Alterations in Cardiometabolic and Vascular Function Measures during the Menopause Transition

by

Saad Samargandy

MBBS, King Abdulaziz University, 2009

MPH, The University of Texas Health Science Center at Houston, 2015

Submitted to the Graduate Faculty of the

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This dissertation was presented

by

Saad Samargandy

It was defended on

October 21, 2020

and approved by

Karen A. Matthews, PhD, Department of Psychiatry

Maria M. Brooks, PhD, Department of Epidemiology

Emma Barinas-Mitchell, PhD, Department of Epidemiology

Jared W. Magnani, MD, MSc, Department of Medicine

Dissertation Director: Samar R. El Khoudary, PhD, MPH, Department of Epidemiology

Alterations in Cardiometabolic and Vascular Function Measures during the Menopause Transition

Saad Samargandy, PhD

University of Pittsburgh, 2020

ABSTRACT

Background: The risk of cardiovascular disease (CVD) in women accelerates after midlife, suggesting a contribution of the menopause transition (MT). A piecewise-linear increase in a CVD predictor close to the final menstrual period (FMP) would be indicative of a menopause contribution.

Objectives: This dissertation included three papers with the following objectives: to determine whether arterial stiffness (paper 1) and abdominal visceral adipose tissue (VAT, paper 2) show piecewise-linear increases close to the FMP, and whether menopause-related VAT changes predict carotid artery intima-media thickness (cIMT, paper 2). In paper 3, we sought to determine whether distinct trajectories of systolic (SBP) and diastolic (DBP) blood pressure over the FMP can be identified, whether any SBP or DBP trajectory shows a piecewise-linear increase close to the FMP, and whether menopause-related factors (age at menopause, vasomotor symptoms, estradiol, and follicle-stimulating hormone [FSH]) predict blood pressure trajectories.

Methods: Participants from the Study of Women's Health Across the Nation (SWAN; n=3,302, age: 46.3±2.7) and SWAN Heart Ancillary Study (n=362, age: 51.1±2.8 years) who had 2 measures of arterial stiffness and VAT and 17 measures of blood pressure over the MT were included. FMP-anchored piecewise-linear mixed effects models and group-based trajectory modeling were used for the analyses. Models were adjusted for age at the FMP, and demographic, lifestyle, and CVD risk factors.

Results: Over 2.3 years of follow-up, both arterial stiffness and VAT showed a piecewise-linear trajectory with significant accelerated increases close to the FMP. Menopause-related VAT increase predicted greater cIMT. Over 19.1 years of follow-up, women experienced three distinct SBP trajectories with 36% of the SWAN cohort experiencing a piecewise-linear increase trajectory with a significant accelerated increase close to the FMP. The other SBP trajectories and all DBP trajectories did not show menopause-related increases. An older age at menopause and vasomotor symptoms predicted a higher SBP trajectory and higher FSH levels predicted a lower SBP overtime.

Conclusions: The MT is associated with increases in cardiometabolic and vascular function measures beyond aging. It is prudent to timely detect increases in CVD risk factors during the MT and emphasize lifestyle changes with the aim of combating such increases.

Table of Contents

Acknowledgments ..... **xiii**

1.0 Introduction **1**

2.0 Specific aims **5**

3.0 Background **7**

3.1 Staging the menopause transition **7**

3.2 Disentangling the contribution of ovarian from chronologic aging **9**

3.3 Central arterial stiffness **11**

3.3.1 Pathophysiology of arterial stiffness, its determinants and relationship with cvd outcomes **11**

3.3.2 Measuring arterial stiffness **12**

3.3.3 Literature review on changes in central arterial stiffness during the menopause transition **14**

3.4 Abdominal visceral adiposity **20**

3.4.1 Pathophysiology of abdominal visceral adipose tissue and relationship with cardiometabolic outcomes **20**

3.4.2 VAT measurement **21**

3.4.3 Literature review on vat changes during the menopause transition **23**

3.5 Blood pressure **26**

3.5.1 Gender and racial/ethnic heterogeneity in hypertension **26**

3.5.2 Blood pressure measurement **27**

3.5.3 Pathophysiology, risk factors, and consequences of hypertension **27**

3.5.4 Literature review on changes in blood pressure during the menopause transition 29

4.0 Manuscript 1: arterial stiffness accelerates within one year of the final menstrual period: the swan heart study 33

4.1 Chapter summary 33

4.2 Introduction 34

4.3 Material and methods 35

4.4 Results 40

4.5 Discussion 42

4.6 Tables and figures (manuscript 1) 47

4.7 Online supplement (manuscript 1) 52

5.0 Manuscript 2: abdominal visceral adipose tissue over the menopause transition and carotid atherosclerosis: the swan heart study 58

5.1 Chapter summary 58

5.2 Introduction 59

5.3 Materials and methods 61

5.4 Results 65

5.5 Discussion 67

5.6 Tables and figures (manuscript 2) 71

5.7 Online supplement (manuscript 2) 76

6.0 Manuscript 3: trajectories of systolic and diastolic blood pressure in women during midlife: is the pattern menopause- or aging-related? 83

6.1 Chapter summary 83

6.2 Introduction 84

6.3 Methods 86

6.4 Results 91

6.5 Discussion 93

6.6 Tables and figures (manuscript 3) 98

6.7 Online supplement (manuscript 3) 105

7.0 Discussion 115

7.1 Summary of the findings 115

7.2 Arterial stiffness, abdominal visceral adiposity, and blood pressure changes accompanying the menopause transition are interrelated 117

7.3 Public health significance 118

7.4 Clinical implications of the findings 120

7.5 Strengths and limitations 123

7.6 Future directions 125

7.7 Conclusions 127

Appendix A: Brief description of the FMP dates imputation process used in SWAN 128

Appendix B: SWAN acknowledgements and sources of funding 130

Bibliography 132

List of Tables

Table 3-1: Definitions of the menopause transition* 10

Table 3-2: Methods used to measure arterial stiffness in research settings 18

Table 3-3: Categories of BP in adults..... 26

Table 4-1: Characteristics of study participant at baseline 47

Table 4-2: Annual percent change in carotid-femoral pulse wave velocity in time segments relative to FMP..... 49

Table 5-1: Characteristics of study participant at baseline (n=362) 71

Table 5-2: Annual percentage change in VAT in time segments relative to FMP..... 72

Table 5-3: Percentage change in carotid IMT measures per 20% greater VAT by time segments..... 73

Table 6-1: Baseline characteristics of study participants by SBP trajectory group..... 101

Table 6-2: Annual change in blood pressure (mmHg) in time segments, by trajectory groups, (n=3302)* 103

Table 6-3: Predictors of SBP trajectory group membership and level across the menopause transition, (n=3302) *..... 104

Appendix Table 4-1: Sensitivity analysis comparing women with observed vs imputed FMP at baseline 54

Appendix Table 4-2: Beta estimates from simple linear regression of univariate associations between baseline study variables and baseline log-transformed carotid-femoral pulse-wave velocity 55

Appendix Table 4-3: Sensitivity analyses of annual percent change in carotid-femoral pulse wave velocity in time segments relative to FMP 57

Appendix Table 5-1: Comparing Women with Observed vs Imputed FMP at Baseline 78

Appendix Table 5-2: Annual Percentage Change in VAT in Time Segments Relative to FMP (women with observed and imputed FMP combined, n=495) 79

Appendix Table 5-3: Annual Percentage Change in VAT in Time Segments Relative to FMP (women who had 2 VAT measurements, n=233) 80

Appendix Table 5-4: Percentage Change in Carotid IMT Measures Per 20% Greater VAT by Time Segments (women with observed and imputed FMP combined, n=495) 81

Appendix Table 5-5: Percentage Change in Carotid IMT Measures Per 20% Greater VAT by Time Segments (women who had 2 VAT measurements, n=233) 82

Appendix Table 6-1: Baseline characteristics of study participants by DBP trajectory group 106

Appendix Table 6-2: Comparing women with observed vs imputed fmp at baseline 108

Appendix Table 6-3: Baseline characteristics of study participants by trajectory group (observed FMP only) 111

Appendix Table 6-4: Annual change in SBP (mmHg) in time segments, by trajectory groups* (observed FMP only) 113

Appendix Table 6-5: Predictors of SBP trajectory group membership and level across the menopause transition, (n=1804) *..... 114

List of Figures

Figure 3-1: The stages of reproductive aging workshop (STRAW) + 10 staging system for reproductive aging in women 8

Figure 3-2: Adjusted population means (95% CI) for segmented mean profiles of follicle-stimulating hormone and estradiol across the final menstrual period 9

Figure 3-3: Carotid-femoral pulse-wave velocity measurement in SWAN 13

Figure 3-4: Measurement of VAT area using CT in SWAN 23

Figure 3-5: Pathophysiologic mechanisms of hypertension 28

Figure 4-1: Means of cfPWV in years around FMP 50

Figure 4-2: Effect modification of race on annual percent change in cfPWV by time segments relative to FMP 51

Figure 5-1: Means of abdominal visceral adipose tissue (VAT) in years around the final menstrual period (FMP). 74

Figure 5-2: Association between abdominal visceral adipose tissue and internal carotid artery IMT by time segments. 75

Figure 6-1: Trajectories of systolic blood pressure over the menopause transition (n=3302) 98

Figure 6-2: Trajectories of diastolic blood pressure over the menopause transition (n=3302) 100

Appendix Figure 6-1: Trajectories of systolic blood pressure over the menopause transition (n=1804), observed FMP only 110

Acknowledgments

I would like to express my appreciation to Dr. Samar El Khoudary. She has been a great mentor and example throughout my program. Her enthusiasm and belief in the work we did fueled my passion for research. Her positive attention to details helped me constantly look at things from different angles. Her constant push for improvement expanded my range of research skills. She has always helped me see the light at the end of the tunnel. I cannot thank her enough.

I would like to thank my dissertation committee members. Their ideas and comments continually brought new insights into this work. I am grateful for the diversity and depth of questions each of them has asked me. I appreciate the time and effort they spent reading and thinking about this project. I certainly have learned from their expertise beyond my expectations.

I am grateful for my wife Safaa. She has given me the warmth and love I needed during this journey. My children, Salma, and Suliman have been my precious source of joy all along. Thank you all!

1.0 Introduction

One in every three women dies of cardiovascular disease (CVD), that is more lives being claimed due to CVD compared with cancer, chronic respiratory lung disease, and Alzheimer disease combined.[1] Despite increases in awareness over the past decade, only 54% of women recognize that CVD is their number one killer.[2] Noticeably, the risk of CVD increases with advancing age in both sexes, with apparent risk acceleration in postmenopausal years in women.[1] This observation suggests that the menopause transition might augment the age-dependent increase in CVD risk. In fact, the risk of CVD and CVD mortality is greater in women who experience early-onset menopause, namely younger than 45 years.[3] The effect of early-onset menopause on CVD risk seems to be more pronounced in women with surgical menopause (bilateral oophorectomy before natural menopause) than in women with a natural menopause.[4] Despite these findings, the menopause transition is not simply the cessation of monthly menstrual cycles and the loss of ovarian estrogen reserve, rather it encompasses complex physiological and pathological changes that could collectively predict future cardiovascular disease risk.[5]

Data from population-based longitudinal studies of changes in hypothalamic-pituitary and ovarian function were desperately needed to critically evaluate the staging of the menopause transition.[6] In 2011, capitalizing on accumulating studies of the menopause transition, the Stages of Reproductive Aging Workshop (STRAW) + 10 reevaluated and proposed the new staging system for ovarian aging, which is now considered the gold standard for characterizing reproductive aging.[6] This system helped standardize the classification of the menopause stage, which facilitated research that aimed at disentangling the relative contributions of ovarian from chronologic aging to women's health. Longitudinal studies that follow premenopausal women

through postmenopause are geared toward this task and they indeed helped identify major risk factors for health that were found to be modified by menopause.[5]

Accumulating evidence supports the clinical importance of the menopause transition as a period of accelerated CVD risk in women when dynamic metabolic and physiologic changes begin to accumulate independent of aging.[7] By midlife, >80% of women have one or more traditional CVD risk factor.[8] When modeled in relation to the number of years before and after the final menstrual period (FMP), total cholesterol, low-density lipoprotein cholesterol (LDL-C), and apolipoprotein B demonstrated substantial increases within the 1-year interval before and after the FMP, consistent with menopause-induced changes.[9,10] Interestingly, greater increases in LDL-C around the FMP were related to greater odds of carotid plaque scores later in life.[11] Furthermore, these lipid changes were accompanied by greater odds of developing the metabolic syndrome in the perimenopausal compared with the postmenopausal period.[12,13] With these adverse changes in CVD risk factors during the years before and after the FMP, it is not surprising that subclinical measures of atherosclerosis will also be concurrently modified. women are subjected to increases in carotid's intima-media thickness and adventitial diameter starting from the late perimenopause.[14,15] These structural vascular changes in the late peri- and post-menopause develop in conjunction with declining function in brachial arteries as measured by flow-mediated dilation.[16,17] These previous findings suggest that midlife women undergo adverse vascular and cardiometabolic changes as they traverse menopause, which may impact their future CVD risk.

An important aspect of vascular function and cardiovascular health is arterial stiffness.[18] Arterial stiffness as a novel subclinical measure of CVD that predicts the development of future CVD events and mortality in the general population,[19] adds predictive value to established risk markers,[20] and changes predicted risk sufficiently to alter recommended therapy.[21] Arterial

stiffness is thought to increase during midlife in women.[22] Interestingly, racial difference has been reported in arterial stiffness, with Black women having greater arterial stiffness comparted with White women.[23] However, studies that used time-oriented analysis to determine when critical arterial stiffness changes occur in relation to the FMP are lacking. Additionally, no previous study has characterized racial differences in arterial stiffness trajectories over the menopause transition. Therefore, given its high predictive capacity, it is crucial to characterize the trajectory of arterial stiffness over the menopause transition. Because modifying arterial stiffness will likely improve CVD outcomes later in life,[24,25] such studies will shed light on an important preventive aspect of cardiovascular health during a critical period of women's life.

Weight gain is frequently reported at midlife and is a major concern for women and their healthcare providers.[26] Indeed, women transitioning through menopause experience fat redistribution toward accumulating more central and perhaps more abdominal visceral fat (VAT).[27,28] From a cardiovascular health perspective, VAT increases and predicts risk of CVD,[29,30] and improves CVD risk prediction when added to a multivariable model that includes traditional CVD risk factors and BMI.[30] Additionally, VAT is associated with worse diabetogenic/atherogenic risk profile.[31,32] Lifestyle changes including increasing physical activity and calorie restriction prove to be beneficial in reducing VAT content in women at midlife.[33,34] However, these interventions needs to be guided by studies that carefully characterize changes in VAT across the menopause transition. Additionally, given the recognized atherosclerotic risks associated with VAT,[32,35] it is crucial to investigate whether VAT changes at midlife are associated with greater risk of subclinical markers of atherosclerosis known to be modified by the menopause transition.

Blood pressure is one of the most important modifiable risk factors contributing to the excess coronary heart disease related deaths in men and women.[36] A higher percentage of men have hypertension compared with women before age 65 years; after that age, the percentage of women with hypertension is higher compared to that of men,[37] an observation suggesting that blood pressure in midlife women has a complex pathophysiology. In addition, hypertension is less controlled in women than in men who are treated for hypertension (14.6% versus 8.3%).[38] Black and Mexican American women have greater prevalence of hypertension and obesity compared with White women.[39] With these racial/ethnic differences in hypertension and obesity,[39] it is expected that not all women would have a common trajectory of blood pressure over the menopause transition. Studying blood pressure trajectory over the menopause transition while considering potential clustering of women based on intrinsic characteristics like race/ethnicity or obesity may help identify a proportion of midlife women that would benefit the most from preventive measures.

2.0 Specific aims

The Study of Women's Health Across the Nation (SWAN) is a multi-ethnic longitudinal cohort study that followed women overtime with detailed description of the menopause transition. SWAN Heart is an ancillary study to SWAN at the Pittsburgh and Chicago sites that focused on subclinical measures of CVD. SWAN and SWAN Heart offer great opportunity for conducting extensive analyses because the women had repeated measures of central arterial stiffness, VAT, and CVD risk factors. Therefore, using data from both SWAN and SWAN Heart, we are interested in assessing the following specific aims corresponding to the three manuscripts for this dissertation:

Specific Aims for Manuscript 1: Determine whether midlife women experience changes in central arterial stiffness relative to the FMP and whether these changes differ between Black and White women.

Hypothesis 1: *Central arterial stiffness will increase close to the FMP.*

Hypothesis 2: *Black women will generally have greater adverse changes in central arterial stiffness at midlife as compared with White women.*

Specific Aims for Manuscript 2: Characterize VAT trajectory over time relative to the FMP, independent of aging, and test whether menopause-related VAT accumulation is associated with greater carotid artery atherosclerosis.

Hypothesis 1: *VAT will follow a non-linear trajectory over time relative to the FMP, with a larger change in VAT around the FMP, compared to prior to the FMP.*

Hypothesis 2: *menopause-related increases in VAT are associated with greater risk of carotid atherosclerosis.*

Specific Aims for Manuscript 3: Determine whether distinct trajectories of systolic and diastolic blood pressure over time relative to the FMP can be identified and test whether age at menopause, and time-varying estradiol, follicle-stimulating hormone, and vasomotor symptoms predict pattern and/or level of systolic and diastolic blood pressure trajectories

Hypothesis 1: *Distinct trajectories for systolic and diastolic blood pressure will be identified over time relative to the FMP that are different in level and pattern.*

Hypothesis 2: *Older age at menopause, greater estradiol, lower follicle-stimulating hormone, greater vasomotor symptoms will predict a higher overall systolic and diastolic blood pressure trajectory pattern and a greater level overtime.*

3.0 Background

3.1 Staging the menopause transition

The hallmark of the menopause transition is changes in menstrual cycle characteristics that results from progressive dysregulation of the hypothalamic pituitary-ovarian axis. During the menopause transition, women go through four different menopausal stages that are determined by frequency and regularity of the menstrual bleeding (Figure 3-1).[6] Based on STRAW, premenopausal or reproductive stage is when women report no or subtle changes in menstrual cycle characteristics. The reproductive stage is followed by the menopause transition stage, which includes early and late menopause transition stages. Early menopause transition (frequently referred to as early peri-menopause) is marked by increased variability in menstrual cycle length. Then, women transition to late menopause transition (frequently referred to as late peri-menopause) that is marked by the occurrence of amenorrhea of 60 days or longer. Menstrual cycles in the late menopause transition are characterized by increased variability in cycle length, extreme fluctuations in hormonal levels, and increased prevalence of anovulation. Finally, women are deemed to have completed the menopause transition and became postmenopausal after 12 consecutive months of amenorrhea (Figure 3-1). Thus, the date of the final menstrual period (FMP) is retrospectively assigned.

Although not used as criteria for staging the menopause transition in STRAW, hormonal biomarker criteria are considered supportive given the lack of international assay standardization, cost, and invasiveness (Figure 3-2).[6] Across the menopause transition, serum follicle-stimulating hormone (FSH) begins to increase 6 years before the FMP, accelerates 2 years before the FMP,

decelerates around the FMP, and attains a stable levels 2 years after the FMP.[40] Serum estradiol (E2) concentration begins to decrease 2 years before the FMP and then decelerates to achieve stability 2 years after the FMP.[40]

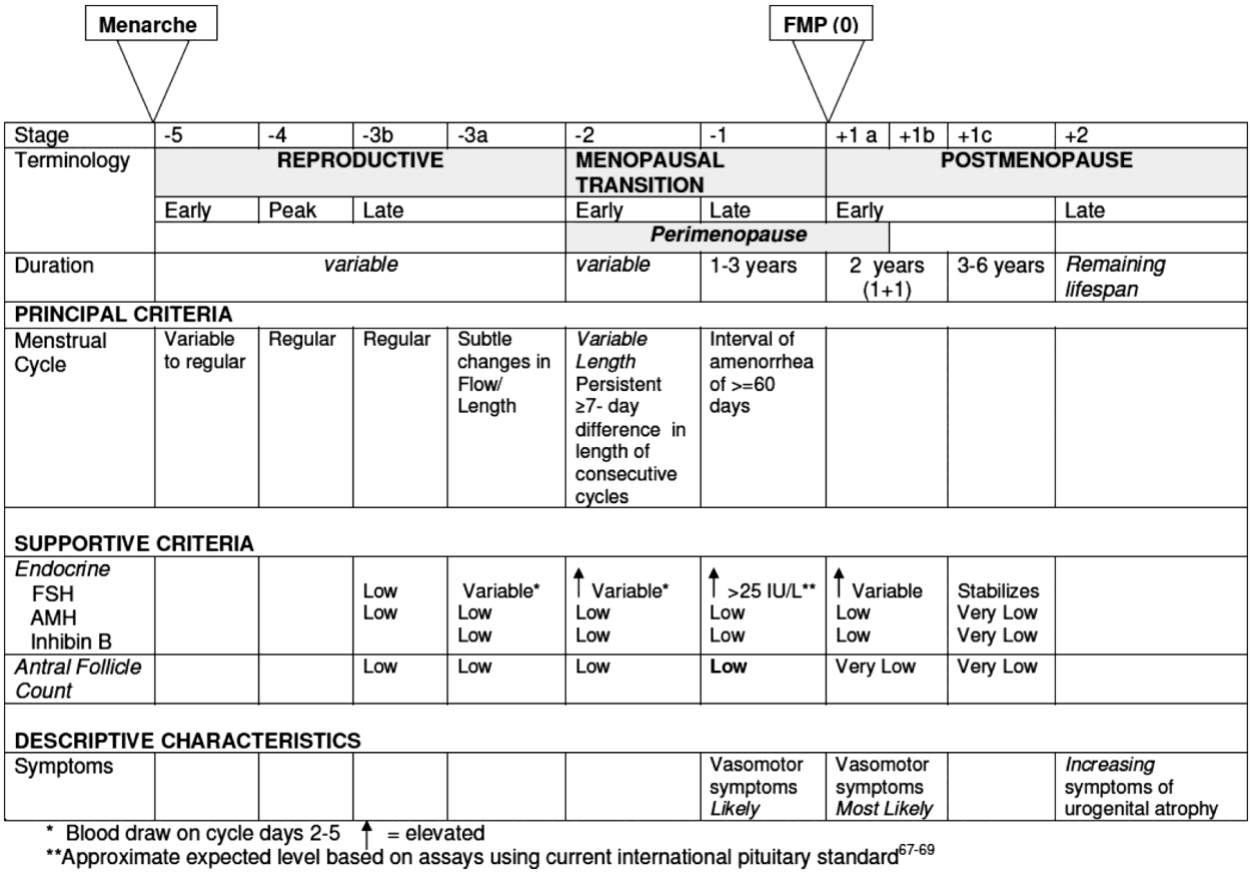


Figure 3-1: The stages of reproductive aging workshop (STRAW) + 10 staging system for reproductive aging in women

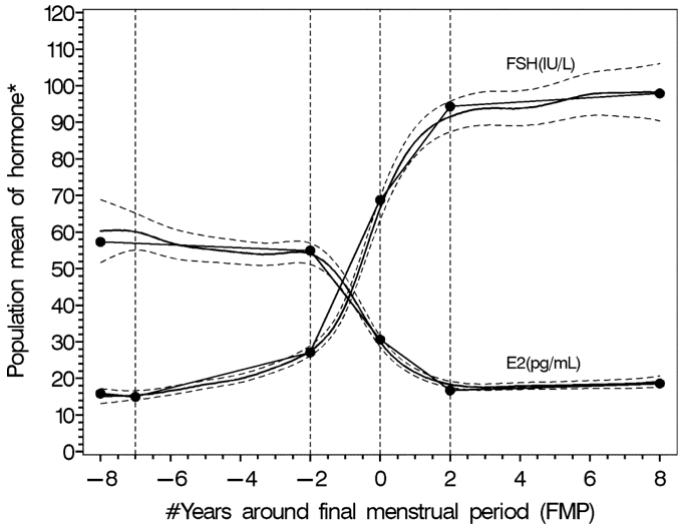


Figure 3-2: Adjusted population means (95% CI) for segmented mean profiles of follicle-stimulating hormone and estradiol across the final menstrual period

The Study of Women's Health Across the Nation (N = 1,215).[40]
* The y axis is unitless. The units of hormone are marked in the corresponding curves.

3.2 Disentangling the contribution of ovarian from chronologic aging

In observational studies, disentangling the effect of ovarian from chronological aging on CVD risk factors is challenging because they occur simultaneously in time. Previous observational studies have used several methods of defining the menopause transition in cross-sectional and longitudinal settings (Table 3-1). In cross-sectional studies, one can recruit a group of midlife women and collect data in a single point in time on factors like menopause stage, time past the FMP, or age at menopause and test associations of these definitions of the menopause transition with a CVD risk factor under question. However, this design will not help us determine if any association we see is due to ovarian or chronologic aging because, for example, by definition,

premenopausal women are younger than their postmenopause counterparts and thus cannot be directly compared.

In longitudinal studies, one can recruit a group of premenopausal women and follow them over time with frequent data collection waves starting from premenopausal years through the menopause transition and into the postmenopause. Data collected over time on endogenous sex hormones and menstrual bleeding patterns can help accurately determine a change in menopause stage,[41] duration of each menopause stage,[14] and document the time of data collection wave relative to the FMP.[9] With appropriate control of chronologic aging, these three methods of defining the menopause transition in longitudinal studies can help determine if there is an ovarian contribution to the risk factor under study. The method of time relative to the FMP can be used to additionally quantify the magnitude and timing of change in a given risk factor relative to the FMP. If the CVD risk factor is indeed related to the menopause transition, this approach will result is a piecewise-linear model with a significant inflection point close to the FMP, strongly supporting an ovarian contribution to the risk factor under consideration independent of chronologic aging. Therefore, key study design features that would help disentangle ovarian from chronologic aging include longitudinal repeated measures design, precisely characterized ovarian aging, and maximizing the number of observed FMPs.

Table 3-1: Definitions of the menopause transition*

Cross-sectional design	Longitudinal
Menopausal stage Endogenous sex hormones Time past the FMP Age at Menopause Early vs late menopause Surgical menopause	Endogenous sex hormones Age at Menopause Early vs late menopause Surgical menopause *Change in menopausal stage since baseline* *Duration of menopause stage* *Time relative to the FMP*

* Definitions in *Italic* can be used to determine if there is an ovarian contribution beyond aging.

3.3 Central arterial stiffness

3.3.1 Pathophysiology of arterial stiffness, its determinants and relationship with cvd outcomes

Arterial stiffening is defined as the loss of elastic distensibility or compliance due to changes in the geometry and microstructure of the vessel wall.[42] The normally functioning aorta regulates the pulsatile blood flow from the heart into steady flow of blood supply to peripheral organs.[43] This vascular function is maintained by the interaction between extracellular matrix components including smooth muscle cells, collagen, and elastin. Smooth muscles regulate tone and flow, and collagen contributes to the strength of the arterial wall, while elastin provides compliance by storing energy during systole to maintain flow during diastole.[44] Several risk factors predicts worsening arterial stiffness in women including aging,[45] increased systolic blood pressure,[46] hyperlipidemia,[47] insulin resistance,[48] central adiposity,[49] and the metabolic syndrome.[50-52] These risk factors may be involved in the pathogenesis of arterial stiffness through increasing vascular wall thickness, loss of vascular elasticity, and increased collagen deposition and turnover.[53,54]

Influence of the menopause transition on arterial stiffness is supported by studies that identified estrogen receptors in the human aorta.[55] Estradiol directly affects arterial wall remodeling by increasing elastin production and decreasing collagen deposition.[56] Additionally, premenopausal women have greater production of the potent vasodilator, nitric oxide (NO), compared with men.[57] Estrogen also has vasodilatory effects that is thought to be mediated through NO.[58] Injection of intracoronary estradiol resulted in improvements in endothelial function and coronary flow in women with coronary artery disease but not in men.[58] Finally, postmenopausal

women develop stiffer arteries as they age.[59] This arterial stiffness may be ameliorated after the administration of hormone therapy and made worse after withdrawal of the treatment.[60,61] These results suggest that arterial stiffness in postmenopausal years may worsen due to progressive estrogen deprivation.

Longstanding arterial stiffness has detrimental implications for cardiovascular, brain, and renal functions. Namely, arterial stiffness can result in left ventricular dysfunction,[62] low coronary perfusion,[63] stroke,[64] and impaired renal function.[65] In the general population, central arterial stiffness independently predicted stroke and coronary heart disease among healthy normotensive population from the Rotterdam Study.[66] These results were consistent with findings from the Framingham Heart Study where arterial stiffness was found to independently predict first CVD event and improve risk prediction when added to traditional CVD risk factors.[20] These results underscore the clinical importance of central arterial stiffness and offer critical information beyond standard CVD risk factors in the prediction of future CVD events.

3.3.2 Measuring arterial stiffness

Arterial stiffness in this dissertation was measured using carotid-femoral pulse-wave velocity (cfPWV), which measures aortic or central arterial stiffness (Figure 3-3). Methods of measuring cfPWV are considered the gold standard for measurement of arterial stiffness.[43] cfPWV measures the distance traveled by the pulse wave over time. Detailed description of the methods used for cfPWV measurement in this dissertation were previously published.[67] In brief, arterial flow waves were simultaneously recorded at the right carotid and femoral arteries of supine participants, using Doppler flow probes. Three runs were performed for each participant, and average of waveforms was used in the analysis. Transit time of the pulse wave was calculated as

the delay between the averaged carotid and femoral waveforms. Distance traveled by the pulse waveform was estimated by measurement over the participant's torso. cfPWV was calculated as distance/transit time (cm/seconds) with higher cfPWV indicating a stiffer artery. Reproducibility study of cfPWV measurement was done in the same laboratory that conducted cfPWV measurements for SWAN participants, with an overall laboratory intraclass correlation of 0.77.[67]

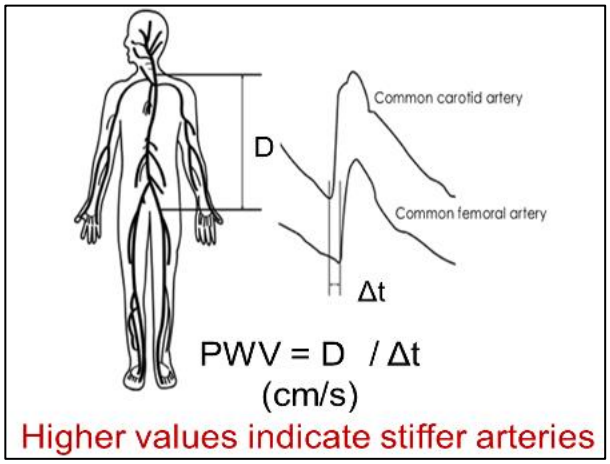


Figure 3-3: Carotid-femoral pulse-wave velocity measurement in SWAN

Numerous other methods and devices are being used to measure arterial stiffness in research settings, selected devices and their features are summarized in Table 3-2.[68] These methods are broadly categorized into four classes depending on the technique being employed.[68] The previously described method of cfPWV measurement falls under the "Ultrasonographic approaches" category. Generally, each of these methods record time delay between the arrival of the foot of the waveform and distance over the body surface between recording sites. Then, the time delay and the distance measured are used to directly calculate arterial stiffness of a vessel segment.[69] The reproducibility of all these methods has been reported to be good.[68]

Other indirect measures of arterial stiffness that are less frequently used include measures of vascular compliance (the absolute change in vessel diameter or area for a given change in

pressure) and distensibility (the relative change in vessel diameter or area for a given change in pressure).[70] Frequently, pulse pressure (PP) measured at the brachial artery is included in the calculations of arterial compliance or distensibility of the aortic artery. However, given the known PP amplification in peripheral arteries, these indirect measures of central arterial stiffness may not be valid, especially in younger populations.[70,71] Augmentation index (AIx) typically measures arterial wave reflections by comparing the first and second systolic peaks in the central aortic waveform. However, AIx is not typically used to measure central arterial stiffness as it is impacted by several other indicators of arterial function including central PP, central systolic pressure, and peripheral vascular resistance.[68]

3.3.3 Literature review on changes in central arterial stiffness during the menopause transition

To disentangle the relative contributions of chronologic and reproductive aging to vascular stiffness, longitudinal repeated-measure studies that follow a cohort of women during the menopause transition are required.[5] Cross-sectional design frequently fail to tackle such a question because, for example, premenopausal women are younger than their postmenopausal counterparts. Therefore, the comparison of arterial stiffness levels among women of different menopausal stages is confounded by age. Additionally, there is minimal, or no age overlap between premenopausal and postmenopausal women making adjusting for age in cross-sectional analyses statistically problematic. Unfortunately, most of our knowledge about the contribution of the menopause transition on arterial stiffness comes from cross-sectional studies. In general, these studies suggest that postmenopausal women have greater arterial stiffness compared with premenopausal women.[72-76] However, adjusting for age seems to account for menopausal status differences in

arterial stiffness.[77-79] A few cross-sectional studies looked at the effect of time since the FMP and found that more time elapsed after menopause is associated with greater arterial stiffness in women with hyperlipidemia,[80] but not in healthy postmenopausal women.[81] In hyperlipidemic women, younger age at menopause is associated with greater arterial stiffness.[80] Nevertheless, these studies should be interpreted in light of the aforesaid inherent limitations of the cross-sectional design as the observed differences between menopausal stages may be due to age differences. Additionally, most of these studies had a small sample size of less than 250 women and did not including women at the menopause transition stage,[72-75,79,81] a period that is characterized by dynamic changes in subclinical atherosclerosis.[14-17]

Limited number of longitudinal studies investigated the relationship between the menopause transition and arterial stiffness.[22,82] Staessen et al. recruited 315 premenopausal and postmenopausal women and followed them for 5 years and measured arterial stiffness once at the study conclusion.[82] The women in the study were categorized based on baseline and follow-up menopausal status into three groups: remained premenopausal, transitioned to postmenopause, and postmenopausal group. The authors found that postmenopausal women had greater arterial stiffness compared with women who remained premenopausal.[82] However, no difference in arterial stiffness was found between postmenopausal women and women who transitioned to postmenopause.[82] Another study by Khan et al. measured arterial stiffness at baseline and after 2.3 years of follow-up found that women who transitioned through menopause during the study follow-up period had greater increase in central arterial stiffness compared with women who remained premenopausal or were postmenopausal at baseline.[22] However, these studies were limited by inadequate covariate adjustment.[22,82] Additionally, no study has evaluated the change

in arterial stiffness by time since the FMP using a longitudinal repeated measure design while controlling for key CVD risk factors.

Few studies that examined the relationship between menopause and arterial stiffness included premenopausal or perimenopausal women and most studies were based largely on postmenopausal women.[73,77,78,80,81] Therefore, the current literature does not support generalizability of the findings to the complete spectrum of the menopause transition. To carry out this, repeated measures study needs to include balanced combinations of women at different menopausal stages, who remained in their menopausal status and those who traversed menopause during the study observation period. Another limitation to the generalizability of the previous findings is that only a few studies were conducted in the United States.[22] The remaining studies were on populations from Asia, Australia, the Middle East, Europe, and the Caribbean.

Arterial stiffness is generally greater in Black than in White women.[83] This racial difference is also evident around the menopause transition, with Black women having greater progression compared with White women.[23] Actually, Black women typically develop CVD risk factors at a younger age compared with White women,[84] which may partially explain why racial difference exist in arterial stiffness. Additionally, 20% of the variance in arterial stiffness is estimated to be heritable;[85] these racial differences may be due in part to Black, but not White, having chromosomal loci that are linked to greater arterial stiffness.[86] However, genome-wide association studies have not yet distinctly identified genetic variants for the racial differences in arterial stiffness.[87] Collectively, these studies suggest that racial differences in arterial stiffness exist but whether Black and White women share different trajectories of arterial stiffness over the menopause transition has not been explored before.

Taken together, the existing literature lacks studies that characterize changes in arterial stiffness across the menopause transition. Proxy definitions for the menopause transition that were used in previous studies include menopausal stage, age at menopause, or change in menopausal status. Although these are valid methods of defining menopause that may answer questions related to menopausal status or age at menopause as risk factors for disease, they do not inform questions related to changes in a risk factor under consideration. Therefore, there is a clear gap in the literature regarding arterial stiffness changes in women transitioning through menopause, with the related potential racial difference remaining unexplored.

Table 3-2: Methods used to measure arterial stiffness in research settings

Device category	Devices using a probe or a tonometer to measure PWV		Devices using cuffs placed around the limbs or the neck that record pulse-wave arrival oscillometrically		Ultrasonographic approaches	MRI-based approaches
Example Device	SphygmoCor	Complior[88]	Omron	Mobil-O-Graph[89]	*	*
Technique	Tonometry-based to record pressure waveform	Mechanotransducer-based to record pressure waveform	Oscillometry-based	Cuff-based device	Doppler ultrasonography to record flow waveform	Phase-contrast MRI
Procedure	Uses a piezoelectric tonometer placed at any 2 pulse detectable sites. Requires 2 sequential readings as only 1 tonometer can attach to the unit. The average transit time is derived with the R wave of the ECG used as a reference point, and PWV is calculated from the manual distance measured.	Similar to SphygmoCor but have simultaneous measurement between sites with distention sensors. The Complior software has an online recording and automatic calculation of PWV.	Uses 4 oscillometric cuffs placed on both arms and ankles to measure baPWV. The subject's demographics are entered into the software, and the distance is calculated statistically based on Japanese population.	Records brachial waveforms to estimate cfPWV. Several parameters from pulse-wave analysis and wave separation analysis are then used to estimate aortic PWV	Similar to PWV based on pressure waveforms. Uses either sequential or simultaneous readings with ECG gating.	Allows measurement of the spatiotemporal flow along the length of the artery to compute PWV.
Example cohorts where device was used	The Anglo-Cardiff Collaborative Study of arterial stiffness.[90] The Chronic Renal	Used extensively in epidemiologic studies in Europe	Used in prospective observational studies, mainly in Asia.		Diabetics,[92] healthy elderly,[93] and the general population.[94]	

Device category	Devices using a probe or a tonometer to measure PWV		Devices using cuffs placed around the limbs or the neck that record pulse-wave arrival oscillometrically		Ultrasonographic approaches	MRI-based approaches
Example Device	SphygmoCor	Complior[88]	Omron	Mobil-O-Graph[89]	*	*
	Insufficiency Cohort study.[91]					
Other measurable vascular indices	cfPWV baPWV Wave reflections	cfPWV baPWV	ankle-brachial index	Brachial BP and central aortic pressure	Local PWV and distensibility	Local PWV and distensibility
Comments		Provided much of the data relating PWV to CVD	Only newer models can measure cfPWV. Used for independently predicting loss of kidney function, CVD, and all-cause mortality.	Uses a proprietary algorithm incorporating age, systolic pressure, and aortic characteristic impedance.	Have independent predictive value for CVD and death.	Can assess almost any vessel and provide accurate distance and area estimates. However, needs long time, has low resolution and high cost

Abbreviations: baPWV: brachial-ankle pulse-wave velocity, cfPWV: carotid-femoral pulse-wave velocity, ECG: electrocardiogram.
* No example provided since ultrasonography and MRI for PWV measurement use technology common to other imaging purposes

3.4 Abdominal visceral adiposity

3.4.1 Pathophysiology of abdominal visceral adipose tissue and relationship with cardiometabolic outcomes

VAT is the fat compartment located in the abdominal cavity and includes intraperitoneal (omental and mesenteric) and retroperitoneal adipose tissues. The intraperitoneal depot drains into the portal circulation while the retroperitoneal depot drains into the systemic circulation.[95] VAT is a metabolically active fat depot that is associated with overflow of portal free fatty acids and increased cytokines secretion.[96] These factors increase insulin resistance, inflammation, and are associated with prothrombotic and hypertensive states.[97] In fact, women accumulating more VAT are at greater risk of developing CVD.[29,30] Additionally, improvement in CVD risk prediction was observed when VAT was added to a multivariable model that included BMI.[30]

Several risk factors are associated with individual differences in VAT among women including age, genetic factors, sex hormones, smoking, level of physical activity, and nutritional factors.[98-100] Abdominal subcutaneous adipose tissue (SAT) acts as a protective metabolic sink that adapts to positive energy balance with expanding by hyperplasia.[96] In cases of adipose tissue hypoxia that results in inability of SAT to expand and store the surplus energy, excess triglyceride molecules accumulate at undesired places including VAT and other ectopic locations (liver, pancreas, skeletal muscle, and heart).[101] Therefore, it has been suggested that excess VAT and ectopic fat deposition is a marker of dysfunctional SAT. This view of VAT/SAT relationship has been supported by studies showing women to have lower postprandial lipemia compared with men

given that women have much larger SAT reservoir than men.[102] Additionally, patients with lipodystrophies that results in dysfunctional SAT have greater volumes of VAT and ectopic fat.[103,104] Experimentally, dietary intervention or endurance exercise that create negative energy balance are shown to induce rapid reductions in VAT and ectopic fat depots.[105] Taken together, the inability of SAT to act as an energy buffer will produce a lipid overflow leading to accumulation of fat at undesired sites, including VAT, that ultimately increase risk of the abovementioned cardiometabolic complications.[97]

As women transition through menopause, they experience fluctuations in E2, relative domination of testosterone (T), and increases in FSH.[40,106] These hormonal changes at midlife create a hormonal milieu favoring greater deposition of body fat and greater central adiposity by influencing appetite, energy expenditure, whole-body thermogenesis, and lipoprotein lipase (LPL) activity.[97,107,108] These findings elucidate pathophysiologic mechanisms by which menopause transition may place midlife women at great risk of accumulating harmful amounts of VAT.

3.4.2 VAT measurement

Waist circumference is a simple and inexpensive method of measuring central adiposity that is found to significantly predicts CVD and mortality.[109] Although waist circumference correlates well with VAT,[110] it cannot distinguish VAT from SAT. Similarly, dual-energy X-ray absorptiometry (DEXA) can be used to measure overall abdominal fat, however, it cannot separate VAT from SAT.[111] Therefore, CT and MRI are used to separately quantify SAT and VAT. Estimation of adipose tissue by CT and MRI are comparable.[112] CT scans can generate sliced images of the body that can be used to quantify areas or volumes of tissues under investigation. CT slices are formed of pixels that take an attenuation value, called Hounsfield units (HU), that

ranges from -1000 HU (air) to +2000 HU (bone) depending on tissue density. Adipose tissues typically have attenuation values between -190 to -30 HU. The reliability of measuring body tissues using CT is excellent with only an error of <1% between paired scans.[113] CT scanning methods frequently quantify abdominal adiposity using a single slice image taken at L4-L5 intervertebral space to limit radiation exposure and decrease cost.[114] Adipose tissue area calculated from a single slice CT image at L4-L5 has a 0.99 correlation coefficient with total abdominal adipose tissue volume.[115]

MRI generates sliced or whole-body images by creating interactions between the protons within biological tissues and magnetic fields generated by the MRI machine. Protons from different tissues return to their equilibrium state at different rates after being exposed to various magnetic fields, a phenomenon that helps construct MRI images. MRI is more appropriate compared with CT in quantification of adiposity over several measurements in the same individual given the absence of radiation exposure with MRI. However, MRI is limited by its high cost and sophisticated equipment and data processing needs.[116] Both CT and MRI machines cannot accommodate severely obese individuals.

A less-frequently used method to exclusively quantify VAT in epidemiologic studies when there is no access to CT or MRI machines is ultrasound.[117,118] The procedure involves measuring the distances between the posterior edge of the abdominal muscles and the lumbar spine using electronic calipers. This is accomplished using a transducer that is placed on a straight line between the left and right midpoint of lower rib and iliac crest, distances are measured from five different angles that include medial, left and right lateral, and half-way in between these positions. Measurements are taken at the end of a quiet expiration while applying minimal pressure without displacement of the intraabdominal contents as observed by the ultrasound image [117,118] Contrary

to CT and MRI that can yield areas and volumes of VAT, ultrasound measures distance of VAT in the abdominal cavity. Nevertheless, the correlation between VAT distance measured using ultrasound and VAT area measured using CT and MRI are 0.81 and 0.80, respectively.[117,118] The coefficient of variation is 5.4% which indicates a good reproducibility of the ultrasound measured VAT.[117]

In this dissertation, VAT was quantified using electron beam CT scan that was obtained between L4 and L5 (Figure 3-4).[119] Attenuation was set to -190 to -30 Hounsfield units to define fat. Intra-observer reliability of this measure was 0.94.

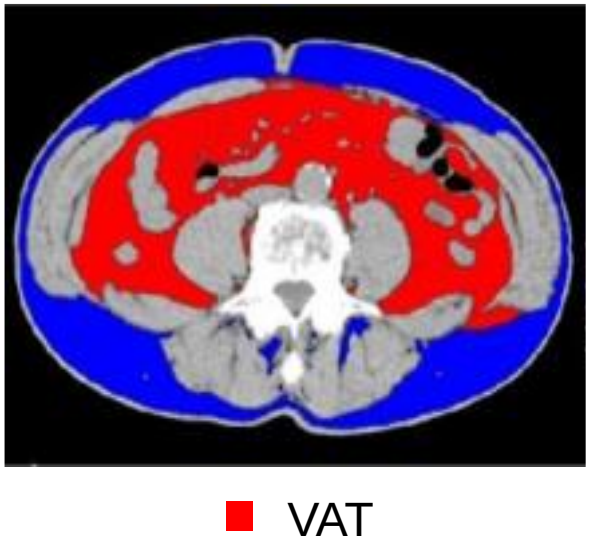


Figure 3-4: Measurement of VAT area using CT in SWAN

3.4.3 Literature review on vat changes during the menopause transition

Among the changes that are argued as being menopause-related is body weight because women frequently report weight gain at midlife.[26] Whether weight gain in women at midlife is due to chronological aging or the menopause transition has been debated. In a survey of around 14,000 midlife women, body-mass index (BMI) increased significantly with older age groups.[120] In the

same cohort of women, however, and after adjusting for chronological age, postmenopausal women did not have greater BMI compared with premenopausal women. These data suggest that weight increases with aging, but it may not be influenced by the menopause transition.

Increases in fat mass and central adiposity at midlife is suggested to be largely menopause-related phenomena.[27,28] Actually, epidemiologic studies show that waist circumference increase during midlife is primarily related to the menopause transition beyond aging.[28,111,121-123] However, waist circumference is a function of SAT and VAT. Studies that measured SAT and VAT found that although both fat depots are associated with deteriorations in cardiometabolic risk factors, when matched for SAT level, individuals with more VAT showed worse diabetogenic/atherogenic risk profile.[31,32]

Few cross-sectional studies explored the association between menopause and VAT by comparing VAT measures between postmenopausal and premenopausal women.[124-126] Results from these cross-sectional studies were not consistent, with some studies showing postmenopausal women having greater VAT compared with premenopausal women,[124] while others showing the reverse or showing no association between VAT and menopausal status.[125,126] The studies showing postmenopausal women to have greater VAT compared with premenopausal women were conducted on healthy women from the general population, while the rest included women with CVD and those on hormone replacement therapy, which may explain the observed inconsistency. As discussed earlier, drawing conclusions about the menopause transition from such observation is problematic because the aging effect cannot be excluded as there is no considerable age overlap between premenopausal and postmenopausal women using a cross-sectional design.

Longitudinal studies that followed midlife women for more than 7 years showed no association between baseline menopausal status and VAT at follow-up.[127,128] However, these

studies offer limited answers to questions about the menopause transition because VAT was compared among women groups that were formed by baseline menopausal status, not change in menopausal status. Additionally, these studies did not adjust for the variations in follow-up periods. Another longitudinal study analyzed within-women change in VAT and found that VAT significantly increases in premenopausal women after 8 years of follow-up, when all women became postmenopausal.[129] This study is limited by the small sample size (n=8) and the absence of a comparison group. Using a similar design with 3 years of follow-up for initially premenopausal women, Abdulnour et al. found that women who became perimenopausal or postmenopausal at follow-up had a significant increase in VAT since baseline.[130] However, the authors did not compare the change in VAT among the groups that were formed by the change in menopausal status since baseline (remained premenopausal, became perimenopausal, and became postmenopausal). Finally, only one study modelled VAT over years before and after the FMP and found that VAT increase started before the FMP and plateaued afterwards.[131] This analysis was limited by the lack of covariate adjustment and the small sample size (n=51) with sparse data at years 3 before and after the FMP. Using larger sample size while adjusting for predictors of adiposity will enable us to better describe VAT trajectory over time since the FMP while controlling the effect of potential confounding bias. Taken together, previous studies on the relationship between the menopause transition and VAT are inconsistent and limited, which highlight the importance of a well-designed repeated measures cohort study that will help characterize VAT change over time since the FMP.

3.5 Blood pressure

3.5.1 Gender and racial/ethnic heterogeneity in hypertension

Definitions of blood pressure (BP) categories are presented in Table 3-3 which are based on the 2017 report from the American College of Cardiology/American Heart Association (AHA) Task Force on Clinical Practice Guidelines.[132] The AHA has identified normal BP category for adults older than 20 years as 1 of the 7 components of ideal cardiovascular health, with 45.4% of the US adults meeting these criteria.[133] The age-adjusted prevalence of hypertension among adult US women in 2016 was 42.8%.[37] Before age 64, women have a lower prevalence of hypertension compared with men; however, this flips starting from age 65 when women shows greater prevalence of hypertension compared with men.[37] Differences in hypertension prevalence also exists among race/ethnic groups. Based on data from NHANES 2016, the prevalence of hypertension in women is the highest in Black, followed by Mexican American, and White have the lowest prevalence.[37]

Table 3-3: Categories of BP in adults

BP Category*	SBP		DBP
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120–129 mm Hg	and	<80 mm Hg
Hypertension			
Stage 1	130–139 mm Hg	*or*	80–89 mm Hg
Stage 2	≥140 mm Hg	*or*	≥90 mm Hg

* Individuals with SBP and DBP in 2 categories should be designated to the higher BP category.
Abbreviations: BP indicates blood pressure (based on an average of ≥2 careful readings obtained on ≥2 occasions); DBP, diastolic blood pressure; and SBP, systolic blood pressure.

3.5.2 Blood pressure measurement

The direct measurement of BP requires an intra-arterial assessment. However, this is not practical as BP can be estimated noninvasively. Traditionally, BP determination relied mostly on measurements taken on the arm that involves auscultation of the brachial artery while watching a sphygmomanometer. However, semiautomated and automated devices that use the oscillometry method that detects the amplitude of the BP oscillations on the arterial wall, have become the norm over the past 2 decades.[134] In this dissertation, systolic and diastolic blood pressure were measured using a standard protocol. Blood pressure measurements were averaged from 2 sequential measures in the right arm with the participant seated.

3.5.3 Pathophysiology, risk factors, and consequences of hypertension

Normal blood pressure is maintained through balance between cardiac output and peripheral vascular resistance. Most patients with primary hypertension, which constitutes about 95% of hypertension cases, have a normal cardiac output but raised peripheral vascular resistance. Peripheral vascular resistance is controlled by the smooth muscles in the walls of small arterioles. These smooth muscle cells contract in response to changes in intracellular calcium levels. With chronic contraction of small arterioles, structural changes and thickening of the arteriolar wall ensue, which leads to irreversible rise in peripheral vascular resistance. Other hypotheses of the pathogenesis of hypertension also exist. For example, it is thought that the sympathetic nervous system over activity causes raise in cardiac output. Subsequently, as a protective mechanism for cellular homeostasis, peripheral arteriolar resistance develops.[135] Body systems that are involved in the regulation and pathogenesis of essential hypertension include the renin-angiotensin-

aldosterone system, the autonomic nervous system, plasma volume (largely mediated by the kidneys), and endothelial function (Figure 3-5).[135,136]

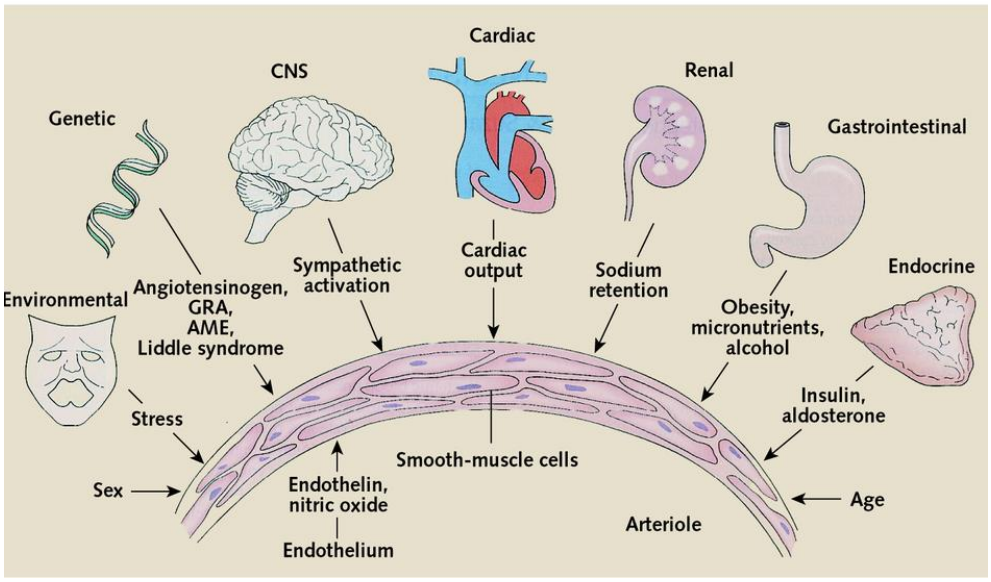


Figure 3-5: Pathophysiologic mechanisms of hypertension

Abbreviations: AME; apparent mineralocorticoid excess, CNS; central nervous system, GRA; glucocorticoid-remediable aldosteronism.[136]

Although the pathogenesis of primary hypertension is multifactorial and complex, several modifiable and non-modifiable risk factors have been identified that are strongly and independently associated with its development. Advancing age is associated with higher systolic blood pressure and increased incidence of hypertension.[137] Overweight is also a major risk factors for hypertension.[138,139] Various environmental exposures such as nutritional factors and physical activity impact BP. Excess sodium and alcohol consumption are linked with greater hypertension risk.[140,141] Physical inactivity also increases the risk of hypertension.[139] Genetic factors were found to account for 30% of the variability in blood pressure, and subjects who have one or two parents with hypertension have double the risk of developing the disease.[142,143] In the aftermath,

hypertension is one the most important and established traditional risk factors for developing CVD. In a meta-analysis that included 95,772 US women, each 10-mm Hg higher systolic blood pressure was associated with 25% (95% CI, 18%-32%) greater risk for CVD.[144] Additionally, among 65,806 females in this meta-analysis, the risk for CVD mortality associated with 10-mm Hg higher systolic blood pressure was 16% (95% CI, 10%-23%).[144]

3.5.4 Literature review on changes in blood pressure during the menopause transition

The effect of menopause stage on systolic and diastolic blood pressure or hypertension has been studied extensively in large samples of diverse healthy midlife women using mainly a cross-sectional study design.[10,145-156] Many of these cross-sectional studies had large sample sizes that allowed age to overlap between premenopausal and postmenopausal women.[10,145,149,155,156] This statistical luxury gained with larger sample sizes made statistically controlling for the confounding effect of age while testing the association between menopause stage and blood pressure appropriate. Even after controlling for the effect of age, previous cross-sectional studies on the association between menopause and blood pressure were inconclusive and findings were inconsistent. Some studies showed that postmenopausal women have greater odds of having hypertension and higher systolic blood pressure compared with premenopausal women after adjusting for age.[145,146,149,150] However, other cross-sectional studies did not support a contribution of menopause on blood pressure or prevalence of hypertension.[151,152,155,156]

Conflicting findings of the association between menopause stage and blood pressure in cross-sectional studies may have resulted from differences in race or obesity status among the studied populations. For example, in White women of normal weight, postmenopausal women had a higher blood pressure compared with their premenopausal counterparts.[146,148] However, a similar

association was not observed in overweight women of the same racial group.[10,153] Contrary to White women, studies on overweight Asian populations tend to show an association between the menopause status and blood pressure,[150,157] while blood pressure was similar across menopausal stages in studies of normal weight Asian women.[147,156] Overall, previous cross-sectional studies suggest the presence of a link between menopause and higher blood pressure or risk of hypertension only in a group of women with certain combinations of racial/ethnic and obesity phenotypes.

Compared with cross-sectional studies, fewer longitudinal studies investigated the potential contribution of the menopause transition to the developmental trajectory of blood pressure.[9,158-162] Most of these longitudinal studies documented menopause stage and measured blood pressure at two time points and then compared blood pressure changes across three groups of women: women who were premenopausal at baseline and remained premenopausal at follow-up, women who were already postmenopausal at baseline, and women who transitioned to postmenopause during follow-up. These studies mainly found similar blood pressure change estimates among the three groups defined by menopause transitioning status.[158-160,162] However, one study found that women who became postmenopausal or were postmenopausal at baseline had a marginally significant greater change in systolic blood pressure (4 mmHg vs no change) compared with women who stayed premenopausal after a median of 5.2 years of follow-up.[161] However, this later study did not adjust for age in their analysis which limits our ability to draw conclusions. In a study conducted on 1,054 women from SWAN, Matthews et al. found that systolic blood pressure increased on average 0.26 mm Hg annually before and after the FMP, while diastolic blood pressure did not change across time. Adjusting for age at FMP and other CVD risk factors, the trajectories of systolic and diastolic blood pressure were found to be linear over time

relative to the FMP without a clear inflection point.[9] Taken together, based on data from longitudinal studies, the general interpretation is that developmental trajectory of blood pressure at midlife is mainly due to chronological aging.

The previous longitudinal studies of blood pressure trajectory in women transitioning to menopause have analyzed the overall sample with the assumption that women share a common blood pressure trajectory. Given the known racial/ethnic differences in hypertension and obesity patterns,[39] it is expected that blood pressure in women may have distinct trajectories over the menopause transition depending on baseline differences among women in terms of premenopausal blood pressure, race, or obesity status. Additionally, the previous conflicting findings in cross-sectional studies suggest that blood pressure is possibly vulnerable to menopause or menopause-related factors only in a group of women with certain combinations of racial/ethnic and obesity phenotypes.

Group-based trajectory modeling (GBTM) can be used to detect distinct trajectories of an outcome of interest over measurement occasions.[163,164] GBTM uses finite mixture modeling to identify distinct groups that share common developmental trajectories. GBTM has been embraced by researchers to study over time development of a wide range of disorders and associated symptoms.[164] Analysis of a set of data using GBTM implies that one size-fits-all growth model may not always be the best approach for characterizing outcomes progression. Rather, GBTM provides an experimental method of identifying clusters of individuals following both typical and atypical courses of development. By dividing the data into trajectory groups for an outcome of interest, GBTM can be used to characterize the distinct trajectories of the outcome separately, test predictive associations of risk factors with the trajectory group membership, and determine predictors of the outcome level over time. Whether blood pressure over the menopause transition

show distinct trajectories that are influenced differently by the menopause transition remains to be explored. Such a hypothesis, if proved to be true, could explain some of the inconsistencies in this topic.

4.0 Manuscript 1: arterial stiffness accelerates within one year of the final menstrual period: the swan heart study

4.1 Chapter summary

Objective: Menopause may augment age-dependent increases in arterial stiffness, with Black women having greater progression in midlife compared with White women. We sought to determine whether and when women experience changes in arterial stiffness relative to the final menstrual period (FMP), and whether these changes differ between Black and White midlife women.

Approach and Results: We evaluated 339 participants from the SWAN Heart Ancillary study. Women had up to two carotid-femoral pulse-wave velocity (cfPWV) exams over a mean±SD of 2.3±0.5 years of follow-up. Annual % changes in cfPWV were estimated in three time segments relative to FMP and compared using piecewise linear mixed-effects models. At baseline, women were 51.1±2.8 years and 36% Black. Annual % change (95% CI) in cfPWV varied by time segments: 0.9% (-0.6%, 2.3%) for >1 year *before* FMP, 7.5% (4.1%, 11.1%) *within* one year of FMP, and -1.0% (-2.8%, 0.8%) for >1 year *after* FMP. Annual % change in cfPWV within one year of FMP was significantly greater than the other two time segments, $p<0.05$ for both comparisons. Adjusting for concurrent CVD risk factors explained part of the change estimates, but did not eliminate the difference. Black women had greater increase in cfPWV compared with White women in the first segment, p for interaction=0.04.

Conclusions: The interval within one year of FMP is a critical period for women when vascular functional alterations occur. These findings underscore the importance of more intensive lifestyle modifications in women transitioning through menopause.

4.2 Introduction

The risk of cardiovascular disease (CVD) increases with age in both sexes, with noticed risk acceleration in postmenopausal years in women.[165] This observation suggests that the menopause transition might augment the age-dependent increase in CVD risk. In fact, women are subjected to increases in carotid intima-media thickness and adventitial diameter starting from late perimenopause,[14,15] a stage that typically starts 1-3 years before the final menstrual period (FMP), and extending to 12 months after FMP.[6] These structural vascular changes in the late peri- and post-menopause are accompanied by declining endothelial function as measured by brachial artery flow-mediated dilation.[17] The Study of Women's Health across the Nation (SWAN) recently showed that women who transitioned through menopause had a significantly greater increase in central arterial stiffness compared with women who remained premenopausal or were postmenopausal at baseline.[41] These findings suggest that women undergo structural and functional vascular changes as they traverse menopause, which may impact their risk of CVD later in life.

The menopause transition, specifically, the few years before and after FMP constitute a critical period in women's lives when dynamic metabolic and physiologic changes begin to accumulate.[7] This period is characterized by adverse structural changes in the carotids,[14,15] and increases in lipids,[9] waist circumference,[28] and metabolic syndrome.[12] Additionally, dynamic

hormonal changes in estradiol and follicle-stimulating hormone (FSH) occur within one year of FMP.[40] Whether concurrent changes in central arterial stiffness, a measure of vascular aging and an independent predictor of CVD risk in the general population,[19] occur in this period remains to be determined.

Racial differences have been reported in arterial stiffness progression in women at midlife, with Black women having greater progression compared with White women.[23] SWAN is a multi-ethnic longitudinal cohort study that concurrently measured central arterial stiffness, CVD risk factors, and endogenous sex hormones as women traversed menopause. This study provides a unique opportunity to characterize vascular functional changes in the years around the FMP while adjusting for other risk factors that are modified during the menopause transition. Therefore, the aims of this study were to determine whether women experience changes in central arterial stiffness, as measured by carotid-femoral pulse-wave velocity (cfPWV), relative to the FMP and whether the magnitude of these changes differ between Black and White women.

4.3 Material and methods

Study participants. SWAN is an ongoing multi-ethnic, multi-site longitudinal study of the physical, biological, and psychosocial changes of the menopause transition. Detailed methods are presented elsewhere.[166] In brief, 3,302 women were recruited between 1996 and 1997 from seven research sites within the US (Detroit, MI; Boston, MA; Chicago, IL; Oakland, CA; Los Angeles, CA; Newark, NJ; and Pittsburgh, PA). To be eligible for SWAN, women had to be 42-52 years old at enrollment, have an intact uterus and at least one ovary with menstrual bleeding within the past three months, not be pregnant or breast-feeding, and not have used hormones

therapy within the past three months. Women self-identified as a member of 1 of 5 ethnic groups depending on the research site: White (all sites), Black (Boston, Chicago, Detroit, Pittsburgh), Chinese/Chinese American (Oakland), Hispanic (Newark) or Japanese/Japanese American (Los Angeles). The institutional review board at each participating site approved the study protocol, and all participants signed informed consent prior to participation.

Central arterial stiffness was measured as part of the SWAN Heart study, an ancillary study to SWAN designed to evaluate subclinical atherosclerosis measures in women at mid-life. Between 2001 and 2003, women from the SWAN Pittsburgh and Chicago sites were recruited to SWAN Heart (N=608) if they had no self-reported clinical CVD. By design, Pittsburgh and Chicago sites recruited only White and Black women. After a mean±SD of 2.3±0.5 years of SWAN Heart baseline visit, women were invited for a follow-up visit.

For the current analysis, we excluded women who reported CVD during the SWAN Heart follow-up period (n=11) or did not have arterial stiffness measurements (n=56). We additionally excluded 202 women for whom FMP date was not observed, see below. The final analytic sample included 339 women who contributed 537 observations with each woman having one or two measurment time points. Compared with the excluded participants, women in analytic sample were less likely to be hormone users (3% vs 24%) at baseline; otherwise, they shared similar baseline clinical characteristics.

Study outcome: central arterial stiffness. Central arterial stiffness was assessed by cfPWV at both SWAN Heart baseline and follow-up visits using a protocol previously described.[67] In brief, arterial flow waves were simultaneously and non-invasively recorded at the right carotid and femoral arteries of supine participants, using unidirectional transcutaneous Doppler flow probes (model 810-a, 10 MHz, Parks Medical Electronics, Aloha, OR). Data were scored using

software developed by the Laboratory of Cardiovascular Science, Gerontology Research Center, National Institute on Aging, which averages 20 seconds worth of waveforms and determines time from the R-wave on the electrocardiogram to the foot of the averaged waveforms. Transit time of the pulse wave was calculated as the delay between the averaged carotid and femoral waveforms. Three runs were performed for each participant, and the average was used in the analysis. Distance traveled by the pulse waveform was estimated by measurement over the participant's torso. Distance from the carotid to aortic site (at the suprasternal notch) was subtracted from the sum of the aortic to umbilicus and umbilicus to femoral sites to adjust for opposite direction of blood flow in that arterial branch. cfPWV was calculated as distance/transit time (cm/seconds) with higher cfPWV indicating a stiffer artery. Reproducibility study of cfPWV measurement was done in the same laboratory that conducted cfPWV measurements for SWAN participants, with an overall laboratory intraclass correlation of 0.77.[67]

Main independent variable: time since FMP. As part of each SWAN annual visit, date of the most recent menstrual period was determined, and FMP was assigned retrospectively as the date of the participant's last menstrual period before 12 consecutive months of amenorrhea. As a sensitivity analysis, we added 128 women to our analytic sample for whom the FMP date could not be observed due to hormone therapy or hysterectomy and/or bilateral oophorectomy and therefore, were multiply imputed as described in the Online Supplement. For observations in our analytic sample, time in years since FMP for cfPWV exam was calculated as the date on which cfPWV exam was performed minus FMP date.

Blood assays. A fasting blood sample was obtained and analyzed with standardized protocols at both the baseline and follow-up visits of the SWAN Heart ancillary study. Lipids, glucose, and insulin were measured at the Medical Research Laboratories (Lexington, KY).

Homeostasis model assessment (HOMA), an index of insulin resistance derived from glucose and insulin measures, was calculated as (fasting insulin (mU/Liter)*fasting glucose (mmoles/Liter))/22.5.[167] Endogenous sex hormones were measured at the University of Michigan Endocrine Laboratory. Since estradiol and FSH show dynamic changes throughout the menstrual cycle, cycle-day of blood draw (within 2-5 days of the menstrual cycle or not) was considered when adjusting for these hormones. A detailed description of blood assays measurement is presented in the Online Supplement.[168]

Study covariates. At visits that coincide with the cfPWV exam dates, participants completed self and interviewer-administered questionnaires that included assessment of sociodemographic and life-style factors and medical history. Age, race, whether woman had financial strain, current smoking status, and ever used hormone therapy were self-reported. Physical activity was assessed via Modified Baecke Scores of Habitual Physical Activity, with higher scores indicating more physical activity.[169] Menopause status was determined based on reported frequency and regularity of menstrual bleeding as follows: (1) premenopause: monthly bleeding with no perceived change in cycle interval, (2) early perimenopause: monthly bleeding with a perceived change in cycle interval, but at least one menstrual period within the past 3 months, (3) late perimenopause: 3 consecutive months of amenorrhea, and natural menopause: 12 consecutive months of amenorrhea [(4) early natural menopause: less than two years since became menopause, (5) late natural menopause: two or more years past menopause]. Medications including cholesterol, blood pressure, and diabetes medications were self-reported and interviewer-verified from medication container label. If a woman reported taking any of these medications, she was labeled as "current medication user". Participants had their physical measurements obtained at each study visit using standardized protocols.

Age at FMP, race, and study site were time-independent variables. For financial strain, we used values that coincided with the first cfPWV scan date. Time-varying physical activity scores that coincided or were closest in time with each participant's cfPWV scan date were used. For all other covariates, we used time-varying values that coincided in time with each participant's cfPWV scan date.

Statistical analysis. Clinical characteristics at baseline were compared between women with observed and imputed FMP. cfPWV was log-transformed due to skewed distribution. A nonparametric LOESS smoothing curve was used to fit cfPWV as a function of time since FMP.[170] From this curve, inflection points associated with increase/decrease in cfPWV were determined.[171] Since the observed inflection points were one year before and one year after the FMP, the cfPWV trajectory was divided into three time segments: segment 1: > one year before FMP, segment 2: within one year before and after FMP, and segment 3: > one year after FMP. Piecewise linear mixed-effects models were used to estimate and compare annual changes of cfPWV among time segments. Annual percent change of cfPWV in each time segment was calculated as: annual % change of cfPWV= $(e^{\text{estimated annual change in log-cfPWV}} -1)*100$.[172] A random intercept for each woman was included to account for baseline heterogeneity. To visualize the cfPWV trajectory, we calculated annual means of cfPWV over time since FMP as well as cfPWV estimates from the piecewise linear model. This figure was truncated at 5 years before and after FMP due to small sample size.

Study covariates were chosen based on *a priori* knowledge of risk factors of arterial stiffness, and by univariate analysis. The first model adjusted for age at FMP and sociodemographic factors including race, study site, and financial strain. The second model further adjusted for hormone therapy and lifestyle factors including current smoking and physical activity.

The third model additionally adjusted for CVD risk factors including systolic blood pressure (SBP), waist circumference, LDL-C, and HOMA to determine if they explain any observed changes in cfPWV around FMP. Finally, we built a parsimonious model (final model) by removing variables from the third model using backward stepwise selection methods with a p-value threshold of >0.2; financial strain, physical activity, and HOMA were removed and their removal did not change the effect estimates (beta) in time segments by more than 10%.

To test whether annual changes in arterial stiffness vary between Black and White women, we included an interaction term of race with each of the three time-segments separately to avoid collinearity. Race-specific annual percent changes in cfPWV were calculated separately for each time segment using the corresponding final model.

In subsequent analyses, we tested separately whether estradiol or FSH can attenuate changes in arterial stiffness relative to time since FMP in the final model controlling for cycle-day of blood draw. Additionally, we reran the final model adjusting separately for CRP, heart rate, and medications to test whether they can explain changes in arterial stiffness relative to time since FMP. As sensitivity analyses, we reran the final model combining women with imputed and observed FMP and on women for whom cfPWV was measured at both SWAN Heart visits.

4.4 Results

Study population. On average, the baseline cfPWV was measured, (mean±SD) 1.0±3.2 years before the FMP and the follow-up cfPWV was 1.1±3.1 years after the FMP. Of the 339 women in our study, 198 (58%) had cfPWV measured at baseline and follow-up visits, 115 (34%)

at baseline visit only, and 26 (8%) at follow-up visit only. Number of women/observations within time segments 1, 2, and 3 were 149 (44%) /189 (35%), 86 (25%) /144 (27%), and 104 (31%) /204 (38%), respectively. Characteristics of all women at baseline are shown in Table 4-1. The largest difference between women with observed and imputed FMP was ever use of hormone therapy (Appendix Table 4-1). The unadjusted association between each study covariate and cfPWV is presented in Appendix Table 4-2.

Changes in cfPWV around menopause, Table 4-2 and Figure 4-1**.** In segment 2, cfPWV increased 6.5% per year and that estimated increase was significantly different from 0% (i.e. "no change") adjusting for study covariates. There was no significant change in cfPWV in segments 1 and 3. The increase in cfPWV in segment 2 was significantly greater than the changes in segments 1 and 3. However, adjusting for midlife CVD risk factors including SBP, waist circumference, and LDL-C explained part of the estimated annual change in segment 2 (from 7.5% in model 2 to 5.9% in model 3). Based on model fit statistics, SBP and waist circumference, but not LDL-C, explained most of the variability. The estimated annual changes in cfPWV in segments 1 and 3 were not statistically different from one another.

Race interaction. Race modified the rate of change in cfPWV only in segment 1 (Figure 4-2); Black and White women showed 2.0% and 0.6% annual increase in cfPWV (p-value for racial difference is 0.04), respectively. Interestingly, only Black women had a statistically significant increase in cfPWV within segment 1.

Additional analyses (data not shown). Adjusting for estradiol, FSH, medications, or heart rate did not attenuate changes in cfPWV relative to the FMP. Adjusting for CRP among women with available CRP measurement (n=259) did not influence the overall conclusions.

Sensitivity analyses. Rerunning the final model to women with imputed and observed FMP and to women who had two cfPWV measurements resulted in similar findings, (Appendix Table 4-3).

4.5 Discussion

The present study characterized functional vascular changes, specifically, central arterial stiffness, in relation to FMP and showed racial differences using a well-characterized cohort of women while adjusting for mid-life covariates. We demonstrated significant increases in central arterial stiffness in the one year period surrounding the FMP in women transitioning through menopause. This increase was independent of aging, mid-life CVD risk factors, estradiol, and FSH. Additionally, we showed that Black women may experience greater adverse changes in arterial stiffness starting early in the transition than White women.

Previous cross-sectional analyses suggest that postmenopausal women have faster PWV measures compared with premenopausal women.[76] However, controlling for age in such analyses is critical as aging may greatly confound these associations.[77] To disentangle the relative contributions of chronologic and reproductive aging to vascular stiffness, longitudinal repeated-measure studies that follow a cohort of women through the menopause transition are required.[5] Using such design, previous results from SWAN have shown that women who transitioned through menopause during the study follow-up period had greater increase in central arterial stiffness compared with women who remained premenopausal or were postmenopausal at baseline.[41] In the

current analysis, we used time-oriented analysis to describe when critical vascular changes occur in relation to the FMP.

A similar, and slightly smaller, estimate of change in central arterial stiffness within one year of the FMP was observed after adding women with imputed FMP to women with observed FMP. However, women with imputed FMP were healthier as they were less likely to smoke and had lower blood pressure compared with women with observed FMP, suggesting that analyses including women with imputed FMP may better reflect the population represented by SWAN Heart.

The aorta regulates the pulsatile blood flow from the heart into steady flow of blood supply to peripheral organs.[43] Aging and other CVD risk factors including hypertension, hyperlipidemia, and central adiposity can increase arterial stiffness through loss of vascular elasticity and increased collagen deposition.[49,52] Consequently, arterial stiffness can result in left ventricular dysfunction,[62] low coronary perfusion,[63] and/or end-organ damage.[64,65] Interestingly, the increase in arterial stiffness within 1 year of FMP remained significantly greater than the increase before 1 year before FMP, however this difference was attenuated after adjusting for CVD risk factors. Although the annual percent increase in cfPWV within 1 year of FMP itself remained significantly different from 0% after adjusting for mid-life CVD risk factors, our results suggest that mid-life CVD risk factors may explain part of the *difference* in arterial stiffness progression across the menopause transition.

Estradiol and FSH dynamically change during the menopause transition.[40] Furthermore, estrogen receptor α and ß are detected on vessel walls in human vasculature, and estradiol is thought to preserve arterial stiffness through vasodilation and vascular-matrix formation.[173] Therefore, one would expect that estradiol or FSH may explain functional changes in central

arteries observed during the menopause transition. In the current study, these hormones did not explain changes or differences in arterial stiffness as women transitioned through menopause. Apart from traditional CVD risk factors, it seems that other mechanisms including inflammation and adipocytokine secretion may contribute to the changes in central arteries during the menopause transition. Several studies suggest that systemic inflammation is involved in the pathogenesis of arterial stiffness,[174] and that the menopause transition is associated with a rise in chronic low-grade inflammation,[175] which may result in accelerating arterial stiffness.[176] However, adjustment for CRP among women with available CRP measurement did not explain our findings. Additionally, women transitioning through menopause experience increased waist circumference and visceral adiposity.[28,131] These ectopic fat depots are thought to alter serum levels of adipocytokines with increase in leptin and decrease in adiponectin, which may have deleterious health effects on mid-life women.[177] Consequently, greater levels of leptin and lower levels of adiponectin in women transitioning through menopause may contribute to worsening arterial stiffness.[178] However, adipocytokine data were not available for most SWAN Heart participants.

Results from the current analysis are in line with previous studies, highlighting the time around menopause as a period when critical changes in the vasculature and CVD risk factors accumulate.[9,12,14,15,28,131] These changes may increase the likelihood of CVD later in life.[11] Our findings emphasize that women should be made aware that their cardiovascular health is expected to be adversely affected during the menopause transition. Therefore, frequent monitoring of CVD risk factors early in the menopause transition may be prudent as women can be counseled to stress lifestyle changes.[179] Interestingly, healthy lifestyle during the midlife is prospectively associated with less carotid atherosclerosis.[180] Future research should examine whether modifying arterial

stiffness in women transitioning through menopause is associated with favorable cardiovascular outcomes later in life.

Racial differences in arterial stiffness. Arterial stiffness is generally greater in Black than in White women.[83] This racial difference is also evident in midlife women, with Black women showing greater progression of arterial stiffness compared with their White counterparts.[23] In the current study, Black and White women did not have uniform trajectories of arterial stiffness around the FMP, such that arterial stiffness increased in Black women starting in the years preceding the one year before the FMP, while a similar increase earlier in the transition was not evident in White women. The reported effect modification of race are supported by previous studies showing racial differences among midlife women in subclinical vascular measures.[23,181,182] Given that Blacks have greater prevalence and earlier onset of CVD risk factors and higher CVD mortality compared with Whites, these results underscore the importance of aggressive and frequent monitoring of risk factors early in the menopause transition among Black women.[84,183]

Study limitations. About 42% of women in this study had a single measurement of arterial stiffness. However, the statistical analysis method we used is appropriate for handling unbalanced data with 1-2 time points of data per subject and yielding unbiased estimates of change.[184] To further support this approach, similar estimates of change and overall conclusions were found from analysis of women with two time points of data. Another limitation is that the follow up was relatively short (mean of 2.3 years). Nevertheless, about 40% of study participants had traversed menopause between the baseline and follow-up visits.

Conclusions. The one year before and after FMP is a critical period in women's lives when vascular functional alterations occur. In our study, arterial stiffness significantly increased within one year of the FMP independent of aging, CVD risk factors, estradiol, and FSH. Our results are

consistent with previous findings showing significant vascular structural changes and CVD risk factors worsening around the time of FMP. Collectively, our findings underscore the importance of frequent and timely monitoring of CVD risk factors and stressing more intensive lifestyle modifications in women transitioning through menopause.

4.6 Tables and figures (manuscript 1)

Table 4-1: Characteristics of study participant at baseline

Variables*	Values
Age (years)	51.10 ± 2.80
Race, N (%)	
White	216 (64)
Black	123 (36)
Financial strain, N (%)	
Hard to pay for basics	107 (34)
No financial strain	222 (66)
Current Smoker, N (%)	64 (19)
Physical activity score †	7.94 ± 1.78
BMI (kg/m^2)	29.40 ± 6.59
Waist circumference (cm)	89.55 ± 14.85
Systolic blood pressure (mmHg)	119.94 ± 16.63
Diastolic blood pressure (mmHg)	76.01 ± 9.58
Heart rate (beats per minute)	71.40 ± 8.93
Total Cholesterol (mg/dl)	201.75 ± 38.75
High-density lipoprotein cholesterol (mg/dl)	57.40 ± 14.22
Low-density lipoprotein cholesterol (mg/dl)	121.62 ± 34.04
Triglycerides (mg/dl), median (Q1, Q3)	97.0 (75.0, 135.0)
Glucose (mg/dL), median (Q1, Q3)	88.0 (83.0, 96.0)
Insulin (µIU/mL), median (Q1, Q3)	9.1 (6.9, 13.5)
HOMA, median (Q1, Q3)	2.0 (1.4, 3.1)
Current medication user ‡, N (%)	66 (20)
Menopausal Status, N (%)	
Premenopause	28 (9)
Early perimenopause	161 (52)
Late perimenopause	36 (11)
Early natural menopause	25 (8)
Late natural menopause	53 (17)
Age at FMP (years)	52.02 ± 2.84
Estradiol (pg/mL), median (Q1, Q3)	28.4 (15.5, 83.6)
FSH (mIU/mL), median (Q1, Q3)	36.8 (16.6, 83.9)
Ever used hormone therapy, N (%)	48 (14)
cfPWV (cm/second), median (Q1, Q3)	774.7 (677.8, 905.2)

* Mean ± standard deviation is presented unless specified.
† Modified Baecke Scores of Habitual Physical Activity, with higher scores indicating more physical activity.
‡ Taking medications for cholesterol, blood pressure, or diabetes.
BMI = body mass index; cfPWV = carotid-femoral pulse-wave velocity; CVD = cardiovascular disease; FMP = final menstrual period; FSH = Follicle stimulating hormone; HOMA = homeostasis model assessment.

Table 4-2: Annual percent change in carotid-femoral pulse wave velocity in time segments relative to FMP

Model*	Annual % Change † in cfPWV (95% CI)			P-Value for Pairwise Difference in Annual Change		
	Segment 1 >1 Year Before FMP	Segment 2 Within 1 Year of FMP	Segment 3 >1 Year After FMP	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Unadjusted	-0.20 (-1.54, 1.16)	6.51 (3.10, 10.04) ‡	-1.31 (-3.11, 0.52)	0.002	0.313	0.001
Model 1	0.95 (-0.47, 2.39)	7.72 (4.30, 11.24) ‡	-0.64 (-2.43, 1.18)	0.002	0.146	0.001
Model 2	0.87 (-0.55, 2.30)	7.54 (4.13, 11.07) ‡	-1.03 (-2.81, 0.79)	0.002	0.082	0.0003
Model 3	0.90 (-0.53, 2.34)	5.94 (2.38, 9.62) ‡	-1.01 (-2.98, 1.00)	0.024	0.102	0.006
Final model	0.88 (-0.50, 2.29)	5.74 (2.37, 9.21) ‡	-0.81 (-2.66, 1.08)	0.023	0.127	0.006

* Model 1: age at FMP, race, study site and financial strain.

Model 2: model 1 + hormone therapy, smoking status, and physical activity.

Model 3: model 2 + systolic blood pressure, waist circumference, low-density lipoprotein cholesterol, and homeostatic model assessment.

Final model: model 3 but without financial strain, physical activity, and homeostatic model assessment.

† Annual % change was calculated as: $e^{\text{estimated annual change in log-cfPWV}} -1)*100$.

‡ P-value < 0.05.

cfPWV = carotid-femoral pulse-wave velocity; FMP = final menstrual period.

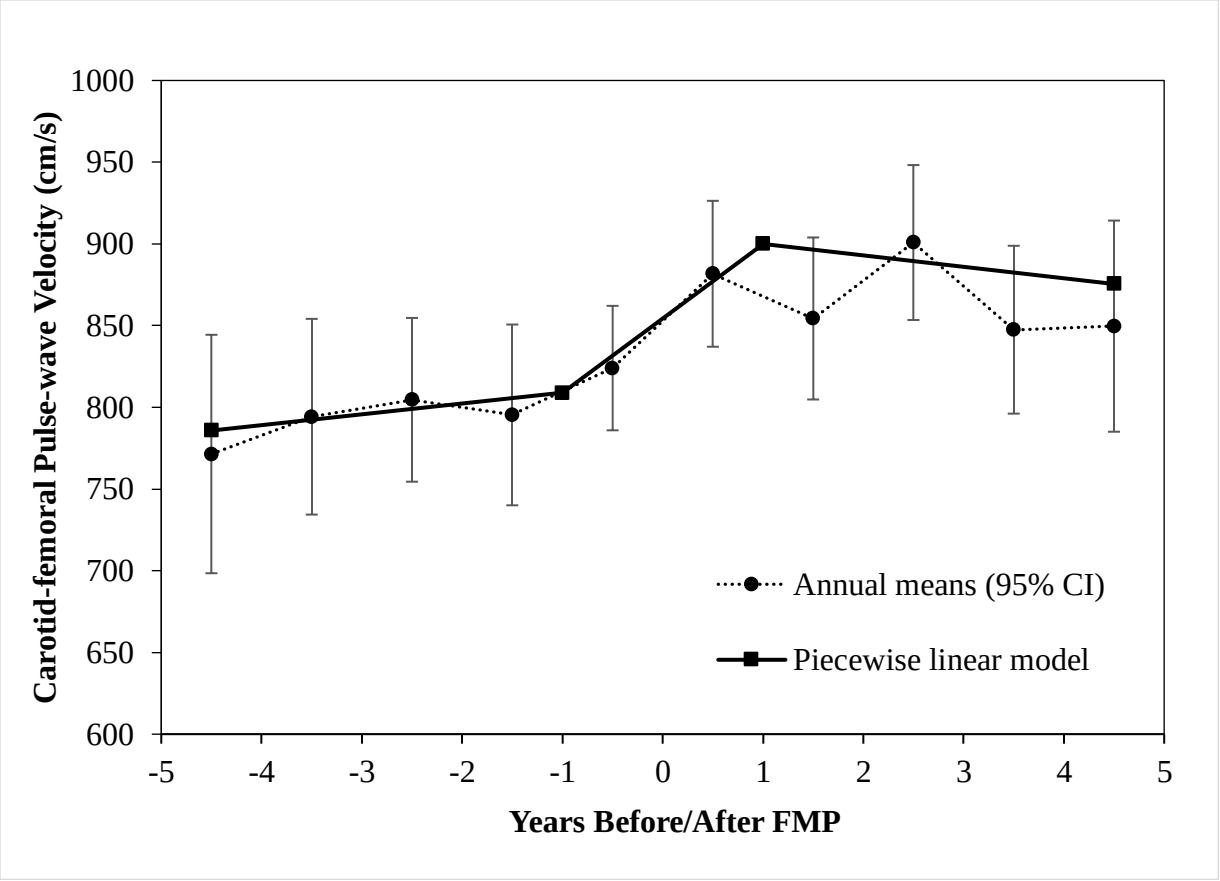


Figure 4-1: Means of cfPWV in years around FMP

Figure showing annual mean values compared with estimated values from piecewise linear model of cfPWV over time since FMP for women from the Study of Women's Health Across the Nation (SWAN) Heart. Model adjusted for age at FMP, race, study site, hormone therapy, smoking status, systolic blood pressure, waist circumference, and low-density lipoprotein cholesterol (final model). Error bars represent 95% CI.
FMP = final menstrual period.

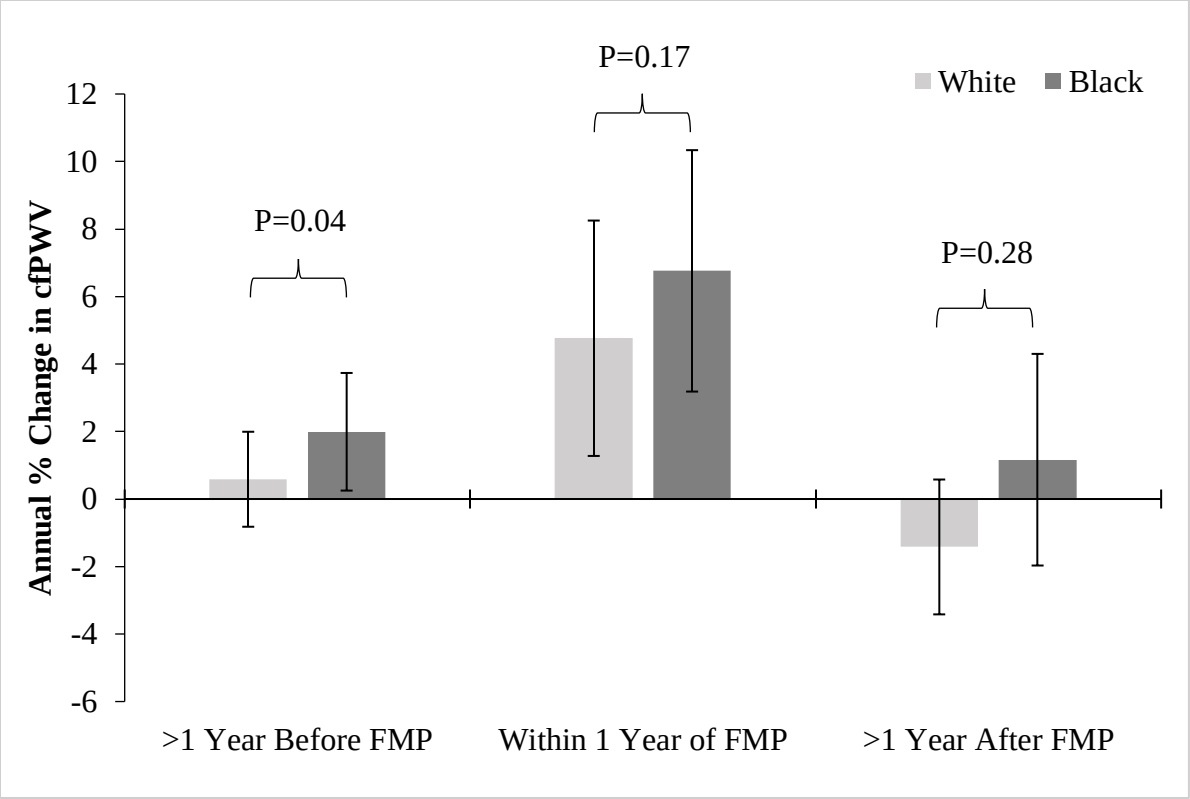


Figure 4-2: Effect modification of race on annual percent change in cfPWV by time segments relative to FMP

Interaction between race with each time segment was tested separately and annual percent change for each segment was estimated from corresponding model. Models adjusted for the other 2 time segments, age at FMP, study site, hormone therapy, smoking status, systolic blood pressure, waist circumference, and low-density lipoprotein cholesterol (final model). Error bars represent 95% CI. cfPWV = carotid-femoral pulse-wave velocity; FMP = final menstrual period.

4.7 Online supplement (manuscript 1)

Blood assays. A fasting blood sample was obtained with standardized protocols at both the baseline and follow-up visits of the SWAN Heart ancillary study. To allow for a standardized hormonal milieu, the blood sample was drawn during early follicular phase of the menstrual cycle (day 2-5) if women were still menstruating. If a timed sample could not be obtained, because menstrual cycles became less regular over time or due to menopause, a random fasting sample was taken within the 90-day period of the corresponding visit. All samples were maintained at 4°C until separated and then were frozen at -80°C and shipped in dry ice to a central certified laboratory (Medical Research Laboratories, Highland Heights, Kentucky).

Lipids, glucose, and insulin were measured at the Medical Research Laboratories (Lexington, KY). Total cholesterol and triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN), and high-density lipoprotein cholesterol was isolated using heparin-manganese. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.[168] Serum insulin was measured by a radioimmunoassay (DPC Coat-a-count) and glucose was measured with a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). C-reactive protein (CRP) was measured using ultrasensitive rate immunonephelometry (Dade Behring, Marburg, Germany). The sensitivity of the assay was 0.03 mg/dl, and the interassay coefficient of variations (CVs) at CRP concentrations of 0.05 and 2.2 mg/dl were 10% to 12% and 5% to 7%, respectively.

Endogenous sex hormones were measured at the University of Michigan Endocrine Laboratory using the Automated Chemilumisence System −180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol was measured using a modified, off-line Automated Chemilumisence System: 180 (E2-6). The lower limit of detection (LLD) was between 1 and 7

pg/mL. The inter- and intra-assay coefficients of variation averaged were 10.6% and 6.4%, respectively. Follicle-stimulating hormone (FSH) was measured by a modification of a manual assay kit (Bayer Diagnostics) utilizing two monoclonal antibodies directed to different regions on the beta subunit. The LLD was between 0.4–1.0 mIU/mL. The inter-and intra-assay coefficients of variation were 11.4% and 3.8%, respectively. Since estradiol and FSH show dynamic changes throughout the menstrual cycle, cycle-day of blood draw (within 2-5 days of the menstrual cycle or not) was considered when adjusting for these hormones.

Study covariates. Participants had their physical measurements obtained at each study visit. Body mass index (BMI) was calculated as weight/height^2. Waist circumference was measured at the level of the natural waist, defined as the narrowest part of the torso as seen from the anterior aspect. Blood pressure was averaged from 2 sequential measures in the right arm with the participant seated after at least 5 minutes of rest using appropriate cuff size that was determined based on arm circumference. Heart rate was created by multiplying beats count per 30 seconds by 2.

Appendix Table 4-1: Sensitivity analysis comparing women with observed vs imputed FMP at baseline

Variables*	Observed N=339	Imputed N=128	P-value
Age (years)	51.1±2.8	52.1±2.8	0.74
Race, N (%)			0.48
White	216 (64)	86 (67)	
Black	123 (36)	42 (33)	
Reported financial strain, N (%)	107 (33)	38 (30)	0.82
Current Smoker, N (%)	64 (19)	9 (7)	0.002
Physical activity score †	7.9±1.8	8.1±1.7	0.32
BMI (kg/m2)	29.4±6.6	28.8±5.8	0.34
Waist circumference (cm)	89.6±14.9	87.5±13.7	0.17
Systolic blood pressure (mmHg)	119.9±16.6	115.8±16.4	0.02
Diastolic blood pressure (mmHg)	76.0±9.6	73.9±9.9	0.04
Heart rate (beats per minute)	71.4±8.9	70.5±10.3	0.39
Total Cholesterol (mg/dl)	201.7±38.8	206.1±34.5	0.27
High-density lipoprotein cholesterol (mg/dl)	57.4±14.2	59.2±15.1	0.22
Low-density lipoprotein cholesterol (mg/dl)	121.6±34.0	120.0±31.8	0.64
Triglycerides (mg/dl), median (Q1, Q3)	97.0 (75.0, 135.0)	106.5 (79, 150.5)	0.03
HOMA index, Median (Q1, Q3)	2.0 (1.4, 3.1)	1.9 (1.4, 3.1)	0.99
Current medication user ‡, N (%)	66 (19)	26 (20)	0.84
Ever used hormone therapy, N (%)	48 (14)	78 (61)	<0.0001
cfPWV (cm/second), median (Q1, Q3)	774.7 (677.8, 905.2)	761.1 (682.4, 895.1)	0.99

* Mean ± standard deviation is presented unless specified.

† Modified Baecke Scores of Habitual Physical Activity, with higher scores indicating more physical activity.

‡ Taking medications for cholesterol, blood pressure, or diabetes.

BMI = body mass index; cfPWV = carotid-femoral pulse-wave velocity; HOMA = homeostasis model assessment.

Appendix Table 4-2: Beta estimates from simple linear regression of univariate associations between baseline study variables and baseline log-transformed carotid-femoral pulse-wave velocity

Variables	Beta (SE)	P-value
Age (years)	0.013 (0.004)	0.0003
Race		
Black	0.09 (0.02)	<.0001
White	Reference	
Financial strain		
Hard to pay for basics	0.03 (0.02)	0.15
No financial strain	Reference	
Current smoking		
Yes	0.03 (0.03)	0.27
No	Reference	
Physical Activity Score	-0.02 (0.01)	0.001
BMI (kg/m^2)	0.01 (0.001)	<.0001
Waist circumference (cm)	0.004 (0.001)	<.0001
Systolic blood pressure (mmHg)	0.004 (0.001)	<.0001
Diastolic blood pressure (mmHg)	0.005 (0.001)	<.0001
Heart rate (beats per minute)	0.002 (0.001)	0.06
Total cholesterol (mg/dl)	0.001 (0.0001)	0.02
High-density lipoprotein cholesterol (mg/dl)	-0.002 (0.001)	0.02
Low-density lipoprotein cholesterol (mg/dl)	0.001 (0.0001)	0.005
Triglycerides* (mg/dl)	0.06 (0.02)	0.008
Glucose* (mg/dl)	0.17 (0.07)	0.01
Insulin* (µIU/mL)	0.08 (0.02)	<.0001
Homeostatic model assessment*	0.07 (0.02)	<.0001
Current medication user †		
Yes	0.11 (0.02)	<.0001
No	Reference	
Age at FMP (years)	0.01 (0.003)	0.02
Estradiol* (pg/mL)	-0.01 (0.01)	0.58
FSH* (mIU/mL)	-0.01 (0.01)	0.22
Ever used hormone therapy		
Yes	0.11 (0.03)	<.0001
No	Reference	

* Log transformed.

† Taking medications for cholesterol, blood pressure, or diabetes.

‡ Unknown due to hormone therapy or hysterectomy.

BMI = body mass index; CVD = cardiovascular disease; FMP = final menstrual period; FSH = follicle stimulating hormone.

Appendix Table 4-3: Sensitivity analyses of annual percent change in carotid-femoral pulse wave velocity in time segments relative to FMP

Final Model*	Annual % Change † in cfPWV (95% CI)			P-Value for Pairwise Difference in Annual Change		
	Segment 1 >1 Year Before FMP	Segment 2 Within 1 Year of FMP	Segment 3 >1 Year After FMP	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Analytic Sample (Women with observed FMP), n=339	0.88 (-0.50, 2.29)	5.74 (2.37, 9.21) ‡	-0.81 (-2.66, 1.08)	0.023	0.127	0.006
Women with imputed and observed FMP, n=467	1.13 (-0.11, 2.39)	4.75 (1.55, 8.05) ‡	0.18 (-1.49, 1.88)	0.076	0.321	0.043
cfPWV measured at both visits, n=198	0.81 (-0.76, 2.40)	6.16 (2.21, 10.26) ‡	-1.64 (-3.78, 0.54)	0.031	0.053	0.005

* Final Model is adjusted for age at FMP, race, study site, hormone therapy, smoking status systolic blood pressure, waist circumference, and low-density lipoprotein cholesterol.

† Annual % change was calculated as: $e^{\text{estimated annual change in log-cfPWV}}$ -1)*100.

‡ P-value < 0.05.

cfPWV = carotid-femoral pulse-wave velocity; FMP = final menstrual period.

5.0 Manuscript 2: abdominal visceral adipose tissue over the menopause transition and carotid atherosclerosis: the swan heart study

5.1 Chapter summary

Background/Objectives: The extents to which the menopause transition, beyond chronological aging, is associated with abdominal visceral adipose tissue (VAT) accumulation and how this accumulation might relate to carotid atherosclerosis are unknown. The objectives were to characterize VAT trajectory relative to the final menstrual period (FMP) independent of aging, and to test whether menopause-related VAT accumulation is associated with greater average (cIMT), common (CCA-IMT), and/or internal (ICA-IMT) carotid artery intima-media thickness.

Subjects/Methods: Participants were 362 women (at baseline: age was (mean±SD) 51.1±2.8 years; 61% White, 39% Black) with no CVD from the SWAN Heart study. Women had up to two measurements of VAT and cIMT overtime. Splines determined potential inflection points of VAT trajectory relative to the FMP. Piecewise-linear random-effects models estimated changes in VAT trajectory. Random-effects models tested associations of menopause-related VAT with each cIMT measure, separately. Estimates were adjusted for age at the FMP, body mass index, and sociodemographic, lifestyle, and CVD risk factors.

Results: The splines revealed a non-linear trajectory of VAT with two inflection points demarcating 3 time segments: segment 1: >2 years before the FMP, segment 2: 2 years before the FMP to the FMP, and segment 3: after the FMP. VAT increased significantly by 8.2% (95% CI: 4.1%, 12.5%) and 5.8% (3.7%, 7.9%) per year in segments 2 and 3, respectively, with no significant change in VAT within segment 1. VAT predicted greater ICA-IMT in segment 2, such

that a 20% greater VAT was associated with a 2.0% (0.8%, 3.1%) greater ICA-IMT. VAT was not an independent predictor of ICA-IMT in the other segments or of the other cIMT measures after adjusting for covariates.

Conclusions: Women experience accelerated increase in VAT starting 2 years before menopause. This menopause-related increase in VAT is associated with greater atherosclerosis in the internal carotid artery.

5.2 Introduction

Waist circumference predicts excess risk of cardiovascular disease (CVD) mortality after menopause irrespective of having normal body weight.[185] With more than 70% of postmenopausal women having central obesity,[186] namely waist circumference ≥88 cm, it is critical to determine whether the menopause transition (MT), independent of chronological aging, puts women at greater risk of accumulating more abdominal fat.

Analyses of regional body fat have suggested that increases in waist circumference during midlife might be related to the MT.[28,111] Waist circumference is an overall measure of abdominal subcutaneous (SAT) and visceral (VAT) adipose tissues. When matching for SAT level, greater VAT is associated with greater risk of carotid artery atherosclerosis.[32,35] VAT-secreted adipocytokines are hypothesized to be the culprit of the heightened atherosclerotic risk.[187] Studies that examined whether the MT is related to midlife VAT accumulation showed inconsistent results and were limited by cross-sectional design,[124,125] small sample size,[130,131] or insufficient covariate adjustment.[131] Moreover, none of these studies assessed whether increases in VAT during the MT

contributed to greater risk of subclinical markers of atherosclerosis known to be influenced by the MT.[14,188]

Midlife women experience increases in average carotid artery intima-media thickness (cIMT) during the late perimenopause stage,[14] which typically starts 1-3 years before the final menstrual period (FMP) and extends to 1 year after the FMP.[6] Averaged cIMT across all carotid artery segments is a strong predictor of CVD events.[189] However, the internal carotid artery (ICA-IMT) has a greater predictive power for CVD risk compared with the common carotid artery (CCA-IMT).[190] Since patterns of carotid artery remodeling are segment-specific,[191] and in young adults greater waist circumference predicts greater progression in ICA-IMT but not the other segments,[192] it is expected that VAT may have distinctive pathological effects on different carotid artery segments. Whether potential menopause-related increases in VAT are associated with measures of carotid atherosclerosis is unknown.

The Study of Women's Health Across the Nation (SWAN) is a unique cohort study of midlife women followed longitudinally with careful measures of the MT and concurrent assessment of VAT and carotid atherosclerosis overtime enabling assessing the following objectives: 1) to characterize VAT trajectory over time relative to the FMP, independent of chronological aging, and 2) to test whether menopause-related increase in VAT was associated with carotid measures of atherosclerosis (average cIMT, CCA-IMT, and ICA-IMT). We hypothesized a non-linear trajectory of VAT over time relative to the FMP, with a larger change in VAT around the FMP, compared to prior to the FMP. We further hypothesized that menopause-related increases in VAT are associated with greater risk of carotid atherosclerosis.

5.3 Materials and methods

Study Participants

SWAN is an ongoing multi-ethnic longitudinal study of the MT. Detailed methods are presented elsewhere.[193] In brief, 3 302 women were recruited between 1996-1997 from: Detroit, MI; Boston, MA; Chicago, IL; Oakland, CA; Los Angeles, CA; Newark, NJ; and Pittsburgh, PA. To be eligible for SWAN, women had to be 42-52 years of age at enrollment, had an intact uterus and at least one ovary with menstrual bleeding within the past three months, not be pregnant or breast-feeding, and not had used hormones therapy within the past three months. The institutional review board at each participating site approved the study protocol, and all participants signed informed consent before participation.

SWAN Heart was an ancillary study to SWAN designed to evaluate women's midlife changes in subclinical atherosclerosis. Between 2001-2003, SWAN Pittsburgh and Chicago women enrolled to SWAN Heart Ancillary study (N=608) if they had no self-reported clinical CVD. By design, Pittsburgh and Chicago sites recruited only White or Black women. After a mean±SD of 2.3±0.5 years following the SWAN Heart baseline visit, women came for a follow-up visit. For the current analysis, we excluded women who reported CVD during the follow-up period (n=11), did not have VAT measurements (n=20), or did not have an observed FMP date (n=215) as described below. The final analytic sample included 362 women who contributed 595 observations with each woman having one or two measurements over time. Women in the analytic sample were older, more likely to be smokers, had higher systolic blood pressure, had larger waist circumference, and were less likely to have ever used hormone therapy at baseline compared with the excluded participants; otherwise, they shared similar baseline clinical characteristics including cIMT measures and VAT.

Time anchored to FMP

At each visit, women provided the date of their most recent menstrual period which enabled retrospectively assigning the FMP date as the date of the participant's last menstrual period before 12 consecutive months of amenorrhea. However, we did not observe the FMP in 215 SWAN Heart women due to hormone therapy, hysterectomy, or bilateral oophorectomy. Applying established methods,[194] 133 (62%) of these women had sufficient data available to allow multiple imputation of their FMP date and were combined with the women who have an observed FMP in a sensitivity analysis (the imputation is described in the Online Supplement).

Visceral Adipose Tissue

VAT area was assessed using electron beam computed tomographic scans. A 6-mm transverse image was obtained between L4 and L5 during breath hold with a C-150 Ultrafast CT Scanner (GE Imatron, San Francisco, CA). The scans were read by a single trained reader at the University of Pittsburgh. The area of adipose tissue was defined using image analysis (AccuImage Diagnostics, South San Francisco, CA) with fat structure determined using a pixel range of -30 to -190 Hounsfield units. A region-of-interest line was drawn at the interior of abdominal musculature along the fascial plane. Adipose tissue within the drawn area was considered to be VAT area. Interobserver reliability was determined by repeat reads on 10 VAT scans, with an intraclass correlation coefficient of 0.94.[195]

Carotid Artery Intima-Media Thickness

cIMT was assessed using a Toshiba SSA-270A scanner (Toshiba American Medical Systems, Tustin, CA) and a Hewlett-Packard 5500 scanner (Hewlett-Packard, Andover, MA). B-mode images were obtained from the following 4 locations in the left and right carotid arteries: the near and far walls of the distal common carotid artery (1 cm proximal to the carotid bulb); the far wall of the carotid bulb (from the point where the near and far walls of the common carotid artery are no longer parallel and extending to the flow divider); and the far wall of the internal carotid artery (distal 1 cm from the flow divider). The lumen-intima interface and the media-adventitia interface across a 1-cm segment of each of these locations were electronically traced. A computer-assisted measurement of each pixel over the traced area was generated for a total of 140 data points in each location. Average of the readings in each location was calculated, and then readings were averaged across all locations to obtain the average cIMT value. The readings of the common and internal carotid artery were averaged to obtain the CCA-IMT and ICA-IMT values, respectively. Interobserver reliability was determined by repeat reads on 20 cIMT scans, with an intraclass correlation coefficient of 0.98.[195]

Covariates

At SWAN visits concurrent with VAT assessment date, participants completed self- and interviewer-administered questionnaires that included assessment of sociodemographic and lifestyle factors and medical history. Participants had their physical and blood pressure measurements and a fasting blood sample obtained at each SWAN visit using standardized protocols. A detailed description of covariates and blood assays measurement is presented in the Online Supplement. Age at FMP, race, study site, and financial strain were time-fixed variables.

For all other covariates, time-varying values that coincided or where the closest in time with each participant's VAT assessment date were used.

Statistical Analysis

VAT and cIMT were log transformed due to skewed distributions. Repeated measures of VAT as a function of time relative to the FMP were plotted using locally weighted scatter-plot smoothing (LOWESS). The plot suggested a non-linear trajectory of VAT and thus piecewise linear random-effect models were used to estimate and compare annual changes of VAT across the identified time segments. Segment-specific annual percentage change of VAT was calculated as $(e^{\text{estimated annual change in log-VAT}}-1)\times 100$. Multivariable analysis adjusted for factors affecting VAT including body mass index (BMI). To visualize VAT trajectory, we plotted annual means of VAT over time relative to the FMP, as well as VAT estimates from the piecewise linear random-effects model.

To assess whether menopause-related increases in VAT are associated with cIMT measures, we created an indicator variable of the time segments identified via LOWESS. Because VAT accelerated significantly 2 years before the FMP, we defined a menopause-related VAT increase as the VAT value between 2 years before the FMP to the FMP (segment 2, see results). We separately modeled the repeated measures of averaged cIMT, CCA-IMT, and ICA-IMT as a function of the repeated measures of VAT, the indicator variable of the time segments, and their interaction (model 1), with segment 1 as the reference level. For more meaningful estimates, we calculated percentage change in each cIMT measure per 20% greater VAT by time segments using $(e^{\text{respective log-VAT beta estimate} \times \log(1.2)}-1)\times 100$. Multivariable analysis adjusted for factors affecting atherosclerosis.

As sensitivity analyses, we reran the previous analyses after combining women with imputed and observed FMP and on women for whom VAT was measured at both SWAN Heart visits. As additional analyses, we adjusted for estradiol in model 3 of the VAT trajectory analysis to explore whether it explains the significant inflection point in VAT trajectory at 2 years before the FMP. Additionally, because we observed a significant association between VAT and ICA-IMT in segment 2, we hypothesized that this association maybe modified by estradiol. For simplicity, we explored this effect modification using linear regression while limiting the analysis to women in segment 2 who do not have repeated measures. All analyses were conducted using SAS 9.4 with a significance level set at 0.05.

5.4 Results

Study population

The baseline VAT was measured 0.8±3.2 years before the FMP, and the follow-up VAT 1.3±3.1 years after the FMP. Of the 362 women in our study, 233 (64%) had VAT and cIMT measured at baseline and follow-up visits, 114 (32%) at baseline visit only, and 15 (4%) at follow-up visit only. Characteristics of the study sample are shown in Table 5-1. Compared with women who had imputed FMP dates, women with an observed FMP were more likely to be smokers, had higher systolic blood pressure, and were less likely to have ever used hormone therapy at baseline (Appendix Table 5-1).

VAT Trajectory over the FMP

LOWESS suggested a non-linear trajectory of VAT with inflection points at 2 years before the FMP and at the FMP, which divided the VAT trajectory into 3 time segments: segment 1: before 2 years before the FMP, segment 2: between 2 years before the FMP to the FMP, and segment 3: after the FMP. In segments 2 and 3, VAT increased 8.2% and 5.8% per year, respectively, and these estimates remained significant after adjusting for study covariates (Table 5-2; Figure 5-1). There was no significant change in VAT within segment 1. VAT increase in segment 2, but not segment 3, was significantly greater than VAT change in segment 1 adjusting for study covariates (model 3). The annual increase in VAT in segments 2 and 3 were not statistically different from each other. Further adjustment for estradiol attenuated the difference between segments 1 and 2, P=0.09 (data not shown).

Menopause-related VAT Increase and cIMT

Menopause-related increases in VAT were associated with increases in ICA-IMT but with modest increases in averaged and CCA-IMT. Each 20% increase in VAT in segment 2 was associated with 2.0% increase in ICA-IMT, 0.9% increase in averaged cIMT, and 0.8% increase in CCA-IMT (Table 5-3). Only the estimate in ICA-IMT of segment 2 remained significant after adjusting for study covariates (Figure 5-2). Associations between VAT and cIMT in segments 1 and 3 were not significant after adjusting for study covariates. Segment-specific difference between segments 1 and 2 in the ICA-IMT analysis was significant. No other segment-specific comparisons were statistically significant.

Limiting the analysis to segment 2 (n=115), although the interaction term was not significant, the reported increase in ICA-IMT with greater VAT was found to be more pronounced

at lower levels of estradiol such that a 20% increase in VAT was associated with a 2.5% (95% CI: 0.50%, 4.5%) increase in ICA-IMT for each one unit decrease in log-estradiol (data not shown).

Sensitivity Analyses

Analyses including women with imputed and observed FMP and women who had 2 VAT measurements resulted in the same conclusions (Appendix Table 5-2, Appendix Table 5-3, Appendix Table 5-4, Appendix Table 5-5).

5.5 Discussion

Using precise data on the timing of the MT, we showed a non-linear increase in VAT as women traverse menopause that accelerated remarkably starting 2 years before the FMP. VAT acceleration was independent of aging, lifestyle factors and overall adiposity. Importantly, menopause-related increases in VAT within 2 years before and up to the FMP was associated with a significant increase in ICA-IMT independent of traditional CVD risk factors and overall adiposity. Results of the current study suggest that the VAT increase during midlife is indeed a menopause-related phenomenon that could contribute to increased risk of carotid atherosclerosis in women during the MT.

Previous studies showed that premenopausal women gained a significant amount of VAT as they transitioned to postmenopause,[130] while others showed no gain mainly after adjusting for age.[127] Analysis [131] grouped VAT measurements to the nearest year relative to the FMP and showed that only mean VAT at years 4 and 3 before the FMP differed from mean VAT at the FMP. However, this analytic approach has limited ability to estimate rate of change in VAT

relative to FMP timing as we have done in our study. We provided adjusted estimates of change and tested differences in VAT trajectory over time relative to the FMP.

As women traverse menopause, they experience fluctuations in estradiol, a relative domination of testosterone, and increases in follicle-stimulating hormone.[40] These hormonal changes favor greater deposition of body fat and greater central adiposity by influencing appetite, energy expenditure, whole-body thermogenesis, and lipoprotein lipase activity.[107] Consistent with this, adjusting for estradiol in our analysis attenuated the inflection point of VAT trajectory at 2 years before the FMP suggesting that estradiol may mediate VAT acceleration.

As VAT accumulates, it acts as a metabolic organ that delivers excess free fatty acids into the portal circulation and secretes proinflammatory adipocytokines.[96] These VAT-derived metabolites are thought to influence atherosclerosis through increasing insulin resistance, inflammation, and blood pressure and viscosity.[97] Epidemiologic studies of the link between VAT and atherosclerosis measures report mixed results, with some showing an independent relationship between VAT and measures of carotid atherosclerosis,[35,196] while others showing the relationship to weaken after adjusting for traditional CVD risk factors.[197] However, none of these studies focused on ICA-IMT.

It is not obvious why menopause-related VAT increase predicted increased cIMT mainly in the internal, but not the common carotid artery. cIMT seems to be impacted by blood flow velocity, with a faster flow through the carotid artery being associated with a thinner cIMT.[198] A lower cerebral vascular resistance is associated with faster blood flow,[199] and estrogen may contribute to decreasing this resistance via its vasodilatory effects.[200] Interestingly, increase in circulating estrogen level in women receiving hormone therapy was associated with faster blood flow within the internal, but not the common carotid artery.[201] Thus, it is possible that estrogen

decline around FMP [40] may reduce flow velocity through the internal carotid artery creating a milieu where excess VAT can synergistically exert its atherosclerotic effects with a higher affinity predominantly on the internal carotid artery. Our exploratory analysis showed that VAT had a larger effect on ICA-IMT at lower levels of estradiol. However, this hypothesis needs to be rigorously tested in future studies.

By midlife, >80% of women have one or more traditional CVD risk factor.[8] Moreover, adverse changes in CVD risk factors and vasculature begin to accumulate during the MT independent of aging.[7,14,17,202] These results and our current findings highlight the importance of frequent monitoring of CVD risk factors early in the MT as women can be counseled to stress lifestyle changes.[203] Results from meta-analyses showed that lifestyle intervention programs including aerobic exercise with or without hypocaloric diets reduced CT scan-measured VAT by 30 cm^2.[204-206] Importantly, healthy lifestyle during midlife is prospectively associated with less carotid atherosclerosis.[180] Future research should assess whether lifestyle factors targeting central adiposity in midlife women is associated with favorable cardiovascular outcomes later in life.

Study Limitations

The excluded women were healthier compared with the women in the current analysis. It is therefore expected that our results may have been overestimated. However, sensitivity analyses run after adding back 62% of the excluded women through multiply imputing their FMP dates resulted in similar conclusions. Although we used repeated measures data in VAT-cIMT associations, the analysis was cross-sectional since VAT and cIMT were measured on the same occasions. Therefore, temporality in VAT-cIMT associations cannot be established. Moreover, our results may only be generalizable to populations similar to SWAN Heart women.

Conclusions

Using a well-characterized woman traversing menopause, we showed that women experience an accelerated increase in VAT starting 2 years before the FMP. Additionally, the increase in VAT between 2 years before the FMP up to the FMP may predispose women to carotid atherosclerosis. The results underscore the importance of frequent and timely monitoring of CVD risk factors including central adiposity and stressing intensive lifestyle modifications in women traversing menopause.

5.6 Tables and figures (manuscript 2)

Table 5-1: Characteristics of study participant at baseline (n=362)

Variables*	Values
Age (years)	51.13 ± 2.77
Race, N (%)	
White	222 (61)
Black	140 (39)
Financial strain, N (%)	117 (36)
Alcohol (drinks/month), N (%)	
1 or less	152 (42)
Between 2 and 4	125 (34)
5 or more	85 (24)
Current Smoker, N (%)	70 (19)
Physical activity score †	7.85 ± 1.77
Total daily calorie intake (kcal)	1731.9 (1355.5, 2204.7)
BMI (kg/m^2)	29.66 ± 6.63
Waist circumference (cm)	89.97 ± 14.79
Systolic blood pressure (mmHg)	120.92 ± 16.89
Diastolic blood pressure (mmHg)	118.41 ± 17.00
Total Cholesterol (mg/dl)	201.86 ± 38.67
High-density lipoprotein cholesterol (mg/dl)	57.39 ± 14.50
Low-density lipoprotein cholesterol (mg/dl)	121.57 ± 33.83
Triglycerides (mg/dl), median (Q1, Q3)	98.5 (75.0, 135.0)
Age at FMP (years)	51.97 ± 2.87
Ever used hormone therapy, N (%)	49 (14)
Estradiol (pg/mL), median (Q1–Q3)	27.2 (15.0, 76.7)
Abdominal VAT area (cm^2)	113.0 (78.1, 166.4)
Mean of average carotid IMT (mm)	0.66 (0.60, 0.73)
Mean of common carotid IMT (mm)	0.66 (0.62, 0.73)
Mean of internal carotid IMT (mm)	0.57 (0.50, 0.65)

* Mean ± standard deviation is presented unless specified.

† Modified Baecke Scores of Habitual Physical Activity, with higher scores indicating more physical activity.

BMI = body mass index; FMP = final menstrual period; IMT = intima-media thickness; VAT = visceral adipose tissue.

Table 5-2: Annual percentage change in VAT in time segments relative to FMP

Model*	Annual Percentage Change in VAT (95% CI)			P-Value for Pairwise Difference		
	Segment 1 >2 Years Before FMP	Segment 2 2 Years Before FMP to FMP	Segment 3 After FMP	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Unadjusted	0.82 (-2.10, 3.82)	**8.20 (4.10, 12.46)**	**5.77 (3.69, 7.89)**	0.01	0.01	0.35
Model 1	**3.43 (0.29, 6.68)**	**8.94 (4.84, 13.19)**	**6.52 (4.42, 8.65)**	0.07	0.11	0.35
Model 2	**3.28 (0.16, 6.49)**	**10.55 (6.37, 14.90)**	**5.74 (3.62, 7.90)**	0.02	0.20	0.07
Model 3	2.25 (-0.22, 4.77)	**7.77 (4.24, 11.42)**	**4.47 (2.72, 6.25)**	0.03	0.15	0.14

* Model 1: age at FMP, race, study site, and hormone therapy. Model 2: model 1 + physical activity, alcohol consumption, and daily calorie intake. Model 3: model 2 + body-mass index.

Bolded estimates indicate P-value < 0.05.

VAT = visceral adipose tissue; FMP = final menstrual period.

Table 5-3: Percentage change in carotid IMT measures per 20% greater VAT by time segments

Carotid-IMT Measure	Model*	Estimate (95% CI)			P-Value for Pairwise Difference		
		Segment 1	Segment 2	Segment 3	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Average Carotid Artery IMT	1	0.59 (-0.04, 1.23)	**0.88 (0.20, 1.56)**	**0.94 (0.39, 1.49)**	0.49	0.40	0.88
	2	0.52 (-0.07, 1.12)	**0.86 (0.21, 1.51)**	**0.72 (0.19, 1.26)**	0.39	0.60	0.72
	3	0.09 (-0.61, 0.80)	0.38 (-0.39, 1.16)	0.27 (-0.41, 0.97)	0.49	0.66	0.78
Common Carotid Artery IMT	1	**0.98 (0.32, 1.64)**	**0.79 (0.08, 1.51)**	**0.79 (0.24, 1.35)**	0.67	0.66	1.00
	2	**0.85 (0.22, 1.48)**	**0.69 (0.01, 1.38)**	**0.54 (0.01, 1.08)**	0.71	0.44	0.70
	3	0.23 (-0.49, 0.95)	0.10 (-0.70, 0.91)	-0.15 (-0.83, 0.55)	0.77	0.37	0.56
Internal Carotid Artery IMT	1	0.54 (-0.47, 1.55)	**1.95 (0.83, 3.07)**	**1.08 (0.22, 1.95)**	0.04	0.41	0.18
	2	0.52 (-0.44, 1.50)	**2.06 (0.98, 3.16)**	**1.01 (0.17, 1.85)**	0.02	0.44	0.10
	3	-0.01 (-1.13, 1.11)	**1.60 (0.34, 2.87)**	0.60 (-0.48, 1.69)	0.02	0.34	0.13

* Model 1: main effects of VAT and time segment indicator and their interaction. Model 2: model 1 + age, race, and study site. Model 3: model 2 + smoking, systolic blood pressure, low-density lipoprotein cholesterol, and body-mass index.

Bolded estimates indicate P-value < 0.05.

IMT = intima-media thickness; VAT = visceral adipose tissue.

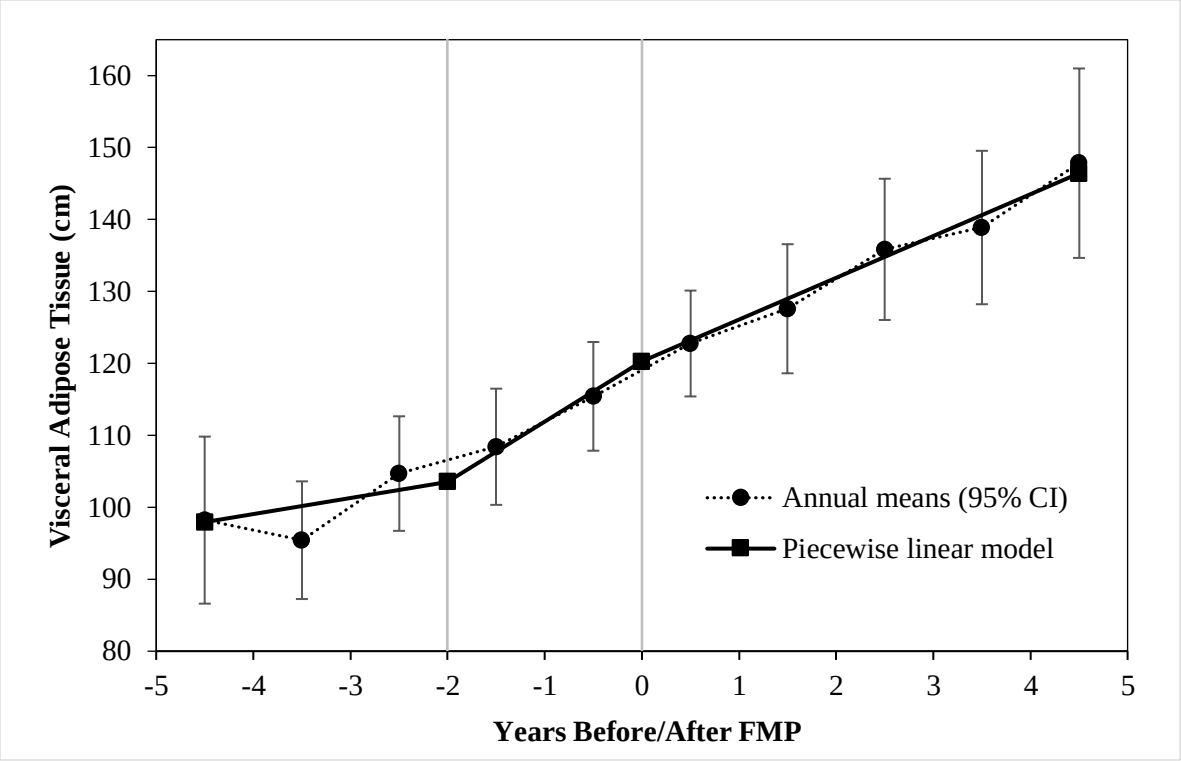


Figure 5-1: Means of abdominal visceral adipose tissue (VAT) in years around the final menstrual period (FMP).

Figure showing annual mean values compared with estimated values from piecewise linear model of VAT over time since FMP for women from the SWAN Heart Study. Model adjusted for age at FMP, race, study site, hormone therapy, physical activity, alcohol consumption, daily calorie intake, and body-mass index (model 3)
Error bars represent 95% CI.

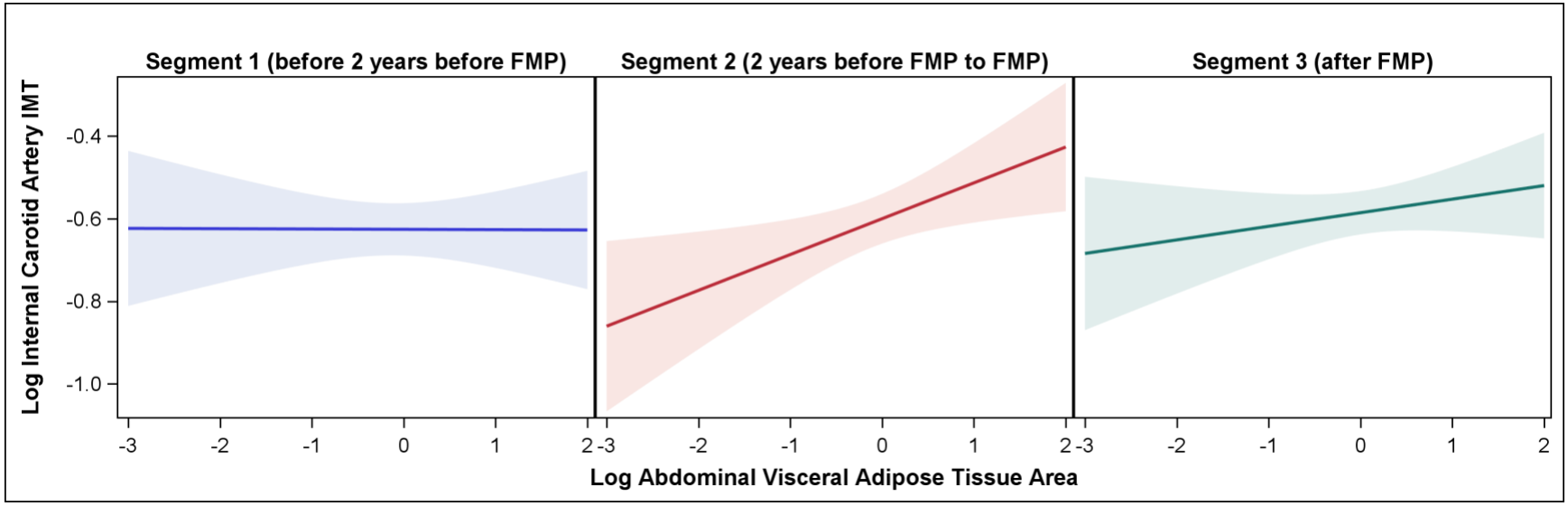


Figure 5-2: Association between abdominal visceral adipose tissue and internal carotid artery IMT by time segments.

Figure showing association between abdominal visceral adipose tissue area and internal carotid artery intima-media thickness (both log-transformed) by time segments relative to the FMP for women from the SWAN Heart Study. Model 3: adjusted for age, race, study site, smoking, systolic blood pressure, low-density lipoprotein cholesterol, and body-mass index.
Bands represent 95 % CI of the prediction.
FMP = final menstrual period; IMT = intima-media thickness.

5.7 Online supplement (manuscript 2)

Supplemental methods

Covariates

A fasting blood sample was obtained with standardized protocols at both the baseline and follow-up visits of the SWAN Heart ancillary study. To allow for a standardized hormonal milieu, the blood sample was drawn during early follicular phase of the menstrual cycle (day 2-5) if women were still menstruating. If a timed sample could not be obtained, because menstrual cycles became less regular over time or due to menopause, a random fasting sample was taken within the 90-day period of the corresponding visit. All samples were maintained at 4°C until separated and then were frozen at -80°C and shipped in dry ice to a central certified laboratory (Medical Research Laboratories, Highland Heights, Kentucky).

Fasting serum lipids were measured at the Medical Research Laboratories (Lexington, KY). Total cholesterol and triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN), and high-density lipoprotein cholesterol (HDL-C) was isolated using heparin-manganese. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald equation.[168] Estradiol was measured at the University of Michigan Endocrine Laboratory using the Automated Chemilumisence System −180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol was measured using a modified, off-line Automated Chemilumisence System: 180 (E2-6). The lower limit of detection was between 1 and 7 pg/mL. The inter- and intra-assay coefficients of variation averaged were 10.6% and 6.4%, respectively. Menstrual cycle day of blood draw (within 2–5 days of the menstrual cycle or not)

was considered when adjusting for estradiol because of its dynamic changes throughout the menstrual cycle.

At SWAN visits concurrent with VAT assessment date, participants completed self- and interviewer-administered questionnaires that included assessment of sociodemographic and lifestyle factors and medical history. Women self-reported age, race, calorie intake, smoking status, alcohol consumption, and whether they had financial strain or ever used hormone therapy. Physical activity was assessed via Modified Baecke scores of habitual physical activity.[169] Participants had their physical and blood pressure measurements obtained at each SWAN visit using standardized protocols.

Appendix Table 5-1: Comparing Women with Observed vs Imputed FMP at Baseline

Variables*	Observed N=362	Imputed N=133	P-value
Age (years)	51.1±2.8	51.3±2.9	0.63
Race, N (%)			0.50
White	222 (61)	86 (65)	
Black	140 (39)	47 (35)	
Financial strain, N (%)	117 (33)	38 (29)	0.31
Alcohol (drinks/month), N (%)			0.89
1 or less	152 (42)	54 (41)	
Between 2 and 4	125 (35)	45 (34)	
5 or more	85 (23)	34 (25)	
Current Smoker, N (%)	70 (19)	9 (7)	0.001
Physical activity score †	7.8±1.8	8.1±1.7	0.10
Total daily calorie intake (kcal)	1731.9 (1355.5, 2204.7)	1685.4 (1288.7, 2174.0)	0.78
BMI (kg/m^2)	29.7±6.6	28.9±5.8	0.22
Waist circumference (cm)	90.0±14.8	87.9±13.8	0.16
Systolic blood pressure (mmHg)	120.9±16.9	116.3±16.6	0.01
Diastolic blood pressure (mmHg)	76.8±10.1	74.1±10.2	0.01
Total Cholesterol (mg/dl)	201.9±38.7	205.8±34.4	0.30
High-density lipoprotein cholesterol (mg/dl)	57.4±14.5	59.3±14.9	0.21
Low-density lipoprotein cholesterol (mg/dl)	121.6±33.8	120.1±31.7	0.67
Triglycerides (mg/dl), median (Q1, Q3)	98.5 (75.0, 135.0)	106.0 (79.0, 146.0)	0.07
Ever used hormone therapy, N (%)	49 (14)	80 (60)	<0.001
Estradiol (pg/mL), median (Q1–Q3)	27.2 (15.0, 76.7)	36.2 (16.3, 63.4)	0.65
Abdominal VAT area (cm^2)	113.0 (78.1, 166.4)	103.5 (65.1, 158.6)	0.18
Mean of average carotid IMT (mm)	0.66 (0.60, 0.73)	0.65 (0.61, 0.70)	0.39
Mean of common carotid IMT (mm)	0.66 (0.62, 0.73)	0.66 (0.61, 0.71)	0.18
Mean of internal carotid IMT (mm)	0.57 (0.50, 0.65)	0.56 (0.51, 0.66)	0.82

* Mean ± standard deviation is presented unless specified.

† Modified Baecke Scores of Habitual Physical Activity, with higher scores indicating more physical activity.

BMI = body mass index; FMP = final menstrual period; IMT = intima-media thickness; VAT = visceral adipose tissue.

Appendix Table 5-2: Annual Percentage Change in VAT in Time Segments Relative to FMP (women with observed and imputed FMP combined, n=495)

Model*	Annual Percentage Change† in VAT (95% CI)			P-Value for Pairwise Difference in Annual Change		
	Segment 1 >2 Years Before FMP	Segment 2 2 Years Before FMP to FMP	Segment 3 After FMP	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Unadjusted	0.84 (-2.01, 3.76)	**7.36 (2.90, 12.00)**	**4.72 (2.70, 6.78)**	0.05	0.03	0.35
Model 1	**3.31 (0.23, 6.48)**	**8.28 (3.83, 12.92)**	**5.78 (3.76, 7.83)**	0.13	0.19	0.36
Model 2	2.63 (-0.38, 5.74)	**9.64 (5.05, 14.44)**	**5.13 (3.07, 7.23)**	0.04	0.18	0.12
Model 3	1.09 (-1.41, 3.65)	**6.81 (2.81, 10.97)**	**3.83 (2.05, 5.65)**	0.05	0.08	0.24

* Model 1: age at FMP, race, study site, and hormone therapy. Model 2: model 1 + physical activity, alcohol consumption, and daily calorie intake. Model 3: model 2 + body-mass index.

† Annual percentage change was calculated as: (eestimated annual change in log-VAT−1) × 100.

Bolded estimates indicate P-value < 0.05.

VAT = visceral adipose tissue; FMP = final menstrual period.

Appendix Table 5-3: Annual Percentage Change in VAT in Time Segments Relative to FMP (women who had 2 VAT measurements, n=233)

Model*	Annual Percentage Change† in VAT (95% CI)			P-Value for Pairwise Difference in Annual Change		
	Segment 1 >2 Years Before FMP	Segment 2 2 Years Before FMP to FMP	Segment 3 After FMP	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Unadjusted	2.02 (-1.20, 5.35)	**8.04 (3.83, 12.41)**	**6.47 (4.28, 8.71)**	0.05	0.03	0.56
Model 1	**4.59 (1.14, 8.16)**	**8.94 (4.73, 13.33)**	**7.36 (5.15, 9.62)**	0.17	0.19	0.55
Model 2	**4.14 (0.69, 7.71)**	**10.44 (6.11, 14.96)**	**6.55 (4.29, 8.85)**	0.05	0.25	0.16
Model 3	**3.33 (0.54, 6.20)**	**8.35 (4.63, 12.20)**	**4.94 (3.06, 6.85)**	0.06	0.35	0.15

* Model 1: age at FMP, race, study site, and hormone therapy. Model 2: model 1 + physical activity, alcohol consumption, and daily calorie intake. Model 3: model 2 + body-mass index.

† Annual percentage change was calculated as: $(e^{\text{estimated annual change in log-VAT}} - 1) \times 100$.

Bolded estimates indicate P-value < 0.05.

VAT = visceral adipose tissue; FMP = final menstrual period.

Appendix Table 5-4: Percentage Change in Carotid IMT Measures Per 20% Greater VAT by Time Segments (women with observed and imputed FMP combined, n=495)

Carotid-IMT Measure	Model *	Estimate† (95% CI)			P-Value for Pairwise Difference		
		Segment 1	Segment 2	Segment 3	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Average Carotid Artery IMT	1	**0.64 (0.12, 1.16)**	**0.87 (0.30, 1.44)**	**0.99 (0.53, 1.45)**	0.51	0.31	0.73
	2	**0.59 (0.09, 1.09)**	**0.88 (0.33, 1.42)**	**0.86 (0.41, 1.31)**	0.39	0.41	0.96
	3	0.19 (-0.38, 0.76)	0.38 (-0.25, 1.01)	0.43 (-0.12, 0.98)	0.60	0.48	0.88
Common Carotid Artery IMT	1	**1.00 (0.46, 1.53)**	**0.75 (0.14, 1.37)**	**0.73 (0.27, 1.19)**	0.53	0.45	0.95
	2	**0.89 (0.37, 1.41)**	**0.66 (0.07, 1.26)**	**0.53 (0.08, 0.98)**	0.54	0.29	0.71
	3	0.38 (-0.20, 0.96)	0.16 (-0.51, 0.83)	-0.02 (-0.56, 0.53)	0.57	0.25	0.64
Internal Carotid Artery IMT	1	0.55 (-0.32, 1.43)	**1.88 (0.85, 2.92)**	**1.26 (0.49, 2.03)**	0.03	0.23	0.32
	2	0.58 (-0.26, 1.42)	**2.04 (1.04, 3.05)**	**1.28 (0.54, 2.04)**	0.02	0.21	0.22
	3	0.06 (-0.89, 1.02)	**1.59 (0.48, 2.72)**	0.89 (-0.04, 1.82)	0.01	0.15	0.25

* Model 1: main effects of VAT and time segment indicator and their interaction. Model 2: model 1 + age, race, and study site. Model 3: model 2 + smoking, systolic blood pressure, low-density lipoprotein cholesterol, and body-mass index.

† Percentage change per 20% higher VAT was calculated as: $(e^{\text{respective log-VAT beta estimate} \times \log(1.2)} - 1) \times 100$.

Bolded estimates indicate P-value < 0.05.

IMT = intima-media thickness; VAT = visceral adipose tissue.

Appendix Table 5-5: Percentage Change in Carotid IMT Measures Per 20% Greater VAT by Time Segments (women who had 2 VAT measurements, n=233)

Carotid-IMT Measure	Model *	Estimate† (95% CI)			P-Value for Pairwise Difference		
		Segment 1	Segment 2	Segment 3	Segment 1 vs. 2	Segment 1 vs. 3	Segment 2 vs. 3
Average Carotid Artery IMT	1	0.64 (-0.04, 1.32)	**0.98 (0.26, 1.71)**	**0.92 (0.33, 1.50)**	0.41	0.51	0.87
	2	0.53 (-0.11, 1.18)	**0.88 (0.18, 1.58)**	**0.65 (0.08, 1.23)**	0.40	0.77	0.57
	3	0.02 (-0.73, 0.78)	0.28 (-0.57, 1.13)	0.08 (-0.67, 0.83)	0.57	0.90	0.64
Common Carotid Artery IMT	1	**0.99 (0.27, 1.72)**	**0.78 (0.00, 1.56)**	**0.73 (0.13, 1.34)**	0.65	0.58	0.92
	2	**0.87 (0.18, 1.57)**	0.59 (-0.16, 1.35)	0.38 (-0.22, 0.98)	0.54	0.27	0.63
	3	0.32 (-0.48, 1.12)	0.01 (-0.89, 0.91)	-0.29 (-1.06, 0.49)	0.53	0.19	0.51
Internal Carotid Artery IMT	1	0.85 (-0.25, 1.97)	**2.35 (1.14, 3.58)**	**1.15 (0.22, 2.09)**	0.04	0.68	0.08
	2	0.77 (-0.29, 1.84)	**2.37 (1.19, 3.56)**	**1.08 (0.17, 2.00)**	0.03	0.64	0.06
	3	0.17 (-1.04, 1.39)	**1.77 (0.38, 3.19)**	0.56 (-0.63, 1.76)	0.03	0.58	0.08

* Model 1: main effects of VAT and time segment indicator and their interaction. Model 2: model 1 + age, race, and study site. Model 3: model 2 + smoking, systolic blood pressure, low-density lipoprotein cholesterol, and body-mass index.

† Percentage change per 20% higher VAT was calculated as: $(e^{\text{respective log-VAT beta estimate} \times \log(1.2)} - 1) \times 100$.

Bolded estimates indicate P-value < 0.05.

IMT = intima-media thickness; VAT = visceral adipose tissue.

6.0 Manuscript 3: trajectories of systolic and diastolic blood pressure in women during midlife: is the pattern menopause- or aging-related?

6.1 Chapter summary

Background: Whether blood pressure trajectory over women's midlife is more driven by chronological aging or the menopause transition (MT) has been debated. Previous studies have assumed a common blood pressure trajectory over the MT. However, this assumption may have masked a menopause contribution.

Objectives: To determine whether distinct trajectories of systolic (SBP) or diastolic (DBP) blood pressure over the MT can be identified, and to test whether menopause-related factors (age at menopause and time-varying estradiol, follicle-stimulating hormone [FSH], and vasomotor symptoms) predict the pattern and/or level of blood pressure trajectories.

Methods: Participants were from the Study of Women's Health Across the Nation (SWAN). Group-based trajectory modeling (GBTM) was used to identify distinct blood pressure trajectories over time relative to the final menstrual period (FMP). Linear (suggestive of an aging effect) or piecewise-linear (suggestive of a menopause contribution) random coefficient models were then utilized to estimate blood pressure trajectory-specific change relative to the FMP. Associations of menopause-related factors with pattern and/or level of blood pressure trajectories were assessed using GBTM.

Results: The analysis included 3,302 women over 17 visits (baseline age[SD]: 46.3[2.7]; 47% White, 28% Black, 8% Chinese, 9% Hispanic, and 8% Japanese) with a median follow up of 19.1 years. Women experienced 3 distinct SBP trajectories: A "low-accelerated rise trajectory" (in

36% of the cohort) with a significant accelerated increase 1 year after the FMP (suggestive of a menopause contribution); a "medium-linear rise trajectory" (47%) with a monotonic increase over the MT (suggestive of an aging-related effect); and a "high-slow decline trajectory" (17%) with an increase up until the FMP followed by a plateau thereafter. An older age at menopause predicted a higher SBP trajectory. A higher FSH level predicted a lower SBP overtime in the low-accelerated and medium-linear rise, while presence of vasomotor symptoms predicted a higher SBP overtime in all SBP trajectories (all $P<0.05$). Estradiol did not predict SBP trajectory or level. Women experienced 3 distinct DBP trajectories but none of these trajectories showed a piecewise-linear increase.

Conclusions: Distinct patterns of SBP trajectories over the MT exist that revealed a group of women whose SBP trajectory is consistent with a menopause contribution. Our findings support frequent monitoring of blood pressure during the MT.

6.2 Introduction

Hypertension is an important modifiable risk factor for premature cardiovascular disease, yet 43% of adult women have hypertension.[39,207] Systolic blood pressure (SBP) increases with age in both sexes, but women's SBP surpasses that of men after midlife.[208] This phenomenon has generated interest in investigating the role the menopause transition (MT) may play in blood pressure trajectory over midlife.[209] The MT occurs contemporaneously as women age, and to investigate whether the MT is associated with blood pressure, longitudinal studies following women as they traverse menopause are needed.

After controlling for chronological aging, previous cross-sectional studies on the association between the MT and blood pressure were inconclusive and findings were inconsistent.[209] Conflicting findings may have resulted from heterogeneity among the studied populations.[209] In fact, in White women of normal weight, postmenopausal women had a higher blood pressure compared with their premenopausal counterparts.[146,148] However, a similar association was not observed in overweight White women.[10,153] In contrast, studies on overweight Asian populations tend to show an association between the MT and blood pressure,[150,157] while blood pressure was similar across MT stages in studies of normal weight Asian women.[147,156] Longitudinal studies of multi-ethnic populations mainly found a linear trajectory of blood pressure over the MT mostly consistent with an aging effect.[9,158,162] However, the literature thus far have analyzed the overall sample assuming that women share a common blood pressure trajectory. With existing racial/ethnic differences in hypertension and obesity patterns,[39] it is expected that blood pressure in women may have distinct trajectories over the MT. The previous conflicting findings suggest that blood pressure is possibly vulnerable to menopause or menopause-related factors only in a group of women with certain intrinsic characteristics.

Declines in estradiol, increases in follicle-stimulating hormone (FSH), and the occurrence of vasomotor symptoms are the hallmarks of the MT.[210,211] It has been hypothesized that these menopause-related factors and age at menopause may influence blood pressure.[157,212-214] However, association between these menopause-related factors and blood pressure trajectories over the MT have not been evaluated thoroughly before. The Study of Women's Health across the Nation (SWAN) is a cohort study with prospective and comprehensive assessment of the MT and concurrent measurements of the associated physical and hormonal changes. Using data from SWAN, we sought to determine whether distinct trajectories of SBP and diastolic blood pressure

(DBP) over time relative to the final menstrual period (FMP) can be identified; any trajectory with age-independent piecewise-linear increase is suggestive of a menopause contribution. We additionally sought to determine whether age at menopause, and time-varying estradiol, FSH, and vasomotor symptoms predicted the pattern and/or level of blood pressure trajectories.

6.3 Methods

Study population

SWAN is an ongoing multi-ethnic, multi-site longitudinal study designed to examine physical, biological, and psychosocial changes in women during midlife. Detailed design and methods were presented elsewhere.[166] In brief, 3,302 women were recruited between 1996 and 1997 from seven sites (Detroit, MI; Boston, MA; Chicago, IL; Oakland, CA; Los Angeles, CA; Newark, NJ; and Pittsburgh, PA). To enroll in SWAN, women had to be aged 42-52 years, have an intact uterus and at least one ovary with menstrual bleeding within the past three months, not be pregnant or breast-feeding, and not have used hormone therapy in the past three months. Women were self-identified as a member of one of five racial/ethnic groups: White (all sites), Black (Boston, Chicago, Detroit, Pittsburgh), Chinese/Chinese American (Oakland), Hispanic (Newark) or Japanese/Japanese American (Los Angeles). The institutional review board at each site approved the study protocol, and all participants signed informed consent before participation. Women in the current study were followed for up to 17 visits. Data from all SWAN women were included (n=3,302) in this analysis.

Time anchored to FMP

At each visit, women provided the date of their most recent menstrual period which enabled retrospectively assigning the FMP date as the date of the participant's last menstrual period before 12 consecutive months of amenorrhea. However, we did not observe the FMP in 1498 (45%) women due to hormone therapy, hysterectomy, or bilateral oophorectomy. Because SWAN is a study of the menopause transition, having FMP dates for all participants is essential to characterize menopause-related changes and be able to generalize results to women represented by SWAN. Therefore, SWAN has dedicated careful attention to multiply imputing the unobserved FMP dates using a wealth of demographic and longitudinal characteristics known in the literature to influence age at the FMP. Our method of imputation accounts for the uncertainty in the imputation by incorporating within and between imputation variability in the estimates.[194] Not to mention the increase in sample size and precision, the advantages of reducing bias by including both women with observed and imputed FMP dates outweigh errors due to imputing the data.[194] A fuller description of the imputation process is in the Online Supplement.

Blood pressure measurement

Blood pressure was measured at all visits except the 14th follow-up visit. Women had not smoked or consumed any caffeinated beverage within 30 minutes of blood pressure measurement. Blood pressure measurements were taken after at least 5 minutes of rest using appropriate cuff size that was determined based on arm circumference. Blood pressure measurements were averaged from 2 sequential measures in the right arm with the participant seated with feet flat on the floor (legs uncrossed) and refraining from talking during the measurements. To account for the effect of blood pressure medications, we imputed untreated values by adding 10 and 5 mmHg to the

observed SBP and DBP values while using medications, respectively.[208,215] SBP and DBP were treated as time-varying variables in analyses.

Blood assays

To allow for a standardized hormonal milieu, a fasting blood sample was drawn during early follicular phase of the menstrual cycle (day 2-5) if women were still menstruating. If a timed sample could not be obtained, because menstrual cycles became less regular over time or due to menopause, a random fasting sample was taken within 90-days of the corresponding visit. All samples were maintained at 4°C until separated and then were frozen at -80°C and shipped in dry ice to a central certified laboratory (Medical Research Laboratories, Highland Heights, Kentucky).

Endogenous sex hormones were measured at the University of Michigan Endocrine Laboratory using the Automated Chemilumisence System −180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol was measured at all SWAN visits except the 11th and 14th-16th follow-up visits. Estradiol was measured using a modified, off-line Automated Chemilumisence System: 180 (E2-6). The lower limit of detection (LLD) was between 1 and 7 pg/mL. The inter- and intra-assay coefficients of variation were 10.6% and 6.4%, respectively.[216] FSH was measured at all SWAN visits except the 11th, 14th, and 16th follow-up visits. FSH was measured by a modification of a manual assay kit (Bayer Diagnostics) utilizing two monoclonal antibodies directed to different regions on the beta subunit. The LLD was between 0.4–1.0 mIU/mL. The inter-and intra-assay coefficients of variation were 11.4% and 3.8%, respectively.[216] Since estradiol and FSH show dynamic changes throughout the menstrual cycle, cycle-day of blood draw (within 2-5 days of the menstrual cycle or not) was considered when analyzing these hormones. Estradiol and FSH were treated as time varying variables in analyses.

Study covariates

At each visit, participants completed self and interviewer-administered questionnaires that included assessment of sociodemographic and life-style factors and medical history. Age, race/ethnicity, education, income, financial strain, smoking status, alcohol consumption, presence of hot flashes or night sweats, and hormone therapy were self-reported. Blood pressure medications were self-reported and interviewer-verified from medication container labels. Body mass index (BMI) was calculated as weight/height^2. Hot flashes and night sweats were measured at all visits except the 16th follow-up visit. Women reporting either hot flashes or night sweats were considered as having vasomotor symptoms. Vasomotor symptoms were treated as a time-varying variable. Unless specified, other study covariates were obtained from the baseline visit.

Statistical analysis

To disentangle the menopause contribution on blood pressure from chronological aging, time in years was anchored to the FMP.[9] Data points 13 years before and 15 years after the FMP were dropped from the analysis due to small number of participants. We used group-based trajectory modeling (GBTM) to detect distinct trajectories of blood pressure over the MT. GBTM uses finite mixture modeling to identify distinct groups that share common developmental trajectory while accounting for classification uncertainty.[163] To allow for flexibility in estimating the shapes of blood pressure trajectories, we tested linear, quadratic, cubic, and quartic terms of time and retained the highest significant term with the corresponding lower terms.[163] We determined the number of groups based upon Bayesian Information Criterion and reasonable

scientific plausibility. For women with imputed FMP dates, we used the mean of the multiply imputed FMP dates for the GBTM.

Within each SBP and DBP trajectory group identified via GBTM, a pattern with inflection points around the FMP was suggestive of a menopause contribution. Potential inflection points around the FMP were identified via plotting repeated measures of SBP and DBP in each trajectory group using locally weighted scatterplot smoothing (LOWESS). Piecewise-linear random coefficients models were then used to test the year of inflection initially identified via LOWESS. To fine tune the identified inflection year, we additionally tested ±6 months. The multivariable analysis adjusted for age at menopause, race/ethnicity, study site, and baseline BMI. Established techniques of analyzing multiply imputed data were used in the random coefficients models.[194]

We explored predictive associations of menopause-related factors with blood pressure trajectories that showed piecewise-linear increases. Multinomial logistic and multiple linear regression within GBTM were used to estimate the odds ratio of trajectory group membership for baseline variables and beta coefficient of blood pressure level for time-varying covariates, respectively. This model adjusted for race/ethnicity, study site, and baseline BMI. Menopause-related factors hypothesized to predict membership in a blood pressure trajectory group were age at menopause (categorized into: before 51 years, between 51 and 53 years, and after 53 years) and baseline estradiol, FSH, and vasomotor symptoms. Menopause-related factors hypothesized to predict blood pressure level within each trajectory group were time-varying estradiol, FSH, and vasomotor symptoms.

We compared baseline characteristics between women with observed and imputed FMP dates and conducted additional sensitivity analyses restricted to women with an observed FMP. An additional sensitivity analysis adjusted further for time-varying hormone therapy. We also ran

models without imputing untreated blood pressure values. All analyses were performed with SAS 9.4 (SAS Institute, Cary NC) with a significance level set at 0.05.

6.4 Results

Distinct blood pressure trajectories

Using 32,967 observations of data spanning 28 years across the MT with a median follow-up of 19.1 years, three trajectories of SBP were identified (**figure 1**): Low-accelerated rise (36%) with an accelerated increase 1 year after the FMP; medium-linear rise (47%) with a monotonic increase over the MT; and high-slow decline (17%) with an accelerated increase up until the FMP followed by a plateau thereafter. White, Chinese, and Japanese women were more likely to follow the low-accelerated rise trajectory. Hispanic women were more likely to follow the medium-linear rise trajectory. Black women were more likely to follow the high-slow decline SBP trajectory. A greater BMI was associated with a higher SBP trajectory. **Table 1** shows other baseline characteristics by SBP trajectory group. About 77% of women showed a low and a medium-linear increase in DBP, while 23% showed a high-accelerated decline (**figure 2**). **Online table 1** shows baseline characteristics by DBP trajectory group.

Blood pressure group-specific rate of change: linear vs piecewise-linear

The SBP low-accelerated rise group showed a piecewise-linear increase with a significant inflection point at 1 year after the FMP consistent with a menopause-related increase (**table 2**). However, the SBP medium-linear rise group showed a linear increase consistent with an aging

effect. The SBP high-slow decline showed a significant inflection point at the FMP. None of the DBP trajectories showed a piecewise-linear increase (**table 2**).

Menopause-related predictors of SBP trajectories

Relative to the SBP medium-linear rise group and women with age at menopause between 51 and 53 years, women with a younger age at menopause were more likely to follow the low-accelerated rise, while women with an older age at menopause were more likely to follow the high-slow decline SBP trajectory (**table 3**). FSH predicted a lower SBP overtime in the low-accelerated and medium-linear rise, while vasomotor symptoms predicted a higher SBP overtime in all the trajectories. Baseline or time-varying estradiol did not predict SBP pattern or level, respectively.

Sensitivity analyses

Women with imputed and observed FMP showed small differences in baseline demographic variables, smoking and blood pressure variables (**online table 2**). Analyses restricted to women with observed FMP resulted in similar conclusions (**online figure 1 and online tables 3-5**). Adjusting for time-varying hormone therapy did not affect results (data not shown). As expected, analyses using observed blood pressure values in women using antihypertensive medications instead of imputed untreated values resulted in lower blood pressure level and change estimates, but the overall conclusions were not affected (data not shown).

6.5 Discussion

Using data from SWAN, one of the largest and longest study-to-date of the menopause transition in the US, we identified three distinct trajectories of SBP: Low-accelerated rise, medium-linear rise, and high-slow decline. We identified a group of women, the low-accelerated rise SBP group, whose SBP showed a piecewise-linear trajectory with an inflection point at 1 year after the FMP consistent with a menopause-related increase. For the majority of women in our study who followed the medium-linear rise, however, SBP followed a linear trajectory that is consistent with a chronological aging effect. In the high-slow decline SBP group, SBP increased up until the FMP followed by a menopause-related plateau thereafter. White, Chinese, and Japanese women were more likely to follow the low-accelerated rise trajectory; Hispanic women were more likely to follow the medium-linear rise trajectory; while Black women were more likely to follow the high-slow decline SBP trajectory. Although we identified three distinct trajectories of DBP, none of these trajectories showed a piecewise linear increase suggesting that increases in DBP were not menopause-related. Contrary to previous studies that found an average linear SBP trajectory over the MT consistent with aging effect, the current findings suggest that women cluster in different SBP trajectories that differ in level, pattern, and relationship with menopause and its related factors. Our findings highlight the period of the MT when SBP is likely to show an accelerated increase. Therefore, frequent and timely monitoring of CVD risk factors in women transitioning through menopause represents a window for counselling and stressing lifestyle changes.[203]

Although the SBP low-accelerated rise trajectory is consistent with a menopause contribution, that does not directly indicate that women following this trajectory are at high risk of elevated blood pressure complications. However, previous studies have shown a graded log-linear association between SBP and CVD death down to a SBP of 115 mmHg without a clear

threshold.[217] Among healthy midlife men and women without previous histories of hypertension or other traditional CVD risk factors, the presence of coronary artery calcium and the risk of incident CVD showed a stepwise increase with increasing SBP starting with SBP as low as 90 mmHg.[218] The increased CVD risk associated with SBP increase also holds across a broad age spectrum, starting as young as 30 years.[219] Whether the acceleration in SBP one year after the FMP in women following the low-accelerated rise trajectory predicts future clinical complications remains to be explored.

Women following the SBP high-slow decline and the DBP high-accelerated decline trajectories showed interesting phenomena of plateau and decrease at the FMP, respectively. A previous NHANES analysis noticed a similar SBP plateauing in Black and Mexican American women and a similar DBP decrease in White, Black, and Mexican American women around age 60.[220] Our study extends these observations into a longitudinal context by showing that Black women with high SBP, who tended to follow the high-slow decline trajectory, may experience a plateau in SBP after menopause. Although requiring further investigation, this plateau in the high-slow decline SBP trajectory appears to be a different phenomenon from the late-life SBP decrease which greatly steeps around age 80.[221]

In other work, greater serum levels of endogenous estradiol during the menstrual cycle or pregnancy are inversely related to SBP.[222] Additionally, surgically induced menopause increases SBP within a few weeks.[223] Evidence suggests vasodilatory effect of endogenous estradiol with subsequent decreases in SBP, thus it was hypothesized that estradiol depletion during the MT would be associated with a higher SBP. However, our results did not support this hypothesis. In fact, previous studies found no link between endogenous estradiol and SBP level or risk of incident hypertension.[224,225] It is hypothesized that the vasodilatory and cardio-protective actions of

endogenous estradiol are mediated in part through its metabolites.[226] 2-methoxyestradiol is an estradiol metabolite that has an estrogen receptor-independent growth inhibitory effects on smooth muscle and endothelial cells.[226] Interestingly, estradiol metabolites, but not estradiol, were associated with a lower SBP in postmenopausal women.[227] These results highlight the complexity of the MT and future studies should compare estradiol metabolite levels and its potential vascular functions across the stages of the MT.

Age at menopause showed no,[156] or even inverse,[157] associations with blood pressure level in previous cross-sectional studies. In a longitudinal study, rate of change in SBP over the MT did not differ among women with different ages at menopause.[228] However, our results did not align with previous studies as we uniquely showed that age at menopause has a direct association with SBP trajectory groups, such that an older age at menopause predicted a higher SBP trajectory. This disagreement across the studies may have resulted from the potential recall bias of age at menopause in cross-sectional studies or from our different analytic approach that assumed the presence of distinct SBP trajectories over the MT.

Women transitioning through menopause frequently report vasomotor symptoms.[229] A previous analysis in SWAN showed that premenopausal vasomotor symptoms predicted a higher risk of incident hypertension.[212] Although the exact mechanism linking vasomotor symptoms with SBP is not completely understood, evidence of greater sympathetic nervous system activation in women reporting vasomotor symptoms may be one plausible mechanism.[230] Our findings extend the previous SWAN analysis by showing vasomotor symptoms predicting a higher SBP overtime regardless of the SBP trajectory.

In women, FSH is known for its gonadal functions of follicular growth initiation and maturation. The presence of extragonadal functions of FSH was hypothesized after finding FSH

receptors expressed on blood vessels,[231] adipose tissues,[232] and the liver.[233] In fact, greater FSH predicted a lower SBP in a cross-sectional analysis among Chinese women.[213] We showed a similar inverse association overtime between FSH and SBP in the low-accelerated and the medium-linear rise trajectories, but not the high-slow decline trajectory. It is hypothesized that FSH has an angiogenesis property that is mediated through increasing vascular endothelial growth factor (VEGF).[234] In turn, VEGF showed an inverted U-shaped association with CVD events with the lowest risk experienced at the lower and upper end of the distribution.[235] However, it is not understood how FSH may reduce SBP; the underlying pathological mechanisms remain to be elucidated in future studies.

Major strengths of our study include the careful prospective characterization of the timing of the FMP, concurrent measurements of endogenous sex hormones and menopause-related factors, the multiethnic composition of SWAN, and the analytic technique that allowed linking menopause related factors with distinct SBP trajectories. This work includes some limitations. We did not observe the FMP in 45% of women. Additionally, for women without observed FMP, GBTM does not offer the option of including uncertainty of the imputation in determining trajectories of blood pressure. However, sensitivity analysis excluding women without observed FMP did not alter our conclusions. By follow-up visit 14, >99% of SWAN women have reached FMP and thus we defined attrition as women lost to follow-up before visit 14. We had 28% attrition rate and blood pressure trajectory groups directly predicted attrition. However, relaxing the assumption that attrition is independent of the trajectories by building the missing data mechanism into the GBTM resulted in a similar group sizes and shapes. As suggested by Haviland et al.[236], when trajectory groups are well separated, as in our case, attrition will effectively be at random and building the missing data structure into the model is not needed.

Conclusions

Distinct patterns of SBP trajectories over the MT exist that revealed a group of women following the low-accelerated rise trajectory consistent with a menopause contribution. Our findings support frequent monitoring of blood pressure during the MT. Additionally, menopause-related factors including age at menopause, vasomotor symptoms, and FSH predicted SBP pattern and level overtime. Future work should investigate potential mechanisms by which the MT may accelerate SBP increase.

6.6 Tables and figures (manuscript 3)

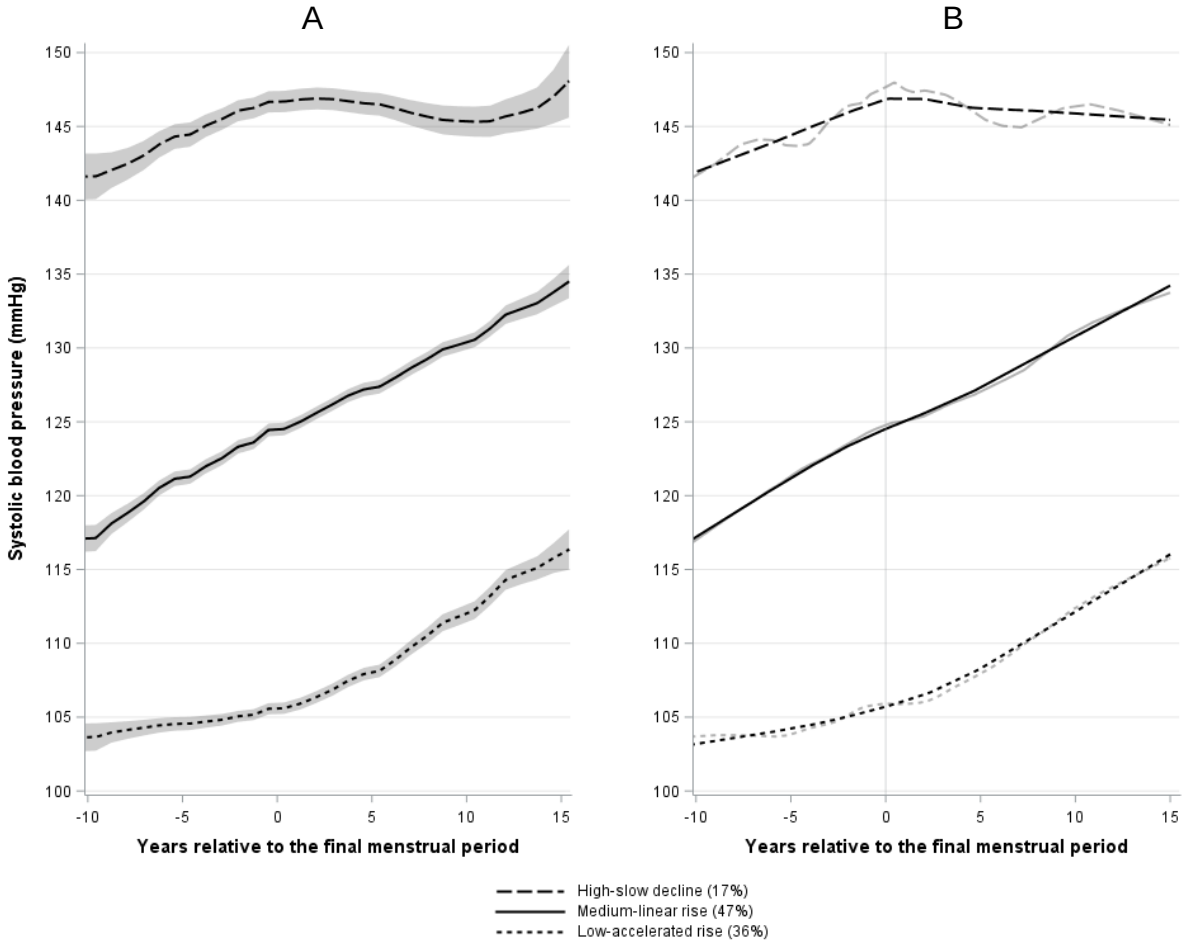


Figure 6-1: Trajectories of systolic blood pressure over the menopause transition (n=3302)

Panel A: Systolic blood pressure represents the predicted mean at each time point within each trajectory group from group-based trajectory modelling. No factors were included in the model.
Panel B: LOWESS curves of systolic blood pressure values over the menopause transition by trajectory groups. Black lines correspond to smoothing level=0.70, while transparent black lines correspond to smoothing level=0.30.

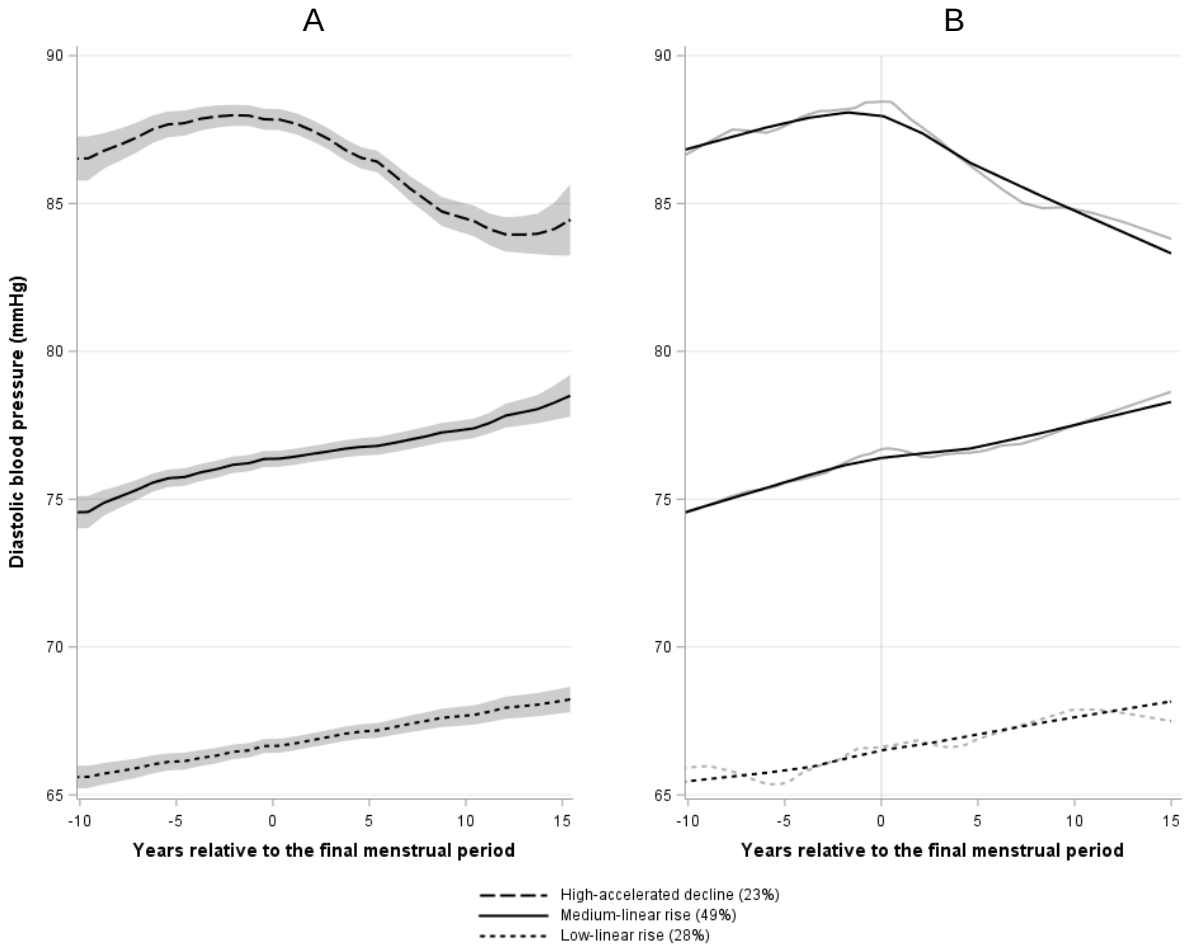


Figure 6-2: Trajectories of diastolic blood pressure over the menopause transition (n=3302)

Panel A: Diastolic blood pressure represents the predicted mean at each time point within each trajectory group from group-based trajectory modelling. No factors were included in the model.
Panel B: LOWESS curves of diastolic blood pressure values over the menopause transition by trajectory groups. Black lines correspond to smoothing level=0.70, while transparent black lines correspond to smoothing level=0.30.

Table 6-1: Baseline characteristics of study participants by SBP trajectory group

Variable*	Total, n=3302	Low-accelerated rise 1185 (36%)	Medium-linear rise 1559 (47%)	High-slow decline 558 (17%)	P value
Age (years)	46.34 ± 2.69	46.04 ± 2.59	46.39 ± 2.68	46.83 ± 2.84	<.0001
Race/ethnicity, N (%)					<.0001
White	1551 (47.0)	716 (60.4)	703 (45.1)	132 (23.7)	
Black	934 (28.3)	154 (13.0)	445 (28.5)	335 (60.0)	
Chinese	250 (7.6)	135 (11.4)	95 (6.1)	20 (3.6)	
Hispanic	286 (8.7)	25 (2.1)	208 (13.3)	53 (9.5)	
Japanese	281 (8.5)	155 (13.1)	108 (6.9)	18 (3.2)	
Education, N (%)					<.0001
Less than high school	238 (7.3)	36 (3.0)	149 (9.7)	53 (9.6)	
Some College	1632 (49.9)	500 (42.3)	799 (51.9)	333 (60.4)	
College Degree or higher	1401 (42.8)	645 (54.6)	591 (38.4)	165 (29.9)	
Income, N (%)					<.0001
<$35,000	1004 (31.3)	241 (20.9)	519 (34.2)	244 (45.1)	
$35,000-$75,000	1308 (40.7)	498 (43.2)	605 (39.9)	205 (37.9)	
>$75,000	900 (28.0)	414 (35.9)	394 (26.0)	92 (17.0)	
Financial Strain, N (%)	1312 (40.0)	359 (30.4)	663 (42.9)	290 (52.2)	<.0001
Smoking Status, N (%)					<.0001
Never	1890 (57.3)	710 (60.0)	895 (57.4)	285 (51.1)	
Past	830 (25.2)	311 (26.3)	390 (25.0)	129 (23.1)	
Current	580 (17.6)	162 (13.7)	274 (17.6)	144 (25.8)	
Alcohol drinks per week, N (%)					0.001
None	1553 (49.6)	532 (47.3)	719 (48.4)	302 (57.9)	

Variable*	Total, n=3302	Low-accelerated rise 1185 (36%)	Medium-linear rise 1559 (47%)	High-slow decline 558 (17%)	P value
<1	326 (10.4)	108 (9.6)	170 (11.4)	48 (9.2)	
1-7	813 (25.9)	306 (27.2)	396 (26.6)	111 (21.3)	
>7	441 (14.1)	179 (15.9)	201 (13.5)	61 (11.7)	
Systolic blood pressure (mmHg)	117.85 ± 17.03	104.80 ± 8.49	119.41 ± 10.96	141.19 ± 17.73	<.0001
Diastolic blood pressure (mmHg)	75.48 ± 10.52	68.91 ± 7.65	76.91 ± 8.77	85.37 ± 11.00	<.0001
Blood pressure medication, N (%)	471 (14.3)	28 (2.4)	182 (11.7)	261 (46.8)	<.0001
BMI	28.26 ± 7.21	24.69 ± 4.98	29.20 ± 6.88	33.34 ± 8.24	<.0001
Age at FMP (years)	52.07 ± 2.85	51.72 ± 2.67	52.30 ± 2.95	52.26 ± 2.93	0.001
Vasomotor symptoms, N (%)	1287 (39.2)	384 (32.7)	622 (40.1)	281 (50.6)	<.0001
Estradiol (pg/mL), Median (Q1, Q3)	55.2 (33.0, 88.7)	58.0 (34.3, 89.7)	55.1 (32.8, 88.7)	50.3 (31.8, 85.2)	0.13
Follicle-stimulating hormone (mIU/mL), Median (Q1, Q3)	15.9 (10.8, 26.4)	16.1 (11.0, 27.1)	15.8 (10.6, 26.2)	16.1 (10.9, 25.4)	0.29
Hormone therapy, N (%)	6 (0.2)	1 (0.1)	4 (0.3)	1 (0.2)	0.58

* Mean ± SD is presented unless otherwise specified

Table 6-2: Annual change in blood pressure (mmHg) in time segments, by trajectory groups, (n=3302)*

Blood pressure	Trajectory Group	n	Inflection point	Change in SBP (95% CI)		P-Value for Difference
				Before inflection	After inflection	
Systolic	**High-slow decline**	558	FMP	0.47 (0.22, 0.73)	-0.13 (-0.33, 0.07)	0.001
	Medium-linear rise	1559	N/A	0.67 (0.62, 0.71)		N/A
	Low-accelerated rise	1185	1 year after FMP	0.24 (0.17, 0.30)	0.78 (0.72, 0.85)	<0.0001
Diastolic	**High-accelerated decline**	763	FMP	0.08 (-0.04, 0.19)	-0.36 (-0.43, -0.28)	<0.0001
	Medium-linear rise	1609	N/A	0.13 (0.10, 0.16)		N/A
	Low-linear rise	930	N/A	0.13 (0.10, 0.16)		N/A

* Models adjusted for age at the FMP, site, race/ethnicity, and SWAN baseline body-mass index

Table 6-3: Predictors of SBP trajectory group membership and level across the menopause transition, (n=3302) *

Variables ‡	SBP trajectory group, n (%)		
	Low-accelerated rise	**Medium-linear rise**	**High-slow decline**
	1158 (36%)	**1559 (47%)**	**558 (17%)**
Predictors of SBP trajectory group membership, OR (95% CI) [a]			
Race/ethnicity			
White	1 [ref]	[ref]	1 [ref]
Black	**0.32 (0.23, 0.43)**	[ref]	**3.35 (2.49, 4.52)**
Hispanic	0.36 (0.12, 1.11)	[ref]	2.30 (0.97, 5.45)
Japanese	**0.46 (0.29, 0.73)**	[ref]	1.53 (0.61, 3.81)
Chinese	**0.39 (0.24, 0.63)**	[ref]	**3.26 (1.21, 8.81)**
Baseline BMI (per 1-unit increase)	**0.86 (0.84, 0.88)**	[ref]	**1.07 (1.05, 1.09)**
Age at menopause			
Before 51 years	**1.79 (1.37, 2.34)**	[ref]	1.18 (0.87, 1.62)
Between 51 and 53 years	1 [ref]	[ref]	1 [ref]
After 53 years	**0.74 (0.58, 0.94)**	[ref]	**1.47 (1.10, 1.96)**
Baseline estradiol † (per 1-SD increase)	0.99 (0.88, 1.11)	[ref]	1.06 (0.92, 1.22)
Baseline FSH † (per 1-SD increase)	0.96 (0.85, 1.09)	[ref]	1.06 (0.93, 1.22)
Baseline vasomotor symptoms	0.83 (0.67, 1.03)	[ref]	**1.37 (1.08, 1.74)**
Predictors of SBP level overtime within each trajectory group, beta (SE) [a]			
Estradiol † (per 1-SD increase)	-0.05 (0.12)	-0.12 (0.12)	0.03 (0.20)
FSH † (per 1-SD increase)	**-0.45 (0.11)**	**-0.39 (0.11)**	-0.19 (0.18)
Vasomotor symptoms	**1.10 (0.26)**	**1.19 (0.26)**	**1.46 (0.44)**

* Bold indicates P-value <0.05

‡ Model further adjusted for site and cycle-day of blood draw

† Log-transformed

[a] Estimates of SBP trajectory group membership and level are from the same GBTM.

6.7 Online supplement (manuscript 3)

Appendix Table 6-1: Baseline characteristics of study participants by DBP trajectory group

Variable*	Low-linear rise 930 (28%)	Medium-linear rise 1609 (49%)	High-accelerated decline 763 (23%)	P value
Age (years)	46.13 ± 2.63	46.28 ± 2.67	46.72 ± 2.76	<.0001
Race/ethnicity, N (%)				<.0001
White	543 (58.4)	774 (48.1)	234 (30.7)	
Black	165 (17.7)	424 (26.4)	345 (45.2)	
Chinese	119 (12.8)	91 (5.7)	40 (5.2)	
Hispanic	4 (0.4)	189 (11.7)	93 (12.2)	
Japanese	99 (10.6)	131 (8.1)	51 (6.7)	
Education, N (%)				<.0001
Less than high school	38 (4.1)	133 (8.4)	67 (8.8)	
Some College	434 (47.0)	780 (49.1)	418 (55.1)	
College Degree or higher	452 (48.9)	676 (42.5)	273 (36.0)	
Income, N (%)				<.0001
<$35,000	216 (23.9)	503 (32.1)	285 (38.4)	
$35,000-$75,000	397 (44.0)	625 (39.9)	286 (38.5)	
>$75,000	289 (32.0)	439 (28.0)	172 (23.1)	
Financial Strain, N (%)	290 (31.3)	658 (41.3)	364 (48.0)	<.0001
Smoking Status, N (%)				0.14
Never	559 (60.2)	897 (55.8)	434 (56.9)	
Past	228 (24.5)	418 (26.0)	184 (24.1)	
Current	142 (15.3)	293 (18.2)	145 (19.0)	
Alcohol drinks per week, N (%)				0.62
None	447 (50.7)	739 (48.4)	367 (50.7)	

Variable*	Low-linear rise 930 (28%)	Medium-linear rise 1609 (49%)	High-accelerated decline 763 (23%)	P value
<1	98 (11.1)	157 (10.3)	71 (9.8)	
1-7	216 (24.5)	403 (26.4)	194 (26.8)	
>7	121 (13.7)	228 (14.9)	92 (12.7)	
Systolic blood pressure (mmHg)	105.56 ± 10.71	117.27 ± 13.03	134.09 ± 17.56	<.0001
Diastolic blood pressure (mmHg)	66.03 ± 7.03	75.63 ± 7.10	86.67 ± 8.84	<.0001
Blood pressure medication, N (%)	43 (4.6)	158 (9.8)	270 (35.4)	<.0001
BMI	25.53 ± 6.01	28.50 ± 7.10	31.14 ± 7.57	<.0001
Age at FMP (years)	51.88 ± 2.76	52.11 ± 2.90	52.24 ± 2.87	0.16
Vasomotor symptoms, N (%)	324 (35.1)	620 (38.8)	343 (45.1)	0.0001
Estradiol (pg/mL), Median (Q1, Q3)	56.3 (34.1, 87.9)	55.4 (33.5, 88.4)	53.2 (31.5, 91.4)	0.67
Follicle-stimulating hormone (mIU/mL), Median (Q1, Q3)	15.9 (11.0, 26.3)	16.0 (10.8, 26.9)	15.7 (10.6, 25.5)	0.78
Hormone therapy, N (%)	0 (0)	4 (0.2)	2 (0.3)	0.31

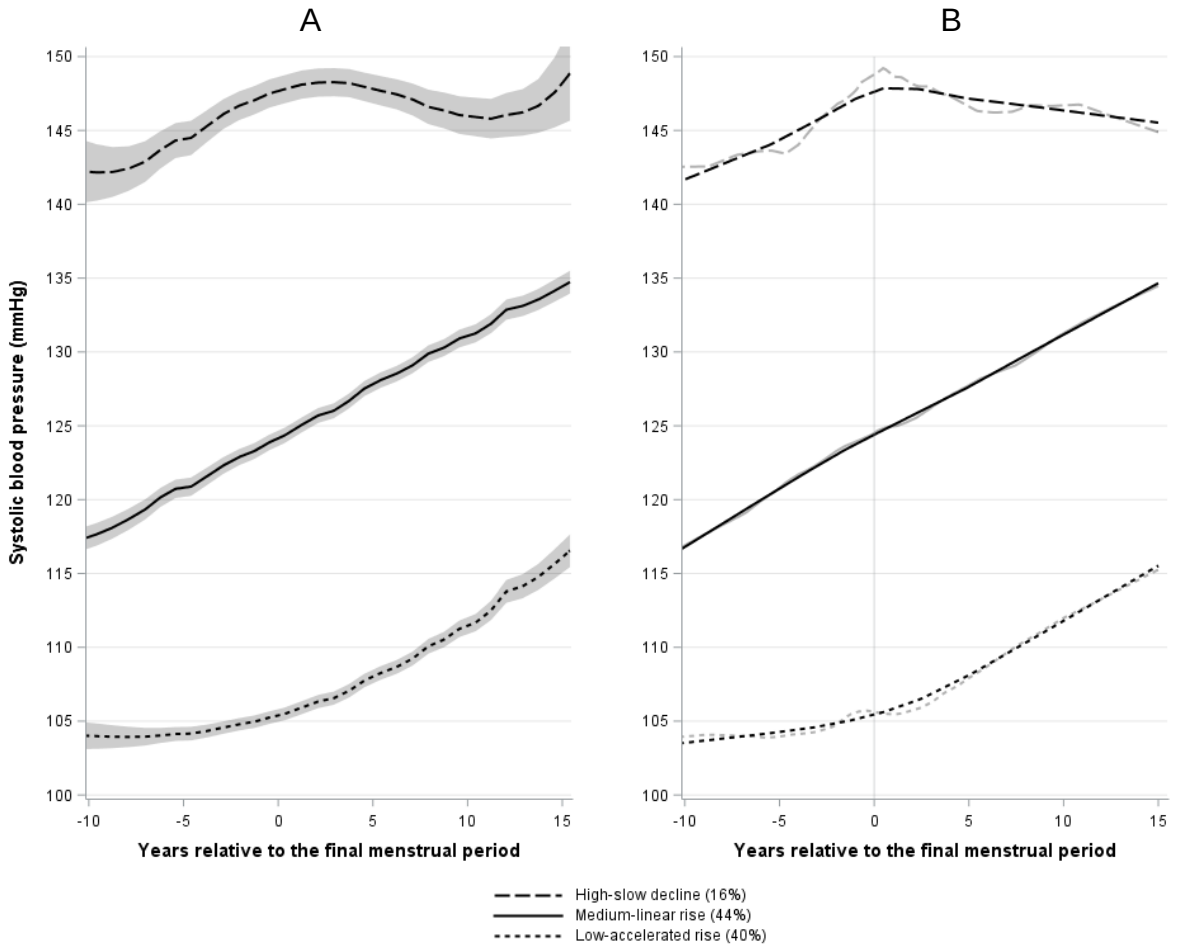
* Mean ± SD is presented unless otherwise specified.

Appendix Table 6-2: Comparing women with observed vs imputed fmp at baseline

Variable*	Observed FMP, n=1804 (55%)	Imputed FMP n= 1498 (45%)	P value
Age (years)	46.36 ± 2.66	46.31 ± 2.72	0.58
Race/ethnicity, N (%)			<.0001
White	820 (45.5)	731 (48.8)	
Black	507 (28.1)	427 (28.5)	
Chinese	174 (9.6)	76 (5.1)	
Hispanic	98 (5.4)	188 (12.6)	
Japanese	205 (11.4)	76 (5.1)	
Education, N (%)			0.01
Less than high school	119 (6.6)	119 (8.0)	
Some College	862 (48.1)	770 (52.1)	
College Degree or higher	811 (45.3)	590 (39.9)	
Income, N (%)			0.04
<$35,000	519 (29.6)	485 (33.2)	
$35,000-$75,000	745 (42.5)	563 (38.6)	
>$75,000	489 (27.9)	411 (28.2)	
Financial Strain, N (%)	681 (37.9)	631 (42.5)	0.01
Smoking Status, N (%)			0.001
Never	1075 (59.6)	815 (54.4)	
Past	447 (24.8)	383 (25.6)	
Current	281 (15.6)	299 (20.0)	
Alcohol drinks per week, N (%)			0.12
None	881 (51.3)	672 (47.5)	
<1	172 (10.0)	154 (10.9)	
1-7	441 (25.7)	372 (26.3)	
>7	224 (13.0)	217 (15.3)	
Systolic blood pressure (mmHg)	117.22 ± 17.36	118.62 ± 16.59	0.02
Diastolic blood pressure (mmHg)	74.96 ± 10.53	76.09 ± 10.49	0.002
Blood pressure medication, N (%)	238 (13.2)	233 (15.6)	0.053
BMI	28.05 ± 7.33	28.53 ± 7.05	0.06
Age at FMP (years)	52.09 ± 2.85	51.81 ± 2.91	0.33
Vasomotor symptoms, N (%)	652 (36.3)	635 (42.7)	0.0002
Estradiol (pg/mL), Median (Q1, Q3)	55.2 (32.8, 87.5)	55.2 (33.2, 91.6)	0.64

Variable*	Observed FMP, n=1804 (55%)	Imputed FMP n= 1498 (45%)	P value
Follicle-stimulating hormone (mIU/mL), Median (Q1, Q3)	15.7 (11.0, 25.6)	15.9 (10.5, 27.4)	0.97
Hormone therapy, N (%)	2 (0.1)	4 (0.3)	0.29

* Mean ± SD is presented unless otherwise specified



Appendix Figure 6-1: Trajectories of systolic blood pressure over the menopause transition (n=1804), observed FMP only

Panel A: Systolic blood pressure represents the predicted mean at each time point within each trajectory group from group-based trajectory modelling. No factors were included in the model.
Panel B: LOWESS curves of systolic blood pressure values over the menopause transition by trajectory groups. Black lines correspond to smoothing level=0.70, while transparent black lines correspond to smoothing level=0.30.

Appendix Table 6-3: Baseline characteristics of study participants by trajectory group (observed FMP only)

Variable*	Total, n=1804	Low-accelerated rise 711 (40%)	Medium-linear rise 799 (44%)	High-slow decline 294 (16%)	P value
Age (years)	46.36 ± 2.66	46.05 ± 2.58	46.41 ± 2.66	46.98 ± 2.74	<.0001
Race/ethnicity, N (%)					<.0001
White	820 (45.5)	411 (57.8)	349 (43.7)	60 (20.4)	
Black	507 (28.1)	74 (10.4)	243 (30.4)	190 (64.6)	
Chinese	174 (9.6)	103 (14.5)	60 (7.5)	11 (3.7)	
Hispanic	98 (5.4)	10 (1.4)	67 (8.4)	21 (7.1)	
Japanese	205 (11.4)	113 (15.9)	80 (10.0)	12 (4.1)	
Education, N (%)					<.0001
Less than high school	119 (6.6)	24 (3.4)	68 (8.6)	27 (9.3)	
Some College	862 (48.1)	289 (40.8)	386 (48.7)	187 (64.3)	
College Degree or higher	811 (45.3)	395 (55.8)	339 (42.7)	77 (26.5)	
Income, N (%)					<.0001
<$35,000	519 (29.6)	145 (21.0)	241 (30.9)	133 (47.0)	
$35,000-$75,000	745 (42.5)	296 (42.8)	340 (43.6)	109 (38.5)	
>$75,000	489 (27.9)	250 (36.2)	198 (25.4)	41 (14.5)	
Financial Strain, N (%)	681 (37.9)	204 (28.8)	318 (40.1)	159 (54.1)	<.0001
Smoking Status, N (%)					<.0001
Never	1075 (59.6)	447 (63.0)	474 (59.3)	154 (52.4)	
Past	447 (24.8)	181 (25.5)	196 (24.5)	70 (23.8)	
Current	281 (15.6)	82 (11.5)	129 (16.1)	70 (23.8)	
Alcohol drinks per week, N (%)					0.01
None	881 (51.3)	330 (48.4)	387 (50.7)	164 (60.3)	

Variable*	Total, n=1804	Low-accelerated rise 711 (40%)	Medium-linear rise 799 (44%)	High-slow decline 294 (16%)	P value
<1	172 (10.0)	61 (8.9)	91 (11.9)	20 (7.4)	
1-7	441 (25.7)	187 (27.4)	192 (25.1)	62 (22.8)	
>7	224 (13.0)	104 (15.2)	94 (12.3)	26 (9.6)	
Systolic blood pressure (mmHg)	117.22 ± 17.36	105.02 ± 8.60	119.30 ± 11.84	141.02 ± 18.73	<.0001
Diastolic blood pressure (mmHg)	74.96 ± 10.53	69.09 ± 7.67	76.52 ± 9.14	84.91 ± 11.04	<.0001
Blood pressure medication, N (%)	238 (13.2)	15 (2.1)	96 (12.0)	127 (43.2)	<.0001
BMI	28.05 ± 7.33	24.31 ± 4.89	29.38 ± 7.09	33.55 ± 8.19	<.0001
Age at FMP (years)	52.09 ± 2.85	51.72 ± 2.68	52.33 ± 2.91	52.32 ± 2.97	0.0004
Vasomotor symptoms, N (%)	652 (36.3)	212 (30.0)	295 (37.0)	145 (49.7)	<.0001
Estradiol (pg/mL), Median (Q1, Q3)	55.2 (32.8, 87.5)	57.6 (34.2, 88.4)	55.8 (32.5, 86.1)	49.8 (32.0, 85.2)	0.40
Follicle-stimulating hormone (mIU/mL), Median (Q1, Q3)	15.7 (11.0, 25.6)	16.1 (11.0, 27.5)	15.2 (10.9, 23.7)	16.6 (11.3, 25.5)	0.07

* Mean ± SD is presented unless otherwise specified

Appendix Table 6-4: Annual change in SBP (mmHg) in time segments, by trajectory groups* (observed FMP only)

Trajectory Group	n	Inflection point	Change in SBP (95% CI)		P-Value for Difference
			Before inflection	After inflection	
High-slow decline	294	FMP	0.72 (0.35, 1.09)	-0.20 (-0.45, 0.05)	0.0003
Medium-linear rise	799	N/A	0.72 (0.66, 0.79)		N/A
Low-accelerated rise	711	1 year after FMP	0.20 (0.12, 0.27)	0.77 (0.69, 0.85)	<0.0001

* Models adjusted for age at the FMP, site, race/ethnicity, and SWAN baseline body-mass index

Appendix Table 6-5: Predictors of SBP trajectory group membership and level across the menopause transition, (n=1804) *

(observed FMP only)

Variables ‡	SBP trajectory group, n (%)		
	Low-accelerated rise	**Medium-linear rise**	**High-slow decline**
	711 (40%)	**799 (44%)**	**294 (16%)**
Predictors of SBP trajectory group membership, OR (95% CI) [a]			
Race/ethnicity			
White	1 [ref]	[ref]	1 [ref]
Black	**0.30 (0.20, 0.44)**	[ref]	**3.58 (2.36, 5.42)**
Hispanic	0.62 (0.16, 2.34)	[ref]	2.02 (0.66, 6.12)
Japanese	**0.26 (0.14, 0.49)**	[ref]	0.99 (0.30, 3.24)
Chinese	**0.44 (0.24, 0.80)**	[ref]	3.34 (0.81, 13.79)
Baseline BMI (per 1-unit increase)	**0.86 (0.83, 0.88)**	[ref]	**1.06 (1.04, 1.09)**
Age at menopause			
Before 51 years	**1.72 (1.23, 2.41)**	[ref]	1.17 (0.77, 1.77)
Between 51 and 53 years	1 [ref]	[ref]	1 [ref]
After 53 years	**0.72 (0.53, 0.99)**	[ref]	1.20 (0.81, 1.78)
Baseline estradiol † (per 1-SD increase)	1.00 (0.86, 1.15)	[ref]	1.15 (0.97, 1.37)
Baseline FSH † (per 1-SD increase)	1.06 (0.91, 1.23)	[ref]	1.17 (0.98, 1.39)
Baseline vasomotor symptoms	0.77 (0.58, 1.01)	[ref]	1.27 (0.92, 1.75)
Predictors of SBP level overtime within each trajectory group, beta (SE) [a]			
Estradiol † (per 1-SD increase)	0.24 (0.13)	0.02 (0.12)	-0.01 (0.25)
FSH † (per 1-SD increase)	**-0.27 (0.10)**	-0.18 (0.10)	-0.25 (0.20)
Vasomotor symptoms	**1.17 (0.31)**	**1.19 (0.33)**	**1.45 (0.59)**

* Bold indicates P-value <0.05

‡ Model further adjusted for site and cycle-day of blood draw

† Log-transformed

[a] Estimates of SBP trajectory group membership and level are from the same GBTM.

7.0 Discussion

7.1 Summary of the findings

This dissertation contains three manuscripts evaluating the contribution of the menopause transition on central arterial stiffness, abdominal visceral adipose tissue, and blood pressure in samples of midlife women transitioning through menopause. The *first manuscript* characterized changes of central arterial stiffness relative to the FMP independent of aging and other CVD risk factors and determined differences between White and Black women in central arterial stiffness trajectories.[202] The *second manuscript* characterized VAT trajectory over time relative to the FMP independent of aging and lifestyle factors and examined the association between menopause-related VAT accumulation and carotid artery atherosclerosis. The *third manuscript* described distinct trajectories of systolic and diastolic blood pressure over time relative to the FMP and investigated predictive associations of age at menopause, and time-varying estradiol, FSH, and vasomotor symptoms with pattern and/or level of blood pressure trajectories.

In the first manuscript, we showed that central arterial stiffness in midlife women accelerated significantly at 1 year before the FMP and then plateaued at 1 year after the FMP.[202] The accelerated increase in central arterial stiffness within one year of the FMP period was independent of traditional CVD risk factors and differed significantly from the changes before and after that period. Interestingly, adjusting for modifiable CVD risk factors explained part of the change estimates in central arterial stiffness at 1 year before the FMP highlighting the potential mediating effects of these risk factors. Additionally, we showed significant racial differences in

arterial stiffness progression early in the transition, such that Black women had a greater increase in arterial stiffness compared with White women in the period 1 year before the FMP.

In the second manuscript, we showed a non-linear trajectory of VAT such that VAT increased significantly starting 2 years before the FMP independent of aging, lifestyle factors and BMI. We showed that this accelerated increase in VAT at 2 years before the FMP was attenuated after adjusting for serum estradiol, further lending support to the contribution of the menopause transition on VAT trajectory in midlife women. Importantly, the increase in VAT between 2 years before the FMP up to the FMP predicted greater atherosclerosis in the internal carotid artery of midlife women.

In the third manuscript, we shed light on the inconsistencies in the literature regarding the contribution of the menopause transition on blood pressure trajectories by showing that women do not share a common trajectory of blood pressure as they transition through menopause. In fact, GBTM revealed 3 distinct trajectories of SBP in midlife women that differed in level and extent to which menopause may influence the pattern: the "Low-accelerated rise trajectory" (36% of the cohort) with a significant accelerated increase 1 year after the FMP consistent with a menopause-related increase; the "medium-linear rise trajectory" (47%) with a monotonic increase over the menopause transition consistent with chronological aging effect; and the "high-slow decline trajectory" (17%) with an accelerated increase up until the FMP followed by a menopause-related plateau thereafter. White, Chinese, and Japanese women were more likely to follow the low-accelerated rise trajectory. Hispanic women were more likely to follow the medium-linear rise trajectory. Black women were more likely to follow the high-slow decline SBP trajectory. DBP trajectories did not demonstrate menopause-related increases. We also showed that an older age at menopause predicted a higher SBP trajectory pattern, FSH predicted a lower SBP overtime in the

low-accelerated and medium-linear rise, while vasomotor symptoms predicted a higher SBP overtime in all SBP trajectories. Estradiol did not predict SBP trajectory pattern or level.

7.2 Arterial stiffness, abdominal visceral adiposity, and blood pressure changes accompanying the menopause transition are interrelated

This dissertation focused on risk factors of CVD during the menopause transition when changes in one may influence the other. For example, VAT and SBP are pathologically interrelated. Increased VAT volume represents a state of dysfunctional adipose tissue metabolism in the forms of adipocyte hypertrophy, impaired adipocyte hyperplasia, and reduced lipid storage capacity.[237] Larger volumes of VAT are associated with overflow of portal free fatty acids and increased cytokines secretion.[96] These factors predispose to increased insulin resistance, inflammation, and endothelial dysfunction and ultimately arterial hypertension.[97] Additionally, VAT seems un upstream factor in the pathogenesis of CVD. A previous meta-analysis of 221,934 people revealed that the HR of CVD was 1.27 (95% CI: 1.20–1.33) for a 1-SD increase in waist circumference, a surrogate marker of VAT and central adiposity.[238] However, in the same study and after adjusting for SBP, history of diabetes, and total and HDL cholesterol, the HR of CVD was attenuated to 1.10 (1.05–1.14) suggesting that the bulk of CVD risk resulting from high central adiposity is largely mediated through the intermediate effect of SBP, insulin resistance, and hyperlipidemia.

We showed that central arterial stiffness and SBP change significantly during the menopause transition. Although the extent to which changes in one of these vascular health measures during the menopause transition affects the other has not been explored here, there has

been a debate in the literature of whether hypertension is a manifestation of arterial stiffness, or vice versa.[42,68] Several longitudinal studies including the Framingham Offspring study, the Atherosclerosis Risk in Communities Study (ARIC), and the Baltimore Longitudinal Study of Aging showed that arterial stiffness measures assessed at baseline predicts progression and incidence of hypertension at follow-up, but not vice versa.[46,239,240] These results lend strong evidence supporting the hypothesis that arterial stiffness is a cause for hypertension. Pathophysiologically, stiffened vasculature is thought to alter blood pressure through its association with impaired baroreceptor sensitivity and inability to buffer short-term alterations in flow.[241,242] In this dissertation, we showed that central arterial stiffness showed accelerated increase one year *before* the FMP, which may possibly have triggered the acceleration in SBP one year *after* the FMP that was observed in the low-accelerated rise SBP trajectory group. Notably, coexisting arterial stiffness and systolic hypertension is a risk factor for treatment-resistance.[243] We showed that the menopause transition is a period of accelerated risk of arterial stiffness and hypertension which may predispose women to develop both conditions, an observation that may explain why more women than men taking antihypertensive medications have blood pressure levels above treatment goals (52% vs 55%).[244]

7.3 Public health significance

In the US, CVD is the number one cause of death where one person dies every 36 seconds from CVD.[39] The major risk factor for CVD in both sexes is aging, but there is a noticeable 10-year lag between men and women.[245] This lag in CVD risk between men and women narrows after midlife suggesting that the menopause transition may play a role. To understand the contribution

of the menopause transition on CVD risk independent of aging in women, we needed studies that determine whether and when the known predictors of CVD are altered during the transition. This dissertation presented time-oriented epidemiologic evidence of the contribution of the menopause transition to CVD risk factors. We not only showed that the menopause transition is a period of accelerated CVD risk, but we also identified windows of time during the menopause transition when central arterial stiffness, VAT, and SBP accelerate. Intervention research may seek guidance from our findings on the best timing during the menopause transition for implementation of lifestyle or pharmacologic interventions targeting CVD risk factors known to be changed by menopause. Our findings encourage timely screening and recognizing and intervening on CVD risk factors early in the menopause transition *before* women reach menopause when primary prevention maybe most effective.

This dissertation work serves an important purpose of raising awareness among midlife women and health care providers that CVD is also a disease of women and that key CVD risk factors including arterial stiffness, VAT, and blood pressure are likely to be adversely modified during the menopause transition. By the 21st century, there have been relative increases in awareness of the importance of CVD in women through educational campaigns and as a result, preventive measures taken to reduce CVD risk in women like physical activity and weight loss have grown.[246] Nevertheless, in 2009 and among women older than 25 years, only 65% know that CVD is their number one killer.[247] Alarmingly, the awareness that CVD is the number one killer of women fell to 44% in 2019.[247] The decline in awareness between 2009 to 2019 was greatest among Hispanic and Black women and in women younger than 65 years.[247] These results advocate for an urgent redoubling of efforts by organizations interested in women's health to reverse these trends and raise awareness of risk and symptoms of CVD in women.

Black women have greater prevalence and earlier onset of CVD risk factors and a higher CVD mortality compared with Whites.[84,183] This dissertation has shown that this disparity may have its roots during the menopause transition because Black women had an earlier acceleration of arterial stiffness and were more likely to be in the high-slow decline SBP trajectory compared with their White counterparts. Given the known strong associations of central arterial stiffness and SBP with CVD risk and mortality, it is expected that the racial differences in these risk factors during the menopause transition we showed in this dissertation may contribute to the racial disparity in CVD risk and mortality reported at an older age. Our results also emphasize the importance of raising awareness of this disparity with aggressive and frequent monitoring of risk factors early in the menopause transition among Black women.

7.4 Clinical implications of the findings

The three studied risk factors in this dissertation are strong predictors of CVD risk. In a meta-analysis conducted on 15,877 subjects who were followed for a mean of 7.7 years, a 100-cm/s increase in cfPWV corresponded to a risk-factors adjusted increase of 14% in CVD events.[19] In 1,387 women from the Health, Aging and Body Composition (Health ABC) Study, a 1-SD (66 cm^2) increase in VAT area corresponded to a 67% increase (95% CI: 28%, 117%) in myocardial infarction events independent of traditional CVD risk factors.[29] Additionally, a 1-SD increase in VAT volume was associated with an OR of 2.1 (1.6, 2.6) for diabetes and 4.7 (3.9, 5.7) for metabolic syndrome in women from the Framingham Heart Study.[32] In a meta-analysis comprising 95,772 midlife women, a 10-mmHg higher systolic blood pressure corresponded to an increase of 25% in CVD events.[144] This effect size in SBP-CVD relationship held in a graded log-linear

fashion starting from a SBP of 115 mmHg without a clear threshold.[217] Additionally, among healthy midlife men and women without previous histories of hypertension or other traditional CVD risk factors, the presence of coronary artery calcium and the risk of incident CVD showed a stepwise increase with increasing SBP starting with SBP as low as 90 mmHg.[218] Taken together, the effect sizes in risk of cardiometabolic outcomes per unit increases in cfPWV, VAT, and SBP are large and clinically significant.

In comparison with unit increases in cfPWV, VAT, and SBP from the literature and their associated increases in cardiometabolic outcomes, we showed that women can experience an ~80-cm/s increase in cfPWV during the period of one year before and after the FMP.[202] We also showed that women may gain ~24 cm^2 of VAT area during the period starting from 2 years before the FMP to the FMP. In the low-accelerated rise SBP trajectory group in this dissertation work, for example, women started gaining a 0.78-mmHg in SBP per year beginning one year after the FMP. Therefore, the amount of changes in cfPWV, VAT, and SBP in women transitioning to menopause in this dissertation are not negligible and may place women at risk of developing CVD later in life. Therefore, early modification of these risk factors either through lifestyle changes or pharmacologic interventions will likely result in cardiometabolic and vascular benefits after midlife. In fact, a previous SWAN analysis showed that healthy lifestyle during midlife is prospectively associated with less carotid atherosclerosis.[180]

Because we showed clinically significant changes in modifiable CVD risk factors during the menopause transition, it is prudent to frequently monitor these risk factors in clinic setting. Challenges surrounding monitoring of CVD risk factors in clinic settings are related to awareness and preparedness among doctors and to economic feasibility. Regardless of the challenges, health

care seeking behaviors in midlife women can be wisely leveraged to increase CVD risk factor monitoring frequency.

Only 40% of women reported having their heart health checked in routine health care visits.[248] This was perhaps because only 39% of primary care physicians (PCPs) would rank CVD a top priority and only 22% of PCPs and 42% of cardiologists felt well prepared to assess CVD risk in women.[248] One approach that may enhance timely and frequent monitoring of CVD risk factors early in the menopause transition is the collaboration with obstetricians and gynecologists.[203] Multifaceted approach that utilizes collaboration between PCPs, obstetricians and gynecologists, and cardiologists may enhance frequent and timely screening of CVD risk factors with counselling for midlife women to stress lifestyle changes. Although guidelines for the prevention of CVD specifically for women were previously published, research into practical application and maintenance of adherence to those guidelines are lacking.[249]

Because VAT is a driving factor for the development of other cardiometabolic risk factors and the metabolic syndrome,[250] particular attention should be given to central adiposity in women transitioning to menopause. Since frequent VAT measurement in clinic settings is economically not feasible, an alternate easily measured surrogate marker of VAT should be used. Waist circumference is a measure of central adiposity that caries joint information about SAT and VAT volumes. Several studies have suggested adding blood level of triglyceride to waistline measurement to distinguishing VAT from SAT.[251] A large waistline in the presence of high serum triglyceride, a condition called “hypertriglyceridemic waist”, is a simple and useful marker of excess VAT.[251] Importantly, prospective studies showed that the presence of hypertriglyceridemic waist was associated with adverse cardiometabolic risk profile predictive of an increased CVD risk.[252]

Women frequently seek medical attention as they transitioning to menopause because of vasomotor symptoms.[253] We showed that vasomotor symptoms is associated with increases in SBP during the menopause transition regardless of the SBP trajectory a woman follows. These findings can be used in the clinic as an easy screening tool to stratify women's risk of SBP acceleration during the menopause transition. Midlife women also seek medical attention for other symptoms like sleeping difficulties, fatigue, weight gain, depression, and vaginal dryness.[254] These frequent encounters between midlife women and healthcare professionals should be leveraged to identify women at risk of CVD and to maximize benefit from preventive measures.

7.5 Strengths and limitations

Major strengths of this dissertation work stem from the design and data collection of SWAN. SWAN is unique because it recruited a multi-ethnic sample of premenopausal and early perimenopausal women and followed them until they became postmenopausal, capturing the complete spectrum of the menopause transition. Data collection in SWAN has been prospective and annually, helping with the exact timing of the FMP and concurrent measurements of CVD risk factors and menopause-related covariates. Gold standard methods of measuring central arterial stiffness and VAT were used in this work. Additional strengths of this work arise from the analytic techniques that separated ovarian from chronologic aging effects while adjusting for essential covariates. Capitalizing on SWAN's data and our analytic techniques, we were able to fill important gaps in the literature regarding the contribution of the menopause transition to midlife changes in central arterial stiffness, VAT, and blood pressure.

However, this work has limitations. The FMP date was not observed in all women included in this work. Reasons for missing the FMP date were hormone therapy use, hysterectomy, or bilateral oophorectomy; these health-related events are expected in longitudinal studies of midlife women. Women with observed FMP showed small differences in baseline demographic variables compared with women without observed FMP. As part of SWAN, the FMP dates were imputed for women for whom the FMP was not observed using multiple imputation while including in the imputation model covariates known in the literature to affect timing of the FMP. To analyze multiply imputed data, one needs to account for the uncertainty in the imputation by incorporating within and between imputation variability in the estimates. Unfortunately, GBTM does not offer the option of including uncertainty of the imputation in determining trajectories of SBP. In an effort to overcome the FMP missingness limitation, we presented all of our results on women with observed FMP only and after combining women with observed and imputed FMPs. Interestingly, our results were similar in both analyses and therefore, our conclusions persisted.

Central arterial stiffness and VAT analyses were based on data from SWAN Heart which by design included two time points of data. Not all SWAN Heart women had two measurements of arterial stiffness and VAT. However, the mixed effects model we used was appropriate for handling unbalanced data with one to two time points of data per subject and should yield unbiased estimates of change.[184] Additionally, similar estimates of change and overall conclusions were found from analyses of women with two time points of data.

Results from this dissertation should only be generalized to women represented by SWAN and SWAN Heart women.

7.6 Future directions

This dissertation work has laid out several areas for future research. These areas include research related to practice guidelines for the prevention of CVD in women, translational research focusing on modifying vascular function and central adiposity during midlife, and research into the underlying mechanisms of our findings.

Almost 60% of midlife women seek medical attention for menopause-related symptoms or other health conditions.[254] These encounters should be leveraged in future research to examine the practical application and maintenance of adherence to previously published guidelines for the prevention of CVD in women among public health professionals.[249] The guidelines on the prevention of CVD in women were last updated in 2011 and in these guidelines, the menopause transition was not considered as a risk factor of CVD or as a period of accelerated CVD risk.[249] However, there has been advances in our understanding of the contribution of the menopause transition on cardiovascular health over the past decade necessitating the need to revise those guidelines.[3,255]

Future research should also determine whether early modification of vascular function during the menopause transition are associated with reduction in future CVD risk. We showed that adjusting for modifiable CVD risk factors explained part of the change estimate in central arterial stiffness at one year before the FMP suggesting that vascular stiffness may be reversed with improved risk factor profile, a hypothesis that requires further testing.

In the second paper of this dissertation, we adjusted for lifestyle factors including physical activity, alcohol consumption, and daily calorie intake but they did not explain the accelerated increases in VAT two years before the FMP. Future research should rigorously assess the role of lifestyle interventions on women's VAT accumulation during midlife. Additionally, future

research should assess whether lifestyle interventions targeting central adiposity in midlife women is associated with favorable cardiovascular outcomes later in life.

A hallmark of the menopause transition is the dynamic decline in serum estradiol. It was therefore hypothesized that this decline in estradiol during the transition maybe responsible, at least in part, for the deterioration in cardiometabolic health seen in older women.[256] Guided by data from the literature, we did adjust for estradiol in the arterial stiffness and SBP analyses in this dissertation. However, estradiol did not explain the inflection points in these vascular health measures during the menopause transition. It is hypothesized that the vasodilatory and cardio-protective actions of endogenous estradiol are mediated in part through its metabolites.[226] 2-methoxyestradiol is an estradiol metabolite that has an estrogen receptor-independent growth inhibitory effects on smooth muscle and endothelial cells.[226] Additionally, 2-methoxyestradiol inhibits the synthesis of extracellular matrix proteins including collagen and improves endothelial function.[226] Interestingly, estradiol metabolites, but not estradiol, were associated with a lower SBP in postmenopausal women.[227] These results highlight the complexity of the MT and future studies should compare estradiol metabolite levels and its potential effects on vascular function across the stages of the menopause transition.

The presence of extragonadal functions of FSH was hypothesized after previous studies found FSH receptors expressed on blood vessels,[231] adipose tissues,[232] and the liver.[233] We showed that FSH was inversely related to SBP level in women following the low-accelerated and medium-linear rise SBP trajectories. Other studies have showed a similar inverse association between FSH and diabetes,[257] obesity,[258] and LDL cholesterol.[213] However, other studies showed a positive relationship between FSH and subclinical measures of atherosclerosis including aortic plaque, adventitial diameter, and CCA-IMT.[259-261] It is not understood why FSH may improve

cardiometabolic risk factors and yet, shows unfavorable effects on subclinical measures of atherosclerosis. Therefore, the underlying pathological mechanisms between FSH and CVD risk factors and subclinical measures of atherosclerosis need to be elucidated in future studies.

7.7 Conclusions

CVD is the number one cause of death in women and the risk of CVD accelerates after the menopause transition. We showed that the menopause transition is associated with accelerated increases in central arterial stiffness, abdominal visceral adiposity, and systolic blood pressure independent of chronological aging. The findings from this dissertation are in line with previous studies that showed other CVD risk factors to be modified during the menopause transition including lipids, the metabolic syndrome, and subclinical measures of CVD. Because the menopause transition is a period of accelerated CVD risk, it is prudent to increase frequency of CVD risk factors monitoring early in the transition when prevention strategies may be most effective.

Appendix A: Brief description of the FMP dates imputation process used in SWAN

FMP dates imputation for women missing their FMP dates was done within the full SWAN cohort (n=3302). For women who had 12 consecutive months of amenorrhea without taking hormone therapy (HT), FMP date was reliably assigned as the date of the last menstrual period (n=1804). For the rest of SWAN cohort (n=1498), FMP date was not assigned because women missed several visits, dropped out, had hysterectomy, had bilateral salpingo-oophorectomy, or received HT.

Analyses of complete data (excluding those with missing FMP date) may produce biased estimates and the results may not be applicable to the community sample represented by the SWAN cohort. Therefore, not to mention the increase in sample size, the advantages of reducing bias by including both women with observed and imputed FMP dates outweigh errors due to imputing data (Little and Rubin, 2019).

The software used for FMP dates imputation was IVEware (https://www.src.isr.umich.edu/wp-content/uploads/iveware-manual-Version-0.3.pdf), a free add-on set of routines for SAS from the University of Michigan. The software uses multivariate sequential regression, also known as Chained Equations; see the above link for additional information. The assumption was that the FMP data are "missing at random" conditional on the observed characteristics. The software imputed 10 FMP sets for women missing the FMP. To account for the uncertainty in the imputation process, our final estimates incorporated within and between imputation variability as recommended by (Little and Rubin, 2019). The utilized approach essentially taking a sample of

size 10 from a woman estimated distribution of FMP date, and incorporating that distribution's variability (imputation uncertainty) in analyses accordingly.

To improve the accuracy of FMP date imputation, the software bases the fill-in values on a participant's own available partial data of menstrual calendar by assigning woman-specific right and left endpoints for the FMP date imputation. Covariates included in imputation model were based on the literature, including papers from SWAN. The following table lists covariates utilized in the imputation:

Demographics/SES	**Reproductive factors**	**Lifestyle and other factors**
Race/ethnicity	Day of menstrual cycle corresponding to blood draw	Prevalent cardiovascular disease diagnosis
Site	Estradiol	Prevalent osteoporosis diagnosis
Age	FSH	Baseline smoking
Educational level	History of oral contraceptive use	Alcohol consumption
Financial strain	History of exogenous hormone use	Total non-work physical activity
Married/partnered status	Number of live births	Body mass index
Employment status	Vasomotor symptom frequency	Self-reported health
	Bleeding patterns	Prevalent diabetes

Appendix B: SWAN acknowledgements and sources of funding

Acknowledgements:

Clinical Centers: University of Michigan, Ann Arbor – Siobán Harlow, PI 2011 – present, MaryFran Sowers, PI 1994-2011; Massachusetts General Hospital, Boston, MA – Joel Finkelstein, PI 1999 – present; Robert Neer, PI 1994 – 1999; Rush University, Rush University Medical Center, Chicago, IL – Howard Kravitz, PI 2009 – present; Lynda Powell, PI 1994 – 2009; University of California, Davis/Kaiser – Ellen Gold, PI; University of California, Los Angeles – Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, NY – Carol Derby, PI 2011 – present, Rachel Wildman, PI 2010 – 2011; Nanette Santoro, PI 2004 – 2010; University of Medicine and Dentistry – New Jersey Medical School, Newark – Gerson Weiss, PI 1994 – 2004; and the University of Pittsburgh, Pittsburgh, PA – Karen Matthews, PI.

NIH Program Office: National Institute on Aging, Bethesda, MD – Winifred Rossi 2012 - present; Sherry Sherman 1994 – 2012; Marcia Ory 1994 – 2001; National Institute of Nursing Research, Bethesda, MD – Program Officers.

Central Laboratory: University of Michigan, Ann Arbor – Daniel McConnell (Central Ligand Assay Satellite Services).

Coordinating Center: University of Pittsburgh, Pittsburgh, PA – Maria Mori Brooks, PI 2012 - present; Kim Sutton-Tyrrell, PI 2001 – 2012; New England Research Institutes, Watertown, MA - Sonja McKinlay, PI 1995 – 2001.

Steering Committee: Susan Johnson, Current Chair. Chris Gallagher, Former Chair.

We thank the study staff at each site and all the women who participated in SWAN.

Sources of Funding:

The Study of Women's Health Across the Nation (SWAN) has grant support from the National Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR) and the NIH Office of Research on Women's Health (ORWH) (Grants U01NR004061; U01AG012505, U01AG012535, U01AG012531, U01AG012539, U01AG012546, U01AG012553, U01AG012554, U01AG012495). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the NIA, NINR, ORWH or the NIH.

SWAN Heart was supported by grants from the NHLBI (HL065581, HL065591).

Bibliography

1. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation.* 2012;125(1):e2-e220.

2. Mosca L, Mochari-Greenberger H, Dolor RJ, Newby LK, Robb KJ. Twelve-year follow-up of American women's awareness of cardiovascular disease risk and barriers to heart health. *Circ Cardiovasc Qual Outcomes.* 2010;3(2):120-127.

3. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. *JAMA cardiology.* 2016;1(7):767-776.

4. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. *Menopause (New York, NY).* 2006;13(2):265-279.

5. Thurston RC, Karvonen-Gutierrez CA, Derby CA, El Khoudary SR, Kravitz HM, Manson JE. Menopause versus chronologic aging: their roles in women's health. *Menopause.* 2018;25(8):849-854.

6. Harlow SD, Gass M, Hall JE, et al. Executive summary of the Stages of Reproductive Aging Workshop +10: addressing the unfinished agenda of staging reproductive aging. *Climacteric.* 2012;15(2):105-114.

7. El Khoudary SR, Thurston RC. Cardiovascular Implications of the Menopause Transition: Endogenous Sex Hormones and Vasomotor Symptoms. *Obstet Gynecol Clin North Am.* 2018;45(4):641-661.

8. Shaw LJ, Bugiardini R, Merz CN. Women and ischemic heart disease: evolving knowledge. *J Am Coll Cardiol.* 2009;54(17):1561-1575.

9. Matthews KA, Crawford SL, Chae CU, et al. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? *Journal of the American College of Cardiology.* 2009;54(25):2366-2373.

10. De Kat AC, Dam V, Onland-Moret NC, Eijkemans MJC, Broekmans FJM, Van Der Schouw YT. Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. *BMC medicine.* 2017;15(1).

11. Matthews KA, El Khoudary SR, Brooks MM, et al. Lipid Changes Around the Final Menstrual Period Predict Carotid Subclinical Disease in Postmenopausal Women. *Stroke; a journal of cerebral circulation.* 2017;48(1):70-76.

12. Janssen I, Powell LH, Crawford S, Lasley B, Sutton-Tyrrell K. Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. *Archives of internal medicine.* 2008;168(14):1568-1575.

13. Gurka MJ, Vishnu A, Santen RJ, DeBoer MD. Progression of Metabolic Syndrome Severity During the Menopausal Transition. *Journal of the American Heart Association.* 2016;5(8).

14. El Khoudary SR, Wildman RP, Matthews K, Thurston RC, Bromberger JT, Sutton-Tyrrell K. Progression rates of carotid intima-media thickness and adventitial diameter during the menopausal transition. *Menopause (New York, NY).* 2013;20(1):8-14.

15. Wildman RP, Colvin AB, Powell LH, et al. Associations of endogenous sex hormones with the vasculature in menopausal women: the Study of Women's Health Across the Nation (SWAN). *Menopause (New York, NY).* 2008;15(3):414-421.

16. Bechlioulis A, Kalantaridou SN, Naka KK, et al. Endothelial function, but not carotid intima-media thickness, is affected early in menopause and is associated with severity of hot flushes. *The Journal of clinical endocrinology and metabolism.* 2010;95(3):1199-1206.

17. Moreau KL, Hildreth KL, Meditz AL, Deane KD, Kohrt WM. Endothelial function is impaired across the stages of the menopause transition in healthy women. *The Journal of clinical endocrinology and metabolism.* 2012;97(12):4692-4700.

18. Laurent S, Briet M, Boutouyrie P. Arterial stiffness as surrogate end point: needed clinical trials. *Hypertension.* 2012;60(2):518-522.

19. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *Journal of the American College of Cardiology.* 2010;55(13):1318-1327.

20. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation.* 2010;121(4):505-511.

21. Ben-Shlomo Y, Spears M, Boustred C, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *Journal of the American College of Cardiology.* 2014;63(7):636-646.

22. Khan ZA, Janssen I, Mazzarelli JK, et al. Serial Studies in Subclinical Atherosclerosis During Menopausal Transition (from the Study of Women's Health Across the Nation). *Am J Cardiol.* 2018.

23. Birru MS, Matthews KA, Thurston RC, et al. African American ethnicity and cardiovascular risk factors are related to aortic pulse wave velocity progression. *American journal of hypertension.* 2011;24(7):809.

24. Daviglus ML, Stamler J, Pirzada A, et al. Favorable Cardiovascular Risk Profile in Young Women and Long-term Risk of Cardiovascular and All-Cause Mortality. *JAMA.* 2004;292(13):1588-1592.

25. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation.* 2001;103(7):987-992.

26. Davis SR, Castelo-Branco C, Chedraui P, et al. Understanding weight gain at menopause. *Climacteric : the journal of the International Menopause Society.* 2012;15(5):419-429.

27. Greendale GA, Sternfeld B, Huang M, et al. Changes in body composition and weight during the menopause transition. *JCI Insight.* 2019;4(5).

28. Sowers M, Zheng H, Tomey K, et al. Changes in body composition in women over six years at midlife: ovarian and chronological aging. *The Journal of clinical endocrinology and metabolism.* 2007;92(3):895-901.

29. Nicklas BJ, Penninx BW, Cesari M, et al. Association of visceral adipose tissue with incident myocardial infarction in older men and women: the Health, Aging and Body Composition Study. *Am J Epidemiol.* 2004;160(8):741-749.

30. Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U, Fox CS. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. *Journal of the American College of Cardiology.* 2013;62(10):921-925.

31. Ross R, Freeman J, Hudson R, Janssen I. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *The Journal of clinical endocrinology and metabolism.* 2002;87(11):5044-5051.

32. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation.* 2007;116(1):39-48.

33. Pramyothin P, Karastergiou K. What Can We Learn from Interventions That Change Fat Distribution? *Current obesity reports.* 2016;5(2):271-281.

34. Dugan SA, Everson-Rose SA, Karavolos K, Avery EF, Wesley DE, Powell LH. Physical activity and reduced intra-abdominal fat in midlife African-American and white women. *Obesity (Silver Spring, Md).* 2010;18(6):1260-1265.

35. Gast KB, den Heijer M, Smit JW, et al. Individual contributions of visceral fat and total body fat to subclinical atherosclerosis: The NEO study. *Atherosclerosis.* 2015;241(2):547-554.

36. Asia Pacific Cohort Studies C. The impact of cardiovascular risk factors on the age-related excess risk of coronary heart disease. *International journal of epidemiology.* 2006;35(4):1025-1033.

37. Benjamin EJ, Muntner P, Alonso A, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation.* 2019;139(10):e56-e66.

38. Kim JK, Alley D, Seeman T, Karlamangla A, Crimmins E. Recent changes in cardiovascular risk factors among women and men. *J Womens Health (Larchmt).* 2006;15(6):734-746.

39. Virani SS, Alonso A, Benjamin EJ, et al. Heart Disease and Stroke Statistics—2020 Update. *Circulation.* 2020.

40. Randolph JF, Zheng H, Sowers MR, et al. Change in Follicle-Stimulating Hormone and Estradiol Across the Menopausal Transition: Effect of Age at the Final Menstrual Period. *The Journal of Clinical Endocrinology & Metabolism.* 2011;96(3):746-754.

41. Khan ZA, Janssen I, Mazzarelli JK, et al. Serial Studies in Subclinical Atherosclerosis During Menopausal Transition (from the Study of Women's Health Across the Nation). *The American journal of cardiology.* 2018;122(7):1161-1168.

42. Oh YS, Berkowitz DE, Cohen RA, et al. A Special Report on the NHLBI Initiative to Study Cellular and Molecular Mechanisms of Arterial Stiffness and Its Association With Hypertension. *Circulation research.* 2017;121(11):1216-1218.

43. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European heart journal.* 2006;27(21):2588-2605.

44. Hayashi K, Naiki T. Adaptation and remodeling of vascular wall; biomechanical response to hypertension. *J Mech Behav Biomed Mater.* 2009;2(1):3-19.

45. AlGhatrif M, Strait JB, Morrell CH, et al. Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore Longitudinal Study of Aging. *Hypertension.* 2013;62(5):934-941.

46. Kaess BM, Rong J, Larson MG, et al. Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA.* 2012;308(9):875-881.

47. de Oliveira Alvim R, Mourao-Junior CA, Magalhaes GL, et al. Non-HDL cholesterol is a good predictor of the risk of increased arterial stiffness in postmenopausal women in an urban Brazilian population. *Clinics (Sao Paulo, Brazil).* 2017;72(2):106-110.

48. Park JS, Nam JS, Cho MH, et al. Insulin resistance independently influences arterial stiffness in normoglycemic normotensive postmenopausal women. *Menopause (New York, NY).* 2010;17(4):779-784.

49. Lee HJ, Hwang SY, Hong HC, et al. Waist-to-hip ratio is better at predicting subclinical atherosclerosis than body mass index and waist circumference in postmenopausal women. *Maturitas.* 2015;80(3):323-328.

50. Lambrinoudaki I, Kazani A, Armeni E, et al. The metabolic syndrome is associated with carotid atherosclerosis and arterial stiffness in asymptomatic, nondiabetic postmenopausal women. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology.* 2018;34(1):78-82.

51. Schillaci G, Pirro M, Vaudo G, et al. Metabolic Syndrome Is Associated With Aortic Stiffness in Untreated Essential Hypertension. *Hypertension.* 2005;45(6):1078-1082.

52. Taquet A, Bonithon-Kopp C, Simon A, et al. Relations of cardiovascular risk factors to aortic pulse wave velocity in asymptomatic middle-aged women. *European journal of epidemiology.* 1993;9(3):298-306.

53. Ogola BO, Zimmerman MA, Clark GL, et al. New Insights into Arterial Stiffening: Does Sex Matter? *Am J Physiol Heart Circ Physiol.* 2018.

54. Humphrey JD. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. *Cell Biochem Biophys.* 2008;50(2):53-78.

55. Campisi D, Cutolo M, Carruba G, et al. Evidence for soluble and nuclear site I binding of estrogens in human aorta. *Atherosclerosis.* 1993;103(2):267-277.

56. Natoli AK, Medley TL, Ahimastos AA, et al. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension.* 2005;46(5):1129-1134.

57. Forte P, Kneale BJ, Milne E, et al. Evidence for a Difference in Nitric Oxide Biosynthesis Between Healthy Women and Men. *Hypertension.* 1998;32(4):730-734.

58. Collins P, Rosano GMC, Sarrel PM, et al. 17β-Estradiol Attenuates Acetylcholine-Induced Coronary Arterial Constriction in Women but Not Men With Coronary Heart Disease. *Circulation.* 1995;92(1):24-30.

59. Staessen J, Van der Heijden-Spek J, Safar M, et al. Menopause and the characteristics of the large arteries in a population study. *Journal of human hypertension.* 2001;15(8):511.

60. Miura S, Tanaka E, Mori A, et al. Hormone replacement therapy improves arterial stiffness in normotensive postmenopausal women. *Maturitas.* 2003;45(4):293-298.

61. Waddell TK, Rajkumar C, Cameron JD, Jennings GL, Dart AM, Kingwell BA. Withdrawal of hormonal therapy for 4 weeks decreases arterial compliance in postmenopausal women. *Journal of hypertension.* 1999;17(3):413-418.

62. Russo C, Jin Z, Palmieri V, et al. Arterial stiffness and wave reflection: sex differences and relationship with left ventricular diastolic function. *Hypertension.* 2012;60(2):362-368.

63. Ikonomidis I, Lekakis J, Papadopoulos C, et al. Incremental value of pulse wave velocity in the determination of coronary microcirculatory dysfunction in never-treated patients with essential hypertension. *American journal of hypertension.* 2008;21(7):806-813.

64. Laurent S, Katsahian S, Fassot C, et al. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke; a journal of cerebral circulation.* 2003;34(5):1203-1206.

65. Ohya Y, Iseki K, Iseki C, Miyagi T, Kinjo K, Takishita S. Increased pulse wave velocity is associated with low creatinine clearance and proteinuria in a screened cohort. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 2006;47(5):790-797.

66. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation.* 2006;113(5):657-663.

67. Sutton-Tyrrell K, Mackey RH, Holubkov R, Vaitkevicius PV, Spurgeon HA, Lakatta EG. Measurement variation of aortic pulse wave velocity in the elderly. *Am J Hypertens.* 2001;14(5 Pt 1):463-468.

68. Townsend RR, Wilkinson IB, Schiffrin EL, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension.* 2015;66(3):698-722.

69. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *American journal of hypertension.* 2002;15(5):426-444.

70. Oliver JJ, Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arteriosclerosis, thrombosis, and vascular biology.* 2003;23(4):554-566.

71. Benetos A, Thomas F, Joly L, et al. Pulse pressure amplification a mechanical biomarker of cardiovascular risk. *Journal of the American College of Cardiology.* 2010;55(10):1032-1037.

72. Cabrera-Rego JO, Navarro-Despaigne D, Staroushik-Morel L, Diaz-Reyes K, Lima-Martinez MM, Iacobellis G. Association between endothelial dysfunction, epicardial fat and subclinical atherosclerosis during menopause. *Clinica e investigacion en arteriosclerosis : publicacion oficial de la Sociedad Espanola de Arteriosclerosis.* 2018;30(1):21-27.

73. Abbas SZ, Sangawan V, Das A, Pandey AK. Assessment of Cardiovascular Risk in Natural and Surgical Menopause. *Indian journal of endocrinology and metabolism.* 2018;22(2):223-228.

74. Shapiro Y, Mashavi M, Luckish E, Shargorodsky M. Diabetes and menopause aggravate age-dependent deterioration in arterial stiffness. *Menopause (New York, NY).* 2014;21(11):1234-1238.

75. Palmiero P, Maiello M, Daly DD, Jr., Ciccone MM, Nanda NC. Aortic stiffness assessed by global pulse wave velocity in postmenopausal women: an ultrasonographic study. *Echocardiography (Mount Kisco, NY).* 2012;29(10):1233-1238.

76. Zaydun G, Tomiyama H, Hashimoto H, et al. Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. *Atherosclerosis.* 2006;184(1):137-142.

77. O'Neill SM, Liu J, O'Rourke MF, Khoo SK. The menopausal transition does not appear to accelerate age-related increases in arterial stiffness. *Climacteric : the journal of the International Menopause Society.* 2013;16(1):62-69.

78. Casiglia E, Tikhonoff V, Boschetti G, et al. Arterial stiffness and related variables across menopausal status: an epidemiologic study. *J Womens Health (Larchmt).* 2013;22(1):75-84.

79. Takahashi K, Miura S, Mori-Abe A, et al. Impact of menopause on the augmentation of arterial stiffness with aging. *Gynecol Obstet Invest.* 2005;60(3):162-166.

80. Gompel A, Boutouyrie P, Joannides R, et al. Association of menopause and hormone replacement therapy with large artery remodeling. *Fertility and sterility.* 2011;96(6):1445-1450.

81. Lambrinoudaki I, Armeni E, Georgiopoulos G, et al. Subclinical atherosclerosis in menopausal women with low to medium calculated cardiovascular risk. *International journal of cardiology.* 2013;164(1):70-76.

82. Staessen JA, van der Heijden-Spek JJ, Safar ME, et al. Menopause and the characteristics of the large arteries in a population study. *J Hum Hypertens.* 2001;15(8):511-518.

83. Din-Dzietham R, Couper D, Evans G, Arnett DK, Jones DW. Arterial stiffness is greater in African Americans than in whites: evidence from the Forsyth County, North Carolina, ARIC cohort. *American journal of hypertension.* 2004;17(4):304-313.

84. Howard G, Safford MM, Moy CS, et al. Racial Differences in the Incidence of Cardiovascular Risk Factors in Older Black and White Adults. *Journal of the American Geriatrics Society.* 2017;65(1):83-90.

85. Sherva R, Miller MB, Lynch AI, et al. A whole genome scan for pulse pressure/stroke volume ratio in African Americans: the HyperGEN study. *American journal of hypertension.* 2007;20(4):398-402.

86. Hall JL, Duprez DA, Barac A, Rich SS. A review of genetics, arterial stiffness, and blood pressure in African Americans. *J Cardiovasc Transl Res.* 2012;5(3):302-308.

87. Musemwa N, Gadegbeku CA. Hypertension in African Americans. *Curr Cardiol Rep.* 2017;19(12):129.

88. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension.* 1995;26(3):485-490.

89. Wei W, Tolle M, Zidek W, van der Giet M. Validation of the mobil-O-Graph: 24 h-blood pressure measurement device. *Blood pressure monitoring.* 2010;15(4):225-228.

90. McEniery CM, Spratt M, Munnery M, et al. An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the Caerphilly prospective study. *Hypertension.* 2010;56(1):36-43.

91. Townsend RR, Wimmer NJ, Chirinos JA, et al. Aortic PWV in chronic kidney disease: a CRIC ancillary study. *American journal of hypertension.* 2010;23(3):282-289.

92. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance. *Circulation.* 2002;106(16):2085-2090.

93. Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated Aortic Pulse Wave Velocity, a Marker of Arterial Stiffness, Predicts Cardiovascular Events in Well-Functioning Older Adults. *Circulation.* 2005;111(25):3384-3390.

94. Wang KL, Cheng HM, Sung SH, et al. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. *Hypertension.* 2010;55(3):799-805.

95. Bergman RN, Van Citters GW, Mittelman SD, et al. Central role of the adipocyte in the metabolic syndrome. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research.* 2001;49(1):119-126.

96. Despres JP, Lemieux I, Bergeron J, et al. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arteriosclerosis, thrombosis, and vascular biology.* 2008;28(6):1039-1049.

97. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. *Physiological reviews.* 2013;93(1):359-404.

98. Barrett-Connor E, Khaw KT. Cigarette smoking and increased central adiposity. *Annals of internal medicine.* 1989;111(10):783-787.

99. Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiological reviews.* 1994;74(4):761-811.

100. Ross R, Bradshaw AJ. The future of obesity reduction: beyond weight loss. *Nature reviews Endocrinology.* 2009;5(6):319-325.

101. Wood IS, de Heredia FP, Wang B, Trayhurn P. Cellular hypoxia and adipose tissue dysfunction in obesity. *The Proceedings of the Nutrition Society.* 2009;68(4):370-377.

102. Couillard C, Bergeron N, Prud'homme D, et al. Gender difference in postprandial lipemia : importance of visceral adipose tissue accumulation. *Arteriosclerosis, thrombosis, and vascular biology.* 1999;19(10):2448-2455.

103. Garg A, Misra A. Lipodystrophies: rare disorders causing metabolic syndrome. *Endocrinology and metabolism clinics of North America.* 2004;33(2):305-331.

104. Hegele RA. Insulin resistance in human partial lipodystrophy. *Current atherosclerosis reports.* 2000;2(5):397-404.

105. Leenen R, van der Kooy K, Deurenberg P, et al. Visceral fat accumulation in obese subjects: relation to energy expenditure and response to weight loss. *The American journal of physiology.* 1992;263(5 Pt 1):E913-919.

106. Burger HG, Dudley EC, Cui J, Dennerstein L, Hopper JL. A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. *The Journal of clinical endocrinology and metabolism.* 2000;85(8):2832-2838.

107. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine reviews.* 2013;34(3):309-338.

108. Sponton CH, Kajimura S. Burning Fat and Building Bone by FSH Blockade. *Cell metabolism.* 2017;26(2):285-287.

109. de Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *European heart journal.* 2007;28(7):850-856.

110. Pouliot MC, Despres JP, Lemieux S, et al. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *The American journal of cardiology.* 1994;73(7):460-468.

111. Feng Y, Hong X, Wilker E, et al. Effects of age at menarche, reproductive years, and menopause on metabolic risk factors for cardiovascular diseases. *Atherosclerosis.* 2008;196(2):590-597.

112. Cornier MA, Despres JP, Davis N, et al. Assessing adiposity: a scientific statement from the American Heart Association. *Circulation.* 2011;124(18):1996-2019.

113. Sjostrom L. A computer-tomography based multicompartment body composition technique and anthropometric predictions of lean body mass, total and subcutaneous adipose tissue. *International journal of obesity.* 1991;15 Suppl 2:19-30.

114. Ross R. Advances in the application of imaging methods in applied and clinical physiology. *Acta diabetologica.* 2003;40 Suppl 1:S45-50.

115. Kvist H, Sjöström L, Tylen U. Adipose tissue volume determinations in women by computed tomography: technical considerations. *International journal of obesity.* 1986;10(1):53-67.

116. Lee SY, Gallagher D. Assessment methods in human body composition. *Current opinion in clinical nutrition and metabolic care.* 2008;11(5):566-572.

117. Stolk RP, Wink O, Zelissen PM, Meijer R, van Gils AP, Grobbee DE. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity.* 2001;25(9):1346-1351.

118. De Lucia Rolfe E, Sleigh A, Finucane FM, et al. Ultrasound measurements of visceral and subcutaneous abdominal thickness to predict abdominal adiposity among older men and women. *Obesity (Silver Spring, Md).* 2010;18(3):625-631.

119. Nagata N, Sakamoto K, Arai T, et al. Effect of Body Mass Index and Intra-Abdominal Fat Measured by Computed Tomography on the Risk of Bowel Symptoms. *PLoS One.* 2015;10(4):e0123993.

120. Matthews KA, Abrams B, Crawford S, et al. Body mass index in mid-life women: relative influence of menopause, hormone use, and ethnicity. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity.* 2001;25(6):863-873.

121. Guthrie JR, Dennerstein L, Dudley EC. Weight gain and the menopause: a 5-year prospective study. *Climacteric : the journal of the International Menopause Society.* 1999;2(3):205-211.

122. Donato GB, Fuchs SC, Oppermann K, Bastos C, Spritzer PM. Association between menopause status and central adiposity measured at different cutoffs of waist circumference and waist-to-hip ratio. *Menopause (New York, NY).* 2006;13(2):280-285.

123. Sternfeld B, Wang H, Quesenberry CP, Jr., et al. Physical activity and changes in weight and waist circumference in midlife women: findings from the Study of Women's Health Across the Nation. *Am J Epidemiol.* 2004;160(9):912-922.

124. Kotani K, Tokunaga K, Fujioka S, et al. Sexual dimorphism of age-related changes in whole-body fat distribution in the obese. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity.* 1994;18(4):207-202.

125. van der Leeuw J, Wassink AM, van der Graaf Y, Westerveld HE, Visseren FL. Age-related differences in abdominal fat distribution in premenopausal and postmenopausal women with cardiovascular disease. *Menopause.* 2013;20(4):409-417.

126. Kanaley JA, Sames C, Swisher L, et al. Abdominal fat distribution in pre- and postmenopausal women: The impact of physical activity, age, and menopausal status. *Metabolism: clinical and experimental.* 2001;50(8):976-982.

127. Trikudanathan S, Pedley A, Massaro JM, et al. Association of female reproductive factors with body composition: the Framingham Heart Study. *The Journal of clinical endocrinology and metabolism.* 2013;98(1):236-244.

128. Whitaker KM, Choh AC, Lee M, Towne B, Czerwinski SA, Demerath EW. Sex differences in the rate of abdominal adipose accrual during adulthood: the Fels Longitudinal Study. *International journal of obesity (2005).* 2016;40(8):1278-1285.

129. Franklin RM, Ploutz-Snyder L, Kanaley JA. Longitudinal changes in abdominal fat distribution with menopause. *Metabolism: clinical and experimental.* 2009;58(3):311-315.

130. Abdulnour J, Doucet E, Brochu M, et al. The effect of the menopausal transition on body composition and cardiometabolic risk factors: a Montreal-Ottawa New Emerging Team group study. *Menopause (New York, NY).* 2012;19(7):760-767.

131. Lovejoy JC, Champagne CM, de Jonge L, Xie H, Smith SR. Increased visceral fat and decreased energy expenditure during the menopausal transition. *International journal of obesity (2005).* 2008;32(6):949-958.

132. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on

Clinical Practice Guidelines. *Journal of the American College of Cardiology.* 2018;71(19):e127-e248.

133. Lloyd-Jones DM, Hong Y, Labarthe D, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation.* 2010;121(4):586-613.

134. Muntner P, Shimbo D, Carey RM, et al. Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association. *Hypertension.* 2019:HYP0000000000000087.

135. Beevers DG, Lip GY, O'Brien ET. *ABC of Hypertension.* John Wiley & Sons; 2014.

136. Oparil S, Zaman MA, Calhoun DA. Pathogenesis of hypertension. *Annals of internal medicine.* 2003;139(9):761-776.

137. Landahl S, Bengtsson C, Sigurdsson JA, Svanborg A, Svärdsudd K. Age-related changes in blood pressure. *Hypertension.* 1986;8(11):1044-1049.

138. CHIANG BN, PERLMAN LV, EPSTEIN FH. Overweight and Hypertension. *Circulation.* 1969;39(3):403-421.

139. Forman JP, Stampfer MJ, Curhan GC. Diet and lifestyle risk factors associated with incident hypertension in women. *Jama.* 2009;302(4):401-411.

140. He FJ, MacGregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials. Implications for public health. *J Hum Hypertens.* 2002;16(11):761-770.

141. Roerecke M, Kaczorowski J, Tobe SW, Gmel G, Hasan OSM, Rehm J. The effect of a reduction in alcohol consumption on blood pressure: a systematic review and meta-analysis. *The Lancet Public health.* 2017;2(2):e108-e120.

142. Wang NY, Young JH, Meoni LA, Ford DE, Erlinger TP, Klag MJ. Blood pressure change and risk of hypertension associated with parental hypertension: the Johns Hopkins Precursors Study. *Archives of internal medicine.* 2008;168(6):643-648.

143. Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. *Lancet (London, England).* 2003;361(9369):1629-1641.

144. Wei YC, George NI, Chang CW, Hicks KA. Assessing Sex Differences in the Risk of Cardiovascular Disease and Mortality per Increment in Systolic Blood Pressure: A Systematic Review and Meta-Analysis of Follow-Up Studies in the United States. *PloS one.* 2017;12(1):e0170218.

145. Amigoni S, Morelli P, Parazzini F, Chatenoud L. Determinants of elevated blood pressure in women around menopause: results from a cross-sectional study in Italy. *Maturitas.* 2000;34(1):25-32.

146. Bonithon-Kopp C, Scarabin PY, Darne B, Malmejac A, Guize L. Menopause-related changes in lipoproteins and some other cardiovascular risk factors. *International journal of epidemiology.* 1990;19(1):42-48.

147. Son MK, Lim NK, Lim JY, et al. Difference in blood pressure between early and late menopausal transition was significant in healthy Korean women. *BMC women's health.* 2015;15:64.

148. Tremollieres FA, Pouilles JM, Cauneille C, Ribot C. Coronary heart disease risk factors and menopause: a study in 1684 French women. *Atherosclerosis.* 1999;142(2):415-423.

149. Zanchetti A, Facchetti R, Cesana GC, Modena MG, Pirrelli A, Sega R. Menopause-related blood pressure increase and its relationship to age and body mass index: the SIMONA epidemiological study. *Journal of hypertension.* 2005;23(12):2269-2276.

150. Zhou Y, Zhou X, Guo X, et al. Prevalence and risk factors of hypertension among pre- and post-menopausal women: a cross-sectional study in a rural area of northeast China. *Maturitas.* 2015;80(3):282-287.

151. Cifkova R, Pitha J, Lejskova M, Lanska V, Zecova S. Blood pressure around the menopause: a population study. *Journal of hypertension.* 2008;26(10):1976-1982.

152. Portaluppi F, Pansini F, Manfredini R, Mollica G. Relative influence of menopausal status, age, and body mass index on blood pressure. *Hypertension.* 1997;29(4):976-979.

153. Kuh D, Langenberg C, Hardy R, et al. Cardiovascular risk at age 53 years in relation to the menopause transition and use of hormone replacement therapy: a prospective British birth

cohort study. *BJOG: An International Journal of Obstetrics and Gynaecology.* 2005;112(4):476-485.

154. Agrinier N, Cournot M, Dallongeville J, et al. Menopause and modifiable coronary heart disease risk factors: a population based study. *Maturitas.* 2010;65(3):237-243.

155. Graff-Iversen S, Thelle DS, Hammar N. Serum lipids, blood pressure and body weight around the age of the menopause. *Eur J Cardiovasc Prev Rehabil.* 2008;15(1):83-88.

156. Lee JS, Hayashi K, Mishra G, Yasui T, Kubota T, Mizunuma H. Independent association between age at natural menopause and hypercholesterolemia, hypertension, and diabetes mellitus: Japan nurses' health study. *Journal of atherosclerosis and thrombosis.* 2013;20(2):161-169.

157. Izumi Y, Matsumoto K, Ozawa Y, et al. Effect of age at menopause on blood pressure in postmenopausal women. *American journal of hypertension.* 2007;20(10):1045-1050.

158. Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR. Menopause and risk factors for coronary heart disease. *N Engl J Med.* 1989;321(10):641-646.

159. Casiglia E, Tikhonoff V, Caffi S, et al. Menopause does not affect blood pressure and risk profile, and menopausal women do not become similar to men. *Journal of hypertension.* 2008;26(10):1983-1992.

160. Lindquist O. Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. *Preventive medicine.* 1982;11(2):162-172.

161. Staessen JA, Ginocchio G, Thijs L, Fagard R. Conventional and ambulatory blood pressure and menopause in a prospective population study. *J Hum Hypertens.* 1997;11(8):507-514.

162. Luoto R, Sharrett AR, Schreiner P, Sorlie PD, Arnett D, Ephross S. Blood pressure and menopausal transition: the Atherosclerosis Risk in Communities study (1987-95). *Journal of hypertension.* 2000;18(1):27-33.

163. Nagin DS, NAGIN D. *Group-based modeling of development.* Harvard University Press; 2005.

164. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. *Annual review of clinical psychology.* 2010;6:109-138.

165. Benjamin EJ, Virani SS, Callaway CW, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation.* 2018;137(12):e67-e492.

166. Sowers MFR, Crawford SL, Sternfeld B, et al. SWAN: a multicenter, multiethnic, community-based cohort study of women and the menopausal transition. 2000.

167. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.

168. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry.* 1972;18(6):499-502.

169. Sternfeld B, Ainsworth BE, Quesenberry CP. Physical activity patterns in a diverse population of women. *Preventive medicine.* 1999;28(3):313-323.

170. Cleveland WS, Devlin SJ. Locally weighted regression: an approach to regression analysis by local fitting. *Journal of the American statistical association.* 1988;83(403):596-610.

171. Greendale GA, Sowers M, Han W, et al. Bone mineral density loss in relation to the final menstrual period in a multiethnic cohort: results from the Study of Women's Health Across the Nation (SWAN). *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research.* 2012;27(1):111-118.

172. Benoit K. Linear regression models with logarithmic transformations. *London School of Economics, London.* 2011;22(1):23-36.

173. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *The New England journal of medicine.* 1999;340(23):1801-1811.

174. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arteriosclerosis, thrombosis, and vascular biology.* 2004;24(5):969-974.

175. Vural P, Akgul C, Canbaz M. Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. *Pharmacological research.* 2006;54(4):298-302.

176. Woodard GA, Mehta VG, Mackey RH, et al. C-reactive protein is associated with aortic stiffness in a cohort of African American and white women transitioning through menopause. *Menopause (New York, NY).* 2011;18(12):1291-1297.

177. Lee CG, Carr MC, Murdoch SJ, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. *J Clin Endocrinol Metab.* 2009;94(4):1104-1110.

178. Zachariah JP, Hwang S, Hamburg NM, et al. Circulating Adipokines and Vascular Function: Cross-Sectional Associations in a Community-Based Cohort. *Hypertension.* 2016;67(2):294-300.

179. Brown HL, Warner JJ, Gianos E, et al. Promoting Risk Identification and Reduction of Cardiovascular Disease in Women Through Collaboration With Obstetricians and Gynecologists: A Presidential Advisory From the American Heart Association and the American College of Obstetricians and Gynecologists. *Circulation.* 2018.

180. Wang D, Jackson EA, Karvonen-Gutierrez CA, et al. Healthy Lifestyle During the Midlife Is Prospectively Associated With Less Subclinical Carotid Atherosclerosis: The Study of Women's Health Across the Nation. *Journal of the American Heart Association.* 2018;7(23):e010405.

181. Peterson LM, Matthews KA, Derby CA, Bromberger JT, Thurston RC. The relationship between cumulative unfair treatment and intima media thickness and adventitial diameter: The moderating role of race in the study of women's health across the nation. *Health Psychol.* 2016;35(4):313-321.

182. Woodard GA, Narla VV, Ye R, et al. Racial differences in the association between carotid plaque and aortic and coronary artery calcification among women transitioning through menopause. *Menopause (New York, NY).* 2012;19(2):157-163.

183. Carnethon MR, Pu J, Howard G, et al. Cardiovascular Health in African Americans: A Scientific Statement From the American Heart Association. *Circulation.* 2017;136(21):e393-e423.

184. Singer JD, Willett JB. *Applied longitudinal data analysis: modeling change and event occurrence.* New York;Oxford;: Oxford University Press; 2003.

185. Sun Y, Liu B, Snetselaar LG, et al. Association of Normal-Weight Central Obesity With All-Cause and Cause-Specific Mortality Among Postmenopausal Women. *JAMA Network Open.* 2019;2(7):e197337.

186. Li C, Ford ES, McGuire LC, Mokdad AH. Increasing trends in waist circumference and abdominal obesity among US adults. *Obesity (Silver Spring, Md).* 2007;15(1):216-224.

187. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology.* 2011;11(2):85-97.

188. Janssen I, Powell LH, Jasielec MS, Kazlauskaite R. Covariation of change in bioavailable testosterone and adiposity in midlife women. *Obesity (Silver Spring, Md).* 2015;23(2):488-494.

189. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness. *Circulation.* 2007;115(4):459-467.

190. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB. Carotid-Wall Intima–Media Thickness and Cardiovascular Events. *New England Journal of Medicine.* 2011;365(3):213-221.

191. Watase H, Sun J, Hippe DS, et al. Carotid Artery Remodeling Is Segment Specific: An In Vivo Study by Vessel Wall Magnetic Resonance Imaging. *Arteriosclerosis, thrombosis, and vascular biology.* 2018;38(4):927-934.

192. Nguyen QM, Toprak A, Xu JH, Srinivasan SR, Chen W, Berenson GS. Progression of segment-specific carotid artery intima-media thickness in young adults (from the Bogalusa Heart Study). *The American journal of cardiology.* 2011;107(1):114-119.

193. Sowers MFR, Crawford SL, Sternfeld B, et al. SWAN: a multicenter, multiethnic, community-based cohort study of women and the menopausal transition. In: Lobo RA, Kelsey J, Marcus R, eds. *Menopause: Biology and Pathology.* 1st ed. USA: Academic Press; 2000:175-188.

194. Little RJ, Rubin DB. *Statistical analysis with missing data.* Vol 793: John Wiley & Sons; 2019.

195. Wildman RP, Janssen I, Khan UI, et al. Subcutaneous adipose tissue in relation to subclinical atherosclerosis and cardiometabolic risk factors in midlife women. *The American journal of clinical nutrition.* 2011;93(4):719-726.

196. Liu KH, Chan YL, Chan JC, Chan WB. Association of carotid intima-media thickness with mesenteric, preperitoneal and subcutaneous fat thickness. *Atherosclerosis.* 2005;179(2):299-304.

197. Lear SA, Humphries KH, Kohli S, Frohlich JJ, Birmingham CL, Mancini GB. Visceral adipose tissue, a potential risk factor for carotid atherosclerosis: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Stroke; a journal of cerebral circulation.* 2007;38(9):2422-2429.

198. Frauchiger B, Schmid HP, Roedel C, Moosmann P, Staub D. Comparison of carotid arterial resistive indices with intima-media thickness as sonographic markers of atherosclerosis. *Stroke; a journal of cerebral circulation.* 2001;32(4):836-841.

199. Kang Y, Park HJ, Kang MI, et al. Adipokines, inflammation, insulin resistance, and carotid atherosclerosis in patients with rheumatoid arthritis. *Arthritis Res Ther.* 2013;15(6):R194.

200. Santizo R, Baughman VL, Pelligrino DA. Relative contributions from neuronal and endothelial nitric oxide synthases to regional cerebral blood flow changes during forebrain ischemia in rats. *Neuroreport.* 2000;11(7):1549-1553.

201. Krejza J, Mariak Z, Huba M, Wolczynski S, Lewko J. Effect of Endogenous Estrogen on Blood Flow Through Carotid Arteries. *Stroke; a journal of cerebral circulation.* 2001;32(1):30-36.

202. Samargandy S, Matthews KA, Brooks MM, et al. Arterial Stiffness Accelerates Within 1 Year of the Final Menstrual Period: The SWAN Heart Study. *Arteriosclerosis, thrombosis, and vascular biology.* 2020;40(4):1001-1008.

203. Brown HL, Warner JJ, Gianos E, et al. Promoting Risk Identification and Reduction of Cardiovascular Disease in Women Through Collaboration With Obstetricians and Gynecologists: A Presidential Advisory From the American Heart Association and the American College of Obstetricians and Gynecologists. *Circulation.* 2018;137(24):e843-e852.

204. Verheggen RJHM, Maessen MFH, Green DJ, Hermus ARMM, Hopman MTE, Thijssen DHT. A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. *Obesity Reviews.* 2016;17(8):664-690.

205. Vissers D, Hens W, Taeymans J, Baeyens J-P, Poortmans J, Van Gaal L. The Effect of Exercise on Visceral Adipose Tissue in Overweight Adults: A Systematic Review and Meta-Analysis. *PloS one.* 2013;8(2):e56415.

206. Kazlauskaite R, Karavolos K, Janssen I, et al. The association between self-reported energy intake and intra-abdominal adipose tissue in perimenopausal women. *Journal of obesity.* 2012;2012.

207. Stanaway JD, Afshin A, Gakidou E, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Stu. *The Lancet.* 2018;392(10159):1923-1994.

208. Ji H, Kim A, Ebinger JE, et al. Sex Differences in Blood Pressure Trajectories Over the Life Course. *JAMA cardiology.* 2020;5(3):19.

209. Coylewright M, Reckelhoff JF, Ouyang P. Menopause and hypertension: an age-old debate. *Hypertension.* 2008;51(4):952-959.

210. Tepper PG, Brooks MM, Randolph JF, Jr., et al. Characterizing the trajectories of vasomotor symptoms across the menopausal transition. *Menopause (New York, NY).* 2016;23(10):1067-1074.

211. Tepper PG, Randolph JF, Jr., McConnell DS, et al. Trajectory clustering of estradiol and follicle-stimulating hormone during the menopausal transition among women in the Study of Women's Health across the Nation (SWAN). *The Journal of clinical endocrinology and metabolism.* 2012;97(8):2872-2880.

212. Jackson EA, El Khoudary SR, Crawford SL, et al. Hot Flash Frequency and Blood Pressure: Data from the Study of Women's Health Across the Nation. *J Womens Health (Larchmt).* 2016;25(12):1204-1209.

213. Wang N, Shao H, Chen Y, et al. Follicle-Stimulating Hormone, Its Association with Cardiometabolic Risk Factors, and 10-Year Risk of Cardiovascular Disease in Postmenopausal Women. *Journal of the American Heart Association.* 2017;6(9).

214. Ashraf MS, Vongpatanasin W. Estrogen and hypertension. *Current hypertension reports.* 2006;8(5):368-376.

215. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med.* 2005;24(19):2911-2935.

216. El Khoudary SR, Santoro N, Chen HY, et al. Trajectories of estradiol and follicle-stimulating hormone over the menopause transition and early markers of atherosclerosis after menopause. *European journal of preventive cardiology.* 2016;23(7):694-703.

217. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet (London, England).* 2002;360(9349):1903-1913.

218. Whelton SP, McEvoy JW, Shaw L, et al. Association of Normal Systolic Blood Pressure Level With Cardiovascular Disease in the Absence of Risk Factors. *JAMA cardiology.* 2020.

219. Rapsomaniki E, Timmis A, George J, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. *Lancet (London, England).* 2014;383(9932):1899-1911.

220. Burt VL, Whelton P, Roccella EJ, et al. Prevalence of Hypertension in the US Adult Population. *Hypertension.* 1995;25(3):305-313.

221. Delgado J, Bowman K, Ble A, et al. Blood Pressure Trajectories in the 20 Years Before Death. *JAMA Internal Medicine.* 2018;178(1):93.

222. Barton M, Meyer MR. Postmenopausal Hypertension. *Hypertension.* 2009;54(1):11-18.

223. Mercuro G, Zoncu S, Saiu F, Mascia M, Melis GB, Rosano GM. Menopause induced by oophorectomy reveals a role of ovarian estrogen on the maintenance of pressure homeostasis. *Maturitas.* 2004;47(2):131-138.

224. Shelley JM, Green A, Smith AM, et al. Relationship of endogenous sex hormones to lipids and blood pressure in mid-aged women. *Annals of epidemiology.* 1998;8(1):39-45.

225. Wang L, Szklo M, Folsom AR, Cook NR, Gapstur SM, Ouyang P. Endogenous sex hormones, blood pressure change, and risk of hypertension in postmenopausal women: The Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis.* 2012;224(1):228-234.

226. Dubey RK, Jackson EK. Potential vascular actions of 2-methoxyestradiol. *Trends in Endocrinology & Metabolism.* 2009;20(8):374-379.

227. Masi CM, Hawkley LC, Xu X, Veenstra TD, Cacioppo JT. Serum estrogen metabolites and systolic blood pressure among middle-aged and older women and men. *American journal of hypertension.* 2009;22(11):1148-1153.

228. Akahoshi M, Soda M, Nakashima E, et al. Effects of age at menopause on serum cholesterol, body mass index, and blood pressure. *Atherosclerosis.* 2001;156(1):157-163.

229. Woods NF, Mitchell ES. Symptoms during the perimenopause: prevalence, severity, trajectory, and significance in women's lives. *The American journal of medicine.* 2005;118 Suppl 12B:14-24.

230. Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flashes. *Fertility and sterility.* 1998;70(2):332-337.

231. Stilley JAW, Christensen DE, Dahlem KB, et al. FSH Receptor (FSHR) Expression in Human Extragonadal Reproductive Tissues and the Developing Placenta, and the Impact of Its Deletion on Pregnancy in Mice1. *Biology of Reproduction.* 2014;91(3):74-74.

232. Cui H, Zhao G, Liu R, Zheng M, Chen J, Wen J. FSH stimulates lipid biosynthesis in chicken adipose tissue by upregulating the expression of its receptor FSHR. *Journal of lipid research.* 2012;53(5):909-917.

233. Song Y, Wang ES, Xing LL, et al. Follicle-Stimulating Hormone Induces Postmenopausal Dyslipidemia Through Inhibiting Hepatic Cholesterol Metabolism. *The Journal of clinical endocrinology and metabolism.* 2016;101(1):254-263.

234. Sun D, Bai M, Jiang Y, et al. Roles of follicle stimulating hormone and its receptor in human metabolic diseases and cancer. *Am J Transl Res.* 2020;12(7):3116-3132.

235. Kaess BM, Preis SR, Beiser A, et al. Circulating vascular endothelial growth factor and the risk of cardiovascular events. *Heart (British Cardiac Society).* 2016;102(23):1898-1901.

236. Haviland AM, Jones BL, Nagin DS. Group-based trajectory modeling extended to account for nonrandom participant attrition. *Sociological Methods & Research.* 2011;40(2):367-390.

237. Piché ME, Tchernof A, Després JP. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. *Circulation research.* 2020;126(11):1477-1500.

238. The Emerging Risk Factors C. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *The Lancet.* 2011;377(9771):1085-1095.

239. Liao D, Arnett DK, Tyroler HA, et al. Arterial stiffness and the development of hypertension. The ARIC study. *Hypertension.* 1999;34(2):201-206.

240. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. *Journal of the American College of Cardiology.* 2008;51(14):1377-1383.

241. Mattace-Raso FU, van den Meiracker AH, Bos WJ, et al. Arterial stiffness, cardiovagal baroreflex sensitivity and postural blood pressure changes in older adults: the Rotterdam Study. *Journal of hypertension.* 2007;25(7):1421-1426.

242. Okada Y, Galbreath MM, Shibata S, et al. Relationship between sympathetic baroreflex sensitivity and arterial stiffness in elderly men and women. *Hypertension.* 2012;59(1):98-104.

243. Chung C-M, Cheng H-W, Chang J-J, et al. Relationship between resistant hypertension and arterial stiffness assessed by brachial-ankle pulse wave velocity in the older patient. *Clinical interventions in aging.* 2014;9:1495-1502.

244. Wenger NK, Arnold A, Bairey Merz CN, et al. Hypertension Across a Woman's Life Cycle. *Journal of the American College of Cardiology.* 2018;71(16):1797-1813.

245. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation.* 2011;123(4):e18-e209.

246. Mosca L, Mochari H, Christian A, et al. National Study of Women's Awareness, Preventive Action, and Barriers to Cardiovascular Health. *Circulation.* 2006;113(4):525-534.

247. Cushman M, Shay CM, Howard VJ, et al. Ten-Year Differences in Women's Awareness Related to Coronary Heart Disease: Results of the 2019 American Heart Association National Survey: A Special Report From the American Heart Association. *Circulation.* 2020.

248. Bairey Merz CN, Andersen H, Sprague E, et al. Knowledge, Attitudes, and Beliefs Regarding Cardiovascular Disease in Women: The Women's Heart Alliance. *Journal of the American College of Cardiology.* 2017;70(2):123-132.

249. Mosca L, Benjamin EJ, Berra K, et al. Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update. *Journal of the American College of Cardiology.* 2011;57(12):1404-1423.

250. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature.* 2006;444(7121):881-887.

251. Neeland IJ, Ross R, Després JP, et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *Lancet Diabetes Endocrinol.* 2019;7(9):715-725.

252. Lemieux I, Poirier P, Bergeron J, et al. Hypertriglyceridemic waist: A useful screening phenotype in preventive cardiology? *Canadian Journal of Cardiology.* 2007;23:23B-31B.

253. Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG. A prospective population-based study of menopausal symptoms. *Obstet Gynecol.* 2000;96(3):351-358.

254. Williams RE, Kalilani L, Dibenedetti DB, Zhou X, Fehnel SE, Clark RV. Healthcare seeking and treatment for menopausal symptoms in the United States. *Maturitas.* 2007;58(4):348-358.

255. El Khoudary SR, Greendale G, Crawford SL, et al. The menopause transition and women's health at midlife. *Menopause (New York, NY).* 2019;Publish Ahead of(10):1213-1227.

256. El Khoudary SR. Gaps, limitations and new insights on endogenous estrogen and follicle stimulating hormone as related to risk of cardiovascular disease in women traversing the menopause: A narrative review. *Maturitas.* 2017;104:44-53.

257. Wang N, Kuang L, Han B, et al. Follicle-stimulating hormone associates with prediabetes and diabetes in postmenopausal women. *Acta diabetologica.* 2016;53(2):227-236.

258. Kohrt WM, Wierman ME. Preventing Fat Gain by Blocking Follicle-Stimulating Hormone. *New England Journal of Medicine.* 2017;377(3):293-295.

259. Munir JA, Wu H, Bauer K, et al. The perimenopausal atherosclerosis transition: relationships between calcified and noncalcified coronary, aortic, and carotid atherosclerosis and risk factors and hormone levels. *Menopause (New York, NY).* 2012;19(1):10-15.

260. El Khoudary SR, Wildman RP, Matthews K, Thurston RC, Bromberger JT, Sutton-Tyrrell K. Endogenous sex hormones impact the progression of subclinical atherosclerosis in women during the menopausal transition. *Atherosclerosis.* 2012;225(1):180-186.

261. Celestino Catão Da Silva D, Nogueira De Almeida Vasconcelos A, Cleto Maria Cerqueira J, et al. Endogenous sex hormones are not associated with subclinical atherosclerosis in menopausal women. *Minerva Ginecol.* 2013;65(3):297-302.