JYU DISSERTATIONS 756

Matti Hyvärinen

Physical Activity, Menopausal Symptoms, and Cardiometabolic Health during the Menopausal Transition

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND
HEALTH SCIENCES

JYU DISSERTATIONS 756

Matti Hyvärinen

Physical Activity, Menopausal Symptoms, and Cardiometabolic Health during the Menopausal Transition

Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi päärakennuksen auditoriossa C1
maaliskuun 28. päivänä 2024 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in Main building, auditorium C1, on March 28, 2024, at 12 o'clock.

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2024

Editors
Anne Viljanen
Faculty of Sport and Health Sciences, University of Jyväskylä
Päivi Vuorio
Open Science Centre, University of Jyväskylä

ISBN 978-951-39-9953-7 (PDF)
URN:ISBN:978-951-39-9953-7
ISSN 2489-9003

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-9953-7

ABSTRACT

Hyvärinen, Matti
Physical Activity, Menopausal Symptoms, and Cardiometabolic Health during the Menopausal Transition
Jyväskylä: University of Jyväskylä, 2024, 77 p. + original articles
(JYU Dissertations
ISSN 2489-9003; 756)
ISBN 978-951-39-9953-7 (PDF)

Menopause is a natural part of the aging process in women that may have a marked influence on health and quality of life. Consequently, menopause and concomitant menopausal symptoms are associated with impaired cardiometabolic health. On the other hand, physical activity is thought to promote cardiometabolic health. The aim of this dissertation was to study the associations between physical activity, menopausal symptoms, and cardiometabolic health in women during the menopausal transition. Secondly, the aim was to investigate the factors that indicate approaching natural menopause in middle-aged women. Cross-sectional and longitudinal data from ERMA and EsmiRs studies were utilized with sample sizes varying from 279 to 1393 across the substudies. Participants were women aged 47–55 years at baseline. Their physical activity was assessed with accelerometers and menopausal symptoms with self-report questionnaires. Metabolic health was assessed based on cholesterol, triglyceride, glucose, and blood pressure levels and body composition was measured with dual-energy X-ray absorptiometry. Lipid, glucose, and blood pressure levels were observed to increase during the 4-year follow-up, and the rate of the increase accelerated near menopause. Higher levels of physical activity and lower prevalence and severity of menopausal symptoms were associated with improved cardiometabolic health, but they did not associate with the magnitude of the changes that occurred during the follow-up. The association between physical activity and menopausal symptoms varied based on the differences in total body mass and lean body mass. Finally, higher estradiol and follicle-stimulating hormone levels, menopausal symptoms, and menstrual cycle irregularity were observed to indicate approaching natural menopause. Based on these results, the decline in cardiometabolic health accelerates during the menopausal transition. Physical activity may not be effective for mitigating this decline, but it seems to promote overall cardiometabolic health in middle-aged women. Furthermore, maintaining healthy weight as well as being physically active, especially in women with lower total and lean body mass, may be beneficial for the management of menopausal symptoms.

Keywords: menopause, physical activity, cardiometabolic disease, cardiovascular disease, menopausal symptoms

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Hyvärinen, Matti
Fyysinen aktiivisuus, vaihdevuosioireet ja kardiometabolinen terveys vaihdevuosien aikana
Jyväskylä: Jyväskylän yliopisto, 2024, 77 s. + alkuperäiset artikkelit
(JYU Dissertations
ISSN 2489-9003; 756)
ISBN 978-951-39-9953-7 (PDF)

Vaihdevuodet ja menopaussi ovat luonnollinen osa naisten ikääntymisprosessia, ja ne voivat vaikuttaa merkittävästi naisten terveyteen ja elämänlaatuun. Vaihdevuodet ja niihin liittyvät vaihdevuosioireet on muun muassa yhdistetty heikentyneeseen kardiometaboliseen terveyteen. Toisaalta fyysisen aktiivisuuden on todettu edistävän kardiometabolista terveyttä. Tämän väitöskirjan tavoitteena oli tutkia fyysisen aktiivisuuden, vaihdevuosioireiden ja kardiometabolisen terveyden välisiä yhteyksiä vaihdevuosien aikana. Lisäksi väitöskirjassa tutkittiin, mitkä tekijät ennustavat lähestyvää luonnollista menopaussia. Tutkimuksessa käytettiin ERMA- ja EsmiRs-tutkimuksissa kerättyä poikittais- ja pitkittäisaineistoa. Tutkittavat olivat alkumittauksissa 47–55-vuotiaita naisia ja tutkittavien määrä vaihteli osatöissä 279:n ja 1393:n välillä. Tutkittavien fyysistä aktiivisuutta arvioitiin aktiivisuusmittausten ja vaihdevuosioireita kyselylomakkeiden avulla. Kardiometabolista terveyttä arvioitiin kolesteroli-, triglyseridi-, verensokeri- ja verenpainetasojen avulla sekä käyttämällä kaksienergiaisen röntgenabsorptiometrian avulla mitattuja kehonkoostumusmuuttujia. Veren rasva-arvojen, verensokerin ja verenpaineen havaittiin kohoavan nelivuotisen seurannan aikana ja näiden muutosten olevan suurempia lähellä menopaussin ajankohtaa. Suurempi fyysinen aktiivisuus sekä vähäisempi vaihdevuosioireiden määrä ja haittaavuus olivat yhteydessä parempaan kardiometaboliseen terveyteen, mutta eivät siinä seurannan aikana tapahtuviin muutoksiin. Kehon kokonaismassan ja rasvattoman kehon massan havaittiin selittävän fyysisen aktiivisuuden ja vaihdevuosioireiden välistä yhteyttä. Kohonneiden estradiolin sekä follikkelia stimuloivan hormonin pitoisuuksien, vaihdevuosioireiden ja kuukautiskierron epäsäännöllisyyden havaittiin ennustavan luonnollisen menopaussin ajankohtaa. Ikääntymiseen liittyvä kardiometabolisen terveyden heikkeneminen vaikuttaa tulosten perusteella kiihtyvän vaihdevuosien aikana. Fyysisen aktiivisuuden avulla ei välttämättä pystytä ehkäisemään näitä muutoksia, mutta se vaikuttaa siitä huolimatta edistävän naisten kardiometabolista terveyttä myös keski-iässä. Tulokset viittaavat siihen, että painonhallinta sekä kehonkoostumuksesta riippuen myös fyysinen aktiivisuus saattavat helpottaa vaihdevuosioireita.

Asiasanat: Vaihdevuodet, menopaussi, fyysinen aktiivisuus, kardiometaboliset riskitekijät, sydän- ja verisuonitaudit, vaihdevuosioireet

Author

Matti Hyvärinen, MSc (Tech.), MSc (Sport)
Gerontology Research Center
Faculty of Sport and Health Sciences
University of Jyväskylä
Finland
matti.v.hyvarinen@jyu.fi
ORCID 0000-0002-5086-3635

Supervisors

Associate Professor Eija Laakkonen, PhD
Gerontology Research Center
Faculty of Sport and Health Sciences
University of Jyväskylä
Finland

Professor Juha Karvanen, DSc (Tech.)
Department of Mathematics and Statistics
University of Jyväskylä
Finland

Academy Research Fellow Timo Rantalainen, PhD
Gerontology Research Center
Faculty of Sport and Health Sciences
University of Jyväskylä
Finland

Reviewers

Consultant Gynecologist Maarit Niinimäki,
MD, PhD, Title of Docent
Department of Obstetrics and Gynecology
University of Oulu
Finland

Associate Professor Imke Janssen, PhD
Department of Preventive Medicine
Rush University
Illinois, The United States of America

Opponent

Professor Marjo Tuppurainen, MD, PhD
Department of Obstetrics and Gynecology
Kuopio University Hospital
Finland

ACKNOWLEDGEMENTS

This dissertation was conducted at the Gerontology Research Center, Faculty of Sport and Health Sciences at the University of Jyväskylä as a part of the ERMA and EsmiRs studies. First, I would like to express my gratitude to my supervisors Associate Professor Eija Laakkonen, Professor Juha Karvanen, and Academy Research Fellow Timo Rantalainen. Your guidance has been invaluable for me during this study, and it has helped my development as a scientist tremendously. I am especially grateful to Eija for providing me the opportunity to conduct this research in a supportive and flexible work environment as a part of the EsmiRs research group. I also want to express my gratitude to Consultant Gynecologist Maarit Niinimäki and Associate Professor Imke Janssen for the preliminary examination of this dissertation and Professor Marjo Tuppurainen for accepting the invite to be my opponent in the public defense of this dissertation.

I want to thank Hanna-Kaarina Juppi, Jari Karppinen, Anna Kankaanpää, Tero Sievänen, Niko Tynkkynen, and all other fellow researchers at the Gerontology Research Center for providing peer support and guidance throughout the study. Furthermore, I want to show my deepest gratitude to the Faculty of Sport and Health Sciences for funding this study and providing a great multidisciplinary working environment. Finally, I would like to thank my lovely family Sari, Markus, and Melody who have always been able to take my mind off the research and helped to get my priorities straight in daily life.

Jyväskylä, 2.2.2024
Matti Hyvärinen

ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTIONS

The dissertation is based on the following original publications, which are referred to by their Roman numbers. The thesis also includes unpublished data.

I **Hyvärinen, M.***, Juppi, H-K.*, Taskinen, S., Karppinen, J. E., Karvinen, S., Tammelin, T. H., Kovanen, V., Aukee, P., Kujala, U. M., Rantalainen, T., Sipilä, S., & Laakkonen, E. K. (2022). Metabolic health, menopause, and physical activity – a 4-year follow-up study. *International Journal of Obesity*, *46*(3), 544–554. https://doi.org/10.1038/s41366-021-01022-x

II **Hyvärinen, M**., Karvanen, J., Juppi, H-K., Karppinen, J. E., Tammelin, T. H., Kovanen, V., Aukee, P., Sipilä, S., Rantalainen, T., & Laakkonen, E. K. (2023). Menopausal symptoms and cardiometabolic risk factors in middle-aged women: A cross-sectional and longitudinal study with 4-year follow-up. *Maturitas, 174*, 39–47. https://doi.org/10.1016/j.maturitas.2023.05.004

III **Hyvärinen, M.,** Karvanen, J., Karppinen, J. E., Karavirta, L., Juppi, H-K., Tammelin, T. H., Kovanen, V., Laukkanen, J., Aukee, P., Sipilä, S., Rantalainen, T., & Laakkonen, E. K. The role of cardiorespiratory fitness and body composition in the association between physical activity and menopausal symptoms. Submitted for publication.

IV **Hyvärinen, M.**, Karvanen, J., Aukee, P., Tammelin T. H., Sipilä, S., Kujala, U. M., Kovanen, V., Rantalainen, T., & Laakkonen, E. K. (2021). Predicting the age at natural menopause in middle-aged women. *Menopause*, *28*(7), 792–799. https://doi.org/10.1097/GME.0000000000001774

For *Study I,* I shared first authorship with Hanna-Kaarina Juppi. We drafted and revised the manuscript together, and I was responsible for investigating the associations of physical activity with blood pressure and blood-based biomarkers. As the lone first author of *Studies II, III,* and *IV*, I drafted the manuscripts and made revisions based on the feedback from the co-authors and reviewers. I was responsible for the statistical analyses in all four studies, and I also participated in data collection. All studies were designed in collaboration with my supervisors, Eija Laakkonen, Juha Karvanen, and Timo Rantalainen.

FIGURES

FIGURE 1 Factors associated with the age at natural menopause (Created with BioRender.com). 17
FIGURE 2 The analytical framework of *Studies I, II,* and *III*. 25
FIGURE 3 The data sets of the study and how they were utilized in individual substudies. 27

TABLES

TABLE 1 The stages of reproductive aging (modified from Harlow et al., 2012). 16
TABLE 2 Criteria for the menopausal group assignment (modified from Kovanen et al., 2018). 29
TABLE 3 Measurements conducted in each stage of the ERMA and EsmiRs studies. 31
TABLE 4 Participant background characteristics in the ERMA and EsmiRs studies. 36
TABLE 5 Participant characteristics in menopausal symptoms, cardiometabolic health, and physical activity. 37
TABLE 6 Pooled linear mixed-effect model estimates for blood-based biomarkers of cardiometabolic health in *Study I* (*n* = 298). 39
TABLE 7 Pooled linear mixed-effect model estimates for blood pressure in *Study I* (*n* = 298). 40
TABLE 8 Pooled linear regression estimates for the association between the number of menopausal symptoms and blood-based biomarker levels in *Study II* (*n* = 1393). 42
TABLE 9 Pooled linear regression estimates for the association of the number of menopausal symptoms with blood pressure, body adiposity, and physical activity levels in *Study II* (*n* = 1393). 43
TABLE 10 Pooled linear regression estimates for the association of physical activity and body composition with the severity of menopausal symptoms in *Study III* (*n* = 298). 44
TABLE 11 Pooled moderator analysis of body composition measures in the association of physical activity and the severity of menopausal symptoms in *Study III* (*n* = 298). 45
TABLE 12 Pooled Cox regression model estimates for the prediction models constructed in *Study IV* (*n* = 279). 46

ABBREVIATIONS

AMH	Anti-Müllerian hormone
ANM	Age at natural menopause
ATC	Anatomical therapeutic chemical
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CMD	Cardiometabolic disease
COVID-19	Coronavirus disease 2019
DBP	Diastolic blood pressure
DXA	Dual-energy X-ray absorptiometry
E2	Estradiol
ERMA	Estrogenic Regulation of Muscle Apoptosis study
EsmiRs	Estrogen, MicroRNAs, and the Risk of Metabolic Dysfunction study
FMP	Final menstrual period
FSH	Follicle-stimulating hormone
HDL-C	High-density lipoprotein cholesterol
ICC	Intraclass correlation coefficient
LDL-C	Low-density lipoprotein cholesterol
LTPA	Leisure-time physical activity
MAD	Mean amplitude deviation
MAE	Mean absolute error
MET	Metabolic equivalent
MetS	Metabolic syndrome
MRS	Menopause Rating Scale
MVPA	Moderate-to-vigorous physical activity
RCT	Randomized controlled trial
SBP	Systolic blood pressure
SWAN	Study of Women's Health Across the Nations
VMS	Vasomotor symptoms

CONTENTS

ABSTRACT
TIIVISTELMÄ (ABSTRACT IN FINNISH)
ACKNOWLEDGEMENTS
ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION
FIGURES AND TABLES
ABBREVIATIONS
CONTENTS

1 INTRODUCTION 13

2 REVIEW OF THE LITERATURE 15
2.1 Menopause and the menopausal transition 15
2.1.1 Age at natural menopause 16
2.1.2 Menopausal symptoms 17
2.2 Physical activity 19
2.2.1 Assessment of physical activity 19
2.2.2 Physical activity and menopause 20
2.3 Cardiometabolic health 21
2.3.1 Assessment of cardiometabolic health 21
2.3.2 Cardiometabolic health and menopause 22
2.3.3 Cardiometabolic health and physical activity 22

3 PURPOSE OF THE STUDY 24

4 MATERIALS AND METHODS 25
4.1 Study design and participants 25
4.2 Ethics 27
4.3 Measurements 28
4.3.1 Menopausal status and age at natural menopause 28
4.3.2 Menopausal symptoms 29
4.3.3 Physical activity 30
4.3.4 Blood-based biomarkers of cardiometabolic health 31
4.3.5 Blood pressure, body composition, and anthropometrics 31
4.3.6 Self-reported background variables 32
4.4 Missing data 32
4.5 Statistical analyses 33
4.5.1 Cross-sectional analyses 33
4.5.2 Longitudinal analyses 33
4.5.3 Variable selection 34

5 RESULTS 35
5.1 Study population 35

5.2 Cardiometabolic health and physical activity around the menopausal transition (*Study I*) 38
5.3 Menopausal symptoms, physical activity, and cardiometabolic health (*Studies II* and *III*) 40
5.4 Predictors of age at natural menopause (*Study IV*) 45

6 DISCUSSION 47
6.1 Cardiometabolic health around menopause 48
6.2 Physical activity and cardiometabolic health 48
6.3 Menopausal symptoms and cardiometabolic health 49
6.4 Physical activity and menopausal symptoms 50
6.5 Prediction of the age at natural menopause 50
6.6 Strengths and limitations 52
6.7 Future directions 53

7 MAIN FINDINGS AND CONCLUSIONS 54

YHTEENVETO (SUMMARY IN FINNISH) 56

REFERENCES 58

APPENDICES 74
SUPPLEMENTARY TABLE 1 Participant characteristics in the last measurement conducted before the last known bleeding period in *Study IV*. 74
SUPPLEMENTARY TABLE 2 Pooled linear mixed-effect model estimates for the association between the number of menopausal symptoms and blood-based biomarker levels for longitudinal study design in *Study II* (n = 298) 75
SUPPLEMENTARY TABLE 3 Pooled linear mixed-effect model estimates for the association between the number of menopausal symptoms with blood pressure, body adiposity, and physical activity levels for longitudinal study design in *Study II* (n = 298). 76
SUPPLEMENTARY TABLE 4 Pooled linear regression estimates for the association of baseline physical activity and body composition with the severity of menopausal symptoms at follow-up measurement in *Study III* for participants that did not report any symptoms at baseline (n = 82). 77

ORIGINAL PAPERS

1 INTRODUCTION

Menopause is a natural part of the aging process and confronted by every woman with functioning ovaries living past middle age. The time around menopause is highlighted by hormonal alterations that induce changes in many biological systems, significantly affecting health and quality of life in women (Monteleone et al., 2018). The importance of menopause-related research is emphasized by the aging populations and increase in life expectancy in Western countries that lead to women spending more than third of their lives after menopause (Kontis et al., 2017).

Increases in the risk of cardiometabolic diseases (CMDs) and menopausal symptoms are two significant public health implications related to menopause. CMD is the leading cause of death worldwide, and its prevalence has been shown to increase in women in middle age (Colpani et al., 2018; Roth et al., 2018). A growing body of research suggests that menopausal alterations in the hormonal milieu are a significant contributor to increased CMD risk in middle age, causing undesirable changes in body adiposity, blood pressure, and lipids (El Khoudary et al., 2020). Menopausal symptoms include a variety of inconveniences that have been shown to significantly impair quality of life in women (Avis et al., 2009; Blumel et al., 2000). These symptoms are particularly distressing because they occur at an age when women tend to have essential roles within the family, at work, and in society (Monteleone et al., 2018). Furthermore, the prevalence of menopausal symptoms has been associated with the increased risk of CMD (Armeni et al., 2023; Muka, Oliver-Williams, Colpani, et al., 2016)

In contrast to menopause, regular physical activity has been shown to promote favorable changes in individual indicators of cardiometabolic health and decrease the overall risk of CMD (Pedersen & Saltin, 2015). However, the effect of physical activity on the changes in cardiometabolic health during the menopausal transition is not well understood. Furthermore, the association between physical activity and menopausal symptoms has been shown to vary based on the type of the symptoms, but overall evidence on the role of physical activity in mitigating menopausal symptoms is inconclusive (Daley et al., 2011; Pettee Gabriel et al., 2015).

One concern that hinders research on the associations between physical activity, cardiometabolic health, and menopausal symptoms across the menopausal transition is the significant interindividual variation in the age when menopause occurs (Schoenaker et al., 2014; Shifren et al., 2014). Consequently, long follow-up times and large-scale studies are needed to include an adequate number of women who encounter menopause during the period of study. This is often not feasible, especially in experimental studies. Therefore, the accurate prediction of the menopausal age would be highly beneficial for future research on menopause-related phenomena. Furthermore, information about the age at natural menopause could be useful in clinical work for family planning and identifying women with increased risk of public health issues associated with menopausal age, such as CMDs, osteoporosis, and cancer (Gold, 2011).

The aim of this dissertation is to investigate the multifaceted associations between physical activity, indicators of cardiometabolic health, and menopausal symptoms in women around the time of menopause. The secondary aim is to identify factors associated with the timing of menopause to create a model to predict age at menopause in middle-aged women.

2 REVIEW OF THE LITERATURE

2.1 Menopause and the menopausal transition

The menopausal transition denotes the shift from the reproductive to the postreproductive phase in a woman's life. It results from the gradual loss of ovarian function commencing with the onset of menstrual irregularities and ending with the final menstrual period (FMP) (Santoro, 2005). The menopausal transition is characterized by the hormonal changes induced by the loss of ovarian function. These changes are highlighted by the decrease in systemic estradiol (E2) and progesterone as well as increase in follicle-stimulating hormone (FSH) and luteinizing hormone levels (Shifren et al., 2014).

Menopause, defined as the FMP, marks the end of the menopausal transition. It is caused by the spontaneous cessation of ovarian function (natural menopause), but it can also result from medical treatments, such as bilateral oophorectomy or chemotherapy (induced menopause). Natural menopause is diagnosed retrospectively after 12 months of amenorrhea (Shifren et al., 2014). The term perimenopause refers to the time of the menopausal transition, but more precisely, it also includes the 12-month period after the menopause (Harlow et al., 2012). However, in this thesis, the terms menopausal transition and perimenopause are used interchangeably.

The different stages of the menopausal transition are commonly described using the Stages of Reproductive Aging Workshop +10 (STRAW +10) guidelines (Harlow et al., 2012), in which the staging is carried out using information on menstrual irregularity and hormonal changes (TABLE 1). A woman's reproductive (premenopausal) phase with a regular menstrual cycle is divided into early, peak, and late stages. The menopausal transition includes early and late perimenopausal stages, which are characterized by menstrual cycle irregularities and elevated FSH levels. Finally, the phase after the FMP consists

of early and late postmenopausal stages, which are highlighted by the absence of menstruation.

TABLE 1 The stages of reproductive aging (modified from Harlow et al., 2012).

Stage		Criteria		Characteristics
		Menstrual cycle	FSH	Menopausal symptoms
Premenopause	Early	Variable to regular		
	Peak	Regular		
	Late	Regular, subtle changes in length	Low, variable	
Perimenopause	Early	≥ 7-day difference in the length of consecutive cycles	Elevated, variable	
	Late	Intervals of amenorrhea ≥ 60 days	Elevated (> 25 IU/L)	Likely VMS
Postmenopause	Early	No menstruation	Elevated, variable	Most likely VMS
	Late	No menstruation	Stabilizes	Symptoms of urogenital atrophy

FSH, follicle-stimulating hormone; VMS, vasomotor symptoms

2.1.1 Age at natural menopause

There is considerable interindividual variation in the age at natural menopause (ANM), but the ANM commonly occurs in women aged 40–58 years (Shifren et al., 2014). Menopause is considered early if it occurs before 45 years and late if it occurs after 55 years (Shifren et al., 2014). In addition to interindividual variation, the ANM varies based on geographical region. Notably, the mean ANM has been reported to vary from 46 to 52 years, with ANM being higher in Western countries than in other regions (Schoenaker et al., 2014). In Finland, the mean ANM is approximately 50 years (Pakarinen et al., 2010).

A significant amount of variation in the ANM is explained by genetic factors (Depmann et al., 2016; Murabito et al., 2005; Ruth et al., 2021). However, lifestyle and socioeconomic factors may also affect the ANM. Especially, smaller body mass index (BMI) (Tao et al., 2015), lower educational level (Schoenaker et al., 2014), and smoking (Parente et al., 2008; Sun et al., 2012) have been associated with an earlier ANM. Furthermore, there is some evidence that lower physical activity, being single, lower parity, and shorter menstrual cycle length associates with an earlier ANM (Gold, 2011; Gold et al., 2001; Schoenaker et al., 2014).

Since menopause is caused by the loss of ovarian function, markers of the ovarian reserve that are not affected by the phase of the menstrual cycle, such as anti-Müllerian hormone (AMH) and antral follicle count are strong predictors the ANM (Finkelstein et al., 2020; C. Kim et al., 2017). The established characteristics

of the menopausal transition, including decline in E2, increase in FSH, and irregular menstrual cycles, also indicate approaching menopause (Greendale et al., 2013). However, E2 and FSH levels vary based on the phase of the menstrual cycle and their trajectories during the menopausal transition are individually distinct, especially with E2, which hampers their use as a predictor of ANM (Sowers et al., 2008; Tepper et al., 2012). The overview of the factors that have been associated with the ANM in previous studies are illustrated in FIGURE 1.

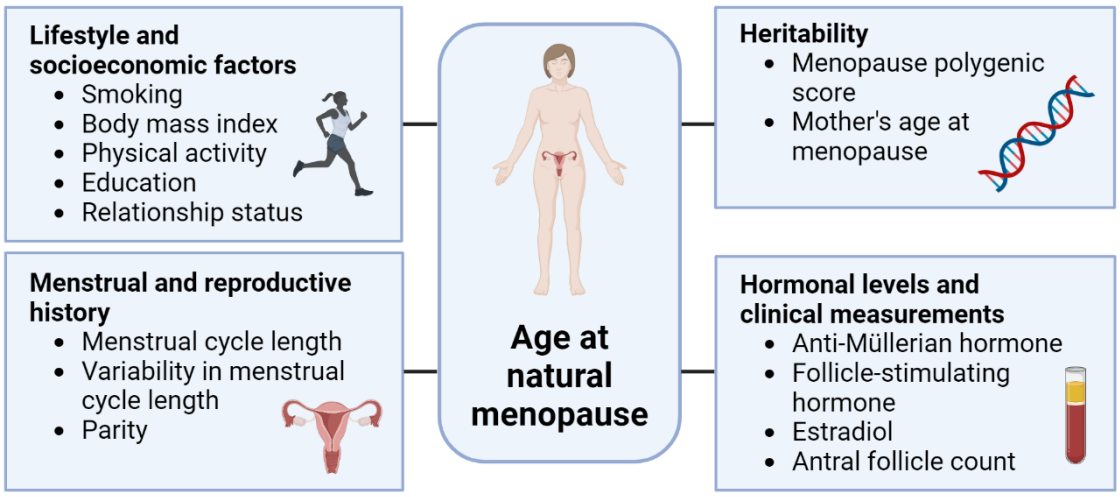

FIGURE 1 Factors associated with the age at natural menopause (Created with BioRender.com).

Menopause marks the beginning of permanent estrogen deficiency that induces bone loss (Sirola et al., 2003) and deterioration in cognition (Russell et al., 2019) and cardiovascular function (Iorga et al., 2017). Consequently, earlier ANM has been associated with increased risk of cardiovascular disease and cardiovascular mortality (Muka, Oliver-Williams, Kunutsor, et al., 2016; Zhu et al., 2019), depression (Georgakis et al., 2016), osteoporosis and fractures (Fistarol et al., 2019; Sullivan et al., 2017), and all-cause mortality (Mondul et al., 2005; Ossewaarde et al., 2005). However, sustained estrogen exposure is a well-established risk factor for various cancers (Liang & Shang, 2013), and therefore later ANM has been associated with increased risk of several cancers, including breast (Collaborative Group on Hormonal Factors in Breast Cancer, 2012; Kelsey et al., 1993), endometrial (Gao et al., 2016), and ovarian cancer (Ossewaarde et al., 2005).

2.1.2 Menopausal symptoms

Menopausal symptoms are the hallmark of the menopausal transition. They are predominantly caused by menopause-related changes in the hormonal milieu, which affect many biological systems (Monteleone et al., 2018). The prevalence and severity of menopausal symptoms increases with the menopausal transition, and up to 85% of women experience them during midlife (Woods & Mitchell,

2005). Menopausal symptoms can significantly impair the quality of life in women and are particularly distressing because they occur at an age when women tend to have essential roles within the family, at work, and in society (Avis et al., 2009; Blümel et al., 2011; Monteleone et al., 2018). Therefore, symptomatic women often seek medical consultation for their symptoms (Guthrie et al., 2003; Williams et al., 2007).

Menopausal symptoms include a variety of complaints that can be classified based on their nature into vasomotor, psychological, urogenital, and somatic symptoms (Laakkonen et al., 2017; Moilanen et al., 2010). Vasomotor symptoms (VMS), such as hot flashes and night sweats, are typically the most frequently reported and bothersome symptom type (Monteleone et al., 2018). VMS can be described as a sudden flare of heat often experienced in the head, neck, and chest area accompanied with sweating, flushing, or trembling (Thurston & Joffe, 2011). The physiology of VMS is not fully understood, but the leading hypothesis is that they are caused by thermoregulatory alterations arising from menopause-related changes in reproductive hormones (Monteleone et al., 2018; Thurston & Joffe, 2011). VMS are most prevalent during the period from 2 years before until about 8 years after menopause, with the median duration of symptoms being slightly over 7 years (Avis et al., 2015; Politi et al., 2008).

Psychological symptoms include a variety of mood disorders, such as anxiety and depression, as well as sleep disruption. The lifetime prevalence of mood disorders is higher in women than in men, but the menopausal transition has been shown to be an especially vulnerable period for their onset in women (Monteleone et al., 2018; Weissman et al., 1996). Notably, postmenopausal women are more likely to report depressed mood and higher levels of anxiety than premenopausal women (Bromberger et al., 2007, 2013; Woods et al., 2008). Furthermore, the increased occurrence of sleep disruption and decline in sleep quality during the menopausal transition has been observed in several studies (Kravitz et al., 2003; Pien et al., 2008). This decline in sleep quality is at least partially explained by the disturbances caused by other menopausal disorders, such as hot flashes and depressive symptoms (Pien et al., 2008).

Decreased estrogen levels contribute to urogenital atrophy, which causes vaginal dryness, dyspareunia, vulvar itching and burning, and urinary incontinence and increases the likelihood of urinary tract infections (Castelo-Branco et al., 2005). Almost half of postmenopausal women report these urogenital symptoms (Parish et al., 2013). Finally, menopause has been associated with the increased prevalence of migraine, headaches, and types of musculoskeletal discomfort, which can be described as somatic menopausal symptoms (Monteleone et al., 2018). These symptoms are also affected by menopausal hormonal changes, and the increase in musculoskeletal symptoms may especially be explained by menopausal estrogen deficiency, which contributes to cartilage and bone degeneration (Lou et al., 2016; Monteleone et al., 2018; Sirola et al., 2003).

2.2 Physical activity

Physical activity is defined as body movement produced by skeletal muscles that results in energy expenditure. Exercise is a subcategory of physical activity, including all planned activities that focus on promoting health and fitness (Caspersen et al., 1985). Consequently, exercise has been shown to improve mood and health-related quality of life (Martin et al., 2009; Mikkelsen et al., 2017). Furthermore, regular physical activity is associated with reduced all-cause mortality, decreased risk of several chronic diseases, and other public health concerns, such as cardiovascular disease, diabetes, cancer, obesity, hypertension, and osteoporosis (Kraus et al., 2019; Warburton et al., 2006).

Physical activity can be characterized using four dimensions—namely, the type or mode, frequency, duration, and intensity of activity (Strath et al., 2013). Of these dimensions, mode determines the specific activity performed, frequency defines the number of activity sessions during a certain time interval, duration is the time of the activity bout, and intensity is the rate of energy expenditure caused by the activity (Strath et al., 2013). Frequency, duration, and intensity determine the amount of energy expenditure caused by physical activity (Caspersen et al., 1985).

Physical activity can be classified based on the domain in which it occurs. Occupational physical activity includes work-related activities, while leisure-time physical activity (LTPA) consists of all activities that are not associated with one's occupation. Domestic and transportation activities are sometimes classified in their own respective domains (Strath et al., 2013), but in this thesis, they are included in LTPA. Interestingly, the health benefits of physical activity have been reported to vary based on the domain, with LTPA being more beneficial than occupational physical activity (Holtermann et al., 2021).

2.2.1 Assessment of physical activity

Physical activity can be assessed by measuring the energy expenditure caused by the activity or by using self-reports and device-based methods to capture the activity level. Indirect calorimetry and doubly labeled water are widely considered the most precise methods for estimating physical activity energy expenditure in the laboratory and in free-living conditions, respectively (Strath et al., 2013). However, these methods are often too costly and time-consuming for large-scale epidemiologic studies.

Self-reported physical activity assessment methods including questionnaires and diaries, are commonly used in epidemiologic studies due to their low cost and ease of implementation (Strath et al., 2013). These methods do not accurately capture low intensity physical activities, such as household chores and occupational activities, and they have limited validity and reliability due to potential response and recall biases (Prince et al., 2008; Shephard, 2003). However, self-reported methods are able to capture how strenuous individual perceives

activity, specific activity dimensions and domains, and past physical activity behaviour (Prince et al., 2008; Warren et al., 2010).

Device-based methods for physical activity assessment include heart rate monitors and motion sensors with accelerometers, which are among the most frequently used devices in population-based studies (Strath et al., 2013; Warren et al., 2010). Accelerometers are used to estimate physical activity by measuring the acceleration of the body parts to which they are attached (Strath et al., 2013). Usually they are attached to the hip, but ankle-, wrist-, and thigh-worn accelerometers can also be used (Warren et al., 2010). For estimating the physical activity level, accelerometer data is traditionally transformed into a count score that represents physical activity intensity using manufacturer-specific closed-source algorithms. However, count scores are highly dependent on the algorithm used, which limits the comparability of the results between studies using different accelerometers (Strath et al., 2013; Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015). Therefore, other methods for processing raw accelerometer data, such as the mean amplitude deviation (MAD) method, have been proposed in recent years (Bakrania et al., 2016; Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). Furthermore, regardless of how the data are processed, accelerometers are often used to assess time spent in different intensities of physical activity using intensity thresholds (Bakrania et al., 2016; Strath et al., 2013; Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015).

Unlike self-reports, accelerometers objectively measure the movement of the body, and thus are able to capture even very light physical activities (Hyvärinen et al., 2020). However, they are only able to capture activities that include the movement of the body parts to which they are attached. Therefore, hip-worn accelerometers, for example, have limited ability to capture upper-body movement or activities such as cycling and rowing. Accelerometers are also often removed before water-based activities such as swimming (Warren et al., 2010). Furthermore, accelerometer-measured physical activity does not account for individual differences in physical activity capacity. Therefore, these measurements are not able to estimate whether a physiological response is evoked by the measured activity without further information about the individual's cardiorespiratory fitness (Fridolfsson et al., 2023). Because both approaches capture different aspects of physical activity, the correlation between self-reported and accelerometer-measured physical activity is low to moderate (Prince et al., 2008).

2.2.2 Physical activity and menopause

Although physical activity has been shown to associate with changes in menstrual cycle (Song et al., 2022; Sternfeld et al., 2002) and decrease in sex hormone levels, including that of E2 (Ennour-Idrissi et al., 2015), few studies have investigated the association between physical activity and ANM. Nonetheless, one meta-analysis of four studies reported a higher level of physical activity to be modestly associated with a later ANM (Schoenaker et al., 2014). However, one large longitudinal study not included in the meta-analysis showed contradictory

results, with higher physical activity being associated with earlier ANM (Gold et al., 2013).

The role of physical activity in the alleviation of menopausal symptoms has been studied widely in both observational and experimental study designs, but the results of these studies were inconclusive (Nguyen et al., 2020; Pettee Gabriel et al., 2015). Observational studies have reported either an indirect or no association between VMS and level of physical activity (Pettee Gabriel et al., 2015). However, exercise and yoga have not been found to be effective treatments for VMS in meta-analyses of randomized controlled trials (RCTs) (Daley et al., 2011). Nonetheless, one more recent RCT found that resistance training intervention decreased the frequency of hot flashes in postmenopausal women more compared to the group with unchanged physical activity (Berin et al., 2019).

The strongest evidence for the positive effect of physical activity on menopausal symptoms has been reported for psychological symptoms. The majority of both observational studies and RCTs have found exercise and physical activity to be associated with better sleep quality as well as fewer and less severe mood disorders (Aibar-Almazán et al., 2019; Pettee Gabriel et al., 2015). The role of physical activity in alleviating urogenital symptoms has been far less studied, and the results of the existing studies are contradictory, with both direct and indirect associations reported between the level of physical activity and urogenital symptoms (Pettee Gabriel et al., 2015). Although the menopausal transition is known to affect body composition, only a few studies with an exercise intervention for the alleviation of menopausal symptoms have included comprehensive assessments of body composition (Ambikairajah, Walsh, Tabatabaei-Jafari, et al., 2019). Therefore, the role of body composition on the association between physical activity and menopausal symptoms is not well known (Aiello et al., 2004).

2.3 Cardiometabolic health

Cardiometabolic health refers to the condition of the cardiovascular system and mechanisms related to cellular and whole-body metabolism. The concept of cardiometabolic health is also closely related to CMDs including a wide range of disorders and medical conditions. Common CMDs with a significant burden of disease are type 2 diabetes and atherosclerotic cardiovascular diseases such as coronary heart disease and stroke (Roth et al., 2018; Sattar et al., 2020). CMD, and cardiovascular disease in particular, is the leading cause of death globally and a major contributor to reduced quality of life (Kyu et al., 2018; Roth et al., 2018).

2.3.1 Assessment of cardiometabolic health

Blood lipid and glucose levels as well as body adiposity are well-established indicators of cardiometabolic health and are known to affect the risk of CMD. For instance, several studies have shown that higher total cholesterol, low-density

lipoprotein cholesterol (LDL-C), and triglyceride levels as well as lower high-density lipoprotein cholesterol (HDL-C) levels contribute to the development of atherosclerotic cardiovascular diseases (Assmann & Gotto, 2004; Ference et al., 2017; Nordestgaard & Varbo, 2014; Peters et al., 2016). Fasting blood glucose is an established biomarker of glucose metabolism, and it is used as a diagnostic criterion for type 2 diabetes (Ely et al., 2017). Furthermore, higher blood glucose level has been shown to associate moderately with increased risk of atherosclerotic cardiovascular disease (Emerging Risk Factors Collaboration, 2010). Additionally, increased total body adiposity, and especially visceral adiposity, is known to contribute to chronic systemic inflammation, which is a significant risk factor for the development of CMD (Berg & Scherer, 2005).

Consequently, one established method to evaluate cardiometabolic health and the risk of CMD is the diagnostic criteria of metabolic syndrome (MetS), which has five different components (Ford et al., 2008; Malik et al., 2004). These include elevated waist circumference, blood pressure, triglyceride levels, and fasting glucose as well as decreased HDL-C levels (Grundy et al., 2004).

2.3.2 Cardiometabolic health and menopause

The risk of CMD has been shown to increase in women after the menopausal transition (Colpani et al., 2018; Janssen et al., 2008). This may be caused by changes in the hormonal milieu during the menopausal transition that accelerate the aging-related deterioration of several CMD risk factors (Carr, 2003; El Khoudary et al., 2020). For instance, the accumulation of visceral adipose tissue (Ambikairajah, Walsh, Tabatabaei-Jafari, et al., 2019) and increased total cholesterol, LDL-C, HDL-C, blood glucose, and blood pressure levels have been reported during the menopausal transition (Ambikairajah, Walsh, & Cherbuin, 2019; Matthews et al., 2009; Otsuki et al., 2007). Furthermore, menopause may induce a decline in the antiatherogenic function of HDL-C (El Khoudary et al., 2016).

Menopausal symptoms have also been associated with increased risk of CMD (Armeni et al., 2023; Carson & Thurston, 2023; Muka, Oliver-Williams, Colpani, et al., 2016). This association is mainly explained by an unfavorable cardiometabolic risk factor profile, such as increased blood pressure, cholesterol levels, and BMI in symptomatic women (Franco et al., 2015; Muka, Oliver-Williams, Colpani, et al., 2016).

2.3.3 Cardiometabolic health and physical activity

There is overwhelming evidence from observational studies that regular physical activity is associated with decreased CMD risk and CMD mortality (Garcia et al., 2023; Kraus et al., 2019; Wahid et al., 2016). Furthermore, RCTs have shown that exercise has a beneficial effect on individual indicators of metabolic health by inducing weight loss and beneficial changes in body composition (Bellicha et al., 2021), lowering systolic and diastolic blood pressure (Cornelissen & Smart, 2013), and increasing HDL-C levels as well as decreasing triglyceride and LDL-C levels

(Halbert et al., 1999; Kelley & Kelley, 2009). Consequently, aerobic exercise in particular has been shown to improve all five components of MetS in participants with MetS (Wewege et al., 2018). Therefore, physical activity is considered to be an effective treatment for MetS as well as elevated blood pressure, dyslipidemia, and obesity (Barone Gibbs et al., 2021; K.-B. Kim et al., 2019; Oppert et al., 2021).

Research on the effect of exercise on cardiometabolic health around menopause is very scarce. Only a few exercise interventions have been conducted in middle-aged women that have accounted for the differences in menopausal status (Ruiz-Rios & Maldonado-Martin, 2022). However, the results from these few experimental and observational studies have indicated that physical activity may reduce the risk of CMD (Ruiz-Rios & Maldonado-Martin, 2022) and associate with favorable levels in blood-based indicators of cardiometabolic health (Karvinen et al., 2019) and body adiposity (Sternfeld et al., 2005) in middle-aged women.

3 PURPOSE OF THE STUDY

The primary purpose of this dissertation is to investigate the associations between physical activity, cardiometabolic health, and menopausal symptoms in women during the menopausal transition. The secondary aim is to identify factors associated with the timing of natural menopause to create a model for predicting the ANM using data-driven approach. The study is conducted using longitudinal data from a Finnish cohort of middle-aged women.

The research questions for this dissertation are as follows:

1. Do aging-related changes in blood pressure, serum lipids, and glucose accelerate around the menopause and can physical activity mitigate the changes in these indicators of metabolic health during the menopausal transition? (*Study I*)

2. Are menopausal symptoms associated with the indicators of metabolic health factors and can the prevalence of these symptoms be used to predict changes in individual metabolic health indicator levels in middle-aged women? (*Study II*)

3. Are body composition and physical activity associated with the severity of menopausal symptoms and does body composition modify the association between physical activity and menopausal symptoms? (*Study III*)

4. What factors are associated with the timing of the natural menopause and can they be used to predict the ANM in middle-aged women? (*Study IV*)

4 MATERIALS AND METHODS

Four substudies were conducted to achieve the aims of this dissertation. *Studies I, II,* and *III* focused on achieving the primary aim of the study by investigating the associations between physical activity, cardiometabolic health, and menopausal symptoms during the menopausal transition. The analytical framework of these studies is illustrated in FIGURE 2. The secondary purpose of this dissertation was addressed in *study IV*, which focused on investigating the factors associated with the timing of natural menopause in middle-aged women.

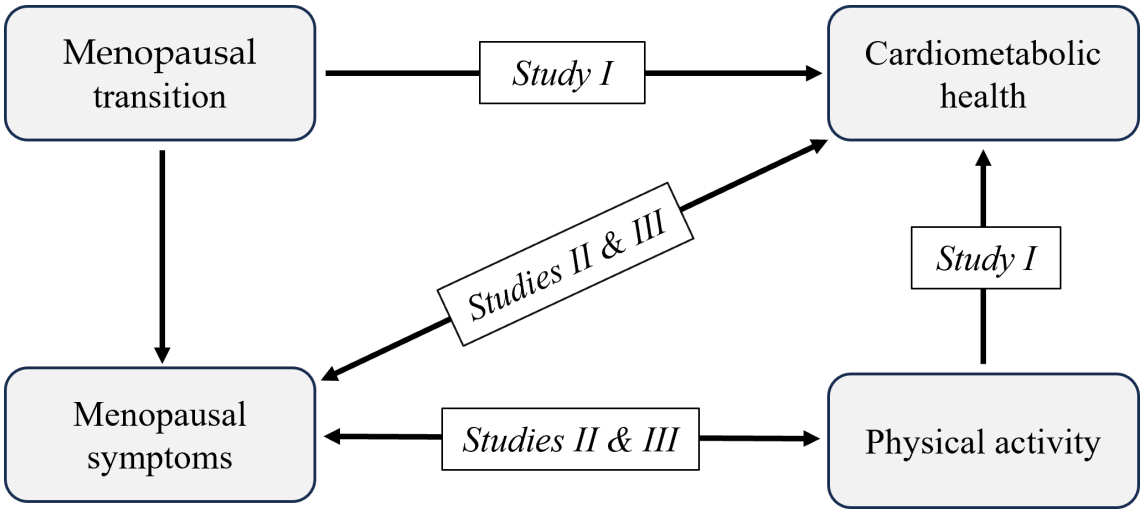

FIGURE 2 The analytical framework of *Studies I, II,* and *III.*

4.1 Study design and participants

This dissertation was conducted using data from the Estrogenic Regulation of Muscle Apoptosis (ERMA) cohort study and its follow-up Esstudiestrogen, MicroRNAs, and the Risk of Metabolic Dysfunction (EsmiRs) study (Laakkonen et al., 2022). In the ERMA study, 6878 randomly selected women aged 47–55 years

living in the city of Jyväskylä and neighboring municipalities were invited to participate in the baseline measurements. This sample was drawn from the Population Information System, and it included 82% of the total age cohort (Kovanen et al., 2018).

Of the women invited, 3649 did not respond and 165 were not willing to participate (Kovanen et al., 2018). Consequently, 3064 women returned the prequestionnaire. Based on the prequestionnaire responses, 334 women did not consent to continue and 992 were excluded. The exclusion criteria for the ERMA baseline measurements were conditions, diseases, and use of medications affecting ovarian and muscle function (e.g., bilateral oophorectomy, use of estrogen-containing preparations, and current pregnancy or lactation) as well as severe obesity (BMI ≥ 35 kg/m^2). Finally, 1627 participants were invited to the first ERMA laboratory visit, and after five women were excluded and 229 chose not to continue, the number of participants in the ERMA baseline measurement was 1393 (FIGURE 3).

The ERMA baseline measurement included menopausal status assignments. Participants were divided into pre-, peri-, and postmenopausal groups, with the respective number of participants in each group being 389, 474, and 530 (TABLE 4). After the menopausal status assignments, participants with conditions or use of medications affecting daily mental or physical function and systemic hormone or inflammatory status were excluded. These exclusion criteria included insulin-treated diabetes, continuous cortisone medication, and cancer diagnosed less than 5 years previously. After the exclusions, all the perimenopausal participants (n = 381) were invited to participate in the ERMA follow-up study (FIGURE 3), for which they kept a menstrual diary and attended the follow-up measurements every 3–6 months until they were postmenopausal.

All ERMA participants with measurements conducted before the last known menstrual period derived from their menstrual bleeding diaries were included in *Study IV* (n = 279). The ANM was defined as the first day of the last known menstrual period for participants who had gone through the ERMA follow-up period and had a complete bleeding diary (n = 105, number of valid measurements = 296). Furthermore, the last known bleeding period was determined for the rest of the pre- and perimenopausal ERMA participants. To ensure that no postmenopausal measurements were included in the analyses, all measurements conducted after the last known menstrual period were excluded. Consequently, the full dataset in *Study IV* included 687 valid measurements from 279 participants. The number of measurements for each participant varied from 1 to 9, with the mean time (± standard deviation) between measurements being 163 ± 44 days. The mean total follow-up time was 0.86 ± 0.97 years, and it varied from 0.0 to 3.7 years.

EsmiRs was a 4-year follow-up study for women who participated in the ERMA baseline measurements. After 582 women did not consent to be contacted, 289 did not respond to the invitation, and 28 were not willing to participate the follow-up study, 494 of the 1393 participants from the ERMA baseline measurements participated in the EsmiRs baseline questionnaire. Subsequently,

based on the questionnaire responses, 56 participants were excluded, 25 did not consent, and 16 were not willing to continue. Furthermore, 99 participants could not be measured due to the early termination of measurements caused by restrictions related to the COVID-19 pandemic. Participants were excluded from the EsmiRs study if they had insulin-treated diabetes, severe cardiovascular dysfunction, cancer diagnosed after the ERMA baseline measurements, or more than 7 years from menopause, based on the self-reports. Consequently, there were 298 participants in the EsmiRs follow-up measurements (FIGURE 3). The time between the ERMA baseline and the EsmiRs measurements varied from 3.6 to 4.7 years, with a mean value of 3.8 years.

Recruitment for the ERMA study was initiated in 2014, and the baseline and follow-up measurements were conducted from 2014 to 2018. The recruitment for the EsmiRs study started in 2018. The EsmiRs laboratory visits were initiated at the beginning of 2019, and they were discontinued on March 16, 2020, due to restrictions caused by the COVID-19 pandemic.

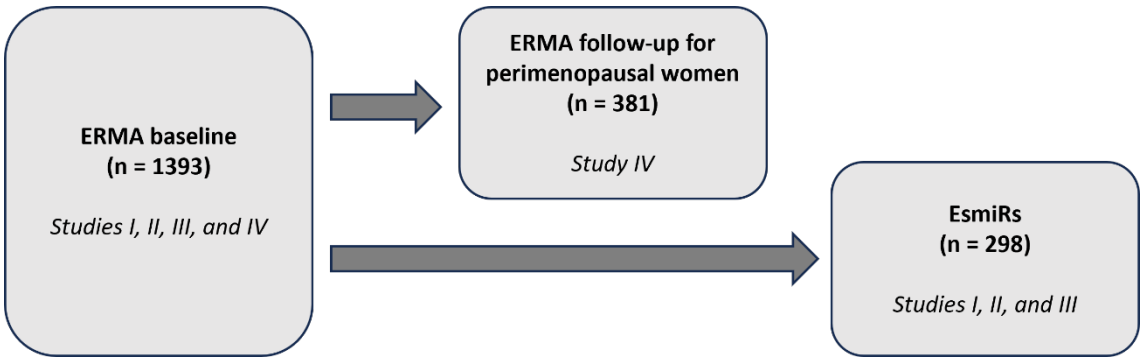

FIGURE 3 The data sets of the study and how they were utilized in individual substudies.

4.2 Ethics

The ERMA and EsmiRs studies were conducted in accordance with the Declaration of Helsinki and they were approved by the Ethics Committee of the Central Finland Health Care District (ERMA 8U/2014 and EsmiRs 9U/2018). All participants provided written informed consent. The safety and anonymity of the participants were protected in all phases of the studies.

4.3 Measurements

4.3.1 Menopausal status and age at natural menopause

Blood sampling after overnight fasting was collected from the antecubital vein between 7 a.m. and 10 a.m. The sampling was scheduled for the first 5 days of the menstrual cycle for the participants with predictable menstrual cycles. Serum concentrations of E2 and FSH were measured with IMMULITE® XPi (Siemens Healthineers, Erlangen, Germany) from serum samples stored at -80 °C. The lower limits of quantification for E2 and FSH immunoassay kits were 0.073 nmol/L and 0.1 IU/L, respectively.

All participants in the ERMA study were instructed to keep menstrual bleeding diaries at least 12 weeks prior to the baseline measurements and, if invited to the follow-up measurements, throughout the follow-up period. Participants reported their menstrual daily bleeding status as bleeding, spotting, or no bleeding. One menstrual bleeding period was defined as at least 1 day of bleeding or three or more consecutive days of spotting. Spotting days preceding or following bleeding days were treated as bleeding days. Furthermore, a single day with no bleeding surrounded by bleeding days was treated as a bleeding day and the surrounding bleeding days were merged into one continuous bleeding period (Harlow et al., 2000).

Menopausal status was determined using slightly modified STRAW +10 guidelines based on the serum FSH levels and menstrual bleeding diaries (Harlow et al., 2012). The criteria for the menopausal group assignment are shown in TABLE 2. In the ERMA follow-up study, participants were deemed as postmenopausal and invited to the final follow-up measurements after two consequently high FSH values and approximately six months of amenorrhea. For women who participated in the ERMA follow-up measurements, were postmenopausal at the final measurement, and had complete menstrual bleeding diaries from the follow-up period, the age at the beginning of the last reported bleeding period was used as the ANM in *Study IV*.

In *Study I*, which was a longitudinal study with follow-up from the ERMA baseline to the EsmiRs measurements, participants were assigned into three groups based on how their menopausal status changed during the follow-up period. The PRE-POST group (n = 149) included participants who experienced the menopause during the follow-up period. In other words, they were categorized as pre- or perimenopausal at the ERMA baseline and postmenopausal at the EsmiRs measurement. Furthermore, participants who were pre- or perimenopausal (n = 56) or postmenopausal (n = 93) at both measurements were assigned to the PRE-PRE and POST-POST groups, respectively.

TABLE 2 Criteria for the menopausal group assignment (modified from Kovanen et al., 2018).

Group	If information about menstrual cycle was available	If information about menstrual cycle was not available
Premenopausal	FSH < 9.5 IU/L or FSH < 17 IU/L and regular menstrual cycle	FSH < 15 IU/L
Perimenopausal	FSH 17–30 IU/L or FSH < 17 IU/L and irregular menstrual cycle (early) or FSH > 30 IU/L and occasional menstrual bleeding during past 3 months (late)	FSH 15–39 IU/L
Postmenopausal	FSH > 30 IU/L and no menstrual bleeding during past 6 months or FSH > 39 IU/L and no menstrual bleeding during past 3 months or very high FSH (>130 IU/L) even if occasional bleeding still occurs	FSH > 39 IU/L

FSH, follicle-stimulating hormone

4.3.2 Menopausal symptoms

Menopausal symptoms were assessed using dichotomous structured questionnaires and the Menopause Rating Scale (MRS) (TABLE 3). In the dichotomous questionnaire, participants were asked to report if they had experienced any of the following predetermined symptoms related to menopause: sweating, hot flashes, sleeplessness, headache, aching joints, tiredness, mood swings, vaginal symptoms, urinary tract symptoms, or lack of sexual desire. The questionnaire also included the option to describe a maximum of three additional symptoms (Kovanen et al., 2018). In *Studies II* and *IV*, all reported symptoms were classified into four categories—namely, vasomotor, psychological, somatic or pain, or urogenital (Laakkonen et al., 2017). VMS included sweating and hot flushes and additionally reported cold flushes, heart palpations, and coldness. Psychological symptoms were sleeplessness, tiredness, and mood swings and additional memory problems, irritability, inability to concentrate, and weepiness. Somatic or pain symptoms were headache and aching joints as well as additional stomach pain, migraine, hip pain, muscle pain, breast pain, dizziness, swelling, and weakness. Finally, vaginal and urinary tract symptoms and lack of sexual desire as well as additional vaginal infection, urinary tract infection, and vaginal dryness were defined as urogenital symptoms. Based on these symptom categories, the prevalence of the symptoms (*Study IV*) and number of symptoms in each category (*Study II*) were utilized. The number of symptoms included the sum of all predetermined symptoms with one

additional symptom for participants who reported one or more additional symptom in that category. Thus, the maximum number of symptoms in each category was three (VMS and somatic or pain symptoms) or four (psychological and urogenital symptoms). The total number of symptoms was defined as the sum of vasomotor, psychological, somatic or pain, and urogenital symptoms.

The MRS used was a standardized and validated measure of health-related quality of life and severity of menopausal symptoms (Potthoff et al., 2000; Schneider et al., 2000), which is used to assess the five-level perceived severity (none, mild, moderate, severe, very severe) of 11 different symptom categories. The individual score of each symptom category varies from 0 (none) to 4 (very severe), and the total symptom severity score is the sum of individual symptom category scores. The three subscales of the MRS (with their respective symptom categories) are somato-vegetative (hot flashes and sweating, heart discomfort, sleep problems, join and muscular discomfort), psychological (depressive mood, irritability, anxiety, physical/mental exhaustion), and urogenital (sexual problems, bladder problems, vaginal dryness).

4.3.3 Physical activity

Physical activity was assessed with accelerometers and self-reports (TABLE 3). For accelerometer-measured physical activity, participants were instructed to wear a device (Actigraph GT3X or wGT3X, Actigraph, Pensacola, FL, USA) on their right hip for seven consecutive days during waking hours, except for water-based activities. Data were collected at a frequency of 60 Hz. Both MAD-based (*Study I, II,* and *III*) and count-based (*Study IV*) variables have been used in this dissertation. To calculate total counts and count-based moderate-to-vigorous physical activity (MVPA), the raw accelerometer data were filtered and converted into 60-second epochs using ActiLife software. Total counts and MVPA were defined as the daily mean of total counts and time spent in moderate-to-vigorous activities normalized to 16-hour wearing time, respectively (Hyvärinen et al., 2020). The triaxial vector magnitude cut-off point of 6166 counts per minute for moderate-to-vigorous activities was used (Laakkonen et al., 2017; Sasaki et al., 2011). For MAD-based physical activity, the Euclidian norm of the resultant acceleration was computed for each time point, MAD values were computed for non-overlapping 5-second epochs, and physical activity level was assessed as the mean value of the 5-second MAD values (Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015). For count- and MAD-based analyses, non-wear time was identified as all continuous periods longer than 60 minutes with zero counts or MAD values continuously less than 0.001 gravitational acceleration on Earth, respectively (Migueles et al., 2017). All measurements with a minimum of 3 days with a wear time more than 10 hours were regarded as valid.

Furthermore, two different self-reported measures of physical activity were used. In the single seven-level scale question, participants chose the statement from the predetermined alternatives that best described their current physical activity level. Alternatives ranged from not moving more than what is

necessary for daily activities to participation in competitive sports (Hirvensalo et al., 1998; Hyvärinen et al., 2020). The five-item questionnaire included questions about the average frequency, intensity, and duration of LTPA as well as the average duration of commuting activity. Based on the responses to these four questions, a continuous variable of MET-hours per day for LTPA was calculated (Kujala et al., 1998).

TABLE 3 Measurements conducted in each stage of the ERMA and EsmiRs studies.

	ERMA baseline	ERMA follow-up	EsmiRs
Bleeding diaries & blood samples			
Bleeding diaries	X	X	
Menopausal status	X	X	X
Blood-based biomarkers	X	X	X
Menopausal symptoms			
Dichotomous questionnaire	X	X	
Menopause Rating Scale			X
Physical activity			
Single seven-level scale question	X	X	X
Five-item questionnaire	X	X	X
Accelerometer	X	X	X
Body composition			
Dual-energy X-ray absorptiometry	X	X[a]	X
Bioelectrical impedance analysis	X	X	X
Anthropometrics & blood pressure			
Anthropometrics	X	X	X
Blood pressure	X	X	X
Self-reported background variables			
Lifestyle	X	X	X
Socioeconomic status	X	X	X
Gynecological history	X	X	X
Use of medications	X	X	X

[a] Measured only in the last ERMA follow-up measurement.

4.3.4 Blood-based biomarkers of cardiometabolic health

Total cholesterol, LDL-C, HDL-C, triglycerides, and glucose were measured using KONELAB 20XTi (Thermo Fischer Scientific, Vantaa, Finland) from all serum samples collected during menopausal status assignments (TABLE 3).

4.3.5 Blood pressure, body composition, and anthropometrics

Blood pressure, body composition, and anthropometrics were measured after overnight fasting (TABLE 3). Blood pressure was measured twice in a sitting position after a 10-minute rest using Omron M6 Comfort (Omron Healthcare, Kyoto, Japan), and the mean value of two measurements was used for systolic (SBP) and diastolic blood pressure (DBP). Waist circumference was measured from the middle of the superior iliac spine and the lower rib margin and hip

circumference at the level of greater trochanters (Snijder et al., 2003). Body mass and height were measured with standard procedures. BMI was computed by dividing the body mass with squared body height. Body composition was measured with bioelectrical impedance analysis (BIA) using InBody 720 (Biospace, Seoul, Korea) and with dual-energy X-ray absorptiometry (DXA) using Lunar Prodigy (GE Healthcare, Chicago, IL, USA).

4.3.6 Self-reported background variables

Lifestyle, socioeconomic status, and gynecological history were assessed using self-reported questionnaires. Responses were used to assess alcohol consumption in measures per week and current smoking status as non-smoker or smoker (*Studies I, II,* and *III*) or never, former, or current smoker (*Study IV*). Diet was assessed using the diet quality score, which was computed based on a food-frequency questionnaire (Juppi et al., 2020). Furthermore, participants reported their education (primary/secondary or tertiary), relationship status (single or in a relationship), physical workload (light, moderate, heavy, or very heavy), age at menarche, parity, and number of pregnancies.

Participants reported their use of regular prescription medications, which were categorized using the Anatomical Therapeutic Chemical (ATC) classification. Participants were categorized separately as nonuser or user of preparations affecting blood pressure (ATC C02–05 and C07–09) and serum lipids (ATC C10). Furthermore, participants were classified based on their use of hormonal contraception (never or former user) in *Study IV* and based on their use of hormonal preparations in *Studies I, II,* and *III* (nonuser, estrogen user, progestogen user, or combined estrogen and progestogen user) according to the self-reported questionnaire responses.

4.4 Missing data

Missing data in ERMA and EsmiRs data sets occurred due to invalid or missing measurements and unclear or incomplete questionnaire responses. The percentage of total missing covariate records across the individual substudies in this dissertation varied from 5% to 16%. Missing data were assumed to occur at random (Seaman et al., 2013). Therefore, multiple imputation with 50 imputed data sets and 50 iterations for chained equations were used in all substudies to reduce bias caused by the missing data. For longitudinal data, variables measured at the same time point as well as the value of the target variable in the previous and following measurements, if available, were used for the imputation of each variable. In the studies where the number of participants differed between measurements (*Studies II* and *IV*), imputation was carried out recursively one measurement at a time. That is, the imputed values of the previous measurement were utilized for the imputation of the current measurement. Passive imputation was used for the imputation of the derived

variables. The parameters of substantive interest were estimated separately in each imputed data set and combined using Rubin's rules (Rubin, 1987). Multiple imputation and pooling of the model estimates were carried out in R using the default settings of the *mice* package (R Core Team, 2023; van Buuren & Groothuis-Oudshoorn, 2011).

4.5 Statistical analyses

4.5.1 Cross-sectional analyses

In the cross-sectional study designs (*Studies II* and *III*), the associations of interest were studied with simple and multiple linear regression models. Residual plots, Q–Q plots, and correlation analysis were used for testing the model assumptions. All analyses were carried out in R using base R functions (R Core Team, 2023), unless otherwise specified. The predictive performance of the models constructed in *Study IV* was studied using leave-one-out cross-validation and by comparing the model predictions with the observed values using mean absolute errors (MAE).

4.5.2 Longitudinal analyses

Linear and mixed-effect models with random intercept were used to study the associations of interest in the longitudinal studies (*Studies I* and *II*). The main effects of the variable of interest, time (baseline = 0, follow-up = 1), and confounders were included in the models. Furthermore, the interaction of time and the variable of interest was included in the models to study the association between the variable of interest and changes in the outcome variable during the follow-up. The model assumptions were tested using residual plots, Q–Q plots, and correlation analysis. Analysis was carried out in R using the *nlme* package (Pinheiro et al., 2021).

Cox proportional hazards regression models with time-varying covariates (Fisher & Lin, 1999) were used to predict ANM in the longitudinal ERMA data set with censored data (*Study IV*). Age was used as the timescale, and the proportional hazards assumption of the models was tested using Schoenfeld residuals. The predictive ability of the models was quantified using *c*-index, which is the probability of concordance between predicted and observed survival. This varies from 0.5 to 1, with $c = 0.5$ for random predictions and $c = 1$ for a perfectly discriminating model (Harrell, 2015, p. 505). Median survival time was used as the model prediction for ANM. Analysis was carried out using the *rms* (Harrell, 2019) and *survival* (Therneau & Grambsch, 2000) packages in R.

4.5.3 Variable selection

For the development of the prediction model in *Study IV*, the set of candidate predictors was identified based on the literature and the potential predictive value of the variables. Two different sets of candidate predictors were used to construct two separate prediction models. The first set of predictors included all 32 candidate predictors identified based on the literature, while the second set included a subset of 16 self-reported or otherwise easy-to-access predictors. The final model predictors were selected using least absolute shrinkage and selection operator (lasso) regression (Tibshirani, 1996). The number of predictors in the models was limited by increasing the tuning parameter lambda based on the average requirement, for which the maximum number of predictors in the model should not exceed the effective sample size divided by 15 (Harrell, 2015, p. 72). Analysis was conducted in R using the *penalized* package (Goeman, 2010).

5 RESULTS

5.1 Study population

Full ERMA baseline data (*n* = 1393) was utilized in *Study II*. The mean age of the participants at baseline was 51.3 years (TABLE 4). The participants were pre-, peri-, and postmenopausal, with the respective share of each menopausal group being 28%, 34%, and 38%, respectively. On average, the participants were slightly overweight, with mean BMI of 25.5 kg/m^2, and had slightly elevated SBP (132 mmHg), DBP (84 mmHg), total cholesterol (5.3 mmol/L), and LDL-C (3.1 mmol/L). VMS were the most frequently reported menopausal symptoms, with 59% of the participants reporting at least one (TABLE 5). At least one psychological, somatic or pain, or urogenital symptom was reported by 50%, 24%, and 35 % of the participants, respectively. The ERMA subsample utilized in *Study IV* included participants who reported at least one bleeding period after measurement (*n* = 279). Their mean age at the ERMA baseline was 51.2 years and the mean BMI 25.8 kg/m^2. The characteristics for the participants in *Study IV* are listed in detail in Supplementary Table 1.

ERMA baseline and EsmiRs follow-up data were used in *Studies I, II*, and *III* for all EsmiRs participants (*n* = 298). Their mean age at the ERMA baseline measurements was 51.3 years, and it was 55.1 years in the EsmiRs measurements (TABLE 4). At baseline, the percentages of the participants in the pre-, peri, and postmenopausal groups were 34, 35, and 31, respectively. After the follow-up period, most participants (81%) were postmenopausal, with only 5% and 14% being pre- or perimenopausal, respectively. Of all the EsmiRs participants, 149 experienced menopause during the follow-up period. In other words, they were categorized as pre- or perimenopausal at the ERMA baseline and postmenopausal at the EsmiRs measurement. Furthermore, 56 participants were peri- or- postmenopausal and 93 postmenopausal in both measurements. In the EsmiRs measurements, the participants reported a total mean MRS score of 8.6,

with the most severe symptoms being reported in the somato-vegetative subscale (TABLE 5).

TABLE 4 Participant background characteristics in the ERMA and EsmiRs studies.

		EsmiRs participants	
Measurement	ERMA baseline (n = 1393)	ERMA baseline (n = 298)	EsmiRs (n = 298)
Age [year]	51.3 ± 2.1	51.3 ± 1.8	55.1 ± 1.8
Menopausal status[a]			
Pre	28 (389)	34 (100)	5 (15)
Peri	34 (474)	35 (105)	14 (2)
Post	38 (530)	31 (93)	81 (241)
Estradiol [nmol/L]	0.34 ± 0.41	0.38 ± 0.53	0.26 ± 0.28
Follicle-stimulating hormone [IU/L]	44.0 ± 38.6	39.9 ± 37.1	69.5 ± 37.5
Body height [m]	1.66 ± 0.06	1.66 ± 0.06	1.66 ± 0.06
Total body mass [kg]	69.7 ± 10.9	69.5 ± 10.8	70.9 ± 11.5
Body mass index [kg/m^2]	25.5 ± 3.7	25.3 ± 3.7	25.8 ± 4.1
Alcohol consumption [portions/week]	3.82 ± 3.75	3.73 ± 3.92	3.24 ± 3.43
Diet quality score	5.79 ± 2.21	5.87 ± 2.45	5.85 ± 2.26
Smoking[a]			
Nonsmoker	93 (1014)	95 (262)	94 (280)
Smoker	7 (78)	5 (13)	6 (18)
Education[a]			
Primary or secondary	59 (643)	56 (155)	55 (165)
Tertiary	41 (455)	44 (121)	45 (133)
Use of hormonal preparations[a]			
Nonuser	61 (676)	62 (186)	60 (180)
Progestogen	39 (426)	38 (112)	19 (56)
Estrogen	0 (0)	0 (0)	3 (10)
Progestogen + Estrogen	0 (0)	0 (0)	18 (52)
Use of antihypertensives[a]			
Nonuser	83 (906)	86 (238	78 (233)
User	17 (192)	14 (38)	22 (65)
Use of lipid-modifying agents[a]			
Nonuser	96 (1055)	97 (267)	92 (274)
User	4 (43)	3 (9)	8 (24)

Data are mean ± standard deviation unless otherwise specified. [a] Data are % (n)

TABLE 5 Participant characteristics in menopausal symptoms, cardiometabolic health, and physical activity.

		EsmiRs participants	
Measurement	ERMA baseline (*n* = 1393)	ERMA baseline (*n* = 298)	EsmiRs (*n* = 298)
Menopausal symptoms			
MRS total symptoms	-	-	8.60 ± 5.00
MRS somato-vegetative symptoms	-	-	3.90 ± 2.18
MRS psychological symptoms	-	-	2.53 ± 2.40
MRS urogenital symptoms	-	-	2.15 ± 1.89
Number of vasomotor symptoms[a]			
No symptoms	41 (454)	49 (135)	-
2 or more symptoms	29 (313)	23 (64)	-
Number of psychological symptoms[a]			
No symptoms	50 (549)	61 (167)	-
2 or more symptoms	28 (305)	22 (61)	-
Number of somatic or pain symptoms[a]			
No symptoms	76 (835)	79 (219)	-
2 or more symptoms	5 (53)	4 (12)	-
Number of urogenital symptoms[a]			
No symptoms	65 (713)	69 (191)	-
2 or more symptoms	15 (167)	12 (33)	-
Indicators of cardiometabolic health			
Total cholesterol [mmol/L]	5.30 ± 0.91	5.23 ± 0.91	5.67 ± 1.00
Low-density lipoprotein cholesterol [mmol/L]	3.05 ± 0.80	3.05 ± 0.80	3.41 ± 0.88
High-density lipoprotein cholesterol [mmol/L]	1.72 ± 0.46	1.72 ± 0.47	1.91 ± 0.50
Glucose [mmol/L]	5.28 ± 0.84	5.15 ± 0.45	5.16 ± 0.62
Triglycerides [mmol/L]	1.09 ± 0.72	1.08 ± 0.61	1.27 ± 0.73
Systolic blood pressure [mmHg]	132 ± 17	132 ± 16	133 ± 18
Diastolic blood pressure [mmHg]	84 ± 10	84 ± 9	82 ± 10
Waist circumference	84 ± 10	83 ± 10	84 ± 10
Waist-to-hip ratio	0.83 ± 0.07	0.83 ± 0.06	0.84 ± 0.06
DXA body fat mass [kg]	25.0 ± 8.5	24.2 ± 8.4	25.9 ± 9.0
DXA lean body mass [kg]	42.1 ± 4.4	42.5 ± 4.1	42.2 ± 4.2
DXA fat percentage [%]	36.4 ± 7.7	35.4 ± 7.6	37.1 ± 7.7
DXA android fat mass	2.24 ± 0.97	2.24 ± 1.01	2.39 ± 1.01
Physical activity			
Mean MAD [mg]	29.2 ± 9.2	30.2 ± 10.0	28.3 ± 8.6
Counts $\times 10^5$	64.5 ± 18.5	66.3 ± 19.7	54.1 ± 23.8
Moderate-to-vigorous [min/day]	52.5 ± 27.4	55.5 ± 29.1	50.3 ± 28.4
Five-item questionnaire [MET-h/day]	4.52 ± 3.91	5.15 ± 4.38	4.91 ± 3.93

Data are mean ± standard deviation unless otherwise specified. MRS, Menopause Rating Scale; DXA, dual-energy X-ray absorptiometry; MAD, mean amplitude deviation; [a] Data are % (n)

5.2 Cardiometabolic health and physical activity around the menopausal transition (*Study I*)

Participants who were already postmenopausal at baseline (POST-POST group) had higher total cholesterol (B = 0.33 mmol/L, 95% CI [0.08, 0.59]) and HDL-C levels (B = 0.18 mmol/L, 95% CI [0.05, 0.31]) than participants who had menopause during the follow-up period from ERMA baseline to EsmiRs (PRE-POST group). The levels of all blood-based biomarkers (TABLE 6) and systolic blood pressure (TABLE 7) increased during the follow-up in the PRE-POST group. The increases in blood-based biomarkers tended to be smaller in participants who did not have menopause during the follow-up period (PRE-PRE and POST-POST groups).

In the full sample, participants with higher physical activity level (Mean MAD [10 mg]) had lower LDL-C (B = -0.11 mmol/L, 95% CI [-0.21, -0.01]) and higher HDL-C levels (B = 0.06 mmol/L, 95% CI [0.01, 0.11]). Physical activity level was not associated with other CMD risk factors during the follow-up. Furthermore, change in physical activity was not associated with a change in any CMD risk factors, except for SBP. Consequently, participants with a higher physical activity level had a smaller increase in SBP (B = -2.40 mmHg, 95% CI [-4.34, -0.46]) during the follow-up.

TABLE 6 Pooled linear mixed-effect model estimates for blood-based biomarkers of cardiometabolic health in *Study I* (*n* = 298).

	Total cholesterol [mmol/L]		**Low-density lipoprotein cholesterol [mmol/L]**		**High-density lipoprotein cholesterol [mmol/L]**		**Glucose [mmol/L]**		**Triglycerides [mmol/L]**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Main effects										
Intercept (PRE-POST)	5.48***	[5.12, 5.83]	3.33***	[3.02, 3.62]	1.54***	[1.38, 1.72]	5.17***	[4.97, 5.38]	1.24***	[0.99, 1.48]
Menopausal group										
PRE-POST (ref.)	–		–		–		–		–	
PRE-PRE	0.03	[-0.27, 0.33]	0.05	[-0.22, 0.32]	-0.05	[-0.20, 0.10]	0.04	[-0.13, 0.22]	0.02	[-0.19, 0.24]
POST-POST	0.33*	[0.09, 0.59]	0.21	[-0.02, 0.43]	0.18**	[0.05, 0.31]	0.06	[-0.08, 0.20]	0.05	[-0.13, 0.22]
PA, Mean MAD [10 mg]	-0.10	[-0.21, 0.01]	-0.11*	[-0.21, -0.01]	0.06*	[0.01, 0.11]	-0.02	[-0.08, 0.04]	-0.06	[-0.13, 0.01]
Time (PRE-POST)	0.45*	[0.07, 0.84]	0.40*	[0.06, 0.74]	0.35*	[0.18, 0.52]	0.32**	[0.08, 0.55]	0.28*	[0.03, 0.52]
Interactions			-		-					
Time × Menopausal group										
Time × PRE-POST (ref.)	–		–		–		–		–	
Time × PRE-PRE	-0.28*	[-0.54, -0.01]	-0.21	[-0.44, 0.02]	-0.07	[-0.18, 0.05]	-0.06	[-0.22, 0.11]	-0.15	[-0.32, 0.01]
Time × POST-POST	-0.42***	[-0.65, -0.20]	-0.34**	[-0.53, -0.14]	-0.15*	[-0.24, -0.05]	-0.22*	[-0.36, -0.08]	-0.09	[-0.23, 0.05]
Time × PA, Mean MAD	0.05	[-0.07, 0.18]	0.04	[-0.07, 0.15]	-0.04	[-0.09, 0.02]	-0.07	[-0.14, 0.01]	-0.01	[-0.09, 0.07]

Models are adjusted with age at baseline and use of hormonal preparations. PRE-POST, participants who were pre- or perimenopausal at follow-up (reference group); PRE-PRE, participants who were pre- or perimenopausal in both measurements; POST-POST, participants who were already postmenopausal at baseline; Time, from ERMA baseline to EsmiRs; CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

TABLE 7 Pooled linear mixed-effect model estimates for blood pressure in *Study I* (*n* = 298).

	Systolic blood pressure [mmHg]		**Diastolic blood pressure [mmHg]**	
	B	95% CI	B	95% CI
Main effects				
Intercept (PRE-POST)	130.9***	[125.1, 136.8]	84.9***	[81.8, 88.0]
Menopausal group				
PRE-POST (ref.)	-		-	
PRE-PRE	1.31	[-4.41, 7.03]	1.10	[-2.07, 4.27]
POST-POST	-1.97	[-6.79, 2.85]	0.01	[-2.66, 2.68]
PA, Mean MAD [10 mg]	0.28	[-1.42, 2.00]	-0.36	[-1.24, 0.51]
Time (PRE-POST)	9.37**	[3.34, 15.39]	-0.16	[-3.11, 2.79]
Interactions				
Time × Menopausal group				
Time × PRE-POST (ref.)	-		-	
Time × PRE-PRE	0.62	[-3.67, 4.91]	-0.81	[-2.88, 1.26]
Time × POST-POST	-0.03	[-3.80, 3.73]	-0.73	[-2.57, 1.12]
Time × PA, Mean MAD	-2.40*	[-4.34, -0.46]	-0.28	[-1.24, 0.68]

Models are adjusted with age at baseline and use of hormonal preparations. PRE-POST, participants who were pre- or perimenopausal at follow-up (reference group); PRE-PRE, participants who were pre- or perimenopausal in both measurements; POST-POST, participants who were postmenopausal already at baseline; Time, from ERMA baseline to EsmiRs; CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

5.3 Menopausal symptoms, physical activity, and cardiometabolic health (*Studies II* and *III*)

The total number of menopausal symptoms as well the total number of vasomotor and urogenital symptoms were directly associated with higher total cholesterol, LDL-C, and HDL-C levels in the cross-sectional analyses with simple linear regression (TABLE 8). However, after adjusting the models for confounders, these associations diminished. Furthermore, total body mass and body adiposity measures were directly associated with total number of menopausal symptoms (TABLE 9) and total severity of menopausal symptoms (TABLE 10) in both the simple and multiple regression models. In the symptom subscales, the severity of somato-vegetative and psychological symptoms also associated directly with total body mass and body fat mass (TABLE 10). Blood glucose, triglycerides, blood pressure, and lean body mass were not statistically significantly associated with menopausal symptoms. The associations between the levels of the cardiometabolic health indicators and the number of menopausal symptoms during 4-year follow-up were somewhat similar to the associations observed in the cross-sectional analyses (Supplementary Tables 2 and 3). However, the number of menopausal symptoms did not associate with changes in the levels of the metabolic health indicators during the 4-year follow-up. The

body composition at baseline did not predict the severity of menopausal symptoms after the 4-year follow-up (Supplementary Table 4).

TABLE 8 Pooled linear regression estimates for the association between the number of menopausal symptoms and blood-based biomarker levels in *Study II* (n = 1393).

	Total cholesterol [mmol/L]		**Low-density lipoprotein cholesterol [mmol/L]**		**High-density lipoprotein cholesterol [mmol/L]**		**Glucose [mmol/L]**		**Triglycerides [mmol/L]**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Total number of symptoms										
Model 1	0.05***	[0.03, 0.07]	0.04***	[0.02, 0.06]	0.01	[0.00, 0.02]	-0.01	[-0.03, 0.01]	0.01	[-0.01, 0.03]
Model 2	0.01	[-0.02, 0.07]	0.00	[-0.02, 0.02]	0.00	[-0.02, 0.01]	-0.01	[-0.04, 0.01]	0.00	[-0.02, 0.02]
Number of vasomotor symptoms										
Model 1	0.13*	[0.07, 0.20]	0.08**	[0.03, 0.14]	0.06***	[0.03, 0.09]	-0.04	[-0.10, 0.02]	0.03	[-0.02, 0.08]
Model 2	0.00	[-0.08, 0.07]	-0.03	[-0.09, 0.03]	0.02	[-0.02, 0.06]	-0.04	[-0.10, 0.03]	0.01	[-0.05, 0.06]
Number of psychological symptoms										
Model 1	0.06*	[0.01, 0.11]	0.04	[0.00, 0.09]	0.01	[-0.02, 0.03]	-0.02	[-0.07, 0.02]	0.02	[-0.02, 0.06]
Model 2	0.01	[-0.04, 0.06]	0.00	[-0.04, 0.05]	-0.01	[-0.03, 0.02]	-0.02	[-0.07, 0.02]	0.01	[-0.03, 0.05]
Number of somatic or pain symptoms										
Model 1	0.10	[-0.01, 0.20]	0.08	[-0.01, 0.17]	0.00	[-0.05, 0.05]	0.01	[-0.08, 0.10]	0.04	[-0.03, 0.12]
Model 2	0.03	[-0.08, 0.13]	0.02	[-0.07, 0.12]	-0.01	[-0.06, 0.03]	0.01	[-0.09, 0.10]	0.03	[-0.05, 0.11]
Number of urogenital symptoms										
Model 1	0.09**	[0.02, 0.15]	0.09**	[0.03, 0.14]	0.01	[-0.02, 0.04]	-0.01	[-0.07, 0.05]	-0.01	[-0.05, 0.04]
Model 2	0.01	[-0.06, 0.08]	0.03	[-0.03, 0.08]	-0.01	[-0.05, 0.02]	-0.02	[-0.08, 0.05]	-0.02	[-0.07, 0.03]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, body mass index, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption. CI, confidence interval; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

TABLE 9 Pooled linear regression estimates for the association of the number of menopausal symptoms with blood pressure, body adiposity, and physical activity levels in *Study II* (*n* = 1393).

	Systolic blood pressure [mmHg]		**Diastolic blood pressure [mmHg]**		**Total fat mass [kg][a]**		**Android fat mass [kg][a]**		**PA, Mean MAD [mg]**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Total number of symptoms										
Model 1	0.18	[-0.33, 0.69]	0.14	[-0.14, 0.42]	0.31*	[0.03, 0.59]	0.04*	[0.01, 0.07]	-0.09	[-0.33, 0.15]
Model 2	-0.07	[-0.58, 0.43]	-0.04	[-0.31, 0.22]	0.35*	[0.03, 0.67]	0.04	[0.00, 0.07]	-0.09	[-0.36, 0.19]
Number of vasomotor symptoms										
Model 1	0.21	[-1.07, 1.49]	0.16	[-0.55, 0.87]	0.44	[-0.33, 1.21]	0.06	[-0.03, 0.14]	-0.26	[-0.91, 0.40]
Model 2	-0.33	[-1.71, 1.05]	-0.23	[-0.98, 0.51]	0.55	[-0.35, 1.45]	0.05	[-0.05, 0.15]	-0.28	[-1.05, 0.49]
Number of psychological symptoms										
Model 1	0.07	[-0.98, 1.13]	0.16	[-0.42, 0.74]	0.49	[-0.16, 1.14]	0.05	[-0.02, 0.13]	-0.13	[-0.71, 0.45]
Model 2	-0.23	[-1.26, 0.80]	-0.07	[-0.62, 0.48]	0.50	[-0.17, 1.16]	0.05	[-0.03, 0.12]	-0.10	[-0.70, 0.50]
Number of somatic or pain symptoms										
Model 1	1.95	[-0.18, 4.09]	0.63	[-0.53, 1.79]	0.91	[-0.34, 2.16]	0.11	[-0.03, 0.24]	-0.21	[-1.17, 0.74]
Model 2	1.36	[-0.65, 3.37]	0.17	[-0.87, 1.22]	0.83	[-0.46, 2.13]	0.09	[-0.05, 0.23]	-0.18	[-1.17, 0.80]
Number of urogenital symptoms										
Model 1	0.13	[-1.19, 1.45]	0.32	[-0.41, 1.05]	0.60	[-0.23, 1.44]	0.08	[-0.01, 0.17]	-0.11	[-0.74, 0.51]
Model 2	-0.41	[-1.77, 0.95]	-0.05	[-0.74, 0.65]	0.60	[-0.26, 1.47]	0.07	[-0.03, 0.17]	-0.08	[-0.74, 0.58]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, body mass index, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption, unless otherwise specified. [a] Model 2 is adjusted for age, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption. CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

TABLE 10 Pooled linear regression estimates for the association of physical activity and body composition with the severity of menopausal symptoms in *Study III* (*n* = 298).

	Total severity of symptoms		**Severity of somato-vegetative symptoms**		**Severity of psychological symptoms**		**Severity of urogenital symptoms**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
PA, Mean MAD [10 mg]								
Model 1	-0.06	[-0.73, 0.62]	-0.17	[-0.46, 0.12]	-0.03	[-0.35, 0.30]	0.14	[-0.11, 0.40]
Model 2	-0.16	[-0.86, 0.55]	-0.23	[-0.53, 0.08]	-0.05	[-0.39, 0.28]	0.12	[-0.15, 0.39]
Total body mass [kg][a]								
Model 1	0.04	[-0.01, 0.09]	0.02	[0.00, 0.04]	0.02	[0.00, 0.05]	0.00	[-0.02, 0.02]
Model 2	0.06*	[0.01, 0.12]	0.03**	[0.01, 0.05]	0.03*	[0.00, 0.05]	0.01	[-0.02, 0.03]
Body fat mass [kg][a]								
Model 1	0.06	[0.00, 0.12]	0.03*	[0.00, 0.06]	0.03	[0.00, 0.06]	0.00	[-0.02, 0.03]
Model 2	0.07*	[0.01, 0.14]	0.04*	[0.01, 0.06]	0.03*	[0.00, 0.06]	0.01	[-0.02, 0.03]
Lean body mass [kg][a]								
Model 1	0.01	[-0.13, 0.15]	0.00	[-0.06, 0.06]	0.05	[-0.02, 0.11]	-0.03	[-0.08, 0.02]
Model 2	0.13	[-0.03, 0.30]	0.05	[-0.02, 0.12]	0.07	[-0.01, 0.15]	0.02	[-0.05, 0.08]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption. [a] Model 2 is additionally adjusted for body height. CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

The level of physical activity was not statistically significantly associated with the number or severity of menopausal symptoms in the cross-sectional (TABLE 9, TABLE 10) or the longitudinal (Supplementary Tables 3 and 4) study design, regardless of the model adjustments. However, both accelerometer-measured and self-reported (data not shown) physical activity tended to be inversely associated with menopausal symptoms. In the moderation analysis, total body and lean body mass showed positive interactions with physical activity, especially in total symptom severity and the severity of psychological symptoms (TABLE 11). Furthermore, after adjusting for the interactions with total body and lean body mass, a higher level of physical activity was associated with less severe menopausal symptoms. Sensitivity analysis indicated that this association was especially prevalent among participants with the lowest total body and lean body mass (data not shown).

TABLE 11 Pooled moderator analysis of body composition measures in the association of physical activity and the severity of menopausal symptoms in *Study III* (*n* = 298).

	Total severity of symptoms		**Severity of somato-vegeta-tive symptoms**		**Severity of psychological symptoms**		**Severity of urogenital symptoms**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Physical activity, Mean MAD [10 mg] & Total body mass [kg]								
Physical activity	-5.83*	[-10.30, -1.37]	-1.33	[-3.29, 0.62]	-2.83**	[-4.96, -0.70]	-1.67	[-3.39, 0.05]
Total body mass	-0.16	[-0.34, 0.02]	-0.02	[-0.10, 0.06]	-0.08	[-0.17, 0.00]	-0.06	[-0.13, 0.01]
Interaction	0.08*	[0.02, 0.15]	0.02	[-0.01, 0.04]	0.04**	[0.01, 0.07]	0.03*	[0.00, 0.05]
Physical activity, Mean MAD [10 mg] & Body fat mass [kg]								
Physical activity	-1.77	[-3.93, 0.39]	-0.39	[-1.33, 0.55]	-0.76	[-1.80, 0.27]	-0.62	[-1.44, 0.21]
Body fat mass	-0.12	[-0.35, 0.12]	0.01	[-0.09, 0.11]	-0.05	[-0.17, 0.06]	-0.07	[-0.16, 0.02]
Interaction	0.07	[-0.01, 0.15]	0.01	[-0.03, 0.04]	0.03	[-0.01, 0.07]	0.03	[0.00, 0.06]
Physical activity, Mean MAD [10 mg] & Lean body mass [kg]								
Physical activity	-8.49*	[-15.20, -1.79]	-2.26	[-5.19, 0.67]	-4.55**	[-7.75, -1.36]	-1.68	[-4.26, 0.91]
Lean body mass	-0.42	[-0.90, 0.05]	-0.09	[-0.29, 0.12]	-0.23*	[-0.46, -0.01]	-0.10	[-0.29, 0.08]
Interaction	0.20*	[0.04, 0.36]	0.05	[-0.02, 0.12]	0.11**	[0.03, 0.18]	0.04	[-0.02, 0.10]

All models are adjusted for age, menopausal group, use of hormonal preparations, smoking, education, alcohol consumption, and body height. CI, confidence interval; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

5.4 Predictors of age at natural menopause (*Study IV*)

In *Study IV*, the number of predictors for each prediction model were limited to seven due to the effective sample size of 105. The final model predictors in model 1, in which the full set of candidate predictors was used, were estradiol and FSH levels, menstrual cycle length standard deviation, alcohol consumption, single seven-level scale physical activity question, the prevalence of VMS, and relationship status (TABLE 12). The predictors in model 2, in which the set of candidate predictors included only the easy-to-access predictors, were alcohol consumption, seven-level scale physical activity question, prevalence of VMS, use of hormonal contraception, relationship status, smoking status, and educational level. Model 1 and model 2 *c*-indices [95% confidence intervals] were 0.76 [0.71, 0.81] and 0.70 [0.65, 0.75], respectively.

For model validation, the MAE (± standard deviation) of the model predictions based on the leave-one-out cross-validation was 0.56 ± 0.49 years for

model 1 and 0.62 ± 0.54 years for model 2. Both models were slightly biased toward predicting too early ANM, with mean bias errors of -0.09 and -0.18 years for models 1 and 2, respectively.

TABLE 12 Pooled Cox regression model estimates for the prediction models constructed in *Study IV* (n = 279).

	Hazard ratio	95% CI
Model 1		
Estradiol [nmol/L]	2.13***	[1.42, 3.18]
Follicle-stimulating hormone [IU/L]	1.01***	[1.01, 1.02]
Cycle length standard deviation [d]	1.02***	[1.01, 1.03]
Alcohol consumption [portions/week]	1.07**	[1.02, 1.12]
Single seven-level scale PA question (linear)	2.29	[0.55, 9.57]
Vasomotor symptoms		
No (ref.)	-	
Yes	2.68**	[1.41, 5.12]
Relationship status		
Single (ref.)	–	
In a relationship	1.42	[0.85, 2.38]
Model 2		
Alcohol consumption [portions/week]	1.06*	[1.01, 1.12]
Single seven-level scale PA question (linear)	1.85	[0.46, 7.49]
Vasomotor symptoms		
No (ref.)	-	
Yes	3.33***	[1.80, 6.19]
Use of hormonal contraception		
Never (ref.)	-	
Former	0.68	[0.38, 1.21]
Relationship status		
Single (ref.)	-	
In a relationship	1.35	[0.81, 2.25]
Smoking		
Never (ref.)	-	
Former	0.99	[0.61, 1.60]
Current	0.37	[0.09, 1.52]
Education		
Primary or secondary (ref.)	–	
Tertiary	1.25	[0.82, 1.91]

CI, confidence interval; PA, physical activity; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

6 DISCUSSION

In this dissertation, my primary aim was to investigate the associations of physical activity, menopausal symptoms, and cardiometabolic health in middle-aged women in a cross-sectional study design as well as a longitudinal study design with 4-year follow-up. I observed unfavorable changes in several blood-based indicators of cardiometabolic health during the follow-up. The magnitude of these changes was greater closer to menopause, which highlights the independent role of menopause in these changes. Furthermore, a higher level of physical activity as well as a lower prevalence and severity of menopausal symptoms were observed to associate with favorable levels in several indicators of metabolic health, especially with lower total cholesterol, LDL-C, and body adiposity. However, for menopausal symptoms, these associations were significantly weaker in longitudinal analyses and after adjusting for confounders. Physical activity was not associated with the prevalence or severity of menopausal symptoms. However, body composition, and especially total and lean body mass, was observed to moderate this association. Consequently, a higher level of physical activity was associated with less severe menopausal symptoms in participants with lower total and lean body mass.

Secondly, I aimed to identify factors associated with the timing of the natural menopause and to utilize this information for creating models for predicting ANM in a longitudinal study design. I found that especially higher E2 and FSH levels, irregular menstrual cycles, and prevalence of vasomotor symptoms are strong indicators of approaching menopause. Additionally, factors related to life habits and socioeconomic status, such as alcohol consumption, smoking habits, relationship status, and physical activity, may provide useful information for assessing the timeline to natural menopause. Overall, our prediction models demonstrated adequate predictive performance with good concordance between predicted and observed ANM.

6.1 Cardiometabolic health around menopause

It is well known that the prevalence of CMD increases around menopause (El Khoudary et al., 2020). The independent role of menopause in this increase was highlighted by the findings of the Study of Women's Health Across the Nations (SWAN) cohort study, which observed that the aging-related increase in lipid levels accelerates significantly in a short time period around menopause (Matthews et al., 2009). Our findings in *Study I* duplicated these results, with the increase in total cholesterol and LDL-C levels being significantly greater in participants who experienced menopause during the follow-up than those who did not. Contrarily, the association between HDL-C and menopause has been found to be more complicated, with studies reporting both increases (Derby et al., 2009; Stevenson et al., 1993) similar to our results and decreases (Gurka et al., 2016) in HDL-C levels around menopause (El Khoudary, 2017). In addition to the change in HDL-C concentration, the antiatherogenic functionality of HDL-C may also deteriorate around menopause, which complicates research on the associations between HDL-C and CMD risk in middle-aged women (El Khoudary et al., 2016, 2021).

Menopause-related hormonal changes may also affect blood pressure and glucose in women. For instance, the decrease in systemic E2 levels may cause deterioration in insulin sensitivity (Mauvais-Jarvis et al., 2013) and impairment in the endothelial function (Yanes & Reckelhoff, 2011). However, due to the previous contradictory findings for both blood pressure (Tikhonoff et al., 2019) and blood glucose levels (Matthews et al., 2009; Otsuki et al., 2007), the independent role of menopause on the change in these levels in middle-aged women has not been confirmed. In *Study I*, we observed significant increases in SBP and blood glucose during the follow-up. The change in SBP observed did not differ in women at different menopausal stages, but in blood glucose, the magnitude of the increase was greater near menopause. These results provide evidence that menopause may have an independent effect on the changes in blood glucose levels in middle-aged women. On the other hand, our results indicate that the increase in SBP observed during the follow-up may primarily be related to aging rather than menopause.

6.2 Physical activity and cardiometabolic health

The findings from previous studies indicate that physical activity is associated with decreased risk of CMD and favorable levels in several indicators of cardiometabolic health in middle-aged women (Karvinen et al., 2019; Mandrup et al., 2017; Ruiz-Rios & Maldonado-Martin, 2022). In agreement with these findings, we observed that a higher level of physical activity was associated with favorable levels in blood lipids in women during the 4-year follow-up period. However, previous studies on this topic have not investigated the independent

role of physical activity in mitigating the detrimental changes in the levels of metabolic health indicators around menopause. In *Study I*, we observed that participants with a higher level of physical activity had a significantly smaller increase in SBP during the follow-up than their less active counterparts. However, physical activity was not associated with a change in blood lipids and glucose levels. Consequently, these results suggest that being physically active may be especially beneficial for the management of hypertension in women around menopause.

6.3 Menopausal symptoms and cardiometabolic health

Previous meta-analyses of observational studies have shown that menopausal symptoms, and especially VMS, are associated with increased risk of CMD and unfavorable levels in blood lipids, blood pressure, and body adiposity (Armeni et al., 2023; Franco et al., 2015; Muka, Oliver-Williams, Colpani, et al., 2016). Nevertheless, the results of these studies are equivocal due to the use of different sets of confounders in the studies included in the meta-analyses (Armeni et al., 2023). The mechanisms behind the linkage between menopausal symptoms and metabolic health are not completely clear, but they could be explained by the menopause-related changes in the hormonal milieu and body adiposity that are known to contribute to the development of menopausal symptoms (Monteleone et al., 2018) and changes in indicators of cardiometabolic health (El Khoudary et al., 2020). However, these mechanisms are directly linked to menopausal status, and therefore they would not explain the potential association between menopausal symptoms and cardiometabolic health independent of menopausal status.

In *Study II*, we observed a higher number of vasomotor, psychological, and urogenital symptoms and that the total number of symptoms was associated with increased total cholesterol, LDL-C, and HDL-C levels. However, the associations diminished after controlling for confounders. This finding is contradictory to the results of the SWAN study, where the frequency of VMS was directly associated with higher LDL-C, HDL-C, and triglyceride levels independent of aging and menopausal status (Thurston et al., 2012). Furthermore, in *Studies II* and *III*, increased body adiposity was associated with a higher prevalence and severity of total menopausal symptoms as well as severity of somato-vegetative and psychological symptoms even after controlling for confounders. Our results are in line with previous results indicating that VMS and other types of menopausal symptoms are associated with unfavorable levels in indicators of cardiometabolic health (Franco et al., 2015). However, it is still unclear if these associations are independent of the menopausal transition or rather reflect the known increase in the prevalence and severity of menopausal symptoms with the menopausal transition (Woods & Mitchell, 2005).

6.4 Physical activity and menopausal symptoms

Regardless of the vast amount of both experimental and observational studies on the topic, the association between physical activity and menopausal symptoms is not well understood (Daley et al., 2011; Nguyen et al., 2020; Pettee Gabriel et al., 2015). Contradictory results on the topic may be partially explained by the diversity of the association between physical activity and different symptoms, which is caused by the heterogeneous nature of menopausal symptoms. Currently, the overall evidence indicates that physical activity may not be beneficial for mitigating VMS, but it may have a positive effect on psychological and urogenital symptoms (Pettee Gabriel et al., 2015). Our results from *Studies II* and *III* provide further evidence that physical activity may not be associated with the prevalence or severity of menopausal symptoms, regardless of the symptom type and model adjustments.

Interestingly, we found that total and lean body mass moderated the association between physical activity and the severity of menopausal symptoms in *Study III*. We observed strong indirect associations between physical activity and the severity of menopausal symptoms in participants with lower total and lean body mass, especially in total and psychological symptoms. This observation may be explained by the differences in the types of activity in which the participants with different lean body mass were engaging—that is, if exercise that does not significantly increase body muscle mass (e.g., aerobic training) is more beneficial for alleviating symptoms than exercise that builds muscle mass (e.g., resistance training). This hypothesis is supported by the results from a small experimental study with aerobic and resistance training interventions, where the aerobic training intervention was more beneficial for alleviating the severity of menopausal symptoms (Ağıl et al., 2010). Other than that, previous experimental studies on this topic have mainly focused on aerobic exercise and yoga rather than resistance training (Daley et al., 2011; Nguyen et al., 2020). Therefore, the effect of different types of exercise on menopausal symptoms is not well known.

6.5 Prediction of the age at natural menopause

The prediction of menopausal age is challenging due to the irregularity of the menopausal transition and significant individual differences in ANM, which is largely controlled by genetic factors (De Bruin et al., 2001; Snieder et al., 1998). Notably, the progression of the menopausal transition is often assessed using hormonal levels, but there is significant inter- and intra-individual variability in hormonal trajectories within the menstrual cycle as well as during the menopausal transition (Harlow et al., 2012; Tepper et al., 2012). In *Study IV*, we observed FSH and E2 to be strong predictors of ANM in middle-aged women. Our finding that higher FSH levels indicates approaching natural menopause is in line with the known increase in FSH levels during the menopausal transition

(Harlow et al., 2012; Shifren et al., 2014). However, although E2 levels are known to decrease around menopause, in our study, higher E2 levels were associated with a shorter time to natural menopause. Similar associations have also been reported previously, and our finding may be explained by the late perimenopausal increase in E2 levels that precedes the menopause-related decrease (Santoro et al., 2007; Sowers et al., 2008).

In addition to direct measures of hormonal levels, menopausal symptoms and irregularity in hormonal cycles have been identified as indicators of approaching menopause in longitudinal studies (Santoro et al., 2007). Similarly, we also observed that a higher prevalence of VMS and increased variability in menstrual cycle length were distinctly associated with shorter time to natural menopause. These findings concur with the well-documented effect of menopause on the menstrual cycle as well as the known increase in the prevalence of menopausal symptoms during the menopausal transition (Harlow et al., 2012; Woods & Mitchell, 2005).

Previous research has shown that factors related to lifestyle and socioeconomic status may also associate with ANM (Schoenaker et al., 2014). However, these findings are mainly derived from cross-sectional studies in which the lifestyle factors assessed reflected current status or lifetime exposure if retrospective assessment was used. Due to our longitudinal study design with multiple measurements and a relatively short follow-up time preceding menopause in *Study IV*, our findings primarily reflect the changes that occur during the menopausal transition rather than lifetime exposure. Based on the feature selection by lasso regression in *Study IV*, we identified several factors related to lifestyle and socioeconomic status, such as smoking, physical activity, relationship status, and education, as potential indicators of approaching natural menopause. However, the most distinct lifestyle-related finding in our study was the indirect association between alcohol consumption and time to natural menopause, indicating that women tend to increase their alcohol consumption during the menopausal transition. Accordingly, the menopausal transition has been shown to be a period of instability regarding alcohol consumption (Peltier et al., 2020), but a previous longitudinal study did not observe an association between alcohol consumption and time to menopause (Santoro et al., 2007).

The prediction models constructed in *Study IV* demonstrated adequate predictive performance with good concordance between predicted and observed ANM as well as with the model predictions being distinctly more accurate than the predicted sample mean. Our results are not comparable with previous studies on this topic, since the previous research used different methodological approaches and predicted ANM using only a few predetermined hormonal measurements (Finkelstein et al., 2020; Greendale et al., 2013; Hefler et al., 2006; C. Kim et al., 2017). Nonetheless, the findings from the previous studies indicate that there are some potentially significant predictors of ANM, such as AMH, antral follicle count, and mother's ANM, that were not available in our dataset (Depmann et al., 2016). Including those into the set of candidate predictors used

in our study could have significantly influenced the set of predictors chosen for the final models and improved the predictive performance of the models.

6.6 Strengths and limitations

The biggest advantage of this dissertation is its use of a unique longitudinal dataset collected from the random sample of middle-aged women. The main strengths of this study are the assessment of physical activity with accelerometers and the accurate definition of menopausal status and the timing of the FMP using repeated FSH measurements and information about menstrual cycles, which enables the precise consideration of the progressing menopausal transition during the follow-up period. Furthermore, the repeated measurements, including DXA-measured body composition, comprehensive assessment of blood-based biomarkers, and the assessment of the full spectrum of menopausal symptoms and confounding factors are the other major upsides of the study. Finally, the significant methodological advantage is the consistent use of multiple imputation throughout the studies to improve the validity of the results and reduce bias caused by the missing values.

The main limitation of this dissertation is related to the generalizability of the results due to the homogenous study sample of white women and the exclusion of women with severe obesity and severe medical disorders. This may limit the generalizability of the findings to more heterogeneous populations with ethnic diversity as well as participants with obesity and disabling conditions. Additionally, causal inference methods were not used in this dissertation, and therefore, it is not possible to distinguish between association and causality based on the results. The assessment of the longitudinal changes in menopausal symptoms was also limited in this dissertation due to the use of different and incompatible symptom questionnaires at different time points. Consequently, the questionnaire used for the assessment of menopausal symptoms in *Study II* had not been validated and captured only the prevalence rather than the frequency or severity of the symptoms.

There are also some limitations related to menopausal status assignments and the assessment of the use of hormonal preparations. The use of a six-month instead of a 12-month period of amenorrhea to verify the timing of menopause may have led to the misclassification of menopausal status for some participants. Although hormonal preparations were used as a confounder in the analyses, the used measure only included information about the current use of hormonal preparations but lacked more detailed information about the dosages and how long they have been used. Furthermore, only including women who were 47 years or older and not postmenopausal in the baseline measurement may have caused selection bias in *Study IV*, since women with earlier menopause were more likely to be excluded.

6.7 Future directions

With respect to previous research, the findings of this dissertation raise the need for more longitudinal studies on multifaceted associations between physical activity, cardiometabolic health, and menopausal symptoms around menopause. The specific emphasis of such studies should be to consider the confounding role of the menopausal transition in these associations. These kinds of studies are warranted to gain more insight on the independent roles of physical activity and different menopausal symptoms in the changes in individual indicators of cardiometabolic health as well as in the overall risk of CMD around menopause. Furthermore, more studies focusing on the physiology behind these associations are needed to increase understanding of the underlying mechanisms.

Another substantive research topic based on our preliminary findings is the potential role of body composition in the association between physical activity and menopausal symptoms. In particular, experimental studies with an emphasis on different types of exercise and menopausal symptoms would be highly beneficial to provide further insight into this topic. For the prediction of ANM, further studies with long-term follow-up time and a carefully implemented selection of predictors are warranted to develop models for women and for clinical work that would be user-friendly and provide more accurate information about the timeline to menopause.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings of this dissertation are as follows:

1. Increases in blood lipid, blood glucose, and blood pressure levels occur in middle-aged women, and the rate of the increase accelerates near menopause. A higher level of physical activity is associated with favorable blood lipid levels, but it may not counteract the changes that occur around menopause. However, being physically active may mitigate the increase in blood pressure in middle-aged women. Although physical activity may not be effective for counteracting menopause-related changes in indicators of cardiometabolic health, our findings highlight the importance of an active lifestyle in women for maintaining a favorable cardiometabolic risk factor profile throughout the lifespan.

2. The prevalence and severity of menopausal symptoms are associated with increased total cholesterol, LDL-C, and HDL-C levels as well as increased body adiposity in middle-aged women. However, the associations between menopausal symptoms and cholesterol levels may not be independent of age and menopausal status. These results suggest that maintaining a healthy weight may be beneficial for the management of menopausal symptoms.

3. Physical activity level is not associated with the prevalence and severity of menopausal symptoms in middle-aged women overall. However, body composition may moderate the association between physical activity and menopausal symptoms. Consequently, being physically active may be more beneficial for the management of menopausal symptoms in women with lower total and lean body mass.

4. Higher estradiol and FSH levels, the prevalence of menopausal symptoms, and menstrual cycle irregularity are strong indicators of approaching menopause in middle-aged women. Furthermore, measures of alcohol consumption, smoking, relationship status, and physical activity may provide

additional information for assessing the timeline to menopause. These findings suggest that, in addition to well-known indicators of approaching menopause, easily accessible measures related to lifestyle, socioeconomic status, and gynecological history could provide useful information for the prediction of the ANM for middle-aged women and the clinicians working with them.

YHTEENVETO (SUMMARY IN FINNISH)

Vaihdevuodet tarkoittavat ajanjaksoa naisten elämässä, jolloin munasarjojen toiminta heikkenee ja lopulta sammuu kokonaan, mikä johtaa myös kuukautisten loppumiseen. Munasarjojen toiminnan heikkenemisen seurauksena vaihdevuosien aikana tapahtuu monia hormonaalisia muutoksia, joista tyypillisimpiä ovat elimistön estradiolipitoisuuden lasku ja follikkelia stimuloivan hormonin pitoisuuden nousu. Menopaussilla tarkoitetaan viimeisten spontaanisti tapahtuvien kuukautisten ajankohtaa. Useimmat naiset kokevat menopaussin 40 ja 58 ikävuoden välissä, ja Suomessa sen keskimääräinen ajankohta on noin 50 vuotta.

Vaihdevuosilla on havaittu olevan merkittäviä vaikutuksia naisten terveyteen ja elämänlaatuun. Vaihdevuosioireita, joista tyypillisimpiä ovat kuumat aallot ja hikoilupuuskat, kokee jopa yli 85 % naisista. Sekä vaihdevuosien että vaihdevuosioireiden on havaittu olevan yhteydessä epäedullisiin muutoksiin monissa aineenvaihdunta- sekä sydän- ja verenkiertoelimistön sairauksien riskitekijöissä. Toisaalta säännöllisellä fyysisellä aktiivisuudella on useissa havainnoivissa ja kokeellisissa tutkimuksissa todettu olevan positiivisia vaikutuksia yksittäisiin kardiometabolista terveyttä kuvaavien muuttujien tasoihin sekä kardiometabolisten sairauksien riskiin. Fyysinen aktiivisuus saattaa myös helpottaa vaihdevuosioireita.

Tämän väitöskirjatutkimuksen tarkoituksena oli tutkia fyysisen aktiivisuuden, vaihdevuosioireiden ja kardiometabolisen terveyden välisiä yhteyksiä vaihdevuosien aikana. Lisäksi väitöskirjassa tutkittiin, mitkä tekijät ennustavat lähestyvää luonnollista menopaussia naisilla keski-iässä. Tutkimuksessa käytettiin Estrogeeni, vaihdevuodet ja toimintakyky (ERMA) -tutkimuksessa sekä sen nelivuotisseurantatutkimuksessa Estrogeeni, mikro-RNA:t ja metabolisten toimintahäiriöiden riski (EsmiRs) kerättyä poikittais- ja pitkittäisaineistoa. Tutkittavat olivat alkumittauksissa 47–55-vuotiaita naisia, ja tutkittavien määrä vaihteli osatöissä 279:n ja 1393:n välillä. Tutkittavien fyysistä aktiivisuutta arvioitiin aktiivisuusmittausten sekä kyselylomakkeiden avulla, ja vaihdevuosioireiden määrää sekä haittaavuutta kyselylomakkeiden avulla. Kardiometabolista terveyttä arvioitiin kolesteroli-, triglyseridi-, verensokeri- ja verenpainetasojen avulla sekä käyttämällä kaksienergiaisen röntgen- absorptiometrian avulla mitattuja kehonkoostumusmuuttujia.

Tutkimuksessa veren rasva-arvojen, verensokerin ja verenpaineen havaittiin kohoavan seurannan aikana ja näiden muutosten olevan suurempia lähellä menopaussin ajankohtaa. Suurempi fyysinen aktiivisuus oli yhteydessä terveydelle edullisten rasva-arvojen kanssa, mutta sen ei havaittu olevan yhteydessä seurannan aikana tapahtuviin muutoksiin muissa kardiometabolista terveyttä kuvaavissa muuttujissa kuin systolisessa verenpaineessa. Vaihdevuosioireiden suurempi määrä ja haittaavuus olivat yhteydessä suurempaan kehon rasvaisuuteen sekä terveydelle epäedullisiin rasva-arvoihin. Vaihdevuosioireiden ja rasva-arvojen yhteydet eivät kuitenkaan olleet itsenäisiä sekoittavista tekijöistä, kuten ikä ja vaihdevuosistatus. Fyysisen aktiivisuuden ei havaittu olevan yhteydessä

vaihdevuosioireisiin, mutta kehonkoostumus-muuttujien havaittiin toimivan moderaattorina fyysinen aktiivisuuden ja vaihdevuosioireiden välisessä yhteydessä. Tutkittaessa luonnollisen menopaussin ajankohtaa ennustavia tekijöitä etenkin korkeampien estradiolin ja follikkelia stimuloivan hormonin pitoisuuksien, vaihdevuosioireiden sekä epäsäännöllisen kuukautiskierron havaittiin ennustavan lähestyvää luonnollista menopaussia. Lisäksi havaittiin, että monet elämäntapoja ja sosioekonomista statusta kuvaavat muuttujat, kuten alkoholinkäyttö, tupakointi, fyysinen aktiivisuuden taso sekä koulutustaso, saattavat auttaa menopaussin ajankohdan ennustamisessa.

Tämä väitöskirjatutkimus osoittaa, että ikääntymiseen liittyvä kardiometabolisen terveyden heikkeneminen kiihtyy naisilla vaihdevuosien aikana. Fyysisen aktiivisuuden avulla ei välttämättä pystytä ehkäisemään näitä vaihdevuosien aikana tapahtuvia muutoksia, mutta säännöllinen fyysinen aktiivisuus vaikuttaa edistävän naisten kardiometabolista terveyttä myös keski-iässä. Tutkimuksen tulokset viittaavat siihen, että painonhallinta saattaa helpottaa vaihdevuosioireita sekä auttaa niiden ennaltaehkäisemisessä. Säännöllinen fyysinen aktiivisuus ei välttämättä helpota vaihdevuosioireita kaikilla naisilla, mutta tulosten perusteella kehonkoostumus saattaa vaikuttaa fyysisen aktiivisuuden ja vaihdevuosioireiden väliseen yhteyteen. Etenkin naisilla, joilla on pienempi kehon massa ja rasvaton kehon massa, fyysinen aktiivisuus saattaa helpottaa vaihdevuosioireita. Tulosten perusteella menopaussin ajankohdasta on mahdollista saada tietoa hormonaalisten mittauksien lisäksi myös helposti määritettävissä olevien itseraportoitavien muuttujien avulla.

REFERENCES

Ağıl, A., Abıke, F., Daşkapan, A., Alaca, R., & Tüzün, H. (2010). Short-term exercise approaches on menopausal symptoms, psychological health, and quality of life in postmenopausal women. *Obstetrics and Gynecology International*, *2010*, e274261. https://doi.org/10.1155/2010/274261

Aibar-Almazán, A., Hita-Contreras, F., Cruz-Díaz, D., de la Torre-Cruz, M., Jiménez-García, J. D., & Martínez-Amat, A. (2019). Effects of Pilates training on sleep quality, anxiety, depression and fatigue in postmenopausal women: A randomized controlled trial. *Maturitas*, *124*, 62–67. https://doi.org/10.1016/j.maturitas.2019.03.019

Aiello, E. J., Yasui, Y., Tworoger, S. S., Ulrich, C. M., Irwin, M. L., Bowen, D., Schwartz, R. S., Kumai, C., Potter, J. D., & McTiernan, A. (2004). Effect of a yearlong, moderate-intensity exercise intervention on the occurrence and severity of menopause symptoms in postmenopausal women. *Menopause*, *11*(4), 382–388. https://doi.org/10.1097/01.GME.0000113932.56832.27

Ambikairajah, A., Walsh, E., & Cherbuin, N. (2019). Lipid profile differences during menopause: A review with meta-analysis. *Menopause*, *26*(11), 1327–1333. https://doi.org/10.1097/GME.0000000000001403

Ambikairajah, A., Walsh, E., Tabatabaei-Jafari, H., & Cherbuin, N. (2019). Fat mass changes during menopause: A metaanalysis. *American Journal of Obstetrics and Gynecology*, *221*(5), 393-409.e50. https://doi.org/10.1016/j.ajog.2019.04.023

Armeni, A., Anagnostis, P., Armeni, E., Mili, N., Goulis, D., & Lambrinoudaki, I. (2023). Vasomotor symptoms and risk of cardiovascular disease in peri- and postmenopausal women: A systematic review and meta-analysis. *Maturitas*, *171*, 13–20. https://doi.org/10.1016/j.maturitas.2023.02.004

Assmann, G., & Gotto, A. M. (2004). HDL cholesterol and protective factors in atherosclerosis. *Circulation*, *109*(23 Suppl 1), III8-14. https://doi.org/10.1161/01.CIR.0000131512.50667.46

Avis, N. E., Colvin, A., Bromberger, J. T., Hess, R., Matthews, K. A., Ory, M., & Schocken, M. (2009). Change in health-related quality of life over the menopausal transition in a multiethnic cohort of middle-aged women: Study of Women's Health Across the Nation (SWAN). *Menopause*, *16*(5), 860–869. https://doi.org/10.1097/gme.0b013e3181a3cdaf

Avis, N. E., Crawford, S. L., Greendale, G., Bromberger, J. T., Everson-Rose, S. A., Gold, E. B., Hess, R., Joffe, H., Kravitz, H. M., Tepper, P. G., & Thurston, R. C. (2015). Duration of menopausal vasomotor symptoms over the menopause transition. *JAMA Internal Medicine*, *175*(4), 531–539. https://doi.org/10.1001/jamainternmed.2014.8063

Bakrania, K., Yates, T., Rowlands, A. V., Esliger, D. W., Bunnewell, S., Sanders, J., Davies, M., Khunti, K., & Edwardson, C. L. (2016). Intensity thresholds on raw acceleration data: Euclidean Norm Minus One (ENMO) and

Mean Amplitude Deviation (MAD) approaches. *PLoS One*, *11*(10), e0164045. https://doi.org/10.1371/journal.pone.0164045

Barone Gibbs, B., Hivert, M.-F., Jerome, G. J., Kraus, W. E., Rosenkranz, S. K., Schorr, E. N., Spartano, N. L., & Lobelo, F. (2021). Physical activity as a critical component of first-line treatment for elevated blood pressure or cholesterol: who, what, and how?: A scientific statement from the American Heart Association. *Hypertension*, *78*(2), e26–e37. https://doi.org/10.1161/HYP.0000000000000196

Bellicha, A., van Baak, M. A., Battista, F., Beaulieu, K., Blundell, J. E., Busetto, L., Carraça, E. V., Dicker, D., Encantado, J., Ermolao, A., Farpour-Lambert, N., Pramono, A., Woodward, E., & Oppert, J.-M. (2021). Effect of exercise training on weight loss, body composition changes, and weight maintenance in adults with overweight or obesity: An overview of 12 systematic reviews and 149 studies. *Obesity Reviews*, *22*(S4), e13256. https://doi.org/10.1111/obr.13256

Berg, A. H., & Scherer, P. E. (2005). Adipose tissue, inflammation, and cardiovascular disease. *Circulation Research*, *96*(9), 939–949. https://doi.org/10.1161/01.RES.0000163635.62927.34

Berin, E., Hammar, M., Lindblom, H., Lindh-Åstrand, L., Rubér, M., & Spetz Holm, A.-C. (2019). Resistance training for hot flushes in postmenopausal women: A randomised controlled trial. *Maturitas*, *126*, 55–60. https://doi.org/10.1016/j.maturitas.2019.05.005

Blumel, J. E., Castelo-Branco, C., Binfa, L., Gramegna, G., Tacla, X., Aracena, B., Cumsille, M. A., & Sanjuan, A. (2000). Quality of life after the menopause: A population study. *Maturitas*, *34*(1), 17–23. https://doi.org/10.1016/S0378-5122(99)00081-X

Blümel, J. E., Chedraui, P., Baron, G., Belzares, E., Bencosme, A., Calle, A., Danckers, L., Espinoza, M. T., Flores, D., Gomez, G., Hernandez-Bueno, J. A., Izaguirre, H., Leon-Leon, P., Lima, S., Mezones-Holguin, E., Monterrosa, A., Mostajo, D., Navarro, D., Ojeda, E., … for the Collaborative Group for Research of the Climacteric in Latin America (REDLINC). (2011). A large multinational study of vasomotor symptom prevalence, duration, and impact on quality of life in middle-aged women. *Menopause*, *18*(7), 778–785. https://doi.org/10.1097/gme.0b013e318207851d

Bromberger, J. T., Kravitz, H. M., Chang, Y., Randolph, J. F., Avis, N. E., Gold, E. B., & Matthews, K. A. (2013). Does risk for anxiety increase during the menopausal transition? Study of Women's Health Across the Nation (SWAN). *Menopause*, *20*(5), 488–495. https://doi.org/10.1097/GME.0b013e3182730599

Bromberger, J. T., Matthews, K. A., Schott, L. L., Brockwell, S., Avis, N. E., Kravitz, H. M., Everson-Rose, S. A., Gold, E. B., Sowers, M., & Randolph, J. F. (2007). Depressive symptoms during the menopausal transition: The Study of Women's Health Across the Nation (SWAN). *Journal of Affective Disorders*, *103*(1), 267–272. https://doi.org/10.1016/j.jad.2007.01.034

Carr, M. C. (2003). The emergence of the metabolic syndrome with menopause. *The Journal of Clinical Endocrinology & Metabolism*, *88*(6), 2404–2411. https://doi.org/10.1210/jc.2003-030242

Carson, M. Y., & Thurston, R. C. (2023). Vasomotor symptoms and their links to cardiovascular disease risk. *Current Opinion in Endocrine and Metabolic Research*, *30*, 100448. https://doi.org/10.1016/j.coemr.2023.100448

Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. *Public Health Reports*, *100*(2), 126–131.

Castelo-Branco, C., Cancelo, M. J., Villero, J., Nohales, F., & Juliá, M. D. (2005). Management of post-menopausal vaginal atrophy and atrophic vaginitis. *Maturitas*, *52*, 46–52. https://doi.org/10.1016/j.maturitas.2005.06.014

Collaborative Group on Hormonal Factors in Breast Cancer. (2012). Menarche, menopause, and breast cancer risk: Individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *The Lancet Oncology*, *13*(11), 1141–1151. https://doi.org/10.1016/S1470-2045(12)70425-4

Colpani, V., Baena, C., Jaspers, L., Dijk, G. van, Farajzadegan, Z., Dhana, K., Tielemans, M., Voortman, T., Freak-Poli, R., Veloso, G., Chowdhury, R., Kavousi, M., Muka, T., & Franco, O. (2018). Lifestyle factors, cardiovascular disease and all-cause mortality in middle-aged and elderly women: A systematic review and meta-analysis. *European Journal of Epidemiology*, *33*(9), 831–845. https://doi.org/10.1007/s10654-018-0374-z

Cornelissen, V. A., & Smart, N. A. (2013). Exercise training for blood pressure: A systematic review and meta-analysis. *Journal of the American Heart Association*, *2*(1), e004473. https://doi.org/10.1161/JAHA.112.004473

Daley, A., Stokes-Lampard, H., & Macarthur, C. (2011). Exercise for vasomotor menopausal symptoms. *The Cochrane Database of Systematic Reviews*, *5*, CD006108. https://doi.org/10.1002/14651858.CD006108.pub3

De Bruin, J. P., Bovenhuis, H., van Noord, P. A. H., Pearson, P., van Arendonk, J. A. M., te Velde, E. R., & Kuurman, W. W. (2001). The role of genetic factors in age at natural menopause. *Human Reproduction*, *16*(9), 2014–2018. https://doi.org/10.1093/humrep/16.9.2014

Depmann, M., Broer, S. L., van der Schouw, Y. T., Tehrani, F. R., Eijkemans, M. J., Mol, B. W., & Broekmans, F. J. (2016). Can we predict age at natural menopause using ovarian reserve tests or mother's age at menopause? A systematic literature review. *Menopause*, *23*(2), 224–232. https://doi.org/10.1097/GME.0000000000000509

Derby, C. A., Crawford, S. L., Pasternak, R. C., Sowers, M., Sternfeld, B., & Matthews, K. A. (2009). Lipid changes during the menopause transition in relation to age and weight: The Study of Women's Health Across the Nation. *American Journal of Epidemiology*, *169*(11), 1352–1361. https://doi.org/10.1093/aje/kwp043

El Khoudary, S. R. (2017). HDL and the menopause. *Current Opinion in Lipidology*, *28*(4), 328–336. https://doi.org/10.1097/MOL.0000000000000432

El Khoudary, S. R., Aggarwal, B., Beckie, T. M., Hodis, H. N., Johnson, A. E., Langer, R. D., Limacher, M. C., Manson, J. E., Stefanick, M. L., & Allison, M. A. (2020). Menopause transition and cardiovascular disease risk: Implications for timing of early prevention: A scientific statement from the American Heart Association. *Circulation*, *142*(25), e506–e532. https://doi.org/10.1161/CIR.0000000000000912

El Khoudary, S. R., Chen, X., Nasr, A., Billheimer, J., Brooks, M. M., McConnell, D., Orchard, T. J., Crawford, S. L., Matthews, K. A., & Rader, D. J. (2021). HDL (High-Density Lipoprotein) subclasses, lipid content, and function trajectories across the menopause transition: SWAN-HDL Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *41*(2), 951–961. https://doi.org/10.1161/ATVBAHA.120.315355

El Khoudary, S. R., Wang, L., Brooks, M. M., Thurston, R. C., Derby, C. A., & Matthews, K. A. (2016). Increase HDL-C level over the menopausal transition is associated with greater atherosclerotic progression. *Journal of Clinical Lipidology*, *10*(4), 962–969. https://doi.org/10.1016/j.jacl.2016.04.008

Ely, E. K., Gruss, S. M., Luman, E. T., Gregg, E. W., Ali, M. K., Nhim, K., Rolka, D. B., & Albright, A. L. (2017). A national effort to prevent type 2 diabetes: Participant-level evaluation of CDC's National Diabetes Prevention Program. *Diabetes Care*, *40*(10), 1331–1341. https://doi.org/10.2337/dc16-2099

Emerging Risk Factors Collaboration. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *The Lancet*, *375*(9733), 2215–2222. https://doi.org/10.1016/S0140-6736(10)60484-9

Ennour-Idrissi, K., Maunsell, E., & Diorio, C. (2015). Effect of physical activity on sex hormones in women: A systematic review and meta-analysis of randomized controlled trials. *Breast Cancer Research*, *17*(1), 139. https://doi.org/10.1186/s13058-015-0647-3

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., Hegele, R. A., Krauss, R. M., Raal, F. J., Schunkert, H., Watts, G. F., Borén, J., Fazio, S., Horton, J. D., Masana, L., Nicholls, S. J., Nordestgaard, B. G., van de Sluis, B., Taskinen, M.-R., … Catapano, A. L. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal*, *38*(32), 2459–2472. https://doi.org/10.1093/eurheartj/ehx144

Finkelstein, J. S., Lee, H., Karlamangla, A., Neer, R. M., Sluss, P. M., Burnett-Bowie, S.-A. M., Darakananda, K., Donahoe, P. K., Harlow, S. D., Prizand, S. H., Joffe, H., Kumar, A., Martin, D. E., McConnell, D.,

Merrilat, S., Morrison, A., Pastore, L. M., Randolph, J. F., Greendale, G. A., & Santoro, N. (2020). Antimullerian hormone and impending menopause in late reproductive age: The Study of Women's Health Across the Nation. *The Journal of Clinical Endocrinology & Metabolism*, *105*(4), e1862–e1871. https://doi.org/10.1210/clinem/dgz283

Fisher, L. D., & Lin, D. Y. (1999). Time-dependent covariates in the Cox proportional-hazards regression model. *Annual Review of Public Health*, *20*(1), 145–157. https://doi.org/10.1146/annurev.publhealth.20.1.145

Fistarol, M., Rezende, C. R., Campos, A. L. F., Kakehasi, A. M., & Geber, S. (2019). Time since menopause, but not age, is associated with increased risk of osteoporosis. *Climacteric*, *22*(5), 523–526. https://doi.org/10.1080/13697137.2019.1634046

Ford, E. S., Li, C., & Sattar, N. (2008). Metabolic syndrome and incident diabetes: Current state of the evidence. *Diabetes Care*, *31*(9), 1898–1904. https://doi.org/10.2337/dc08-0423

Franco, O. H., Muka, T., Colpani, V., Kunutsor, S., Chowdhury, S., Chowdhury, R., & Kavousi, M. (2015). Vasomotor symptoms in women and cardiovascular risk markers: Systematic review and meta-analysis. *Maturitas*, *81*(3), 353–361. https://doi.org/10.1016/j.maturitas.2015.04.016

Fridolfsson, J., Arvidsson, D., Ekblom-Bak, E., Ekblom, Ö., Bergström, G., & Börjesson, M. (2023). Accelerometer-measured absolute versus relative physical activity intensity: Cross-sectional associations with cardiometabolic health in midlife. *BMC Public Health*, *23*(1), 2322. https://doi.org/10.1186/s12889-023-17281-4

Gao, Y., Zhao, M., Dai, X., Tong, M., Wei, J., & Chen, Q. (2016). The prevalence of endometrial cancer in pre- and postmenopausal Chinese women. *Menopause*, *23*(8), 884–887. https://doi.org/10.1097/GME.0000000000000684

Garcia, L., Pearce, M., Abbas, A., Mok, A., Strain, T., Ali, S., Crippa, A., Dempsey, P. C., Golubic, R., Kelly, P., Laird, Y., McNamara, E., Moore, S., de Sa, T. H., Smith, A. D., Wijndaele, K., Woodcock, J., & Brage, S. (2023). Non-occupational physical activity and risk of cardiovascular disease, cancer and mortality outcomes: A dose-response meta-analysis of large prospective studies. *British Journal of Sports Medicine*, *57*(15), 979–989. https://doi.org/10.1136/bjsports-2022-105669

Georgakis, M. K., Thomopoulos, T. P., Diamantaras, A.-A., Kalogirou, E. I., Skalkidou, A., Daskalopoulou, S. S., & Petridou, E. T. (2016). Association of age at menopause and duration of reproductive period with depression after menopause: A systematic review and meta-analysis. *JAMA Psychiatry*, *73*(2), 139–149. https://doi.org/10.1001/jamapsychiatry.2015.2653

Goeman, J. J. (2010). L-1 penalized estimation in the Cox proportional hazards model. *Biometrical Journal*, *52*(1), 70–84. https://doi.org/10.1002/bimj.200900028

Gold, E. B. (2011). The timing of the age at which natural menopause occurs. *Obstetrics and Gynecology Clinics of North America*, *38*(3), 425–440. https://doi.org/10.1016/j.ogc.2011.05.002

Gold, E. B., Bromberger, J., Crawford, S., Samuels, S., Greendale, G. A., Harlow, S. D., & Skurnick, J. (2001). Factors associated with age at natural menopause in a multiethnic sample of midlife women. *American Journal of Epidemiology*, *153*(9), 865–874. https://doi.org/10.1093/aje/153.9.865

Gold, E. B., Crawford, S. L., Avis, N. E., Crandall, C. J., Matthews, K. A., Waetjen, E. L., Lee, J. S., Thurston, R., Vuga, M., & Harlow, S. D. (2013). Factors related to age at natural menopause: Longitudinal analyses from SWAN. *American Journal of Epidemiology*, *178*(1), 70–83. https://doi.org/10.1093/aje/kws421

Greendale, G. A., Ishii, S., Huang, M.-H., & Karlamangla, A. S. (2013). Predicting the timeline to the final menstrual period: The Study of Women's Health Across the Nation. *The Journal of Clinical Endocrinology & Metabolism*, *98*(4), 1483–1491. https://doi.org/10.1210/jc.2012-3732

Grundy, S. M., Hansen, B., Smith, S. C., Cleeman, J. I., & Kahn, R. A. (2004). Clinical management of metabolic syndrome: Report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Circulation*, *109*(4), 551–556. https://doi.org/10.1161/01.CIR.0000112379.88385.67

Gurka, M. J., Vishnu, A., Santen, R. J., & DeBoer, M. D. (2016). Progression of metabolic syndrome severity during the menopausal transition. *Journal of the American Heart Association*, *5*(8), e003609. https://doi.org/10.1161/JAHA.116.003609

Guthrie, J. R., Dennerstein, L., Taffe, J. R., & Donnelly, V. (2003). Health care-seeking for menopausal problems. *Climacteric*, *6*(2), 112–117. https://doi.org/10.1080/713605371

Halbert, J. A., Silagy, C. A., Finucane, P., Withers, R. T., & Hamdorf, P. A. (1999). Exercise training and blood lipids in hyperlipidemic and normolipidemic adults: A meta-analysis of randomized, controlled trials. *European Journal of Clinical Nutrition*, *53*(7), 514–522. https://doi.org/10.1038/sj.ejcn.1600784

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., Sherman, S., Sluss, P. M., & Villiers, T. J. de. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: Addressing the unfinished agenda of staging reproductive aging. *Menopause*, *19*(4), 387–395. https://doi.org/10.1097/gme.0b013e31824d8f40

Harlow, S. D., Lin, X., & Ho, M. J. (2000). Analysis of menstrual diary data across the reproductive life span Applicability of the bipartite model approach and the importance of within-woman variance. *Journal of Clinical Epidemiology*, *53*(7), 722–733. https://doi.org/10.1016/S0895-4356(99)00202-4

Harrell, F. E. (2015). *Regression modeling strategies* (2nd ed.). Springer.

Harrell, F. E. (2019). *rms: regression modeling strategies*. https://CRAN.R-project.org/package=rms

Hefler, L. A., Grimm, C., Bentz, E.-K., Reinthaller, A., Heinze, G., & Tempfer, C. B. (2006). A model for predicting age at menopause in white women. *Fertility and Sterility*, *85*(2), 451–454. https://doi.org/10.1016/j.fertnstert.2005.07.1300

Hirvensalo, M., Lampinen, P., & Rantanen, T. (1998). Physical exercise in old age: An eight-year follow-up study on involvement, motives, and obstacles among persons age 65-84. *Journal of Aging and Physical Activity*, *6*(2), 157–168.

Holtermann, A., Schnohr, P., Nordestgaard, B. G., & Marott, J. L. (2021). The physical activity paradox in cardiovascular disease and all-cause mortality: The contemporary Copenhagen General Population Study with 104 046 adults. *European Heart Journal*, *42*(15), 1499–1511. https://doi.org/10.1093/eurheartj/ehab087

Hyvärinen, M., Sipilä, S., Kulmala, J., Hakonen, H., Tammelin, T. H., Kujala, U. M., Kovanen, V., & Laakkonen, E. K. (2020). Validity and reliability of a single question for leisure-time physical activity assessment in middle-aged women. *Journal of Aging and Physical Activity*, *28*(2), 231–241. https://doi.org/10.1123/japa.2019-0093

Iorga, A., Cunningham, C. M., Moazeni, S., Ruffenach, G., Umar, S., & Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biology of Sex Differences*, *8*(1), 33. https://doi.org/10.1186/s13293-017-0152-8

Janssen, I., Powell, L. H., Crawford, S., Lasley, B., & Sutton-Tyrrell, K. (2008). Menopause and the metabolic syndrome: The Study of Women's Health Across the Nation. *Archives of Internal Medicine*, *168*(14), 1568–1575. https://doi.org/10.1001/archinte.168.14.1568

Juppi, H.-K., Sipilä, S., Cronin, N. J., Karvinen, S., Karppinen, J. E., Tammelin, T. H., Aukee, P., Kovanen, V., Kujala, U. M., & Laakkonen, E. K. (2020). Role of menopausal transition and physical activity in loss of lean and muscle mass: A follow-up study in middle-aged Finnish women. *Journal of Clinical Medicine*, *9*(5), 1588. https://doi.org/10.3390/jcm9051588

Karvinen, S., Jergenson, M. J., Hyvärinen, M., Aukee, P., Tammelin, T., Sipilä, S., Kovanen, V., Kujala, U. M., & Laakkonen, E. K. (2019). Menopausal status and physical activity are independently associated with cardiovascular risk factors of healthy middle-aged women: Cross-sectional and longitudinal evidence. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00589

Kelley, G. A., & Kelley, K. S. (2009). Impact of progressive resistance training on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. *Preventive Medicine*, *48*(1), 9–19. https://doi.org/10.1016/j.ypmed.2008.10.010

Kelsey, J. L., Gammon, M. D., & John, E. M. (1993). Reproductive factors and breast cancer. *Epidemiologic Reviews*, *15*(1), 36–47. https://doi.org/10.1093/oxfordjournals.epirev.a036115

Kim, C., Slaughter, J. C., Wang, E. T., Appiah, D., Schreiner, P., Leader, B., Calderon-Margalit, R., Sternfeld, B., Siscovick, D., & Wellons, M. (2017). Anti-Müllerian hormone, follicle stimulating hormone, antral follicle count, and risk of menopause within 5 years. *Maturitas*, *102*, 18–25. https://doi.org/10.1016/j.maturitas.2017.04.018

Kim, K.-B., Kim, K., Kim, C., Kang, S.-J., Kim, H. J., Yoon, S., & Shin, Y.-A. (2019). Effects of exercise on the body composition and lipid profile of individuals with obesity: A systematic review and meta-analysis. *Journal of Obesity & Metabolic Syndrome*, *28*(4), 278–294. https://doi.org/10.7570/jomes.2019.28.4.278

Kontis, V., Bennett, J. E., Mathers, C. D., Li, G., Foreman, K., & Ezzati, M. (2017). Future life expectancy in 35 industrialised countries: Projections with a Bayesian model ensemble. *The Lancet*, *389*(10076), 1323–1335. https://doi.org/10.1016/S0140-6736(16)32381-9

Kovanen, V., Aukee, P., Kokko, K., Finni, T., Tarkka, I. M., Tammelin, T., Kujala, U. M., Sipilä, S., & Laakkonen, E. K. (2018). Design and protocol of Estrogenic Regulation of Muscle Apoptosis (ERMA) study with 47 to 55-year-old women's cohort: Novel results show menopause-related differences in blood count. *Menopause*, *25*(9), 1020–1032. https://doi.org/10.1097/GME.0000000000001117

Kraus, W. E., Powell, K. E., Haskell, W. L., Janz, K. F., Campbell, W. W., Jakicic, J. M., Troiano, R. P., Sprow, K., Torres, A., Piercy, K. L., & 2018 Physical Activity Guidelines Advisory Committee. (2019). Physical activity, all-cause and cardiovascular mortality, and cardiovascular disease. *Medicine and Science in Sports and Exercise*, *51*(6), 1270–1281. https://doi.org/10.1249/MSS.0000000000001939

Kravitz, H. M., Ganz, P. A., Bromberger, J., Powell, L. H., Sutton-Tyrrell, K., & Meyer, P. M. (2003). Sleep difficulty in women at midlife: A community survey of sleep and the menopausal transition. *Menopause*, *10*(1), 19.

Kujala, U. M., Kaprio, J., Sarna, S., & Koskenvuo, M. (1998). Relationship of leisure-time physical activity and mortality: The Finnish Twin Cohort. *JAMA*, *279*(6), 440–444. https://doi.org/10.1001/jama.279.6.440

Kyu, H. H., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, M., Abebe, Z., Abil, O. Z., Aboyans, V., Abrham, A. R., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., … Murray, C. J. L. (2018). Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *392*(10159), 1859–1922. https://doi.org/10.1016/S0140-6736(18)32335-3

Laakkonen, E. K., Kovanen, V., & Sipilä, S. (2022). *Data from Estrogenic Regulation of Muscle Apoptosis (ERMA) study* [Data set]. https://doi.org/10.17011/jyx/dataset/83491

Laakkonen, E. K., Kulmala, J., Aukee, P., Hakonen, H., Kujala, U. M., Lowe, D. A., Kovanen, V., Tammelin, T., & Sipilä, S. (2017). Female reproductive factors are associated with objectively measured physical activity in middle-aged women. *PLoS One*, *12*(2), e0172054. https://doi.org/10.1371/journal.pone.0172054

Liang, J., & Shang, Y. (2013). Estrogen and cancer. *Annual Review of Physiology*, *75*(1), 225–240. https://doi.org/10.1146/annurev-physiol-030212-183708

Lou, C., Xiang, G., Weng, Q., Chen, Z., Chen, D., Wang, Q., Zhang, D., Zhou, B., He, D., & Chen, H. (2016). Menopause is associated with articular cartilage degeneration: A clinical study of knee joint in 860 women. *Menopause*, *23*(11), 1239–1246. https://doi.org/10.1097/GME.0000000000000697

Malik, S., Wong, N. D., Franklin, S. S., Kamath, T. V., L'Italien, G. J., Pio, J. R., & Williams, G. R. (2004). Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*, *110*(10), 1245–1250. https://doi.org/10.1161/01.CIR.0000140677.20606.0E

Mandrup, C. M., Egelund, J., Nyberg, M., Lundberg Slingsby, M. H., Andersen, C. B., Løgstrup, S., Bangsbo, J., Suetta, C., Stallknecht, B., & Hellsten, Y. (2017). Effects of high-intensity training on cardiovascular risk factors in premenopausal and postmenopausal women. *American Journal of Obstetrics and Gynecology*, *216*(4), 384.e1-384.e11. https://doi.org/10.1016/j.ajog.2016.12.017

Martin, C. K., Church, T. S., Thompson, A. M., Earnest, C. P., & Blair, S. N. (2009). Exercise dose and quality of life: A randomized controlled trial. *Archives of Internal Medicine*, *169*(3), 269–278. https://doi.org/10.1001/archinternmed.2008.545

Matthews, K. A., Crawford, S. L., Chae, C. U., Everson-Rose, S. A., Sowers, M. F., Sternfeld, B., & Sutton-Tyrrell, K. (2009). 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*, *54*(25), 2366–2373. https://doi.org/10.1016/j.jacc.2009.10.009

Mauvais-Jarvis, F., Clegg, D. J., & Hevener, A. L. (2013). The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine Reviews*, *34*(3), 309–338. https://doi.org/10.1210/er.2012-1055

Migueles, J., Cadenas-Sanchez, C., Ekelund, U., Nyström, C. D., Mora-Gonzalez, J., Löf, M., Labayen, I., Ruiz, J., & Ortega, F. (2017). Accelerometer data collection and processing criteria to assess physical activity and other outcomes: A systematic review and practical considerations. *Sports Medicine*, *47*(9), 1821–1845. https://doi.org/10.1007/s40279-017-0716-0

Mikkelsen, K., Stojanovska, L., Polenakovic, M., Bosevski, M., & Apostolopoulos, V. (2017). Exercise and mental health. *Maturitas*, *106*, 48–56. https://doi.org/10.1016/j.maturitas.2017.09.003

Moilanen, J., Aalto, A.-M., Hemminki, E., Aro, A. R., Raitanen, J., & Luoto, R. (2010). Prevalence of menopause symptoms and their association with lifestyle among Finnish middle-aged women. *Maturitas*, *67*(4), 368–374. https://doi.org/10.1016/j.maturitas.2010.08.007

Mondul, A. M., Rodriguez, C., Jacobs, E. J., & Calle, E. E. (2005). Age at natural menopause and cause-specific mortality. *American Journal of Epidemiology*, *162*(11), 1089–1097. https://doi.org/10.1093/aje/kwi324

Monteleone, P., Mascagni, G., Giannini, A., Genazzani, A. R., & Simoncini, T. (2018). Symptoms of menopause—Global prevalence, physiology and implications. *Nature Reviews Endocrinology*, *14*(4), 199–215. https://doi.org/10.1038/nrendo.2017.180

Muka, T., Oliver-Williams, C., Colpani, V., Kunutsor, S., Chowdhury, S., Chowdhury, R., Kavousi, M., & Franco, O. H. (2016). Association of vasomotor and other menopausal symptoms with risk of cardiovascular disease: A systematic review and meta-analysis. *PLoS One*, *11*(6), e0157417. https://doi.org/10.1371/journal.pone.0157417

Muka, T., Oliver-Williams, C., Kunutsor, S., Laven, J. S. E., Fauser, B. C. J. M., Chowdhury, R., Kavousi, M., & Franco, O. H. (2016). 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*, *1*(7), 767–776. https://doi.org/10.1001/jamacardio.2016.2415

Murabito, J. M., Yang, Q., Fox, C., Wilson, P. W. F., & Cupples, L. A. (2005). Heritability of age at natural menopause in the Framingham Heart Study. *The Journal of Clinical Endocrinology and Metabolism*, *90*(6), 3427–3430. https://doi.org/10.1210/jc.2005-0181

Nguyen, T. M., Do, T. T. T., Tran, T. N., & Kim, J. H. (2020). Exercise and quality of life in women with menopausal symptoms: A systematic review and meta-analysis of randomized controlled trials. *International Journal of Environmental Research and Public Health*, *17*(19), Art. 19. https://doi.org/10.3390/ijerph17197049

Nordestgaard, B. G., & Varbo, A. (2014). Triglycerides and cardiovascular disease. *The Lancet*, *384*(9943), 626–635. https://doi.org/10.1016/S0140-6736(14)61177-6

Oppert, J.-M., Bellicha, A., & Ciangura, C. (2021). Physical activity in management of persons with obesity. *European Journal of Internal Medicine*, *93*, 8–12. https://doi.org/10.1016/j.ejim.2021.04.028

Ossewaarde, M. E., Bots, M. L., Verbeek, A. L. M., Peeters, P. H. M., Graaf, Y. van der, Grobbee, D. E., & van der Schouw, Y. T. (2005). Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology*, *16*(4), 556–562. https://doi.org/10.1097/01.ede.0000165392.35273.d4

Otsuki, M., Kasayama, S., Morita, S., Asanuma, N., Saito, H., Mukai, M., & Koga, M. (2007). Menopause, but not age, is an independent risk factor for fasting plasma glucose levels in nondiabetic women. *Menopause*, *14*(3), 404. https://doi.org/10.1097/01.gme.0000247014.56254.12

Pakarinen, M., Raitanen, J., Kaaja, R., & Luoto, R. (2010). Secular trend in the menopausal age in Finland 1997–2007 and correlation with socioeconomic, reproductive and lifestyle factors. *Maturitas*, *66*(4), 417–422. https://doi.org/10.1016/j.maturitas.2010.04.005

Parente, R. C., Faerstein, E., Celeste, R. K., & Werneck, G. L. (2008). The relationship between smoking and age at the menopause: A systematic review. *Maturitas*, *61*(4), 287–298. https://doi.org/10.1016/j.maturitas.2008.09.021

Parish, S. J., Nappi, R. E., Krychman, M. L., Kellogg-Spadt, S., Simon, J. A., Goldstein, J. A., & Kingsberg, S. A. (2013). Impact of vulvovaginal health on postmenopausal women: A review of surveys on symptoms of vulvovaginal atrophy. *International Journal of Women's Health*, 437–447. https://doi.org/10.2147/IJWH.S44579

Pedersen, B. K., & Saltin, B. (2015). Exercise as medicine – evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scandinavian Journal of Medicine & Science in Sports*, *25*(S3), 1–72. https://doi.org/10.1111/sms.12581

Peltier, M. R., Verplaetse, T. L., Roberts, W., Moore, K., Burke, C., Marotta, P. L., Phillips, S., Smith, P. H., & McKee, S. A. (2020). Changes in excessive alcohol use among older women across the menopausal transition: A longitudinal analysis of the Study of Women's Health Across the Nation. *Biology of Sex Differences*, *11*(1), 1–37. https://doi.org/10.1186/s13293-020-00314-7

Peters, S. A. E., Singhateh, Y., Mackay, D., Huxley, R. R., & Woodward, M. (2016). Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. *Atherosclerosis*, *248*, 123–131. https://doi.org/10.1016/j.atherosclerosis.2016.03.016

Pettee Gabriel, K., Mason, J. M., & Sternfeld, B. (2015). Recent evidence exploring the associations between physical activity and menopausal symptoms in midlife women: Perceived risks and possible health benefits. *Women's Midlife Health*, *1*, 1. https://doi.org/10.1186/s40695-015-0004-9

Pien, G. W., Sammel, M. D., Freeman, E. W., Lin, H., & DeBlasis, T. L. (2008). Predictors of sleep quality in women in the menopausal transition. *Sleep*, *31*(7), 991–999.

Pinheiro, J., Bates, D., BebRoy, S., Sarkar, D., & R Core Team. (2021). *Linear and Nonlinear Mixed Effects Models.* R package version 3.1-152. https://cran.r-project.org/web/packages/nlme/index.html

Politi, M. C., Schleinitz, M. D., & Col, N. F. (2008). Revisiting the duration of vasomotor symptoms of menopause: A meta-analysis. *Journal of General Internal Medicine*, *23*(9), 1507–1513. https://doi.org/10.1007/s11606-008-0655-4

Potthoff, P., Heinemann, L. A., Schneider, H. P., Rosemeier, H. P., & Hauser, G. A. (2000). Menopause-rating skala (MRS): Methodische standardisierung in der deutschen bevölkerung [The Menopause Rating Scale (MRS): Methodological standardization in the German population]. *Zentralblatt Fur Gynakologie*, *122*(5), 280–286.

Prince, S. A., Adamo, K. B., Hamel, M. E., Hardt, J., Gorber, S. C., & Tremblay, M. (2008). A comparison of direct versus self-report measures for assessing physical activity in adults: A systematic review. *The International Journal of Behavioral Nutrition and Physical Activity*, *5*(1), 56. https://doi.org/10.1186/1479-5868-5-56

R Core Team. (2023). *R: A language and environment for statistical computing*. Version 4.3.1. R Foundation for Statistical Computing. https://www.R-project.org/

Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, H. T., Abebe, M., Abebe, Z., Abejie, A. N., Abera, S. F., Abil, O. Z., Abraha, H. N., … Murray, C. J. L. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *392*(10159), 1736–1788. https://doi.org/10.1016/S0140-6736(18)32203-7

Rubin, D. B. (1987). *Multiple imputation for nonresponse in surveys*. Wiley.

Ruiz-Rios, M., & Maldonado-Martin, S. (2022). Physical activity on cardiorespiratory fitness and cardiovascular risk in premenopausal and postmenopausal women: A systematic review of randomized controlled trials. *Menopause*, *29*(10), 1222–1229. https://doi.org/10.1097/GME.0000000000002037

Russell, J. K., Jones, C. K., & Newhouse, P. A. (2019). The role of estrogen in brain and cognitive aging. *Neurotherapeutics*, *16*(3), 649–665. https://doi.org/10.1007/s13311-019-00766-9

Ruth, K. S., Day, F. R., Hussain, J., Martínez-Marchal, A., Aiken, C. E., Azad, A., Thompson, D. J., Knoblochova, L., Abe, H., Tarry-Adkins, J. L., Gonzalez, J. M., Fontanillas, P., Claringbould, A., Bakker, O. B., Sulem, P., Walters, R. G., Terao, C., Turon, S., Horikoshi, M., … Perry, J. R. B. (2021). Genetic insights into biological mechanisms governing human ovarian ageing. *Nature*, *596*(7872), 393–397. https://doi.org/10.1038/s41586-021-03779-7

Santoro, N. (2005). The menopausal transition. *The American Journal of Medicine*, *118*(12), 8–13. https://doi.org/10.1016/j.amjmed.2005.09.008

Santoro, N., Brockwell, S., Johnston, J., Crawford, S., Gold, E., Harlow, S., Matthews, K., & Sutton-Tyrrell, K. (2007). Helping midlife women predict the onset of the final menses: SWAN, the Study of Women's

Health Across the Nation. *Menopause*, *14*(3), 415–424. https://doi.org/10.1097/gme.0b013e31802cc289
Sasaki, J. E., John, D., & Freedson, P. S. (2011). Validation and comparison of ActiGraph activity monitors. *Journal of Science and Medicine in Sport*, *14*(5), 411–416. https://doi.org/10.1016/j.jsams.2011.04.003
Sattar, N., Gill, J. M. R., & Alazawi, W. (2020). Improving prevention strategies for cardiometabolic disease. *Nature Medicine*, *26*(3), 320–325. https://doi.org/10.1038/s41591-020-0786-7
Schneider, H. P., Heinemann, L. A., Rosemeier, H. P., Potthoff, P., & Behre, H. M. (2000). The Menopause Rating Scale (MRS): Comparison with Kupperman index and quality-of-life scale SF-36. *Climacteric*, *3*(1), 50–58. https://doi.org/10.3109/13697130009167599
Schoenaker, D. A., Jackson, C. A., Rowlands, J. V., & Mishra, G. D. (2014). Socioeconomic position, lifestyle factors and age at natural menopause: A systematic review and meta-analyses of studies across six continents. *International Journal of Epidemiology*, *43*(5), 1542–1562. https://doi.org/10.1093/ije/dyu094
Seaman, S., Galati, J., Jackson, D., & Carlin, J. (2013). What is meant by "missing at random"? *Statistical Science*, *28*(2), 257–268. https://doi.org/10.1214/13-STS415
Shephard, R. J. (2003). Limits to the measurement of habitual physical activity by questionnaires. *British Journal of Sports Medicine*, *37*(3), 197–206. https://doi.org/10.1136/bjsm.37.3.197
Shifren, J. L., Gass, M. L. S., & NAMS Recommendations for Clinical Care of Midlife Women Working Group. (2014). The North American Menopause Society Recommendations for clinical care of midlife women. *Menopause*, *21*(10), 1038. https://doi.org/10.1097/GME.0000000000000319
Sirola, J., Kröger, H., Honkanen, R., Jurvelin, J. S., Sandini, L., Tuppurainen, M. T., & Saarikoski, S. (2003). Factors affecting bone loss around menopause in women without HRT: A prospective study. *Maturitas*, *45*(3), 159–167. https://doi.org/10.1016/S0378-5122(03)00150-6
Snieder, H., MacGregor, A. J., & Spector, T. D. (1998). Genes control the cessation of a woman's reproductive Life: A twin study of hysterectomy and age at menopause. *The Journal of Clinical Endocrinology and Metabolism*, *83*(6), 1875–1880. https://doi.org/10.1210/jc.83.6.1875
Snijder, M. B., Dekker, J. M., Visser, M., Bouter, L. M., Stehouwer, C. D. A., Kostense, P. J., Yudkin, J. S., Heine, R. J., Nijpels, G., & Seidell, J. C. (2003). Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: The Hoorn Study. *The American Journal of Clinical Nutrition*, *77*(5), 1192–1197. https://doi.org/10.1093/ajcn/77.5.1192
Song, S., Choi, H., Pang, Y., Kim, O., & Park, H.-Y. (2022). Factors associated with regularity and length of menstrual cycle: Korea Nurses' Health Study. *BMC Women's Health*, *22*(1), 361.

https://doi.org/10.1186/s12905-022-01947-z
Sowers, M. R., Zheng, H., McConnell, D., Nan, B., Harlow, S. D., & Randolph, J. F. (2008). Estradiol rates of change in relation to the final menstrual period in a population-based cohort of women. *The Journal of Clinical Endocrinology and Metabolism*, *93*(10), 3847–3852. https://doi.org/10.1210/jc.2008-1056
Sternfeld, B., Bhat, A. K., Wang, H., Sharp, T., & Quesenberry, C. P. J. (2005). Menopause, physical activity, and body composition/fat distribution in midlife women. *Medicine & Science in Sports & Exercise*, *37*(7), 1195. https://doi.org/10.1249/01.mss.0000170083.41186.b1
Sternfeld, B., Jacobs, M. K., Quesenberry, C. P., Jr., Gold, E. B., & Sowers, M. (2002). Physical activity and menstrual cycle characteristics in two prospective cohorts. *American Journal of Epidemiology*, *156*(5), 402–409. https://doi.org/10.1093/aje/kwf060
Stevenson, J. C., Crook, D., & Godsland, I. F. (1993). Influence of age and menopause on serum lipids and lipoproteins in healthy women. *Atherosclerosis*, *98*(1), 83–90. https://doi.org/10.1016/0021-9150(93)90225-J
Strath, S. J., Kaminsky, L. A., Ainsworth, B. E., Ekelund, U., Freedson, P. S., Gary, R. A., Richardson, C. R., Smith, D. T., Swartz, A. M., & American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health and Cardiovascular, Exercise, Cardiac Rehabilitation and Prevention Committee of the Council on Clinical Cardiology, and Council. (2013). Guide to the assessment of physical activity: Clinical and research applications: a scientific statement from the American Heart Association. *Circulation*, *128*(20), 2259–2279. https://doi.org/10.1161/01.cir.0000435708.67487.da
Sullivan, S. D., Lehman, A., Nathan, N. K., Thomson, C. A., & Howard, B. V. (2017). Age of menopause and fracture risk in post-menopausal women randomized to calcium + vitamin D, hormone therapy, or the combination: Results from the Women's Health Initiative Clinical Trials. *Menopause*, *24*(4), 371–378. https://doi.org/10.1097/GME.0000000000000775
Sun, L., Tan, L., Yang, F., Luo, Y., Li, X., Deng, H.-W., & Dvornyk, V. (2012). Meta-analysis suggests that smoking is associated with an increased risk of early natural menopause. *Menopause*, *19*(2), 126–132. https://doi.org/10.1097/gme.0b013e318224f9ac
Tao, X., Jiang, A., Yin, L., Li, Y., Tao, F., & Hu, H. (2015). Body mass index and age at natural menopause: A meta-analysis. *Menopause*, *22*(4), 469–474. https://doi.org/10.1097/GME.0000000000000324
Tepper, P. G., Randolph, J. F., McConnell, D. S., Crawford, S. L., El Khoudary, S. R., Joffe, H., Gold, E. B., Zheng, H., Bromberger, J. T., & Sutton-Tyrrell, K. (2012). 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, *97*(8), 2872–2880. https://doi.org/10.1210/jc.2012-1422

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling survival data: Extending the Cox model*. Springer.

Thurston, R. C., El Khoudary, S. R., Sutton-Tyrrell, K., Crandall, C. J., Gold, E., Sternfeld, B., Joffe, H., Selzer, F., & Matthews, K. A. (2012). Vasomotor symptoms and lipid profiles in women transitioning through menopause. *Obstetrics and Gynecology*, *119*(4), 753–761. https://doi.org/10.1097/AOG.0b013e31824a09ec

Thurston, R. C., & Joffe, H. (2011). Vasomotor symptoms and menopause: Findings from the Study of Women's Health Across the Nation. *Obstetrics and Gynecology Clinics of North America*, *38*(3), 489–501. https://doi.org/10.1016/j.ogc.2011.05.006

Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society: Series B*, *58*(1), 267–288. https://doi.org/10.1111/j.2517-6161.1996.tb02080.x

Tikhonoff, V., Casiglia, E., Gasparotti, F., & Spinella, P. (2019). The uncertain effect of menopause on blood pressure. *Journal of Human Hypertension*, *33*(6), 421–428. https://doi.org/10.1038/s41371-019-0194-y

Vähä-Ypyä, H., Vasankari, T., Husu, P., Mänttäri, A., Vuorimaa, T., Suni, J., & Sievänen, H. (2015). Validation of cut-points for evaluating the intensity of physical activity with accelerometry-based Mean Amplitude Deviation (MAD). *PLoS One*, *10*(8), e0134813. https://doi.org/10.1371/journal.pone.0134813

Vähä-Ypyä, H., Vasankari, T., Husu, P., Suni, J., & Sievänen, H. (2015). A universal, accurate intensity-based classification of different physical activities using raw data of accelerometer. *Clinical Physiology and Functional Imaging*, *35*(1), 64–70. https://doi.org/10.1111/cpf.12127

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: multivariate imputation by chained equations in R. *Journal of Statistical Software*, *45*(3), 1–67. https://doi.org/10.18637/jss.v045.i03

Wahid, A., Manek, N., Nichols, M., Kelly, P., Foster, C., Webster, P., Kaur, A., Friedemann Smith, C., Wilkins, E., Rayner, M., Roberts, N., & Scarborough, P. (2016). Quantifying the association between physical activity and cardiovascular disease and diabetes: A systematic review and meta-analysis. *Journal of the American Heart Association*, *5*(9), e002495. https://doi.org/10.1161/JAHA.115.002495

Warburton, D. E. R., Nicol, C. W., & Bredin, S. S. D. (2006). Health benefits of physical activity: The evidence. *CMAJ*, *174*(6), 801–809. https://doi.org/10.1503/cmaj.051351

Warren, J. M., Ekelund, U., Besson, H., Mezzani, A., Geladas, N., & Vanhees, L. (2010). Assessment of physical activity – a review of methodologies with reference to epidemiological research: A report of the exercise physiology section of the European Association of Cardiovascular Prevention and Rehabilitation. *European Journal of Cardiovascular*

Prevention and Rehabilitation, *17*(2), 127–139. https://doi.org/10.1097/HJR.0b013e32832ed875

Weissman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S., Hwu, H.-G., Joyce, P. R., Karam, E. G., Lee, C.-K., Lellouch, J., Lépine, J.-P., Newman, S. C., Rubio-Stipec, M., Wells, J. E., Wickramaratne, P. J., Wittchen, H.-U., & Yeh, E.-K. (1996). Cross-national epidemiology of major depression and bipolar disorder. *JAMA*, *276*(4), 293–299. https://doi.org/10.1001/jama.1996.03540040037030

Wewege, M. A., Thom, J. M., Rye, K.-A., & Parmenter, B. J. (2018). Aerobic, resistance or combined training: A systematic review and meta-analysis of exercise to reduce cardiovascular risk in adults with metabolic syndrome. *Atherosclerosis*, *274*, 162–171. https://doi.org/10.1016/j.atherosclerosis.2018.05.002

Williams, R. E., Kalilani, L., DiBenedetti, D. B., Zhou, X., Fehnel, S. E., & Clark, R. V. (2007). Healthcare seeking and treatment for menopausal symptoms in the United States. *Maturitas*, *58*(4), 348–358. https://doi.org/10.1016/j.maturitas.2007.09.006

Woods, N. F., & Mitchell, E. S. (2005). Symptoms during the perimenopause: Prevalence, severity, trajectory, and significance in women's lives. *The American Journal of Medicine*, *118*(12), 14–24. https://doi.org/10.1016/j.amjmed.2005.09.031

Woods, N. F., Smith-DiJulio, K., Percival, D. B., Tao, E. Y., Mariella, A., & Mitchell, E. S. (2008). Depressed mood during the menopausal transition and early postmenopause: Observations from the Seattle Midlife Women's Health Study. *Menopause*, *15*(2), 223. https://doi.org/10.1097/gme.0b013e3181450fc2

Yanes, L. L., & Reckelhoff, J. F. (2011). Postmenopausal hypertension. *American Journal of Hypertension*, *24*(7), 740–749. https://doi.org/10.1038/ajh.2011.71

Zhu, D., Chung, H.-F., Dobson, A. J., Pandeya, N., Giles, G. G., Bruinsma, F., Brunner, E. J., Kuh, D., Hardy, R., Avis, N. E., Gold, E. B., Derby, C. A., Matthews, K. A., Cade, J. E., Greenwood, D. C., Demakakos, P., Brown, D. E., Sievert, L. L., Anderson, D., … Mishra, G. D. (2019). Age at natural menopause and risk of incident cardiovascular disease: A pooled analysis of individual patient data. *The Lancet Public Health*, *4*(11), e553–e564. https://doi.org/10.1016/S2468-2667(19)30155-0

APPENDICES

SUPPLEMENTARY TABLE 1 Participant characteristics in the last measurement conducted before the last known bleeding period in *Study IV*.

	Participants with known ANM (*n* = 105)	**All participants (*n* = 279)**
Hormone levels (n)	105	279
Estradiol [nmol/L]	0.44 ± 0.37	0.42 ± 0.40
Follicle-stimulating hormone [IU/L]	44.4 ± 30.0	31.6 ± 29.7
Menstrual cycle characteristics (n)	67	181
Cycle length mean [d]	49.8 ± 21.9	39.9 ± 19.3
Cycle length standard deviation [d]	30.1 ± 22.5	20.6 ± 21.7
Menopausal symptoms and gynecological history (n)	105	271
Vasomotor symptoms[a]		
No	10 (11)	34 (93)
Yes	90 (94)	66 (178)
Somatic or pain symptoms[a]		
No	56 (59)	73 (197)
Yes	44 (46)	27 (74)
Psychological symptoms[a]		
No	31 (33)	45 (123)
Yes	69 (72)	55 (148)
Urogenital symptoms[a]		
No	56 (59)	64 (174)
Yes	46 (44)	36 (97)
Use of hormonal contraception[a]		
Never	85 (89)	64 (174)
Former	15 (16)	36 (97)
Lifestyle and socioeconomic status (n)	103	268
Alcohol consumption [portions/week]	4.30 ± 4.21	3.62 ± 3.72
Single physical activity question[a,b]		
1	1 (1)	4 (10)
2	5 (5)	8 (22)
3	10 (10)	9 (24)
4	30 (31)	24 (63)
5	42 (43)	38 (102)
6	12 (12)	16 (44)
7	1 (1)	1 (3)
Smoking[a]		
Never	73 (75)	67 (180)
Former	25 (26)	27 (72)
Current	2 (2)	6 (16)
Education[a]		
Primary or secondary	50 (52)	52 (140)
Tertiary	50 (53)	48 (130)
Relationship status[a]		
Single	17 (18)	23 (61)
In a relationship	83 (85)	77 (207)

Data are mean ± standard deviation unless otherwise specified. ANM, Age at natural menopause; [a] Data are % (n); [b] Greater number indicates higher level of physical activity.

SUPPLEMENTARY TABLE 2 Pooled linear mixed-effect model estimates for the association between the number of menopausal symptoms and blood-based biomarker levels for longitudinal study design in *Study II* (n = 298).

		Total cholesterol [mmol/l]		**Low-density lipo-protein cholesterol [mmol/l]**		**High-density lipo-protein cholesterol [mmol/l]**		**Glucose [mmol/l]**		**Triglycerides [mmol/l]**	
		B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Total number of symptoms											
Model 1	Main effect	0.04	[-0.01, 0.09]	0.04	[0.00, 0.08]	0.00	[-0.03, 0.02]	0.01	[-0.02, 0.03]	0.03	[-0.01, 0.06]
	Interaction with time	0.00	[-0.04, 0.05]	0.00	[-0.04, 0.04]	-0.01	[-0.03, 0.01]	-0.01	[-0.04, 0.02]	0.02	[-0.01, 0.05]
Model 2	Main effect	0.00	[-0.05, 0.06]	0.02	[-0.03, 0.06]	-0.02	[-0.04, 0.01]	0.00	[-0.03, 0.03]	0.02	[-0.02, 0.05]
	Interaction with time	0.02	[-0.03, 0.07]	0.01	[-0.03, 0.06]	0.00	[-0.02, 0.02]	-0.01	[-0.04, 0.02]	0.02	[-0.01, 0.05]
Number of vasomotor symptoms											
Model 1	Main effect	0.15*	[0.02, 0.28]	0.12*	[0.01, 0.24]	0.03	[-0.04, 0.09]	-0.02	[-0.10, 0.05]	0.05	[-0.04, 0.15]
	Interaction with time	-0.03	[-0.16, 0.09]	-0.02	[-0.13, 0.09]	-0.02	[-0.08, 0.03]	-0.04	[-0.12, 0.03]	0.07	[-0.01, 0.14]
Model 2	Main effect	0.03	[-0.11, 0.18]	0.05	[-0.08, 0.17]	-0.03	[-0.10, 0.04]	-0.04	[-0.12, 0.04]	0.01	[-0.09, 0.11]
	Interaction with time	0.04	[-0.09, 0.17]	0.03	[-0.08, 0.15]	0.01	[-0.04, 0.07]	-0.02	[-0.11, 0.06]	0.09*	[0.01, 0.17]
Number of psychological symptoms											
Model 1	Main effect	0.05	[-0.06, 0.15]	0.06	[-0.03, 0.16]	-0.01	[-0.07, 0.04]	0.01	[-0.05, 0.07]	0.04	[-0.03, 0.12]
	Interaction with time	0.03	[-0.07, 0.13]	0.02	[-0.06, 0.11]	-0.01	[-0.05, 0.03]	0.00	[-0.06, 0.06]	0.01	[-0.05, 0.07]
Model 2	Main effect	0.01	[-0.10, 0.11]	0.04	[-0.06, 0.13]	-0.03	[-0.09, 0.02]	0.01	[-0.05, 0.07]	0.02	[-0.05, 0.10]
	Interaction with time	0.04	[-0.05, 0.14]	0.03	[-0.06, 0.12]	0.01	[-0.04, 0.05]	0.00	[-0.06, 0.06]	0.00	[-0.06, 0.07]
Number of somatic or pain symptoms											
Model 1	Main effect	0.12	[-0.09, 0.33]	0.09	[-0.09, 0.28]	0.00	[-0.11, 0.11]	0.02	[-0.10, 0.14]	0.11	[-0.04, 0.26]
	Interaction with time	0.00	[-0.20, 0.20]	0.00	[-0.17, 0.17]	-0.04	[-0.13, 0.05]	-0.06	[-0.19, 0.06]	-0.04	[-0.17, 0.08]
Model 2	Main effect	0.05	[-0.16, 0.26]	0.04	[-0.14, 0.23]	-0.03	[-0.14, 0.08]	0.00	[-0.12, 0.12]	0.09	[-0.07, 0.24]
	Interaction with time	0.05	[-0.15, 0.25]	0.03	[-0.14, 0.20]	0.00	[-0.10, 0.09]	-0.06	[-0.19, 0.06]	-0.05	[-0.18, 0.08]
Number of urogenital symptoms											
Model 1	Main effect	0.04	[-0.10, 0.17]	0.04	[-0.08, 0.15]	-0.01	[-0.08, 0.06]	0.04	[-0.04, 0.11]	0.04	[-0.05, 0.14]
	Interaction with time	-0.01	[-0.14, 0.11]	0.00	[-0.11, 0.11]	-0.05	[-0.11, 0.00]	-0.01	[-0.09, 0.06]	0.06	[-0.02, 0.14]
Model 2	Main effect	-0.03	[-0.17, 0.11]	-0.01	[-0.13, 0.11]	-0.04	[-0.11, 0.03]	0.04	[-0.04, 0.11]	0.02	[-0.08, 0.12]
	Interaction with time	0.01	[-0.11, 0.14]	0.01	[-0.10, 0.12]	-0.03	[-0.09, 0.03]	-0.01	[-0.09, 0.07]	0.07	[-0.01, 0.15]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, body mass index, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption. CI, confidence interval; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

SUPPLEMENTARY TABLE 3 Pooled linear mixed-effect model estimates for the association between the number of menopausal symptoms with blood pressure, body adiposity, and physical activity levels for longitudinal study design in *Study II* (*n* = 298).

		Systolic blood pressure [mmHg]		**Diastolic blood pressure [mmHg]**		**Total fat mass [kg][a]**		**Android fat mass [kg][a]**		**PA, Mean MAD [mg]**	
		B	95% CI	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Total number of symptoms											
Model 1	Main effect	0.58	[-0.37, 1.53]	0.40	[-0.12, 0.92]	0.34	[-0.18, 0.85]	0.03	[-0.03, 0.09]	-0.14	[-0.65, 0.38]
	Interaction with time	-0.40	[-1.29, 0.49]	-0.13	[-0.59, 0.34]	0.06	[-0.37, 0.49]	0.01	[-0.04, 0.06]	-0.29	[-0.82, 0.23]
Model 2	Main effect	0.34	[-0.63, 1.31]	0.18	[-0.35, 0.71]	0.18	[-0.36, 0.72]	0.01	[-0.05, 0.07]	0.00	[-0.55, 0.54]
	Interaction with time	-0.35	[-1.25, 0.55]	-0.03	[-0.48, 0.43]	0.15	[-0.30, 0.59]	0.02	[-0.03, 0.07]	-0.30	[-0.82, 0.23]
Number of vasomotor symptoms											
Model 1	Main effect	-0.91	[-3.52, 1.69]	-0.43	[-1.87, 1.02]	0.74	[-0.72, 2.21]	0.08	[-0.08, 0.24]	-0.67	[-2.06, 0.72]
	Interaction with time	-0.15	[-2.68, 2.37]	0.01	[-1.33, 1.35]	-0.21	[-1.49, 1.07]	-0.03	[-0.18, 0.12]	-0.27	[-1.72, 1.19]
Model 2	Main effect	-1.71	[-4.39, 0.98]	-1.16	[-2.62, 0.30]	0.29	[-1.28, 1.86]	0.02	[-0.15, 0.20]	-0.47	[-1.93, 1.00]
	Interaction with time	0.37	[-2.18, 2.91]	0.59	[-0.71, 1.90]	0.09	[-1.26, 1.44]	0.01	[-0.15, 0.16]	-0.35	[-1.80, 1.11]
Number of psychological symptoms											
Model 1	Main effect	1.30	[-0.80, 3.41]	0.93	[-0.22, 2.07]	0.53	[-0.62, 1.67]	0.04	[-0.09, 0.17]	0.03	[-1.07, 1.12]
	Interaction with time	-1.18	[-3.18, 0.83]	-0.55	[-1.61, 0.50]	0.46	[-0.52, 1.45]	0.05	[-0.06, 0.16]	-0.87	[-1.95, 0.22]
Model 2	Main effect	0.96	[-1.11, 3.03]	0.68	[-0.42, 1.78]	0.38	[-0.78, 1.54]	0.02	[-0.10, 0.15]	0.28	[-0.85, 1.42]
	Interaction with time	-1.22	[-3.16, 0.71]	-0.54	[-1.52, 0.45]	0.52	[-0.48, 1.51]	0.06	[-0.05, 0.17]	-0.81	[-1.91, 0.29]
Number of somatic or pain symptoms											
Model 1	Main effect	2.83	[-1.28, 6.94]	0.91	[-1.34, 3.17]	1.06	[-1.22, 3.35]	0.08	[-0.18, 0.34]	-0.02	[-2.20, 2.16]
	Interaction with time	-1.93	[-5.79, 1.93]	-0.31	[-2.35, 1.74]	-0.07	[-2.08, 1.94]	-0.02	[-0.25, 0.22]	0.31	[-1.98, 2.59]
Model 2	Main effect	1.95	[-2.09, 5.98]	0.23	[-1.93, 2.40]	0.62	[-1.69, 2.93]	0.03	[-0.23, 0.29]	0.24	[-1.96, 2.44]
	Interaction with time	-1.86	[-5.76, 2.04]	-0.08	[-2.08, 1.92]	0.15	[-1.90, 2.20]	0.01	[-0.23, 0.25]	0.34	[-1.90, 2.58]
Number of urogenital symptoms											
Model 1	Main effect	1.89	[-0.71, 4.48]	1.47	[0.00, 2.93]	0.43	[-1.00, 1.87]	0.05	[-0.11, 0.22]	-0.39	[-1.80, 1.03]
	Interaction with time	-0.07	[-2.56, 2.42]	0.10	[-1.25, 1.46]	-0.07	[-1.37, 1.22]	0.00	[-0.15, 0.15]	-0.61	[-2.05, 0.84]
Model 2	Main effect	1.45	[-1.12, 4.01]	1.08	[-0.32, 2.48]	0.16	[-1.30, 1.62]	0.02	[-0.14, 0.19]	-0.21	[-1.62, 1.20]
	Interaction with time	0.08	[-2.36, 2.53]	0.26	[-1.00, 1.52]	0.08	[-1.20, 1.36]	0.02	[-0.13, 0.17]	-0.57	[-2.00, 0.85]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, body mass index, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption unless otherwise specified. [a] Model 2 is adjusted for age, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption. CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

SUPPLEMENTARY TABLE 4 Pooled linear regression estimates for the association of baseline physical activity and body composition with the severity of menopausal symptoms at follow-up measurement in *Study III* for participants that did not report any symptoms at baseline (n = 82).

	Total severity of symptoms		**Severity of somato-vegetative symptoms**		**Severity of psychological symptoms**		**Severity of urogenital symptoms**	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
PA, Mean MAD [10 mg]								
Model 1	-0.25	[-1.12, 0.63]	-0.18	[-0.58, 0.23]	0.08	[-0.32, 0.48]	-0.15	[-0.49, 0.20]
Model 2	-0.18	[-1.08, 0.72]	-0.13	[-0.55, 0.29]	0.03	[-0.38, 0.44]	-0.08	[-0.43, 0.27]
Total body mass [kg][a]								
Model 1	0.01	[-0.07, 0.10]	-0.01	[-0.05, 0.03]	0.02	[-0.01, 0.06]	0.00	[-0.04, 0.03]
Model 2	-0.03	[-0.13, 0.06]	-0.03	[-0.07, 0.02]	0.01	[-0.03, 0.05]	-0.02	[-0.05, 0.02]
Body fat mass [kg][a]								
Model 1	0.01	[-0.09, 0.12]	0.00	[-0.06, 0.05]	0.02	[-0.03, 0.06]	0.00	[-0.04, 0.04]
Model 2	-0.04	[-0.39, 0.31]	0.01	[-0.16, 0.17]	-0.05	[-0.20, 0.10]	0.01	[-0.13, 0.14]
Lean body mass [kg][a]								
Model 1	0.03	[-0.09, 0.12]	-0.03	[-0.15, 0.09]	0.08	[-0.02, 0.19]	-0.03	[-0.13, 0.07]
Model 2	0.04	[-0.29, 0.37]	0.00	[-0.16, 0.16]	0.06	[-0.09, 0.20]	-0.02	[-0.15, 0.11]

Model 1 is a simple linear regression model. Model 2 is adjusted for age, menopausal status, use of hormonal preparations, education, smoking, and alcohol consumption. [a] Model 2 is additionally adjusted for body height. CI, confidence interval; PA, physical activity; MAD, mean amplitude deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

ORIGINAL PAPERS

I

METABOLIC HEALTH, MENOPAUSE, AND PHYSICAL ACTIVITY – A 4-YEAR FOLLOW-UP STUDY

by

Hyvärinen, M., Juppi, H.-K., Taskinen, S., Karppinen, J. E., Karvinen, S., Tammelin, T. H., Kovanen, V., Aukee, P., Kujala, U. M., Rantalainen, T., Sipilä, S., & Laakkonen, E. K. 2022

International Journal of Obesity, *46*(3), 544-554

https://doi.org/10.1038/s41366-021-01022-x

ARTICLE OPEN

Check for updates

Epidemiology and Population Health

Metabolic health, menopause, and physical activity—a 4-year follow-up study

Matti Hyvärinen[1,6✉], Hanna-Kaarina Juppi[1,6], Sara Taskinen[2], Jari E. Karppinen[3], Sira Karvinen[1], Tuija H. Tammelin[4], Vuokko Kovanen[3], Pauliina Aukee[5], Urho M. Kujala[3], Timo Rantalainen[1], Sarianna Sipilä[1] and Eija K. Laakkonen[1]

BACKGROUND: In women, metabolic health deteriorates after menopause, and the role of physical activity (PA) in mitigating the change is not completely understood. This study investigates the changes in indicators of metabolic health around menopause and evaluates whether PA modulates these changes.
METHODS: Longitudinal data of 298 women aged 48–55 years at baseline participating in the ERMA and EsmiRs studies was used. Mean follow-up time was 3.8 (SD 0.1) years. Studied indicators of metabolic health were total and android fat mass, waist circumference, waist-to-hip ratio (WHR), systolic (SBP) and diastolic (DBP) blood pressure, blood glucose, triglycerides, serum total cholesterol, and high- (HDL-C) and low-density (LDL-C) lipoprotein cholesterol. PA was assessed by accelerometers and questionnaires. The participants were categorized into three menopausal groups: PRE-PRE (pre- or perimenopausal at both timepoints, $n = 56$), PRE-POST (pre- or perimenopausal at baseline, postmenopausal at follow-up, $n = 149$), and POST-POST (postmenopausal at both timepoints, $n = 93$). Analyses were carried out using linear and Poisson mixed-effect models.
RESULTS: At baseline, PA associated directly with HDL-C and inversely with LDL-C and all body adiposity variables. An increase was observed in total ($B = 1.72$, 95% CI [0.16, 3.28]) and android fat mass (0.26, [0.06, 0.46]), SBP (9.37, [3.34, 15.39]), and in all blood-based biomarkers in the PRE-POST group during the follow-up. The increase tended to be smaller in the PRE-PRE and POST-POST groups compared to the PRE-POST group, except for SBP. The change in PA associated inversely with the change in SBP (−2.40, [−4.34, −0.46]) and directly with the change in WHR (0.72, [0.05, 1.38]).
CONCLUSIONS: In middle-aged women, menopause may accelerate the changes in multiple indicators of metabolic health. PA associates with healthier blood lipid profile and body composition in middle-aged women but does not seem to modulate the changes in most of the studied metabolic health indicators during the menopausal transition.

International Journal of Obesity (2022) 46:544–554; https://doi.org/10.1038/s41366-021-01022-x

INTRODUCTION

Metabolic health is an umbrella term for factors that combine several aspects of cellular, cardiovascular, and cardiorespiratory health and well-being. Body adiposity, anthropometrics, blood pressure, and blood-based biomarkers, such as serum lipids and blood glucose, can be clinically used to evaluate metabolic health. One established method is to use the diagnostic criteria of metabolic syndrome (MetS) [1], a multifaceted disorder predisposing individuals to severe health concerns, such as atherosclerotic heart disease [2] and type II diabetes [3]. Although there is a significant genetic component in the individual variance of metabolic health and emergence of MetS risk factors [4], unhealthy lifestyle habits, such as physical inactivity, are proposed to be a major contributor.

The effect of menopause on metabolic health and the development of MetS has been an increasing area of interest, as nowadays women in Western countries are expected to live in the postmenopausal state for more than one third of their lives [5–7]. Menopausal transition and the accompanying changes in the hormonal milieu (e.g., decrease in the systemic estradiol (E2) levels) have been associated with unfavorable changes in several indicators of metabolic health [8, 9]. For instance, increased blood glucose [10], accumulation of abdominal adiposity [11] as well as unhealthy changes in serum lipids [12] have been reported during menopausal transition. Additionally, menopause-related increase in inflammation marker levels [13] and decrease in muscle mass [14] have an additive negative impact on metabolic health. Therefore, it is not surprising that in women the incidence of MetS and cardiovascular disease increases after menopause [8, 15].

Physical activity (PA) has been widely proposed to improve the metabolic risk factor profile and cardiovascular health. Literature suggests that regular PA decreases total and visceral fat mass,

[1]Gerontology Research Center and Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland. [2]Department of Mathematics and Statistics, University of Jyväskylä, Jyväskylä, Finland. [3]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland. [4]LIKES Research Centre for Physical Activity and Health, Jyväskylä, Finland. [5]Department of Obstetrics and Gynecology, Pelvic Floor Research and Therapy Unit, Central Finland Health Care District, Jyväskylä, Finland. [6]These authors contributed equally: Hyvärinen Matti, Juppi Hanna-Kaarina. ✉email: matti.v.hyvarinen@jyu.fi

Received: 19 July 2021 Revised: 28 October 2021 Accepted: 4 November 2021
Published online: 20 November 2021

improves insulin sensitivity, prevents dyslipidemia, and decreases systolic (SBP) and diastolic (DBP) blood pressure [16–18]. Thus, it can be used for the prevention and treatment of MetS. However, the associations between PA and changes in indicators of metabolic health around menopause are understudied as only few longitudinal studies have been conducted using device-measured PA [12, 19]. Moreover, these studies included only women transitioning from pre- or perimenopause to postmenopause and therefore could not address the contemporaneous aging-related changes.

The objective of this study was to investigate the changes around menopause in serum lipids and glucose, blood pressure, and body adiposity as indicators of metabolic health. Additionally, the aim was to evaluate whether PA modulates these changes using unique longitudinal data from the study of middle-aged women with different menopausal status.

MATERIALS AND METHODS

Study design and population

This study utilized the data from the observational Estrogenic Regulation of Muscle Apoptosis (ERMA) and Estrogen, MicroRNAs and the Risk of Metabolic Dysfunction (EsmiRs) studies. The participant selection for the ERMA study has been described in detail elsewhere [20]. Briefly, out of the 6 878 randomly selected women aged 47–55 years living in Central Finland, 1393 consented and met the inclusion criteria for the baseline measurements (Fig. 1). Exclusion criteria included conditions and the use of medications affecting ovarian function and systemic hormone or inflammatory status, such as bilateral oophorectomy, pregnancy, lactating, severe obesity (self-reported body mass index (BMI) ≥ 35 kg/m^2), or the use of estrogen-containing medications and continuous cortisone or inflammatory drug treatment [20].

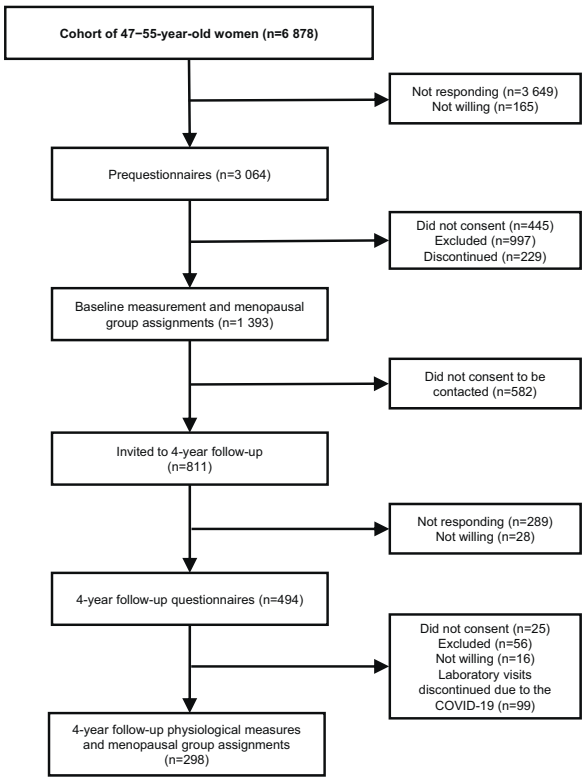

Fig. 1 Flow chart of the study. The flow chart describes the participant enrollment and selection procedure of the ERMA and EsmiRs studies with detailed information about the exclusions and discontinuations during each phase of the study.

The 4-year follow-up measurements were carried out in the EsmiRs study. Out of the 811 participants measured in the ERMA baseline who consented to be contacted, 494 were willing to participate in the EsmiRs questionnaire. Of these participants, 56 were excluded, 25 did not consent, and 16 were not willing to continue to physiological measurements. The participants were excluded due to having more than 7 years from menopause based on the self-reports ($n = 46$), diabetes requiring regular insulin therapy ($n = 2$), severe cardiovascular dysfunction ($n = 2$), or diagnosed with cancer during the follow-up ($n = 6$). Furthermore, 99 participants could not be measured because of the COVID-19 lockdown. Consequently, the final study sample included 298 white women (Fig. 1). To estimate potential selection bias, sensitivity analyses comparing the included sample to the rest of the measured participants ($n = 1095$) at the ERMA baseline for all outcome variables and accelerometer-measured PA were conducted.

The recruiting for the ERMA study was conducted in 2014. The baseline measurements were initiated at the beginning of 2015, and they lasted until the end of 2016. The recruiting for the EsmiRs study started in November 2018 and laboratory measurements were initiated in January 2019. They were discontinued on March 16, 2020 due to the COVID-19 pandemic. The study was performed in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the study was approved by the ethical committee of the Central Finland Health Care District (ERMA 8U/2014 and EsmiRs 9U/2018).

Menopausal status assignments

Blood sampling after overnight fasting was performed in a supine position from the antecubital vein during days 1–5 of menstrual cycle if the cycle was predictable. Serum was separated from whole blood and stored at −80 °C before analysis. Serum concentrations of E2 and follicle-stimulating hormone (FSH) were determined using IMMULITE® 2000 XPi (Siemens Healthineers, Erlangen, Germany) according to the manufacturer's instructions.

Participants were categorized as pre-, peri-, or postmenopausal in both measurements based on the FSH concentrations and self-reported menstrual bleeding diaries using the adapted Stages of Reproductive Aging Workshop (STRAW + 10) guidelines [20]. The participants were divided into three groups based on how their menopausal status changed during the study. PRE-POST group ($n = 149$) consisted of women who experienced menopause during the follow-up period. That is, they were categorized as pre- or perimenopausal in the baseline and postmenopausal in the follow-up measurement. Furthermore, women that were pre- or perimenopausal (PRE-PRE, $n = 56$) or postmenopausal (POST-POST, $n = 93$) in both measurements were designated to their respective groups.

Indicators of metabolic health

Blood pressure and anthropometrics were measured after overnight fasting. SBP and DBP was measured twice in a sitting position after 10 min rest using Omron M6 Comfort (Omron Healthcare, Kioto, Japan) with a standard size cuff and the mean values of the measurements were used. Waist circumference was measured in light underwear midway between the superior iliac spine and the lower rib margin, and hip circumference at the level of the greater trochanters [21]. Body mass and height were measured with standard procedures and BMI was computed by dividing the body mass with squared body height. Total body fat mass and percentage, android fat mass, and fat free mass were assessed with dual-energy X-ray absorptiometry (DXA; LUNAR, GE Healthcare, Chicago, IL, USA).

Serum samples collected during menopausal status assignment were also used for outcome variable analysis. Serum glucose, high- (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, and triglycerides were measured with KONELAB 20 XTi analyzer (Thermo Fischer Scientific, Vantaa, Finland).

The updated ATP III criteria for MetS risk factors was used [1]. The defining levels for risk factors were ≥88 cm for waist circumference, ≥130/≥85 mmHg for blood pressure, ≥1.69 mmol/l for serum triglycerides, ≥5.6 mmol/l for blood glucose, and <1.29 mmol/l for HDL-C.

Physical activity

Accelerometry-measured PA was assessed in both timepoints by triaxial ActiGraph GT3X and wGT3X accelerometers (ActiGraph LLC, Pensacola, FL, USA) with an accompanied diary. Participants were instructed to wear the accelerometers for seven consecutive days on their right hip during

waking hours, except during water-based activities. The data were collected at 60 Hz and the Euclidian norm of the resultant acceleration was computed for each timepoint. Consequently, the mean amplitude deviations (MAD) were computed for non-overlapping 5 s epochs, and the mean MAD value for 1 min epochs were determined based on the 5 s MAD values [22]. The accelerometer-measured MAD (ACC-MAD) reflects the directly measured acceleration and captures the volume of the activity in the entire intensity profile [23] and has been validated against oxygen consumption [24]. Non-wear time was identified as any epoch of at least 60 min with 1 min MAD continuously less than 0.001 g (g denotes the gravitational acceleration on Earth).[1] A minimum of 3 days with a wear time of 10 h or more was regarded as a valid measurement.[2] Finally, the ACC-MAD was determined for wear time for each measurement. For supplementary information, we defined activity with intensity higher or equal to 0.091 g as moderate-to-vigorous physical activity (MVPA) [24]. The ACC-MAD was strongly associated ($r = 0.88$ and $r = 0.79$) with the amount of MVPA and ActiGraph counts [25], respectively.

Additionally, PA was assessed by a self-reported questionnaire (SR-PA) [26]. Briefly, the questionnaire included four questions about the average frequency, intensity, and duration of leisure time PA bouts as well as the average duration of the commuting activity. Based on the responses, the metabolic equivalent (MET) hours per day for leisure time PA was calculated.

Covariates

The use of medications and lifestyle habits were assessed by a structured questionnaire at baseline and follow-up measurements. Responses were used to assess alcohol consumption in portions per week and current smoking status (nonsmoker/smoker). Participants also reported their use of regular prescription medications that were categorized using the Anatomical Therapeutic Chemical (ATC) classification [27]. The use of medications was assessed (non-user/user) separately in preparations affecting blood pressure (ATC C02–05 and C07–09), serum lipids (ATC C10), and thyroid function (ATC H03).

Based on self-reports, participants were classified as being either non-user, only estrogen, only progestogen or combined estrogen and progestogen users. Exogenous sex hormone preparations for contraceptive and hormone replacement therapy use, such as pills, intra-uterine device, patches, and transdermal gels but not intravaginal local estrogen therapy were included. Diet quality score (DQS) was computed based on a food-frequency questionnaire as reported previously [14]. Shortly, DQS consisted of 11 elements characteristic to a healthy diet by the Nordic Nutrition Recommendations 2012. A higher intake of whole-grains, vegetables, fruits and berries, low-fat dairy, fish, and nuts and seeds, and a lower intake of processed grains, processed meats, sugary beverages, fast foods, and sweet or salty snacks were regarded as beneficial. Each component accounted for was worth of 1 point, and the maximum score available was therefore 11 points. A higher DQS score was regarded to reflect a healthier diet. The DQS was partly adapted from Masip et al. [28].

Missing data

The percentage of missing values across the variables separately for each timepoint varied from 0 to 21%. The number of valid measurements for 298 participants in each variable is presented in Table 1. The total number of missing data values was 741 out of 13,708 (5%). Missing data occurred due to invalid or missing measurements as well as unclear or incomplete questionnaire responses. Missing data were assumed to occur at random and multiple imputation was used to create and analyse 50 multiply imputed data sets. Multiple imputation was carried out in R [29] using the "mice" package [30]. All variables measured at the same timepoint and the target variable measurement from the other timepoint were used for imputation of each variable. The number of iterations was set to 50 and passive imputation was used for the derived waist-to-hip ratio (WHR) variable. The model parameters were estimated in each imputed dataset separately and pooled using Rubin's rules [31]. For comparison, we also performed the complete case analysis and there was no notable difference in the results that would have led to different conclusions.

Statistical analysis

The main analyses were carried out using linear and Poisson mixed-effect models with random intercept [32]. For each outcome variable, the fixed effects were time (0 = baseline, 1 = follow-up), menopausal group, ACC-MAD, and interactions between time and group as well as time and ACC-MAD. The interactions were included in the models to study how the change in PA associate with the change in outcome variables during the follow-up. Furthermore, the covariates included as fixed effects were mean centered age at baseline and the use of hormonal preparations. Residual plots, Q–Q plots, and correlation analysis were used for testing the model assumptions. The analyses were carried out in R using the "nlme" [33] and "lme4" [34] packages.

Based on the literature, we identified candidate covariates related to lifestyle habits and the use of medications that may be associated with the outcome variables. Their distributions in the study population are presented in detail in Supplementary Table 1. However, to our consideration, lifestyle habits and the use of antihypertensives, lipid-modifying agents or thyroid therapy do not significantly affect the progression of menopausal transition, and the use may even be caused by the menopause-induced changes in the outcome variables. Thus, only the use of sex hormone therapy was controlled for confounding. Nonetheless, we also performed the analysis including the relevant variables and their interaction with time as covariates, but it did not have a notable effect on the results. Furthermore, we conducted sensitivity analyses for blood lipids and blood pressure by excluding the participants who used lipid-modifying agents and antihypertensives, respectively.

RESULTS

Characteristics of the study population

The average follow-up-time was 3.8 years in all groups (Table 1). At baseline, the participants were slightly overweight with mean BMI of 25.3 ± SD 3.7 and had slightly elevated SBP (132.0 ± 3.7), DBP (84.1 ± 9.2), total cholesterol (5.23 ± 0.91), and LDL-C (3.05 ± 0.80). Other outcome variable means were within the normal range [35, 36]. Participants in the PRE-PRE group were the youngest and had the lowest FSH and highest E2 levels at baseline. Respectively, the participants in the POST-POST group were the oldest and had the highest FSH and lowest E2 levels. The most notable changes in E2 and FSH levels occurred in the PRE-POST group during the follow-up. The percentage of the participants with three or more MetS risk factors was 16% at baseline and at follow-up. The sensitivity analyses using unpaired *T*-test indicated the study sample to have slightly lower blood glucose (5.15 ± 0.45 and 5.28 ± 0.63, t (1387) = 3.319, $p = 0.001$) and higher ACC-MAD (30.2 ± 10.0 and 28.8 ± 8.8, t (782) = −2.044, $p = 0.041$) compared to participants that did not participate in the follow-up. No differences were observed for other outcome variables (data not shown).

Blood-based biomarkers

The PRE-POST group had lower total cholesterol and HDL-C compared to the POST-POST group (Table 2). In the full sample, ACC-MAD was directly associated with HDL-C ($B = 0.06$, 95% CI [0.01, 0.11]) and inversely with LDL-C ($B = -0.11$, 95% CI [−0.21, −0.01]). The levels of all blood-based biomarkers increased during the follow-up in the PRE-POST group and the increase tended to be smaller in the PRE-PRE and, especially, in the POST-POST group. The change in ACC-MAD was not associated with the change in any of the outcome variables measured from blood. The use of progestogen was associated with lower HDL-C, while the combined progestogen and estrogen use was associated with a lower blood glucose. The results did not differ notably when using SR-PA as a PA measure (Supplementary Table 2) or excluding participants using lipid-modifying agents (Supplementary Table 3).

[1]The threshold of 0.001 g was determined based on the correspondence with the self-reported wear time in this population ($r = 0.70$). Self-reported wear time was not used in the analysis due to the invalid and missing entries in the diaries.

[2]Seven valid days were recorded in 89% (462/528) of the measurements.

Table 1. Characteristics of the study population in full sample and separately for each group.

	Full sample			PRE-POST			PRE-PRE			POST-POST		
	BL	**FU**	***Change*[a]**	**BL**	**FU**	***Change*[a]**	**BL**	**FU**	***Change*[a]**	**BL**	**FU**	***Change*[a]**
Age and blood-based biomarkers [*n*]	298	298	*298*	149	149	*149*	56	56	*56*	93	93	*93*
Age [year]	51.3 ± 1.8	55.1 ± 1.8	*3.8 ± 0.1*	51.3 ± 1.7	55.2 ± 1.7	*3.8 ± 0.2*	50.0 ± 1.4	53.8 ± 1.4	*3.8 ± 0.1*	52.1 ± 1.8	55.9 ± 1.8	*3.8 ± 0.1*
Estradiol [nmol/l]	0.38 ± 0.53	0.26 ± 0.28	*−0.12 ± 0.62*	0.47 ± 0.49	0.20 ± 0.21	*−0.27 ± 0.54*	0.52 ± 0.98	0.58 ± 0.39	*0.06 ± 1.09*	0.15 ± 0.10	0.17 ± 0.13	*0.02 ± 0.10*
Follicle-stimulating hormone [IU/l]	39.9 ± 37.1	69.5 ± 37.5	*29.5 ± 40.5*	24.2 ± 21.8	80.3 31.9	*56.0 ± 36.3*	11.7 ± 16.6	18.9 ± 12.3	*7.1 ± 21.5*	82.0 ± 29.1	82.6 ± 29.6	*0.6 ± 24.7*
Total cholesterol [mmol/l]	5.23 ± 0.91	5.67 ± 1.00	*0.43 ± 0.88*	5.14 ± 0.90	5.75 ± 1.02	*0.61 ± 0.75*	5.07 ± 0.78	5.41 ± 0.99	*0.34 ± 0.92*	5.50 ± 0.95	5.69 ± 0.94	*0.20 ± 0.98*
HDL-C [mmol/l]	1.72 ± 0.47	1.91 ± 0.50	*0.19 ± 0.39*	1.68 ± 0.42	1.93 ± 0.48	*0.25 ± 0.39*	1.61 ± 0.38	1.78 ± 0.41	*0.17 ± 0.29*	1.86 ± 0.55	1.97 ± 0.56	*0.11 ± 0.42*
LDL-C [mmol/l]	3.05 ± 0.80	3.41 ± 0.88	*0.37 ± 0.76*	2.98 ± 0.75	3.49 ± 0.91	*0.51 ± 0.67*	2.97 ± 0.75	3.27 ± 0.86	*0.30 ± 0.80*	3.20 ± 0.89	3.37 ± 0.85	*0.17 ± 0.82*
Glucose [mmol/l]	5.15 ± 0.45	5.16 ± 0.62	*0.02 ± 0.55*	5.12 ± 0.43	5.22 ± 0.70	*0.10 ± 0.64*	5.18 ± 0.42	5.20 ± 0.45	*0.03 ± 0.36*	5.18 ± 0.48	5.05 ± 0.55	*−0.13 ± 0.45*
Triglycerides [mmol/l]	1.08 ± 0.61	1.27 ± 0.73	*0.19 ± 0.53*	1.06 ± 0.53	1.31 ± 0.70	*0.25 ± 0.52*	1.03 ± 0.49	1.12±0.54	*0.10 ± 0.41*	1.13 ± 0.76	1.29 ± 0.88	*0.16 ± 0.60*
Blood pressure and anthropometrics [*n*]	249	298	*249*	139	149	*139*	46	56	*46*	64	93	*64*
Systolic blood pressure [mmHg]	132.0 ± 16.3	133.2 ± 18.3	*2.0 ± 13.4*	132.2 ± 17.4	133.6 ± 18.0	*2.0 ± 13.4*	132.0 ± 15.8	133.6 ± 19.1	*2.2 ± 13.6*	131.4 ± 14.6	132.2 ± 18.4	*1.8 ± 13.4*
Diastolic blood pressure [mmHg]	84.1 ± 9.2	81.9 ± 10.0	*−2.1 ± 6.5*	83.9 ± 9.7	82.2 ± 10.4	*−1.5 ± 7.1*	84.3 ± 8.6	81.5 ± 9.3	*−3.1 ± 5.9*	84.3 ± 8.8	81.7 ± 10.0	*−2.8 ± 5.3*
Waist circumference [cm]	82.9 ± 9.7	83.7 ± 10.4	*1.2 ± 4.2*	83.0 ± 10.3	83.8 ± 11.1	*1.3 ± 3.9*	83.2 ± 8.8	84.6 ± 9.6	*0.9 ± 4.8*	82.3 ± 9.0	83.1 ± 9.8	*1.1 ± 4.4*
Waist-to-hip ratio × 100	82.5 ± 6.4	84.2 ± 5.5	*1.7 ± 3.7*	82.4 ± 6.7	83.8 ± 5.4	*1.4 ± 4.1*	82.8 ± 6.0	85.1 ± 5.8	*2.1 ± 3.7*	82.7 ± 6.0	84.4 ± 5.4	*2.2 ± 2.5*
Weight [kg]	69.5 ± 10.8	70.9 ± 11.5	1.8 ± 3.9	69.8 ± 11.0	71.7 ± 12.3	2.3 ± 3.6	70.0 ± 10.3	72.0 ± 10.4	1.5 ± 3.3	68.3 ± 10.8	69.1 ± 10.6	1.0 ± 4.5
Body mass index [kg/m^2]	25.3 ± 3.7	25.8 ± 4.1	0.7 ± 1.4	25.5 ± 3.9	26.2 ± 4.4	0.9 ± 1.4	25.1 ± 3.1	25.9 ± 3.3	0.5 ± 1.2	24.9 ± 3.6	25.3 ± 3.8	0.4 ± 1.6
Body mass index [kg/m^2][b]												
<18.5	0 (1)	1 (3)		0 (0)	1 (1)		0 (0)	0 (0)		2 (1)	2 (2)	
18.5–24.9	53 (131)	44 (131)		51 (71)	44 (66)		52 (24)	38 (21)		56 (36)	47 (44)	
25–29.9	36 (90)	39 (117)		36 (50)	36 (54)		39 (18)	48 (27)		34 (22)	39 (36)	
≥30	11 (27)	16 (47)		13 (18	19 (28)		9 (4)	14 (8)		8 (5)	12 (11)	
Body composition [*n*]	244	292	*240*	137	145	*134*	44	55	*44*	63	92	*62*
Total fat mass [kg]	24.2 ± 8.4	25.9 ± 9.1	*2.0 ± 3.3*	24.7 ± 8.9	26.7 ± 9.8	*2.6 ± 2.8*	23.5 ± 7.3	25.1 ± 7.9	*1.2 ± 3.3*	23.7 ± 7.9	25.1 ± 8.4	*1.3 ± 4.0*
Android fat mass [kg]	2.14 ± 0.91	2.39 ± 1.01	*0.27 ± 0.42*	2.18 ± 0.96	2.47 ± 1.09	*0.35 ± 0.38*	2.06 ± 0.81	2.26 ± 0.87	*0.15 ± 0.35*	2.11 ± 0.88	2.34 ± 0.96	*0.17 ± 0.50*
Total fat percentage [%]	34.0 ± 7.4	35.7 ± 7.6	2.0 ± 2.8	34.3 ± 8.0	36.4 ± 8.0	2.5 ± 2.3	32.9 ± 5.9	34.2 ± 6.6	1.1 ± 3.0	34.1 ± 7.0	35.5 ± 7.4	1.4 ± 3.3
Fat free mass [kg]	45.2 ± 4.3	44.8 ± 4.4	−0.4 ± 1.5	45.2 ± 4.3	44.6 ± 4.5	−0.5 ± 1.6	46.6 ± 4.5	46.8 ± 4.0	0.1 ± 1.3	44.2 ± 4.0	44.0 ± 4.2	−0.5 ± 1.5
	249	298		139	149		46	56		64	93	

Table 1 continued

	Full sample			PRE-POST			PRE-PRE			POST-POST		
	BL	FU	*Change*[a]	BL	FU	*Change*[a]	BL	FU	*Change*[a]	BL	FU	*Change*[a]
Metabolic syndrome risk factors[b] [*n*]												
0	31 (77)	29 (87)		31 (43)	28 (42)		28 (13)	29 (16)		33 (21)	31 (29)	
1	32 (79)	35 (103)		30 (42)	35 (52)		37 (17)	39 (22)		31 (20)	31 (29)	
2	22 (54)	21 (61)		24 (33)	20 (29)		15 (7)	18 (10)		22 (14)	24 (22)	
3	10 (25)	8 (25)		10 (14)	8 (12)		9 (4)	11 (6)		11 (7)	8 (7)	
4	4 (11)	5 (15)		4 (6)	6 (9)		7 (3)	4 (2)		3 (2)	4 (4)	
5	1 (3)	2 (7)		1 (1)	3 (5)		4 (2)	0 (0)		0 (0)	2 (2)	
Accelerometer-measured PA [*n*]	235	283	*222*	134	141	*126*	43	55	*43*	58	87	*53*
ACC-MAD [mg]	30.2 ± 10.0	28.3±8.6	*−1.9 ± 7.2*	29.7 ± 11.1	28.0 ± 8.3	*−1.8 ± 7.8*	31.7 ± 7.8	29.1 ± 8.8	*−3.3 ± 5.8*	30.3 ± 8.6	28.4 ± 9.0	*−0.9 ± 6.7*
Use of hormonal preparations[b] [*n*]	298	298		149	149		56	56		93	93	
Non-user	62 (186)	60 (180)		62 (101)	66 (99)		39 (22)	34 (19)		68 (63)	67 (62)	
Progestogen	38 (112)	19 (56)		32 (48)	15 (23)		61 (34)	41 (23)		32 (30)	11 (10)	
Estrogen	0 (0)	3 (10)		0 (0)	3 (4)		0 (0)	4 (2)		0 (0)	4 (4)	
Progestogen + Estrogen	0 (0)	18 (52)		0 (0)	15 (23)		0 (0)	21 (12)		0 (0)	18 (17)	
Lifestyle habits [*n*]	276	298	*276*	144	149	*144*	53	56	*53*	79	93	*79*
Alcohol consumption [portions/wk]	3.73 ± 3.92	3.24 ± 3.43	*−0.53 ± 2.63*	3.93 ± 3.32	3.68 ± 3.69	*−0.26 ± 2.32*	3.00 ± 2.43	2.54 ± 1.94	*−0.58 ± 1.91*	3.86 ± 5.43	2.98 ± 3.62	*−0.99 ± 3.45*
Diet quality score	5.87 ± 2.45	5.85 ± 2.26	*−0.02 ± 1.90*	5.84 ± 2.52	5.70 ± 2.33	*−0.04 ± 1.90*	5.77 ± 2.28	6.20 ± 2.34	*0.38 ± 1.91*	5.99 ± 2.46	5.88 ± 2.08	*−0.27 ± 1.89*
Smoking[b]												
Non-smoker	95 (262)	94 (280)		95 (136)	94 (140)		96 (51)	96 (54)		95 (75)	92 (86)	95 (262)
Smoker	5 (13)	6 (18)		5 (7)	6 (9)		4 (2)	4 (2)		5 (4)	8 (7)	5 (13)

Data are mean ± SD unless otherwise specified.

PRE-POST participants who were pre- or perimenopausal at baseline and postmenopausal at follow-up, *PRE-PRE* participants who were pre- or perimenopausal in both measurements, *POST-POST* participants who were postmenopausal already at baseline, *BL* baseline measurement, *FU* follow-up measurement, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *PA* physical activity, *ACC-MAD* accelerometer-measured mean amplitude deviation, *mg* milligravity (0.00981 m/s^2).

[a]For participants with baseline and follow-up measurement.

[b]Data are % (*n*).

Table 2. Pooled fixed effect estimates for blood-based biomarkers ($n = 298$).

	Total cholesterol [mmol/l]		HDL-C [mmol/l]		LDL-C [mmol/l]		Glucose [mmol/l]		Triglycerides [mmol/l]	
	B	**95% CI**	***B***	**95% CI**	***B***	**95% CI**	***B***	**95% CI**	***B***	**95% CI**
Intercept (PRE-POST)	5.48***	[5.12, 5.83]	1.54***	[1.38, 1.72]	3.33***	[3.02, 3.62]	5.17***	[4.97, 5.38]	1.24***	[0.99, 1.48]
Main effects										
Group										
PRE-POST (ref.)	–		–		–		–		–	
PRE-PRE	0.03	[−0.27, 0.33]	−0.05	[−0.20, 0.10]	0.05	[−0.22, 0.32]	0.04	[−0.13, 0.22]	0.02	[−0.19, 0.24]
POST-POST	0.33*	[0.09, 0.59]	0.18**	[0.05, 0.31]	0.21	[−0.02, 0.43]	0.06	[−0.08, 0.20]	0.05	[−0.13, 0.22]
ACC-MAD [10 mg]	−0.10	[−0.21, 0,01]	0.06*	[0.01, 0.11]	−0.11*	[−0.21, −0.01]	−0.02	[−0.08, 0.04]	−0.06	[−0.13, 0.01]
Age at baseline [year]	0.03	[−0.03, 0.09]	−0.00	[−0.03, 0.03]	0.02	[−0.03, 0.07]	−0.00	[−0.03, 0.03]	0.03	[−0.01, 0.07]
Use of hormonal preparations										
Non-user (ref.)	–		–		–		–		–	
Progestogen	−0.14	[−0.32, 0.04]	−0.11**	[−0.20, −0.03]	−0.05	[−0.21, 0.10]	0.06	[−0.05, 0.16]	−0.03	[−0.15, 0.09]
Estrogen	−0.19	[−0.68, 0.30]	0.16	[−0.07, 0.38]	−0.16	[−0.59, 0.27]	0.12	[−0.18, 0.42]	−0.13	[−0.45, 0.18]
Progestogen + Estrogen	−0.17	[−0.42, 0.07]	−0.08	[−0.19, 0.03]	−0.15	[−0.37, 0.06]	−0.19*	[−0.33, −0.04]	−0.02	[−0.17, 0.14]
Time (PRE-POST)	0.45*	[0.07, 0.84]	0.35***	[0.18, 0.52]	0.40*	[0.06, 0.74]	0.32**	[0.08, 0.55]	0.28*	[0.03, 0.52]
Interactions										
Time × Group										
Time × PRE-POST (ref.)	–		–		–		–		–	
Time × PRE-PRE	−0.28*	[−0.54, −0.01]	−0.07	[−0.18, 0.05]	−0.21	[−0.44, 0.02]	−0.06	[−0.22, 0.11]	−0.15	[−0.32, 0.01]
Time × POST-POST	−0.42***	[−0.65, −0.20]	−0.15*	[−0.24, −0.05]	−0.34**	[−0.53, −0.14]	−0.22*	[−0.36, −0.08]	−0.09	[−0.23, 0.05]
Time × ACC-MAD	0.05	[−0.07, 0.18]	−0.04	[−0.09, 0.02]	0.04	[−0.07, 0.15]	−0.07	[−0.14, 0.01]	−0.01	[−0.09, 0.07]

HDL-C high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *CI* Confidence interval, *PRE-POST* participants who were pre- or perimenopausal at baseline and postmenopausal at follow-up (reference group), *PRE-PRE* participants who were pre- or perimenopausal in both measurements, *POST-POST* participants who were postmenopausal already at baseline, *ACC-MAD* accelerometer-measured mean amplitude deviation, *mg* milligravity (0.00981 m/s^2), *Time* from baseline to follow-up.
*$p \leq 0.05$; **$p \leq 0.01$; ***$p < 0.001$.

Table 3. Pooled fixed effect estimates for body composition and anthropometrics ($n = 298$).

	Fat mass [kg]		Android fat mass [kg]		Waist circumference [cm]		Waist-to-hip ratio × 100	
	B	**95% CI**	***B***	**95% CI**	***B***	**95% CI**	***B***	**95% CI**
Intercept (PRE-POST)	26.66***	[24.59, 28.73]	2.46***	[2.19, 2.73]	85.41***	[82.81, 88.00]	84.95***	[82.85, 87.04]
Main effects								
Group								
PRE-POST (ref.)	–		–		–		–	
PRE-PRE	0.46	[−2.33, 3.26]	0.04	[−0.27, 0.35]	1.96	[−1.30, 5.22]	1.10	[−0.92, 3.12]
POST-POST	−0.74	[−3.05, 1.57]	−0.04	[−0.30, 0.21]	−0.75	[−3.48, 1.97]	−0.05	[−1.78, 1.68]
ACC-MAD [10 mg]	−0.77**	[−1.27, −0.26]	−0.11**	[−0.18, −0.03]	−0.92**	[−1.60, −0.24]	−0.89**	[−1.51, −0.27]
Age at baseline [year]	0.40	[−0.18, 0.98]	0.04	[−0.02, 0.11]	0.61	[−0.07, 1.29]	0.32	[−0.08, 0.72]
Use of hormonal preparations								
Non-user (ref.)	–		–		–		–	
Progestogen	−0.61	[−1.55, 0.34]	−0.06	[−0.18, 0.05]	−0.28	[−1.48, 0.92]	−0.12	[−1.19, 0.94]
Estrogen	1.02	[−1.08, 3.13]	0.09	[−0.17, 0.36]	1.14	[−1.58, 3.86]	0.19	[−2.37, 2.75]
Progestogen + Estrogen	−0.60	[−1.81, 0.62]	−0.14	[−0.29, 0.01]	−0.47	[−1.94, 0.99]	0.58	[−0.76, 1.92]
Time (PRE-POST)	1.72*	[0.16, 3.28]	0.26**	[0.06, 0.46]	0.44	[−1.64, 2.51]	−0.75	[−2.77, 1.26]
Interactions								
Time × Group								
Time × PRE-POST (ref.)	–		–		–		–	
Time × PRE-PRE	−1.33*	[−2.41, −0.26]	−0.15*	[−0.29, −0.02]	−0.19	[−1.61, 1.22]	0.61	[−0.81, 2.03]
Time × POST-POST	−1.20*	[−2.12, −0.28]	−0.12	[−0.24, 0.00]	−0.47	[−1.76, 0.81]	0.36	[−0.92, 1.64]
Time × ACC-MAD	0.24	[−0.28, 0.75]	0.03	[−0.04, 0.09]	0.23	[−0.46, 0.91]	0.72*	[0.05, 1.38]

CI Confidence interval, *PRE-POST* participants who were pre- or perimenopausal at baseline and postmenopausal at follow-up (reference group), *PRE-PRE* participants who were pre- or perimenopausal in both measurements, *POST-POST* participants who were postmenopausal already at baseline, *ACC-MAD* accelerometer-measured mean amplitude deviation, *mg* milligravity (0.00981 m/s^2), *Time* from baseline to follow-up.
*$p \le 0.05$; **$p \le 0.01$; ***$p < 0.001$.

Body composition and anthropometrics

ACC-MAD was inversely associated with total fat mass ($B = -0.77$, 95% CI [−1.27, −0.26]) and android fat mass ($B = -0.12$, 95% CI [−0.18, −0.03]), waist circumference ($B = -0.92$, 95% CI [−1.60, −0.24]), and WHR ($B = -0.89$, 95% CI [−1.51, −0.27]) in the full sample (Table 3). Total ($B = 1.72$, 95% CI [0.16, 3.28]) and android fat mass ($B = 0.26$, 95% CI [0.06, 0.46]) increased during the follow-up in the PRE-POST group and the change was smaller in the PRE-PRE and POST-POST groups compared to the PRE-POST group. The change in ACC-MAD was directly associated with the change in WHR ($B = 0.72$, 95% CI [0.05, 1.38]). Combined progestogen and estrogen use was associated with lower android fat mass when compared to non-hormone users. The results were relatively similar when using SR-PA, however, SR-PA was not associated with the change in WHR (Supplementary Table 4).

Blood pressure

ACC-MAD was not associated with SBP and DBP in the full sample (Table 4). SBP increased during the follow-up in the PRE-POST group ($B = 9.37$, 95% CI [3.34, 15.39]) and the change did not differ between the groups. Additionally, the change in ACC-MAD was inversely associated with the change in SBP ($B = -2.40$, 95% CI [−4.34, −0.46]), but this association was not observed with SR-PA (Supplementary Table 5). The combined progestogen and estrogen use was associated with lower SBP and DBP. The results did not differ notably when excluding participants using antihypertensives (Supplementary Table 6).

Number of MetS risk factors

In the Poisson mixed-effect models (Table 5), age at baseline was directly associated with the number of MetS risk factors at baseline (exp(B) = 1.07, 95% CI [1.00, 1.14]) in the full sample. The number of risk factors at baseline and the change in the number of risk factors during the follow-up did not differ between the groups. Furthermore, ACC-MAD was not associated with the number of risk factors at baseline nor with the change in the number. The results did not differ notably when using SR-PA (Supplementary Table 7) or excluding participants using lipid-modifying agents or antihypertensives (Supplementary Table 8).

DISCUSSION

In this longitudinal study of middle-aged women, an increase in several indicators of metabolic health, ranging from blood-based biomarkers and SBP to body adiposity, were observed during the follow-up. The increase was greater during menopausal transition, and the rate of change decelerated after menopause, especially in blood-based biomarkers. Higher PA was associated with favorable levels in metabolic health indicators; however, the change in PA did not associate with the rate of change during the follow-up in most of the studied metabolic health indicators. Nonetheless, associations of higher PA with a greater increase in WHR and a smaller increase in SBP were observed. PA was not associated with the number of MetS risk factors.

We observed a significant increase in total cholesterol, HDL-C, LDL-C, triglycerides, and blood glucose in women going through menopause during the follow-up. Several other longitudinal studies have also reported an increase in serum total cholesterol, LDL-C, and triglycerides during the menopausal transition [37–40]. However, the literature on the associations of menopause and HDL-C is more inconsistent. Previous studies have reported HDL-C to increase [12, 19, 39, 41], peak right before menopause [40], as

Table 4. Pooled fixed effect estimates for blood pressure ($n = 298$).

	Systolic blood pressure [mmHg]		Diastolic blood pressure [mmHg]	
	B	**95% CI**	***B***	**95% CI**
Intercept (PRE-POST)	130.91***	[125.01, 136.80]	84.90***	[81.83, 87.96]
Main effects				
Group				
PRE-POST (ref.)	–		–	
PRE-PRE	1.31	[−4.41, 7.03]	1.10	[−2.07, 4.27]
POST-POST	−1.97	[−6.79, 2.85]	0.01	[−2.66, 2.68]
ACC-MAD [10 mg]	0.28	[−1.42, 2.00]	−0.36	[−1.24, 0.51]
Age at baseline [year]	1.10	[−0.03, 2.23]	0.42	[−0.22, 1.06]
Use of hormonal preparations				
Non-user (ref.)	–		–	
Progestogen	0.46	[−2.66, 3.59]	−0.18	[−1.80, 1.43]
Estrogen	1.76	[−6.24, 9.76]	−0.95	[−4.91, 3.01]
Progestogen + Estrogen	−5.55**	[−9.61, −1.49]	−4.33***	[−6.36, −2.30]
Time (PRE-POST)	9.37**	[3.34, 15.39]	−0.16	[−3.11, 2.79]
Interactions				
Time × Group				
Time × PRE-POST (ref.)	–		–	
Time × PRE-PRE	0.62	[−3.67, 4.91]	−0.81	[−2.88, 1.26]
Time × POST-POST	−0.03	[−3.80, 3.73]	−0.73	[−2.57, 1.12]
Time × ACC-MAD	−2.40*	[−4.34, −0.46]	−0.28	[−1.24, 0.68]

CI Confidence interval, *PRE-POST* participants who were pre- or perimenopausal at baseline and postmenopausal at follow-up (reference group), *PRE-PRE* participants who were pre- or perimenopausal in both measurements, *POST-POST* participants who were postmenopausal already at baseline, *ACC-MAD* accelerometer-measured mean amplitude deviation, *mg* milligravity (0.00981 m/s^2), *Time* from baseline to follow-up.
*$p \le 0.05$; **$p \le 0.01$, ***$p < 0.001$.

well as continuously decline during menopausal transition [42]. In addition to increase in HDL-C in the PRE-POST group, higher baseline HDL-C levels and lower increase rate in the postmenopausal group were also observed. These conflicting results suggest that the change in HDL-C during menopausal transition is a complicated process related to, e.g., aging and genetic background. As HDL-C and its antiatherogenic functionality have a major role in promoting cardiovascular health, it is obvious that more detailed longitudinal studies are needed to clarify this process.

Previous findings on associations of menopausal transition and blood glucose are also contradictory. Some longitudinal studies have reported a decrease [15, 19] during the menopausal transition, but in cross-sectional design postmenopausal women have been reported to have higher blood glucose compared to pre- and perimenopausal women [10, 43]. We observed an increase in fasting blood glucose in women going through menopause and the increase was attenuated in the POST-POST group. Our findings indicate that in addition to aging, the increase in blood glucose may be explained by the decreasing E2 levels during menopausal transition, since E2 is known to enhance insulin sensitivity and glucose disposal in women [44].

The observed increase in total and android fat masses in this study are consistent with previous literature [45–47]. The decrease in female sex hormone levels during menopausal transition is proposed to lead to increased accumulation of adipose tissue especially in the waist and visceral area [11, 48], yet the association of menopause to total adipose tissue accumulation is somewhat debated [9]. Although android fat mass increased during the follow-up, we did not observe a change in waist circumference. Similar results have also been reported by others [19, 49]. This indicates a change in the ratio between android lean and fat masses during the follow-up. A comparable change in muscle-to-fat ratio is also observed in total body level during the menopausal transition [11, 47]. Furthermore, we observed an increase in SBP that did not differ between the groups. This finding is supported by the previous review by Taddei [50] that suggested the changes in SBP to be more dependent on age than menopausal status in middle-aged women.

Regular PA is a well-established contributor to a healthier blood lipid profile and body composition also in menopausal women [12, 51]. With both accelerometry-measured and self-reported measures, higher PA was associated with lower levels in blood-based biomarkers and body composition variables but, surprisingly [52], not in blood pressure. When exploring the combined effect of PA and follow-up time, increased PA was associated with an accelerated increase in WHR. This result suggests accelerated decrease in hip circumference in more active women, since the change in PA was not associated with the change in waist circumference. While estradiol levels are associated with both gluteal adipose [53] and muscle mass [14, 54], we suspect that the pronounced decrease in more physically active women is caused especially by the loss of muscle mass due to the potentially higher muscle mass on their gluteal area at baseline. However, in the current study, we were not able to accurately identify the lost tissue type at the hip area. We also observed higher PA to be associated with a smaller increase in SBP during the follow-up. As discussed earlier, the observed changes in SBP may have been related to aging rather than menopausal transition [50], but our results indicate that regular PA may be efficient for controlling SBP in menopausal women similar to other populations [55, 56].

Table 5. Pooled fixed effect estimates for number of metabolic syndrome risk factors ($n = 298$).

	Number of metabolic syndrome risk factors	
	exp (B)	**95% CI**
Intercept (PRE-POST)	1.41	[0.91, 2.16]
Main effects		
Group		
PRE-POST (ref.)	–	
PRE-PRE	1.31	[0.93, 1.84]
POST-POST	0.95	[0.71, 1.28]
ACC-MAD [10 mg]	0.91	[0.79, 1.04]
Age at baseline [year]	1.07*	[1.00, 1.14]
Use of hormonal preparations		
Non-user (ref.)	–	
Progestogen	0.98	[0.78, 1.23]
Estrogen	0.85	[0.41, 1.74]
Progestogen + Estrogen	0.71	[0.50, 1.02]
Time (PRE-POST)	1.59	[0.93, 2.73]
Interactions		
Time × Group		
Time × PRE-POST (ref.)	–	
Time × PRE-PRE	0.77	[0.53, 1.14]
Time × POST-POST	0.93	[0.67, 1.30]
Time × ACC-MAD	0.88	[0.73, 1.06]

CI Confidence interval, *PRE-POST* participants who were pre- or perimenopausal at baseline and postmenopausal at follow-up (reference group), *PRE-PRE* participants who were pre- or perimenopausal in both measurements, *POST-POST* participants who were postmenopausal already at baseline, *ACC-MAD* accelerometer-measured mean amplitude deviation, *mg* milligravity (0.00981 m/s^2), *Time* from baseline to follow-up.
*$p \le 0.05$; **$p \le 0.01$; ***$p < 0.001$.

Although PA was associated with individual indicators of metabolic health, no associations with PA and the number of MetS risk factors or change in the number were observed. This may be caused by the strict cutoff points used in the clinical identification of MetS that does not capture the change unless the cutoff point is reached. Our findings are somewhat contradictory to a recent longitudinal study [57] in which higher PA was associated with lower incidence and better recovery from MetS in middle-aged women. Nonetheless, also in our study, the number of MetS factors tended to be smaller and the increase in the number was slightly lower in more active participants. Thus, PA might be beneficial for preventing the unwanted changes in individual MetS risk factors, but more studies on the associations of PA and number of MetS risk factors during menopause are required.

An interesting additional finding of the study was the observed associations of external hormone use with multiple indicators of metabolic health, highlighted by the distinctive association between the combined use of estrogen and progestogen and lower SBP. The use of hormone replacement therapy has been previously shown to reduce abdominal fat, blood glucose, LDL-to-HDL ratio and blood pressure [58], similar to our results. The individual effects of progestogen use on body composition and metabolic health are less studied, but estrogen is recognized to associate directly with gynoid adipose tissue volume [48, 59], better insulin sensitivity [44], and beneficial effects on vasodilatation and LDL-C concentration [60]. Although our results from exogenous hormone use are mostly in agreement with previous results, the results need to be interpreted with caution, since we did not consider the dosage, the duration of use, or form of the exogenous hormones.

One of the limitations was that the measurements were repeated only once. The homogenous sample of white, middle-aged women with exclusion of women with severe obesity and different medical disorders may limit the generalizability of the results for more heterogeneous populations including participants with disabling conditions. Furthermore, based on the sensitivity analysis, dropouts during the study have caused healthy selection bias particularly towards slightly better glucose control and higher PA which also limits the generalizability of the results. However, this is unlikely to have caused overestimation of the observed unhealthy menopause-related changes in outcome variables. The strengths of the study included the use of accelerometers for PA and DXA for body composition measurements. Additionally, the study design in which women of similar age but different menopausal status were followed for the same amount of time allowed to study the menopause-related changes in outcome variables while taking into account the simultaneous aging.

In conclusion, the results indicate that undesirable changes in blood lipids, body adiposity, and blood pressure occur in middle-aged women, and the rate of change accelerates near menopause, especially in blood lipids. Although habitual PA associated with a healthier blood lipid profile and lower body adiposity in middle-aged women in this study, it did not significantly modulate the menopause-related changes in most of the studied metabolic health indicators. However, higher PA may attenuate the increase in SBP and associate with an accelerated increase in WHR. These results indicate that significant increases in PA around menopause may be needed to counteract the menopause-related changes in blood-based biomarkers and body adiposity. Nonetheless, our findings could encourage professionals working with menopausal women to highlight the importance of PA in the early prevention of hypertension and cardiovascular disease. Further longitudinal studies on the role of PA on the metabolic health during the menopausal transition are needed.

REFERENCES

1. Grundy SM, Hansen B, Smith SC Jr, Cleeman JI, Kahn RA. Clinical Management of Metabolic Syndrome: Report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association Conference on Scientific Issues Related to Management. Circulation. 2004;109:551–6. https://doi.org/10.1161/01.CIR.0000112379.88385.67.
2. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. Circulation. 2004;110:1245–50. https://doi.org/10.1161/01.CIR.0000140677.20606.0E.
3. Ford ES, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. Diabetes Care. 2008;31:1898–904. https://doi.org/10.2337/dc08-0423.
4. Poulsen P, Vaag A, Kyvik K, Beck-Nielsen H. Genetic versus environmental aetiology of the metabolic syndrome among male and female twins. Diabetologia. 2001;44:537–43. https://doi.org/10.1007/s001250051659.
5. Gold EB. The timing of the age at which natural menopause occurs. Obstet Gynecol Clin North Am. 2011;38:425–40. https://doi.org/10.1016/j.ogc.2011.05.002.
6. Dratva J, Gómez Real F, Schindler C, Ackermann-Liebrich U, Gerbase MW, Probst-Hensch NM, et al. Is age at menopause increasing across Europe? Results on age at menopause and determinants from two population-based studies. Menopause. 2009;16:385–94. https://doi.org/10.1097/gme.0b013e31818aefef.
7. Kontis V, Bennett JE, Mathers CD, Li G, Foreman K, Ezzati M. Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble. Lancet. 2017;389:1323–35. https://doi.org/10.1016/S0140-6736(16)32381-9.
8. Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab. 2003;88:2404–11. https://doi.org/10.1210/jc.2003-030242.
9. El Khoudary SR, Aggarwal B, Beckie TM, Hodis HN, Johnson AE, Langer RD, et al. Menopause transition and cardiovascular disease risk: implications for timing of early prevention: a scientific statement from the American Heart Association. Circulation. 2020;142:e506–32. https://doi.org/10.1161/CIR.0000000000000912.

10. Otsuki M, Kasayama S, Morita S, Asanuma N, Saito H, Mukai M, et al. Menopause, but not age, is an independent risk factor for fasting plasma glucose levels in nondiabetic women. Menopause. 2007;14:404–7. https://doi.org/10.1097/01.gme.0000247014.56254.12.
11. Ambikairajah A, Walsh E, Tabatabaei-Jafari H, Cherbuin N. Fat mass changes during menopause: a metaanalysis. Am J Obstet Gynecol. 2019;221:393–409.e50. https://doi.org/10.1016/j.ajog.2019.04.023.
12. Karvinen S, Jergenson MJ, Hyvärinen M, Aukee P, Tammelin T, Sipilä S, et al. Menopausal status and physical activity are independently associated with cardiovascular risk factors of healthy middle-aged women: cross-sectional and longitudinal evidence. Front Endocrinol. 2019;10. https://doi.org/10.3389/fendo.2019.00589.
13. Wang Q, Ferreira DLS, Nelson SM, Sattar N, Ala-Korpela M, Lawlor DA. Metabolic characterization of menopause: cross-sectional and longitudinal evidence. BMC Med. 2018;16:17. https://doi.org/10.1186/s12916-018-1008-8.
14. Juppi H-K, Sipilä S, Cronin NJ, Karvinen S, Karppinen JE, Tammelin TH, et al. Role of menopausal transition and physical activity in loss of lean and muscle mass: a follow-up study in middle-aged Finnish Women. J Clin Med. 2020;9. https://doi.org/10.3390/jcm9051588.
15. 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. Arch Intern Med. 2008;168:1568–75. https://doi.org/10.1001/archinte.168.14.1568.
16. Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: the evidence. CMAJ. 2006;174:801–9. https://doi.org/10.1503/cmaj.051351.
17. Lakka TA, Laaksonen DE. Physical activity in prevention and treatment of the metabolic syndrome. Appl Physiol Nutr Metab. 2007;32:76–88. https://doi.org/10.1139/h06-113.
18. Wewege MA, Thom JM, Rye K, Parmenter BJ. Aerobic, resistance or combined training: a systematic review and meta-analysis of exercise to reduce cardiovascular risk in adults with metabolic syndrome. Atherosclerosis. 2018;274:162–71. https://doi.org/10.1016/j.atherosclerosis.2018.05.002.
19. Abdulnour J, Doucet É, Brochu M, Lavoie J, Strychar I, Rabasa-Lhoret R, et al. The effect of the menopausal transition on body composition and cardiometabolic risk factors: a Montreal-Ottawa New Emerging Team group study. Menopause. 2012;19:760–7. https://doi.org/10.1097/gme.0b013e318240f6f3.
20. Kovanen V, Aukee P, Kokko K, Finni T, Tarkka IM, Tammelin T, et al. Design and protocol of Estrogenic Regulation of Muscle Apoptosis (ERMA) study with 47 to 55-year-old women's cohort: novel results show menopause-related differences in blood count. Menopause. 2018;25:1020–32. https://doi.org/10.1097/GME.0000000000001117.
21. Snijder MB, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Kostense PJ, et al. Associations of hip and thigh circumferences independent of waist circumference with the incidence of type 2 diabetes: the Hoorn Study. Am J Clin Nutr. 2003;77:1192–7. https://doi.org/10.1093/ajcn/77.5.1192.
22. Vähä-Ypyä H, Vasankari T, Husu P, Suni J, Sievänen H. A universal, accurate intensity-based classification of different physical activities using raw data of accelerometer. Clin Physiol Funct Imaging. 2015;35:64–70. https://doi.org/10.1111/cpf.12127.
23. Rowlands AV. Moving forward with accelerometer-assessed physical activity: two strategies to ensure meaningful, interpretable, and comparable measures. Pediatr Exerc Sci. 2018;30:450–6. https://doi.org/10.1123/pes.2018-0201.
24. Vähä-Ypyä H, Vasankari T, Husu P, Mänttäri A, Vuorimaa T, Suni J, et al. Validation of cut-points for evaluating the intensity of physical activity with accelerometry-based mean amplitude deviation (MAD). Plos ONE. 2015;10:e0134813. https://doi.org/10.1371/journal.pone.0134813.
25. Hyvärinen M, Sipilä S, Kulmala J, Hakonen H, Tammelin TH, Kujala UM, et al. Validity and reliability of a single question for leisure-time physical activity assessment in middle-aged women. J Aging Phys Act. 2019;28:231–41. https://doi.org/10.1123/japa.2019-0093.
26. Kujala UM, Kaprio J, Sarna S, Koskenvuo M. Relationship of leisure-time physical activity and mortality: the Finnish twin cohort. JAMA. 1998;279:440–4. https://doi.org/10.1001/jama.279.6.440.
27. The WHO Collaborating Centre for Drug Statistics Methodology. ATC: structure and principles. 2018. https://www.whocc.no/atc/structure_and_principles/. Accessed 29 April 2021.
28. Masip G, Keski-Rahkonen A, Pietiläinen KH, Kujala UM, Rottensteiner M, Väisänen K, et al. Development of a Food-Based Diet Quality Score from a Short FFQ and Associations with Obesity Measures, Eating Styles and Nutrient Intakes in Finnish Twins. Nutrients. 2019;11. https://doi.org/10.3390/nu11112561.
29. R Core Team. R: a language and environment for statistical computing. Version 4.0.5. Vienna, Austria: R Foundation for Statistical Computing; 2021. https://www.R-project.org/.
30. van Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45:1–67. https://doi.org/10.18637/jss.v045.i03.
31. Rubin DB. Multiple imputation for nonresponse in surveys. New Jersey, USA: John Wiley & Sons; 2004.
32. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982;38:963–74. https://doi.org/10.2307/2529876.
33. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. nmle: linear and nonlinear mixed effects models. R package version 3.1–152. 2021. https://cran.r-project.org/web/packages/nlme/index.html.
34. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67:1–48. https://doi.org/10.18637/jss.v067.i01.
35. WHO. Prevention of cardiovascular disease: guidelines for assessment and management of total cardiovascular risk. World Health Organization; 2007. https://apps.who.int/iris/handle/10665/43685.
36. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). Eur Heart J. 2018;39:3021–104. https://doi.org/10.1093/eurheartj/ehy339.
37. Jensen J, Nilas L, Christiansen C. Influence of menopause on serum lipids and lipoproteins. Maturitas. 1990;12:321–31. https://doi.org/10.1016/0378-5122(90)90012-U.
38. Stevenson JC, Crook D, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis. 1993;98:83–90. https://doi.org/10.1016/0021-9150(93)90225-j.
39. Derby CA, Crawford SL, Pasternak RC, Sowers M, Sternfeld B, Matthews KA. Lipid changes during the menopause transition in relation to age and weight: the Study of Women's Health Across the Nation. Am J Epidemiol. 2009;169:1352–61. https://doi.org/10.1093/aje/kwp043.
40. Matthews KA, Crawford SL, Chae CU, Everson-Rose SA, Sowers MF, Sternfeld B, et al. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? J Am Coll Cardiol. 2009;54:2366–73. https://doi.org/10.1016/j.jacc.2009.10.009.
41. Do K-A, Green A, Guthrie JR, Dudley EC, Burger HG, Dennerstein L. Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am J Epidemiol. 2000;151:584–93. https://doi.org/10.1093/oxfordjournals.aje.a010246.
42. Gurka MJ, Vishnu A, Santen RJ, DeBoer MD. Progression of metabolic syndrome severity during the menopausal transition. J Am Heart Assoc. 2016;5. https://doi.org/10.1161/JAHA.116.003609.
43. Lynch NA, Ryan AS, Berman DM, Sorkin JD, Nicklas BJ. Comparison of VO2max and disease risk factors between perimenopausal and postmenopausal women. Menopause. 2002;9:456–62. https://doi.org/10.1097/00042192-200211000-00012.
44. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev. 2013;34:309–38. https://doi.org/10.1210/er.2012-1055.
45. Ho SC, Wu S, Chan SG, Sham A. Menopausal transition and changes of body composition: a prospective study in Chinese perimenopausal women. Int J Obes. 2010;34:1265–74. https://doi.org/10.1038/ijo.2010.33.
46. Razmjou S, Abdulnour J, Bastard J, Fellahi S, Doucet É, Brochu M, et al. Body composition, cardiometabolic risk factors, physical activity, and inflammatory markers in premenopausal women after a 10-year follow-up: a MONET study. Menopause. 2018;25:89–97. https://doi.org/10.1097/GME.0000000000000951.
47. Greendale GA, Sternfeld B, Huang M, Han W, Karvonen-Gutierrez C, Ruppert K, et al. Changes in body composition and weight during the menopause transition. JCI Insight. 2019;4. https://doi.org/10.1172/jci.insight.124865.
48. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 2009;297:271–88. https://doi.org/10.1152/ajpendo.90920.2008.
49. Franklin RM, Ploutz-Snyder L, Kanaley JA. Longitudinal changes in abdominal fat distribution with menopause. Metabolism. 2009;58:311–5. https://doi.org/10.1016/j.metabol.2008.09.030.
50. Taddei S. Blood pressure through aging and menopause. Climateric. 2009;12:36–40. https://doi.org/10.1080/13697130903004758.
51. Sternfeld B, Bhat AK, Wang H, Sharp T, Quesenberry CP. Menopause, physical activity, and body composition/fat distribution in midlife women. Med Sci Sports Exerc. 2005;37:1195–202. https://doi.org/10.1249/01.mss.0000170083.41186.b1.
52. Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. Hypertension. 2005;46:667–75. https://doi.org/10.1161/01.HYP.0000184225.05629.51.
53. Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating α2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor α. implications for the female fat distribution. J Clin Endocrinol Metab. 2004;89:1869–78. https://doi.org/10.1210/jc.2003-031327.
54. Sipilä S, Törmäkangas T, Sillanpää E, Aukee P, Kujala UM, Kovanen V, et al. Muscle and bone mass in middle-aged women: role of menopausal status and physical

activity. J Cachexia Sarcopenia Muscle. 2020;11:678–709. https://doi.org/10.1002/jcsm.12547.
55. Hu G, Barengo NC, Tuomilehto J, Lakka TA, Nissinen A, Jousilahti P. Relationship of physical activity and body mass index to the risk of hypertension: a prospective study in Finland. Hypertension. 2004;43:25–30. https://doi.org/10.1161/01.HYP.0000107400.72456.19.
56. Börjesson M, Onerup A, Lundqvist S, Dahlöf B. Physical activity and exercise lower blood pressure in individuals with hypertension: narrative review of 27 RCTs. Br J Sports Med. 2016;50:356–61. https://doi.org/10.1136/bjsports-2015-095786.
57. Ward E, Gold EB, Johnson WO, Ding F, Chang P, Song P, et al. Patterns of cardiometabolic health as midlife women transition to menopause: a prospective multiethnic study. J Clin Endocrinol Metab. 2019;104:1404–12. https://doi.org/10.1210/jc.2018-00941.
58. Salpeter SR, Walsh JME, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab. 2006;8:538–54. https://doi.org/10.1111/j.1463-1326.2005.00545.x.
59. Lindberg UB, Crona N, Silfverstolpe G, Björntorp P, Rebuffé-Scrive M. Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. Horm Metab Res. 1990;22:345–51. https://doi.org/10.1055/s-2007-1004917.
60. Dubey RK, Jackson EK. Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Renal Physiol. 2001;280. https://doi.org/10.1152/ajprenal.2001.280.3.F365.

ACKNOWLEDGEMENTS

The authors would like to thank Anja Ahlgren, Eeva-Maija Palonen, Mervi Matero, Hanne Tähti, and the other laboratory staff in Faculty of Sport and Health Sciences for their invaluable help with the data collection, as well as the participants in ERMA and EsmiRs studies who volunteered their time and effort.

AUTHOR CONTRIBUTIONS

MH and HKJ prepared the original draft and share the first authorship of the paper. MH was the major contributor in statistical analyses, while ST offered guidance. MH analyzed the raw accelerometer data and HKJ processed the DXA data. JEK was responsible for forming the diet analysis used. VK and EKL obtained funding for the project. ST, JEK, SK, THT, VK, PA, UMK, TR, SS, and EKL all gave their professional effort in the writing process. All authors read and approved the final paper.

FUNDING

The study was supported by the Academy of Finland (grant numbers 275323 to VK and 309504, 314181 and 335249 to EKL). TR was an Academy Research Fellow during the preparation of this paper (Academy of Finland grant numbers 321336 and 328818). Open Access funding provided by University of Jyväskylä (JYU).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41366-021-01022-x.

Correspondence and requests for materials should be addressed to Matti Hyvärinen.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

II

MENOPAUSAL SYMPTOMS AND CARDIOMETABOLIC RISK FACTORS IN MIDDLE-AGED WOMEN: A CROSS-SECTIONAL AND LONGITUDINAL STUDY WITH 4-YEAR FOLLOW-UP

by

Hyvärinen, M., Karvanen, J., Juppi, H.-K., Karppinen, J. E., Tammelin, T. H., Kovanen, V., Aukee, P., Sipilä, S., Rantalainen, T., & Laakkonen, E. K. 2023

Maturitas, *174*, 39-47

https://doi.org/10.1016/j.maturitas.2023.05.004

Maturitas 174 (2023) 39–47

Contents lists available at ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

Original article

Menopausal symptoms and cardiometabolic risk factors in middle-aged women: A cross-sectional and longitudinal study with 4-year follow-up

Matti Hyvärinen [a,*], Juha Karvanen [b], Hanna-Kaarina Juppi [a], Jari E. Karppinen [c], Tuija H. Tammelin [d], Vuokko Kovanen [c], Pauliina Aukee [e], Sarianna Sipilä [a], Timo Rantalainen [a], Eija K. Laakkonen [a]

[a] Gerontology Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35, 40014, Finland
[b] Department of Mathematics and Statistics, University of Jyväskylä, P.O. Box 35, 40014, Finland
[c] Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35, 40014, Finland
[d] LIKES, JAMK University of Applied Sciences, Piippukatu 2, 40100 Jyväskylä, Finland
[e] Department of Obstetrics and Gynecology, The Wellbeing Services County of Central Finland, 40620 Jyväskylä, Finland

ARTICLE INFO

Keywords:
Menopause
Night sweats
Hot flashes
Cardiovascular disease
Obesity
Inactivity

ABSTRACT

Objective: To study associations of menopausal symptoms with cardiometabolic risk factors.
Study design: A cross-sectional and longitudinal study of a representative population sample of 1393 women aged 47–55 years with a sub-sample of 298 followed for four years. The numbers of vasomotor, psychological, somatic or pain, and urogenital menopausal symptoms were ascertained at baseline through self-report. Their associations with cardiometabolic risk factors were studied using linear regression and linear mixed-effect models. Models were adjusted for age, menopausal status, body mass index, the use of hormonal preparations, education, smoking, and alcohol consumption.
Main outcome measures: Cardiometabolic risk factors included total cholesterol, low-density and high-density lipoprotein cholesterol, blood pressure, glucose, triglycerides, total and android fat mass, and physical activity.
Results: All cholesterol and fat mass measures had modest positive associations with menopausal symptoms. The number of vasomotor symptoms, in particular, was associated with total cholesterol (B = 0.13 mmol/l, 95 % CI [0.07, 0.20]; 0.15 mmol/l [0.02, 0.28]) and low-density lipoprotein cholesterol (0.08 mmol/l [0.03, 0.14]; 0.12 mmol/l [0.01, 0.09]) in cross-sectional and longitudinal analyses, respectively. However, these associations disappeared after adjusting for confounders. The number of symptoms was not associated with blood pressure, glucose, triglycerides, and physical activity. Menopausal symptoms at baseline did not predict the changes in the risk factors during the follow-up.
Conclusions: Menopausal symptoms may not be independently associated with cardiometabolic risk, and they do not seem to predict the changes in risk factors during the menopausal transition.

1. Introduction

The menopausal transition marks the time in women's life characterized by the hormonal changes including decrease in systemic estradiol (E2) and increase in follicle-stimulating hormone (FSH) levels, and it has been linked to adverse metabolic changes and a significant increase in the risk of cardiovascular disease [1]. During the menopausal transition, women experience various symptoms, such as sleep disturbances, depression, anxiety, sexual dysfunction, and vasomotor symptoms (VMS) including hot flushes and night sweats [2].

The incidence of cardiovascular disease and metabolic syndrome increases during the menopausal transition [3,4]. This is at least partially due to the unfavorable changes in cardiometabolic disease (CMD) risk factors that are independent of the effect of aging [5,6]. For instance, the menopausal transition has been associated with an increase in blood lipids, blood glucose, blood pressure, and total and abdominal

* Corresponding author at: Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35 (LL232), 40014, Finland.
E-mail addresses: matti.v.hyvarinen@jyu.fi (M. Hyvärinen), juha.t.karvanen@jyu.fi (J. Karvanen), hanna.k.juppi@jyu.fi (H.-K. Juppi), jari.e.j.karppinen@jyu.fi (J.E. Karppinen), tuija.tammelin@jamk.fi (T.H. Tammelin), pauliina.aukee@hyvaks.fi (P. Aukee), sarianna.sipila@jyu.fi (S. Sipilä), timo.rantalainen@jyu.fi (T. Rantalainen), eija.k.laakkonen@jyu.fi (E.K. Laakkonen).

https://doi.org/10.1016/j.maturitas.2023.05.004
Received 27 October 2022; Received in revised form 8 May 2023; Accepted 17 May 2023
Available online 23 May 2023

fat mass [5,6]. As CMD is one of the leading causes of death in women [3], the early identification of individuals with highlighted risk of developing CMD after menopause could have significant clinical and public health implications.

Menopausal symptoms have been associated with increased CMD risk. This association may differ with age and is mainly explained by the unfavorable risk factor profile in symptomatic women [7–9]. However, it is still unclear whether menopausal symptoms are an independent risk factor for metabolic syndrome [10]. Furthermore, the link between menopausal symptoms and CMD risk is mostly derived from cross-sectional studies, highlighting the need for longitudinal studies on this topic [9–11]. Only one previous study with three different sub-studies has explored longitudinal changes in CMD risk factors during the menopausal transition based on the menopausal symptoms and the focus of those studies was only on the VMS [12–14].

The objective of this study was to investigate the associations of diverse menopausal symptoms with CMD risk factors in cross-sectional and longitudinal study designs. The risk factors of interest included several blood-based biomarkers, blood pressure, and measures of body composition and physical activity. The focus of the study was to explore if the prevalence of menopausal symptoms could be used to predict future cardiometabolic health in middle-aged women.

2. Materials and methods

2.1. Study design and population

This observational study was part of the Estrogenic regulation of Muscle Apoptosis (ERMA) and its follow-up the Estrogen MicroRNAs and the risk of Metabolic Dysfunction (EsmiRs) study (dataset: 10.17011/jyx/dataset/83491). The baseline measurements in ERMA were conducted in 2015–2016. The follow-up measurements in EsmiRs were initiated at the beginning of 2019, and they were discontinued on March 16, 2020 due to the pandemic. The studies were performed in accordance with the Declaration of Helsinki, and they were approved by the ethical committee of the Central Finland Health Care District. All participants provided written informed consent.

The participant selection procedures for both cross-sectional [15] and longitudinal [6] parts of the study have been described elsewhere. Briefly, 1393 women aged 47–55 years living in the Central Finland participated in the baseline ERMA measurements. The ERMA exclusion criteria included conditions affecting ovarian function, systemic hormone levels or inflammatory status, such as bilateral oophorectomy, pregnancy, lactating and severe obesity (self-reported body mass index (BMI) $\geq 35\ kg/m^2$). Additionally, participants using estrogen-containing medications and continuous cortisone or inflammatory drug treatment were excluded. The loss of participants during the study is illustrated in Fig. 1.

The cross-sectional analyses of the study were carried out using the data from the ERMA baseline measurement (n = 1393), and the longitudinal analyses utilize data from the ERMA baseline and EsmiRs 4-year follow-up measurements (n = 298). Menopausal symptoms were only assessed at baseline, but all other covariates of the study are assessed in both time points.

2.2. Menopausal symptoms

At baseline, participants were asked to report if they had experienced any of the symptoms related to menopause based on the list of 10 predetermined symptoms (Table 1) [15,16]. The questionnaire also included the option to describe a maximum of three additional symptoms [15]. Reported symptoms were classified into four categories (vasomotor, psychological, somatic or pain, and urogenital) [16]. To consider the quantity of the distinct symptoms as well as the prevalence

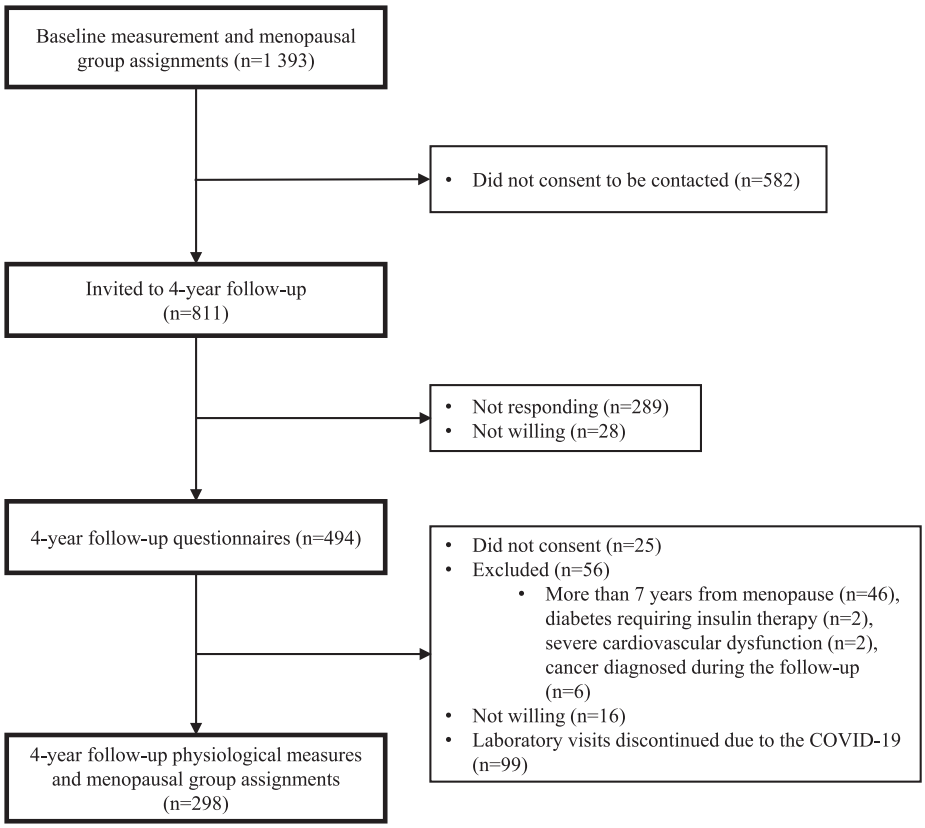

Fig. 1. The flow chart of the study.

Table 1
Classification of menopausal symptoms.

Symptom category (maximum number of symptoms[a])	Symptoms included in the questionnaire	Additional self-described symptoms
Vasomotor symptoms (3)	Sweating Hot flashes	Cold flashes Heart palpations Coldness
Psychological symptoms (4)	Sleeplessness Tiredness Mood swings	Memory problems Irritability Inability to concentrate Weepiness
Somatic or pain symptoms (3)	Headache Aching joints	Stomach pain Migraine Hip pain Muscle pain Breast pain Dizziness Swelling Weakness
Urogenital symptoms (4)	Vaginal symptoms Urinary tract symptoms Lack of sexual desire	Vaginal infection Urinary tract infection Vaginal dryness

[a] The number of symptoms was determined as the sum of all reported predetermined symptoms in each category with one additional symptom for participants who reported one or more additional self-described symptoms in that category.

of the symptoms, the number of reported symptoms in each category was determined.

2.3. Cardiometabolic risk factors

Total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), glucose, and triglycerides were measured from the serum samples using KONELAB 20XTi analyzer (Thermo Fischer Scientific, Vantaa, Finland). Blood pressure was measured in a sitting position after a 10-minute rest using Omron M6 Comfort (Omron Healthcare, Kioto, Japan) and the mean value of two measurements was used for systolic (SBP) and diastolic blood pressure (DBP). Total and android fat mass were assessed with dual energy X-ray absorptiometry (DXA; LUNAR, GE Healthcare, Chicago, IL, USA). All measurements were carried out after overnight fasting and during the first five days of the menstrual cycle for participants with a predictable menstrual cycle.

Physical activity was assessed with accelerometers using the mean amplitude deviation method (ACC-MAD). The detailed description of the procedure is described elsewhere [6]. Briefly, participants used hip-worn accelerometers (ActiGraph GT3X and wGT3X; Actigraph LLC, Pensacola, FL, USA) for seven consecutive days during waking hours, except for water-based activities. The data were collected at 60 Hz, and the Euclidian norm of the resultant acceleration was computed for each time point. Finally, mean amplitude deviation values were computed for non-overlapping five-second epochs, and ACC-MAD was computed as their mean value.

2.4. Covariates

Serum concentrations of E2 and FSH were measured using IMMULITE® 2000 XPi (Siemens Healthineers, Erlangen, Germany). Participants were categorized as pre-, peri-, or postmenopausal based on the FSH concentrations and self-reported menstrual bleeding diaries using the adapted Stages of Reproductive Aging Workshop (STRAW +10) guidelines [15]. BMI was calculated using body mass and height measured with standard procedures.

Structured questionnaires were used to assess education (primary or secondary/tertiary), smoking status (non-smoker/smoker) and alcohol consumption in portions per week. The use of hormonal preparations and medications was assessed based on self-reports. Regarding hormonal preparations, the participants were classified as non-user, only progestogen, only estrogen, and combined estrogen and progestogen users. All exogenous sex hormone preparations for contraceptive and hormone therapy use were included, except for the intravaginal local estrogen therapy. The regular prescription medication users were classified using the Anatomical Therapeutic Chemical (ATC) classification separately for participants using preparations affecting serum lipids (ATC C10) and blood pressure (ATC C02-C05 and C07-C09).

2.5. Missing data

The percentage of missing values across the variables varied from 0 to 44 at baseline (n = 1393) and from 0 to 7 at follow-up (n = 298) (Table 2). The missing data values were 7238 out of 43,869 (16 %). Missing data occurred due to invalid or missing measurements and unclear and incomplete questionnaire responses. Missing data were assumed to occur at random and multiple imputation was used to create 50 multiply imputed data sets. Multiple imputation was carried out recursively. That is, baseline values for each variable were imputed first, and the imputed baseline measurement values were then utilized for the imputation of follow-up measurement [17]. Variables measured at the same timepoint and the target variable measurement from the other timepoint were used for the imputation of each variable. The number of iterations for chained equations [18] was set to 50, and passive imputation was used for the derived variable BMI. The model parameters were estimated separately for each data set. Multiple imputation and pooling of the model estimates were carried out in R [19] using the standard settings of the "mice" package [18]. We also performed the complete case analysis and there were no notable differences in the results.

2.6. Statistical analyses

Linear regression models and linear mixed-effect models with random intercept were used for the cross-sectional and longitudinal analyses, respectively. Models were created separately for each CMD risk factor as the outcome variable (total cholesterol, LDL-C, HDL-C, glucose, triglycerides, SBP, DBP, total fat mass, android fat mass, and physical activity). We constructed models both with and without confounders (Model 1 and model 2, respectively). In linear mixed-effect models, the main effect of the menopausal symptoms at baseline, time (0 = baseline, 1 = follow-up), and their interaction was included in all models to study the changes in outcome variable over time based on the menopausal symptoms. The confounding factors in the model 2 were age, BMI, menopausal status, use of hormonal preparations, education, smoking status, and alcohol consumption. However, BMI was not included in the total fat mass and android fat mass models due to its strong association with the outcome variable. We also constructed models that were additionally adjusted with E2 and FSH. Furthermore, the confounding of symptoms from other categories was taken into account by including all symptom categories in the same models as predictors. Finally, we conducted sensitivity analyses for blood lipids and blood pressure by excluding the participants who used lipid modifying agents and antihypertensives, respectively. Residual plots, Q–Q plots, and correlation analysis were used for testing the model assumptions. The analyses were carried out using base R and the "nlme" package [20].

3. Results

3.1. Characteristics of the study population

The characteristics of the study population are shown in Table 2 and Fig. S1. At baseline, percentages of the participants in pre-, peri-, and postmenopausal groups were 28, 34, and 38, respectively. On average,

Table 2
Characteristics of the study population.

	Cross-sectional study	Longitudinal study		
	Baseline	Baseline	Follow-up	Change[a]
Menopausal symptoms[b]	1097	276		
Number of vasomotor symptoms[c]				
No symptoms	41 (454)	49 (135)		
2 or more symptoms	29 (313)	23 (64)		
Number of psychological symptoms[c]				
No symptoms	50 (549)	61 (167)		
2 or more symptoms	28 (305)	22 (61)		
Number of somatic or pain symptoms[c]				
No symptoms	76 (835)	79 (219)		
2 or more symptoms	5 (53)	4 (12)		
Number of urogenital symptoms[c]				
No symptoms	65 (713)	69 (191)		
2 or more symptoms	15 (167)	12 (33)		
Total number of symptoms[c]				
No symptoms	24 (260)	30 (82)		
2 or more symptoms	60 (657)	52 (143)		
Age & menopausal status (n)	1393	298	298	298
Age [years]	51.3 ± 2.1	51.3 ± 1.8	55.1 ± 1.8	3.8 ± 0.2
Menopausal status[c]				
Pre	28 (389)	34 (100)	5 (15)	
Peri	34 (474)	35 (105)	14 (42)	
Post	38 (530)	31 (93)	81 (241)	
Blood-based biomarkers (n)	1393	298	298	298
Estradiol [nmol/l]	0.34 ± 0.41	0.38 ± 0.57	0.26 ± 0.28	−0.12 ± 0.63
Follicle-stimulating hormone [IU/l]	44.0 ± 38.6	39.9 ± 37.1	69.5 ± 37.5	29.5 ± 40.5
Total cholesterol [mmol/l]	5.30 ± 0.91	5.24 ± 0.91	5.67 ± 1.00	0.43 ± 0.88
HDL-C [mmol/l]	1.72 ± 0.46	1.72 ± 0.47	1.91 ± 0.50	0.19 ± 0.39
LDL-C [mmol/l]	3.05 ± 0.80	3.05 ± 0.80	3.41 ± 0.88	0.37 ± 0.76
Glucose [mmol/l]	5.28 ± 0.84	5.15 ± 0.45	5.16 ± 0.62	0.02 ± 0.55
Triglycerides [mmol/l]	1.09 ± 0.72	1.08 ± 0.61	1.27 ± 0.73	0.19 ± 0.53
Dual-energy X-ray absorptiometry (n)	902	244	292	240
Fat mass [kg]	25.0 ± 8.5	24.2 ± 8.4	25.9 ± 9.1	2.0 ± 3.3
Android fat mass [kg]	2.24 ± 0.97	2.14 ± 0.91	2.39 ± 1.01	0.27 ± 0.42
Blood pressure & body mass index (n)	932	249	298	249
Systolic blood pressure [mm Hg]	132.5 ± 17.5	132.0 ± 16.3	133.2 ± 18.3	2.0 ± 13.4
Diastolic blood pressure [mm Hg]	84.5 ± 9.7	84.1 ± 9.2	81.9 ± 10.0	−2.1 ± 6.5
Body mass index [kg/m^2]	25.5 ± 3.7	25.3 ± 3.7	25.8 ± 4.1	0.7 ± 1.4
Accelerometer-measured PA (n)	784	235	283	222
ACC-MAD [mg]	29.2 ± 9.2	30.2 ± 10.0	28.3 ± 8.6	−1.9 ± 7.2
Lifestyle habits & medications (n)	1098	276	298	276
Alcohol consumption [portions/week]	3.82 ± 3.75	3.73 ± 3.92	3.24 ± 3.43	−0.53 ± 2.63
Smoking[c]				
Non-smoker	93 (1014)	95 (262)	94 (280)	
Smoker	7 (78)	5 (13)	6 (18)	
Education[c]				
Primary or secondary	59 (643)	55 (165)	55 (165)	
Tertiary	41 (455)	45 (133)	45 (133)	
Use of hormonal preparations[c]				
Non-user	61 (676)	62 (186)	60 (180)	
Progestogen	39 (426)	38 (112)	19 (56)	
Estrogen	0 (0)	0 (0)	3 (10)	
Progestogen + estrogen	0 (0)	0 (0)	18 (52)	

Data are mean ± standard deviation unless otherwise specified. HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, PA physical activity, ACC-MAD, accelerometer-measured physical activity mean amplitude deviation, mg milligravity (0.00981 m/s^2).
[a] For participants with baseline and follow-up measurement.
[b] Measured only at baseline.
[c] Data are % (n).

participants had slight overweight at baseline with a mean BMI of 25.5 kg/m^2 and had slightly elevated SBP (132.5 mm Hg), DBP (84.5 mm Hg), total cholesterol (5.23 mmol/l), and LDL-C (3.05 mmol/l). Vasomotor and psychological symptoms were the most frequently reported symptoms, with 59 % and 50 % of the participants reporting at least one symptom of those categories in the full sample, respectively. One or more somatic or pain symptoms and urogenital symptoms were reported by 24 % and 35 % of the participants, respectively. Consequently, 76 % of the participants reported at least one symptom from any category. At baseline, 4 % (n = 43) and 17 % (n = 192) of the participants reported using lipid modifying agents and antihypertensives.

3.2. Cross-sectional analyses

In simple linear regression models, the number symptoms in most categories were positively associated with cholesterol levels (Table 3). Especially, vasomotor, urogenital, and the total number of symptoms were associated with higher total cholesterol and LDL-C. Also, VMS associated with higher HDL-C. However, after adjusting the models with confounders, all associations between symptoms and cholesterol levels disappeared. In unadjusted models, a higher total number of symptoms associated positively with higher total (B = 0.31 kg, 95 % CI [0.03, 0.59]) and android fat mass (B = 0.04 kg, 95 % [0.01, 0.07]). Adjusting the models did not notably affect these results. Participants who reported more somatic or pain symptoms tended to have higher SBP in unadjusted (B = 1.95 mm Hg, 95 % CI [−0.18, 4.09]) and adjusted (B = 1.36 mm Hg, 95 % CI [−0.65, 3.37]) models. Including all symptom categories in the same models (Tables S1 and S2), adjusting the models with E2 and FSH (Table S3), and removing the participants using medications directly affecting the outcome variables (Table S4) did not change the results notably. No interactions between symptoms and other covariates were observed, and omitting BMI from the list of confounders did not significantly affect the results in adjusted models (data not shown). The full models shown in Tables S1 and S2 indicate that BMI was positively associated with most of the outcome variables, while age and menopausal status were positively associated with cholesterol levels.

3.3. Longitudinal analyses

The number of menopausal symptoms in each category tended to

Table 3
Associations of menopausal symptoms with cardiometabolic risk factors in cross-sectional study design (n = 1393).

	Total cholesterol [mmol/l]		LDL-C [mmol/l]		HDL-C [mmol/l]		Glucose [mmol/l]		Triglycerides [mmol/l]		Systolic blood pressure [mm Hg]		Diastolic blood pressure [mm Hg]		Total fat mass [kg][a]		Android fat mass [kg][a]		Physical activity[b] [mg]	
	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI
Vasomotor symptoms																				
Model 1	0.13***	[0.07, 0.20]	0.08**	[0.03, 0.14]	0.06***	[0.03, 0.09]	−0.04	[−0.10, 0.02]	0.03	[−0.02, 0.08]	0.21	[−1.07, 1.49]	0.16	[−0.55, 0.87]	0.44	[−0.33, 1.21]	0.06	[−0.03, 0.14]	−0.26	[−0.91, 0.40]
Model 2	0.00	[−0.08, 0.07]	−0.03	[−0.09, 0.03]	0.02	[−0.02, 0.06]	−0.04	[−0.10, 0.03]	0.01	[−0.05, 0.06]	−0.33	[−1.71, 1.05]	−0.23	[−0.98, 0.51]	0.55	[−0.35, 1.45]	0.05	[−0.05, 0.15]	−0.28	[−1.05, 0.49]
Psychological symptoms																				
Model 1	0.06*	[0.01, 0.11]	0.04	[0.00, 0.09]	0.01	[−0.02, 0.03]	−0.02	[−0.07, 0.02]	0.02	[−0.02, 0.06]	0.07	[−0.98, 1.13]	0.16	[−0.42, 0.74]	0.49	[−0.16, 1.14]	0.05	[−0.02, 0.13]	−0.13	[−0.71, 0.45]
Model 2	0.01	[−0.04, 0.06]	0.00	[−0.04, 0.05]	−0.01	[−0.03, 0.02]	−0.02	[−0.07, 0.02]	0.01	[−0.03, 0.05]	−0.23	[−1.26, 0.80]	−0.07	[−0.62, 0.48]	0.50	[−0.17, 1.16]	0.05	[−0.03, 0.12]	−0.10	[−0.70, 0.50]
Somatic or pain symptoms																				
Model 1	0.10	[−0.01, 0.20]	0.08	[−0.01, 0.17]	0.00	[−0.05, 0.05]	0.01	[−0.08, 0.10]	0.04	[−0.03, 0.12]	1.95	[−0.18, 4.09]	0.63	[−0.53, 1.79]	0.91	[−0.34, 2.16]	0.11	[−0.03, 0.24]	−0.21	[−1.17, 0.74]
Model 2	0.03	[−0.08, 0.13]	0.02	[−0.07, 0.12]	−0.01	[−0.06, 0.03]	0.01	[−0.09, 0.10]	0.03	[−0.05, 0.11]	1.36	[−0.65, 3.37]	0.17	[−0.87, 1.22]	0.83	[−0.46, 2.13]	0.09	[−0.05, 0.23]	−0.18	[−1.17, 0.80]
Urogenital symptoms																				
Model 1	0.09**	[0.02, 0.15]	0.09**	[0.03, 0.14]	0.01	[−0.02, 0.04]	−0.01	[−0.07, 0.05]	−0.01	[−0.05, 0.04]	0.13	[−1.19, 1.45]	0.32	[−0.41, 1.05]	0.60	[−0.23, 1.44]	0.08	[−0.01, 0.17]	−0.11	[−0.74, 0.51]
Model 2	0.01	[−0.06, 0.08]	0.03	[−0.03, 0.08]	−0.01	[−0.05, 0.02]	−0.02	[−0.08, 0.05]	−0.02	[−0.07, 0.03]	−0.41	[−1.77, 0.95]	−0.05	[−0.74, 0.65]	0.60	[−0.26, 1.47]	0.07	[−0.03, 0.17]	−0.08	[−0.74, 0.58]
Total number of symptoms																				
Model 1	0.05***	[0.03, 0.07]	0.04***	[0.02, 0.06]	0.01	[0.00, 0.02]	−0.01	[−0.03, 0.01]	0.01	[−0.01, 0.03]	0.18	[−0.33, 0.69]	0.14	[−0.14, 0.42]	0.31*	[0.03, 0.59]	0.04*	[0.01, 0.07]	−0.09	[−0.33, 0.15]
Model 2	0.01	[−0.02, 0.03]	0.00	[−0.02, 0.02]	0.00	[−0.02, 0.01]	−0.01	[−0.04, 0.01]	0.00	[−0.02, 0.02]	−0.07	[−0.58, 0.43]	−0.04	[−0.31, 0.22]	0.35*	[0.03, 0.67]	0.04	[0.00, 0.07]	−0.09	[−0.36, 0.19]

Model 1 is simple linear regression model. Model 2 is adjusted with age, body mass index, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption unless otherwise specified. LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol. CI confidence interval, mg milligravity (0.00981 m/s^2).

[a] Model 2 is adjusted with age, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption.

[b] Accelerometer-measured physical activity mean amplitude deviation.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

associate positively with total cholesterol, LDL-C, total fat mass, and android fat mass (Tables 4 and 5). Notably, a higher number of VMS associated with higher total cholesterol (B = 0.15 mmol/l, 95 % CI [0.02, 0.28]) and LDL-C (B = 0.12 mmol/l, 95 % CI [0.01, 0.24]). However, adjusting the models with confounders diminished the associations, especially with total cholesterol and LDL-C. Participants reporting more psychological, somatic or pain, and urogenital symptoms tended to have higher SBP with an estimated increase per reported symptom varying from 0.96 to 2.83 mm Hg. Participants reporting more VMS tended to have lower SBP, with an estimated decrease per reported symptom varying from 0.91 to 1.71 mm Hg. A higher number of VMS was associated with a 0.09 mmol/l (95 % CI [0.01, 0.17]) greater increase in triglycerides during the follow-up. The results did not differ notably when including all symptoms in the same models (Tables S5 and S6), adjusting the models with E2 and FSH (Table S7), or removing participants using lipid modifying agents and antihypertensives (Table S8). The full models in Tables S5 and S6 show that all studied cholesterol levels increased and DBP decreased during the follow-up. Also, BMI associated positively with LDL-C, glucose, triglyceride, and blood pressure levels and negatively with HDL-C and physical activity. Menopausal status was positively associated with total cholesterol, LDL-C, and HDL-C.

4. Discussion

In this study, menopausal symptoms were observed to have modest positive associations with cholesterol levels and body fat mass measures. Remarkably, most of the observed associations disappeared after adjusting for confounders. Menopausal symptoms were not associated with blood pressure, blood glucose, triglycerides, and physical activity. Furthermore, menopausal symptoms at baseline did not predict the changes in most of the CMD risk factors during the follow-up.

In both cross-sectional and longitudinal analyses, we observed that menopausal symptoms, especially VMS, were positively associated with total cholesterol, LDL-C, and HDL-C but not with triglycerides and blood glucose. However, the observed associations disappeared after controlling for confounders with a higher number of VMS accounting only for −0.03 to 0.04 mmol/l change in all cholesterol levels. The previous evidence from cross-sectional and longitudinal studies focusing on night sweats and hot flashes has demonstrated positive associations of VMS with total cholesterol, LDL-C, HDL-C, and triglycerides [9,12], as well as with blood glucose [13,21] even after controlling for confounders. For instance, higher total cholesterol levels have been reported for women with hot flashes (0.27 mmol/l, 95 % CI [0.15, 0.39]) in large cross-sectional study [22] and for women with night sweats (0.17 mmol/l, 95 % CI [0.03, 0.31]) in meta-analysis including two large cross-sectional studies [9]. However, similarly to our results, one small study of Nordic women did not observe associations between VMS and blood lipids [23].

Previous meta-analysis of cross-sectional studies reported women with hot flashes and night sweat to have higher SBP and DBP [9]. Similar findings were also observed in one large-scale longitudinal study [14]. However, our longitudinal analyses indicated that women with a higher number of vasomotor symptoms might even have lower SBP and DBP. On the other hand, in all other symptoms categories, a higher number of symptoms tended to associated with higher SBP and DBP in our analyses. Furthermore, a higher physical activity level has previously been found to associate with fewer somatic and mood symptoms but the evidence of its association with VMS is contradictory [24]. Our analyses demonstrated a trend that the number of symptoms in all categories

Table 4
Associations of menopausal symptoms with blood-based biomarkers in longitudinal study design (n = 298).

		Total cholesterol [mmol/l]		LDL-C [mmol/l]		HDL-C [mmol/l]		Glucose [mmol/l]		Triglycerides [mmol/l]	
		B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI
Vasomotor symptoms											
Model 1	Main effect	0.15*	[0.02, 0.28]	0.12*	[0.01, 0.24]	0.03	[−0.04, 0.09]	−0.02	[−0.10, 0.05]	0.05	[−0.04, 0.15]
	Interaction with time	−0.03	[−0.16, 0.09]	−0.02	[−0.13, 0.09]	−0.02	[−0.08, 0.03]	−0.04	[−0.12, 0.03]	0.07	[−0.01, 0.14]
Model 2	Main effect	0.03	[−0.11, 0.18]	0.05	[−0.08, 0.17]	−0.03	[−0.10, 0.04]	−0.04	[−0.12, 0.04]	0.01	[−0.09, 0.11]
	Interaction with time	0.04	[−0.09, 0.17]	0.03	[−0.08, 0.15]	0.01	[−0.04, 0.07]	−0.02	[−0.11, 0.06]	0.09*	[0.01, 0.17]
Psychological symptoms											
Model 1	Main effect	0.05	[−0.06, 0.15]	0.06	[−0.03, 0.16]	−0.01	[−0.07, 0.04]	0.01	[−0.05, 0.07]	0.04	[−0.03, 0.12]
	Interaction with time	0.03	[−0.07, 0.13]	0.02	[−0.06, 0.11]	−0.01	[−0.05, 0.03]	0.00	[−0.06, 0.06]	0.01	[−0.05, 0.07]
Model 2	Main effect	0.01	[−0.10, 0.11]	0.04	[−0.06, 0.13]	−0.03	[−0.09, 0.02]	0.01	[−0.05, 0.07]	0.02	[−0.05, 0.10]
	Interaction with time	0.04	[−0.05, 0.14]	0.03	[−0.06, 0.12]	0.01	[−0.04, 0.05]	0.00	[−0.06, 0.06]	0.00	[−0.06, 0.07]
Somatic or pain symptoms											
Model 1	Main effect	0.12	[−0.09, 0.33]	0.09	[−0.09, 0.28]	0.00	[−0.11, 0.11]	0.02	[−0.10, 0.14]	0.11	[−0.04, 0.26]
	Interaction with time	0.00	[−0.20, 0.20]	0.00	[−0.17, 0.17]	−0.04	[−0.13, 0.05]	−0.06	[−0.19, 0.06]	−0.04	[−0.17, 0.08]
Model 2	Main effect	0.05	[−0.16, 0.26]	0.04	[−0.14, 0.23]	−0.03	[−0.14, 0.08]	0.00	[−0.12, 0.12]	0.09	[−0.07, 0.24]
	Interaction with time	0.05	[−0.15, 0.25]	0.03	[−0.14, 0.20]	0.00	[−0.10, 0.09]	−0.06	[−0.19, 0.06]	−0.05	[−0.18, 0.08]
Urogenital symptoms											
Model 1	Main effect	0.04	[−0.10, 0.17]	0.04	[−0.08, 0.15]	−0.01	[−0.08, 0.06]	0.04	[−0.04, 0.11]	0.04	[−0.05, 0.14]
	Interaction with time	−0.01	[−0.14, 0.11]	0.00	[−0.11, 0.11]	−0.05	[−0.11, 0.00]	−0.01	[−0.09, 0.06]	0.06	[−0.02, 0.14]
Model 2	Main effect	−0.03	[−0.17, 0.11]	−0.01	[−0.13, 0.11]	−0.04	[−0.11, 0.03]	0.04	[−0.04, 0.11]	0.02	[−0.08, 0.12]
	Interaction with time	0.01	[−0.11, 0.14]	0.01	[−0.10, 0.12]	−0.03	[−0.09, 0.03]	−0.01	[−0.09, 0.07]	0.07	[−0.01, 0.15]
Total number of symptoms											
Model 1	Main effect	0.04	[−0.01, 0.09]	0.04	[0.00, 0.08]	0.00	[−0.03, 0.02]	0.01	[−0.02, 0.03]	0.03	[−0.01, 0.06]
	Interaction with time	0.00	[−0.04, 0.05]	0.00	[−0.04, 0.04]	−0.01	[−0.03, 0.01]	−0.01	[−0.04, 0.02]	0.02	[−0.01, 0.05]
Model 2	Main effect	0.00	[−0.05, 0.06]	0.02	[−0.03, 0.06]	−0.02	[−0.04, 0.01]	0.00	[−0.03, 0.03]	0.02	[−0.02, 0.05]
	Interaction with time	0.02	[−0.03, 0.07]	0.01	[−0.03, 0.06]	0.00	[−0.02, 0.02]	−0.01	[−0.04, 0.02]	0.02	[−0.01, 0.05]

All models include menopausal symptoms, time, and their interaction as predictors. Model 2 is additionally adjusted with age, body mass index, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption. LDL-C Low-density lipoprotein cholesterol, HDL-C High-density lipoprotein cholesterol, CI Confidence interval.

* $p \leq 0.05$.

Table 5
Associations of menopausal symptoms with blood pressure, body composition, and physical activity measures in longitudinal study design (n = 298).

		Systolic blood pressure [mm Hg]		Diastolic blood pressure [mm Hg]		Total fat mass [kg][a]		Android fat mass [kg][a]		Physical activity[b] [mg]	
		B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI	B	95 % CI
Vasomotor symptoms											
Model 1	Main effect	−0.91	[−3.52, 1.69]	−0.43	[−1.87, 1.02]	0.74	[−0.72, 2.21]	0.08	[−0.08, 0.24]	−0.67	[−2.06, 0.72]
	Interaction with time	−0.15	[−2.68, 2.37]	0.01	[−1.33, 1.35]	−0.21	[−1.49, 1.07]	−0.03	[−0.18, 0.12]	−0.27	[−1.72, 1.19]
Model 2	Main effect	−1.71	[−4.39, 0.98]	−1.16	[−2.62, 0.30]	0.29	[−1.28, 1.86]	0.02	[−0.15, 0.20]	−0.47	[−1.93, 1.00]
	Interaction with time	0.37	[−2.18, 2.91]	0.59	[−0.71, 1.90]	0.09	[−1.26, 1.44]	0.01	[−0.15, 0.16]	−0.35	[−1.80, 1.11]
Psychological symptoms											
Model 1	Main effect	1.30	[−0.80, 3.41]	0.93	[−0.22, 2.07]	0.53	[−0.62, 1.67]	0.04	[−0.09, 0.17]	0.03	[−1.07, 1.12]
	Interaction with time	−1.18	[−3.18, 0.83]	−0.55	[−1.61, 0.50]	0.46	[−0.52, 1.45]	0.05	[−0.06, 0.16]	−0.87	[−1.95, 0.22]
Model 2	Main effect	0.96	[−1.11, 3.03]	0.68	[−0.42, 1.78]	0.38	[−0.78, 1.54]	0.02	[−0.10, 0.15]	0.28	[−0.85, 1.42]
	Interaction with time	−1.22	[−3.16, 0.71]	−0.54	[−1.52, 0.45]	0.52	[−0.48, 1.51]	0.06	[−0.05, 0.17]	−0.81	[−1.91, 0.29]
Somatic or pain symptoms											
Model 1	Main effect	2.83	[−1.28, 6.94]	0.91	[−1.34, 3.17]	1.06	[−1.22, 3.35]	0.08	[−0.18, 0.34]	−0.02	[−2.20, 2.16]
	Interaction with time	−1.93	[−5.79, 1.93]	−0.31	[−2.35, 1.74]	−0.07	[−2.08, 1.94]	−0.02	[−0.25, 0.22]	0.31	[−1.98, 2.59]
Model 2	Main effect	1.95	[−2.09, 5.98]	0.23	[−1.93, 2.40]	0.62	[−1.69, 2.93]	0.03	[−0.23, 0.29]	0.24	[−1.96, 2.44]
	Interaction with time	−1.86	[−5.76, 2.04]	−0.08	[−2.08, 1.92]	0.15	[−1.90, 2.20]	0.01	[−0.23, 0.25]	0.34	[−1.90, 2.58]
Urogenital symptoms											
Model 1	Main effect	1.89	[−0.71, 4.48]	1.47	[0.00, 2.93]	0.43	[−1.00, 1.87]	0.05	[−0.11, 0.22]	−0.39	[−1.80, 1.03]
	Interaction with time	−0.07	[−2.56, 2.42]	0.10	[−1.25, 1.46]	−0.07	[−1.37, 1.22]	0.00	[−0.15, 0.15]	−0.61	[−2.05, 0.84]
Model 2	Main effect	1.45	[−1.12, 4.01]	1.08	[−0.32, 2.48]	0.16	[−1.30, 1.62]	0.02	[−0.14, 0.19]	−0.21	[−1.62, 1.20]
	Interaction with time	0.08	[−2.36, 2.53]	0.26	[−1.00, 1.52]	0.08	[−1.20, 1.36]	0.02	[−0.13, 0.17]	−0.57	[−2.00, 0.85]
Total number of symptoms											
Model 1	Main effect	0.58	[−0.37, 1.53]	0.40	[−0.12, 0.92]	0.34	[−0.18, 0.85]	0.03	[−0.03, 0.09]	−0.14	[−0.65, 0.38]
	Interaction with time	−0.40	[−1.29, 0.49]	−0.13	[−0.59, 0.34]	0.06	[−0.37, 0.49]	0.01	[−0.04, 0.06]	−0.29	[−0.82, 0.23]
Model 2	Main effect	0.34	[−0.63, 1.31]	0.18	[−0.35, 0.71]	0.18	[−0.36, 0.72]	0.01	[−0.05, 0.07]	0.00	[−0.55, 0.54]
	Interaction with time	−0.35	[−1.25, 0.55]	−0.03	[−0.48, 0.43]	0.15	[−0.30, 0.59]	0.02	[−0.03, 0.07]	−0.30	[−0.82, 0.23]

All models include menopausal symptoms, time, and their interaction as predictors. Model 2 is additionally adjusted with age, body mass index, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption unless otherwise specified. CI confidence interval.

[a] Model 2 is additionally adjusted with age, menopausal status, use of hormonal contraception, education, smoking status, and alcohol consumption.

[b] Accelerometer-measured physical activity mean amplitude deviation, mg milligravity (0.00981 m/s^2).

might be associated with higher total and android fat mass. These results are partially in line with previous results showing associations of VMS with higher body fat percentage [25] and BMI [9].

The mechanisms behind the linkage between menopausal symptoms and CMD risk factors are not completely clear. However, this linkage could be explained by the change in the hormonal milieu and especially the decrease in systemic E2 levels during the menopausal transition. These hormonal changes are often considered to be one major contributor to the development of menopausal symptoms [2], and they may also contribute to the unhealthy changes in CMD risk factors during the menopausal transition [1,6]. Some potential mechanisms for how the hormonal changes could affect the relationship between menopausal symptoms and CMD risk factors are the menopause-related changes in endothelial function and sympathetic activity that are also associated with VMS [26,27] and may result in changes in blood pressure [28] and lipids [29]. Our findings, in which the studied associations diminished after adjusting for menopausal status and other confounders, support the hypothesis about the confounding role of the change in the hormonal milieu and its derivatives in associations between menopausal symptoms and CMD risk factors.

Additionally, menopausal transition and varied menopausal symptoms have been linked to changes in body adiposity and obesity [2]. The increased amount of adipose tissue that contributes to CMD risk and the development of a pro-inflammatory adipokine profile is associated with a variety of menopausal symptoms and may play a role in the development of VMS [2,30]. BMI has been reported to moderate the associations between VMS with blood lipids [12], which indicates that the amount of adipose tissue may play a role in the associations between menopausal symptoms and CMD risk factors. However, contradictory to our results, several previous studies have reported these associations to persist even after adjusting for BMI [9,12,14]. Also, in our analyses, BMI did not moderate the associations between menopausal symptoms and CMD risk factors.

Strengths of the study include the longitudinal study design, where a total of 148 out of 298 participants experienced menopause during the follow-up. Another upside of this study is the relatively large sample with a comprehensive set of CMD risk factors and measured confounders. Furthermore, the assessment of menopausal symptoms in our study was not limited only to VMS, but included a variety of other symptom types as well. However, the substantive limitation of the study is the assessment of the menopausal symptoms only at baseline with a questionnaire that has not been validated and does not capture the frequency or the severity of the symptoms. Furthermore, the homogeneous sample of white women with several health-related exclusion criteria limits the generalizability of the results and may affect the associations between menopausal symptoms and CMD risk factors.

5. Conclusion

This study shows that menopausal symptoms are associated with higher cholesterol levels and body adiposity in middle-aged women. However, the associations between menopausal symptoms and cholesterol levels were diminished after controlling for confounders, indicating that these associations are, at least partially, explained by differences in age, menopausal status, BMI, socioeconomic status, and lifestyle habits. According to our results, menopausal symptoms are not associated with blood glucose, triglycerides, blood pressure, and physical activity levels, and they do not predict changes in CMD risk factors

during the menopausal transition. The independent role of menopausal symptoms in explaining the CMD risk factors may therefore be smaller than what has been previously suggested. More large-scale longitudinal studies with a comprehensive set of confounders are needed to clarify the independent role of menopausal symptoms in CMD risk in women.

Contributors

Matti Hyvärinen prepared the data and original manuscript, and was the major contributor to the statistical analyses.

Juha Karvanen offered guidance for statistical analyses and contributed to the writing process.

Hanna-Kaarina Juppi contributed to the writing process.

Jari E. Karppinen contributed to the writing process.

Tuija H. Tammelin contributed to the writing process.

Vuokko Kovanen obtained funding for the project and contributed to the writing process.

Pauliina Aukee contributed to the writing process.

Sarianna Sipilä contributed to the writing process.

Timo Rantalainen contributed to the writing process.

Eija K. Laakkonen obtained funding for the project and contributed to the writing process.

All authors saw and approved the final version, and no other person made a substantial contribution to the paper.

Funding

This work was supported by the Academy of Finland (grant numbers 275323 to VK and 309504, 314181 and 335249 to EKL). TR was an Academy Research Fellow during the preparation of this manuscript (Academy of Finland grant numbers 321336 and 328818).

Ethical approval

The study was performed in accordance with the Declaration of Helsinki. Both ERMA and EsmiRs studies were approved by the ethical committee of the Central Finland Health Care District (ERMA 8U/2014 and EsmiRs 9U/2018).

Provenance and peer review

This article was not commissioned and was externally peer reviewed.

Research data (data sharing and collaboration)

There are no linked datasets for this paper. The datasets generated and/or analyzed during the current study are not publicly available due to EU and Finnish legislation and the consent provided by the participants does not permit open access to individual-level personal data. However, they are available from the corresponding author on reasonable request. More information about the datasets: 10.17011/jyx/dataset/83491.

Declaration of competing interest

The authors declare that they have no competing interest.

Acknowledgements

The authors would like to thank Anja Ahlgren, Eeva-Maija Palonen, Mervi Matero, Hanne Tähti, and the other laboratory staff in Faculty of Sport and Health Sciences for their invaluable help with the data collection, as well as the participants in ERMA and EsmiRs studies who volunteered their time and effort.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.maturitas.2023.05.004.

References

[1] S.R.E. Khoudary, B. Aggarwal, T.M. Beckie, H.N. Hodis, A.E. Johnson, R.D. Langer, et al., Menopause transition and cardiovascular disease risk: implications for timing of early prevention: a scientific statement from the American Heart Association, Circulation. 142 (25) (2020) e506–e532, https://doi.org/10.1161/CIR.0000000000000912.

[2] P. Monteleone, G. Mascagni, A. Giannini, A.R. Genazzani, T. Simoncini, Symptoms of menopause — global prevalence, physiology and implications, Nat. Rev. Endocrinol. 14 (4) (2018) 199–215, https://doi.org/10.1038/nrendo.2017.180.

[3] A.S. Go, D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, M.J. Blaha, et al., Heart disease and stroke statistics—2014 update: a report from the American Heart Association, Circulation. 129 (3) (2014) e28–292, https://doi.org/10.1161/01.cir.0000441139.02102.80.

[4] M.C. Carr, The emergence of the metabolic syndrome with menopause, J. Clin. Endocrinol. Metab. 88 (6) (2003) 2404–2411, https://doi.org/10.1210/jc.2003-030242.

[5] K.A. Matthews, S.L. Crawford, C.U. Chae, A. Everson-Rose Susan, M.F. Sowers, B. Sternfeld, et al., Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? J. Am. Coll. Cardiol. 54 (25) (2009) 2366–2373, https://doi.org/10.1016/j.jacc.2009.10.009.

[6] M. Hyvärinen, H.-K. Juppi, S. Taskinen, J.E. Karppinen, S. Karvinen, T. H. Tammelin, et al., Metabolic health, menopause, and physical activity—a 4-year follow-up study, Int. J. Obes. 46 (3) (2022) 544–554, https://doi.org/10.1038/s41366-021-01022-x.

[7] T. Muka, C. Oliver-Williams, V. Colpani, S. Kunutsor, S. Chowdhury, R. Chowdhury, et al., Association of vasomotor and other menopausal symptoms with risk of cardiovascular disease: a systematic review and meta-analysis, PLoS One 11 (6) (Jun 2016), e0157417, https://doi.org/10.1371/journal.pone.0157417.

[8] A. Armeni, P. Anagnostis, E. Armeni, N. Mili, D. Goulis, I. Lambrinoudaki, Vasomotor symptoms and risk of cardiovascular disease in peri- and postmenopausal women: a systematic review and meta-analysis, Maturitas. 171 (2023) 13–20, https://doi.org/10.1016/j.maturitas.2023.02.004.

[9] O.H. Franco, T. Muka, V. Colpani, S. Kunutsor, S. Chowdhury, R. Chowdhury, et al., Vasomotor symptoms in women and cardiovascular risk markers: systematic review and meta-analysis, Maturitas. 81 (3) (2015) 353–361, https://doi.org/10.1016/j.maturitas.2015.04.016.

[10] P. Tuomikoski, H. Savolainen-Peltonen, Vasomotor symptoms and metabolic syndrome, Maturitas. 97 (2017) 61–65, https://doi.org/10.1016/j.maturitas.2016.12.010.

[11] G.M. van Dijk, M. Maneva, V. Colpani, K. Dhana, T. Muka, L. Jaspers, et al., The association between vasomotor symptoms and metabolic health in peri- and postmenopausal women: a systematic review, Maturitas. 80 (2) (2015) 140–147, https://doi.org/10.1016/j.maturitas.2014.11.016.

[12] R.C. Thurston, S.R. El Khoudary, K. Sutton-Tyrrell, C.J. Crandall, E. Gold, B. Sternfeld, et al., Vasomotor symptoms and lipid profiles in women transitioning through menopause, Obstet. Gynecol. 119 (4) (2012) 753–761, https://doi.org/10.1097/AOG.0b013e31824a09ec.

[13] R.C. Thurston, S.R. El Khoudary, K. Sutton-Tyrrell, C.J. Crandall, B. Sternfeld, H. Joffe, et al., Vasomotor symptoms and insulin resistance in the study of women's health across the nation, J. Clin. Endocrinol. Metab. 97 (10) (2012) 3487–3494, https://doi.org/10.1210/jc.2012-1410.

[14] E.A. Jackson, S.R. El Khoudary, S.L. Crawford, K. Matthews, H. Joffe, C. Chae, et al., Hot flash frequency and blood pressure: data from the Study of Women's Health Across the Nation, J. Women's Health (Larchmt) 25 (12) (2016) 1204–1209, https://doi.org/10.1089/jwh.2015.5670.

[15] V. Kovanen, P. Aukee, K. Kokko, T. Finni, I.M. Tarkka, T. Tammelin, et al., Design and protocol of Estrogenic Regulation of Muscle Apoptosis (ERMA) study with 47 to 55-year-old women's cohort: novel results show menopause-related differences in blood count 25 (9) (2018) 1020–1032, https://doi.org/10.1097/GME.0000000000001117.

[16] E.K. Laakkonen, J. Kulmala, P. Aukee, H. Hakonen, U.M. Kujala, D.A. Lowe, et al., Female reproductive factors are associated with objectively measured physical activity in middle-aged women, PLoS One 12 (2) (2017), e0172054, https://doi.org/10.1371/journal.pone.0172054.

[17] M. Hyvärinen, J. Karvanen, P. Aukee, T.H. Tammelin, S. Sipilä, U.M. Kujala, et al., Predicting the age at natural menopause in middle-aged women, Menopause. 28 (7) (2021) 792–799, https://doi.org/10.1097/GME.0000000000001774.

[18] S. van Buuren, K. Groothuis-Oudshoorn, mice: multivariate imputation by chained equations in R, J. Stat. Softw. 45 (3) (2011) 1–67, https://doi.org/10.18637/jss.v045.i03.

[19] R Core Team, R: A Language and Environment for Statistical Computing. Version 4.0.5, R Foundation for Statistical Computing, Vienna, Austria, 2021. Available from: https://www.R-project.org/.

[20] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, R Core Team, Linear and nonlinear mixed effects models. R package version 3.1-152, Available from: https://cran.r-project.org/web/packages/nlme/index.html, 2021.

[21] G.C.M. Gast, G.N. Samsioe, D.E. Grobbee, P.M. Nilsson, Y.T. van der Schouw, Vasomotor symptoms, estradiol levels and cardiovascular risk profile in women, Maturitas. 66 (3) (2010) 285–290, https://doi.org/10.1016/j.maturitas.2010.03.015.
[22] G.C.M. Gast, D.E. Grobbee, V.J.M. Pop, J.J. Keyzer, C.J.M. Wijnands-van Gent, G. N. Samsioe, et al., Menopausal complaints are associated with cardiovascular risk factors, Hypertension. 51 (6) (2008) 1492–1498, https://doi.org/10.1161/HYPERTENSIONAHA.107.106526.
[23] P. Tuomikoski, T.S. Mikkola, E. Hämäläinen, M.J. Tikkanen, U. Turpeinen, O. Ylikorkala, Biochemical markers for cardiovascular disease in recently postmenopausal women with or without hot flashes, Menopause. 17 (1) (2010) 145–151, https://doi.org/10.1097/gme.0b013e3181acefd5.
[24] B. Sternfeld, S. Dugan, Physical activity and health during the menopausal transition, Obstet. Gynecol. Clin. N. Am. 38 (3) (2011) 537–566, https://doi.org/10.1016/j.ogc.2011.05.008.
[25] R.C. Thurston, M.R. Sowers, Y. Chang, B. Sternfeld, E.B. Gold, J.M. Johnston, et al., Adiposity and reporting of vasomotor symptoms among midlife women: the Study of Women's Health Across the Nation, Am. J. Epidemiol. 167 (1) (2008) 78–85, https://doi.org/10.1093/aje/kwm244.
[26] A. Bechlioulis, S.N. Kalantaridou, K.K. Naka, A. Chatzikyriakidou, K.A. Calis, A. Makrigiannakis, et al., Endothelial function, but not carotid intima-media thickness, is affected early in menopause and is associated with severity of hot flushes, J. Clin. Endocrinol. Metab. 95 (3) (2010) 1199–1206, https://doi.org/10.1210/jc.2009-2262.
[27] R.C. Thurston, I.C. Christie, K.A. Matthews, Hot flashes and cardiac vagal control: a link to cardiovascular risk? Menopause. 17 (3) (2010) 456–461, https://doi.org/10.1097/gme.0b013e3181c7dea7.
[28] W. Vongpatanasin, Autonomic regulation of blood pressure in menopause, Semin. Reprod. Med. 27 (4) (2009) 338–345, https://doi.org/10.1055/s-0029-1225262.
[29] E. Lambert, N. Straznicky, C.I. Sari, N. Eikelis, D. Hering, G. Head, et al., Dyslipidemia is associated with sympathetic nervous activation and impaired endothelial function in young females, Am. J. Hypertens. 26 (2) (2013) 250–256, https://doi.org/10.1093/ajh/hps016.
[30] R.C. Thurston, Y. Chang, P. Mancuso, K.A. Matthews, Adipokines, adiposity, and vasomotor symptoms during the menopause transition: findings from the Study of Women's Health Across the Nation, Fertil. Steril. 100 (3) (2013) 793–800, https://doi.org/10.1016/j.fertnstert.2013.05.005.

III

THE ROLE OF CARDIORESPIRATORY FITNESS AND BODY COMPOSITION IN THE ASSOCIATION BETWEEN PHYSICAL ACTIVITY AND MENOPAUSAL SYMPTOMS

by

Hyvärinen, M., Karvanen, J., Karppinen, J. E., Karavirta, L., Juppi, H-K., Tammelin, T. H., Kovanen, V., Laukkanen, J., Aukee, P., Sipilä, S., Rantalainen, T., & Laakkonen, E. K.

Submitted manuscript.

Request a copy from the author.

IV

PREDICTING THE AGE AT NATURAL MENOPAUSE IN MIDDLE-AGED WOMEN

by

Hyvärinen, M., Karvanen, J., Aukee, P., Tammelin T. H., Sipilä, S., Kujala, U. M., Kovanen, V., Rantalainen, T., & Laakkonen, E. K. 2021

Menopause, *28*(7), 792-799

https://doi.org/10.1097/GME.0000000000001774

Menopause: The Journal of The North American Menopause Society
Vol. 28, No. 7, pp. 792-799
DOI: 10.1097/GME.0000000000001774

OPEN

ORIGINAL STUDY

Predicting the age at natural menopause in middle-aged women

Matti Hyvärinen, MSc,[1] Juha Karvanen, DSc,[2] Pauliina Aukee, PhD, MD,[3] Tuija H. Tammelin, PhD,[4] Sarianna Sipilä, PhD,[1] Urho M. Kujala, PhD, MD,[5] Vuokko Kovanen, PhD,[5] Timo Rantalainen, DSc,[1] and Eija K. Laakkonen, PhD[1]

Abstract

Objective: To predict the age at natural menopause (ANM).

Methods: Cox models with time-dependent covariates were utilized for ANM prediction using longitudinal data from 47 to 55-year-old women ($n = 279$) participating in the Estrogenic Regulation of Muscle Apoptosis study. The ANM was assessed retrospectively for 105 women using bleeding diaries. The predictors were chosen from the set of 32 covariates by using the lasso regression (model 1). Another easy-to-access model (model 2) was created by using a subset of 16 self-reported covariates. The predictive performance was quantified with *c*-indices and by studying the means and standard deviations of absolute errors (MAE $\pm$ SD) between the predicted and observed ANM.

Results: Both models included alcohol consumption, vasomotor symptoms, self-reported physical activity, and relationship status as predictors. Model 1 also included estradiol and follicle-stimulating hormone levels as well as SD of menstrual cycle length, while model 2 included smoking, education, and the use of hormonal contraception as additional predictors. The mean c-indices of 0.76 (95% CI 0.71-0.81) for model 1 and 0.70 (95% CI 0.65-0.75) for model 2 indicated good concordance between the predicted and observed values. MAEs of 0.56 ± 0.49 and 0.62 ± 0.54 years respectively for model 1 and 2 were clearly smaller than the MAE for predicted sample mean (1.58 ± 1.02).

Conclusions: In addition to sex hormone levels, irregularity of menstrual cycle, and menopausal symptoms, also life habits and socioeconomic factors may provide useful information for ANM prediction. The suggested approach could add value for clinicians' decision making related to the use of contraception and treatments for menopausal symptoms in perimenopausal women.

Key Words: Final menstrual period – Menopausal transition – Menopause prediction – Perimenopause – Premenopause.

Video Summary: http://links.lww.com/MENO/A743.

Factors related to age at natural menopause (ANM) have been one of the most frequently studied topics in menopause-related research in recent decades due to the many potential clinical implications of ANM. For instance, accurate prediction of ANM would be beneficial for women who are making decisions related to family planning and treatments for menopausal symptoms. Moreover, accurate prediction of ANM would help to identify women likely to have early menopause, which may put them at increased risk for cardiovascular disease, cardiovascular mortality,[1,2] depression[3] as well as osteoporosis[4] and fractures.[5] On the other hand, later ANM has been associated with

Received December 21, 2020; revised and accepted February 10, 2021.
From the [1]Gerontology Research Center and Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [2]Department of Mathematics and Statistics, University of Jyväskylä, Jyväskylä, Finland; [3]Department of Obstetrics and Gynecology, Pelvic Floor Research and Therapy Unit, Central Finland Central Hospital, Jyväskylä, Finland; [4]LIKES Research Centre for Physical Activity and Health, Jyväskylä, Finland; and [5]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland.
Funding/support: The ERMA study was financially supported by the Academy of Finland (grant no. 275323 for V.K., grant no. 309504 and 314181 for E.K.L.), European Commission Horizon 2020 (ref. 15-0667 for S.S.) and the Juho Vainio Foundation (E.K.L.). Gerontology Research Center is a joint effort between the University of Jyväskylä and the University of Tampere.

Financial disclosure/conflicts of interest: J.K. has received funding from Biogen Finland and Tale Ltd. The other authors have nothing to disclose.

Supplemental digital content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's Website (www.menopause.org).

Address correspondence to: Matti Hyvärinen, MSc, Gerontology Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35 (LL232), Jyväskylä 40014, Finland. E-mail: matti.v.hyvarinen@jyu.fi

increased risk of breast cancer[6,7] as well as endometrial[8] and ovarian[9] cancer. However, due to the considerable interindividual variation in the ANM as well as the irregularity and long duration of the menopausal transition, predicting the ANM accurately is challenging.[10]

Previous research in predicting the ANM has mostly focused on investigating the predictive performance of only a few predetermined blood-based biomarkers, such as estradiol, follicle-stimulating hormone, and anti-Müllerian hormone levels, or utilized data and methods with time-invariant or categorized continuous covariates.[11-14] Therefore, the objectives of the study was to use longitudinal study design with repeated covariate measurements to 1) investigate the factors associated with the ANM and 2) develop prospective models for predicting the ANM. A comprehensive set of potential predictors were considered and the predictors for the final models were chosen with an automated selection method. In addition to laboratory-assessed characteristics, the predictive performance of easily accessible self-defined covariates that could be useful for clinicians estimating the time to approaching menopause was evaluated.

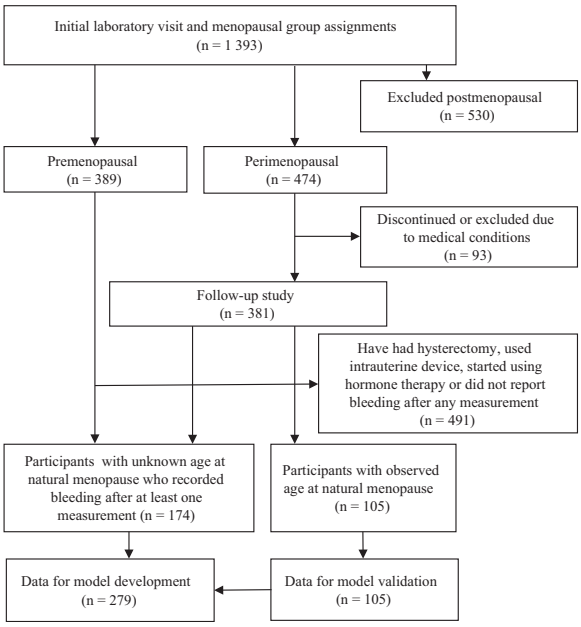

FIG. 1. Flow chart for the study participant selection.

METHODS

Study design

The study was part of the Estrogenic Regulation of Muscle Apoptosis study that has been described in detail elsewhere.[15] In brief, participants were randomly selected from the Digital and Population Data Services Agency (dvv.fi) and a postal invitation were sent to 6,878 women aged 47 to 55 years living in the city of Jyväskylä or neighboring municipalities. Of 2,390 women who responded, decided to continue, and consented, 997 were excluded based on the exclusion criteria. These criteria included several factors and medical conditions that could affect the timing of the final menstrual period or hinder the menopausal group definitions, such as the use of estrogen containing medications, bilateral oophorectomy, pregnancy, lactating, polycystic ovary syndrome, and severe obesity (body mass index $\geq$ 35).

The sample of 1,393 White women were categorized as pre-, peri-, or postmenopausal based on serum follicle-stimulating hormone (FSH) concentrations and self-reported menstrual diaries.[16] The categorization follows the Stages of Reproductive Aging Workshop +10 guidelines[16] although due to technical research reasons a minimum of 6 months follow-up period was used instead of 12 months to verify the postmenopausal status.[15] The participants assigned to the perimenopausal group were invited to a follow-up phase that included keeping menstrual diaries as well as laboratory visits every 3 to 6 months until the participant was categorized as postmenopausal. To avoid misclassification, the FSH concentration was verified with a second measurement about a month after the participant first met the postmenopausal criteria. The participants who have had a hysterectomy, used hormonal intrauterine device, or started using hormone therapy during the follow-up period were excluded from the current study (Fig. 1). The measurements were initiated in the beginning of 2015 and the follow-up period lasted until the end of 2018. All participants provided written informed consent and the study was approved by the ethical committee of the Central Finland Health Care District (No. 8U/2014).

Outcome

All participants in the Estrogenic Regulation of Muscle Apoptosis study were instructed to keep a menstrual diary throughout the study starting at least 12 weeks prior to the baseline measurement. In menstrual diaries, participants reported their daily menstrual bleeding status as bleeding, spotting, or no menstrual bleeding. Bleeding period was defined as at least 1 day of bleeding or 3 or more consecutive days of spotting and spotting days preceding or following bleeding days were considered bleeding days. A single bleeding-free day surrounded by bleeding days was treated as a bleeding day and the surrounding bleeding days were merged into one continuous bleeding period.[17]

For the participants who were perimenopausal in the baseline measurement, went through the follow-up period and had monthly complete bleeding diary, ANM was determined by the age when the last reported bleeding period had started (n = 105). Only the measurements carried out prior to the ANM could be utilized in the analysis and, therefore, all measurements carried out before the menopause were excluded. Finally, there were 296 valid measurements from the participants with known ANM that were used for model development as well as model validation. Additionally, for other participants who were pre- or perimenopausal in the baseline measurement, the last known menstrual period was determined. To make sure that no postmenopausal measurements were included in the analysis,

measurements that were carried out after the last reported bleeding period were excluded. Thus, 391 measurements from 174 participants with unknown time to menopause were also used for model development as censored observations.

Consequently, in addition to data from participants with known ANM, also the data from participants with unknown ANM were used for the development of the models. This data set included 687 separate measurements from 279 participants. However, for the validation of the models, only the data from 105 participants with known ANM could be utilized.

Covariates

The data set consisted of 32 covariates describing characteristics that have been associated with ANM or have been reported to fluctuate during menopausal transition.[18-20] They included several blood-based biomarkers, body composition variables, objectively measured physical activity, menstrual cycle characteristics as well as self-reported variables related to gynecologic history, menopausal symptoms, life habits, and socioeconomic status. They were assessed during every laboratory visit in baseline and follow-up measurements with self-report questionnaires and various measurements. The baseline visits were scheduled to occur at the beginning of the menstrual cycle for participants with regular or predictable menstrual cycles. Most of the candidate predictors were time varying and their values were updated after each laboratory visit. However, self-report variables such as age at menarche, parity, number of pregnancies, and education level (secondary, tertiary) were considered time-invariant and their baseline measurement value was used throughout the study. The questionnaire and measurements that were used are described in more detail elsewhere.[15]

During the laboratory visits, blood samples were taken, and anthropometrics were measured after overnight fasting. IMMULITE 2000 XPi (Siemens Healthineers AG, Erlangen, Germany) was used to measure FSH and estradiol (E2) levels from which the FSH/E2 -ratio was also computed. Furthermore, fasting blood glucose, triglyceride, total cholesterol as well as high- and low-density lipoprotein cholesterol levels were measured. Fat free mass, fat mass, and body fat percentage were assessed using InBody 720 multifrequency bioelectrical impedance analyzer (Biospace Co. Ltd, Seoul, Korea). Body mass and height were measured with standard procedures and body mass index (BMI) was computed by dividing the body mass with the square of the body height.

Menstrual cycle characteristics were determined by using the menstrual diaries and included covariates were menstrual cycle length mean and standard deviation as well as range of menstrual cycle length that was determined as the difference between the longest and shortest recorded menstrual cycle. The length of one cycle was determined in days from the start of bleeding period to the end of the following bleeding-free period and at least two fully recorded cycles was required for a valid covariate value.

Menopausal symptoms were recorded using structured questionnaires in which the participants were asked to report what kind of menopausal symptoms they had experienced. The reported symptoms were merged into four categories that were vasomotor symptoms (eg, sweating and hot flashes), somatic or pain (eg, headache and joint pain), psychological symptoms (eg, sleeping problems and mood swings) and urogenital problems (eg, vaginal symptoms and urinary tract problems).[21] Furthermore, the self-report questionnaires were used to determine the use of hormonal contraception (never, former), relationship status (single, in relationship), smoking status (never, former, current), alcohol consumption in portions per week and physical workload (light, moderate, heavy, very heavy) that describes the occupational physical activity.

Physical activity level was assessed using ActiGraph GT3X and wGT3X accelerometers (ActiGraph LLC, Pensacola, FL). The data were collected at 60 Hz, filtered, and converted into 60-second epochs. The daily mean of total counts and time spent in moderate-to-vigorous activities with tri-axial vector magnitude cut-off point of 6,166 counts per minute normalized to 16-hour wearing time was used.[22,23] Valid measurements included three or more days with more than 10 hours of wear time. Additionally, since self-report physical activity measures capture different aspects of physical behavior compared with accelerometers, two self-report measures focusing on the leisure-time physical activity were included in the analysis. They were a single seven-level scale question [23] and a four-item questionnaire with questions related to commuting as well as average intensity, duration, and frequency of leisure time physical activity that were used for assessing physical activity in MET-hours per day.[24]

Missing data

Of 279 participants in the study, the ANM was determined from 105. The other 174 participants were treated as censored observations in the analysis. The percentage of missing values across the 32 covariates varied between 0% and 28% (Table 1). In total 1,602 covariate records out of 23,358 (7%) were incomplete. Missing data occurred due to incomplete or unclear questionnaire responses and bleeding diaries as well as invalid or missing measurements. Missing data were assumed to occur at random and multiple imputation was used to create and analyze 50 multiply imputed data sets. Multiple imputation was carried out in R (R Foundation for Statistical Computing, Vienna, Austria) using the ''mice'' package.[25] The parameters of substantive interest were estimated in each imputed dataset separately and combined using Rubin's rules.[26]

Multiple imputation was carried out recursively one measurement at a time starting from the baseline measurement. That is, the imputed values of the previous measurement were utilized for the imputation of current measurement. The predictors for the imputation of each variable measured at the same time point were chosen based on their associations with the target variable and missing data values.[27] Additionally, the value of target variables in the previous and following time points were used as the predictors in imputation if they existed. The number of iterations in the imputation algorithm

TABLE 1. *Characteristics of study population, the distribution of all candidate predictors, and the number of missing data values*

	Participants with known ANM ($n = 105$)	All participants ($n = 279$)	All measurements ($n = 687$)	Missing data value[a] n (%)
Blood-based biomarkers				
Total cholesterol [mmol/L]	5.33 ± 0.92	5.31 ± 0.89	5.31 ± 0.90	19 (2.8)
Low-density lipoprotein cholesterol [mmol/L]	2.96 ± 0.77	3.05 ± 0.80	3.03 ± 0.80	19 (2.8)
High-density lipoprotein cholesterol [mmol/L]	1.83 ± 0.42	1.73 ± 0.44	1.75 ± 0.43	19 (2.8)
Blood glucose [mmol/L]	5.24 ± 0.59	5.21 ± 0.60	5.25 ± 0.57	19 (2.8)
Triglycerides [mmol/L]	1.06 ± 0.44	1.09 ± 0.54	1.06 ± 0.53	20 (2.9)
Estradiol [nmol/L]	0.44 ± 0.37	0.42 ± 0.40	0.43 ± 0.45	0 (0.0)
Follicle-stimulating hormone [IU/L]	44.4 ± 30.0	31.6 ± 29.7	32.0 ± 27.6	0 (0.0)
FSH/E2 [IU/pmol]	0.41 ± 1.41	0.28 ± 1.00	0.23 ± 0.69	0 (0.0)
Anthropometrics and body composition				
Body mass index [kg/m^2][b]	25.7 ± 4.1	25.9 ± 3.8	25.9 ± 3.8	17 (2.5)
Fat free mass [kg]	46.6 ± 5.3	47.3 ± 5.3	47.4 ± 5.2	17 (2.5)
Fat mass [kg]	23.1 ± 9.0	22.7 ± 8.2	22.7 ± 8.3	17 (2.5)
Body fat percentage	32.3 ± 7.9	31.6 ± 7.7	31.5 ± 7.6	17 (2.5)
Menstrual cycle characteristics				
Cycle length mean [d]	49.8 ± 21.9	39.9 ± 19.3	38.0 ± 17.1	189 (27.5)
Cycle length standard deviation [d]	30.1 ± 22.5	20.6 ± 21.7	18.7 ± 17.8	189 (27.5)
Cycle length range [d]	71.7 ± 50.5	57.1 ± 56.4	52.6 ± 46.8	189 (27.5)
Menopausal symptoms and gynecological history				
Vasomotor symptoms[b,c]				8 (1.2)
No	11 (10.5)	93 (34.3)	214 (31.5)	
Yes	94 (89.5)	178 (65.7)	465 (68.5)	
Somatic or pain symptoms[b,c]				8 (1.2)
No	59 (56.2)	197 (72.7)	446 (65.7)	
Yes	46 (43.8)	74 (27.3)	233 (34.3)	
Psychological symptoms[b,c]				8 (1.2)
No	33 (31.4)	123 (45.4)	294 (43.3)	
Yes	72 (68.6)	148 (54.6)	385 (56.7)	
Urogenital symptoms[b,c]				8 (1.2)
No	59 (56.2)	174 (64.2)	433 (63.8)	
Yes	46 (43.8)	97 (35.8)	246 (36.2)	
Use of hormonal contraception[b,c]				10 (1.5)
Never	89 (84.8)	216 (80.0)	536 (79.2)	
Former	16 (15.2)	54 (20.0)	141 (20.8)	
Age at menarche [yr.][b,d]	13 (1.3)	13 (2)	13 (2)	18 (2.6)
Parity[b,d]	2 (2)	2 (2)	2 (2)	12 (1.7)
Pregnancies[b,d]	2 (2)	2 (2)	2 (3)	14 (2.0)
Physical activity				
Accelerometer, MVPA [min/d]	50.6 ± 31.8	48.7 ± 27.3	51.5 ± 29.6	114 (16.6)
Accelerometer, Counts $\times 10^5$	6.11 ± 2.01	6.19 ± 1.78	6.34 ± 1.90	114 (16.6)
PA questionnaire [MET×h/d][b]	4.43 ± 4.13	4.32 ± 4.18	4.41 ± 4.37	18 (2.6)
Single seven-level scale question[b,c]				17 (2.5)
1	1 (1.0)	10 (3.7)	30 (4.5)	
2	5 (4.9)	22 (8.2)	49 (7.3)	
3	10 (9.7)	24 (9.0)	53 (7.9)	
4	31 (30.1)	63 (23.5)	168 (25.1)	
5	43 (41.7)	102 (38.1)	244 (36.4)	
6	12 (11.7)	44 (16.4)	120 (17.9)	
7	1 (1.0)	3 (1.1)	6 (0.9)	
Life habits and other self-report variables				
Alcohol consumption [portions/wk][b]	4.30 ± 4.21	3.62 ± 3.72	3.60 ± 3.73	17 (2.5)
Smoking[b,c]				21 (3.1)
Never	75 (72.8)	180 (67.2)	457 (68.6)	
Former	26 (25.2)	72 (26.9)	169 (25.4)	
Current	2 (1.9)	16 (5.9)	40 (6.0)	
Education[b,c]				10 (1.5)
Secondary	52 (49.5)	140 (51.9)	366 (54.1)	
Tertiary	53 (50.5)	130 (48.1)	311 (45.9)	
Relationship status[b,c]				18 (2.6)
Single	18 (17.5)	61 (22.8)	163 (24.4)	
In relationship	85 (82.5)	207 (77.2)	506 (75.6)	
Physical workload[b,c]				78 (11.4)
Light	48 (51.1)	135 (55.1)	316 (51.9)	
Moderate	23 (24.5)	53 (21.6)	139 (22.8)	
Heavy	20 (21.3)	52 (21.2)	148 (24.3)	
Very heavy	3 (3.2)	5 (2.1)	6 (1.0)	

The first two data columns include only the values from the last valid measurement from each participant. Data are mean ± SD unless otherwise specified.

ANM, age at natural menopause; FSH/E2, follicle-stimulating hormone/estradiol; MVPA, moderate-to-vigorous physical activity; PA, physical activity.

[a]Missing data rates illustrated in data set that includes all measurements.

[b]Easily accessible predictor.

[c]Data are n (%).

[d]Data are median (IQR).

was set to 50 and passive imputation was used for derived variables, such as FSH/E2 -ratio and body fat percentage.

Model selection and validation

Cox regression models with time-varying covariates[28] and age as time scale[29] were used for predicting the ANM. The proportional hazards assumption of the Cox models was tested using Schoenfeld residuals and the model selection was carried out using the lasso (Least Absolute Shrinkage and Selection Operator) regression[30] in two separate sets of candidate predictors. The first set included all 32 candidate predictors and the second set included only 16 of them that could be measured without any clinical measurement devices and long-term diaries. Thus, the second set included all self-report covariates as well as BMI (Table 1) and the aim of using this set of candidate predictors was to investigate the predictive performance of easily accessible covariates that a woman can effortlessly provide herself without expert assistance.

The model selection was done in R using the ''penalized'' package.[31] The optimal value of the tuning parameter lambda was initially chosen separately for all 50 imputed data sets using cross-validation and the covariates of interest were selected as the intersection of all predictor sets. However, the optimal lambda value resulted in 19 covariates of interest with all candidate predictors and in 11 covariates of interest with easily accessible candidate predictors which might lead to overfitting with current data set with effective sample size of 105. Thus, based on the good average requirement presented by Harrell[32] in which the maximum number of predictors should be less than effective sample size divided by 15, we increased the lambda value to limit the number of covariates of interest to seven.

The predictive performance of fitted models was quantified using pooled concordance index c[32] and leave-one-out cross-validation that was used for studying the errors between the predicted and observed ANM. The mean of the median survival times from all 50 complete data sets was used for prediction. Bootstrap validation with 200 resamples in 1 randomly selected imputed data set was used to estimate how accurately the models predict the ANM on future subjects or subjects not used to develop the model. Additionally, the leave-one-out cross-validation was also used for sensitivity analysis by utilizing only the first measurement from each participant to investigate the predictive performance of the constructed models with longer time from measurement to ANM. Analysis was carried out in R using the ''rms'' package.[33]

RESULTS

Characteristics of study population

With the notation of mean ± standard deviation, the age of the participants in the baseline measurement was 51.2 ± 1.8 with minimum of 48.6 and maximum of 57.4 years. The number of valid measurement time points varied from 1 to 9 for each participant. In the complete data set of 279 participants, the number of participants with specific number of measurement time points in order from 1 to 9 was 143, 37, 24, 23, 26, 13, 8, 3, and 2. Respective numbers for participants with known ANM ($n = 105$) were 27, 31, 16, 12, 11, 3, 3, 1, and 1. The mean time between repeated measurements was 163 ± 44 days and the time from the last measurement to ANM varied from 4 to 196 days with the mean of 70 ± 49. Valid full follow-up time varied from 0.00 to 3.67 years with the mean of 0.86 ± 0.97 years. The mean ANM in the study population was 52.8 ± 1.9 years. Other characteristics of the candidate predictors and the number of missing values is given in Table 1.

Constructed models

Among all 50 imputed data sets, the median c-index for models in which the predictors were selected from the set of all available covariates (model 1) was 0.762 with the minimum of 0.755 and maximum of 0.783. Respectively, the median c-index for the model in which the predictors were selected from the set of easily accessible covariates (model 2) was 0.701 with the minimum of 0.694 and maximum of 0.706. The model predictors as well as pooled model hazard ratios, P values, and c-indices are given in Table 2.

Model validation

The predictive performance of the models was measured by the mean absolute error (MAE) between the predicted and observed values (Table 3). The MAEs for model 1 (0.56 y) and model 2 (0.62 y) were clearly smaller than the MAE (1.58 y) for the model that used the sample mean as the prediction. The distributions of model errors are illustrated in Figure 2. Furthermore, leave-one-out cross-validation indicated that both models 1 and 2 were slightly biased toward predicting younger ANM compared with the observed values. However, mean bias errors were approximately only 1 month (0.09 y) for model 1 and 2 months (0.18 y) for model 2. The bootstrap validation with one randomly selected data set and 200 resamples demonstrates that there was no significant overfitting present with either one of the conducted models with bootstrap estimate of c-index being 0.74 and 0.65 for models 1 and 2, respectively (Table 4).

The sensitivity analysis indicated that the time from measurement to ANM varied from 0.01 to 2.94 years with mean of 0.90 ± 0.72 and the MAEs were 0.55 ± 0.44 years for model 1 and 0.61 ± 0.54 years for model 2 (Supplemental Digital Content 1, http://links.lww.com/MENO/A742). For both models, the MAEs were distinctly smaller for the participants with the time from measurement to ANM varying from 0 to 0.5 years (0.52 ± 0.38 and 0.44 ± 0.32 y, respectively) or 0.5 to 1.5 years (0.36 ± 0.26 and 0.39 ± 0.29 y, respectively) compared to participants with intervals longer than 1.5 years (1.00 ± 0.57 and 1.41 ± 0.56 y, respectively).

DISCUSSION

Our longitudinal study of 279 pre- and perimenopausal women from whom 105 had observed ANM demonstrated that especially higher estradiol and FSH levels, irregular menstrual bleeding, vasomotor symptoms, and higher level

TABLE 2. *Pooled model characteristics*

	Hazard ratio (95% CI)	*P* value	*c*-index (95% CI)[a]
Model 1: Predictors selected from set of all available predictors			
Estradiol [nmol/L]	2.13 (1.42-3.18)	<0.001	0.76 (0.71-0.81)
Follicle-stimulating hormone [IU/L]	1.01 (1.01-1.02)	<0.001	
Cycle length standard deviation [d]	1.02 (1.01-1.03)	<0.001	
Alcohol consumption [portions/wk]	1.07 (1.02-1.12)	0.009	
SR-PA7 (linear)	2.29 (0.55-9.57)	0.257	
Vasomotor symptoms		0.003	
No	1 reference		
Yes	2.68 (1.41-5.12)		
Relationship status		0.182	
Single	1 reference		
In relationship	1.42 (0.85-2.38)		
Model 2: Predictors selected from the set of easily accessible predictors			
Alcohol consumption [portions/wk]	1.06 (1.01-1.12)	0.016	0.70 (0.65-0.75)
SR-PA7 (linear)	1.85 (0.46-7.49)	0.388	
Vasomotor symptoms		<0.001	
No	1 reference		
Yes	3.33 (1.80-6.19)		
Use of hormonal contraception		0.184	
Never	1 reference		
Former	0.68 (0.38-1.21)		
Relationship status		0.245	
Single	1 reference		
In relationship	1.35 (0.81-2.25)		
Smoking			
Never	1 reference		
Former	0.99 (0.61-1.60)	0.959	
Current	0.37 (0.09-1.52)	0.169	
Education		0.297	
Secondary	1 reference		
Tertiary	1.25 (0.82-1.91)		

CI, confidence interval; SR-PA7, single seven-level scale question for physical activity assessment.
[a]Pooled using Rubin's rules for logit transformed index values.

of alcohol consumption are indicators of approaching natural menopause. The two models constructed in the study demonstrated adequate performance for predicting the ANM by reaching the threshold of good ($c \geq 0.7$) but not strong ($c < 0.8$) concordance with the observed values. Furthermore, the predictions of both models were distinctly more accurate compared with using sample mean ANM as the prediction for all participants.

Mostly, the associations observed in the study are in agreement with the literature. However, a novel observation in the study was that the participants tended to increase their self-reported alcohol consumption when approaching the ANM, although previous studies have reported no association[34] or positive association between alcohol consumption and ANM.[35,36] On the other hand, menopausal transition has been previously shown to be a period of instability regarding alcohol consumption[37] and increasing alcohol consumption could potentially be influenced by negative affect, such as depressive symptoms, caused by hormonal changes during the menopausal transition.[38] Furthermore, the observed association between higher estradiol levels and shorter time to natural menopause was interesting considering that estradiol levels are known to decrease during the menopausal transition. Nonetheless, similar associations have been reported previously[34] and they may result from estradiol levels remaining relatively constant until the late perimenopause and even slightly increasing before they start to decrease toward postmenopause.[39]

TABLE 3. *Differences between the predicted and observed age at natural menopause computed using leave-one-out cross-validation (n = 104[a])*

	Model 1	Model 2	Predicted sample mean
Observed ANM	52.77 ± 1.89	52.77 ± 1.89	52.77 ± 1.89
Predicted ANM	52.68 ± 1.79	52.59 ± 1.72	52.77 ± 0.00
Bias error[b]	−0.09 ± 0.74	−0.18 ± 0.80	0.00 ± 1.89
Absolute error[b]	0.56 ± 0.49	0.62 ± 0.54	1.58 ± 1.02
Squared error[b]	0.55 ± 0.97	0.67 ± 1.12	3.55 ± 4.01
Pairwise *t* test[b]	*t* = −1.298	*t* = −2.245	
	df = 103	df = 103	
	p = 0.197	p = 0.027	

Data are mean ± SD in years unless otherwise specified.
ANM, age at natural menopause.
[a]Number of participants with valid ANM prediction.
[b]Between observed and predicted values. Pairwise *t* test was not carried out for predicted sample mean since the results would not be meaningful.

Covariates, such as educational level, relationship status, physical activity, BMI, parity, and age at menarche, have been frequently associated with ANM,[36,40,41] yet contradictory results have also been reported.[19,34,42] In this study, educational level, relationship status, and physical activity were only weakly associated with ANM; however, they still increased the accuracy of the models if included as predictors. On the other hand, BMI, parity, and age at menarche were not chosen by the lasso regression as predictors in either one of the models. These results that are partially contradictory to

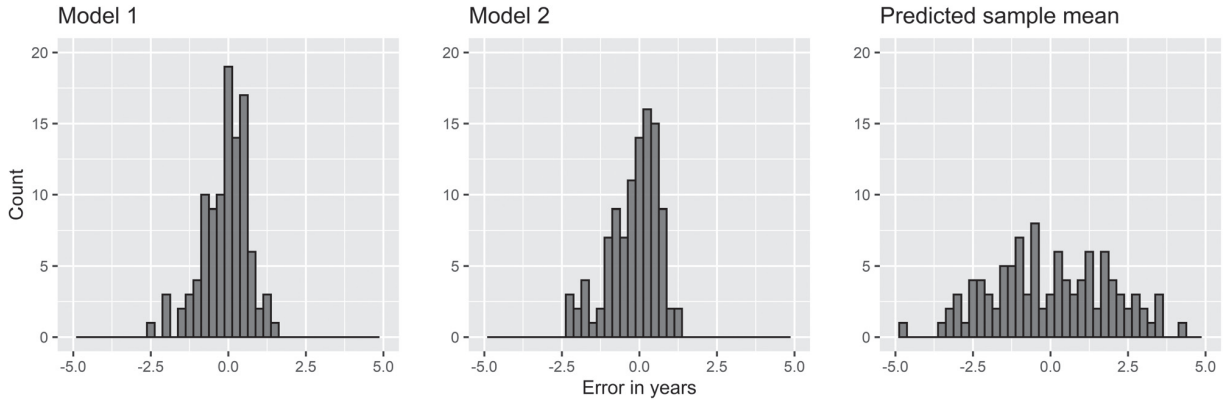

FIG. 2. Histograms illustrating the distributions of model errors.

previous findings may result from the unique study design that utilizes information from several measurements carried out during the perimenopause. Thus, unlike most of the previous studies, this study also captures the changes that occur during the menopausal transition in addition to associations of certain covariates with ANM. Furthermore, the predictors for the models were not chosen based on their statistical significance but by optimizing the constructed models.

The greatest strength of the study is the methodological approach including dealing with the missing data values, using models that encompass time-varying covariates, and utilizing an extensive set of candidate predictors with automated model selection. As the participants were a homogenous sample of Finnish middle-aged women, the generalizability of the results is good for populations consisting of Scandinavian White women but poorer for more heterogeneous populations. Furthermore, although assessing the postmenopausal status using bleeding diaries and FSH measurements is considered advantageous compared with retrospective self-reports, the use of a 6-month follow-up period instead of 12 months to verify to postmenopausal status may have led to misclassification for some participants. The age range of 47 to 55 years as well as the exclusion of postmenopausal women in the baseline may have also caused selection bias since women with younger ANM were more likely to be excluded. Other limitations of the study are the data set with relatively small sample size and follow-up time less than 4 years for all participants. This increases the uncertainty in the results and keeps the constructed models from being suited for predicting the ANM for women in their 30s or early 40s. Additionally, some potentially strong predictors of ANM, such as anti-Müllerian hormone levels, follicle counts, or mother's ANM,[43] were not included in the set of candidate predictors because they were not available.

Although the models were constructed with a data set in which the time from last measurement to ANM were less than 7 months for all participants, the sensitivity analysis using only one measurement from each participant demonstrated that the models provide adequate prediction accuracy when using measurements that are carried out up to 18 months before the ANM (Supplemental Digital Content 1, http://links.lww.com/MENO/A742). These are encouraging results indicating that by utilizing a training data set with longer follow-up time and possibly a few additional candidate predictors, the suggested predictors and methodological approach could also be used for discovering the diagnostic rules for predicting ANM for younger premenopausal women.

CONCLUSIONS

Higher estradiol and FSH levels, irregular menstrual cycles, and menopausal symptoms are strong indicators of approaching menopause in middle-aged women. Additionally, information related to life habits and socioeconomic factors, such as alcohol consumption, smoking habits, relationship status, physical activity, and the use of hormonal contraception may provide useful information for assessing the time to natural menopause. The suggested approach for predicting ANM

TABLE 4. *Bootstrap validation of constructed models*

	Original sample	Training sample	Test sample	Optimism	Corrected index	*n*
Model 1						
c-index	0.763	0.775	0.747	0.028	0.735	200
R^2	0.162	0.190	0.135	0.056	0.106	200
Slope[a]	1.000	1.000	0.766	0.233	0.766	200
Model 2						
c-index	0.697	0.715	0.672	0.043	0.654	200
R^2	0.097	0.121	0.070	0.051	0.046	200
Slope[a]	1.000	1.000	0.696	0.304	0.696	200

[a]Calibration slope (slope of predicted log odds vs true log odds).

could be useful for clinicians when making decisions related to the use of hormonal contraception and treatment for menopausal symptoms in perimenopausal women. However, further studies with a similar methodological approach, long-term follow-up time and a more comprehensive set of covariates are warranted to develop models with improved predictive performance that would be applicable in more heterogeneous populations and for women in their 30s or early 40s.

Acknowledgments: The authors thank the participants of the ERMA study who volunteered their time and effort.

REFERENCES

1. Zhu D, Chung H, Dobson AJ, et al. Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data. *Lancet Public Health* 2019;4:e553-e564.
2. 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 Cardiol* 2016;1:767-776.
3. Georgakis MK, Thomopoulos TP, Diamantaras A, et al. Association of age at menopause and duration of reproductive period with depression after menopause: a systematic review and meta-analysis. *JAMA Psychiatry* 2016;73:139-149.
4. Fistarol M, Rezende CR, Figueiredo Campos AL, Kakehasi AM, Geber S. Time since menopause, but not age, is associated with increased risk of osteoporosis. *Climacteric* 2019;22:523-526.
5. Sullivan SD, Lehman A, Nathan NK, Thomson CA, Howard BV. Age of menopause and fracture risk in post-menopausal women randomized to calcium + vitamin D, hormone therapy, or the combination: results from the women's health initiative clinical trials. *Menopause* 2017;24:371-378.
6. Collaborative Group on Hormonal Factors in Breast Cancer. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol* 2012;13:1141-1151.
7. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiol Rev* 1993;15:36-47.
8. Gao Y, Zhao M, Dai X, Tong M, Wei J, Chen Q. The prevalence of endometrial cancer in pre- and postmenopausal Chinese women. *Menopause* 2016;23:884-887.
9. Ossewaarde ME, Bots ML, Verbeek ALM, et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology* 2005; 16:556-562.
10. McKinlay SM. The normal menopause transition: an overview. *Maturitas* 1996;23:137-145.
11. Greendale GA, Ishii S, Huang M, Karlamangla AS. Predicting the timeline to the final menstrual period: the study of women's health across the nation. *J Clin Endocrinol Metab* 2013;98:1483-1491.
12. Kim C, Slaughter JC, Wang ET, et al. Anti-müllerian hormone, follicle stimulating hormone, antral follicle count, and risk of menopause within 5 years. *Maturitas* 2017;102:18-25.
13. Finkelstein JS, Lee H, Karlamangla A, et al. Antimullerian hormone and impending menopause in late reproductive age: the study of women's health across the nation. *J Clin Endocrinol Metab* 2020;105:e1862-e1871.
14. Hefler LA, Grimm C, Bentz E, Reinthaller A, Heinze G, Tempfer CB. A model for predicting age at menopause in white women. *Fertil Steril* 2006;85:451-454.
15. Kovanen V, Aukee P, Kokko K, et al. Design and protocol of estrogenic regulation of muscle apoptosis (ERMA) study with 47 to 55-year-old women's cohort: novel results show menopause-related differences in blood count. *Menopause* 2018;25:1020-1032.
16. 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. *Menopause* 2012;19:387-395.
17. Harlow SD, Lin X, Ho MJ. Analysis of menstrual diary data across the reproductive life span applicability of the bipartite model approach and the importance of within-woman variance. *J Clin Epidemiol* 2000; 53:722-733.
18. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 2003;88:2404-2411.
19. Gold EB. The timing of the age at which natural menopause occurs. *Obstet Gynecol Clin North Am* 2011;38:425-440.
20. Monteleone P, Mascagni G, Giannini A, Genazzani AR, Simoncini T. Symptoms of menopause—global prevalence, physiology and implications. *Nat Rev Endocrinol* 2018;14:199-215.
21. Laakkonen EK, Kulmala J, Aukee P, et al. Female reproductive factors are associated with objectively measured physical activity in middle-aged women. *PLoS One* 2017;12:e0172054.
22. Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. *J Sci Med Sport* 2011;14:411-416.
23. Hyvärinen M, Sipilä S, Kulmala J, et al. Validity and reliability of a single question for leisure-time physical activity assessment in middle-aged women. *J Aging Phys Act* 2020;28:231-241.
24. Kujala UM, Kaprio J, Sarna S, Koskenvuo M. Relationship of leisure-time physical activity and mortality: the Finnish twin cohort. *JAMA* 1998;279:440-444.
25. Buuren Sv, Groothuis-Oudshoorn K. Mice: Multivariate imputation by chained equations in R. *J Stat Softw* 2011;45:1-67.
26. Rubin DB. *Multiple Imputation for Nonresponse in Surveys*. New York: Wiley; 1987.
27. van Buuren S. *Flexible Imputation of Missing Data.*, 2nd ed. Boca Raton, FL: CRC Press; 2018.
28. Fisher LD, Lin DY. Time-dependent covariates in the cox proportional-hazards regression model. *Annu Rev Public Health* 1999;20:145-157.
29. Thiébaut ACM, Bénichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. *Stat Med* 2004;23:3803-3820.
30. Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc Series B Stat Methodol* 1996;58:267-288.
31. Goeman JJ. L-1 penalized estimation in the cox proportional hazards model. *Biom J* 2010;52:70-84.
32. Harrell FE. *Regression Modeling Strategies.*, 2nd ed. New York: Springer; 2015.
33. Harrell FE. Rms: Regression modeling strategies. 2019. Available at: https://CRAN.R-project.org/package=rms. Accessed November 11, 2020.
34. Santoro N, Brockwell S, Johnston J, et al. Helping midlife women predict the onset of the final menses: SWAN, the study of women's health across the nation. *Menopause* 2007;14:415-424.
35. Kinney A, Kline J, Levin B. Alcohol, caffeine and smoking in relation to age at menopause. *Maturitas* 2006;54:27-38.
36. Gold EB, Crawford SL, Avis NE, et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. *Am J of Epidemiol* 2013; 178:70-83.
37. Peltier MR, Verplaetse TL, Roberts W, et al. Changes in excessive alcohol use among older women across the menopausal transition: a longitudinal analysis of the study of women's health across the nation. *Biol Sex Differ* 2020;11:1-37.
38. Peltier MR, Verplaetse TL, Mineur YS, et al. Sex differences in stress-related alcohol use. *Neurobiol Stress* 2019;10:100149.
39. Sowers MR, Zheng H, McConnell D, Nan B, Harlow SD, Randolph JF. Estradiol rates of change in relation to the final menstrual period in a population-based cohort of women. *J Clin Endocrinol Metab* 2008;93: 3847-3852.
40. Schoenaker DA, Jackson CA, Rowlands JV, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. *Int J Epidemiol* 2014;43:1542-1562.
41. Rödström K, Bengtsson C, Milsom I, Lissner L, Sundh V, Bjoürkelund C. Evidence for a secular trend in menopausal age: a population study of women in gothenburg. *Menopause* 2003;10:538-543.
42. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. *Am J of Epidemiol* 1997;145:124-133.
43. Depmann M, Broer SL, van der Schouw. Yvonne T, et al. Can we predict age at natural menopause using ovarian reserve tests or mother's age at menopause? A systematic literature review. *Menopause* 2016;23:224-232.