examining these changes in black African women.

- Bone density has been studied in black South African women (293). Research studies dating from approximately 30 years ago suggested black South African women have a lower risk of decreased bone density, than their white counterparts (294, 295). Although there are studies comparing bone density in black and white South African women, those that are available suggest there are fewer differences than previously thought (296-298). However, these studies were not controlled for menopausal stage.

- Earlier age at FMP is related to increased risk of metabolic disease (212, 213), and those women who have experienced economic hardship throughout childhood and into adulthood are more likely to experience earlier onset of menopause (66, 67, 299), but this has not been studied amongst black urban South African women. In addition, many women, across the socioeconomic spectrum, have difficulty in recalling FMP age accurately (69, 72)This may be compounded by the fact that given the inequality between private and public healthcare in South Africa (300, 301), the majority of midlife black urban women do not have annual medical or gynecological checkups, so there are few accurate records of their ovarian aging.

- Studies have described how HIV infection may affect both age (257) at menopause and severity and prevalence of VMS (262). In addition, ART can affect metabolic factors (8). However the MT has not been studied in African HIV-positive women who are currently on treatment. Research has also shown that HIV infection and ART may each affect fat distribution (261), which may enhance risk for MetS (302), and low BMD (268, 270). A wide body of research has shown that changes in body composition are significantly associated with the MT (146, 303, 304), but since the MT has not been accurately staged in black urban South African women, it is difficult

to explain whether changes in body composition are related to HIV infection, ART, reproductive aging, or to a combination of these factors.

1.11 Study aims and objectives

The principal aim of this study is to investigate the metabolic, hormonal and anthropometric characteristics in black urban South African women at different stages of the MT.

1.11.1 Objectives

The objectives of this study, which pertain only to black, urban South African women, are therefore as follows:

1. To accurately stage reproductive aging in a population of urban African women using bleeding patterns and to assess whether STRAW + 10 criteria are appropriate to stage ovarian aging in this population, and to measure FSH and E2 levels as supportive criteria in determining MT stage.

2. To determine whether obesity is associated with age at FMP and menopausal symptoms, particularly VMS, and whether HIV infection is associated with age at FMP and menopausal symptoms.

3. To determine whether general body adiposity, BFD, lean muscle mass and BMD are associated with stages of the MT and if so what factors may play a role in these associations.

4. To determine the prevalence of the MetS, the level of its individual components and associated metabolic factors in African females at different stages of the MT and what factors play a role in modulating the levels of these variables.

1.11.2 Study Hypotheses

1. STRAW+ 10 is a valid instrument to stage reproductive aging in women in a low resource country

2. Body composition changes are associated with both reproductive and chronological aging in black urban South African women

3. Black postmenopausal South African women are at greater risk for cardiometabolic diseases than those women in the late reproductive stage

1.11.3 Study relevance

Studies have shown that there is a high prevalence of cardiometabolic diseases in midlife black urban South African women (5, 7, 134). A more wide-ranging and in-depth understanding of the risk factors associated with these diseases is needed to address the gaps in the literature, and the lack of information on the relationship of cardiometabolic diseases to the MT in these women. Data from the women in SWEET should help to clarify whether the MT is related to the high prevalence of obesity and metabolic disease in this population. Establishing whether a staging tool, using bleeding criteria, can accurately stage the MT is the first step in investigating the relationship between reproductive aging and cardiometabolic disease. In addition, assays of several sex steroid hormones that have not been previously collected from black South African women during the MT will enable us to determine whether these hormones are associated with differences in body composition and levels of cardiometabolic disease risk factors in . The examination of body composition will include an exploration of BMD in women at different stages of the MT, since there is a perception that bone fragility and its attendant health risks is lower in these black African women than in Caucasians (294, 295), so a deeper understanding of BMD across the MT

will be useful. Assessing HIV status and ART use may provide additional insight into the risk of metabolic diseases in HIV-infected menopausal women. An analysis of socioeconomic factors, lifestyle and education will examine how these affect both anthropometric and metabolic variables in this group of women. This information may help provide information that can be used to reduce the risk of cardiometabolic disease among black South African women. The research in this study may also inform public policy and improve the quality of health care available to midlife black South African women.

1.11.4 Conceptual Framework

The conceptual framework shown below (Figure 1.12) attempts to highlight the proposed relationships between the variables examined in this investigation of the hormonal, metabolic and anthropometric differences in African females at different stages of the MT, and to act as a guide to the experimental components that have been utilized in this PhD. The relationships shown in this diagram have been more fully described in the preceding literature review.

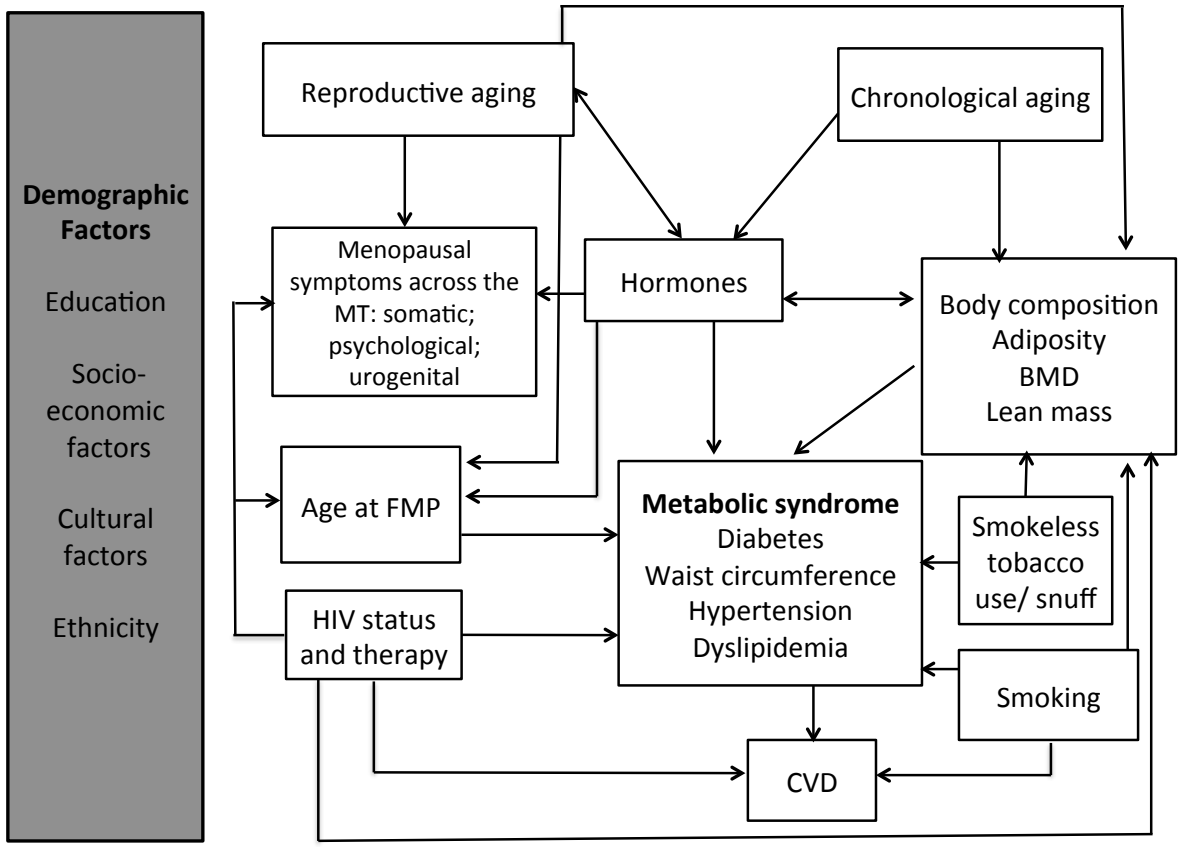

Figure 1.12 Conceptual framework for the SWEET study

Chapter 2: Methodology and design

2 Methodology and Design

2.1 Historical context of study

From 1948 -1994, black South Africans lived under the system of apartheid, which segregated people by ethnicity and discriminated against people of colour; politically, legally, socially and economically. White people, who were considered racially superior, benefitted exponentially (300). Apartheid had a significant effect on the South African healthcare system (305). When the African National Congress (ANC) came to power in 1994, they promised changes to healthcare and created a National Health Plan (301, 306). However, the healthcare system continues to face challenges. The focus, since 1994, has been on improving primary healthcare, but though access to healthcare has improved, the quality of healthcare continues to deteriorate due to several reasons. These include the legacy of apartheid; years of poverty and inequality that compromised the health of black South Africans, incompetent administrators, the burden of NCDs, HIV and tuberculosis, lack of training and poor leadership, management and administrative skills (300, 305). Administrative problems occurred after 1994 due to changes at senior management levels, when white administrators were replaced by their black counterparts, which resulted in what Coovadia et al described as a 'loss of institutional memory' (300).

The public health sector is under pressure to deliver services to about 84% of the population. In contrast, private healthcare caters to the higher income groups in the country, who happen to also have health insurance. Approximately 16 % of South Africans belong to medical aid schemes and have private healthcare (307). In 2011, the ANC published a green paper proposing a National Health Insurance (NHI) plan to address the inequalities in the healthcare system (308).

South Africa is one of the most unequal countries in the world, and this is underlined by its healthcare system (305). Black mid-life South African women generally attend satellite clinics (Figure 2.1), which are often poorly equipped and under staffed by inadequately trained personnel (300). Many of the country's healthcare professionals prefer to work in the private sector rather than in these public entities and by 2007, over 70% of South African doctors were working in private healthcare, while many others had immigrated. Furthermore, they were not specifically trained to work in rural areas with poor primary healthcare conditions (300), although, since 2005/2006 graduating doctors must complete a two year compulsory community service internship program that was implemented by the Health Professions Council of South Africa (HSPCA) (309, 310). An understanding of doctor shortages in the public healthcare sector is illustrated by the following statistic, which shows that although the doctor to population ratio in South Africa is estimated at 0.77 per 1000, there is only one doctor per 4219 people because 73% of doctors practice in the private sector (311).

Primary healthcare nurses, who mainly work in the satellite clinics described above, have very basic degrees in primary healthcare and full responsibility for diagnosing and prescribing medicine for a wide range of medical conditions. Training and quality of nurses was compromised when nursing colleges were closed towards the end of the 90's as a reaction to the apartheid legacy (301). In addition, there is a perception that many nurses are disinterested in their patients, abusive and uncaring (312). The need to address this problem is critical (300).

In South Africa 81% of women are black, and over 4 million women are 40 to 59 years old. Life expectancy in females rose from 55.7 in 2002 to 63.1 years in 2014 (313). In addition, a recent South African national health survey confirmed that the prevalence of all NCDs increases with age (6). Research discussed in Chapter 1 has shown that risk for NCDs is high in midlife women, and a need for both information and management of menopause has

been identified by the Department of Health (314), but it does not appear that specific guidelines have been given. However, in the public health sector in South Africa, there appear to be very few clinics specializing in menopause. Research examining metabolic, hormonal and anthropometric factors in the MT in this population group is urgently needed.

Figure 2.1 A satellite clinic in Johannesburg, South Africa. (Source: Mooney et al) (305)

2.2 Study setting and cohort background

The cohort is part of the well-known Birth to 20 (BT20) study in the MRC/ Developmental Pathways for Health Research Unit, Department of Paediatrics, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand. In 2004, it was described as the 'largest and longest running longitudinal birth cohort study in Africa' (315). The cohort population derives from Soweto (South Western township), a black urban area known as a 'township', bordering the gold mines of Johannesburg (316). It was created in line with the policy of apartheid, implemented by the Nationalist government of the time, to separate black and white South Africans (317). There are approximately 1.3 million people in Soweto

(Figure 2.2) (318). Although many houses in Soweto have been upgraded, large sections are still informal, and issues of poverty, housing, unemployment and inadequate health services present significant challenges (319).

Figure 2.2 A view of Soweto (Source: Antioch University, USA)(320)

Towards the end of the 1980's, there was rapid socio-political change in South Africa, and growing urbanization in the form of shack and informal settlements adjacent to designated white areas. As described earlier, the legacy of apartheid had impacted healthcare (305), and there was concern amongst clinicians and researchers that mortality and morbidity of the children, growing up in these informal settlements, where health was already compromised by the apartheid system, would experience further deterioration. This would be as a result of continuing administrative problems and rapid migration from rural to urban areas, which would significantly compromise the available but inadequate healthcare services. There was already a high prevalence of HIV/AIDS and tuberculosis in this

population, and increasing obesity, diseases of lifestyle and traumatic injuries could further exacerbate the situation (300, 321).

In 1989, funding was granted to implement a birth cohort study in Soweto, and enrollment began in mid-1990. Several pilot studies were developed to examine the viability of this longitudinal research in exploring the ongoing health of these mothers and children. The 3275 children in the study were those born to women who had been resident in Soweto for six months or more prior to the child's birth (315). Initially, the children, known as 'Mandela's children', since they were born in the period from February 11 – March 30, 1990; the 7-week period following Mandela's release from incarceration, were to be studied until they turned ten. The study was named Birth to Ten (BT10). However, in 2000, the name was changed to Birth to Twenty (BT20) when the investigators decided to continue the study until the children turned 20 (321).

There has been a low attrition rate in BT20, and by 2011 about 2200 participants were still in touch with the study (322). One of the reasons for the decreased numbers is difficulty in contacting the participants. It has been noted that the participants in BT20 move frequently; within the first 16 years of the study approximately ten percent had changed their addresses four or more times. Other reasons for this attrition include mortality, refusal to be part of related studies and possibly a perception that they were not benefitting from the data collection process. However, it is postulated that these women, who do not generally have annual medical check-ups, may perceive data collection to be similar to healthcare monitoring, and may be encouraged to continue to participate (323). The term, 'caregivers', is used in the BT20 cohort profiles. The explanation is that in this population children may be raised by a close female relative in order for the biological mothers to find work in another area, or for school-age mothers to continue their education (300).

2.3 Data collection process

2.3.1 Recruitment

The black urban African women in this cross sectional study were participants in SWEET. They were the biological mothers and caregivers of the children in the BT20 cohort. Owing to infrastructure and timeline constraints, not all of the 2,200 participants, who were still in contact with BT20, could be recruited into the study and the maximum sample size used in the study was based on feasibility and costs of recruitment within the 3-year recruitment time period. The minimum number of participants in the study cohort would be defined as that at which at least 100 women were present in each of the following four study subgroups (based on menopause staging using STRAW+10 guidelines (56): late reproductive (stages - 3b and -3a), the MT (stages - 2 and - 1), early postmenopause (stages 1a, 1b, and 1c), and late postmenopause (stage +2).

As shown in Figure 2.3, a group of 902 women (this was the maximum number of women we could contact within the timeframe and infrastructural limits of the study), were randomly chosen from the available 2200 women and contacted by a team member who described the study and data collection process, and extended an invitation to participate in the study. Exclusion criteria were < 40 years and >60 years, pregnancy and ethnicity other than black African. Within this group of 902 women, 200 women did not participate. The reasons were as follows: 35 were now older than 60 years, 79 refused to participate, for several reasons including an inability to take time off from work, lack of interest in the study, or that they had had their blood tests taken at a local clinic and did not believe the study would benefit them. In addition, 37 women were deceased, 3 were terminally ill, and 46 had become untraceable, or now lived outside the study area. Every effort was made to trace the last group of women. Team members made field visits to the addresses listed on the contact sheets, but found in many cases that the participants had moved and were not contactable because several occupants had lived at the address since the participant moved. Many had

left no forwarding address, and in some cases the initial address that had been given was incorrect. Ultimately, 702 women agreed to participate in the study.

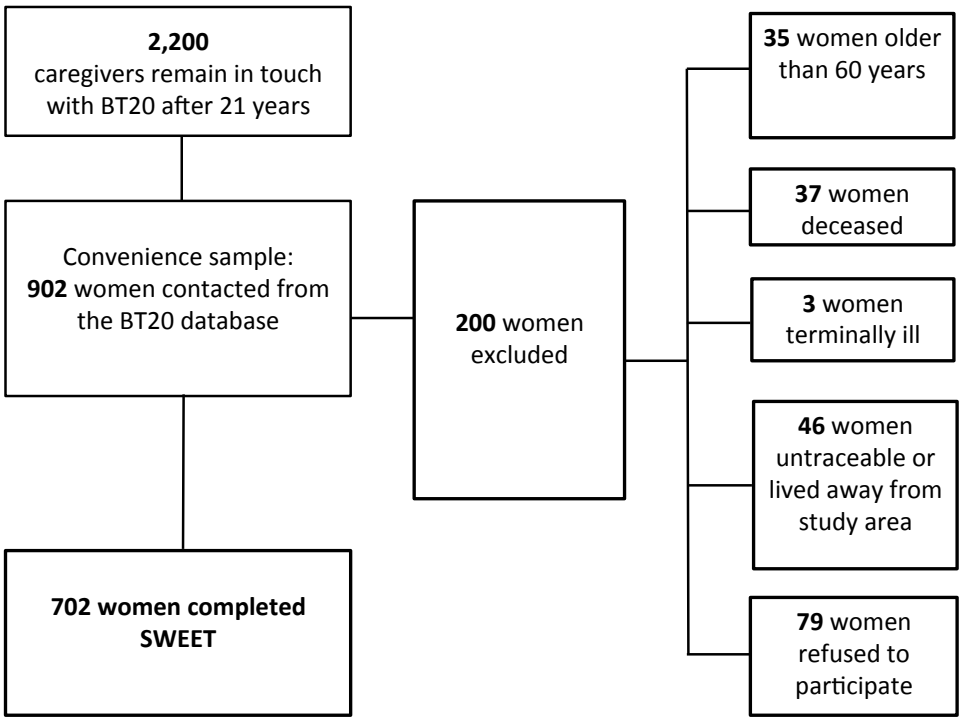

Figure 2.3 Flow diagram showing SWEET cohort recruitment

2.3.2 Ethics

Confidentiality for all women was maintained because the participants in BT20 had been given an identity number that was also used in the SWEET study. All participants signed informed consent forms (Appendices; Page 170). As per the informed consent form, it was agreed that if participants' results were out of the normal range, they would receive a referral letter to a primary health care clinic, medical practitioner or specialist for further testing and treatment. At the end of the data collection process, participants were given information sheets on menopause and cervical cancer (Appendices; Page 184) The Human Research Ethics Committee (Medical) of the University of the Witwatersrand approved the protocol (ethics certificate number M090620) (Appendices; Page 165-166).

2.3.3 Data management

The data for each SWEET participant was collected in a separate folder. Each participant was allocated a BT20 ID number. Each participant had a contact sheet and the information on the contact sheet was updated during the check in process. Separate barcode stickers with the participant's ID number were stuck onto each one of the data collection documents. Once the data collection was completed the folder containing the documents, and subsequently the results of the blood analytes were filed under lock and key under the auspices of DPHRU. Data was uploaded into an Excel spreadsheet and transferred to Statistica for data cleaning and analysis. All spreadsheets contained only BT20 ID numbers and not participant names.

2.3.4 Data capture

Since the participant folders were not allowed, at any stage, to leave the room where they were stored, all data was captured on a designated DPHRU computer. Only those with access to the identifier code and password were able to capture the data, so that confidentiality was maintained at all times

2.4 Questionnaire

2.4.1 General questionnaire

In this study there was one general questionnaire (Appendices; page 166) that incorporated all the questionnaires described below. There is a list on the front of the general questionnaire showing all the questionnaires and collection procedures that participants in SWEET needed to complete. The Food Frequency Questionnaire, the Caregiver peripheral quantitative computed tomography (pQCT) and the Caregiver carotid intima-media thickness (cIMT) measurements have been excluded from the description below because they do not pertain to this study. Data from those were used in the study of another PhD candidate, who

was collecting data at that time from the cohort. Data from the Utian Quality of Life Scale (UQoL) questionnaire, which is included within the general questionnaire, was collected and has not yet been analyzed.

There are 11 official languages in South Africa and although English is not the first language of most of the participants, it is the language most commonly used, and the majority of the participants speak and understand English. It was therefore decided that the questionnaires should be administered in English during face-to-face interviews, by a single researcher. When necessary, clarification and translation, in the home language of the participants, were available by trained members of the team whose first language corresponded with those of the participants. A general questionnaire included questions on reproductive health, menstrual history, general health and employment, educational level and tobacco and smokeless tobacco (snuff) use. Current and former smokers were grouped together and compared to subjects who had never smoked. This questionnaire was derived from the general health questionnaire formulated and validated in a previous study of the same population group (324).

2.4.2 Assessment of menopause transition stage

Within the general questionnaire (Section B: Menstrual history), women were asked close-ended questions about bleeding patterns to determine their MT stage. Participants were asked the date of their last menstrual period. If they had skipped a menstrual period, they were asked when they last had a menstrual period (3 months, 6 months, 12 months, or more than 1 year ago). These questions were followed by open-ended questions about bleeding patterns, to more specifically ascertain cycle changes. Where possible the date of the FMP was obtained. Hormone therapy and contraceptive use were determined; the participant was asked whether she knew the names of the medication she was using. Information on hysterectomy and oophorectomy was obtained whenever possible. STRAW + 10 criteria (56)

were used to ascertain menopause stage. The participants were assigned to a STRAW+10 stage as follows: late reproductive (-3b, -3a); early and late menopausal transition (-2, -1); early postmenopause (+1a, +1b, +1c), and late postmenopause (+2) as shown in Figure 2.4.

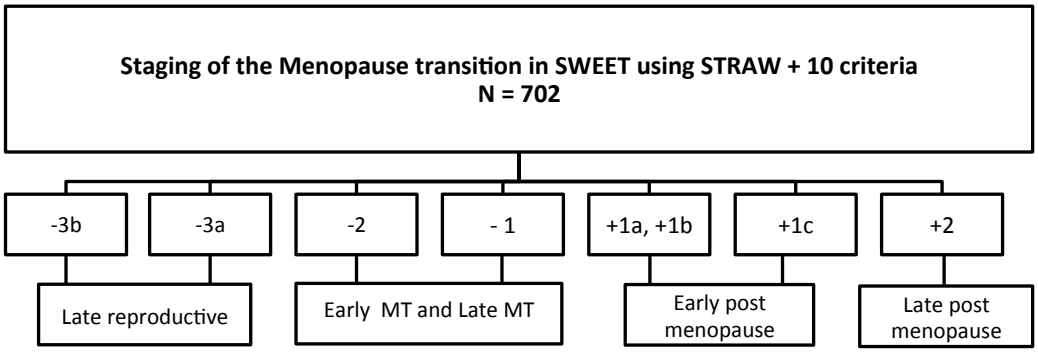

Figure 2.4 Diagram showing the staging groups for SWEET participants

2.4.3 Menopause Rating Scale (MRS)

Within the general questionnaire, the presence, prevalence and severity of menopausal symptoms were measured using the Menopause Rating Scale (MRS). This is an internationally validated, standardized scale and has been fully described in (Chapter 1, section 1.5.3). The scale allows the participant to rate the presence and intensity of 11 symptoms on a scale of 0-4, and was graded as absent = 0, mild = 1 or 2, severe = 3 or 4 (98). A single interviewer administered the MRS, and when necessary, team members were trained to interpret the questions in the home language of the participant (Appendices; Page 173).

2.5 Anthropometric measurements

2.5.1 Simple measures of body anthropometry

Participants wearing light clothing and without shoes, were weighed and their height measured, using respectively, a calibrated electronic scale and a fixed-wall stadiometer, (Holtain, Crymych, UK). A soft measuring tape was used to measure waist and hip circumferences to the nearest 0.5cm; the former at the smallest girth above the umbilicus and the latter at the greatest circumference of the hips. The intra-observer CVs for height, weight, and hip circumference was less than 1% and less than 2% for waist circumference. Inter-observer CV for height, weight, hip circumference and waist circumference was less than 1%. Body mass index (BMI) was calculated. Blood pressure was recorded on the left arm, while the participant was seated using a digital reader (Omron M6; Omron, Kyoto, Japan) and appropriate cuffs. Three readings were taken with a 2-minute interval time between each reading. The first reading was discarded and the remaining two values were averaged. BMI was calculated as an estimate of obesity by dividing weight (kilograms) by height (meters) squared.

2.5.2 Dual-energy X-ray absorptiometry (DXA) measurements

Whole body DXA scans were performed by a single trained technician using a Hologic Discovery A (S/N 83145) DXA machine (Bedford, MA, USA). The participants removed their clothing and all metal objects and surgical gowns were worn for the procedure. Whole body scans for fat and lean mass were analyzed using whole body less head, since many participants wore wigs and hair weaves that could not be removed; these hairpieces are similar in density to soft tissue and may have caused measurement artifact, as described in a previous study (325). The terms ‘total’ or ‘whole body’ are used in the study papers when referring to these sub-total (i.e. whole body minus head) measures of lean and fat mass. During data collection a DXA phantom was scanned each morning to examine the CV of the DXA machine and the CV was found to be less than 0.5% for all parameters.

2.5.3 Ultrasound measurements of VAT and SAT

A trained operator measured visceral and subcutaneous adipose tissue using a GE LOGIQ e ultrasound machine with a 2-5.5 MHz 4C-RS curved transducer (GE Healthcare, Piscataway, NJ, USA). The VAT thickness was defined as the distance in centimeters from the peritoneum to the vertebral bodies and SCAT thickness as the depth in centimeters from the skin to the linea alba. In order to visualize the relevant anatomical structures the scan depth was set at 15 cm for the VAT and 9 cm for SCAT. The site for both measurements was where the xyphoid line and waist circumference meet. The CV for the ultrasound measurement for VAT and SCAT was less than 2%.

2.6 Assays of blood analytes

Fasting blood samples were obtained in the morning before 11am during the 4-hour data collection period. Where possible, seven tubes of venous blood were drawn, using standard venipuncture techniques. Four SST 10ml tubes were drawn for serum analysis, two EDTA 5ml tubes were drawn for plasma analysis, and one Fluoride 5ml tube was drawn for glucose analysis. The blood was centrifuged (Beckman Coulter, USA) at 642 g (2,500 rpm) for 10 minutes at 24°C. Serum and plasma samples were collected and aliquoted into corresponding cryovials and immediately stored at -80ºC until assays were performed. Levels of follicle stimulating hormone (FSH), estradiol (E2), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone, lipids, blood glucose, leptin, adiponectin and sex hormone binding globulin (SHBG) were measured. Immunoassays were performed for E2, FSH and insulin as per manufacturer's instructions (ADVIA Centaur XP Systems, Siemens Healthcare Diagnostics, Tarrytown, NY). The principle for E2 is a competitive chemiluminescent immunoassay and the assay range is 43.6 -11,010 pmol/L (11.8–3000 pg/ml), and intra- and inter-assay coefficients of variation (CVs) are 4.2% and 1.9% respectively. For FSH, the principle is a two-site sandwich

chemiluminescent immunoassay and the assay range is 0.3 – 200 IU/L, and the intra- and inter-assay CVs are 2.4% and 1.5% respectively. The assay range for insulin is 0.5mU/L-300mU/L, and the intra- and inter-assay CV's were 5.9% - 4.8% and 4.6%-3.3% respectively. The immunoassays for SHBG and DHEAS were performed on the Immulite 2000 Systems analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). The SHBG was performed using solid phase two-site chemiluminescent immunometric assay. The intra-assay and total CV for SHBG was 3.06% and 4.40% respectively, while the assay range was 0.02 - 180 nmol/L. DHEAS was performed using a solid phase competitive chemiluminescent enzyme assay. The intra-assay and total CV for DHEAS was 7.1% and 9.8% respectively and the reported assay range was 0.41 - 27.0 µmol/L (3.0 - 1000µg/dL). The homeostasis model assessment (HOMA) method was used to calculate insulin resistance (326). Total cholesterol, HDL, triglycerides, glycated haemoglobin (HbA1C) and glucose were measured using the prescribed enzymatic methods on the Advia 1800 Chemistry Systems analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). Low density lipoprotein (LDL) levels were calculated using the Friedwald formula (327). The analytical performance of each assay is as follows: cholesterol, assay range 0.26 -17.48mmol/L (10.0 - 675mg/dL), and intra-assay CV is 0.3%; HDL assay range is 0.1-3.0mmol/L (5.0 - 115mg/dL) and the intra-assay CV is 1.0 - 2.3%; triglyceride analytical range was 0.0 - 6.22mmol/L (0.0 - 550mg/dL) with an intra-assay CV of 0.5% - 1.6%; HbA1C assay range is 0.23% - 17.8% and the intra-assay CV is 0.8% - 1.3% ; the assay range for glucose is 0.2 - 38.9mmol/L (4.0 - 700mg/dL) with an intra-assay CV of 0.4 - 0.5% and a total CV ranging from 0.9% at a lower concentration to 0.7% at the higher concentration of 16.5mmol/L.

The DHEA immunoassay (ELISA) was performed with the solid phase competitive binding, enzyme-linked immunosorbent assay, (DRG instruments Gmb-H, Marburg, Germany). The assay range is 0.0 – 30.0ng/mL, and the intra- and inter-assay CVs were 5.1% and 6.8% respectively. Leptin was measured with an ELISA (Biovendor Research and Diagnostic Products, Candler, NC). The assay range is 0.2 - 50ng/mL, and the inter-assay CV is 4.4% -

6.7% and the intra-assay CV is 4.2 - 7.6%. Adiponectin was measured with an ELISA Quantikine kit (R&D systems, Boston Biochem, Cambridge, MA). The assay range is 0.079 - 250ng/mL, and the intra-assay CV is 5.8% - 6.9% and the inter-assay CV is 2.5% - 4.7%. Testosterone extraction was performed using a liquid-liquid extraction method according to Benton et al (328). Ten microliters of sample was injected onto an ultraperformance liquid chromatography mass spectrometer (Micromass Quattro micro API Mass Spectrometer, Waters, Milford, MA). The lower limit of detection was 0.25nmol/L (7.2ng/dL) and average intra- and inter-assay CVs were 7.85% and 9.23% respectively. Free and bioavailable testosterone levels were calculated using the method of Sodergard et al (329).

All biochemical analyses were performed in the laboratory of Department of Chemical Pathology/ National Health Laboratory Services, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa.

2.7 HIV testing

All participants were offered a voluntary HIV antibody test, Alere Determine ™ HIV-1/2 (Alere San Diego, Inc. San Diego, CA). This test has been shown to be easy to use and has a very high sensitivity and specificity (330).

Participants who knew their status and disclosed this information were noted. Those women who wished to know their status were offered pre- and post-test counseling by a trained nurse/HIV counselor in accordance with recommendations from the Department of Health (331) as shown in Figure 2.5, and were assured of full confidentiality (Appendices; Page 180)

If the result was positive, referrals were made to a local HIV clinic for confirmatory serological testing and CD4 count and management. Both HIV positive women who were

being treated with antiretroviral medication and HIV positive women, who were not, were maintained in the study.

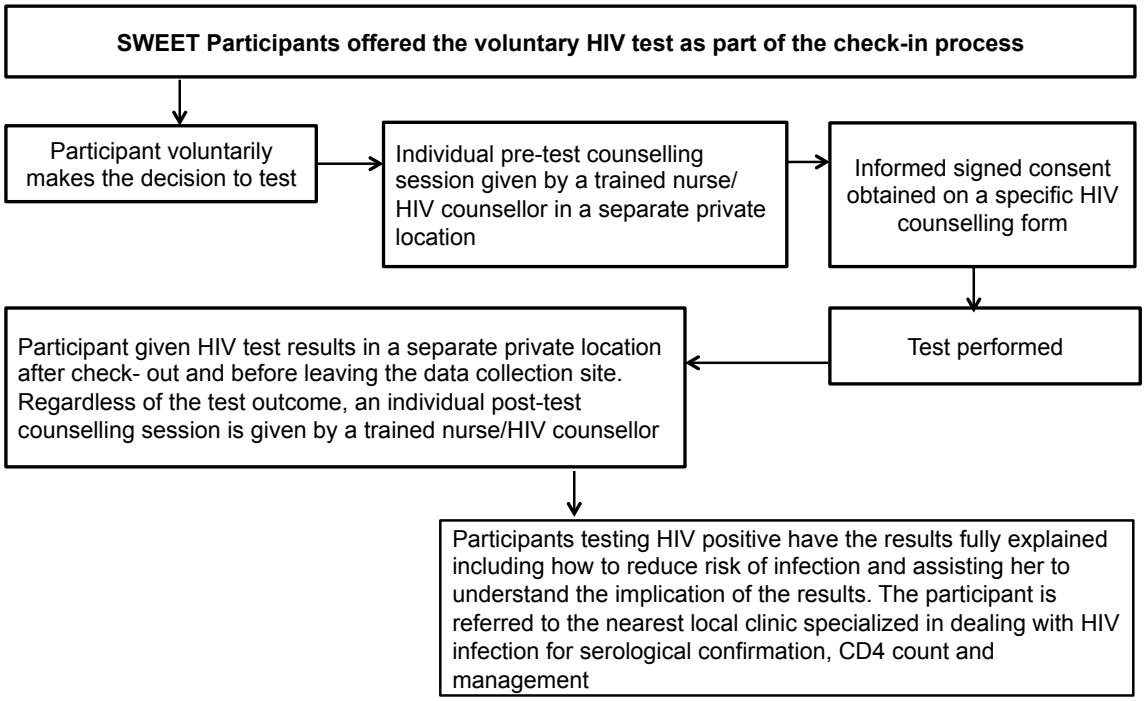

Figure 2.5 Procedure for voluntary HIV testing of SWEET participants: adapted from policy guidelines for HIV testing and counseling adapted from the policy guidelines from the National Department of Health, South Africa (331)

2.8 Statistical analysis

The statistical analyses used in this investigation have been fully described in chapters 3, 4 and 5 as the statistical methodology used was specific to each results chapter.

Chapter 3: Staging reproductive aging using STRAW+10 in black urban African women in the Study of Women Entering and in Endocrine Transition

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3 Staging reproductive aging using STRAW+10 in black urban African women in the Study of Women Entering and in Endocrine Transition

3.1 Introduction

The World Health Organization estimates that by 2030, 1.2 billion women will be 50 years or older. This is nearly triple the number of women who belonged to that age bracket in 1990. A growing number of these women can expect to live for several decades after menopause, and it is estimated that 76% of postmenopausal women will be living in developing countries by 2030 (2).

Research shows that the menopause transition (MT) is accompanied by clear physiological changes, some of which are temporary whereas others are long term (9). The Study of Women Entering and in Endocrine Transition (SWEET), was developed to examine changes in metabolic, hormonal and anthropometric parameters in black urban South African women across the MT, because research suggests that these changes may increase the risk for cardiometabolic disease.(137). Obesity is highly prevalent in middle-aged, urban African females (244) but very few studies have analyzed the relationship between obesity and the MT in this population. In addition, data on obesity and its effects on the prevalence of vasomotor symptoms (VMS) in this population have been limited; studies of Western women have shown a strong correlation (332). VMS are one of the defining symptoms of the MT (333), and have been linked to an increased risk of cardiovascular diseases(79, 80, 332). Accurate staging of reproductive aging is fundamental to understanding the relationships discussed above (112). However, there is a paucity of research on accurately determining mean age at final menstrual period (FMP) and on MT staging in sub-Saharan African women. In 2001, the Stages of Reproductive Aging Workshop (STRAW) (113) identified

criteria defining the different stages of reproductive aging, to help clinicians and research scientists stage the reproductive cycle. Harlow et al (114) considered these criteria to be the gold standard for staging the menopause transition. However, research in the 10 years following STRAW has enabled clinicians in the field to have a wider understanding of the hypothalamic-pituitary and ovarian function of the MT. At a recent workshop, 'STRAW+ 10: Addressing the Unfinished Agenda of Staging Reproductive Aging', the criteria for staging menopause were revised and updated based on the latest data (56). Bleeding patterns (menstrual cycles) remain the most important criteria to determine the stage in the MT, since there is no international standardization of biomarker assays (56, 115, 118). In addition, low or middle-income countries do not have the resources for the wide use of blood assays in assessing specific stages in the reproductive cycle. In 2001, STRAW recommended that follicle stimulating hormone (FSH) levels could be used as a biomarker in the late transition stage. Given the improved understanding of endocrine changes involved in ovarian aging (51), Stages of Reproductive Aging Workshop + 10 (STRAW +10) suggested that FSH and E2 - specifically the concentrations before and after the final menstrual period (FMP) - be used as supportive criteria to verify the stage of MT.(57) In addition, VMS and vaginal atrophy may be used to support other measures to determine the stage of MT (56).

It appears that no prior study in sub-Saharan Africa has used the STRAW+10 guidelines for staging MT. In this study, the reliability of this method for classifying ovarian status in a population with a high prevalence of obesity was assessed (6). Data from this same population of women (mean age, 43 years) showed that diabetes and metabolic syndrome are very common (7). A high incidence of human immunodeficiency virus (HIV) infection has been observed in this group of women (334) and some studies show that being HIV- positive may affect the symptoms and conditions of the MT in midlife women.(257, 335)

This study aims to examine reproductive aging in a population of urban African women to assess the usefulness of STRAW + 10 criteria in staging ovarian aging in these women by

determining bleeding patterns. Vasomotor symptoms (VMS) severity, FSH levels and E2 levels were determined as supportive criteria. It was hoped that the research would determine whether obesity has any effect on the MT, FMP and menopausal symptoms, particularly VMS. Age at FMP was noted, and HIV status was obtained (where possible) to analyze whether HIV infection was associated with age at FMP and menopausal symptoms.

3.2 Methods

The following methods were used: questionnaires for determining education level, use of ART, understanding of menopause, FMP, menopausal stage (using STRAW+10 criteria) and frequency and severity of menopausal symptoms (using the MRS); measurement of waist circumference, weight and height; BMI calculated; assays of serum E2 and FSH levels; measurement of HIV status. Each of these methods has been fully described in Chapter 2.

3.2.1 Statistical analyses

Non-normally distributed data were log transformed to normality before being used in any of the statistical analyses. These data are presented as median (interquartile range) in the tables and text, whereas normally distributed data are expressed as mean ± SD. Continuous variables were analyzed across groups using analysis of variance (ANOVA; or analysis of covariance [ANCOVA] with adjustment for possible confounders), and paired means were compared using Tukey post hoc test. Hormone levels, BMI, waist circumference and age were compared across the 7 menopausal stage groups shown in Figure 3.1. Menopause symptoms were compared across the 4 menopausal stage groups shown in Figure 3.1, to increase the power of the analysis. Percentage values were compared using the χ^2 test. Age at FMP was calculated using probit analysis. Logistic regression was used to identify the risk of selected menopausal symptoms across menopausal stages with and without adjustment for possible confounding variables (ie. age, BMI, FSH level and E2 level). These variables

were added individually to each model and also added all together. A multiple regression model was developed to identify the principal determinants of age at FMP. Independent variables included in the initial model were chosen based on biological plausibility and previous statistical analyses. These variables were: BMI (used as a categorical variable with BMI<25.0 coded as 0 and BMI≥25.0 coded as 1), waist circumference, education (coded with dummy variables using as the reference the group who did not attend high school), FSH level and E2 level. Backward, stepwise regression was performed, with variables removed one at a time (based on their p-level) until only variables with $p<0.05$ remained in the model. Pearson univariate correlation analysis was used to determine the relationship between the reported age at FMP and the length of time that had elapsed up until this age was reported to the study investigators (i.e. years post-FMP). Participants were also divided into quartiles of years post-FMP and the reported age at FMP for each quartile was compared by ANOVA

3.3 Results

3.3.1 Descriptive data for study population

Table 3.1 provides the descriptive data of the study cohort. The analytical sample contained 702 women; HIV status was recorded for 404 of these women, of whom 21.3% were HIV-positive. The frequency of antiretroviral use was 55.3% within the HIV-positive women. Participants had a mean age of 49.2 ± 5.29 years (Table 3.1). The mean age at FMP (n = 234) was 46.0 ± 4.63 years, which was very low. The data were therefore analyzed in greater detail in subsequent statistical analyses (see Table 3.2 and its associated description). The prevalence of obesity (BMI ≥ 30.0 kg/m^2) and extreme obesity (BMI ≥ 40 kg/m^2) were 67.8% and 16.8% respectively. Within the study population 61.9% had an understanding of menopause. Only 30% had finished high school.

Table 3.1 Descriptive data of study cohort

Variables	Values[a]	Ranges
Age (years)	49.2 ± 5.29	40.0 - 61.0
Age at FMP (years)[b]	46.0 ± 4.63	27.0 - 57.0
Waist (cm)	99.1 ± 14.6	47.0 - 151
BMI (kg/m^2)	33.4 ± 7.32	16.6 - 61.6
BMI ≥ 30 (%)	67.8	-
BMI ≥ 40 (%)	16.8	-
HIV-positive (%)[c]	21.3	-
ARV use (%)[d]	55.3	-
Education (%): - Junior school only - High school, but did not finish - Finished high school with or without higher education	 12.3 57.7 30.0	 - - -
Understand menopause (%)	61.9	-

FMP = final menstrual period; BMI= body mass index; HIV = human immunodeficiency virus; ARV = antiretroviral; [a] Data are presented as mean ± SD or percentage; [b] n = 234; [c] n = 404; [d] n = 85 (n = 702 for the remaining variables)

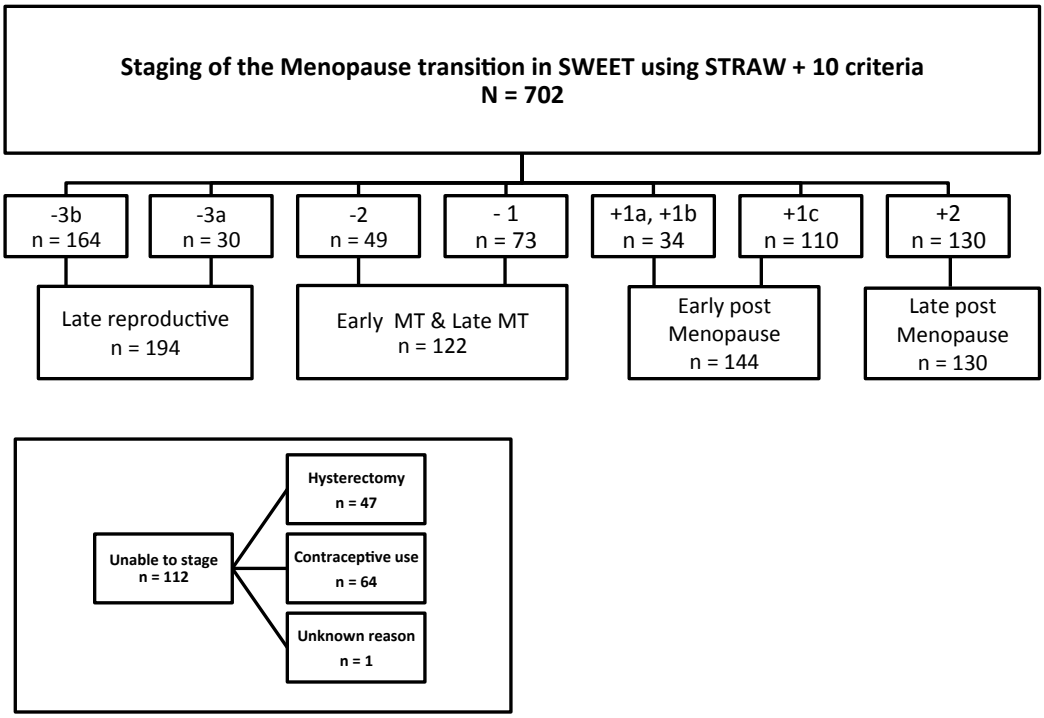

Figure 3.1 Diagram showing menopause staging groups for SWEET participants

3.3.2 Age, BMI, waist, E2 level, and FSH level by menopause stage

Menopausal stage could not be ascertained in 112 (15.5%) of the women because of contraceptive use (n= 64), hysterectomies (n=47), and unknown reason (n=1). The number of women in each menopausal stage, as assessed using STRAW+10 was as follows: -3b, 164; -3a, 30; -2, 49; -1,73; 1a, 1b, 34; 1c, 110; +2, 130 (Figure 3.1). Serum FSH levels (Figure 3.2 [A]) increased across seven groups, and this trend was highly significant ($p<0.0005$ from ANCOVA adjusted for age and BMI). As shown in Figure 3.2 [A], mean serum E2 levels decreased across the menopause stages ($p<0.0005$ from ANCOVA adjusted for age and BMI). Figure 3.2 [B] shows, as expected, that age was strongly related to menopause stage ($p<0.0005$ from ANOVA). BMI, adjusted for age, tended to fall across the menopause stages ($p=0.003$ for trend from ANCOVA) and there was a weak, non-significant relationship between menopause stage and waist circumference ($p=0.07$ from ANCOVA adjusted for age; data not shown).

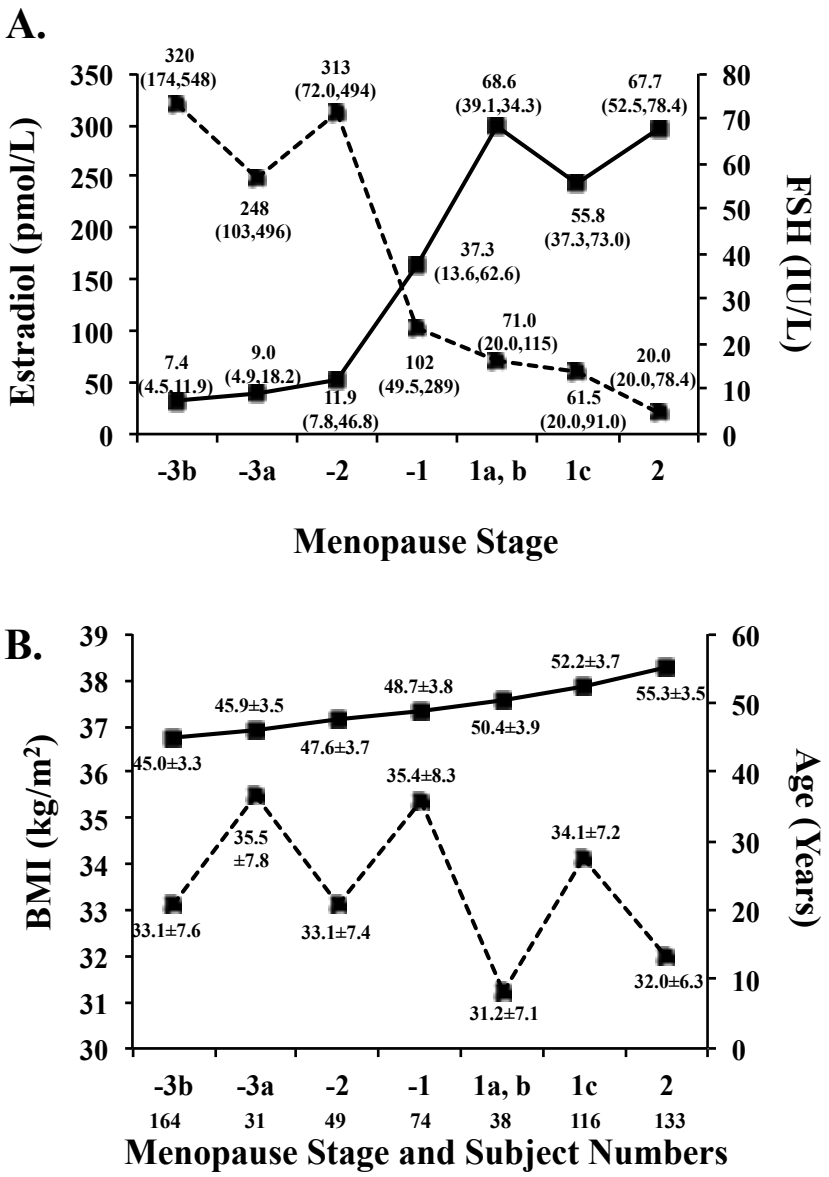

Figure 3.2 E2 (- - -) and FSH (—) levels by menopause stage: -3b, (n=164); -3a, (n=30); -2, (n=49); -1, (n=73); 1a, 1b, (n=34); 1c, (n=110); +2, (n=130). The p-values for trend (ANCOVA) are p<0.0005 for both E2 and FSH, and are adjusted for age and BMI (A). Age (—) and BMI (- - -) by menopause stage (the n's are the same as for A). The p-value for trend (ANOVA) for age is p<0.0005, whilst for BMI p=0.003 (ANCOVA, adjusted for age) (B). Data given as mean ± SD or median (interquartile range)

Given the very low age at FMP that was observed for all women who reported an age for FMP (n=234), further statistical analysis of this data was undertaken. Probit analysis gave a median age at FMP of 46 years. However, Pearson univariate analysis demonstrated a significant inverse relationship between reported age at FMP and the number of years that had elapsed since that event (i.e. years after FMP; r=-0.59, p<0.0005, n=234). This suggests

that women who had their FMP recently reported an older age at FMP than those who had their FMP much earlier. This is confirmed by the data in Table 3.2 that shows that women in the lowest quartile of years post-FMP (≤ 3 years post-FMP), reported a significantly higher age at FMP than women in each of the other quartiles. Within the lowest quartile there was no significant relationship between reported age at FMP and years after FMP (r=-0.04, p=0.77, n=58). When women were divided into tertiles of years post-FMP and a similar univariate analysis was performed for women in the lowest tertile (≤ 4 years post-FMP) a near-significant inverse relationship was observed (r=-0.22, p=0.05, n=78). These women reported a mean age at FMP of 48.5 ± 3.92 years. The data suggest that the reported age for FMP in women within the lowest quartile for years after FMP (49.0 ± 3.80 years; Table 3.2) is the most reliable. When age at FMP was calculated for women who were 2 years or less from FMP (49.1 ± 3.94 years; n=36), it was found to be very similar to that of women who were 3 years or less from FMP. Data in Figure 3.2 [B] confirm that the age at FMP is 49.0 years. This shows that women at menopause stages occurring immediately before and immediately after the FMP (i.e. stages -1, 1a, and 1b), have mean ages of 48.7 ± 3.76 and 50.4 ± 3.94 years, respectively. Age at FMP must fall between these two ages, and 49.0 years does.

Table 3.2 Age at FMP by quartiles of years after FMP

Years post FMP	n	Age at FMP (years)
≤ 3 years	59	49.0 ± 3.80[a]
> 3 and ≤ 6 years	62	46.6 ± 3.50[a,b]
> 6 and ≤ 9 years	50	46.6 ± 4.00[a,b]
> 9 years	63	42.0 ± 4.06
Combined	234	46.0 ± 4.63

Data are presented as mean ± SD; FMP = final menstrual period; [a]p<0.0005 versus > 9 years, [b]p<0.005 versus ≤ 3 years

Table 3.3 Age at FMP by BMI group in women 3 years or less after FMP

BMI group	n	Age at FMP (years)
<25	11	45.7 ± 3.00
≥ 25 and < 30	13	49.8 ± 4.32[a]
≥ 30 and < 35	18	49.0 ± 3.27[a]
≥ 35	17	49.0 ± 3.3.75[a]

Data given as mean ± SD; FMP = final menstrual period; BMI = body mass index; [a]p<0.05 versus < 25

Among participants with reliable estimates of age at FMP (i.e. those ≤ 3 years after FMP), age at FMP was lower in women with a BMI < 25 kg/m2 compared with women in the higher BMI groups ($p<0.05$ for all comparisons; Table 3.3).

3.3.3 Determinants of age at FMP

Multiple regression analysis was used to identify the principal determinants of age at FMP. The women included in this analysis were those who provided details of age at FMP within 3 years of this event (n=59). The variables that showed a significant association with age at FMP within the final multiple regression model were BMI ($\beta=0.41$, $p=0.0007$) and education (completed high school vs. not gaining entry to high school: $\beta=-0.31$, $p=0.009$). The r^2 value for the full regression model was 0.27 ($p<0.0005$).

3.3.4 Prevalence of menopause symptoms at any level by menopause stage

There was a significantly higher prevalence of VMS and sexual problems in early postmenopause than in the late reproductive stage ($p<0.05$; Table 3.4). Irritability was more prevalent in late postmenopause than in the late reproductive and early postmenopause stages ($p<0.05$ for both). None of the other menopausal symptoms assessed using the MRS showed any significant trends across the menopause stages.

Table 3.4 Prevalence of selected menopause symptoms by menopause stage

Menopause stage	n[a]	Vasomotor symptoms	Sexual problems	Irritability
-3b and -3a	194	55.7	65.8	60.8
-2 and -1	123	64.2	75.5	68.3
1a, 1b & 1c	153	69.3[b]	79.6[b]	57.9
+2	133	58.6	77.8	72.9[b,c]
All groups combined	603	61.5	72.2	64.2

Data expressed as percentage; [a]n for subjects who answered question on sexual problems within each menopausal stage group: -3b and -3a (n= 161);stages -2 and -1 (n=90); stages 1a, 1b and 1c (n= 103), stage +2 (n= 72); total (n= 426); [b]p<0.05 versus stage -3b and 3a; [c]p<0.05 versus stages 1a, 1b, 1c

3.3.1 Prevalence of symptoms by BMI group and by HIV status

Table 3.5 shows that although the prevalence of VMS of any level did not change across the BMI groups, the prevalence of severe/very severe VMS was significantly higher in the group with BMI ≥35.0 kg/m^2 (28.2%) compared with both the group with BMI of 30.0 kg/m^2 to 34.9 kg/m^2 (20.2%%; p<0.05) and the group with BMI < 30.0 kg/m^2 (20.1%; p<0.05). Sleep problems were significantly more common in women with a BMI of ≥35 kg/m^2 compared with women with BMI between 30.0 kg/m^2 and 34.9 kg/m^2. Irritability was significantly more common in the highest BMI group compared with the lowest BMI group, whereas joint problems were less common in the lowest BMI group when compared with both of the higher BMI groups.

No significant difference in the prevalence of any of the menopause symptoms was noted between HIV-negative women (n=318) and HIV-positive women receiving (n=47) or not receiving (n=39) antiretrovirals. However, owing to the low number of HIV-positive women it

is possible that the study had insufficient power to detect differences in prevalence.

Table 3.5 Prevalence of symptoms by BMI group

BMI group	n	Vasomotor symptoms	Irritability	Sleep problems	Joint problems
< 30.0	225	61.6	59.5[a]	62.7	62.9[b,c]
≥ 30.0, < 35.0	208	56.7	65.7	56.0[*]	76.3
≥ 35.0	266	61.6	69.8	65.0	76.8
All groups combined	699	60.2	65.3	61.6	72.2

Data expressed as percentages; BMI = body mass index; [a]p<0.05, [b]p<0.005 vs ≥35 kg/m^2; [c]p<0.005 vs ≥30 kg/m^2, <35 kg/m^2

3.3.2 Effects of menopause stage on symptom risk

Table 3.6 shows that the odds ratio (OR) for VMS was significantly increased in early postmenopause compared with the late reproductive stage, but that the OR was attenuated after adjustment for FSH levels but not after adjustment for age, BMI or E2 levels. When all four of these possible confounding variables were included together in this model, the results were similar to those found for the model in which only FSH levels were included (Table 3.6).

The OR for sexual problems was significantly elevated in the early postmenopause stage, and there was also an increased OR in late menopause but this failed to reach statistical significance (p=0.07). These associations were significantly weakened after adjustment for age, but not after adjustment for FSH level, E2 level or BMI. When all four of these possible confounding variables were added together to model 2, the results found were similar to those observed with just the inclusion of age in the model (Table 3.6).

The OR for irritability was significantly increased at stage +2 (Table 3.6), and adjusting for all possible confounders (ie. age, BMI, FSH level or E2 level, either individually or all together), did not weaken this relationship. Although BMI did not attenuate the significant OR for irritability observed in stage +2 of menopause, it was itself associated with a significant OR for irritability (1.03; 95% CI, 1.00-1.06; p=0.03). This confirms the data in Table 3.5, which shows an increasing prevalence of irritability with rising BMI.

Table 3.6 Logistic regression showing effects of menopause stage on symptom risk

Model number and adjustments	Dependent variable	Independent variable	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]
Model 1 without and with adjustment for FSH	Vasomotor symptoms	Stage -2 & -1: Stage1a,b,c: Stage +2: FSH:	1.43 (0.90, 2.28); 0.13 1.80 (1.15, 2.81); 0.01 1.13 (0.72, 1.77); 0.59 -	1.22 (0.73, 2.03); 0.44 1.12 (0.64, 1.95); 0.68 0.74 (0.41, 1.35); 0.33 1.01 (1.00, 1.02); 0.02
Model 2 without and with adjustment for age	Sexual problems	Stage -2 & -1: Stage 1a,b, c: Stage +2: Age:	1.60 (0.90, 2.87); 0.11 2.02 (1.13, 3.62); 0.02 1.82 (0.95, 3.46); 0.07 -	1.26 (0.68, 2.32); 0.46 1.19 (0.59, 2.37); 0.65 0.82 (0.35, 1.93); 0.65 1.09 (1.03, 1.16); 0.005
Model 3 with no adjustment	Irritability	Stage -2 & -1: Stage 1a,b,c: Stage +2:	1.39 (0.86, 2.24); 0.18 0.88 (0.57, 1.37); 0.58 1.73 (1.07, 2.80); 0.02 -	- - - - - - - - -

The reference group is stage -3b, -3a (late reproductive); all models were adjusted for each possible confounder i.e. FSH, estradiol, body mass index and age one at a time. Vasomotor symptoms and irritability: stages -3b and -3a (n = 194); stages -2 and -1 (n = 123); stages 1a, 1b, and 1c (n = 153); stage +2 (n = 133). Sexual problems: stages -3b and -3a (n = 161); stages -2 and -1 (n = 90); stages 1a, 1b, and 1c (n = 103); stage +2 (n = 72); OR = odds ratio; FSH = follicle-stimulating hormone. Only the models in which adjusting for a possible confounder affected the outcomes, are shown

3.4 Discussion

The aims of this study were to examine reproductive aging in black urban South African women, and to determine whether STRAW +10 is an accurate and reliable method of

staging reproductive aging, as this method has not previously been used in sub-Saharan women. The age at which FMP occurred and whether obesity has any effect on the MT or has any association with menopause symptoms, especially VMS, were also noted.

3.4.1 STRAW+10 criteria in staging the MT using FSH and E2 as supportive criteria

As suggested by Harlow et al (56), reproductive aging was staged based on reported changes in the menstrual cycles of participants. Bleeding pattern definitions as described in STRAW+10 were technically complicated and the participants in the pilot study found the terminology difficult to understand. Therefore the menstrual history and bleeding change related questions used in the final questionnaire were basic, and the interviewer used open-ended questions to clarify the responses. This mixed approach to self-reported VMS has been recommended in studies and reviews where cultural differences are apparent (123, 158). The accuracy of menopause staging is strongly dependent on the participants understanding of the terms used when asked about bleeding patterns as described by Smith-DiJulio et al (77). Although more than two thirds of the participants had a very low educational level and nearly half of the cohort did not understand the meaning of the term menopause, the women were able to give reasonably precise information about changes in bleeding patterns, such that their reproductive aging could be staged using STRAW + 10 criteria. This correct staging was confirmed by the FSH and E2 trends. The results showed a strong association between the reproductive stages, as described by STRAW+10 and serum FSH level, serum E2 level, and age. The two defining endocrine changes that occur during the MT are rising FSH levels and falling E2 levels (59, 336). In this study, FSH levels increased gradually from the late reproductive stage through the late MT, accelerated rapidly around the time of FMP, and reached a plateau during the postmenopause stages. The E2 levels decreased consistently from the early MT stage to the last postmenopause stage. These changes in serum FSH and E2 levels across the MT were comparable with those

observed in a large longitudinal investigation of menopause-associated endocrine changes, the Study of Women's Health Across the Nation (SWAN) (51).

3.4.2 Age and recall of age at FMP

The mean age at FMP was lower than that observed in Western women. In SWAN, the median age at natural menopause was 51.4 years (64); in a recent European study where age at menopause was estimated in 5,288 women, it was 54.0 years (337). When recall bias was taken into account, the mean age at FMP in SWEET was 49.0 years. This is similar to that of other African studies (273). A very small study of 88 women in Zaria, Nigeria, reported a mean age for FMP of 46.1 years (338). However, in a larger study of Nigerian women (N=402), Ozumba et al reported mean age at FMP at 49.4 years (339), whereas in a smaller study of Ghanaian women (N=152), mean age for FMP was 48.05 years (285).

It seems that the number of years since FMP affects accurate recall of FMP among SWEET participants; those 3 years or less from FMP reported a mean age at FMP of 49.0 years, whereas those who were interviewed more than 9 years after FMP had a reported mean age at FMP of 42.0 years. Hahn et al (69) found that inaccurate recall of FMP increases with years since menopause and is greater in women with natural menopause. A study in Sweden found that 565 women who first reported age at FMP in 1992 recalled it reasonably accurately after nearly 20 years. In a cross-sectional study, Rödström et al (340), suggested that recall of age at menopause among women aged 60 years or younger is more reliable than recall of age at menopause among women long past menstruation. Other studies have also shown that age at menopause is recalled less accurately with increasing time from FMP, but none has observed a systematic lowering of the reported age at FMP with increasing time from the FMP (69-71, 73). However, all these studies have involved a comparison of recalled age at menopause at two different time points after menopause (i.e. to test for reproducibility) compared with the current study where age at FMP was obtained

at one time point only. Sievert (75) suggested that the transition into menopause is gradual; thus, when women are asked to recall age at FMP, they often have to rely on memory, looking back across a long time continuum to recall the exact time of cessation. A limitation in the estimation of age at menopause is that this current study was a cross-sectional analysis. Age at FMP can be more accurately predicted in longitudinal studies, where the participants use menstrual calendars (76). A limitation in the estimation of age at menopause is that this current study was a cross-sectional analysis. Age at FMP can be more accurately predicted in longitudinal studies, where the participants use menstrual calendars (76).

It is not clear why the women in the current study had poor recall of FMP. A lack of understanding about the menopause transition and a poor primary health care system with limited access to gynecologists may affect this recall, because these women were not questioned about their MT and therefore did not need to recall information about it. In addition, economic status and cultural values on menopause and aging may also have played a role in the women in the study. Further examination, as to whether the MT has the same level of importance for black urban South African women as it seems to have in Western women, is needed.

A strong negative association between level of education and age at FMP was found in SWEET participants. This finding differs from other studies, where low levels of education are associated with younger age at menopause (64, 341). This association was difficult to explain, but the small sample number in this group (n=59) may be a factor; it is possible that this association could be explained by a confounding variable that was not measured in the study. Another factor that has been shown to modulate age at FMP is smoking status. Research has shown that current smoking is associated with earlier age at natural menopause (64, 342). The effect of smoking status on age at FMP was not analyzed since only 3% of the women in the study were current smokers.

3.4.3 BMI and age at FMP

The reported FMP was significantly lower in women with a BMI less than 25 kg/m^2 compared with those participants with higher BMI. Akahoshi et al (343) found that higher BMI was associated with later menopause unlike SWAN, where Gold et al (64) found that BMI was not related to age at natural menopause. However, this negative association between age at FMP with BMI was observed in a small sub-sample of the SWEET participants (n=59), and needs to be confirmed in a much larger cohort with accurate recall of age at FMP.

3.4.4 Prevalence and severity of menopause symptoms across the MT

In the current study, the prevalence of severe/very severe VMS was significantly higher in very obese women. Data from SWAN also showed a positive association between VMS and body fat level (231). Thurston and Joffe (332) found that obesity is a strong risk factor for VMS, especially in the late MT and early postmenopause stage. In the SWEET participants, VMS was also strongly associated with the early MT, late MT, early postmenopause stage, and increased FSH levels. Thurston et al report findings (332) similar to Cray et al (344), who found similar results in participants from the Seattle Midlife Women's Health Study. The reproductive staging criteria from STRAW +10 describe VMS in the late MT and early menopause (56). Other than VMS, only two other symptoms were strongly associated with menopause stage in the SWEET participants: sexual problems and irritability. Sexual problems manifested most strongly in the early and late postmenopause stages and were associated with age. There was a significant risk of increased irritability in late menopause, which is similar to the findings of Rahman et al (345) who also used the MRS to determine prevalence and severity of menopausal symptoms in their participants. However, in a similar study in Omani women, the association of increased irritability was not significant at this stage (102). In the current study, no possible confounders (BMI, age, or FSH level, or E2 level) weakened the association of irritability with late postmenopause, although increased BMI was associated with a higher prevalence and a higher OR for irritability. Among the

symptoms described in the psychological domain of the MRS (depression, irritability, anxiety, mental exhaustion), irritability was the only symptom in the cohort, associated with menopause stage. Several studies have shown that there is an increased risk of depression and mood problems during the perimenopause (346), but Kornstein et al (347) found that irritability was more prevalent in premenopausal women, whereas postmenopausal women were more likely to experience depressive episodes.

The prevalence of VMS reported in the current study for women in the late reproductive stage was higher than those in other studies that used the MRS to determine frequency and severity. Blumel et al (100) reported a VMS prevalence of 37.1% in the premenopause stage in their participants, and a similar prevalence (35.4%) was reported in Malaysian women (348), compared to the 55.7% VMS prevalence in the participants. The prevalence of VMS in the current study is associated with increased levels of FSH. However, there may also be a cultural determinant in the way that women from SWEET describe hot flashes. Many cross-cultural studies have suggested that there are both biological and cultural determinants in describing VMS, and they recommend using a biocultural approach (123, 132). Gold et al (333) suggested that vasomotor symptoms are highly associated with menopause stage, whereas differences in absolute rates are related to cultural differences. Other studies suggested that night sweats and hot flashes be reported and analyzed separately, rather than being grouped together as VMS (349).

3.4.5 Effect of HIV-infection on VMS

No difference was found in the prevalence of menopausal symptoms between HIV-positive and HIV-negative women. However, several studies reported that HIV infection is associated with increased risk of VMS (335, 350, 351). Boonyanurak et al (257) found that the HIV-positive women in their study had a high prevalence of hot flushes, but the number of HIV-positive women in the current study was small (n =86) compared with their sample number

(N=268). Therefore, it is possible that the current study had insufficient power to detect differences in the prevalence of VMS and other menopausal symptoms between HIV-positive and HIV-negative women. Although some studies found that HIV infected women had younger age at menopause (352), the effect of HIV-infection on age at FMP, could not be analyzed, as the women in the current study became infected or were tested for HIV antibodies after FMP. In their comprehensive review of HIV and menopause, Kanapathipillai et al (8) suggested that research to date has failed to show that age at menopause in HIV-positive is younger or older than that in HIV-negative women, and more research is needed. The high prevalence of HIV infection found in this study may be a reflection of population bias. Thus, a large proportion of subjects refused to have their HIV-status assessed and it is therefore possible that those who agreed to be tested may represent a biased sample.

3.4.6 Study limitations and advantages

A limitation of this study was its cross-sectional format. However, the sample numbers were large, and menopause status was accurately determined. A further limitation of the present research was that English was not the first language of the participants, and although interpreters were available, some idiomatic meaning may have been lost in translation (127). An additional limitation was that information on HIV status was only available in 58% of the participants. A strength of the study was the consistency in interpreting reported information on menstrual cycle changes (each participant was interviewed by a single interviewer).

3.5 Conclusions

STRAW+10 is appropriate for staging menopause in resource-limited countries that use information on self-reported bleeding criteria, but there is a need for validated interviewer questions and simplification of technical terms to improve accuracy. The terminology used by STRAW +10 may not be easily generalizable to other groups of women in rural and

informal urban settlements. Years since FMP affects accurate recall and is most accurate in women less than 4 years from FMP. In this group, lower education levels are associated with older age at FMP, whereas leanness is related to an earlier menopause. Only three symptoms (VMS, sexual problems and irritability) are significantly related to menopausal stage. As expected, FSH levels affect the prevalence of VMS across the menopausal stages, and obesity is strongly associated with a risk for severe VMS. Longitudinal investigations in this group of women are needed to further clarify the issues discussed above.

Several of the stated aims of this research, including the usefulness and suitability of using STRAW +10 criteria to accurately stage the MT, an understanding of whether changes in FSH and E2 levels might be used as supportive criteria in defining MT stage, whether obesity and HIV infection have an effect on age at FMP, and menopausal symptoms in black urban South African women, have been explored and described in this chapter. The research in the following chapter examines the differences in body composition across groups of black sub-Saharan African women at different stages of the menopause transition, and the relationship between the anthropometric measures and possible modifying variables, including hormone levels, menopausal stage and HIV-ART status.

Chapter 4. Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): an African perspective

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4 Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): an African perspective

4.1 Introduction

The menopause transition (MT) is closely associated with changes in body composition including lower bone mineral density (BMD) at many skeletal sites (181), an increase in obesity (148), a decrease in lean muscle mass (353), increases in body mass index (BMI) (148), and changes in body fat distribution (BFD) (150), particularly increased central adiposity (155). Abdominal obesity is a principal risk factor for cardiometabolic disease (163). Some studies suggest changes in the abdominal deposition of visceral (VAT) and subcutaneous adipose tissue (SAT) during the MT are related to chronological aging (157), while others find a strong association with reproductive aging (292), suggesting that central adiposity is a result of the changing hormonal milieu (155). Other data show that both may explain changes in body adiposity and a decrease in lean muscle mass (sarcopenia) during the MT (146).

Obesity is widely prevalent amongst mid-life, black South African women (244). The data from the Study of Women Entering and in Endocrine Transition (SWEET) show a high rate of obesity at menopause (68%) (354) and previous investigations have shown that diabetes and metabolic syndrome are very prevalent in these women (7). The causes of this are not known, but given the strong association between MT and changes in BFD and lean muscle mass reported in non-African populations (152), the MT may play a role. The subject of central adiposity and its strong association with non-communicable diseases (NCDs) (165), the relationship between androgens and VAT deposition during MT (355), and the fall in BMD at various skeletal sites observed during the MT is well reported in women from high-income countries (181) but no such data appear to be available in sub-Saharan African menopausal women. A recent study from South Africa demonstrated a strong relationship

between lean mass and BMD in African male and female subjects (325). However, it is not known whether this relationship occurs across the MT and whether changes in lean mass during this period will affect BMD. In addition, the prevalence of HIV infection is high in populations of urban, mid-life black South African females (11), but it is not known whether this contributes to changes in body composition.

The aims of the study were to determine whether general body adiposity, lean muscle mass, BFD and BMD are associated with stages of the MT in these women, and if so, whether this association is related to differences in the serum concentrations of follicle stimulating hormone (FSH), estradiol (E2), androgens and sex hormone binding globulin (SHBG). An additional aim was to determine if HIV-infection and the related antiretroviral therapy influenced body composition and hormone levels in mid-life African females.

4.2 Methods

The following methods were used: questionnaires for determining education level, use of ART, tobacco and smokeless tobacco (snuff) use, menopausal stage (using STRAW+10 criteria); assays of serum E2 and FSH, DHEA, DHEAS, testosterone and SHBG levels; measurement of HIV status; measurement of waist circumference, weight and height; BMI calculated; measurement of body composition (using dual-energy X-ray absorptiometry (DXA); measurement of VAT and SAT (using ultrasound). Each of these methods has been fully described in Chapter 2

4.2.1 Statistical analyses

Data that were not normally distributed (all hormone measures) were log transformed to normality before being used in the statistical analyses. These data are presented as median (interquartile range) in the tables and text, whilst data with a normal distribution (all

anthropometric measures) are expressed as mean ± SD. Continuous variables were analyzed across groups using ANOVA and ANCOVA, and paired means were compared using Tukeys post hoc test. Multivariate linear regression analysis was used to identify the principal correlates of each of the body anthropometry variables. Independent variables included in each model were chosen based on scientific plausibility and correlation with the outcome variable in univariate analyses, with $p<0.50$. Backward, stepwise regression was performed. Height, total fat mass and total lean mass were included in all initial regression models to reduce the chance of confounding, but exceptions to this were the models for BMI (only total lean mass included), total fat mass (only height and total lean mass included), and total lean mass (only height and total fat mass included). Collinearity was tested using the variance inflation factor (VIF) and variables were excluded if VIF was greater than 10.0. Collinearity was present for total, free and bioavailable testosterone and SHBG, and from these 4 highly inter-related variables only SHBG and free testosterone could be included in the same regression models. Collinearity was also observed for BMI and total fat mass and therefore only the latter variable was included in the relevant regression model.

4.3 Results

4.3.1 Subject characteristics

Menopausal stage was not determined in 112 women due to hysterectomy (n=47), contraceptive use (n=64) and unknown reason (n=1). Participants in each stage were as follows: late reproductive (n=194); early and late menopausal transition (n =122); early postmenopause (n=144) and late postmenopause (n=130) (Figure 4.1).

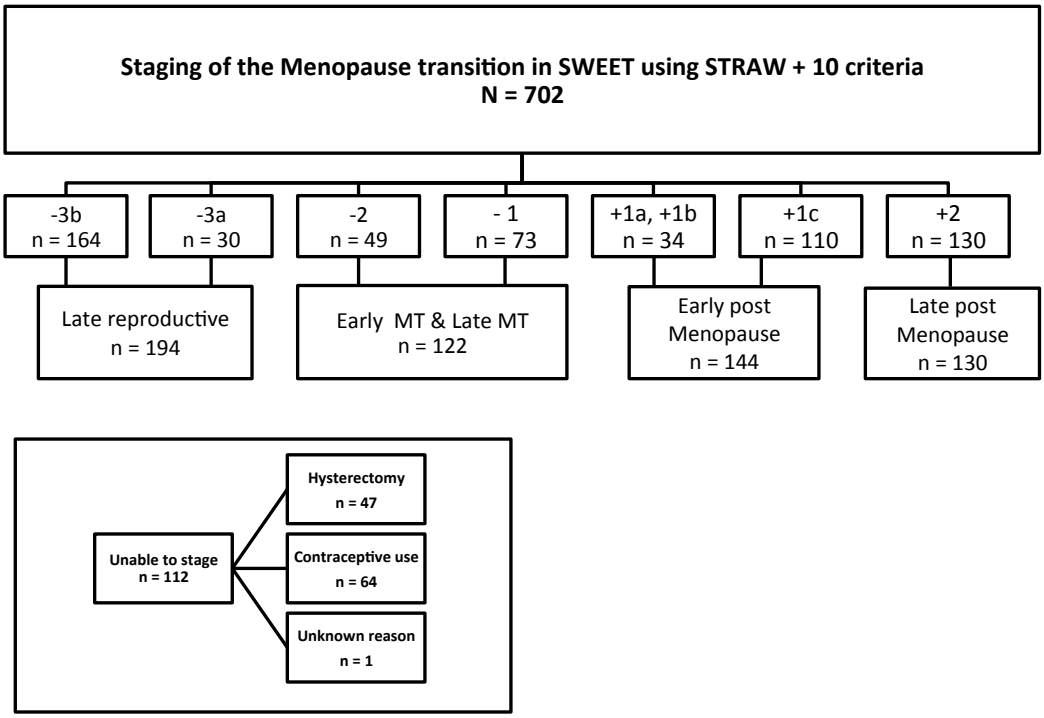

Figure 4.1 Diagram showing menopause staging groups for SWEET participants

The characteristics of this study population have been reported previously (11). However, a brief overview of this population will be provided here, as this is relevant to the current study. Thus, obesity was highly prevalent at 67.8%, with a mean BMI (±SD) of 33.4 ± 7.32. The mean age (±SD) was 49.2 ± 5.29 years, and was much higher ($p<0.0005$) in the postmenopausal than the premenopausal groups (Table 4.1). Amongst the participants, 404 knew or agreed to have their HIV status measured and 21.3% were HIV positive, of whom 55.3% were receiving antiretroviral therapy (ART). Table 4.1 shows that HIV-positivity was less common in the group at menopause stage +2 than at menopause stage -3b, -3a ($p<0.05$). The use of ART did not differ significantly between the menopausal stages (Table 4.1). Within the study cohort 30% had finished high school, as previously reported (354), the employment level was 57% and 20.9% of the participants were snuff users.

Table 4.1 Age, HIV status and ART use, and anthropometric measurements across menopausal stages

Anthropometric variables	Menopausal stages (from STRAW+10) and n's				P-value for trend
	-3b & -3a n = 194	-2 & -1 n = 122	1a, 1b & 1c n = 144	+2 n = 130	
Age (years)	45.1 ± 3.30	48.3 ± 3.75	51.8 ± 3.86	55.3 ± 3.50	<0.0005
HIV infection (%)[a]	28.8	24.2	17.3	14.8	0.02[c]
ART use (%)[b]	59.4	50.0	57.1	69.2	NS[d]
BMI (kg/m^2)	33.5 ± 7.64	34.5 ± 8.03	33.4 ± 7.28	32.0 ± 6.32	0.06
Waist (cm)	99.3 ± 14.9	101 ± 16.3	99.6 ± 14.8	98.3 ± 12.6	0.60
Hip (cm)	118 ± 15.7	120 ± 15.8	119 ± 16.4	116 ± 13.2	0.22
Visceral fat (cm^2)	94.4 ± 40.6	97.7 ± 42.8	96.3 ± 38.9	89.4 ± 34.3	0.35
Subcutaneous fat (cm^2)	400 ± 134	408 ± 133	395 ± 121	367 ± 109	0.05
Arm fat (kg)	3.77 ± 1.31	3.88 ± 1.38	3.68 ± 1.19	3.70 ± 1.24	0.61
Arm lean (kg)	4.62 ± 0.86	4.48 ± 0.95	4.31 ± 0.83	4.29 ± 0.70	0.001
Leg fat (kg)	14.3 ± 5.00	14.2 ± 4.49	14.1 ± 4.75	14.0 ± 4.48	0.97
Leg lean (kg)	16.1 ± 2.88	15.6 ± 3.19	15.2 ± 3.01	15.1 ± 2.38	0.007
Trunk fat (kg)	14.3 ± 5.09	15.1 ± 5.54	14.6 ± 5.14	14.2 ± 4.88	0.55
Trunk lean (kg)	22.4 ± 3.39	22.1 ± 3.84	21.2 ± 3.39	21.3 ± 2.93	0.003
Whole body fat (kg)	32.3 ± 10.4	33.2 ± 10.3	32.4 ± 10.3	32.0 ± 9.63	0.82
Whole body lean (kg)	43.2 ± 6.74	42.2 ± 7.68	40.7 ± 6.93	40.7 ± 5.62	0.002
Whole body BMD (mg/cm^2)	935 ± 77.9	931 ± 80.5	889 ± 77.6	866 ± 76.2	<0.0005
Whole body BMC (g)	1702 ± 232	1661 ± 247	1566 ± 242	1539 ± 232	<0.0005

Data expressed as percentage or mean ± SD; BMD = bone mineral density, BMC = bone mineral content, NS = non-significant; [a] subject numbers per menopausal stage who agreed to an HIV test were 111, 66, 81 and 88 respectively; [b] subject numbers per menopausal stage who were HIV-positive were 32, 16, 14 and 13, respectively; [c] p-value from χ^2 test for menopausal stage -3b, -3a versus stage +2; [d] using the χ^2 test no differences were noted in frequency of ART use across the 4 groups

4.3.2 Anthropometric variables and menopause transition stages

Anthropometric measurements (Table 4.1) show a trend of lower BMI (p=0.06) and SAT (p=0.05) in females in the postmenopausal groups, whilst whole body lean mass (p=0.002),

and lean mass at all body sites were lower. Whole body BMD and BMC were significantly lower in the postmenopausal groups ($p<0.0005$ for both).

4.3.3 Hormone levels and menopause transition stages

As expected E2 levels were lower ($p<0.0005$) and FSH ($p<0.0005$) levels were higher in the postmenopausal groups (Table 4.2). These data are shown in detail in a previous publication (354). Both SHBG and DHEAS respectively, were significantly lower ($p<0.0005$ and $p=0.007$) in the postmenopausal groups.

Table 4.2 Hormone concentrations across menopausal stages

Hormones	Menopausal stages (from STRAW+10)				P-value for trend
	-3b & -3a n = 194	-2 & -1 n = 122	1a, 1b & 1c n = 144	+2 n = 130	
Estradiol (pmol/L)	315 (369)	130 (362)	63.0 (74.0)	20.0 (52.0)	<0.0005
FSH (IU/L)	7.55 (7.80)	26.2 (52.4)	57.6 (41.7)	67.7 (25.9)	<0.0005
Total T (pmol/L)	650 (430)	610 (410)	612 (340)	595 (460)	0.18
Bioavail. T (pmol/L)	256 (155)	255 (133)	267 (128)	254 (138)	0.88
Free T (pmol/L)	10.0 (6.79)	10.3 (5.56)	10.3 (4.59)	10.1 (6.52)	0.74
SHBG (nmol/L)	61.0 (33.8)	52.2 (36.3)	49.7 (28.1)	50.3 (28.3)	<0.0005
DHEA (ng/ml)	2.90 (2.80)	2.50 (2.50)	2.50 (3.30)	2.45 (3.20)	0.73
DHEAS (µmol/L)	1.40 (1.40)	1.30 (1.00)	1.10 (1.10)	0.95 (1.00)	0.007

Data expressed as median interquartile range (IQR); T= testosterone, and Bioavail = bioavailable

4.3.4 Effect of HIV on anthropometric and hormonal variables

Table 4.3 shows that the groups of HIV-positive, ART-naïve ($p<0.05$) and HIV-positive, ART-treated ($p<0.0005$) women were both younger than the HIV-negative women, and also had higher total testosterone ($p<0.005$ for both groups) and SHBG ($p<0.05$ and $p<0.005$ respectively) levels. The HIV-positive, ART-treated subjects had higher BMI ($p<0.0005$ and $p<0.005$ respectively), hip circumference ($p<0.0005$ and $p<0.005$ respectively), subcutaneous fat area ($p<0.0005$ and $p<0.05$ respectively), whole body fat ($p<0.0005$ and $p<0.05$ respectively) and lean mass ($p<0.005$ and $p<0.05$ respectively), whole body BMD ($p<0.05$ for both) and BMC levels ($p<0.0005$ and $p<0.005$ respectively) and serum DHEAS levels ($p<0.005$ and $p<0.05$ respectively) than the HIV-negative and HIV-positive, ART-naive groups. The HIV-negative group had a higher waist circumference ($p<0.0005$) and more visceral fat ($p<0.005$) than the HIV-positive, ART-treated group. As shown by ANCOVA none of the differences in hormonal levels across the groups were explained by the differences in age or anthropometry.

Table 4.3 Anthropometric measures and hormone levels according to HIV status and therapy

Variables	HIV-negative (n=318)	HIV-positive, ART-naïve (n=39)	HIV-positive, ART-treated (n=47)
Age (years)	49.7 ± 5.33	47.6 ± 5.19*	46.3 ± 5.04***
BMI (kg/m^2)	33.2 ± 6.25†††	33.5 ± 7.88††	28.8 ± 7.86
Waist (cm)	99.7 ± 13.4†††	96.7 ± 13.3	91.4 ± 16.1
Hip (cm)	118 ± 13.2†††	119 ± 16.6††	109 ± 16.7
Visceral fat (cm^2)	94.3 ± 37.5††	82.8 ± 36.9	73.5 ± 42.4
Subcutaneous fat (cm^2)	389 ± 106†††	387 ± 137†	315 ± 129
Whole body fat (kg)	32.8 ± 9.17†††	31.7 ± 9.98†	25.6 ± 12.3
Whole body lean (kg)	42.0 ± 6.76††	41.9 ± 7.22†	38.1 ± 6.66
Whole body BMD (mg/cm^2)	911 ± 84.0†	916 ± 77.4†	872 ± 70.8
Whole body BMC (g)	1634 ± 247†††	1654 ± 228††	1482 ± 235
Estradiol (pmol/L)	85.0 (272)	88.0 (322)	116 (306)
FSH (IU/L)	29.8 (60.2)	41.5 (53.5)	24.5 (60.1)
Total T (pmol/L)	580 (360)	770 (470)**	760 (600)**
Bioavail. T (pmol/L)	250 (136)	272 (139)	286 (160)
Free T (pmol/L)	9.92 (5.66)	11.8 (5.52)	11.2 (7.16)
SHBG (nmol/L)	51.6 (36.5)	67.9 (34.2)*	77.1 (56.3)**
DHEA (ng/ml)	2.40 (2.30)	2.40 (2.30)	2.20 (1.60)
DHEAS (µmol/L)	1.20 (1.00)††	1.10 (1.0)†	0.80 (1.00)

Data expressed as mean ± SD or median interquartile range (IQR); BMD = bone mineral density, BMC = bone mineral content, T = testosterone, and Bioavail = bioavailable; *p<0.05, **p<0.005, ***p<0.0005 vs HIV-negative; †p<0.05, ††p<0.005, †††p<0.0005 vs HIV-positive with ART; all analyses adjusted for age

4.3.5 Correlates of anthropometric variables

In the univariate analyses preceding multiple regression (Table 4.4), age correlated significantly only with visceral fat (r=0.08, p=0.04). Models 1 and 2 show that ART use and SHBG have negative relationships with BMI and total fat mass, whilst DHEAS serum levels show a negative correlation with total fat mass only. Total lean mass correlated with BMI and total fat mass, whilst height correlated negatively with total fat mass. Model 3 demonstrates

that levels of FSH and SHBG correlate negatively with total lean mass, whilst DHEAS correlated positively. Height and total fat mass also correlated with total lean mass. The FSH levels also correlated negatively with BMI (outcome variable) in a univariate regression model (β=-1.26, p=0.006) but this relationship was severely attenuated (β=0.43, p=0.15) if total lean mass was included as an independent variable. Models 4 and 5 show that women with a high school education, compared to those who did not, have a smaller waist circumference and less visceral fat. A negative effect of employment and serum SHBG levels is observed for both variables, and a positive effect of age and snuff use on waist and visceral fat area respectively, was noted. Model 6 demonstrates a positive correlation of serum DHEA levels with subcutaneous adipose area. Height (negatively), total fat and total lean mass all correlated significantly ($p<0.0005$) with waist, visceral and subcutaneous fat area. Model 7 shows that total BMD displayed a negative but weak relationship with SHBG and positive correlations with E2 and DHEAS. BMD was lower in the postmenopausal stages of the MT when compared to the late reproductive stage, and was significantly reduced in women who used snuff when compared to those who did not. Total lean mass correlated strongly with BMD. In regression model 8, total BMC was shown to correlate negatively with ART use and positively with serum E2 levels. Height and total lean mass both correlated strongly with BMC.

Table 4.4 Multivariate regression models for anthropometric measures

Model number	Dependent variable	Independent variable with unstandardised β (p-value)		Adjusted R^2 (p–value) for full model
1	BMI (kg/m^2)	Lean mass	0.68 (<0.0005)	0.64 (<0.0005)
		Use of ART	-1.49 (0.02)	
		SHBG (log)	-1.71 (0.04)	
2	Total fat mass (kg)	Height	-0.12 (0.04)	0.60 (<0.0005)
		Lean mass	1.13, (<0.0005)	
		Use of ART	-2.92 (0.008)	
		SHBG (log)	-3.29 (0.02)	
		DHEAS (log)	-1.61 (0.04)	
3	Total lean mass (kg)	Height	0.29 (<0.0005)	0.64 (<0.0005)
		Fat mass	0.42 (<0.0005)	
		FSH (log)	-1.65 (<0.0005)	
		SHBG (log)	-2.19 (0.003)	
		DHEAS (log)	1.06 (0.005)	
4	Waist (cm)	Height	-0.31 (<0.0005)	0.70 (<0.0005)
		Fat mass	0.60 (<0.0005)	
		Lean mass	0.86 (<0.0005)	
		SHBG (log)	-5.05 (<0.0005)	
		Employed	-1.44 (0.02)	
		Age	0.15 (0.009)	
		Graduated HS	-1.77 (0.008)	
5	Visceral fat (cm^2)	Height	-0.51 (0.007)	0.46 (<0.0005)
		Fat mass	1.11 (<0.0005)	
		Lean mass	2.32 (<0.0005)	
		SHBG (log)	-17.9 (0.0007)	
		Employed	-5.37 (0.02)	
		Graduated HS	-7.02 (0.006)	
		Take snuff	6.66 (0.02)	
6	Subcutaneous fat (cm^2)	Height	-6.64 (<0.0005)	0.91 (<0.0005)
		Fat mass	7.16 (<0.0005)	
		Lean mass	6.37 (<0.0005)	
		DHEA (log)	12.7 (0.003)	
7	Total BMD (mg/cm^2)	Lean mass	4.79 (<0.0005)	0.33 (<0.0005)
		SHBG (log)	-31.5 (0.05)	
		DHEAS (log)	16.1 (0.01)	
		Estradiol (log)	20.0 (0.002)	
		Stage 1a,1b,1c	-20.2 (0.01)	
		Stage 2	-37.9 (<0.0005)	
		Take snuff	-14.6 (0.04)	
8	Total BMC (g)	Height	9.65 (<0.0005)	0.59 (<0.0005)
		Lean mass	20.6 (<0.0005)	
		Estradiol (log)	69.5 (<0.0005)	
		Use of ART	-78.8 (0.003)	

Variable coding: employed were compared to unemployed subjects, those who used snuff were compared against those who did not and subjects who used ART were compared with those who were ART naïve; subjects who attended but did not graduate and subjects who graduated high school (HS) were compared with those who did not attend; for menopausal stages, stage -3b with -3a was used as the reference group.

4.3.6 Investigation of explanatory anthropometric variables across different menopause transition stages

Differences across the MT observed for lean mass, BMD and BMC, which were analyzed using ANOVA (Table 4.1), were further analyzed using ANCOVA to determine which variables may confound these results. Variables correlating with lean mass, BMD and BMC in the regression models (Table 4.4) were likely to be possible confounders and these were used as co-variates in the ANCOVA, with menopausal stage used as the grouping variable and lean mass and BMD as the dependent variables. The ANCOVA for lean mass demonstrated that including FSH as a co-variate dramatically reduced the unadjusted F-value for the grouping variable from 4.90 ($p=0.002$) (Table 4.1) to 0.05 ($p=0.98$). The ANCOVA for BMD showed that including E2 as a co-variate reduced the unadjusted F-value from 25.2 ($p<0.0005$; (Table 4.1)) to 7.89, but with no major lessening of significance ($p<0.0005$). With regards to BMC, the unadjusted F-value ($F=15.5$, $p<0.0005$; (Table 4.1)) was reduced by including ART use ($F=7.77$, $p<0.0005$) or estradiol ($F=5.31$, $p=0.001$), but including both variables in the same ANCOVA had a far more dramatic effect ($F=1.89$, $p=0.13$). Adjusting for other co-variates in any of the three ANCOVAs had minimal effects. It is possible that the relationships between hormones and body composition are bi-directional and therefore to test this hypothesis an ANCOVA was performed in which the dependent variable was FSH or E2 and the co-variate was lean mass or BMD, respectively. An ANCOVA with E2 as the dependent variable and BMC as the co-variate was set up. These analyses (data not shown) showed that the presence of lean mass, BMD or BMC as a co-variate had minimal effects on the F- or p-values within these ANCOVAs, suggesting that the relationships are unidirectional with body composition being influenced by hormone levels and not vice versa.

4.4 Discussion

This is the first study to analyze body composition and hormone levels during the MT in sub-Saharan black African females. This cross-sectional study, demonstrates that within this population group, and as shown previously in other populations, significant relationships exist between hormone levels and body adiposity, BFD (204), lean mass (356) and skeletal measures (357). However, despite these relationships minimal differences were observed between the MT groups in body adiposity or BFD, whilst lean mass and skeletal measures (BMC and BMD) were significantly lower in the postmenopausal compared to the premenopausal groups. With regards to hormone levels, FSH was higher, and E2, SHBG and DHEAS were lower, in post- compared to premenopausal women.

4.4.1 Body composition and the menopause transition

There was a tendency ($p=0.06$) for BMI to be lower in the postmenopausal than the premenopausal women in the cohort. This is in contrast to a large longitudinal multi-ethnic study in the USA found an increase in BMI across the MT (358), as did a sizeable European cross-sectional study (359). A multi-ethnic cross-sectional survey found, after adjusting for age, that women with natural menopause did not have increased BMI compared to premenopausal women (360), and some longitudinal studies found no difference in BMI between pre- and postmenopausal women, although differences in BFD with reproductive aging were noted (9). Thus, data from various studies, both longitudinal and cross-sectional, have produced conflicting results with regards to the change in BMI observed across the MT. This may be due to differences in study methodology, sample size and lack of adjustment for possible confounding variables. Also these conflicting results may be caused by cross-sectional period effects versus longitudinal changes in women in cohort studies, Furthermore, there may be ethnic differences in the response of adipose tissue to the changing metabolic and hormonal milieu that is characteristic of the MT, and this requires further detailed investigation. Mauriége et al (361) found a fall in SAT levels across the MT,

as was found in the current study, although the effect was quite small (p=0.05). As shown in the SWAN study (166), no effect of the MT on BFD and waist circumference was found in the SWEET participants, although other investigators have found an association (155). It was observed that lean mass was lower at all body sites in the postmenopausal groups in the current study, which has been observed previously in several studies (9, 146, 184), although this was not found in one small cross-sectional study (152). Bone mineral density and BMC were both lowest in the postmenopausal groups in the current study, a trend that has been observed in a number of other studies (362).

4.4.2 Hormone levels, the menopause transition and HIV infection

As expected, the results showed a trend of significantly higher FSH and lower E2 serum levels when moving from pre- to postmenopausal groups. While no difference was found across the subject groups in concentrations of total or bioavailable testosterone, or DHEA, levels of both SHBG and DHEAS were significantly lower in the postmenopausal women. These results are similar to data from the longitudinal Melbourne Women's Midlife Health Project, which found that SHBG decreased, whilst levels of total testosterone did not change across the MT (53). However, not all studies have shown a fall in SHBG levels across the MT (363). It was observed that DHEAS was lower in the postmenopausal groups in the present study, but there was a transitory rise in the late menopausal transition group; these results mimic those described in a large longitudinal study (52), although this was not shown in another large cross-sectional study (363).

The prevalence of HIV-infection was lower in postmenopausal than premenopausal females, which is probably a consequence of the age difference between these groups. The frequency of use of ART did not differ between the different stages of the MT as defined using the STRAW+10 criteria.

The data found that total testosterone and SHBG levels were higher in HIV-infected than non-infected women, irrespective of ART. A previous study has shown that SHBG levels are higher but total testosterone levels are lower in HIV-positive, premenopausal women (364), but there are no data on androgen levels for HIV-infected, menopausal or postmenopausal women. These findings must therefore be confirmed in future studies. The present study demonstrated lower DHEAS levels in HIV-positive women receiving ART than in both HIV-negative and HIV-positive, ART-treated women. There is no comparable data on the effect of ART on DHEAS levels in menopausal women.

4.4.3 Age and body composition

Within univariate analyses, the only variable correlating with age was visceral fat, and this relationship was lost after inclusion in a multiple regression model. The reason for the lack of association between age and adipose tissue measures may be that adiposity (as measured using BMI) in black South African women aged 40-60 years is fairly static, as described in a large nationwide health survey (244).

4.4.4 Relationship between hormone levels and body composition

The current study demonstrated that levels of SHBG were negatively correlated with a number of anthropometric variables, confirming data from other studies showing relationships of SHBG with BMI, total fat mass, lean mass, waist (355) and VAT (154). The negative relationship observed between SHBG levels and BMD in this study, has been described previously (357).

The data showed a positive association of DHEAS with lean mass and BMD, and a negative relationship with fat mass, although this relationship was quite weak ($p=0.04$). This finding differs from that of a small, multi-ethnic cohort which showed that although DHEAS levels were lower in postmenopausal women, they were not associated with body adiposity (240).

As with the current study's findings, results from SWAN show that DHEAS levels are negatively associated with BMI (365) and these data confirm the association between BMD and DHEAS found in a large longitudinal study (366).

The research identified a strong inverse association between FSH levels and total lean mass, as observed in previous studies (146, 367). A large longitudinal study showed a negative correlation between BMI and serum FSH levels in menopause (365), however, total lean mass was not reported in this study. The current study showed that a significant negative relationship between BMI and FSH exists but is rendered non-significant with the addition of lean body mass to the regression model. The negative association between FSH and lean mass is currently not fully understood. It is hypothesized that FSH may be related to lean mass through an indirect mechanism since there appears to be no evidence that FSH receptors exist in lean tissue.

4.4.5 Education, ART and snuff and body composition

There appears to be little research on the relationship of waist circumference with levels of education and employment in subjects during the MT. Donato et al found no association between educational level and waist circumference in menopausal females (155), but a large European study agreed with the present study's findings, showing a negative association between waist circumference and education levels (368). The data from both ANOVA and multiple regression analysis demonstrate a negative relationship of ART with both total body fat and BMC in the current study. In a recent systematic review of the lipodystrophic effects of ART it was shown that the use of antiretroviral agents is associated with lipoatrophy, and thus lower BMI (267). Studies have also shown that ART is associated with reduced bone mass (369). It has also been shown that ART-naive women with HIV experience decreases in bone density (272), but this was not observed in the current study. Amongst the participants, snuff use was significantly associated with lower BMD and elevated visceral fat

area. Snuff use is prevalent among black South African women, and nicotine in popular snuff brands used by these women appears to be very high (255) and potentially more detrimental to bone health than snuff products used in other countries. Lower BMD was shown with combined use of cigarettes and snuff in black South African women (252) and data from a small cross-sectional study (370) suggested smokeless tobacco use may be an additional risk for decreased BMD. Snuff use has been linked to increased obesity and thus may increase risk of metabolic syndrome (254), and it has also been observed that smoking increases abdominal girth (249). Therefore, it is plausible that nicotine-laden snuff may have an effect on visceral fat.

As reported earlier, levels of DHEA correlated positively with subcutaneous fat area in the participants. No other studies appear to have analyzed the relationship between subcutaneous fat area and DHEA levels during the MT. However, one Italian study performed in 28 premenopausal females did show a negative relationship between serum DHEA levels and subcutaneous fat measures (371). Further studies are required to fully investigate the effect of DHEA on body fat distribution during the MT. Estradiol levels in the current study correlated positively with BMC and BMD, as observed in other studies (181), and statistical analyses suggested that the fall in both bone measures observed across the MT groups may be related to the decline in E2 levels. It is well recognized that E2 plays an important role in bone maintenance through effects mediated by bone estrogen receptors (372). The data further showed, in multiple regression models and ANCOVA, that BMD correlates strongly with menopause stage independently of other variables, suggesting that factors in addition to E2, but not measured in this study, may be involved in BMD differences across the MT.

The relationship between hormones and body composition during the MT is complex. It is uncertain whether differences in adiposity are driven by an altered hormonal milieu or vice versa, although recent longitudinal data from SWAN suggest that alterations in body fat

modulate hormone levels (373). However, testosterone and E2 receptors are expressed in adipocytes (204), thus the subcellular machinery necessary for sex steroids to influence adipose tissue deposition is in place. Within the present study, body adiposity appeared to fall instead of rising across the MT groups, in the face of lower E2 levels. The cross sectional nature of the study allows associations to be noted, but does not allow the causal direction of these relationships to be determined, however ANCOVA showed that differences in BMD and lean mass across the MT groups are strongly related to serum levels of E2 and FSH, respectively, and these relationships are unidirectional. These are only statistical relationships and should be verified in longitudinal studies.

4.4.6 Study limitations and advantages

A limitation of this study was its cross-sectional format. However, the sample numbers were large, and menopause status was accurately determined (354). Another limitation was the inability to collect serum samples during the follicular stage of the menstrual cycle (days 2-5) in premenopausal women. However, ovarian function becomes progressively more dysfunctional in the late reproductive and early menopause transition stages (50), making it more difficult to determine the follicular stage, so timing of E2 assessment may become less important. In spite of the fact that a timed serum sample was not obtained, women in the early stages of the MT were characterized by much higher levels of E2 and much lower levels of FSH than observed in women in the early and late postmenopausal stages, as would be expected. An additional limitation was that information on HIV status was only available in 58% of the participants. Studies have shown that intra-abdominal fat can be accurately measured by ultrasound measurements (374). Although computed tomography (CT) scans and magnetic resonance imaging (MRI) are gold standard measurements in assessing visceral fat, they are expensive and often unavailable, while results from ultrasound have been shown to be highly reproducible (374). The Sodergard equation was used to calculate free and bioavailable testosterone (329), which has been shown to be

reliable (375). Immunoassays for testosterone can be compromised by cross reaction with related steroids and a lack of sensitivity (376), therefore testosterone levels were measured using liquid chromatography-mass spectrometry, which has been shown to be an accurate assay method (328). Further positive aspects of this investigation are that the serum levels of a wide variety of relevant hormones and a number of anthropometric and demographic variables were measured within a menopausal population group for which no such data was previously available.

4.5 Conclusions

In conclusion, the principal body composition outcomes observed in this study were lower lean mass, BMD and BMC in post- than in premenopausal women. The lower lean and bone mass in the postmenopausal groups may be related to higher levels of FSH and lower levels of E2, respectively. Antiretroviral treatment was associated with lower body adiposity and BMC whilst snuff use was associated with lower BMD but higher levels of visceral fat. Thus, both physiological and environmental factors appear to modulate body composition during the MT in this population group. These findings may help to determine whether MT-related sarcopenia is associated with changes in cardiometabolic disease risk factors. This research has implications for the use of behavioral interventions to lower morbidity and mortality in this population group. Exercise programs to help maintain lean mass and reduce adiposity and an education campaign to explain the health risks associated with snuff use may be beneficial. In addition, recommendations for the use of ART regimens with a more bone sparing effect may be considered

The differences in body composition across groups of black sub-Saharan African women at different stages of the menopause transition and the relationship between these anthropometric measures and possible modifying variables have been explored and described in this chapter. The prevalence of the MetS, the level of its individual components,

and associated metabolic factors at different stages of the MT in these women, and factors that play a role in modulating the levels of these variables, are examined in Chapter 5.

Chapter 5: Reproductive aging and associated hormonal changes are related to metabolic syndrome and cardiovascular disease risk factors in menopausal African women

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5 Reproductive aging and associated hormonal changes are related to metabolic syndrome and cardiovascular disease risk factors in menopausal African women

5.1 Introduction

Research suggests that the metabolic syndrome (MetS) increases the risk of cardiovascular disease (CVD) (377). Further studies show that the risk of MetS increases in midlife women (141), and that heart disease is more prevalent as women transition into menopause (378, 379). Physiological changes occurring across the menopause transition (MT) stages may increase the risk of MetS, although other studies have shown that aging attenuates the association of reproductive aging with the MetS (139). These findings are predominantly derived from studies of Western women, but whilst investigations have shown that obesity is prevalent in midlife black South African women (244, 354), and that they are at risk for both MetS and heart disease in middle age (5, 7), this has not, except in a small Ghanaian study (274, 380), been studied in relation to the menopause transition in sub-Saharan African women.

Research shows that several factors may be responsible for the increased risk of MetS in midlife women. Some data show that menopausal status, may increase this risk (135), while data from the Study of Women Across the Nation (SWAN), a large longitudinal study, showed that 13.7% of a cohort of 949 women had MetS at the time of the final menstrual period (FMP), but suggested that this risk seemed to lessen after FMP (110). Studies have shown that the individual components of the MetS i.e. high-density lipoprotein (HDL) (381), triglycerides (9), glucose levels (382), blood pressure (383), and waist circumference (384) do change across the MT, leading to an increased prevalence of the MetS. Hormonal changes across the MT, including those of the adipose tissue-derived hormones leptin and adiponectin, may also be associated with a higher risk of MetS (193, 385). Data from SWAN

showed that an increasing androgenic milieu, as defined by the ratio of testosterone to estrogen, across the MT appears to predispose women to an increased risk of MetS (189). In terms of changes in body anthropometry that occur during the MT, a study has shown that changes in visceral fat may drive the increased risk for MetS in midlife women (240). In addition, lean mass has been shown to fall across the MT (354), and research has shown that sarcopenia, a combination of low lean mass and obesity, may also be related to metabolic disease (133, 172).

As described above, data suggest that changes in metabolic, hormonal, and anthropometric parameters across the MT may increase the risk of metabolic disease in midlife women. Therefore, the aims of this research were to: (i) determine the prevalence of metabolic syndrome, its individual components and related metabolic disorders; (ii) to measure the levels of these variables across the MT groups; and (iii) to determine the principle correlates of these cardiovascular risk factors in a population of midlife, urban African women for whom accurate menopausal staging was available.

5.2 Methods

The following methods were used: questionnaires for determining education level, use of ART, tobacco and smokeless tobacco (snuff) use, menopausal stage (using STRAW+10 criteria), and frequency and severity of menopausal symptoms (using the MRS); assays of serum E2 and FSH, DHEA, DHEAS, testosterone, SHBG, lipids and blood glucose levels, leptin and adiponectin levels; insulin resistance calculated; blood pressure recorded; measurement of HIV status; measurement of waist circumference, weight and height; BMI calculated; measurement of body composition (using dual-energy X-ray absorptiometry (DXA); measurement of VAT and SAT (using ultrasound). Each of these methods has been fully described in Chapter 2

5.2.1 Statistical analyses

Normally distributed data are expressed as mean ± SD in the text and tables, whilst data that is not normally distributed are expressed as median [interquartile range]. The latter data was log transformed to normality before being analyzed.

The principal correlates of all the metabolic variables, and blood pressure, were isolated using univariate correlation analyses (Pearson test) for continuous variables and ANOVA for categorical variables followed by backward, stepwise linear regression analyses. The independent variables included in the regression models were those that correlated with the dependent variable at $p<0.50$ in the univariate analysis. Only scientifically plausible variables were used in the initial univariate analyses. Collinearity was tested using the variance inflation factor (VIF) where any variable with a VIF>10.0 was excluded from the regression model. Total, free and bioavailable testosterone, and SHBG were highly collinear and from these 4 variables only SHBG and free testosterone could be included in the same regression models. Within the multivariate regression models menopausal stage was defined as pre- or post FMP as this produced more significant effects than using the four STRAW+10 groups. Collinearity between the anthropometric variables was minimal as was that between menopausal stages and FSH and E2 levels (VIFs<3.0).

Mean values were compared across the four STRAW+10 menopause groups or between pre- (STRAW+10 groups -3a, -3b, -2 and -1) and post FMP (groups 1a, 1b, 1c and 2) women using ANOVA or Students t test respectively, whilst categorical data was analyzed using the χ^2 test. If significant trends were detected in the ANOVA, an ANCOVA was performed using as a co-variable any independent variable that correlated significantly with the respective dependent variable in the multiple regression models described previously. If more than one co-variable was used, each one was added to the ANCOVA on its own.

5.3 Results

5.3.1 Description of the study cohort

The mean age of the participants (±SD) was 49.2 ± 5.29 years. Reproductive aging could not be staged in 15.9% (n=112) of the participants due to hysterectomy (n=47), contraceptive use (n=64), and reason not known (n =1) (Figure 3.1). There was a high prevalence of obesity at 67.8% and the mean BMI was 33.4 ± 7.32. Mean waist circumference was 99.1 ± 14.6 cm. Amongst the 404 women who knew or agreed to have their HIV status measured, 21.3% (n= 86) were HIV positive and of those, 55.3% (n=47) were receiving antiretroviral therapy (ART). Within the study cohort, 8.0% of the participants described themselves as previous or current smokers and 20.9% of the women used snuff. With regards to menopausal symptoms, 60.1% of the women had vasomotor symptoms (VMS), while 61.7% were experiencing sleep problems. The data on educational status showed that 12.3% of participants had only completed junior school while 30% had finished high school, and 57% had attended high school but did not graduate.

Table 5.1 Prevalence of diabetes, metabolic syndrome* and related disorders

Disorder	N	Prevalence (%)
Diabetes (fasting glucose ≥7mmol/L)	643	8.86
Impaired fasting glucose (≥5.6mmol/L)	643	7.62
High waist (>80cm)	698	89.7
Hypertension (SBP ≥130 and/or DBP ≥85mmHg)	686	65.0
Hypertriglyceridemia (≥1.7mmol/L)	651	15.4
Low HDL (<1.3mmol/L)	651	59.7
Elevated LDL (≥3.0mmol/L)	649	36.4
Hypercholesterolemia (≥5.0mmol/L)	649	29.9
Metabolic syndrome	645	49.6

* Metabolic syndrome is defined in the SWEET study using the harmonized guidelines (fully described in 1.7.1)

5.3.2 Prevalence of metabolic disorders

Table 5.1 summarizes the prevalence of diabetes, MetS and related disorders in the cohort. The prevalence of impaired fasting glucose and diabetes was 7.6% and 8.9% respectively (16.5% combined), and MetS was high at 49.6%. The most common components of the MetS were high waist circumference (89.7%), hypertension (65.0%) and low HDL levels (59.7%). Hypercholesterolemia was highly prevalent (29.9%), as was the presence of high LDL levels (36.4%), whilst hypertriglyceridemia (15.4%) was less common.

5.3.3 Trends across menopausal stages

Table 5.2 describes the level of metabolic variables and the prevalence of MetS at each stage of the MT, with the stages of menopause defined using the STRAW+10 guidelines (56). There was some evidence for glucose levels to be higher in the post- than the premenopausal group (F=2.65, p=0.05), but this was not observed for HbA1c and HOMA levels. In an ANCOVA, adjustment for age caused the trend for increasing glucose levels to become non-significant (F=0.89, p=0.44). Both levels of total cholesterol and LDL became significantly higher (F=8.05 and 7.80, respectively; p<0.0005 for both trends) in the postmenopausal groups, with a weaker trend for higher triglyceride levels (F=3.72, p=0.01). Within an ANCOVA, adjustment for FSH severely attenuated the trend for higher cholesterol levels (F=1.51, p=0.21). This same adjustment in an ANCOVA for LDL also attenuated the trend (F=1.58, p=0.19), as did adjustment for age (F=0.43, p=0.73). The trend for higher triglyceride levels was not significantly attenuated by any co-variable adjustment. Leptin did not vary across the stages of menopause whilst there was a slight trend for serum adiponectin levels to become higher (p=0.08). Systolic blood pressure was significantly higher in the postmenopausal groups (F=6.43, p=<0.0005) with a weaker trend for higher diastolic blood pressure (F=3.83, p=0.01). The ANCOVA for systolic blood pressure demonstrated that inclusion of age as a co-variable dramatically reduced the significant trend observed in the ANOVA (F=0.60, p=0.62). A similar analysis for diastolic blood pressure showed that the inclusion of E2 as a co-variable led to a weakening of the trend (F=1.30, p=0.27). There was a tendency for a greater prevalence of MetS across the MT stages (p=0.05).

Table 5.2 Metabolic measurements and prevalence of metabolic syndrome across menopausal stages

Metabolic variables	Menopausal stages (from STRAW+10)				F-value (p-level)[a]	F-value (p-level)[b]
	-3b & -3a n = 194	-2 & -1 n = 122	1a, 1b & 1c n = 144	+2 n = 130		
Glucose (mmol/L)	4.72 ± 0.51	4.84 ± 0.58	4.79 ± 0.49	4.89 ± 0.55	2.65 (0.05)	0.89 (0.44)[c]
HbA1c (%)	5.77 ± 0.64	5.93 ± 0.54	5.82 ± 0.66	5.89 ± 0.67	1.42 (0.23)	-
HOMA	2.08 [1.88]	2.27 [1.78]	1.84 [1.70]	1.99 [1.48]	0.24 (0.87)	-
Cholesterol (mmol/L)	4.26 ± 0.92	4.42 ± 1.02	4.67 ± 1.01	4.80 ± 1.23	8.05 (<0.0005)	1.51 (0.21)[d]
LDL (mmol/L)	2.53 ± 0.83	2.67 ± 0.90	2.81 ± 0.85	3.01 ± 0.99	7.80 (<0.0005)	1.58 (0.19)[e] 0.43 (0.73)[f]
HDL (mmol/L)	1.20 [0.40]	1.20 [0.50]	1.20 [0.45]	1.20 [0.40]	0.37 (0.78)	-
Triglyceride (mmol/L)	1.10 [0.60]	1.00 [0.60]	1.15 [0.65]	1.20 [0.60]	3.72 (0.01)	-
Leptin (ng/ml)	29.3 [33.9]	28.9 [26.7]	27.6 [26.4]	24.2 [23.8]	1.37 (0.25)	-
Adiponectin (µg/ml)	6.67 [6.06]	6.64 [4.71]	7.73 [6.51]	7.32 [5.49]	2.22 (0.08)	-
Systolic bp (mmHg)	129 ± 19.4	135 ± 20.6	137 ± 21.6	139 ± 24.4	6.43 (<0.0005)	0.60 (0.62)[g]
Diastolic bp (mmHg)	85.8 ± 12.8	89.0 ± 11.9	89.8 ± 12.1	89.5 ± 12.9	3.83 (0.01)	1.30 (0.27)[h]
Metabolic syndrome (%)	42.3	48.6	52.4	53.6	- (0.05)[b]	-

Data expressed as median [interquartile range], mean ± SD or percentage; [a]p-value from ANOVA except for [b]p-value from χ^2, stage -3b, -3a versus stage 2; [b]p-values from ANCOVA with adjustments for [c,f,g]age, [d,e]FSH and [h]E2

5.3.4 Multivariable linear regression models

Table 5.3 shows the multiple linear regression data for the metabolic variables. Model 1 demonstrates that age and waist circumference correlate positively with levels of glucose, whilst levels of adiponectin and SHBG correlate negatively. Model 2 demonstrates adiponectin has a negative association with HbA1c levels, whilst age and visceral fat both correlate positively. Model 3 demonstrates that there is a strong positive correlation of both lean mass and leptin with HOMA. There was a strong negative association of adiponectin and hip circumference with HOMA. Waist and employment (negatively) were weakly associated with HOMA. Model 4 shows that women who are HIV positive, but ART naïve (n=39) tend to have lower cholesterol levels, whilst FSH levels correlate positively with

cholesterol. In model 5, a strong positive relationship of FSH, and a strong negative relationship of adiponectin with LDL were observed, whilst age was weakly related. Regression model 6 demonstrates that levels of HDL are strongly associated with both adiponectin and lean mass (negatively). There was a weak negative relationship of HDL with both contraceptive use and the presence of HIV infection. Model 7 shows that triglyceride levels correlated positively and strongly with visceral fat, smoking and being post FMP, whilst adiponectin, SHBG and DHEAS all associated strongly and negatively with triglycerides. Triglyceride correlated negatively with BMI. This was not due to collinearity as all independent variables in model 7 had VIFs < 2.00. Removal of visceral fat from model 7 attenuated the relationship between BMI and triglyceride levels (BMI β=-0.001, p=0.30) whilst the removal of any other independent variable had a minimal effect. As seen in model 8 both age and waist circumference were strongly associated with systolic blood pressure whilst there was a weaker relationship with VMS, smoking and employment (negative). Model 9 shows that diastolic blood pressure has a strong positive association with waist and smoking, and a strong negative relationship with E2 levels. Education was weakly related to diastolic blood pressure.

Table 5.3 Multivariate regression models for metabolic variables

Model number	Dependent variable	Independent variable with unstandardized β (p-value)	Adjusted R^2 (p–value) for full model
1	Glucose (mmol/L)	Age 0.01 (0.009) Waist 0.005 (0.003) Adiponectin (log) -0.31 (0.0006) SHBG (log) -0.31 (0.005)	0.10 (<0.0005)
2	HbA1c (%)	Age 0.02 (<0.0005) Visceral fat 0.002 (0.005) Adiponectin (log) -0.26 (0.02)	0.06 (<0.0005)
3	HOMA (log)	Lean mass 0.008 (0.001) Waist 0.003 (0.02) Hip -0.007 (<0.0005) Leptin (log) 0.34 (<0.0005) Adiponectin (log) -0.25 (<0.0005) Employment -0.06 (0.02)	0.28 (<0.0005)
4	Cholesterol (mmol/L)	FSH (log) 0.52 (<0.0005) HIV positive -0.47 (0.009)	0.08 (<0.0005)
5	LDL (mmol/L)	Age 0.02 (0.01) Adiponectin (log) -0.47 (<0.0005) FSH (log) 0.29 (<0.0005)	0.08 (<0.0005)
6	HDL (log; mmol/L)	Lean mass -0.004 (<0.0005) Adiponectin (log) 0.15 (<0.0005) HIV positive -0.05 (0.01) Contraception use -0.04 (0.04)	0.21 (<0.0005)
7	Triglycerides (log; mmol/L)	BMI -0.004 (0.002) Visceral fat 0.001 (<0.0005) Adiponectin (log) -0.17 (<0.0005) SHBG (log) -0.15 (<0.0005) DHEAS (log) -0.05 (<0.0005) Smoking 0.07 (0.008) Postmenopausal 0.04 (0.003)	0.19 (<0.0005)
8	Systolic bp (mmHg)	Age 0.87 (<0.0005) Waist 0.25 (<0.0005) Smoking 6.14 (0.03) Employment -3.28 (0.04) VM symptoms 3.96 (0.01)	0.09 (<0.0005)
9	Diastolic bp (mmHg)	Waist 0.24 (<0.0005) Estradiol (log) -2.58 (0.002) Smoking 4.83 (0.006) Education 1.99 (0.04)	0.09 (<0.0005)

Variable coding: employed were compared to unemployed subjects; current and former smokers were combined and compared to those who never smoked; HIV-positive subjects who used ART or were ART-naïve were each compared with those who were HIV-negative; subjects who attended but did not graduate and subjects who graduated high school were each compared with those who did not attend; postmenopausal were compared to premenopausal subjects; women who used contraceptives were compared to non-users; women who experienced vasomotor (VM) symptoms were compared to those who did not

5.3.5 Multivariate regression models for metabolic variables

Table 5.4 shows the results of a multiple logistic regression analysis to isolate the major correlates of MetS. The model demonstrates that age, BMI, visceral fat, insulin resistance and smoking are positively associated with MetS, whilst raised adiponectin levels are negatively associated. A univariate logistic regression model demonstrated that being post FMP was positively associated with MetS (odds ratio with 95% CIs: 1.40 [1.00, 1.96]; p=0.05), and this effect was attenuated only by age (0.79 [0.50, 1.25]; p=0.32).

Table 5.4 Logistic regression analysis for identification of risk factors for metabolic syndrome

Categorical variable	Independent variables with odds ratio (95% CI's); p-value
Presence of metabolic syndrome	Age 1.07 (2,15, 1.32); <0.0005 BMI 1.05 (1.02, 1.09); 0.003 Visceral fat 1.007 (1.001, 1.01); 0.03 Adiponectin 0.95 (0.91, 0.99); 0.008 HOMA 1.32 (1.17, 1.48); <0.0005 Smoking 2.15 (1.11, 4.18); 0.02

5.4 Discussion

Although there is a plethora of research examining the prevalence of MetS, its individual components and related metabolic disorders across the MT in Western literature (386, 387), there is very little research on this subject in sub-Saharan African (SSA) women, although the relationship between metabolic variables and the MT has been studied in Ghanaian women (274). The current study is the largest and most comprehensive study of metabolic, hormonal and anthropometric parameters ever undertaken in an indigenous sub-Saharan African (SSA) population of menopausal females using accurate estimation of menopausal staging.

5.4.1 Prevalence of diabetes, metabolic syndrome components, metabolic syndrome and related factors.

There was a high prevalence of diabetes in this population, however this may be an underestimate as diabetes was diagnosed using fasting glucose levels only, rather than by the use of an oral glucose tolerance test. Given the high prevalence of obesity in the study population the high frequency of both diabetes and MetS is not unexpected. Hypertension was highly prevalent amongst these participants (65%), which has been described previously in South African women (6, 7), and appears to be increasing amongst sub-Saharan African women (388).

Nearly 90% of women in this study had a waist circumference ≥80.0 cm, whilst the prevalence of low HDL levels was also very high at 59.7%. Similar results have been observed in other sub-Saharan African studies with raised waist circumference and low HDL levels being the commonest components of the MetS (41,42).

The low prevalence of hypertriglyceridemia in this study cohort has also been observed in other investigations of the metabolic syndrome in African populations (389, 390). Elevated LDL levels and hypercholesterolemia were quite prevalent amongst the women in the study. This is an interesting observation because previous studies have noted low serum LDL and total cholesterol levels in African women (7, 391) . This difference may be because these studies were conducted in younger, premenopausal females and the current study clearly demonstrates that rising FSH levels correlate strongly and positively with both LDL and total cholesterol serum concentrations.

5.4.2 Differences in cardiometabolic variables across the MT stages

Glucose, triglyceride, total cholesterol, LDL, systolic and diastolic blood pressure were significantly higher in post- than premenopausal women as shown by ANOVA, and these differences were related to age (glucose, LDL & systolic blood pressure), FSH (cholesterol and LDL), and estradiol (diastolic blood pressure). This suggests that differences in cholesterol and blood pressure levels across the menopause may be related to hormone levels. Previous research has shown an association between FSH and lipid levels in premenopausal females (182), and between estradiol and diastolic blood pressure (392). The finding that differences in glucose levels are related to age in the current study has been confirmed in a retrospective analysis (393), while other research shows that increased levels of LDL and systolic blood pressure are also related to age (394). A large longitudinal study found that glucose and blood pressure were not related to reproductive aging, but total cholesterol and LDL levels were, independent of age (395). However, that study did not measure FSH or estradiol levels and therefore could not adjust for their effects. The higher triglyceride levels in the postmenopausal females in SWEET were not explained by the other variables measured, including age. Similarly, a large longitudinal study has shown that triglycerides rose across the MT independent of the effect of age (396).

The MT does not appear to be a risk factor for insulin resistance, as observed in this and previous studies (393, 397), though others have shown that it is positively associated (378). Leptin levels did not change between pre- and postmenopausal women in this present cohort, whilst serum adiponectin levels tended to be slightly higher in the postmenopausal groups, which has been seen in other studies (238).

5.4.3 Relationship between anthropometric and cardiometabolic variables

In this study waist circumference was positively associated with glucose, HbA1c, HOMA, and systolic and diastolic blood pressure, visceral fat was positively associated with HBA1c

and triglyceride levels and hip circumference was negatively associated with HOMA; BMI correlated negatively with triglyceride level. These data suggest that in this population body fat distribution rather than total body fat mass is the principle anthropometric correlate of cardiometabolic disease risk factors, confirming data from many other studies in pre- and postmenopausal females (239). The negative association of hip circumference with HOMA suggests that gluteofemoral fat may protect against insulin resistance, a finding supported by other studies (398). An unusual finding in the participants was the negative relationship between BMI and triglycerides, and the dependence of this relationship on visceral fat. The positive relationship between body fat and triglycerides is well described in the literature and the reverse phenomenon observed in this investigation may therefore be specific to this population and/or may be a result of the unique structure of the regression model used in the present study. Further analyses are required to explain these findings.

Lean mass correlated positively with HOMA and negatively with HDL levels in multivariate regression models. Previous studies have shown that lean mass is positively associated with metabolic syndrome risk, independently of fat mass in a Chinese population (399) and that HDL levels correlate negatively with body lean mass (400).These data suggest that the assessment of body composition is important for isolating the true anthropometric markers of disease risk in this population. These findings highlight the fact that fat mass is not the only relevant anthropometric measure when studying the etiology of cardiometabolic diseases, and that the use of variables such as BMI, that do not discriminate between lean and fat mass, must be used with caution.

5.4.4 Relationship between adipokines and cardiometabolic variables

Leptin levels correlated positively with HOMA in the SWEET participants, confirming previous studies demonstrating the correlation of leptin with insulin levels (401), and the worsening of insulin resistance (206). In the current study adiponectin levels were

associated with several metabolic variables and may therefore play an important role in the etiology of MetS in this population. As shown in other research studies, adiponectin was negatively associated with glucose, HbA1c, HOMA, LDL and triglycerides and positively associated with HDL levels (402).

5.4.5 Relationship between hormones and cardiometabolic variables

The current study shows that SHBG was negatively associated with glucose and triglyceride levels. A previous study has shown that in both pre- and postmenopausal obese females serum SHBG correlated negatively with glucose levels (403). It has also been shown that SHBG correlates negatively with triglyceride levels in postmenopausal females (201). Other research has shown that lower levels of SHBG may increase the risk of MetS in menopausal women (193).

Cholesterol and LDL but not HDL or triglyceride levels both correlated positively with FSH levels in this population of midlife African females. An American study of premenopausal females has shown that cholesterol and LDL levels were higher in those with the highest FSH levels, but this was not observed for HDL and triglyceride levels (54). These data in combination with the ANOVA results described previously strongly suggest that rising FSH levels during the menopause may drive the increase in cholesterol levels observed during the MT. The molecular mechanisms involved in the association between FSH and cholesterol levels are not known.

A negative association was found between triglyceride and DHEAS levels within the current study. A similar relationship was observed in a cross sectional study of 217 obese Italian women, but this association was not maintained in a multivariable regression analysis (404), whilst in another Italian study, DHEAS levels correlated negatively with triglyceride levels in women over 90-years-of-age (405).

In the present study, estradiol levels were found to correlate negatively with diastolic blood pressure. A number of studies have shown similar relationships, and estradiol is known to have a vasodialatory effect (61). These results suggest that increasing blood pressure across the MT is related to falling estradiol levels, and this is confirmed by the ANCOVA results described earlier.

5.4.6 Relationship of HIV and vasomotor symptoms with cardiometabolic variables

Cholesterol levels were negatively associated with HIV infection in the participants, as were HDL levels. Infection with HIV is often associated with reduced HDL, LDL and total cholesterol levels (261). In this investigation, higher systolic blood pressure was associated with VMS. Increased cardiovascular disease risk has been linked to VMS in previous studies (233). However, the mechanisms involved in this association are not fully understood.

5.4.7 Relationship of socioeconomic status, smoking and contraceptive use with cardiometabolic variables

Both HOMA and systolic blood pressure were negatively related to employment status in the participants, and in a very large cross-sectional study of Korean women, it was shown that employment status was associated with a lower risk of metabolic syndrome (406). Diastolic blood pressure was positively but weakly associated with education level in the SWEET participants, as has been shown in women from a sub-sample of a large longitudinal South African study (407). These current data also showed that smoking was positively associated with triglycerides, as described in a population-based study (248), and with hypertension, as shown in a very large prospective cohort study (408). In the present study, there was a weak negative association between HDL levels and contraceptive use, and data have shown that, depending on the formulation, oral contraceptive use may reduce HDL levels (409).

5.4.8 Risk factors for metabolic syndrome

Similar risk factors for MetS, as described in other studies, were found in the SWEET participants These were age (139), BMI, visceral fat, HOMA ((410) and smoking (249). Lower levels of adiponectin have been shown to increase risk of MetS (411), as also observed in the current study. As described in other studies, women in the postmenopausal group appeared to be at greater risk for metabolic disease (139), but this effect was attenuated with the addition of age to the regression model. While some researchers have not found menopause to be a risk factor for MetS (144), others, which include a study describing an increased prevalence of MetS in a group of sub-Saharan African women, suggested it plays a significant role (380, 395). However, MT was not accurately staged in those African women. Although MetS risk in the current study may not be higher in women post FMP than those who are pre FMP, individual components of the syndrome are elevated in the former group. Thus, triglyceride levels were higher in women post FMP, whilst diastolic blood pressure was also higher due to lower estradiol levels. Furthermore, the MetS-related factors cholesterol and LDL were higher in postmenopausal women due to high FSH levels.

5.4.9 Study limitations

A limitation of this study was its cross-sectional design, although the sample number was large and menopause stage was accurately verified (354). (354). Another limitation was the inability to collect serum FSH and E2 samples during the primary follicular stage of the menstrual cycle (days 2- 5) in premenopausal women, but timing of E2 and FSH assessment may not be crucial since in the menopause transition stage, ovarian function becomes increasingly dysfunctional making it difficult to accurately determine follicular stage (50). Despite this, women in the early stages of the MT had much higher levels of E2 and much lower levels of FSH than found in women in the early and late postmenopausal stages (6). An additional limitation was that information on HIV status was only available in 58% of the participants. Another limitation of the study was that the HOMA method to calculate

insulin resistance was used rather than the glucose clamp method, which is the gold standard (412). However this procedure is both invasive and time consuming (413), making it impractical for large population sizes, such as that of this cohort. Furthermore, the HOMA method has been shown to be an accurate procedure for assessing the level of insulin resistance (414) Computed tomography (CT) scans and magnetic resonance imaging (MRI) are gold standard measurements in assessing visceral fat, however they are expensive and often unavailable, while results from ultrasound have been shown to be highly reproducible (374). A positive aspect of the current study was the wide variety of relevant serum hormones that were assessed, and that cardiometabolic, anthropometric, and demographic variables were measured in a population group of late reproductive and menopausal women, for whom no such data was formerly available.

5.5 Conclusions

Certain MetS components and related factors were present at higher levels in women post menopause and were related to changes in FSH (LDL and cholesterol) and estradiol (diastolic blood pressure). These data suggest that the hormonal changes that characterize menopause may play a role in the etiology of specific MetS components in this population. Furthermore, lower levels of adiponectin were associated with MetS and its individual components. Although the association between reproductive and chronological aging and MetS is complex, age attenuated the already weak association of the MT with MetS in the current study. Thus, within this population the MetS is associated with chronological aging, whereas individual components of the syndrome are associated with reproductive aging. Lifestyle interventions to reduce obesity, smoking prevention programs and more regular monitoring of metabolic risk factors in this group may be useful in lowering cardiometabolic risk factors and hence morbidity. Furthermore, a previous study has shown a poor understanding of the health consequences of the menopause in this population (6) and therefore education programs covering this issue would be appropriate.

Chapter 6: Discussion

6 Discussion

The final chapter of this thesis presents a consolidation of the findings discussed in the empirical research found in chapters 3, 4 and 5. Four key objectives were identified (section 1.11.1) and each will be analyzed and discussed. The relevance of this body of research will be examined and a revised conceptual framework will be presented. The relevance of the research from a South African perspective will be described, and the generalizability of the findings, in relation to other sub–Saharan African countries, will be discussed. The limitations and opportunities for future research in this field will be examined, followed, finally, by the conclusion.

6.1 Consolidated findings

The SWEET study aimed to examine the metabolic, anthropometric and hormonal effects of the MT in urban black women. These objectives were described in four research questions, and the results obtained from studies set up to meet these objectives were described in scientific papers in chapters 3, 4 and 5. A summary of these findings is shown in Table 6.1.

Table 6.1 Summary of consolidated findings

Number	Objectives	Main Study Findings	Chapter
1	To accurately stage reproductive aging using bleeding patterns and assess usefulness of STRAW + 10 criteria in staging MT in black urban South African women, and measure FSH and E2 levels as supportive criteria to determine MT stage	• Menopause can be accurately staged in black urban African women using self-reported menstrual cycle changes • Recollected age at FMP difficult to assess accurately • Serum FSH levels significantly higher, and serum E2 levels lower in post- compared to premenopausal women	3
2	To determine whether obesity or HIV infection has an effect on the FMP and menopausal symptoms, particularly VMS	• Obesity highly prevalent but not related to the MT • BMI lower in the post- than premenopausal women • Age at FMP appears to be related to a lower BMI (<25 kg/m^2) • Prevalence of severe/very severe VMS, sleep problems, irritability and joint pain significantly higher in obese women (BMI>35.0 kg/m^2) • HIV-positive women did not have worse VMS than HIV- negative women	3
3	To determine whether general body adiposity, BFD, lean muscle mass and BMD are associated with stages of the MT and if so what factors may play a role in these associations	• Whole body BMD and BMC significantly lower in the postmenopausal groups • BMI lower in post- than premenopausal women in SWEET women • Lean mass lower at all body sites in the menopausal transition and post FMP stages • Levels of SHBG and DHEAS significantly lower in postmenopausal women • SHBG and DHEAS serum levels both negatively associated with total fat mass. SHBG correlates negatively with total lean mass while DHEA correlates positively with it • Differences in BMD and lean mass across the MT groups strongly related to serum levels of E2 and FSH, respectively • BMC correlated positively with serum E2 levels	4
4	To determine the prevalence of the MetS, the level of its individual components and associated metabolic factors in African females at different stages of the MT and what factors play a role in modulating the levels of these variables	• High prevalence of MetS, IFG and diabetes • High waist circumference and dyslipidemia common components of MetS • FSH levels during MT associated with increased cholesterol levels • E2 levels negatively related to diastolic blood pressure	5

6.2 Emerging research themes from the SWEET findings

This section will examine the study findings in relation to the objectives delineated in Chapter 1 and described in Table 6.1, and the specific research themes that have emerged from the study. These emerging themes include: valid and reliable measurements to accurately stage the MT in black urban South African women; body composition changes and their relationship to the MT in this population, the prevalence of metabolic syndrome, its association with chronological and /or reproductive aging, and the increased risk of CVD in midlife sub-Saharan African women.

6.2.1 Crucial aspects in understanding and staging the menopause transition in black, urban South African women

This section explores selected findings related to staging reproductive aging accurately in black urban South African women, and some of the difficulties encountered when staging ovarian aging, determining accurate recall of age at menopause. In addition, the cultural perceptions that may impact the understanding and description of menopausal symptoms in this population will be examined.

6.2.1.1 STRAW+10

Accurate staging of reproductive aging is vital in understanding the impact of the MT stage and its relationship to a wide variety of variables, which may in turn be related to an increased risk of morbidity and mortality in midlife women. In a low-resource setting, where the public healthcare system is overburdened, it is costly and impractical to use biomarkers to assess MT. Bleeding criteria appear a more appropriate method to stage ovarian aging. This study has shown that STRAW+10 criteria are a cost-effective and easy way to stage the MT for application in large epidemiological studies examining reproductive and chronological aging in black African women. Although less than half the women in the

cohort understood what menopause meant, most were able to describe changes in their bleeding patterns accurately enough to be allocated to a specific MT stage group.

If, however, this method of staging ovarian aging becomes commonly used, several issues of concern emerged during the research process, which should be addressed. Clinical knowledge in interpreting the responses of the SWEET participants cannot be underestimated and it is important that questionnaires in future studies incorporating the STRAW +10 criteria should be both simple to use by team members without a clinical background, and easily understood by the participants. The terminology recommended by STRAW +10 is often medical, making it difficult for a layperson to understand. Initially, when piloting the questionnaires for SWEET, it was found that the terms used were complex and difficult to understand by the demographic group representative of the women who would be recruited into SWEET. English was the second language for these women, and in this pilot group, even when the respondents had a tertiary education or a nursing background, the criteria were difficult to interpret. As described earlier, each participant in SWEET was questioned about her menstrual cycle, during one-on-one interviews, by a single interviewer, who had a clinical background of dealing with women in the MT. The close-ended questions of the questionnaire that asked the participant to describe her bleeding patterns were followed by open-ended questions that enabled the interviewer to define these bleeding patterns more accurately. However, in future research, where researchers may not have the requisite clinical background, the terminology needs to be simplified and all questions should be contextualized. An explanation of what is being asked and the reason for this should be given. Clarification could be achieved first by explaining the norms of a regular cycle, and then by asking the participants how their cycles have changed. Contraceptive use may be a confounder in response to questions on menstrual cycle regularity, and this should be specifically addressed in questions on bleeding patterns.

In addition, since the purpose of the STRAW +10 criteria is to stage reproductive aging using bleeding patterns, there should be a section in the staging questionnaire that addresses the possibility of a surgical or chemically induced menopause, and a clear description of what researchers mean by 'natural menopause'. Reasons for interrupted or changing menstrual cycles should be included in questions about changes in bleeding patterns. There are many explanations as to why menstrual cycles change; for example stress, medications or breast-feeding. Different causes should all be included. Subsequent to the presentation of the STRAW +10 criteria at the NAMS meeting in 2013, several discussions on how to improve the validity of the STRAW + 10 document have been held between researchers at different international universities, who are conducting studies which rely on accurate staging of the MT using bleeding criteria.

A disturbing revelation of the data collection process was the imperfect understanding that many of the women had of reproductive aging and fertility, and a belief that after the age of 40 they were no longer fertile, especially when their menstrual cycles began to alter. In fact, several of the women were older primigravida, and had unexpectedly become pregnant in their mid to late forties because they thought their age would be a contraceptive. In regards to this, it is suggested that satellite clinics could be provided with simple information sheets explaining what happens to fertility during late reproductive aging.

6.2.1.2 Age at FMP

An interesting finding of this study was the difficulty these women had in recalling their age at FMP, and the apparent difference in age at FMP between sub-Saharan African women and their European and Western counterparts. The latter groups experience their FMP at least 2 -3 years later than the African women (64, 288). Early menopause may be related to socioeconomic factors such as poverty, education, inadequate healthcare and ethnic differences. Recall of age at FMP is complex and questionnaires using improved bleeding

criteria terminology, as suggested above, should yield more relevant data explaining why some women experience it earlier. The Department of Health, as discussed in Chapter 2, has identified a need for midlife women to be given more information about menopause, but there have been no clear guidelines on how this can be achieved. Primary health care providers in South Africa should be trained in this area of reproductive health so that they are able to elicit the appropriate information from their patients, which may improve accuracy of recall of age at FMP. Since an early menopause may be related to greater morbidity and mortality as explained in Chapters 1 and 3, more emphasis on extensive questioning about the age of the mothers or siblings of participants, trauma, sexual abuse, childhood and adult poverty may help to better understand why some women have an earlier age at FMP. If, bleeding criteria become more commonly used to help black African women become aware of their reproductive status, in spite of the fact that annual medical or gynecological check-ups are not the norm as in many high-resource countries, this knowledge may help to improve their recall of age at FMP, which has been shown to be important in predicting future morbidity and mortality. As discussed above, primary health care nurses, equipped with adequate training and information about the MT, could, during a routine clinic visit, use a brief questionnaire, relating to a patient's menstrual cycle, to gather information about their reproductive status. This information could be included in the patients' medical records for future reference.

6.2.1.3 Menopause symptoms.

In examining the symptoms that are most prevalent in cross-cultural comparisons, VMS was the defining symptom of the MT in the SWEET participants, as found in Western women. However, it is not clear why more late reproductive stage black African women described themselves as having hot flushes, as compared to other late reproductive women in different population groups (99, 100), where the MRS was used to determine prevalence and severity of VMS. Hot flushes generally become more prevalent in the late menopausal transition

stage (56). It was thought that many of the women in this study, especially those who were more obese, might not have distinguished a hot flush from general body temperature changes and feelings of heat. They appeared to understand what VMS were, especially night sweats, and their description of VMS correlated significantly with FSH levels. Some researchers have suggested that hot flushes and night sweats should not be combined into one term under the heading of VMS (349). However, the women in SWEET appeared to be very clear about the difference between these two when questioned, so the reason for the finding remains unclear. It is possible that cultural perceptions and expectations may play a role in the responses of these women.

The problem of comparing symptomology across different ethnicities and cultures was highlighted in the fact that vaginal atrophy, which is a common complaint in menopause in Western women, and is usually very prevalent in late menopause (56), did not appear to be of significance to the women in SWEET. This may be related to their partners' sexual preferences and cultural perceptions, where older women may be seen as less desirable than those who are younger. Many women reported that they had 'finished' with sex and had not been sexually active for many years and did not know if they had vaginal atrophy or the urinary complications that often occur if this is present. No connection was made between any vaginal atrophy or urinary issues they were experiencing and the MT. Unlike their Western counterparts, where a wide body of research reports distress at sexual inactivity and loss of libido, which is often related to vaginal atrophy (415, 416), this did not appear to concern many of the SWEET participants. Although sexually active women reported sexual problems in early postmenopause, they did not generally relate these to dyspareunia.

The fact that the participants in SWEET appeared to experience VMS earlier than was found in other women where data on menopausal symptoms were collected using the MRS, may also be related to both idiomatic and translation issues as well as cultural perspectives.

Apart from VMS, only two menopausal symptoms were significantly associated with MT stage in SWEET participants. These were sexual problems and irritability

In Western research, depression and mood swings during the menopause transition have been well described, and an increase in depression and mood changes in the perimenopause has been found (346). However, apart from irritability, none of the symptoms described in the psychological domain of the MRS (depression, irritability, anxiety, and mental exhaustion), were associated with menopause stage in the study participants. An unexpected outcome of the research was that irritability appeared to significantly increase in the late postmenopausal stage. This differs from other research where irritability is more generally shown in premenopausal women and the symptom of depression is more commonly found in late menopausal women (347). The fact that the symptom of irritability rather than depression was associated with MT stage in this population, and this symptom seemed to increase in the late menopausal stage, where it is not usually described, may have been due to a misinterpretation of the word, 'irritability' and/or a different cultural perception of the meaning of 'depression'. Several research studies describe the differences in the way in which different women from different cultures experience and identify menopausal symptoms (122), particularly those in the psychological domain (226), and recommend that cultural differences should be taken into account when identifying symptoms (128).

Mental health does not appear to have been studied in the context of reproductive aging in these black African women, although in Western studies, there is a greater risk of depression at onset of menopause, even in women with no previous history of depression (417), and there a strong association between mood and the effect of falling estrogen levels on key neurotransmitters has been shown (346). If greater attention is paid to the physiology of the MT and its biomarkers in this population, it may follow that attendant psychological aspects may emerge, and may be found to play a far greater role than has been shown in

this study. However, it is essential to understand whether menopause and its accompanying symptoms have the same meaning and importance for black African women, as they appear to have for Western women.

6.2.2 Body composition changes and the MT

The overarching theme arising from this study was the very high prevalence of obesity in these midlife black urban women, which has been fully discussed in Chapters 3-5. It has been well described how obesity affects many aspects of reproductive aging and increases the risk of metabolic disease in these aging black African women. It is an issue of paramount importance in the healthcare sector (242, 245) and issues contributing to obesity in these women should be addressed.

6.2.2.1 Obesity and lifestyle

Addressing and dealing with the problem of obesity in these women is difficult. The nutrition transition has meant that there has been an increase in the consumption of both high carbohydrate and high fat diets (418), and these diets are very common in urban black South African populations (245). Fast foods, extremely high in fat and carbohydrates are widely available in Soweto, which means they are easy to obtain and relatively inexpensive (419). The perception of obesity amongst South African women is also problematic, since they are less likely than their white counterparts to view themselves as obese (420). In addition the stigma attached to HIV infection and its accompanying weight loss means that thinness is associated with ill-health, and a knock-on effect of this perception is that being obese is viewed as a sign of health and wealth in African communities (244).

Women in SWEET who had a BMI ≥ 30kg/m^2 were referred to the Dietetic Clinic, in the Department of Nutrition, at the Chris Hani Baragwanath Hospital, for nutritional counselling and information to help them lose weight. However, this recommendation although well

intentioned, may not have been effective for several reasons, including lack of easy access to the clinic, the long waiting period due to over-crowding and lack of efficient monitoring and evaluation of the dietetic regimen. In addition, there has been no follow-up in the SWEET participants to see whether this intervention was helpful. It may be more effective to train both primary healthcare nurses and community organizations to explain the principals of healthy eating and benefits of regular exercise to women who are premenopausal rather than wait until they are postmenopausal, as these interventions may help to lower the future risk of metabolic disease. Studies have shown that weight loss during the MT and post menopause may also result in unhealthy loss of lean mass (171). The high energy, low nutrient diets that are economically viable in this population (421), may lead to further loss of lean mass. In addition these obese women may not exercise, as they should. Socioeconomic factors such as the cost of high protein, nutritionally balanced diets, lack of places to exercise and concerns about safety in many areas in Soweto may compromise recommended weight loss and exercise regimens. The importance of adapting dietary recommendations to specific socioeconomic and diverse ethnic food preferences should also be considered. However, weight loss regimens used with appropriate lifestyle interventions should be seriously considered as they may improve health in these aging women (422).

6.2.2.2 Lean mass

The loss of lean mass across the MT described in SWEET participants has significant health implications in black African women. Loss of skeletal muscle has been shown to be related to loss of strength in aging women, which in turn are related to infirmity and weakness (423). In addition, the loss of BMD, which was also described in these women may increase bone fragility (269). A further problem that may arise is that the loss of muscle strength and functioning which may result from loss of skeletal muscle mass may increase risk of falling. Data have shown that physical activity and resistance exercise is recommended to retain lean mass. Although data from this cohort have shown that these women are physically

active (424), the intensity of the exercise was not measured, and there appear to be few studies identifying the levels and type of exercise, including resistance training, undertaken by these women (245). Thus, weight loss regimens in these women must be carefully controlled to prevent increased loss of skeletal muscle mass, and the type and intensity of exercise should also be monitored.

6.2.3 Metabolic Syndrome and morbidities

The high prevalence of MetS and related metabolic variables described in this cohort are of concern. Little attention has been paid to health risks in midlife black African women, consequently they and their primary healthcare providers, have scant understanding of the association between obesity and risk for metabolic disease, which are commonly explained to aging women in healthcare systems in high resource countries. The effect of obesity in these women has been discussed and the importance of weight loss regimens and increased exercise in lowering the risk for this has been explored. However, these alone may not be enough in preventing MetS and related NCDs. Annual check-ups, which include anthropometric measures and relevant biomarkers may help to increase awareness of health risks and prevent the high rate of morbidity in this population of midlife women. However, the costs of these and the lack of adequate trained healthcare practitioners may be beyond the scope of the public healthcare system in implementing this. Therefore, workshops to train community organizations and primary healthcare nurses to explain the risks of reproductive and chronological aging to women may be more practical.

6.2.3.1 Aging, mortality and morbidity

It is well described in the literature that chronological aging is related to an increased risk of NCDs, including diabetes, hypertension, osteoporosis and cancers (425). However, the SWEET study has clearly shown that risks for some of these NCDs are related to the trajectory of reproductive aging in these women, and the importance of age at FMP on future

health risks cannot be underestimated (214), although age at FMP and the association with cardiometabolic risk factors were not examined in the SWEET participants due to poor recall of the date of the FMP in the study participants. Many research studies suggest that an HT regimen may ameliorate the health consequences of an early menopause (202) but international menopause and endocrine societies, though they are considering the importance of the timing hypothesis in prescribing HT in early, healthy postmenopausal women, are clear that HT should not be prescribed to prevent osteoporosis, or cardiovascular disease. The consensus is that the primary use of HT is to alleviate VMS and vaginal atrophy (61). While recent research suggests that older women may benefit from judicious use of HT (426), women using HT should be regularly monitored with annual gynecological examinations (38), which is not the norm in the this population as discussed in Chapter 2.

6.2.3.2 Lifestyle interventions

Research has shown that lifestyle interventions, such as healthy eating and regular exercise in obese midlife women can improve risk for MetS and future CVD (422). There are also other lifestyle interventions that can reduce the risk of MetS. Thus, there was a high prevalence of SWEET participants who used a smokeless tobacco (snuff), high in nicotine, which was related to both low bone density and higher visceral fat levels in this population, suggesting that it is imperative to educate these women in the dangers of this product. However, this may present some difficulties. Depending on their motivation for using snuff, religious or cultural reasons may trump healthcare concerns in these women, and care should be taken to understand cultural sensitivities in this regard. In addition, although smoking in this cohort is not as prevalent as snuff use, it is associated, as discussed in Chapter 5, with several metabolic risk factors including MetS, increased triglyceride levels, and raised systolic and diastolic blood pressure. Regulations in South Africa have helped to

reduce the incidence of smoking, but a recent survey recommended that these regulations should be more strongly enforced (6).

6.3 Conceptual relevance

The conceptual framework diagram (Figure 1.12) in Chapter 1, attempted to give an overview of the theoretical relationship of cardiometabolic disease and risk factors with the MT. It was hoped that this framework would serve as a guide to the various experimental components that have been discussed in this study. However, after this study, certain adjustments were necessary (Figure 6.1).

It was hypothesized that HIV infection would affect age at FMP, prevalence and severity of VMS but this was not found. Although no effect of HIV infection or treatment on the prevalence and severity of VMS was seen, this may have been due to the small sample of HIV-positive women in the cohort. In addition the effect of HIV infection on age at FMP could not be tested, because all the women were diagnosed after onset of menopause. However, body composition and metabolic variables were related to HIV and ART.

Although a wide range of menopausal symptoms are generally associated with the MT only three were significant in these women. These were VMS, irritability and sexual problems, and these three menopausal symptoms have been added to Figure 6.1.

The effect of snuff use on BMD was significant, as was its effect on visceral fat. In addition, smoking was associated with triglycerides, hypertension and MetS (Figure 6.1).

Figure 1.12 showed a theoretical relationship of certain demographic variables on MT risk factors. This study found that demographic factors such as employment status and levels of education were associated with certain metabolic variables such as blood pressure, visceral

adiposity and waist circumference and this has been shown in Figure 6.1. . The role that cultural differences may play in the description and perception of menopause symptoms has been discussed earlier.

As expected, the roles of both chronological and reproductive aging, as shown in the initial conceptual framework diagram (Figure 1.12) were significant.

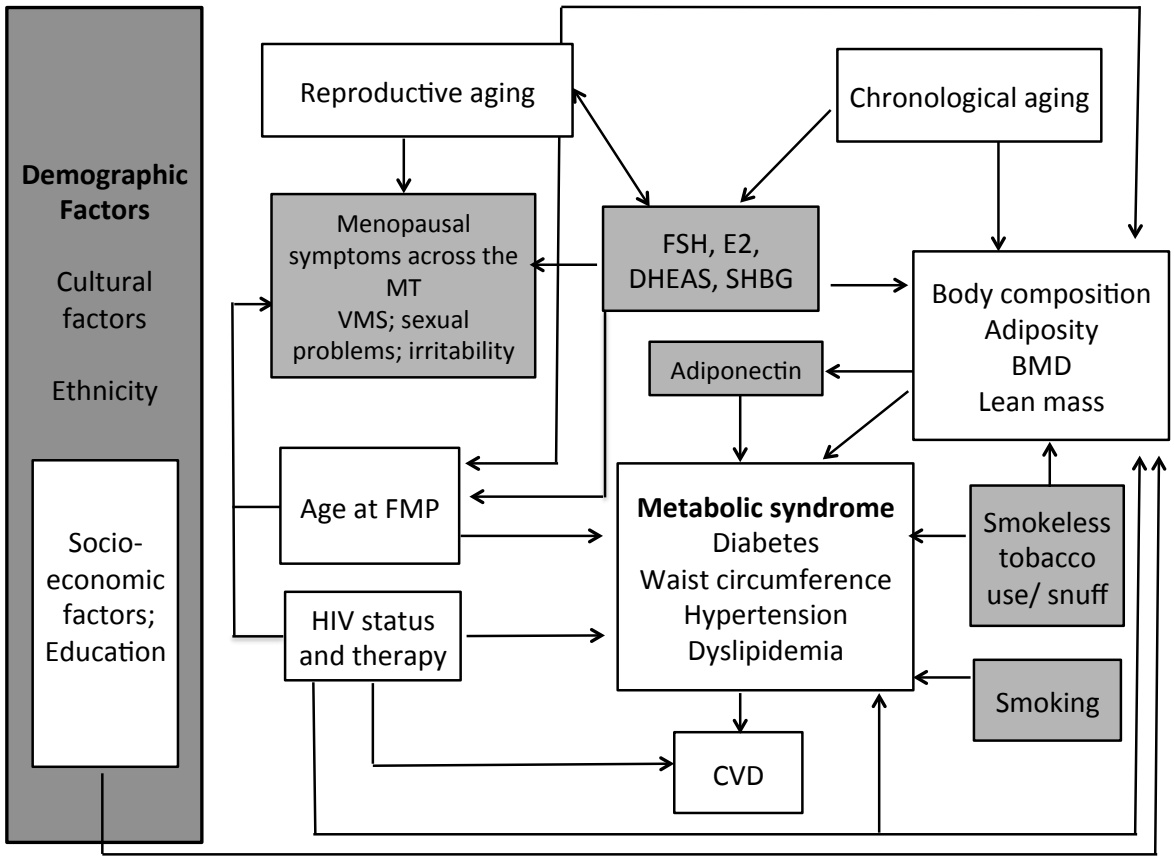

Figure 6.1 Adapted conceptual framework describing the findings of the research.

6.3.1 Novel findings

This research highlighted several new and important findings in midlife black, urban South African women.

While an increase in BMI across the MT has been widely described in Western literature (358, 359), this was not observed in the current study. Indeed, BMI was lower in postmenopausal compared to premenopausal females.

Only three menopausal symptoms, VMS, sexual problems and irritability were significantly related to the MT. It was expected that VMS would be related to the MT, but the finding that sexual problems and irritability were related to MT was surprising. Irritability is usually found in premenopausal women (347), not in late postmenopausal women, while sexual problems and low libido are generally related to vaginal atrophy (415). In addition, data have shown that VMS are associated with sleep disorders and mood problems (427), but these problems were not shown to be related to MT stage in this study population.

Higher serum FSH levels were associated with lower lean mass, as previously reported in only 2 other studies (146, 367) and with higher levels of total cholesterol and LDL-cholesterol, as also reported in only one other study that was conducted in premenopausal females (182).

A further novel finding was the effect of snuff use on both visceral fat and lowered BMD in midlife women.

6.4 Contextual relevance

6.4.1 Local, national and international relevance

The SWEET findings may help clinicians and researchers in the field of women's health, to have a better understanding of the association between reproductive aging in black midlife South African women and their increased risk of cardiometabolic disease. There is a high prevalence of obesity and MetS in these women, but until now there was no information as to whether the MT is related to NCDs.

There are different methods to stage the MT, but many use blood assays, which are invasive and costly, especially in a low-resource country like South Africa, where funding for healthcare is limited. This research has shown that STRAW +10 criteria are accurate in staging reproductive aging and do not rely on blood assays for verification of MT stage, resulting in savings of both resources and manpower. Furthermore, STRAW+10 criteria have not previously been used to stage reproductive aging in sub-Saharan African women and this method may be generalizable to populations of both rural and urban women in these countries.

The inability of most of the study participants to accurately recall age at FMP has highlighted a neglected area in women's health care in this country and possibly in other sub-Saharan countries. Annual screening, identifying important biomarkers, metabolic risk factors, and informing women of their healthcare risks as they transition into menopause do not appear to be the norm in South Africa or other sub-Saharan countries. However, an understanding of midlife women's health risks is vital, especially as improved care for HIV-infected populations means that aging women are living longer and are thus at greater risk for NCDs. In addition, ART use was related to lower BMC. This, in conjunction with the high prevalence of obesity in these women, and the finding that lean mass and BMD were lower in premenopausal than postmenopausal women is of concern, but may help to emphasize the need for lifestyle interventions such as appropriate muscle strengthening exercise, weight loss regimens and bone density screening.

Poor education and high unemployment are both challenges in South Africa, and the finding that these are related to metabolic risk factors in midlife black urban women, is of concern, highlighting a need to be cognisant of the socioeconomic and education status of aging women in this population.

This study has shown that certain lifestyle variables are related to metabolic disease, although smokeless tobacco use has been investigated in black South Africans, its effect on visceral adiposity and BMD have not been shown specifically in aging black women, who are already at risk for metabolic disease and osteoporosis. Smoking, though less prevalent in the study participants, is also related to metabolic variable, underlying the importance of continuing to monitor and educate this population about the risks of any type of tobacco use.

6.5 Limitations and advantages of the study

The limitations and advantages of this study have been fully described in chapters 3-5. However other shortcomings and positive attributes of the study should be explored. The fact that English was the second language of the participants meant that aspects of both the understanding of the question and the response might have been 'lost in translation'. In addition, those team members who were trained to interpret the questions and answers did not speak English as their first language. However, rigorous training in the description and interpretation of the answers was given to team members, who were available to act as interpreters. The importance of not adding additional explanations or 'improving' the question or answers was stressed. Since English is the most commonly used language in South Africa, loss of idiomatic meaning and misinterpretation was rare and the team members were all widely experienced in the research field. The questionnaires were piloted among women who spoke English as a second language and where possible, words and terminology were simplified. An additional limitation was that CD-4 counts were not obtained.

In terms of the positive aspects of this study, this is the first time that the effects of a wide range of hormonal, anthropometric and metabolic variables and their relationship to reproductive aging in black urban South African women have been assessed. The large sample number provided sufficient statistical power to allow us to observe multiple

associations between these different variables and the MT.

6.6 Future research studies

Due to the fact that research into reproductive aging has not previously been undertaken in this population, a wide range of possible future research studies became apparent during the course of this investigation. The following topics may generate some useful data:

6.6.1 Longitudinal research to confirm validity of the SWEET findings

The development of a large, longitudinal study investigating both chronological and reproductive aging in this group of women would be useful to confirm whether the findings from SWEET, which is a cross-sectional study, are valid and thus generalizable to other sub-Saharan women.

6.6.2 Improved research tools to accurately stage age at FMP

Use of an improved staging instrument should help to define the menopause transition stages more accurately and allow more accurate recall of age at FMP. Studies have shown that earlier age at menopause is related to increased morbidity and mortality (62), so research examining the relationship between metabolic risk factors that are prevalent in these women and age at FMP using more accurate recall data may be important. Additional information relating to the age at menopause of the mothers, close female relatives or siblings of participants should be obtained, since data have shown that heritability is strongly associated with age at FMP (65). Trauma, sexual abuse, childhood and adult poverty are also associated with earlier menopause (62), and data on these are necessary to better understand why some women have an earlier age at FMP. Use of menstrual calendars in a longitudinal study may be helpful, and possibly an examination of genetic factors which

might affect age at FMP. Ethics permission to collect DNA from this group of women was given and the DNA has been stored for future analysis.

6.6.3 Research using objective hot flush measurements to assess MRS validity and the effect of cultural perception on the description of menopause symptoms

The MRS, which was used to assess prevalence and severity of menopausal symptoms in the research, has been internationally validated, However language differences and cultural perceptions of the menopause may affect responses, so a study investigating objective hot flushes, using a small external monitoring device (428), as compared with self-reported hot flushes in midlife black African women, may be helpful in assessing the validity of the MRS, and improving understanding of cultural differences in the perception of hot flushes.

6.6.4 HIV and the MT

The high prevalence of HIV infection in this group of women suggests that the effect of the disease on both chronological and reproductive aging in these midlife black women is highly relevant. Furthermore, only 55% of woman with HIV were receiving ART suggesting that the effects of the virus and of ART both require further investigation in this population. Research on the effect of HIV infection on reproductive aging and its related variables was an important aspect of the study, but the small sample number of subjects who allowed us to perform an HIV test limited the interpretation of the results. Future studies may have a longitudinal design and a large sample of premenopausal women, aged ≥ 45 years should be recruited. Voluntary HIV testing should be offered but the numbers should be large enough to allow sufficient power. Both HIV-positive and HIV-negative women should be followed up to FMP and 12 months beyond. Given the association of ART with BMC and BFD in some SWEET participants, research should determine the effects of different ART regimens on hormone levels, FMP, cardiometabolic and anthropometric variables in this

population. In addition, the role of HIV and ART in reproductive aging should be investigated.

6.6.5 Contraceptive use and body composition in sub-Saharan African women

Contraceptive use was determined in this study, and was shown to be associated with lower HDL levels, but previous treatment and type of contraceptive was not ascertained. It would be important to determine if particular contraceptives were responsible for this association. Given the trend for lower BMD across all MT groups, and the fact that Depo-Provera, which is known to affect bone density (429, 430), was widely used by the public healthcare system in South Africa, an investigation into length of use and bone density may further clarify any association between contraceptive use and bone loss.

6.6.6 HT regimens and VMS, bone density and CVD

Research into the possibility of prescribing HT to this population group, and the accompanying risks and benefits is important, especially in relation to bone density loss and alleviation of VMS. The use of HT may also lower risk of metabolic disease and improve quality of life in this group of women.

6.6.7 The role of FSH in sarcopenia in black African women

Lower lean mass in postmenopausal women in this population was significantly related to rising levels of FSH. This finding is interesting and requires further investigation because the molecular mechanisms involved are not understood. There is no research indicating that FSH receptors are present in lean muscle tissue. Thus, studies have shown that the FSH receptors are found only in the tissues of the testes or ovaries (431). It is possible that FSH receptors may be expressed in low levels in muscle and this requires further investigation. It is also possible that there may be an indirect mechanism by which FSH affects lean mass.

In addition, further longitudinal research on the lower lean mass observed in the postmenopausal women in this study is needed to examine whether these women are at greater risk of falling due to the decrease in muscle function that has been shown to occur with skeletal muscle loss. In addition, further research could investigate whether decreased muscle strength may lead to greater bone fragility in this population.

6.6.8 BMD and BMC in midlife black African women

The lower BMC and BMD in postmenopausal SWEET participants suggest a need for additional research. There are several factors that must be investigated. Firstly, DXA machines have no set parameters for scans in black South African women. The parameters for African American women are not appropriate as they are generally taller with heavier bones, so Caucasian parameters have been used in black South Africans for convenience, but there are clear ethnic differences in micro bone architecture, bone density and bone mineral metabolism (432) that may impact data. Popular medical wisdom from the last century assumed that black South African women had higher bone density than their Caucasian counterparts (294, 295). Studies have now disproved this, but menopause was not accurately staged in the latter studies (296, 298). It seems as though lower levels of estrogen are implicated in decreased bone density shown in the SWEET women, but HT, which protects against bone loss in the MT, is not usually prescribed in this population. In addition, the negative effects of snuff use on BMD in this population should be further investigated.

6.6.9 Aspects of cognition and executive function in black midlife South African women.

Data on cognition, verbal memory and learning, working memory and executive function are widely available in midlife Western women (82). Relationships of these variables to

reproductive aging, hormonal levels and VMS, have not yet been determined in black urban African women. Further research using appropriately validated instruments for this population group may improve the understanding of the above functions in aging women, and clarify whether reproductive or chronological aging, or both have an effect on them in sub-Saharan women. Mechanisms for these potential changes should also be examined.

6.7 Conclusions

This study found that STRAW+10 criteria can be used to stage the MT in black urban South African women. Obesity is not associated with the MT in this population. However, the MT is characterized by sarcopenia, reduced BMD and increased cardiometabolic risk, all of which are driven by changes in hormone levels, specifically E2 and FSH, and by chronological aging. Lifestyle factors, including smoking, snuff use and ART also play a role in the increased risk of MetS in these women.

The public healthcare system in South Africa has not focused on the effects of chronological and reproductive aging in midlife black women, but given the indication of increased morbidity in this group, further research is urgently needed to determine the interventions needed to lower these health risks. These data suggest that midlife African females require more frequent screening for cardiometabolic disease and intervention studies to reduce prevalence of obesity and snuff use.

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Appendices

Ethics clearance certificate
Approval of ethics changes

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mrs Nicole Jaff

CLEARANCE CERTIFICATE	**M090620**
PROJECT	An Investigation of the Metabolic, Hormonal and anthropometric Characteristics of the Menopausal Transition in Black Urban South African Women
INVESTIGATORS	Mrs Nicole Jaff.
DEPARTMENT	Department of Chemical Pathology
DATE CONSIDERED	09.06.26
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 2009/12/14 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof N Crowther

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

18 July 2011

Mrs Nicole Jaff
Department of Chemical Pathology
Medical School
University

Dear Mrs Jaff

RE: Protocol M090620: 'An Investigation of the Metabolic, Hormonal and Anthropometric Characteristics of the Menopausal Transition in Black urban South African Women

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved the following amendments on the abovementioned protocol as detailed in your letter dated July 5, 2011:

- Title of Project: ;'Study of Women entering and in Endocrine Transition (SWEET Study): an Investigation of the Metabolic, Hormonal and Anthropometric Characteristics of the Menopausal Transition in Black Urban South African Women' **an updated clearance certificate will be issued in due course**
- The research sites
- Additional blood measurements

Thank you for keeping us informed and updated.

Yours sincerely,

Anisa Keshav
Secretary
Human Research Ethics Committee (Medical)

SWEET questionnaire

University of the Witwatersrand, Johannesburg
Developmental Pathways for Health Research Unit
Department of Paediatrics and Child Health

SWEET STUDY: Study of Women in and Entering Endocrine Transition
CAREGIVER QUESTIONNAIRE 2011

DATE: **Day** ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER: ☐☐☐☐☐☐☐

BONE ID NUMBER: ☐☐☐

Consent Table

Components	Yes	No
Caregiver Questionnaire		
Food Frequency Questionnaire		
SDMT Cognitive Test		
Caregiver Anthropometric Measurements		
Caregiver DXA		
Caregiver PQCT		
Caregiver Ultrasound		
Caregiver cIMT		
Caregiver Bloods		
HIV Testing		

Contact details of a relative or a friend who will **always** know where you live (different to information on contact sheet):

Name: _____ Relationship: _____

Landline number: _____ Cell number: _____

Work number: _____ Other: _____

Address: _____

INFORMED CONSENT FOR DNA SAMPLING AND STORAGE

INFORMED CONSENT FOR DNA SAMPLING AND STORAGE

As mentioned in the information sheet, I have requested that an extra tube of blood (5ml = I teaspoon) will be taken for DNA studies. The DNA samples will be stored in a safe place in my department where I will be the only person who will have access to them. The sample of blood for DNA studies will be used to look for DNA changes that are caused by obesity and also to look for other DNA changes that are involved in causing diabetes, obesity and cardiovascular diseases. The blood samples will be used for no other purposes other than those I have described to you. However, if at some future date we realize there are other studies that we would like to carry out on your DNA samples, such tests would only be performed if permission is given to us by the Human Research Ethics Committee of the University of the Witwatersrand.

Your identity will be anonymous as your sample will be identified by a number (as described in the information sheet).

I agree to take part in the above clinical study and to give a blood sample for DNA studies. The procedures to be carried out have been explained to me. The possible discomforts, risks and benefits involved in taking part in the test have also been described to me. I understand that I can leave the study at any point. I also understand that if I have any questions concerning the test then the investigator will explain these to me.

Contact details of researcher:

If you have any questions or queries at any time during the study, please contact me at 011-8851498 or 0828524857or call our department at 011-4898500.

Contact details of Human Research Ethics Committee (Medical)/ HREC:

If you have any other queries or wish to complain about the conduct of the study or researcher, you may contact the HREC chair, Prof Cleaton-Jones through the secretary Ms Anisa Keshav at 011-717 1234.

Date: Patient:

INFORMED CONSENT

I agree to myself being a participant in the SWEET study, a sub-study of Birth to Twenty
The goals and methods of SWEET study are clear to me.
I understand that the study will involve interviews, some routine body measurements and some blood tests. All the details and purposes of this study have been explained to me. I understand that I have the right to refuse to participate in the study.

I agree to participation in the study on the condition that:

1. I can withdraw from the study at any time voluntarily and that no adverse consequences will follow on withdrawal from the study.

2. I have the right not to answer any or all questions posed in the interviews and not to participate in any or all of the procedures / assessments.

3. The University of the Witwatersrand Human Ethics committee has approved the study protocol and procedures.

4. All results will be treated with the strictest confidentiality.

5. Only group results, and not my individual results, will be published in scientific journals and in the media.

6. The SWEET study scientific team are committed to treating participants with respect and privacy through interviews conducted in private and follow-up counselling available on request.

7. I will receive a referral note to a health service if any result is out of the normal range or a problem is detected in the course of the study.

PARTICIPANT (Caregiver)

Printed Name	**Signature / Mark or Thumbprint**	**Date and Time**

RESEARCH ASSISTANT:

Printed Name	**Signature**	**Date and Time**

SECTION A: GENERAL INFORMATION, EMPLOYMENT AND INCOME

The information you give us from this section will give us general information about you

1. Are you currently employed? | Y | N |

1.1 If YES, what type of employment?

_____

1.2 If NO to question 1, do you get any income from other sources? | Y | N |

If YES to question 1.2, what type of sources do these include?

_____

SECTION B: MENSTRUAL HISTORY

This section will tell us more about your menstrual cycle.

1. Are you currently pregnant? | Y | N |

2. Have you had a hysterectomy? | Y | N |

2.1IF YES, what date was it? _____

2.2IF YES, do you know whether you had a partial hysterectomy or a full hysterectomy?

| Partial | Full |

3. Do you have regular periods? | Y | N |

3.1 IF YES, when did you have your last period? (month and year)

_____

3.2 IF NO, when was your last period?

3 months ago	6 months ago	1 year ago	more than one year

4. Have you ever had a pap smear? | Y | N |

4.1 IF YES, when did you have it? _____

4.2 IF YES, did you get the results? | Y | N |

5. Do you understand what menopause (change of life) means? | Y | N |

6. Where did you learn about the menopause?

mother	friend	doctor	nurse	family member	other
If other describe where _____					

7. Do you feel comfortable discussing the menopause? | Y | N |

SECTION C: MENOPAUSE RATING-SCALE (MRS II)

The aim of this questionnaire is to see whether you have certain symptoms, signs or body changes that happen to you as you move into menopause and to understand how strong or mild they are compared with other women in the world.

Which of the following complaints do you experience at **this time or recently?**
We will mark the following symptoms with a tick in the box which best describes the severity of your symptom. If you are not experiencing that symptom the interviewer will tick the box saying "none"

	None	slight	medium	strong	very strong
	0	1	2	3	4
1. Body Temperature Changes..... (hot flushes, sweats and night sweats)	☐	☐	☐	☐	☐
2. Heart Complaints..... (irregular heartbeat, palpitations, chest pains,)	☐	☐	☐	☐	☐
3. Sleep problems..... (interrupted sleep, trouble in sleeping through the night, waking too early)	☐	☐	☐	☐	☐
4. Mood problems..... (listlessness,'finished", sadness, tearfulness, no energy, mood swings - mood changes quickly)	☐	☐	☐	☐	☐
5. Irritability..... (argue all the time, get cross quickly nervous, feeling tense, aggressiveness)	☐	☐	☐	☐	☐
6. Fearfulness..... (being afraid, panic attacks and anxiety, being very anxious)	☐	☐	☐	☐	☐
7. Physical and mental tiredness..... (lack of energy, forgetting often, not concentrating well, confused about time or place)	☐	☐	☐	☐	☐
8. Sexual Problems..... (change in sexual desire, change in sexual activity and sexual satisfaction)	☐	☐	☐	☐	☐
9. Urinary Problems – problems passing water..... (passing water more often, increased urgency – need to go urgently – can't wait, leaking, stress incontinence - passing water during physical activity, like coughing, sneezing, laughing, or exercise.)	☐	☐	☐	☐	☐
10. Vaginal dryness..... (feeling of dryness or burning, itchiness pain during sexual intercourse)	☐	☐	☐	☐	☐
11. Joint and muscle complaints..... (pain near the joints, arthritic complaints)	☐	☐	☐	☐	☐

SECTION F: GENERAL HEALTH (tick the most relevant box in each section)

1. How often did you use the following over the **past 6 months?(Mark each line)**

	Never	Occa-sionally	Often
Laxatives			
Medication to lose weight			
Antibiotics			
Pain killers			
Aspirin			
Vitamins and minerals			

2. How often do you experience the following complaints?**(Mark each line)**

	Never	Occa-sionally	Often
Easily tired			
Difficult in concentrating			
Nervous, anxious, irritable			
Painful muscles/cramps			
Constipation			
Colds/flu			
Headaches			

3. Indicate whether you and your biological parents have/have had the following: **(Mark every line)**

	No-one	Self	Mother	Father
Weight problem/ obesity				
High blood pressure				
Heart problems				
Stroke				
High cholesterol (a lot of fat in your blood)				
Diabetes (sugar)				
Thyroid problems				
Breast cancer				
Other cancer/s				
Depression				
Arthritis				

3.1 IF YES to self, do you currently use medication/s for these? **Y** **N**

3.2 IF YES to 3.1, what medication/s do you regularly use?

_____ _____ _____

4. How many days have you been off "sick in bed" during the past 6 months? (**Mark only one)**

Never	
1 – 2 days	
3 – 4 days	
5 or more days	

SECTION E: LIFE STYLE

1. Have you ever smoked? | Y | N |

1.1 IF YES to question 1, do you currently smoke? | Y | N |

1.2 IF YES 1.1, how many cigarettes do you smoke a day?

_____

1.3 IF NO to question 1.1, when did you stop smoking? (year and month)

_____

2. Do you use snuff? | Y | N |

2.1 IF YES to question 2, do you use snuff for?

Religious/cultural purposes	
Medical purposes	
Relaxation	

2.2 IF YES to question 2, how do you take snuff?

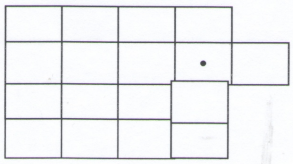
Through your nose	
Through your mouth/on your lip	

2.3 IF YES, how often do you use snuff?

Once a day	
Twice a day	
Three times a day	
More than three times a day	
Other: Specify _____	

SECTION J: CAREGIVER MEASUMENTS

- STANDING HEIGHT: (mm)
- WEIGHT: (kg)
- WAIST CIRCUMFERENCE: (mm)
- HIP CIRCUMFERENCE (mm)



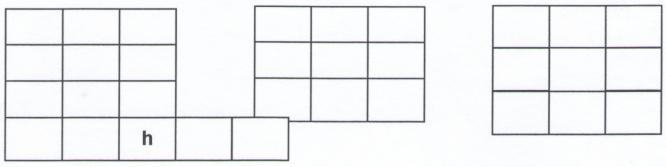
SKIN COLOR CHART

1	2	3	4	5	6

BLOOD PRESSURE

- SYSTOLIC BP
- DIASTOLIC BP
- PULSE
- TIME OF BP

(1) (2) (3)



RESEARCH ASSISTANT: _____ **DATE:** _____

COLLECTION OF SPECIMENS

- ROUTINE BLOOD SAMPLE

Y	N

RESEARCH NURSE: _____ **DATE:** _____

DXA	Y / N	**OPERATOR**		**DATE:**	
PQCT	Y / N	**OPERATOR**		**DATE:**	
ULTRASOUND	Y / N	**OPERATOR**		**DATE:**	
cIMT	Y / N	**OPERATOR**		**DATE:**	

FFQ	Y / N	RESEARCH ASSISTANT	
STMD	Y / N	RESEARCH ASSISTANT	

QUALITY CHECKED BY:		DATE:	

NOTES:

HIV testing consent form

Bt20 ID
SWEET study

HIV COUNSELLING FORM

Hello! My name is _____. First, we would like to discuss some matters with you. Information collected will be not be used in any identification form outside this facility. Therefore confidentiality will be maintained. We will provide you with information you need to know about HIV and AIDS. This will then be followed by information to help you understand your risk exposure to HIV and then you could be able to take an HIV test.

CLIENTS HIV HISTORY

Have you been tested for HIV before?	Yes	No	If Yes, when did you test? What was the HIV results		Negative	Positive
If positive, do you have a copy of the results	Yes	No	If no, would you like to do another test?		No	Yes
What was your reason for testing	Illness	Insurance	Partner died	Pregnancy (Females Only)	Employment	General Check up
If other please state reason						

CLIENT SUPPORT SYSTEM

Have you ever had a loss in your life?	Yes	No				
If yes,	Who					
	When					
If the test is HIV positive, will you tell someone?	Yes	No	If Yes Who?			
Who else will you tell if you are HIV positive?	Family	Partner	Friend	Other (State)		
How will you tell this person you trust?						
Do you think you will get support from that person?	Yes	No	Would you like us to offer support?		Yes	No

PRE COUNSELLING SESSION

UNDERSTANDING HIV AND AIDS

COUNSELLOR TO USE CUE CARDS FOR COUNSELLING

Understanding of HIV/AIDS, client should be explaining mode of transmission and exchange of fluids.		Meaning of Window Period (What is it?)	
Benefits for HIV Testing		Importance of knowing ones HIV status (What does it mean?)	
Meaning of HIV testing		Meaning of HIV Negative Result	
Meaning of Confidentiality. (Counsellor to clarify confidentiality)		Meaning of HIV Positive Result	
		Perception of risk to HIV exposure. (Does the client think they are at risk to HIV infection?)	

HIV TESTING

Counsellor: Explain rapid testing processes. A rapid test for HIV will be done by the DPHRU lab. About a teaspoon full of blood will be collected (5ml to 10ml) and tested on specific HIV testing kits to check for HIV antibodies. Test results will be given to you in private when you check out today by a registered trained counsellor. If the report states negative it means that there are no antibodies to HIV. The window period will be explained. If the report states positive, it means that you are HIV positive and that there are antibodies to HIV. You will be given a letter to refer you to a clinic specialising in HIV treatment and you will be given a second test at the clinic to confirm this result. Sometimes we cannot clearly tell if the results are negative or positive. We will then repeat the test and if it is still indeterminate we will refer you to a clininc for a second testing that will help confirm the results

PATIENT CONSENT

I agree to have the HIV Rapid test. The procedure to be carried out has been explained to me. The possible discomforts, risks and benefits involved in taking part in the test have also been described to me. I understand that I can leave the study at any point. I also understand that if I have any questions concerning the test then the investigator will explain these to me

Contact details of researcher:

If you have any questions or queries at any time during the study, please contact me at 011-8851498 or 0828524857or call our department at 011-4898500.

Contact details of Human Research Ethics Committee (Medical)/ HREC:

If you have any other queries or wish to complain about the conduct of the study or researcher, you may contact the HREC chair, Prof Cleaton-Jones through the secretary Ms Anisa Keshav at 011-717 1234.

Date: Patient:

POST TEST COUNSELING SESSION

NB. COUNSELOR: Identify Client with Name and ID number against HIV Test Results

HIV NEGATIVE TEST RESULT

We spoke earlier about what HIV positive and HIV negative results mean. Explain again. Your results are back and you are HIV negative; you do not have the HIV virus in your body

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Explain the implications of the negative test result		
Identify and prioritize the behaviours that correspond to the client's risk		
Motivate the client to develop a risk reduction plan		
Encourage clients to discuss their HIV status with current and future partners		

POST TEST COUNSELLING

HIV POSITIVE TEST RESULTS

We spoke earlier about what HIV positive and HIV negative results mean. Explain again. Your results are back and you are HIV positive; you do have the HIV virus in your body

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Inform client that the test results are available		
Provide results clearly and simply		
Review the meaning of the result		
Allow the client time to absorb the meaning of the result		
Explore the client's understanding of the result		
Assess how client is coping with the result		
Acknowledge the challenges of dealing with an initial positive result		

IDENTIFY SOURCES OF SUPPORT

COUNSELORS KEY TASKS	CLIENTS NOTES	COMMENTS
Identify current health care resources		
Address the need for health care providers to know client's test result		
Explore client's access to medical services		
Identify needed medical referrals		
Discuss situations in which the client may want to consider protecting his or her own confidentiality		
Discuss options of support groups (i.e. post test club)		
Provide appropriate referrals		

REFERRAL TO OTHER PROGRAMS

Refer the client with letter to Thandekile Essien and the clinic (ZAZI VCT service at Baragwanath Hospital); she will then ensure that the client has the appropriate support.

COUNSELLORS NOTES:

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Counsellor's Signature:……

THANK THE CLIENT FOR USING OUR SERVICES

Menopause information sheet for SWEET participants

MENOPAUSE AND CERVICAL CANCER INFORMATION SHEET

What is menopause?
The word menopause means the last day of a woman's last period ever. Some women call it the 'change of life'.

What happens when you are in menopause?
When you reach menopause you can't become pregnant any more.
You don't usually become menopausal suddenly. The changes in your body happen slowly as you move towards the time of menopause. This time is called perimenopause and during it you may notice physical and emotional changes happening to you. These happen as the hormones in your body that are connected to childbearing start to rise and fall. Some hormones are responsible in helping you have regular periods and causing ovulation (when you are able to have a baby).
During perimenopause you may experience uncomfortable symptoms:

- ✓ Hot flushes and night sweats,
- ✓ Tender breasts.
- ✓ Irritability and emotional feelings
- ✓ Battling to sleep well or to stay asleep.
- ✓ Weight gain
- ✓ Changes in periods – they can be very heavy, much lighter or stop for a few months and then start again.

The time it takes to become menopausal can be short - up to two years, or long - up to 14 years. All women are different, so your menopause experience may not be the same as your friends. Although you and many other women may struggle in the years leading up to menopause, some women have no problems at all. Once you are fully into menopause the hot flushes and other symptoms usually stop, though some women may suffer from a dry vagina, a low sex drive (not wanting to have sex) and sometimes urinary problems (pain when urinating or urinary infections)

Don't become pregnant by mistake
Even if a your periods are not regular you can still **become pregnant, so until you are sure that you are fully menopausal and haven't had a period for at least 12 months, you should still use contraception during sex**

How do you know when you are menopausal?
If you are in your mid-40's or older and haven't had a period for 12 months you can usually be confident that you are menopausal. However, certain things such as losing too much weight, lots of stress and unhealthy life style, habits like smoking and drinking too much alcohol, can also cause your periods to stop. So be aware if there are other reasons for your periods to stop or change. It is important to know that menopause symptoms can be very similar to some changes caused by certain health problems, and may **not** be caused as your ovaries are getting older. If you don't think that you are moving towards your menopause, you must explain this to your doctor or clinic sister and they may do some blood tests to help confirm whether you are in menopause

Dealing with hot flushes and night sweats

- ❖ Wear loose comfortable clothes that are made of pure cotton, so that the fabric breathes.

Wear layers so you can remove something if you get too hot.

- Carry a small spray bottle of water and spray it on the back of your neck and wrists and knees when you feel a hot flush coming on. Keep one next to your bed at night.
- Don't have spicy or very hot food and drink tea or coffee at room temperature.
- Don't overeat or drink too much alcohol or coffee.
- Don't smoke.
- Exercise regularly.
- Stress can trigger a hot flush, so try to relax and stay calm.
- Hormone Therapy (HT) is the most effective treatment if you are really battling with hot flushes. Your doctor will advise you of the risks and benefits.
- If you take HT, you must have a thorough medical examination and then an annual gynaecological examination and a mammogram. Tell your doctor your family and medical history. Take the smallest, effective amount of HT for the shortest possible time. Each year check to see whether you still have hot flushes and need to use it.
- For women who can't take HT, there are other treatments; ask your doctor or clinic sister about them.

INFORMATION ABOUT THE PAP SMEAR AND CERVICAL CANCER

What is a pap smear?

The Pap smear is the best way to see whether you have cancer or pre cancer of your cervix. The cervix is the part of your body that connects your vagina to your womb. Most invasive cancers of the cervix can be detected early if you have Pap tests. In this procedure some cells from your cervix are scraped off painlessly. These are then sent to be checked.

You are at risk for cervical cancer if you:

- Smoke
- Have many sexual partners
- If your partner has many sexual partners

If possible, you should have a Pap smear once a year to check for cervical cancer. You can ask at your local clinic for them to do the pap smear. You can have **3(three)** pap smears in your lifetime at the ages of **30, 40** and **50. These are free of charge.** If you or your sexual partner has another new partner or partners, then you should have a Pap smear once a year.

Signs that you may have a problem (if you have any of these signs you should see to a doctor):

- Painful sex
- Bleeding after sex
- Pain while urinating
- Pain in your lower back
- Unusual bleeding
- Unusual discharge from your vagina

If you or your sexual partner have other new partners, then you should have a Pap smear **once a year**. After age 65-70, you can stop having Pap smears as long as you have had three **negative** tests within the past 10 years. If you have a new sexual partner after age 65, you should begin having Pap smear screening again.

Authors’ agreement

Authors agreement letter

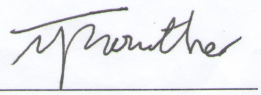
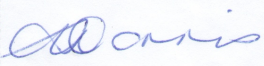
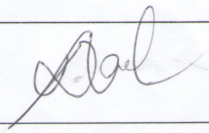
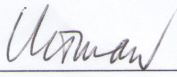
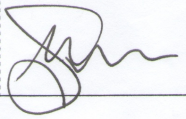
The scientific research papers, titles listed below, form part of a PhD thesis:

Titles:
1) Staging reproductive aging using Stages of Reproductive Aging Workshop + 10 in black urban African women in the Study of Women Entering and in Endocrine Transition
2) Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): an African perspective
3) Reproductive aging and associated hormonal changes are related to metabolic syndrome and cardiovascular disease risk factors in menopausal African women,

Nicole Jaff had the following role in each scientific research paper: Design and conduct of the study, data collection, data analysis, data interpretation and manuscript writing

Authors Agreement

We, the co-authors agree with the role played by Nicole Jaff as first author of these papers as stated above, and agree to the inclusion of these papers in the thesis

Author(s)	Signature
Nigel J Crowther	
Shane A Norris	
Frederick J Raal	
Marketa Toman	
Tracy Snyman	

Original papers

Menopause: The Journal of The North American Menopause Society
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Staging reproductive aging using Stages of Reproductive Aging Workshop + 10 in black urban African women in the Study of Women Entering and in Endocrine Transition

Nicole G. Jaff, NCMP,[1,2] Tracy Snyman, BScHon,[2] Shane A. Norris, PhD,[1] and Nigel J. Crowther, PhD[2]

Abstract

Objective: There has been limited research on accurate staging of the menopausal transition in sub-Saharan African women. Our aim was to assess the usefulness of the Stages of Reproductive Aging Workshop + 10 (STRAW + 10) criteria in staging ovarian aging in black South African women, examining whether obesity has any effect on the menopausal transition.

Methods: The study enrolled 702 women aged 40 to 60 years. STRAW + 10 criteria were used to categorize the stages of reproductive aging. The Menopause Rating Scale was used to measure the prevalence of vasomotor symptoms. Follicle-stimulating hormone (FSH) and estradiol levels were used as supportive criteria for staging. Human immunodeficiency virus status was assessed using a point-of-care method.

Results: Reported age at final menstrual period (FMP) was higher in women interviewed within 4 years of FMP (mean [SD], 49.0 [3.80] y) than in women interviewed 10 years or more after FMP (mean [SD], 42.0 [4.06] y; $P < 0.0005$). In women within 4 years of FMP, lower body mass index was associated with earlier age at FMP. FSH levels increased and estradiol levels decreased ($P < 0.0005$ for both trends) across seven staging groups. Human immunodeficiency virus status had no effect on menopause symptoms. Obesity (body mass index ≥35.0 kg/m^2) was associated with severe vasomotor symptoms.

Conclusions: Reporting of age at FMP is unreliable in women interviewed 4 years or more after the event. STRAW + 10 seems accurate in staging reproductive aging, as confirmed by the strong association of FSH and estradiol levels with the menopausal transition stage. STRAW + 10 may be appropriate for use in resource-limited settings in the absence of biomarkers. Biocultural methods may be useful in assessing the menopausal transition in culturally diverse women.

Key Words: Menopausal transition – Black African women – Obesity – Stages of Reproductive Aging Workshop + 10 – Vasomotor symptoms – Cultural differences.

The World Health Organization estimates that, by 2030, 1.2 billion women will be 50 years or older. This is nearly triple the number of women who belonged to that age bracket in 1990. A growing number of these women can expect to live for several decades after menopause, and it is estimated that 76% of postmenopausal women will be living in developing countries by 2030.[1]

Research shows that the menopausal transition (MT) is accompanied by clear physiological changes, some of which are temporary whereas others are long term.[2] The Study of Women Entering and in Endocrine Transition (SWEET) was developed to examine changes in metabolic, hormonal, and anthropometric parameters in black urban South African women across the MT because research suggests that these changes may increase the risk for cardiometabolic diseases.[3] Obesity is highly prevalent in middle-aged, urban African women,[4] but very few studies have analyzed the relationship between obesity and the MT in this population. In addition, data on obesity and its effects on the prevalence of vasomotor symptoms (VMS) in this population have been limited; studies of Western women have shown a strong correlation.[5] VMS are one of the defining symptoms of the MT[6] and have been linked to an increased risk of cardiovascular diseases.[5,7,8]

Accurate staging of reproductive aging is fundamental to understanding the relationships discussed above.[9] However, there is a paucity of research on accurately determining mean age at final menstrual period (FMP) and on MT staging in sub-Saharan African women. In 2001, the Stages of Reproductive Aging Workshop (STRAW)[10] identified criteria defining the different stages of reproductive aging to help clinicians and research scientists stage the reproductive cycle. Harlow et al[11] considered these criteria as the gold standard for staging the MT.

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However, research in the 10 years following STRAW has enabled clinicians in the field to have a wider understanding of the hypothalamic-pituitary and ovarian functions of the MT. At a recent workshop, "STRAW + 10: Addressing the Unfinished Agenda of Staging Reproductive Aging," the criteria for staging menopause were revised and updated based on the latest data.[12] Bleeding patterns (menstrual cycles) remain the most important criteria for determining the stage of the MT because there is no international standardization of biomarker assays.[12-14] In addition, low- or middle-income countries do not have the resources for the wide use of blood assays in assessing specific stages in the reproductive cycle. In 2001, STRAW recommended that follicle-stimulating hormone (FSH) level be used as a biomarker in the late transition stage. Given the improved understanding of endocrine changes involved in ovarian aging,[15] Stages of Reproductive Aging Workshop + 10 (STRAW + 10) suggested that FSH and estradiol (E_2)—specifically their concentrations before and after the FMP—be used as supportive criteria to verify the stage of the MT.[16] In addition, VMS and vaginal atrophy may be used to support other measures to determine the stage of the MT.[12]

To our knowledge, no prior study in sub-Saharan Africa has used the STRAW + 10 guidelines for staging the MT. In this study, we assess the reliability of this method for classifying ovarian status in a population with a high prevalence of obesity.[17] Data from this same population of women (mean age, 43 y) showed that diabetes and metabolic syndrome are very common.[18] A high incidence of human immunodeficiency virus (HIV) infection has been observed in this group of women,[19] and some studies have shown that being HIV-positive may affect the symptoms and conditions of the MT in midlife women.[20,21]

This study aims to examine reproductive aging in a population of urban African women to assess the usefulness of STRAW + 10 criteria in staging ovarian aging in these women by determining bleeding patterns. VMS severity, FSH levels, and E_2 levels were determined as supportive criteria. We also wanted to determine whether obesity has any effect on the MT, FMP, and menopausal symptoms, particularly VMS. Age at FMP was noted, and HIV status was obtained (where possible) to analyze whether HIV infection was associated with age at FMP and menopausal symptoms.

METHODS

Participants

Data were collected for a period of 2 years from August 2011 to July 2013. Participants in this cross-sectional study are biological mothers of the children in the Birth to Twenty Plus (BT20) cohort,[22] which is the largest and longest-running longitudinal birth cohort study of child health and development in Africa. A cohort of 3,275 children who were born in 1990 during a 7-week period to women residents in Soweto, South Africa, were recruited into the Birth to Twenty Plus study. After 21 years, 2,200 of these women are still in contact with the study. The sample size used for the current study was based on convenience. Owing to infrastructure and timeline constraints, not all of the 2,200 participants could be recruited into the study. We calculated that a maximum of 1,000 women could be studied and that the minimal number of participants would be defined as that at which at least 100 women were present in each of the following four study subgroups (based on menopause stage): late reproductive (stages −3b and −3a), the MT (stages −2 and −1), early postmenopause (stages 1a, 1b, and 1c), and late postmenopause (stage +2). Therefore, 902 women were invited to participate in the study, and the following inclusion criteria were applied: age between 40 and 60 years, not pregnant, black African women. The following women did not, or could not, participate in the study: older than 60 years, 35; refused to participate, 79; deceased, 37; terminally ill, 3; untraceable or living out of the study area, 46 (leaving a total of 702 participants). All participants signed an informed consent form. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand approved the protocol (certificate M090620).

Questionnaires, menopause staging, and symptomology

General questionnaires

Questionnaires were administered in English during face-to-face interviews; however, where necessary, members of the research team whose first language corresponded with those of the participants were trained to translate the questions. The questionnaire included close-ended questions on reproductive health, menstrual history and understanding of menopause, and educational level. Hormone therapy and contraceptive use were determined. Where possible, information on hysterectomy and oophorectomy was obtained. Tobacco use was noted.

Women were asked close-ended questions about bleeding patterns to determine the MT. Participants were asked the date of their last menstrual period. If they had skipped a menstrual period, they were asked when they last had a menstrual period (3 mo, 6 mo, 1 y, or >1 y ago). These questions were followed by open-ended questions to more specifically ascertain the cycle changes they were experiencing.

MT stage

STRAW + 10 criteria[12] were used to categorize the women into menopause stages (Fig.): late reproductive (stages −3b and −3b), early MT (stage −2), late MT (stage −1), early postmenopause (stages 1a, 1b, and 1c), and late postmenopause (stage +2).

Menopause Rating Scale

The Menopause Rating Scale (MRS), an internationally validated standardized questionnaire,[23] was used to determine menopause status and to identify symptoms as they occurred in the past 4 weeks. It was used to measure the prevalence and severity of symptoms.[24] The MRS allows the participant to rate the presence and intensity of 11 symptoms—which have been divided into three domains: psychological (depression, irritability, anxiety, and mental exhaustion), somatic (sweating/flashing, cardiac complaints, sleep problems, joint and muscle complaints), and urogenital (sexual problems, urinary complaints, vaginal atrophy)—on a scale from 0 to 4.[25] Although the scale can

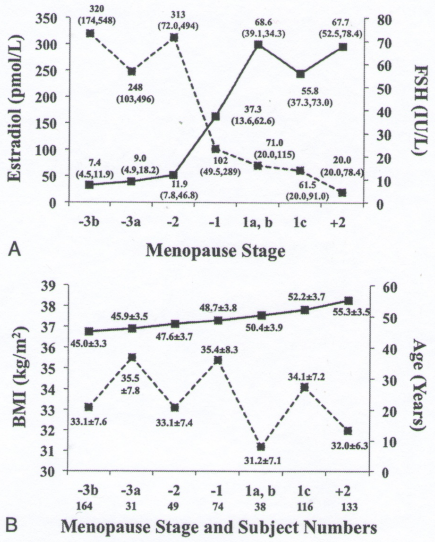

FIG. Estradiol (- - -) and FSH (—) levels by menopause stage: stage −3b (n = 164); stage −3a (n = 31); stage −2 (n = 49); stage −1 (n = 74); stages 1a and 1b (n = 38); stage 1c (n = 116); stage +2 (n = 133). The *P* values for trend (analysis of covariance) are $P < 0.0005$ for both estradiol and FSH and are adjusted for age and BMI **(A)**. Age (—) and BMI (- - -) by menopause stage (n's are the same as for **A**). The *P* value for trend (analysis of variance) is $P < 0.0005$ for age and $P = 0.003$ for BMI (analysis of covariance adjusted for age) **(B)**. Data are presented as mean (SD) or median (interquartile range). FSH, follicle-stimulating hormone; BMI, body mass index.

be self-administered, the MRS—to maintain consistency—was administered by a single interviewer and, where appropriate, team members were trained to interpret the questions in the home language of the participant. The prevalence of symptoms at any level (ie, rating of 1-4) was calculated for each of the 11 symptoms, as was the prevalence of symptoms rated at a level of 3 (severe) or higher (level 4; very severe).

Anthropometric measurements

The weight and height of participants (without shoes and wearing light clothing) were measured using, respectively, a calibrated electronic scale and a fixed-wall stadiometer (Holtain, Crymych, UK). Waist circumference and hip circumference were measured to the nearest 0.5 cm using a soft measuring tape. Body mass index (BMI) was calculated as an estimate of obesity by dividing weight (kilograms) by height (meters) squared. With the participant seated, blood pressure was measured three times, using a digital machine (Omron M6; Omron, Kyoto, Japan) and appropriate cuffs, at 2-minute intervals between readings. The first reading was discarded, and the remaining two values were averaged.

Hormone levels and HIV status

As supportive criteria for self-reported bleeding patterns, serum FSH and E_2 levels were measured in serum samples obtained at the collection site during the 4-hour data collection period. The blood samples were aliquoted into cryovials and stored at 4°C until specialized assays had been performed. Immunoassays were performed for E_2 and FSH as per the manufacturer's instructions (ADVIA Centaur XP Systems; Siemens Healthcare Diagnostics, Tarrytown, NY). Sensitivity (assay range) was 43.6 to 11,010 pmol/L (11.8-3,000 pg/mL) for E_2 and 0.3 to 200 IU/L for FSH.

A voluntary HIV antibody test (Alere Determine HIV-1/2; Alere San Diego Inc, San Diego, CA), using whole blood collected from a fingerstick, was offered to all participants. If the test result was positive, the participant was referred to a local HIV clinic for confirmatory serological testing, CD4 count, and management.

Statistical analyses

Non–normally distributed data were log-transformed to normality before being used in any of the statistical analyses. These data are presented as median (interquartile range), whereas normally distributed data are presented as mean (SD). Continuous variables were analyzed across groups using analysis of variance (ANOVA; or analysis of covariance [ANCOVA] with adjustment for possible confounders), and paired means were compared using Tukey post hoc test. Percentages were compared using χ^2 test. Logistic regression was used to identify the risk of selected menopausal symptoms across menopause stages, with and without adjustment for possible confounding variables (ie, age, BMI, FSH level, and E_2 level). These variables were added individually to each model and also added altogether. A multiple regression model was developed to identify variables associated with age at FMP. Independent variables included in the initial model were chosen based on biological plausibility and previous statistical analyses: BMI (used as a categorical variable with BMI <25.0 kg/m^2 coded as 0 and with BMI ≥25.0 kg/m^2 coded as 1), waist circumference, education (coded with dummy variables using as reference the group that did not attend high school), FSH level, and E_2 level. Backward stepwise regression was performed, with variables removed one at a time (based on their *P* level) until only variables with $P < 0.05$ remained in the model. Pearson univariate correlation analysis was used to determine the relationship between the reported age at FMP and the length of time that had elapsed up until this age was reported to the study investigators (ie, years after FMP). Participants were also divided into quartiles of years after FMP, and the reported age at FMP for each quartile was compared by ANOVA.

RESULTS

Descriptive data of the study population

Table 1 provides the descriptive data of the study cohort. The analytical sample included 702 women; HIV status was recorded for 404 of these women, of whom 21.3% were HIV-positive.

TABLE 1. *Descriptive data of the study cohort*

Variables	Values[a]	Ranges
Age, y	49.2 (5.29)	40.0-61.0
Age at FMP, y[b]	46.0 (4.63)	27.0-57.0
Waist circumference, cm	99.1 (14.6)	47.0-151
BMI, kg/m^2	33.4 (7.32)	16.6-61.6
BMI ≥30 kg/m^2, %	67.8	–
BMI ≥40 kg/m^2, %	16.8	–
HIV-positive, %[c]	21.3	–
ARV use, %[d]	55.3	–
Education, %		
Junior school only	12.3	–
High school, but did not finish	57.7	–
Finished high school with or without higher education	30.0	–
Understand menopause, %	61.9	–

FMP, final menstrual period; BMI, body mass index; HIV, human immunodeficiency virus; ARV, antiretroviral.
[a]Data are presented as mean (SD) or percentage.
[b]n equals 234.
[c]n equals 404.
[d]n equals 85 (n = 702 for the remaining variables).

The frequency of antiretroviral use was 55.3% in HIV-positive women. Participants had a mean (SD) age of 49.2 (5.29) years (Table 1). The mean (SD) age at FMP (n = 234) was 46.0 (4.63) years, which was very low. The data were therefore analyzed in greater detail in subsequent statistical analyses (see Table 2 and its associated description). The prevalence of obesity (BMI ≥30.0 kg/m^2) and extreme obesity (BMI ≥40 kg/m^2) were 67.8% and 16.8%, respectively. Within the study population, 61.9% had an understanding of menopause. Only 30% had finished high school.

Age, BMI, waist circumference, E_2 level, and FSH level by menopause stage

Menopause stage could not be ascertained in 109 (15.5%) of the women because of contraceptive use (n = 64), hysterectomy (n = 44), and unknown reason (n = 1). The number of women in each menopause stage, as assessed using STRAW + 10, was as follows: stage −3b (n = 164); stage −3a (n = 31); stage −2 (n = 49); stage −1 (n = 74); stages 1a and 1b (n = 38); stage 1c (n = 116); stage +2 (n = 133). Figure (A) shows that serum FSH levels increased across the seven groups, and this trend was highly significant ($P < 0.0005$ from ANCOVA adjusted for age and BMI). Mean serum E_2 levels decreased across menopause stages ($P < 0.0005$ from ANCOVA adjusted for age and BMI). Figure (B) shows that, as expected, age was strongly related to menopause stage ($P < 0.0005$ from ANCOVA). BMI, adjusted for age, tended to fall across the menopause stages ($P = 0.003$ for trend from ANCOVA), and there was a weak nonsignificant relationship between menopause stage and waist circumference ($P = 0.07$ from ANCOVA adjusted for age; data not shown).

Age at FMP across quartiles of years after FMP and across BMI groups

Given the very low age at FMP that was observed for all women who reported an age at FMP (n = 234), we undertook further statistical analysis of the data. Thus, Pearson univariate analysis demonstrated a significant inverse relationship between reported age at FMP and the number of years that had elapsed since that event (ie, years after FMP; $r = -0.59$, $P < 0.0005$, n = 234). This suggests that women who had their FMP recently reported an older age at FMP than those who had their FMP much earlier. This is confirmed by the data in Table 2, which show that women in the lowest quartile of years after FMP (≤3 y after FMP) reported a significantly higher age at FMP than women in each of the other quartiles. Within the lowest quartile, there was no significant relationship between reported age at FMP and years after FMP ($r = -0.04$, $P = 0.77$, n = 58). When women were divided into quartiles of years after FMP and a similar univariate analysis was performed for women in the lowest quartiles (≤4 y after FMP), a near-significant inverse relationship was observed ($r = -0.22$, $P = 0.05$, n = 78). These women reported a mean (SD) age at FMP of 48.5 (3.92) years. The data suggest that the reported age at FMP among women within the lowest quartile of years after FMP (mean [SD], 49.0 [3.80] y; Table 2) is the most reliable. When age at FMP was calculated for women who were 2 years or less from FMP (mean [SD], 49.1 [3.94] y; n = 36), it was found to be very similar to that of women who were 3 years or less from FMP. Data in Figure (B) confirm that the age at FMP is 49.0 years. This shows that women at menopause stages occurring immediately before and immediately after the FMP (ie, stages −1, 1a, and 1b) have mean (SD) ages of 48.7 (3.76) and 50.4 (3.94) years, respectively. Age at FMP must fall between these two ages, and 49.0 years does.

Among participants with reliable estimates of age at FMP (ie, those ≤3 y after FMP), age at FMP was lower in women with a BMI less than 25 kg/m^2 compared with women in the higher BMI groups ($P < 0.05$ for all comparisons; Table 3).

TABLE 2. *Age at FMP by quartiles of years after FMP*

Years after FMP	n	Age at FMP, y
≤3 y	59	49.0 (3.80)[a]
>3 to ≤6 y	62	46.6 (3.50)[a,b]
>6 to ≤9 y	50	46.6 (4.00)[a,b]
>9 y	63	42.0 (4.06)
Combined	234	46.0 (4.63)

Data are presented as mean (SD).
FMP, final menstrual period.
[a]$P < 0.0005$ versus ">9 years."
[b]$P < 0.005$ versus "≤3 years."

TABLE 3. *Age at FMP by BMI group in women 3 years or less after FMP*

BMI group	n	Age at FMP, y
<25 kg/m^2	11	45.7 (3.00)
≥25 to <30 kg/m^2	13	49.8 (4.32)[a]
≥30 to <35 kg/m^2	18	49.3 (3.27)[a]
≥35 kg/m^2	17	50.3 (3.39)[a]

Data are presented as mean (SD).
FMP, final menstrual period; BMI, body mass index.
[a]$P < 0.05$ versus "<25 kg/m^2."

Determinants of age at FMP

Multiple regression analysis was used to identify the principal determinants of age at FMP. The women included in this analysis were those who provided details of age at FMP within 3 years of this event (n = 59). The variables that showed a significant association with age at FMP within the final multiple regression model were BMI ($\beta = 0.41$, $P = 0.0007$) and education (completed high school vs did not gain entry to high school; $\beta = -0.31$, $P = 0.009$). The r^2 value for the full regression model was 0.27 ($P < 0.0005$).

Prevalence of menopause symptoms at any level by menopause stage

There was a significantly higher prevalence of VMS and sexual problems in early postmenopause than in the late reproductive stage ($P < 0.05$; Table 4). Irritability was more prevalent in late postmenopause than in the late reproductive and early postmenopause stages ($P < 0.05$ for both). None of the other menopausal symptoms assessed using the MRS showed any significant trends across the menopause stages.

Prevalence of symptoms by BMI group and HIV status

Table 5 shows that although the prevalence of VMS at any level did not change across the BMI groups, the prevalence of severe/very severe VMS was significantly higher in the BMI group with 35.0 kg/m^2 or higher (28.2%) compared with the groups 30.0 kg/m^2 or higher, less than 35.0 kg/m^2 (20.2%; $P < 0.05$), and less than 30.0 kg/m^2 (20.1%; $P < 0.05$). Sleep problems were significantly more common in women with a BMI of 35 kg/m^2 or higher compared with women with BMI between 30.0 and 34.9 kg/m^2. Irritability was significantly more common in the highest BMI group compared with the lowest BMI group, whereas joint problems were less common in the lowest BMI group compared with both of the higher BMI groups.

No significant difference in the prevalence of any of the menopause symptoms was noted between HIV-negative women (n = 318) and HIV-positive women receiving (n = 47) or not receiving (n = 39) antiretrovirals. However, owing to the low number of HIV-positive women, it is possible that the study had insufficient power to detect differences in prevalence.

TABLE 4. *Prevalence of selected menopause symptoms by menopause stage*

Menopause stage	n[a]	Vasomotor symptoms	Sexual problems	Irritability
−3b and −3a	194	55.7	65.8	60.8
−2 and −1	123	64.2	75.5	68.3
1a, 1b, and 1c	153	69.3[b]	79.6[b]	57.9
+2	133	58.6	77.8	72.9[b,c]
All groups combined	603	61.5	72.2	64.2

Data are expressed as percentages.
[a]Sexual problems: stages −3b and −3a (n = 161); stages −2 and −1 (n = 90); stages 1a, 1b, and 1c (n = 103); stage +2 (n = 72); total (n = 426).
[b]$P < 0.05$ versus stages −3b and −3a.
[c]$P < 0.05$ versus stages 1a, 1b, and 1c.

TABLE 5. *Prevalence of symptoms by BMI group*

BMI group	n	Vasomotor symptoms	Irritability	Sleep problems	Joint problems
<30.0 kg/m^2	225	61.6	59.5[a]	62.7	62.9[b,c]
≥30.0 to <35.0 kg/m^2	208	56.7	65.7	56.0[a]	76.3
≥35.0 kg/m^2	266	61.6	69.8	65.0	76.8
All groups combined	699	60.2	65.3	61.6	72.2

Data are expressed as percentages.
BMI, body mass index.
[a]$P < 0.05$.
[b]$P < 0.005$ versus "≥35 kg/m^2."
[c]$P < 0.005$ versus "≥30 kg/m^2" and "<30 kg/m^2."

Effects of menopause stage on symptom risk

Table 6 shows that the odds ratio (OR) for VMS was significantly increased in early postmenopause compared with the late reproductive stage, but that the OR was attenuated after adjustment for FSH levels, but not after adjustment for age, BMI, or E_2 levels. When all four of these possible confounding variables were included together in this model, the results were similar to those found for the model in which only FSH levels were included (Table 6).

The OR for sexual problems was significantly elevated in the early postmenopause stage, and there was also an increased OR in late menopause, but this failed to reach statistical significance ($P = 0.07$). These associations were significantly weakened after adjustment for age, but not after adjustment for FSH level, E_2 level, or BMI. When all four of these possible confounding variables were added together to model 2, the results obtained were similar to those observed with just the inclusion of age in the model (Table 6).

The OR for irritability was significantly increased in stage +2 (Table 6), and adjusting for all possible confounders (ie, age, BMI, FSH level, or E_2 level, either individually or altogether) did not weaken this relationship. Although BMI did not attenuate the significant OR for irritability observed in stage +2 of menopause, it was itself associated with a significant OR for irritability (1.03; 95% CI, 1.00-1.06; $P = 0.03$). This confirms the data in Table 5, which show an increasing prevalence of irritability with rising BMI.

DISCUSSION

The aims of this study were to examine reproductive aging in black urban South African women and to determine whether STRAW + 10 is an accurate and reliable method for staging reproductive aging, as this method has not previously been used in sub-Saharan women. We also noted the age at which FMP occurred and whether obesity has any effect on the MT or has any association with menopause symptoms, especially VMS.

As suggested by Harlow et al,[12] we staged reproductive aging based on reported changes in the menstrual cycles of participants. Bleeding pattern definitions, as described in STRAW + 10, were technically complicated, and participants

TABLE 6. *Logistic regression showing the effects of menopause stage on symptom risk*

Model number and adjustments	Dependent variable	Independent variable	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]
Model 1 without and with adjustment for FSH	Vasomotor symptoms	Stages −2 and −1	1.43 (0.90-2.28); $P = 0.13$	1.22 (0.73-2.03); $P = 0.44$
		Stages 1a, 1b, and 1c	1.80 (1.15-2.81); $P = 0.01$	1.12 (0.64-1.95); $P = 0.68$
		Stage +2	1.13 (0.72-1.77); $P = 0.59$	0.74 (0.41-1.35); $P = 0.33$
		FSH	–	1.01 (1.00-1.02); $P = 0.02$
Model 2 without and with adjustment for age	Sexual problems	Stages −2 and −1	1.60 (0.90-2.87); $P = 0.11$	1.26 (0.68-2.32); $P = 0.46$
		Stages 1a, 1b, and 1c	2.02 (1.13-3.62); $P = 0.02$	1.19 (0.59-2.37); $P = 0.65$
		Stage +2	1.82 (0.95-3.46); $P = 0.07$	0.82 (0.35-1.93); $P = 0.65$
		Age	–	1.09 (1.03-1.16); $P = 0.005$
Model 3 with no adjustment	Irritability	Stages −2 and −1	1.39 (0.86-2.24); $P = 0.18$	–
		Stages 1a, 1b, and 1c	0.88 (0.57-1.37); $P = 0.58$	–
		Stage +2	1.73 (1.07-2.80); $P = 0.02$	–

The reference group is the late reproductive stage (stages −3b and −3a). All models were adjusted for each possible confounder (ie, FSH, estradiol, body mass index, and age) one at a time.
Vasomotor symptoms and irritability: stages −3b and −3a (n = 194); stages −2 and −1 (n = 123); stages 1a, 1b, and 1c (n = 153); stage +2 (n = 133).
Sexual problems: stages −3b and −3a (n = 161); stages −2 and −1 (n = 90); stages 1a, 1b, and 1c (n = 103); stage +2 (n = 72).
OR, odds ratio; FSH, follicle-stimulating hormone.
[a]Only the models in which adjusting for a possible confounder affected the outcomes are shown.

in the pilot study found the terminology difficult to understand. Therefore, the menstrual history and bleeding change related questions that we used in our final questionnaire were basic, and the interviewer used open-ended questions to clarify the responses. This mixed approach to self-reported VMS has been recommended in studies and reviews where cultural differences are apparent.[26,27] The accuracy of menopause staging is strongly dependent on the participants' understanding of the terms used when asked about bleeding patterns, as described by Smith-DiJulio et al.[28] Although more than two thirds of our participants had a very low educational level and nearly half of the cohort did not understand the meaning of the term *menopause*, the women were able to give reasonably precise information about changes in bleeding patterns such that we could stage their reproductive aging using STRAW + 10 criteria. This correct staging was confirmed by the FSH and E_2 trends. A strength of our study was the consistency in interpreting reported information on menstrual cycle changes (each participant was interviewed by a single interviewer). Our results showed a strong association between the reproductive stages, as described by STRAW + 10, and serum FSH level, serum E_2 level, and age. The two defining endocrine changes that occur during the MT are rising FSH levels and falling E_2 levels.[29,30] In our study, FSH levels increased gradually from the late reproductive stage through the late MT, accelerated rapidly around the time of FMP, and reached a plateau during the postmenopause stages. E_2 levels decreased consistently from the early MT stage to the last postmenopause stage. These changes in serum FSH and E_2 levels across the MT were comparable with those observed in a large longitudinal investigation of menopause-associated endocrine changes—the Study of Women's Health Across the Nation (SWAN).[15]

The mean age at FMP was lower than that observed in Western women. In SWAN, the median age at natural menopause was 51.4 years[31]; in a recent European study where age at menopause was estimated in 5,288 women, it was 54.0 years.[32] When we took recall bias into account, the mean age at FMP in SWEET was 49.0 years. This is similar to that of other African studies.[33] A very small study of 88 women in Zaria, Nigeria, reported a mean age at FMP of 46.1 years.[34] However, in a larger study of Nigerian women (N = 402), Ozumba et al[35] reported a mean age at FMP of 49.4 years, whereas in a smaller study of Ghanaian women (N = 152), the mean age at FMP was 48.05 years.[36]

It seems that the number of years since FMP affects accurate recall of FMP among SWEET participants; those 3 years or less from FMP reported a mean age at FMP of 49.0 years, whereas those who were interviewed more than 9 years after FMP had a reported mean age at FMP of 42.0 years. Hahn et al[37] found that inaccurate recall of FMP increases with years since menopause and is greater in women with natural menopause. A study in Sweden found that 565 women who first reported age at FMP in 1992 recalled it reasonably accurately after nearly 20 years. Rödström et al,[38] in a cross-sectional study, suggested that recall of age at menopause among women aged 60 years or younger is more reliable than recall of age at menopause among women long past menstruation. Other studies have also shown that age at menopause is recalled less accurately with increasing time from FMP, but none has observed a systematic lowering of the reported age at FMP with increasing time from FMP.[37-40] However, all these studies have involved a comparison of recalled age at menopause at two different time points after menopause (ie, to test for reproducibility) compared with the current study where age at FMP was obtained at one time point only. Sievert[41] suggested that the transition into menopause is gradual; thus, when women are asked to recall their age at FMP, they often have to rely on memory, looking back across a long time continuum to recall the exact time of cessation. A limitation in our estimation of age at menopause is that it was a cross-sectional analysis. Age at FMP can be more accurately predicted in longitudinal studies, where the participants use menstrual calendars.[42]

It is not clear why the women in our study had poor recall of FMP. A lack of understanding of the MT and a poor primary healthcare system with limited access to gynecologists may have affected recall because these women were not questioned about their MT and therefore did not need to recall information about it. In addition, economic status and cultural values on menopause and aging may have also played a role in

the women in our study. We need to further examine whether the MT has the same level of importance for black urban South African women as it seems to have in Western women.

We found that there was a strong negative association between level of education and age at FMP in SWEET participants. This finding differs from other studies, where low levels of education were associated with younger age at menopause.[31,43] We were unable to explain this association, but the small sample in this group (n = 59) may have been a factor; it is possible that this association could be explained by a confounding variable that was not measured in our study. Another factor that has been shown to modulate age at FMP is smoking status. Research has shown that current smoking is associated with earlier age at natural menopause.[31,44] Only 3% of the women in our study were current smokers; therefore, we were unable to analyze the effects of smoking status on age at FMP.

The reported FMP was significantly lower in women with a BMI less than 25 kg/m^2 compared with those participants with higher BMI. Akahoshi et al[45] found that higher BMI was associated with later menopause, unlike SWAN, where Gold et al[31] found that BMI was not related to age at natural menopause. However, this negative association between age at FMP and BMI was observed in a small subsample of our study participants (n = 59) and needs to be confirmed in a much larger cohort with accurate recall of age at FMP.

In the current study, the prevalence of severe/very severe VMS was significantly higher in very obese women. Data from SWAN also showed a positive association between VMS and body fat level.[46] Thurston and Joffe[5] found that obesity is a strong risk factor for VMS, especially in the late MT and early postmenopause stage. In our participants, VMS were also strongly associated with early MT, late MT, early postmenopause, and increased FSH levels. Thurston and Joffe[5] reported findings similar to those of Cray et al,[47] who found similar results in participants from the Seattle Midlife Women's Health Study. The reproductive staging criteria from STRAW + 10 describe VMS in the late MT and early menopause.[12] Other than VMS, only two other symptoms were strongly associated with menopause stage in SWEET participants: sexual problems and irritability. Sexual problems manifested most strongly in the early and late postmenopause stages and were associated with age. There was a significant risk of increased irritability in late menopause, which is similar to the findings of Rahman et al,[48] who also used the MRS to determine the prevalence and severity of menopausal symptoms in their participants. However, in a similar study in Omani women, the association of increased irritability was not significant at this stage.[49] In our study, no possible confounders (BMI, age, FSH level, or E_2 level) weakened the association of irritability with late postmenopause, although increased BMI was associated with a higher prevalence of irritability and a higher OR for irritability. Among the symptoms described in the psychological domain of the MRS (depression, irritability, anxiety, and mental exhaustion), irritability was the only symptom in our cohort that was associated with menopause stage. Several studies have shown that there is an increased risk of depression and mood problems during perimenopause,[50] but Kornstein et al[51] found that irritability was more prevalent in premenopausal women, whereas postmenopausal women were more likely to experience depressive episodes.

The prevalence of VMS reported in the current study for women in the late reproductive stage was higher than those in other studies that used the MRS to determine frequency and severity. Blumel et al[52] reported a VMS prevalence of 37.1% in the premenopause stage in their participants, and a similar prevalence (35.4%) was reported in Malaysian women,[48] compared with the 55.7% VMS prevalence in our participants. The prevalence of VMS in our study is associated with increased levels of FSH. However, there may also be a cultural determinant in the way that women from SWEET describe hot flashes. Many cross-cultural studies have suggested that there are biological and cultural determinants in describing VMS, and they recommended using a biocultural approach.[27,53] Gold et al[6] suggested that VMS are highly associated with menopause stage, whereas differences in absolute rates are related to cultural differences. Other studies suggested that night sweats and hot flashes be reported and analyzed separately, rather than being grouped together as VMS.[54] A limitation of our study was that English was not the first language of the participants, and although interpreters were available, some idiomatic meanings may have been lost in translation.[55]

We found no differences in the prevalence of menopausal symptoms between HIV-positive and HIV-negative women. However, several studies reported that HIV infection is associated with an increased risk of VMS.[20,56,57] Boonyanurak et al[21] found that HIV-positive women in their study had a high prevalence of hot flashes, but the number of HIV-positive women in our study was small (n = 86) compared with their sample (N = 268). Therefore, it is possible that we had insufficient power to detect differences in the prevalence of VMS and other menopausal symptoms between HIV-positive and HIV-negative women. Although some studies found that HIV-infected women had younger age at menopause,[58] we were unable to analyze whether HIV affected age at FMP, as the women in our study became infected with HIV or were tested for HIV antibodies after FMP. In their comprehensive review of HIV and menopause, Kanapathipillai et al[59] suggested that research to date has failed to show that age at menopause in HIV-positive women is younger or older than that in HIV-negative women, and that more research is needed.

CONCLUSIONS

STRAW + 10 is appropriate for staging menopause in resource-limited countries that use information on self-reported bleeding criteria, but there is a need for validated interviewer questions and simplification of technical terms to improve accuracy. The terminology used by STRAW + 10 may not be easily generalizable to other groups of women in rural and informal urban settlements. Years since FMP affects accurate recall and is most accurate in women less than 4 years from FMP. In this group, lower education levels are associated with older age at FMP, whereas leanness is related to

earlier menopause. Only three symptoms (VMS, sexual problems, and irritability) are significantly related to menopause stage. As expected, FSH levels affect the prevalence of VMS across the menopause stages, and obesity is strongly associated with a risk for severe VMS. Longitudinal investigations in this group of women are needed to further clarify the issues discussed above.

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Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): A perspective of African women who have a high prevalence of obesity and HIV infection

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ABSTRACT

Objectives. Little data are available for sub-Saharan African women on changes in body composition in menopause transition (MT). The study aimed to determine whether there are differences in body adiposity, lean muscle mass, and bone mineral density (BMD) across MT groups in urban African women, who have a high prevalence of obesity and HIV infection, and if this is related to an altered hormonal milieu.

Design. Participants were 702 black urban women. Menopause stage was defined using STRAW+10 criteria. Levels of follicle stimulating hormone (FSH), estradiol (E2), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone (T) and sex hormone blinding globulin (SHBG) were measured. Body composition was measured with dual-energy X-ray absorptiometry (DXA) and ultrasound scans.

Results. Whole body lean mass ($p = 0.002$) and BMD ($p < 0.0005$) were significantly lower in postmenopausal compared to premenopausal groups. Estradiol ($p < 0.0005$), SHBG ($p < 0.0005$) and DHEAS ($p = 0007$) were significantly lower in post- than premenopausal groups, while FSH was higher ($p < 0.0005$). FSH correlated negatively ($\beta = -2.06$, $p < 0.0005$) with total lean mass while E2 correlated positively ($\beta = 20.0$, $p = 0.002$) with BMD. Use of antiretroviral therapy (ART) correlated negatively with total fat mass ($\beta = -2.92$, $p = 0.008$) and total bone mineral content (BMC; $\beta = -78.8$, $p = 0.003$).

Conclusions. The MT in this population is characterized by lower whole body lean mass and BMD in post- compared to premenopausal subjects but there are minimal differences in fat mass. Lower lean mass and BMD were associated with higher FSH and lower E2 serum levels, respectively. Use of ART was associated with lower fat mass and BMC.

Abbreviations: MT, menopause transition; BMD, bone mineral density; BMI, body mass index; BMC, bone mineral content; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; FSH, follicle-stimulating hormone; E2, estradiol; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; T, testosterone; SHBG, sex hormone-binding globulin; STRAW+10, stages of reproductive aging workshop; ART, antiretroviral therapy.

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1. Introduction

The menopause transition (MT) is closely associated with changes in body composition including lower bone mineral density (BMD) at many skeletal sites [1], an increase in obesity [2], a decrease in lean muscle mass [3], increases in body mass index (BMI) [2], and changes in body fat distribution (BFD) [4], particularly increased central adiposity [5]. Abdominal obesity is a principal risk factor for cardiometabolic disease [6]. Some studies suggest changes in the abdominal deposition of visceral (VAT) and subcutaneous adipose tissue (SAT) during the MT is related to chronological aging [7], while others find a strong association with reproductive aging [8], suggesting that central adiposity is a result of the changing hormonal milieu [5]. Other data show that both may explain changes in body adiposity and a decrease in lean muscle mass (sarcopenia) during the MT [9].

Obesity is widely prevalent among mid-life, black South African women [10]. The data from the Study of Women Entering and in Endocrine Transition (SWEET) show a high rate of obesity at menopause (68%) [11] and previous investigations have shown that diabetes and metabolic syndrome are very prevalent in these women [12]. The causes of this are not known, but given the strong association between MT and changes in BFD and lean muscle mass reported in non-African populations [13], the MT may play a role. The subject of central adiposity and its strong association with non-communicable diseases (NCDs) [14], the relationship between androgens and VAT deposition during MT [15], and the fall in BMD at various skeletal sites observed during the MT are well reported in women from high-income countries [1] but no such data appear to be available in sub-Saharan African menopausal women. A recent study from South Africa demonstrated a strong relationship between lean mass and BMD in African male and female subjects [16]. However, it is not known whether this relationship occurs across the MT and whether changes in lean mass during this period will affect BMD. In addition, the prevalence of HIV infection is high in populations of urban, mid-life black South African females [11], but it is not known whether this contributes to changes in body composition.

The aims of our study were to determine whether general body adiposity, lean muscle mass, BFD and BMD are associated with stages of the MT in these women, and if so, whether this association is related to differences in the serum concentrations of follicle stimulating hormone (FSH), estradiol (E2), androgens and sex hormone binding globulin (SHBG).

2. Methods

2.1. Subjects

The women in this cross sectional study are participants in SWEET and are the biological mothers and caregivers of the children in the Birth to Twenty Plus (BT20) cohort, the largest and longest-running longitudinal birth cohort study of child health and development in Africa [17]. After 21 years, 2200 of these women are still in contact with the study. From that group, we contacted a convenience sample of 902 women, ensuring that the minimum number of participants in the study cohort would be defined as that at which at least 100 women were present in each of the following four study subgroups (based on menopause staging using STRAW+10 guidelines (18)): late reproductive (stages −3b and −3a), the MT (stages −2 and −1), early postmenopause (stages 1a, 1b, and 1c), and late postmenopause (stage +2). Exclusion criteria were: <40 years and >60 years, pregnancy and ethnicity other than black African. Within this group of 902 women, 35 were older than 60 years, 37 were deceased, 3 were terminally ill, and 46 had become untraceable, or now lived outside the study area. Other women (n = 79) refused to participate for 2 main reasons; they were unable to take time off from work, or were no longer interested in the study and did not believe it would benefit them. Therefore 702 women took part in the study. All participants signed informed consent forms. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand approved the protocol (ethics certificate number M090620).

2.2. Questionnaires and Menopausal Transition Stage

There are 11 official languages in South Africa, and English is the language most commonly used though it is not the first language of the majority of the participants. Questionnaires were administered in English by a single researcher, with members of our research team whose first language corresponded with those of the participants being available to help those women who were unable to understand any question. Reproductive health, menstrual history, educational level and tobacco and smokeless tobacco (snuff) use were determined and questions were derived from the general health questionnaire formulated and validated in a previous study of the same population group [19]. The STRAW+10 questionnaire derived from STRAW+ 10 criteria [18] and was used to ascertain menopause stage. These stages are late reproductive (−3b, −3b); early menopausal transition (−2); late menopausal transition (−1); early postmenopause (+1a, +1b, +1c) and late postmenopause (stage +2). This questionnaire has not previously been used on African females but a previous publication has shown that it is valid for staging menopause in this population [11].

2.3. Hormone Assays

Fasting blood samples were obtained in the morning before 11 am, during the 4-h data collection period. Serum and plasma samples were collected and aliquoted into corresponding cryovials and immediately stored at −80°C until the assays were performed. Levels of FSH, E2, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone and SHBG were measured in serum samples. Immunoassays were performed for E2 and FSH as per manufacturer's instructions (ADVIA Centaur XP Systems, Siemens Healthcare Diagnostics, Tarrytown, NY). The E2 assay is a competitive chemiluminescent immunoassay and assay range is 43.6–11,010 pmol/L. Intra- and interassay coefficients of variation (CVs) for E2 averaged 4.2% and 1.9% respectively. The FSH assay is a two-site sandwich chemiluminometric immunoassay and the assay range is 0.3–200 IU/L, with the intra- and interassay CVs averaging 2.4% and 1.5% respectively. The immunoassays for SHBG and DHEAS were performed on the Immulite 2000 Systems

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analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). The SHBG measurements were performed using a solid phase two-site chemiluminometric immunoassay. The intra-assay and total CV for SHBG was 3.06% and 4.40% respectively, while the assay range was 0.02–180 nmol/L. The DHEAS measurements were performed using a solid phase competitive chemiluminescent enzyme assay. The intra-assay and total CV for DHEAS was 7.1% and 9.8% respectively and the assay range was 0.41–27.0 μmol/L. DHEA was measured with a solid phase competitive binding, enzyme-linked immunosorbent assay (DRG instruments, Marburg, Germany). Reported assay range is 0–30 ng/mL. Intra- and interassay CVs were 5.1% and 6.8% respectively. Testosterone extraction was performed using a liquid–liquid extraction method according to Benton et al. [20]. Ten microliters of sample was injected onto an ultraperformance liquid chromatography mass spectrometer (Micromass Quattro micro API Mass Spectrometer, Waters, Milford, MA). The lower limit of detection was 0.25 nmol/L and average intra- and interassay CVs were 7.85% and 9.23% respectively. Free and bioavailable testosterone levels were calculated using the method of Sodergard et al. [21].

2.4. *HIV Testing*

A voluntary HIV antibody test, Alere Determine™ HIV-1/2 (Alere San Diego, Inc. San Diego, CA), was offered to all participants. If found positive, the participant was referred to a local HIV clinic for confirmatory serological testing and CD4 count and management. Both HIV positive women who were being treated with antiretroviral medication, and HIV positive women, who were not, were maintained in the study.

2.5. *Simple Measures of Body Anthropometry*

Participants without shoes, wearing light clothing, were weighed and their height measured, using respectively, a calibrated electronic scale and a fixed-wall stadiometer (Holtain, Crymych, UK). Waist and hip circumferences were measured with a soft measuring tape to the nearest 0.5 cm; the former at the smallest girth above the umbilicus and the latter at the greatest circumference of the hips. Trained technicians performed all measurements. The intra-observer coefficient of variation (CV) for height, weight, and hip circumference was less than 1% and less than 2% for waist circumference. Inter-observer CV for height, weight, hip circumference and waist circumference was less than 1%. Body mass index was calculated by dividing weight (kilograms) by height (meters) squared.

2.6. *Dual-Energy X-ray Absorptiometry (DXA) Measurements*

A single trained technician carried out whole body DXA scans using a Hologic Discovery A (S/N 83145) DXA machine (Bedford, MA, USA software version 12.5:7). The women removed clothing and all metal objects and wore surgical gowns for the procedure. Whole body scans were analyzed using whole body less head because many participants wear wigs and hair weaves that could not be removed; these hairpieces are similar in density to soft tissue and may have caused measurement artefact. For this study we measured sub-total (whole body less head) fat mass, lean mass, BMD and bone mineral content (BMC), as described in a previous study [16]. The terms 'whole body' are used in the remainder of this paper when referring to these sub-total measures of lean and fat mass and BMD and BMC. During data collection the DXA phantom was scanned each morning to examine the CV of the DXA machine and we found the CV to be less that 0.5% for all parameters.

2.7. *Ultrasound Measurements of VAT and SAT*

Visceral and subcutaneaous adipose tissues were measured by a trained operator using a GE LOGIQ e ultrasound machine with a 2–5.5 MHz 4C-RS curved transducer (GE Healthcare, Piscataway, NJ, USA). We defined VAT thickness as the distance in centimeters (cm) from the peritoneum to the vertebral bodies and SAT thickness as the depth in cm from the skin to the linea alba. In order to visualize the relevant anatomical structures the scan depth was set at 15 cm for the VAT and 9 cm for SAT. The site for both measurements was where the xyphoid line and waist circumference meet. The CV for our ultrasound measurements was less than 2%. The SAT and VAT thicknesses were converted to areas using published and validated equations [22].

2.8. *Statistical Analyses*

Data that were not normally distributed (all hormone measures) were log transformed to normality before being used in the statistical analyses. These data are presented as median (interquartile range) in the tables and text, while data with a normal distribution (all anthropometric measures) are expressed as mean ± SD. Continuous variables were analyzed across groups using ANOVA and ANCOVA, and paired means were compared using Tukey's *post hoc* test. Multivariate linear regression analysis was used to identify the principal correlates of each of the body anthropometry variables. Independent variables included in each model were chosen based on scientific plausibility and correlation with the outcome variable in univariate analyses, with $p < 0.50$. Backward, stepwise regression was performed. Height, total fat mass and total lean mass were included in all initial regression models to reduce the chance of confounding, but exceptions to this were the models for BMI (only total lean mass included), total fat mass (only height and total lean mass included), and total lean mass (only height and total fat mass included). Collinearity was tested using the variance inflation factor (VIF) and variables were excluded if VIF was greater than 10.0. Collinearity was present for total, free and bioavailable testosterone and SHBG, and from these 4 highly inter-related variables only SHBG and free testosterone could be included in the same regression models. Collinearity was also observed for BMI and total fat mass and therefore only the latter variable was included in the relevant regression model.

3. Results

3.1. *Subject Characteristics*

Menopausal stage was not determined in 112 women due to hysterectomy (n = 47), contraceptive use (n = 64) and unknown reason (n = 1). Participants in each stage were as

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follows: late reproductive (n = 194); early and late menopausal transition (n = 122); early postmenopause (n = 144) and late postmenopause (n = 130).

The characteristics of this study population have been reported previously [11]. However, a brief overview of this population will be provided here as this is relevant to the current study. Thus, obesity was highly prevalent at 67.8%, with a mean BMI (±SD) of 33.4 ± 7.32. The mean age (±SD) was 49.2 ± 5.29 years, and was much higher ($p < 0.0005$) in the postmenopausal than the premenopausal groups (Table 1). Among the participants, 404 knew or agreed to have their HIV status measured and 21.3% were HIV positive, of whom 55.3% were receiving antiretroviral therapy (ART). Table 1 shows that HIV-positivity was less common in the group at menopause stage +2 than at menopause stage −3b and −3a ($p < 0.05$). The use of ART did not differ significantly between the menopausal stages (Table 1). Within the study cohort 30% had finished high school, as previously reported [11], the employment level was 57% and 20.9% of the participants were snuff users.

3.2. *Anthropometric Variables and Menopause Transition Stages*

Anthropometric measurements (Table 1) show a trend of lower BMI ($p = 0.06$) and SAT ($p = 0.05$) in females in the postmenopausal groups, while whole body lean mass ($p = 0.002$), and lean mass at all body sites were lower. Whole body BMD and BMC were significantly lower in the postmenopausal groups ($p < 0.0005$ for both).

3.3. *Hormone Levels and Menopause Transition Stages*

As expected E2 levels were lower ($p < 0.0005$) and FSH ($p < 0.0005$) levels were higher in the postmenopausal groups (Table 2). These data are shown in detail in a previous publication [11]. Both SHBG and DHEAS respectively, were significantly lower ($p < 0.0005$ and $p = 0.007$) in the postmenopausal groups.

3.4. *Effect of HIV on Anthropometric and Hormonal Variables*

Table 3 shows that the groups of HIV-positive, ART-naïve ($p < 0.05$) and HIV-positive, ART-treated ($p < 0.0005$) women were both younger than the HIV-negative women, and also had higher total testosterone ($p < 0.005$ for both groups) and SHBG ($p < 0.05$ and $p < 0.005$ respectively) levels. The HIV-positive, ART-treated subjects had lower BMI ($p < 0.0005$ and $p < 0.005$ respectively), hip circumference ($p < 0.0005$ and $p < 0.005$ respectively), subcutaneous fat area ($p < 0.0005$ and $p < 0.05$ respectively), whole body fat ($p < 0.0005$ and $p < 0.05$ respectively) and lean mass ($p < 0.005$ and $p < 0.05$ respectively), whole body BMD ($p < 0.05$ for both) and BMC levels ($p < 0.0005$ and $p < 0.005$ respectively) and serum DHEAS levels ($p < 0.005$ and $p < 0.05$ respectively) than the HIV-negative and HIV-positive, ART-naive groups. The HIV-negative group had a higher waist circumference ($p < 0.0005$) and more visceral fat ($p < 0.005$) than the HIV-positive, ART-treated group. As shown by ANCOVA none of the differences in hormonal levels across the groups were explained by the differences in age or anthropometry.

Table 1 – Age, HIV status and ART use, and anthropometric measurements across menopausal stages.

Anthropometric variables	Menopausal stages (from STRAW+10) and n's				P-value for trend
	−3b & −3a n = 194	−2 & −1 n = 122	1a, 1b & 1c n = 144	+2 n = 130	
Age (years)	45.1 ± 3.30	48.3 ± 3.75	51.8 ± 3.86	55.3 ± 3.50	<0.0005
HIV infection (%) [a]	28.8	24.2	17.3	14.8	0.02 [c]
ART use (%) [b]	59.4	50.0	57.1	69.2	NS [d]
BMI (kg/m^2)	33.5 ± 7.64	34.5 ± 8.03	33.4 ± 7.28	32.0 ± 6.32	0.06
Waist (cm)	99.3 ± 14.9	101 ± 16.3	99.6 ± 14.8	98.3 ± 12.6	0.60
Hip (cm)	118 ± 15.7	120 ± 15.8	119 ± 16.4	116 ± 13.2	0.22
Visceral fat (cm^2)	94.4 ± 40.6	97.7 ± 42.8	96.3 ± 38.9	89.4 ± 34.3	0.35
Subcutaneous fat (cm^2)	400 ± 134	408 ± 133	395 ± 121	367 ± 109	0.05
Arm fat (kg)	3.77 ± 1.31	3.88 ± 1.38	3.68 ± 1.19	3.70 ± 1.24	0.61
Arm lean (kg)	4.62 ± 0.86	4.48 ± 0.95	4.31 ± 0.83	4.29 ± 0.70	0.001
Leg fat (kg)	14.3 ± 5.00	14.2 ± 4.49	14.1 ± 4.75	14.0 ± 4.48	0.97
Leg lean (kg)	16.1 ± 2.88	15.6 ± 3.19	15.2 ± 3.01	15.1 ± 2.38	0.007
Trunk fat (kg)	14.3 ± 5.09	15.1 ± 5.54	14.6 ± 5.14	14.2 ± 4.88	0.55
Trunk lean (kg)	22.4 ± 3.39	22.1 ± 3.84	21.2 ± 3.39	21.3 ± 2.93	0.003
Whole body fat (kg)	32.3 ± 10.4	33.2 ± 10.3	32.4 ± 10.3	32.0 ± 9.63	0.82
Whole body lean (kg)	43.2 ± 6.74	42.2 ± 7.68	40.7 ± 6.93	40.7 ± 5.62	0.002
Whole body BMD (mg/cm^2)	935 ± 77.9	931 ± 80.5	889 ± 77.6	866 ± 76.2	<0.0005
Whole body BMC (g)	1702 ± 232	1661 ± 247	1566 ± 242	1539 ± 232	<0.0005

Data expressed as percentage or mean ± SD; BMD = bone mineral density, BMC = bone mineral content, NS = non-significant.
[a] Subject numbers per menopausal stage who agreed to an HIV test were 111, 66, 81 and 88 respectively.
[b] Subject numbers per menopausal stage who were HIV-positive were 32, 16, 14 and 13, respectively.
[c] p-value from χ^2 test for menopausal stage −3b, −3a vs stage +2.
[d] Using the χ^2 test no differences were noted in frequency of ART use across the 4 groups.

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Table 2 – Hormone concentrations across menopausal stages.

Hormones	Menopausal stages (from STRAW+10)				P-value for trend
	−3b & −3a n = 194	−2 & −1 n = 122	1a, 1b & 1c n = 144	+2 n = 130	
Estradiol (pmol/L)	315 (369)	130 (362)	63.0 (74.0)	20.0 (52.0)	<0.0005
FSH (IU/L)	7.55 (7.80)	26.2 (52.4)	57.6 (41.7)	67.7 (25.9)	<0.0005
Total T (pmol/L)	650 (430)	610 (410)	612 (340)	595 (460)	0.18
Bioavail. T (pmol/L)	256 (155)	255 (133)	267 (128)	254 (138)	0.88
Free T (pmol/L)	10.0 (6.79)	10.3 (5.56)	10.3 (4.59)	10.1 (6.52)	0.74
SHBG (nmol/L)	61.0 (33.8)	52.2 (36.3)	49.7 (28.1)	50.3 (28.3)	<0.0005
DHEA (ng/ml)	2.90 (2.80)	2.50 (2.50)	2.50 (3.30)	2.45 (3.20)	0.73
DHEAS (μmol/L)	1.40 (1.40)	1.30 (1.00)	1.10 (1.10)	0.95 (1.00)	0.007

Data expressed as median interquartile range (IQR); T = testosterone, and Bioavail = bioavailable.

3.5. *Correlates of Anthropometric Variables*

In the univariate analyses preceding multiple regression, age correlated significantly only with visceral fat ($r = 0.08$, $p = 0.04$). Linear multiple regression analyses demonstrated that SHBG correlated negatively with 6 of the 8 anthropometric dependent variables i.e. BMI, total fat and lean masses, waist circumference, visceral fat and total BMD, while DHEAS correlated negatively with total fat mass and positively with total lean mass and total BMD (Table 4). Serum levels of DHEA correlated only with subcutaneous fat area. Estradiol correlated positively with BMD and BMC, while regression model 3 demonstrates that levels of FSH correlated negatively with total lean mass. The FSH levels also correlated negatively with BMI (outcome variable) in a univariate regression model ($\beta = -1.26$, $p = 0.006$) but this relationship was severely attenuated ($\beta = 0.43$, $p = 0.15$) if total lean mass was included as an independent variable. Models 1 and 2 show that ART use was negatively associated with BMI and total fat mass. Employment and education level both correlated negatively with waist circumference and visceral fat area whereas use of snuff was positively associated with visceral fat and negatively associated with total BMD.

3.6. *Investigation of Explanatory Anthropometric Variables Across Different Menopause Transition Stages*

Differences across the MT observed for lean mass, BMD and BMC, which were analyzed using ANOVA (Table 2), were further analyzed using ANCOVA to determine which variables

Table 3 – Anthropometric measures and hormone levels according to HIV status and therapy.

Variables	HIV-negative (n = 318)	HIV-positive, ART-naïve (n = 39)	HIV-positive, ART-treated (n = 47)
Age (years)	49.7 ± 5.33	47.6 ± 5.19 *	46.3 ± 5.04 ***
BMI (kg/m^2)	33.2 ± 6.25 †††	33.5 ± 7.88 ††	28.8 ± 7.86
Waist (cm)	99.7 ± 13.4 †††	96.7 ± 13.3	91.4 ± 16.1
Hip (cm)	118 ± 13.2 †††	119 ± 16.6 ††	109 ± 16.7
Visceral fat (cm^2)	94.3 ± 37.5 ††	82.8 ± 36.9	73.5 ± 42.4
Subcutaneous fat (cm^2)	389 ± 106 †††	387 ± 137 †	315 ± 129
Whole body fat (kg)	32.8 ± 9.17 †††	31.7 ± 9.98 †	25.6 ± 12.3
Whole body lean (kg)	42.0 ± 6.76 ††	41.9 ± 7.22 †	38.1 ± 6.66
Whole body BMD (mg/cm^2)	911 ± 84.0 †	916 ± 77.4 †	872 ± 70.8
Whole body BMC (g)	1634 ± 247 †††	1654 ± 228 ††	1482 ± 235
Estradiol (pmol/L)	85.0 (272)	88.0 (322)	116 (306)
FSH (IU/L)	29.8 (60.2)	41.5 (53.5)	24.5 (60.1)
Total T (pmol/L)	580 (360)	770 (470) **	760 (600) **
Bioavail. T (pmol/L)	250 (136)	272 (139)	286 (160)
Free T (pmol/L)	9.92 (5.66)	11.8 (5.52)	11.2 (7.16)
SHBG (nmol/L)	51.6 (36.5)	67.9 (34.2) *	77.1 (56.3) **
DHEA (ng/ml)	2.40 (2.30)	2.40 (2.30)	2.20 (1.60)
DHEAS (μmol/L)	1.20 (1.00) ††	1.10 (1.0) †	0.80 (1.00)

Data expressed as mean ± SD or median interquartile range (IQR); BMD = bone mineral density, BMC = bone mineral content, T = testosterone, and Bioavail = bioavailable.

* $p < 0.05$ vs HIV-negative.
** $p < 0.005$ vs HIV-negative.
*** $p < 0.0005$ vs HIV-negative.
† $p < 0.05$ vs HIV-positive with ART.
†† $p < 0.005$ vs HIV-positive with ART.
††† $p < 0.0005$ vs HIV-positive with ART.

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Table 4 – Multivariate regression models for anthropometric measures.

Model number	Dependent variable	Independent variable with unstandardized β (p-value)	Adjusted R^2 (p-value) for full model
1	BMI (kg/m^2)	Lean mass 0.68 (<0.0005) Use of ART −1.49 (0.02) SHBG (log) −1.71 (0.04)	0.64 (<0.0005)
2	Total fat mass (kg)	Height −0.12 (0.04) Lean mass 1.13, (<0.0005) Use of ART −2.92 (0.008) SHBG (log) −3.29 (0.02) DHEAS (log) −1.61 (0.04)	0.60 (<0.0005)
3	Total lean mass (kg)	Height 0.29 (<0.0005) Fat mass 0.42 (<0.0005) FSH (log) −1.65 (<0.0005) SHBG (log) −2.19 (0.003) DHEAS (log) 1.06 (0.005)	0.64 (<0.0005)
4	Waist (cm)	Height −0.31 (<0.0005) Fat mass 0.60 (<0.0005) Lean mass 0.86 (<0.0005) SHBG (log) −5.05 (<0.0005) Employed −1.44 (0.02) Age 0.15 (0.009) Graduated HS −1.77 (0.008)	0.70 (<0.0005)
5	Visceral fat (cm^2)	Height −0.51 (0.007) Fat mass 1.11 (<0.0005) Lean mass 2.32 (<0.0005) SHBG (log) −17.9 (0.0007) Employed −5.37 (0.02) Graduated HS −7.02 (0.006) Take snuff 6.66 (0.02)	0.46 (<0.0005)
6	Subcutaneous fat (cm^2)	Height −6.64 (<0.0005) Fat mass 7.16 (<0.0005) Lean mass 6.37 (<0.0005) DHEA (log) 12.7 (0.003)	0.91 (<0.0005)
7	Total BMD (mg/cm^2)	Lean mass 4.79 (<0.0005) SHBG (log) −31.5 (0.05) DHEAS (log) 16.1 (0.01) Estradiol (log) 20.0 (0.002) Stage 1a, 1b, 1c −20.2 (0.01) Stage 2 −37.9 (<0.0005) Take snuff −14.6 (0.04)	0.33 (<0.0005)
8	Total BMC (g)	Height 9.65 (<0.0005) Lean mass 20.6 (<0.0005) Estradiol (log) 69.5 (<0.0005) Use of ART −78.8 (0.003)	0.59 (<0.0005)

Variable coding: employed subjects were compared to unemployed subjects, those who used snuff were compared against those who did not and subjects who used ART were compared with those who were ART naïve; subjects who attended but did not graduate and subjects who graduated high school (HS) were compared with those who did not attend; for menopausal stages, stage −3b with −3a was used as the reference group.

may confound these results. Variables correlating with lean mass, BMD and BMC in the regression models (Table 4) were likely to be possible confounders and these were used as co-variates in the ANCOVA, with menopausal stage used as the grouping variable and lean mass and BMD as the dependent variables. The ANCOVA for lean mass demonstrated that including FSH as a co-variate dramatically reduced the unadjusted F-value for the grouping variable from 4.90 ($p = 0.002$) (Table 2) to 0.05 ($p = 0.98$). The ANCOVA for BMD showed that including E2 as a co-variate reduced the unadjusted F-value from 25.2 ($p < 0.0005$; (Table 2)) to 7.89, but with no major lessening of significance ($p < 0.0005$). With regard to BMC, the unadjusted F-value ($F = 15.5$, $p < 0.0005$; (Table 2)) was reduced by including ART use ($F = 7.77$, $p < 0.0005$) or estradiol ($F = 5.31$, $p = 0.001$), but including both variables in the same ANCOVA had a far more dramatic effect ($F = 1.89$, $p = 0.13$). Adjusting for other co-variates in any of the three ANCOVAs had minimal effects. It is possible that the relationships between hormones and body composition are bi-directional and therefore to test this hypothesis we performed ANCOVA in which the dependent variable was FSH or E2 and the co-variate was lean mass or BMD, respectively. We also set up an ANCOVA with E2 as the dependent variable and BMC as the co-variate. These analyses (data not shown) showed that the presence of lean mass, BMD or BMC as a co-variate had minimal effects on the F- or p-values within these ANCOVAs, suggesting that the relationships are unidirectional with body composition being influenced by hormone levels and not vice versa.

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4. Discussion

This is the first study to analyze body composition and hormone levels during the MT in sub-Saharan black African females. This cross-sectional study, demonstrates that within this population group, and as shown previously in other populations, significant relationships exist between hormone levels and body adiposity, BFD [23], lean mass [24] and skeletal measures [25]. However, despite these relationships minimal differences were observed between the MT groups in body adiposity or BFD, while lean mass and skeletal measures (BMC and BMD) were significantly lower in the postmenopausal compared to the premenopausal groups. With regard to hormone levels, FSH was higher, and E2, SHBG and DHEAS were lower, in post- compared to premenopausal women.

4.1. *Body Composition and the Menopause Transition*

There was a tendency ($p = 0.06$) for BMI to be lower in the postmenopausal than the premenopausal women in our cohort. This is in contrast to a large longitudinal multi-ethnic study in the USA that found an increase in BMI across the MT [26], as did a sizeable European cross-sectional study [27]. A multi-ethnic cross-sectional survey found, after adjusting for age, that women with natural menopause did not have increased BMI compared to premenopausal women [28], and some longitudinal studies found no difference in BMI between pre- and postmenopausal women, although differences in BFD with reproductive aging were noted [29]. Thus, data from various studies, both longitudinal and cross-sectional, have produced conflicting results with regard to the change in BMI observed across the MT. This may be due to differences in study methodology including sample size and lack of adjustment for possible confounding variables. Furthermore, there may be ethnic differences in the response of adipose tissue to the changing metabolic and hormonal milieu that is characteristic of the MT, and this requires further detailed investigation. Mauriége et al. [30] found a fall in SAT levels across the MT, as we did, although the effect was quite small ($p = 0.05$). As shown in the SWAN study [31] we found no effect of the MT on BFD and waist circumference, although other investigators have found an association [5]. We observed that lean mass was lower at all body sites in the postmenopausal groups, which has been observed previously in several studies [9,29,32], although this was not observed in one small cross-sectional study [13]. Bone mineral density and BMC were both lowest in the postmenopausal groups, a trend that has been observed in a number of other studies [33].

4.2. *Hormone Levels, the Menopause Transition and HIV Infection*

As expected, our results showed a trend of significantly higher FSH and lower E2 serum levels when moving from pre- to postmenopausal groups. While we found no difference across the subject groups in concentrations of total or bioavailable testosterone, or DHEA, levels of both SHBG and DHEAS were significantly lower in the postmenopausal women. Our results are similar to data from the longitudinal Melbourne Women's Midlife Health Project, which found that SHBG decreased, while levels of total testosterone did not change across the MT [34]. However, not all studies have shown a fall in SHBG levels across the MT [35]. We observed that DHEAS was lower in the postmenopausal group, as also described in a large longitudinal study in which levels rose from premenopause to reach a peak by the late menopausal transition/early postmenopause stages, with levels then falling to a nadir by the late postmenopause stage [36]. In a large cross-sectional study DHEAS levels were lower in post- compared to premenopausal females, but this was related to chronological rather than reproductive aging [35].

The prevalence of HIV-infection was lower in postmenopausal than premenopausal females, which is probably a consequence of the age difference between these groups. The frequency of use of ART did not differ between the different stages of the MT as defined using the STRAW+10 criteria.

We demonstrated that total testosterone and SHBG levels were higher in HIV-infected than non-infected women, irrespective of ART. A previous study has shown that SHBG levels are higher but total testosterone levels are lower in HIV-positive, premenopausal women [37], but there are no data on androgen levels for HIV-infected, menopausal or postmenopausal women. These findings must therefore be confirmed in future studies. The present study demonstrated lower DHEAS levels in HIV-positive women receiving ART than in both HIV-negative and HIV-positive, ART-treated women. There are no comparable data on the effect of ART on DHEAS levels in menopausal women.

4.3. *Age and Body Composition*

Within univariate analyses, the only variable correlating with age was visceral fat, and this relationship was lost after inclusion in a multiple regression model. Within the multiple regression models, age correlated only with waist. The reason for the lack of association between age and adipose tissue measures may be that adiposity (as measured using BMI) in black South African women aged 40–60 years is fairly static, as described in a large nationwide health survey [10].

4.4. *Relationship Between Hormone Levels and Body Composition*

The current study demonstrated that levels of SHBG were negatively correlated with a number of anthropometric variables, confirming data from other studies showing relationships of SHBG with BMI, total fat mass, lean mass, waist [15] and VAT [38]. The negative relationship observed between SHBG levels and BMD has been described previously [25].

Our data showed a positive association of DHEAS with lean mass and BMD, and a negative relationship with fat mass, although this relationship was quite weak ($p = 0.04$). Our finding differs from that of a small, multi-ethnic cohort which showed that although DHEAS levels were lower in postmenopausal women, they were not associated with body adiposity [39]. As with our findings, results from SWAN show that DHEAS levels are negatively associated with BMI [40] and our data confirm the association between BMD and DHEAS found in a large longitudinal study [41].

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Our research identified a strong inverse association between FSH levels and total lean mass, as observed in previous studies [9,42]. A large longitudinal study showed a negative correlation between BMI and serum FSH levels in menopause [40], however, total lean mass was not reported in this study. Our study showed that a significant negative relationship between BMI and FSH exists but is rendered non-significant with the addition of lean body mass to the regression model. The negative association between FSH and lean mass is currently not fully understood. We hypothesize that FSH may be related to lean mass through an indirect mechanism since there appears to be no evidence that FSH receptors exist in lean tissue.

As reported earlier, levels of DHEA correlated positively with subcutaneous fat area in our participants. No other studies appear to have analyzed the relationship between subcutaneous fat area and DHEA levels during the MT. However, one Italian study performed in 28 premenopausal females did show a negative relationship between serum DHEA levels and subcutaneous fat measures [43]. Further studies are required to fully investigate the effect of DHEA on body fat distribution during the MT. Estradiol levels correlated positively with BMC and BMD, as observed in other studies [1], and statistical analyses suggested that the fall in both bone measures observed across the MT groups may be related to the decline in E2 levels. It is well recognized that E2 plays an important role in bone maintenance through effects mediated by bone estrogen receptors [44]. Our data further showed, in multiple regression models and ANCOVA, that BMD correlates strongly with menopause stage independently of other variables, suggesting that factors in addition to E2, but not measured in this study, may be involved in BMD differences across the MT.

The relationship between hormones and body composition during the MT is complex. It is uncertain whether differences in adiposity are driven by an altered hormonal milieu or vice versa, although recent longitudinal data from SWAN suggest that alterations in body fat modulate hormone levels [45]. However, testosterone and E2 receptors are expressed in adipocytes [23], thus the subcellular machinery necessary for sex steroids to influence adipose tissue deposition is in place. Within our study body adiposity appeared to fall instead of rising across the MT groups, in the face of lower E2 levels. The cross sectional nature of our study allows us to note associations but does not allow us to determine the causal direction of these relationships, however ANCOVA showed that differences in BMD and lean mass across the MT groups are strongly related to serum levels of E2 and FSH, respectively, and these relationships are unidirectional. These are only statistical relationships and should be verified in longitudinal studies.

4.5. *Education, ART and Snuff and Body Composition*

There appears to be little research on the relationship of waist circumference with levels of education and employment in subjects during the MT. Donato et al. found no association between educational level and waist circumference in menopausal females [5], but a large European study agreed with our findings, showing a negative association between waist circumference and education levels [46]. Our data from both ANOVA and multiple regression analysis demonstrate a negative relationship of ART with both total body fat and BMC. In a recent systematic review of the lipodystrophic effects of ART it was shown that the use of antiretroviral agents is associated with lipoatrophy, and thus lower BMI [47]. Studies have also shown that ART is associated with reduced bone mass [48]. It has also been shown that ART-naive women with HIV experience decreases in bone density [49], but we did not observe this in our study. In our participants, snuff use was significantly associated with lower BMD and elevated visceral fat area. Snuff use is prevalent among black South African women, and nicotine in popular snuff brands used by these women appears to be very high [50] and potentially more detrimental to bone health than snuff products used in other countries. Lower BMD was shown with combined use of cigarettes and snuff in black South African women [51] and data from a small cross-sectional study [52] suggested that smokeless tobacco use may be an additional risk for decreased BMD. Snuff use has been linked to increased obesity and thus may increase risk of metabolic syndrome [53], and it has also been observed that smoking increases abdominal girth [54]. Therefore, it is plausible that nicotine-laden snuff may have an effect on visceral fat.

4.6. *Study Limitations and Advantages*

A limitation of our study was its cross-sectional format. However, our sample numbers were large, and menopause status was accurately determined [11]. Another limitation was our inability to collect serum samples during the follicular stage of the menstrual cycle (days 2–5) in premenopausal women. However, ovarian function becomes progressively more dysfunctional in the late reproductive and early menopause transition stages [55], making it more difficult to determine the follicular stage, so timing of E2 assessment may become less important. In spite of the fact that a timed serum sample was not obtained, women in the early stages of the MT were characterized by much higher levels of E2 and much lower levels of FSH than observed in women in the early and late postmenopausal stages, as would be expected. Studies have shown that intra-abdominal fat can be accurately measured by ultrasound measurements [56]. Although computed tomography (CT) scans and magnetic resonance imaging (MRI) are gold standard measurements in assessing visceral fat, they are expensive and often unavailable, while results from ultrasound have been shown to be highly reproducible [56]. We used the Sodergard equation to calculate free and bioavailable testosterone [21], which has been shown to be reliable [57]. Immunoassays for testosterone can be compromised by cross reaction with related steroids and a lack of sensitivity [58], therefore we measured testosterone levels using liquid chromatography–mass spectrometry, which has been shown to be an accurate assay method [20]. Further positive aspects of this investigation are that we measured the serum levels of a wide variety of relevant hormones and a number of anthropometric and demographic variables within a menopausal population group for which no such data were previously available.

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4.7. Conclusions

In conclusion, the principal body composition outcomes observed in this study were lower lean mass, BMD and BMC in post- than in premenopausal women. The lower lean and bone mass in the postmenopausal groups may be related to higher levels of FSH and lower levels of E2, respectively. Antiretroviral treatment was associated with lower body adiposity and BMC while snuff use was associated with lower BMD but higher levels of visceral fat. Thus, both physiological and environmental factors appear to modulate body composition during the MT in this population group. These findings may help to determine whether MT-related sarcopenia is associated with changes in cardiometabolic disease risk factors. This research has implications for the use of behavioral interventions to lower morbidity and mortality in this population group. Exercise programs to help maintain lean mass and reduce adiposity and an education campaign to explain the health risks associated with snuff use may be beneficial. In addition, recommendations for the use of ART regimens with a more bone sparing effect may be considered.

Author Contributions

Design and conduct of the study: Nicole Jaff, Shane Norris, Nigel Crowther; data collection: Nicole Jaff, Tracy Snyman, Marketa Toman; data analysis Nicole Jaff, Shane Norris, Nigel Crowther; data interpretation and manuscript writing: Nicole Jaff, Shane Norris, Nigel Crowther.

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Conflict of interest

The authors report no conflict of interest.

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