'This is a **man's** *world...'*

Cardiovascular disease in women:
female-specific risk factors
and risk prediction

Veerle Dam

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Colofon

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“This is a man’s world…”

Cardiovascular disease in women: female-specific risk factors and risk prediction

“Dit is een mannen wereld…”

Hart- en vaatziekten in vrouwen: vrouwspecifieke risico factoren en risico voorspelling
(met een samenvatting in het Nederlands)

Proefschrift

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Veerle Dam

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CHAPTER 1

General introduction

Formerly, cardiovascular disease (CVD) was seen as a male disease, and for many years research on CVD was conducted in men only. In 1897, the typical heart disease patient was described as a "… keen and ambitious man, the indicator of whose engine is always at full speed ahead."[1]. In 1991 dr. Bernadine Healy 'coined' the term 'the Yentl Syndrome' to describe sex differences in coronary heart disease (CHD) management, referring to Isaac Bashevis Singer's short story in which the Ashkenazi Jewish woman Yentl had to disguise herself as a man to attend school and study. Healy showed that women are less likely to get treated for angina or chest pain compared to men. However, when studying women after cardiac catheterization, there were no sex differences in the likelihood of getting coronary surgery[2]. So, only when women were seen as men (after severe CHD was diagnosed), women were treated equally. Nowadays, CVD is still the leading cause of death in both sexes in Western countries with 17.7 million deaths worldwide in 2015. Sex differences are recognized more often and incidence rates show that women accounted for more new cases of CVD compared to men (~5.7 million cases versus ~5.3 million cases) in 2015[3]. Moreover, in the past four decades CHD deaths even appeared to increase in women between 35 and 54, likely because of the obesity epidemic[4]. Therefore, research has now shifted towards a more sex-specific approach, with an increased role for female-specific risk factors.

Studies show that women have similar risk factors compared to men, although their impact is different for some in women. The association between total cholesterol and low density lipoprotein cholesterol and CHD death is less strong in women compared to men and prolonged smoking seems to have a potentially stronger association with CVD risk in women than in men. However, elevated blood pressure does not seem to have a different effect on CVD risk in men or women and the association between body mass index (BMI) and CHD is approximately similar in men and women as well[5,6]. So, these generally known risk factors cannot completely explain the difference in CVD risk between men and women. Therefore, female-specific risk factors, like early age at menarche and menopause, pregnancy complications and polycystic ovary syndrome (PCOS) are receiving increasing attention in cardiovascular research[7].

Female-specific risk factors

CHD risk increases in women after the age of 50, leading to suggestions that menopause may be a contributing factor[8–10]. A recent-meta analysis suggested that women with an age at menopause before age 45 had a 50% higher coronary heart disease (CHD) risk compared to those with later menopause[11]. However, not all studies could be included, because of differences in categorization of age at menopause or because studies analysed age at menopause linearly, while in some of these studies an association was not convincingly demonstrated. Furthermore, the mechanisms underlying the association between menopause and CVD are not fully understood yet. The decline in oestrogens after menopause has been suggested to cause the increase in

CVD risk[12]. Therefore, randomized clinical trials (RCTs) have been initiated that provide women with postmenopausal hormone therapy to protect them against CVD after menopause. However, these RCTs could not confirm the protective effect of exogenous oestrogens, some even finding an increased risk for CHD and stroke[13], while others do not find an association at all[14], casting doubt on the exact role of oestrogens in CHD development.

Besides menopause itself, also vasomotor menopausal symptoms (VMS), both hot flushes and night sweats, have been associated with cardiovascular risk factors. Studies show that women who experience VMS have a higher systolic and diastolic blood pressure, higher total cholesterol levels and higher BMI[15]. Furthermore, VMS have also been associated with intermediate CVD outcomes like lower flow mediated dilation, higher aortic calcification, higher carotid intima media thickness and plaque[16–19]. These results indicate that also VMS might increase CVD risk. However, studies that investigate hard clinical endpoints are scarce and show contradictory results[20–24].

Another female-specific risk factor that might increase CVD risk is PCOS. PCOS is the most common endocrine disorder in women of reproductive age with a prevalence varying between 6%-15%, depending on the criteria used for diagnosis of PCOS[25,26]. Recent meta-analyses showed that women with PCOS had a statistically significantly higher CVD risk compared to women without CVD. However, the results for CHD and stroke are less robust[27–29].

Cardiovascular disease risk prediction

Now research on CVD is more often focused on sex differences and female-specific risk factors, guidelines are trying to implement the findings. For example, the American guidelines for the prevention of cardiovascular diseases in women define women with preeclampsia, gestational diabetes or hypertensive disorders of pregnancy (HDP) as 'at risk' for CVD and they advise that these women need close monitoring. However, this is often not routinely done in women, and, as symptoms of CVD are more subtle in women, there is often delayed diagnosis, and thus delayed treatment, consequently leading to poorer prognosis in women[30,31]. In order to monitor and assess risk in women, physicians need properly validated CVD risk prediction models that are useful in women and specific female-populations. Based on predictions of those models, physicians can decide whether or not to treat women for CVD risk factors. However, even though there is an abundance of CVD risk prediction models available for the general population[32], and a lot of these models seem to have a separate risk equation for women, there is no proper evidence available on the performance of these models in women, making it hard for physicians to decide on the right model for the female patient. The general cardiovascular prevention guidelines often recommend using the Framingham Risk Score[33], Pooled Cohort Equations[34] or, in Europe, the

Systematic Coronary Risk Evaluation model (SCORE)[35], but these models are not validated in populations with a high prevalence of some female-specific risk factors.

Objectives of this thesis

This thesis studies female specific risk factors for cardiovascular disease and CVD risk prediction in women. The aims of this thesis are 1) to study whether female-specific risk factors (menopausal status, age at menopause, VMS and PCOS) are (causally) associated with CVD, CHD and stroke; 2) to investigate possible the role of cardiovascular risk factors as mechanisms underlying this association; and 3) to assess the availability and performance of existing CVD risk prediction models for women in the general population and to establish whether the performance of a subset of commonly used models differs between women with and without a history of HDP.

Outline of this thesis

Part one

In the first part of this thesis we investigated the etiologic (causal) associations between female specific risk factors and CVD and we tried to identify possible mechanisms underlying this association with cardiovascular risk factors. Chapter 2.1 starts with unravelling the association of menopausal status with cardiovascular risk factors, independent of age in the LifeLines Cohort study. Subsequently, we studied the association of menopausal status, and in postmenopausal women, age at menopause and type of menopause with CHD (Chapter 2.2). Furthermore, this chapter also investigates whether cardiovascular risk factors mediate this association, both using the pan-European EPIC-CVD case-cohort study. As a third step, we studied the causal association between age at natural menopause and CHD and CVD risk factors using a Mendelian Randomization approach with 56 Single Nucleotide Polymorphisms (SNPs) associated with younger age at natural menopause as instrumental variables (Chapter 2.3). This part uses summary data from the UK Biobank, a modified version of CARDIoGRAMplusC4D, EPIC-CVD, the Global Lipids Genetics Consortium and MAGIC. Besides menopause, we also studied the association between VMS (both hot flushes and night sweats) with CVD, CHD and cerebrovascular disease using data from the Australian Longitudinal Study on Women's Health (Chapter 2.4). Lastly, we investigated whether PCOS was associated with CVD, CHD and stroke in the EPIC-NL cohort (Chapter 2.5).

Part two

The second part of this thesis was focused on CVD risk prediction in women. The first chapter (Chapter 3.1) systematically reviews risk prediction models that are developed in a female population or that included sex as a predictor. In the second chapter, we validated three of these

models, the Framingham Risk Score, Pooled Cohort Equations and SCORE, in women with and without a history of HDP and subsequently recalibrated and refitted the models.

References

1 Osler W. Lectures on Angina Pectoris and Allied States. 1897.

2 Healy B. The Yentl Syndrome. *N Engl J Med* 1991;**325**:274–6.

3 Timmis A, Townsend N, Gale C, *et al.* European Society of Cardiology: Cardiovascular disease statistics 2017. *Eur Heart J* 2018;**39**:508–77. doi:10.1093/eurheartj/ehx628

4 Mosca L, Benjamin EJ, Berra K, *et al.* Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update. *J Am Coll Cardiol* 2011;**57**:1404–23. doi:10.1016/j.jacc.2011.02.005

5 Appelman Y, van Rijn BB, Ten Haaf ME, *et al.* Sex differences in cardiovascular risk factors and disease prevention. *Atherosclerosis* 2015;**241**:211–8. doi:10.1016/j.atherosclerosis.2015.01.027

6 Collins P, Webb CM, de Villiers TJ, *et al.* Cardiovascular risk assessment in women – an update. *Climacteric* 2016;**19**:329–36. doi:10.1080/13697137.2016.1198574

7 Bairey Merz CN, Ramineni T, Leong D. Sex-specific risk factors for cardiovascular disease in women-making cardiovascular disease real. *Curr Opin Cardiol* 2018;:1. doi:10.1097/HCO.0000000000000543

8 Kannel WB, Hjortland MC, McNamara PM, *et al.* Menopause and risk of cardiovascular disease: the Framingham study. *AnnInternMed* 1976;**85**:447–52.

9 Gordon T, Kannel WB, Hjortland MC, *et al.* Menopause and coronary heart disease. The Framingham Study. *Ann Intern Med* 1978;**89**:157–61.http://www.ncbi.nlm.nih.gov/pubmed/677576

10 Colditz GA, Willett WC, Stampfer MJ, *et al.* Menopause and the risk of coronary heart disease in women. *NEnglJMed* 1987;**316**:1105–10.

11 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**:767–76. doi:10.1001/jamacardio.2016.2415

12 Van Der Graaf Y, van der Schouw YT. Menopause and cardiovascular disease. *J Psychosom Obstet Gynecol* 1997;**18**:113–20. doi:10.1177/2053369117749675

13 Beral V, Banks E, Reeves G. Evidence from randomised trials on the long-term effects of hormone replacement therapy. *Lancet* 2002;**360**:942–4. doi:10.1016/S0140-6736(02)11032-4

14 Benkhadra K, Mohammed K, Al Nofal A, *et al.* Menopausal Hormone Therapy and Mortality: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* 2015;**100**:4021–8. doi:10.1210/jc.2015-2238

15 Franco OH, Muka T, Colpani V, *et al.* Vasomotor symptoms in women and cardiovascular risk markers: Systematic review and meta-analysis. *Maturitas* 2015;**81**:353–61. doi:10.1016/j.maturitas.2015.04.016

16 Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, *et al.* Hot flashes and subclinical cardiovascular disease: Findings from the Study of Women's Health Across the Nation Heart Study. *Circulation* 2008;**118**:1234–40. doi:10.1161/CIRCULATIONAHA.108.776823

17 Thurston RC, Kuller LH, Edmundowicz D, *et al.* History of hot flashes and aortic calcification among postmenopausal women. *Menopause* 2010;**17**:256–61. doi:10.1097/gme.0b013e3181c1ad3d

18 Thurston RC, El Khoudary SR, Tepper PG, *et al.* Trajectories of Vasomotor Symptoms and Carotid Intima Media Thickness in the Study of Women's Health Across the Nation. *Stroke* 2016;**47**:12–7. doi:10.1161/STROKEAHA.115.010600

19 Jackson EA, El Khoudary SR, Crawford SL, *et al.* Hot Flash Frequency and Blood Pressure: Data from the Study of Women's Health Across the Nation. *J Women's Health* 2016;**25**:1204–9. doi:10.1089/jwh.2015.5670

20 Svartberg J, von Mühlen D, Kritz-Silverstein D, *et al.* Vasomotor symptoms and mortality. *Menopause* 2009;**16**:888–91. doi:10.1097/gme.0b013e3181a4866b

21 Gast G-CM, Pop VJM, Samsioe GN, *et al.* Vasomotor menopausal symptoms are associated with increased risk of coronary heart disease. *Menopause* 2011;**18**:146–51. doi:10.1097/gme.0b013e3181f464fb

22 Szmuilowicz ED, Manson JE, Rossouw JE, *et al.* VAsomotor symptoms and vascular events in postmenopausal women. *Menopause* 2012;**18**:603–10. doi:10.1097/gme.0b013e3182014849. Vasomotor

23 Herber-Gast GCM, Brown WJ, Mishra GD. Hot flushes and night sweats are associated with coronary heart disease risk in midlife: A longitudinal study. *BJOG An Int J Obstet Gynaecol* 2015;**122**:1560–7. doi:10.1111/1471-0528.13163

24 Thurston RC, Johnson BD, Shufelt CL, *et al.* Menopausal symptoms and cardiovascular disease mortality in the Women's Ischemia Syndrome Evaluation (WISE). *Menopause* 2017;**24**:126–32. doi:10.1097/GME.0000000000000731

25 Fauser BCJM. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;**81**:19–25. doi:10.1016/j.fertnstert.2003.10.004

26 March WA, Moore VM, Willson KJ, *et al.* The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;**25**:544–51. doi:10.1093/humrep/dep399

27 Zhao L, Zhu Z, Lou H, *et al.* Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. *Oncotarget* 2016;**7**:33715–21. doi:10.18632/oncotarget.9553

28 Anderson SA, Barry JA, Hardiman PJ. Risk of coronary heart disease and risk of stroke in women with polycystic ovary syndrome: A systematic review and meta-analysis. *Int J Cardiol* 2014;**176**:486. doi:10.1016/j.ijcard.2014.06.079

29 de Groot PCM, Dekkers OM, Romijn JA, *et al.* PCOS, coronary heart disease, stroke and the influence of obesity: A systematic review and meta-analysis. *Hum Reprod Update* 2011;**17**:495–500. doi:10.1093/humupd/dmr001

30 Mosca L, Linfante AH, Benjamin EJ, *et al.* National study of physician awareness and adherence to cardiovascular disease prevention guidelines. *Circulation* 2005;**111**:499–510. doi:10.1161/01.CIR.0000154568.43333.82

31 Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovascular Disease. *Physiol Rev* 2017;**97**:1–37. doi:10.1152/physrev.00021.2015

32 Damen JAAG, Hooft L, Schuit E, *et al.* Prediction models for cardiovascular disease risk in the general population: systematic review. *BMJ* 2016;**353**:i2416. doi:10.1136/bmj.i2416

33 D'Agostino RB, Vasan RS, Pencina MJ, *et al.* General cardiovascular risk profile for use in primary care: The Framingham heart study. *Circulation* 2008;**117**:743–53. doi:10.1161/CIRCULATIONAHA.107.699579

34 Goff DC, Lloyd-Jones DM, Bennett G, *et al.* 2013 ACC/AHA guideline on the assessment of cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. *J Am Coll Cardiol* 2014;**63**:2935–59. doi:10.1016/j.jacc.2013.11.005

35 Conroy RM, Pyörälä K, Fitzgerald a. P, *et al.* Estimation of ten-year risk of fatal cardiovascular disease in Europe: The SCORE project. *Eur Heart J* 2003;**24**:987–1003. doi:10.1016/S0195-668X(03)00114-3

PART ONE

Female-specific risk factors for cardiovascular disease in the general population

CHAPTER 2.1

Unravelling the associations of age and menopause with cardiovascular risk factors in a large population-based study

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Abstract

Background: Although the association between menopause and cardiovascular disease (CVD) risk has been studied extensively, the simultaneous role of chronological aging herein remains underexposed. This study aims to disentangle the relationships of menopausal status and chronological aging with CVD risk factors in the largest study population to date.

Methods: In this cross-sectional study, CVD risk factors were compared between women with a different menopausal status within the same yearly age strata. The study population comprised female participants of the baseline visit of the population-based LifeLines Cohort Study. A total of 63,466 women, aged between 18 and 65 years, was included. Of them, 39,379 women were considered to be premenopausal, 8669 were perimenopausal, 14,514 were naturally postmenopausal, and 904 women were surgically postmenopausal.

Results: Compared to postmenopausal women aged 45 years, average total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) were 0.5 and 0.4 mmol/L higher, respectively, in postmenopausal women aged 50. Systolic and diastolic blood pressure levels were 4 and 1 mmHg higher, respectively. At all ages between 46 and 55 years, and after adjustment for confounders, naturally postmenopausal women had 0.2 to 0.4 mmol/L higher TC and 0.1 to 0.3 mmol/L higher LDL-c levels compared to premenopausal women in the same age range. Systolic blood pressure levels were up to 4 mmHg lower in naturally post- compared to premenopausal women at all ages between 29 and 52 years. Body mass index levels were up to 3.2 kg/m^2 higher in women with surgical menopause compared to all other women between ages 32 and 52 years. All aforementioned results were statistically significant.

Conclusions: Chronological age and menopausal status are both independently associated with CVD risk factors. Based on the comparatively smaller observed differences associated with menopausal status than chronological aging, the significance of a more unfavourable lipid profile in a later reproductive stage may be less obvious than previously thought.

Introduction

Menopause is the final result of the continuous decline of ovarian reserve, marking the end of a woman's reproductive lifespan. An earlier age of reaching menopause is considered to be associated with an increased risk of cardiovascular disease (CVD)[1,2], but the mechanisms through which menopause is associated with CVD remain unclear. The menopausal transition and postmenopausal status have been associated with adverse CVD risk factor levels[3–10], but studies have recently contended that chronological aging or prior CVD risk play a more important role[11–13].

As postmenopausal women are, by definition, older than premenopausal women, it is challenging to separate the effects of biological aging from the various phases of the reproductive aging process[14]. This problem was previously circumvented by exclusively studying 53-year-old women born within the same week[7], longitudinally estimating the rate of change of CVD risk factors in the time surrounding the final menstrual period[6,15], or comparing blood pressure levels between women in biannual age strata[16]. However, as the menopausal transition occurs over several years, its longitudinal effects can be ascribed to both aging and menopausal status in the same participant. The currently available studies were furthermore not able to assess the individual effects of chronological and reproductive aging over a large age interval.

In this study, we aimed to disentangle the associations of menopausal status and chronological aging with CVD risk factors over a wide age range. To this end, we compared levels of CVD risk factors with menopausal status, within and between yearly age strata, in the largest study population to date.

Methods

Cohort profile

For our study population, there were 80,853 potentially eligible women between 18 and 65 years old who participated in the baseline examination of the LifeLines Cohort Study. LifeLines is a multi-disciplinary prospective population-based cohort study examining, in a unique three-generation design, the health and health-related behaviours of 167,729 persons living in the north of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics[17,18]. The cohort participants were recruited through general practitioner registrations between 2006 and 2013. Cohort members are examined at baseline and will be prospectively followed up with visits in 5-year intervals and questionnaires every 1.5 years. The current study was based on information from the baseline examination, which includes a questionnaire, anthropometric measurements and blood withdrawal. All participants gave written informed

consent[19] and ethical approval was granted by the medical ethics committee of University Medical Centre Groningen[18]. LifeLines is a facility that is open for all researchers. Information on application and data access procedure is summarized on www.lifelines.net.

Menopausal status assessment

Women with an intra-uterine contraceptive device (n=2445, 3.0%), who previously underwent a hysterectomy (n=4937, 6.2%), and/or who reported never having had a regular menstrual cycle (n=4780, 5.9%) were excluded, leaving 73,662 women. Participants were then divided into groups based on menopausal status, which were defined as premenopausal, perimenopausal, naturally postmenopausal or surgically menopausal. Group allocation was based on baseline questionnaire information and followed the Stages of Reproductive Aging Workshop (STRAW) criteria[20]. Women with a currently regular menstrual cycle (n=39,379, 53.4%) were classified as premenopausal. Women with an irregular menstrual cycle since several months (n=7661) or years (n=1260; total n=8669, 11.8%) were considered to be perimenopausal. Women who answered that they were postmenopausal when asked about cycle regularity, and with a date of last menstruation being more than 1 year before the visit (n=14,514, 19.7%), were considered to be naturally postmenopausal. Women who reported having had a bilateral oophorectomy (n=904, 6.7%) were classified as surgically postmenopausal. The reproductive status of 5293 (7.2%) women could not be determined. This left 63,466 women in the study population.

Cardiovascular risk factor assessment

At the baseline examination, height and weight were measured by trained staff, from which body mass index (BMI; in kg/m^2) was calculated. Systolic and diastolic blood pressure (SBP and DBP) were measured 10 times during 10 minutes using a Dynamap PRO (GE Healthcare, Freiburg, Germany) [18], from which the average values were used. The baseline examination furthermore included fasting venous blood withdrawal. Directly after blood withdrawal, prespecified biomarkers in each fasting blood sample were routinely assessed at the in-house laboratory of the University Medical Centre Groningen. Serum levels of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) were assessed with an enzymatic colorimetric method, low-density lipoprotein cholesterol (LDL-c) was assessed with a colorimetric method and triglyceride (TG) levels were measured with a colorimetric ultraviolet method, with a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland). Fasting blood glucose was assessed with a hexokinase method[21].

Other variables

The questionnaires additionally contained questions about hormonal contraception or postmenopausal hormone therapy (HT) use and smoking status. Participants were asked whether

they had ever or were currently using oral contraceptives, a hormonal intrauterine device, contraceptive injection (henceforth altogether referred as hormonal contraception) or HT. Current use included any use in the prior month. Smoking status was assessed by asking participants whether they were current smokers or had smoked the previous month. Current and ever smokers were furthermore asked about the total duration and daily frequency of smoking. For this study, smoking status was defined as current smoker (yes or no), including women who had smoked up until the prior month.

Women who were pregnant at the time of examination (n=109, 0.1%) were asked to fill out the questionnaire about the period preceding their pregnancy. They completed their baseline visit at least 6 months after their pregnancy and 3 months after ceasing to breastfeed, at which point the questionnaire was handed in and blood withdrawal occurred.

Data analysis

For all variables of interest, the number of complete cases was 60,811 (96%) and missing information per variable did not exceed 1%. Missing values were imputed by conditional multiple imputation with 10 iterations, through predictive mean matching for continuous variables and proportional odds for categorical variables. All CVD risk factor variables, with the exception of TG, were normally distributed. As the distribution of TG levels was right-skewed, TG levels were log-transformed. Baseline characteristics were presented across menopausal status groups as mean ± SD or n (%), unless stated otherwise.

To gain a first insight in differences in CVD risk factor levels between the menopausal status categories independently of age, a linear regression analysis was performed within each 1-year age stratum for each outcome, with premenopausal women as the reference category. Women below the age of 34 were all included in a 34-years and younger group, due to the relative lack of postmenopausal women before this age. In similar fashion, women above age 56 were all included in a 56-years and older age stratum. The regression analyses were adjusted for smoking status, current hormonal contraception and BMI due to their potential association with both menopausal status and CVD risk factors. Because BMI was considered to be both a potential confounder and CVD risk factor, a model with BMI as an outcome was also fit, which adjusted for smoking and hormonal contraception use only. Models were furthermore adjusted for antihypertensive and lipid-lowering medication.

The objective of investigating an independent association of both calendar age and menopausal status with CVD risk factor levels was addressed by creating a linear regression model for each CVD risk factor as an outcome, with menopausal status and age as independent covariables. In order to adjust for smoking status, hormonal contraception use, antihypertensive or lipid-lowering medication and BMI (except in the case of BMI as a CVD risk factor outcome), these parameters

were additionally added to the model. To test whether the association with age differed between the menopausal status groups, we included an interaction term of menopausal status with age in the model and tested its significance with an analysis of variance (ANOVA). Furthermore, in order to take into account a potential non-linear relationship of age with CVD risk factors, restricted cubic splines for age were added to the model[22,23]. The model was then tested for non-linearity with an ANOVA analysis. Using the resulting best fitting model (excluding the interaction term or splines if the interaction term or test for non-linearity were non-significant), the adjusted values for each outcome were plotted against age for each menopausal status group.

All statistical analyses were performed with R (www.r-project.org), version 3.1.3. Multiple imputation was done using the 'mice' library, using a prediction matrix with all determinants, outcomes and confounders[24]. The regression models were fitted with the fit.mult.impute function from the 'Hmisc' library.

Sensitivity analyses

We performed four sensitivity analyses. First, the analyses described above were repeated after including women with missing reproductive status information, by assigning them to menopausal groups based on their age, similar to the methods by Clavel-Chapelon *et al.*[25]. Secondly, the analyses were repeated after excluding women who reported current use of cholesterol- or blood pressure-lowering medication. Thirdly, the analyses were performed with only inclusion of women who reported an irregular cycle 'since several months' as the perimenopausal group. Finally, as the classification of the STRAW criteria for the whole study population was based on the answers to the question of cycle regularity and menopause, hormonal contraception and HT use were not taken into account for this determination. To assess the differences between the menopausal status groups independently from exogenous hormone use, women who had ever used HT or currently used hormonal contraception were excluded from analysis.

Patient involvement

The development of the research question and study design occurred without the involvement of patients. The research question fits within the scope of healthy aging in the general population, an objective set by LifeLines.

Results

In Table 1, the number of women in each age stratum and menopausal status group is listed. Characteristics for women in each reproductive category are presented in Table 2. Mean age increased over the pre-, peri-, and postmenopausal groups, and so did the mean levels of all of CVD risk factors. Hormonal contraception usage decreased over the pre-, peri- and postmenopausal

groups, with the lowest percentage of users in the surgically postmenopausal group. The vast majority of women who reported ever using HT (3% of the study population) were postmenopausal (77%), with the highest percentage (64%) in the surgical menopause group. In the premenopausal group, 203 (0.5%) women said to have ever used HT, but reported a currently regular menstrual cycle. In the naturally postmenopausal group, median age (interquartile range, IQR) at menopause was 51 (46-53) years.

For all CVD risk factors studied, the association between age and risk factor level was significantly non-linear (P value for non-linearity <0.001 in all cases), so all models included restricted cubic splines for age. In addition, for all CVD risk factors besides SBP and glucose there was a significant interaction between age and menopausal status (P values for the interaction term ranged between <0.001 and 0.01), indicating that the magnitude of the differences in these risk factor levels between menopausal status groups varied with age. The models including cubic splines and the interaction term had a better fit than the models without, assessed by comparison of the Akaike's Information Criterion. All model residuals were furthermore normally distributed. Since a single regression coefficient cannot be estimated due to the splines and interactions, the fully adjusted mean levels with 95% confidence interval (CI) bands of all CVD risk factors are displayed for each menopausal status group with age in figure 1 (A-H).

Table 1. Number of study participants in each menopausal status group per annual age stratum

Age stratum	Premenopausal	Perimenopausal	Naturally post-menopausal	Surgically post-menopausal	Total
18	726	32	1	0	759
19	561	19	2	0	582
20	552	35	3	0	590
21	638	29	8	1	676
22	655	50	4	0	709
23	670	50	7	0	727
24	704	51	14	0	769
25	813	79	10	0	902
26	1138	119	26	1	1284
27	1064	120	25	0	1209
28	982	101	19	0	1102
29	971	119	19	1	1110
30	950	107	27	0	1084
31	995	116	28	2	1141
32	1028	119	42	3	1192
33	1070	110	32	1	1213
34	1118	99	39	2	1258

Table 1. Continued

Age stratum	Premenopausal	Perimenopausal	Naturally post-menopausal	Surgically post-menopausal	Total
35	1151	113	54	1	1319
36	1235	138	70	5	1448
37	1369	134	83	8	1594
38	1464	145	63	6	1678
39	1606	153	108	15	1882
40	1731	187	95	7	2020
41	1796	245	119	11	2171
42	1860	248	121	16	2245
43	1783	352	135	22	2292
44	1781	380	137	19	2317
45	1740	490	157	28	2415
46	1577	535	208	40	2360
47	1489	659	290	39	2477
48	1337	743	392	43	2515
49	1148	788	508	61	2505
50	898	795	703	51	2447
51	467	565	691	37	1760
52	134	233	464	18	849
53	69	169	556	22	816
54	57	123	653	32	865
55	29	62	802	24	917
56	14	32	886	23	955
57	4	16	870	28	918
58	1	6	902	38	947
59	3	1	876	47	927
60	1	1	900	29	931
61	0	0	883	30	913
62	0	1	817	52	870
63	0	0	821	37	858
64	0	0	790	48	808
65	0	0	84	56	140
Total	**39397**	**8669**	**14514**	**904**	**63466**

Between ages 29 and 52 mean SBP levels adjusted for hormonal contraception use, smoking and BMI were significantly lower in the naturally postmenopausal group compared to the three

other menopausal status groups, as there was no overlap of CIs (Figure 1A). Compared to the premenopausal group, fully adjusted SBP levels were between 2.6 and 4.0 mmHg lower in the naturally postmenopausal group. Similar results were found with the regression analyses within each age stratum (Appendix 1: Table S1 displays the regression coefficients with 95% CI for the linear regression analyses in each age stratum for SBP). With regard to chronological aging, compared to age 45, adjusted SBP levels at age 50 were between 3.0 to 3.8 mmHg higher on average (Table 3). No distinct pattern of differences between menopausal stages within the age bands was observed for DBP (Figure 1B, Appendix 1: Table S2). Adjusted DBP levels in all menopausal status groups were between 0.9 and 1.6 mmHg higher at age 50 compared to age 45 (Table 3).

Table 2. Characteristics per menopausal status group

	Premenopausal ***n = 39,397***	Perimenopausal ***n = 8,669***	Naturally post-menopausal ***n = 14,514***	Surgically post-menopausal ***n = 904***
	Baseline			
Age (years)	36.9 ± 8.1	45.0 ± 8.1	55.3 ± 7.4	52.7 ± 8.1
Age range (years)	18-60	18-62	18-65	21-65
Current OC use	18,526 (47.6)	1,938 (22.7)	1,787 (12.6)	825 (2.6)
Current smoker	8,125 (21.0)	1,969 (22.9)	2,751 (19.1)	165 (18.4)
Antihypertensive med.	1,559 (4.0)	608 (7.0)	2,356 (20.3)	182 (20.3)
Lipid-lowering med.	388 (1.0)	178 (2.1)	1,222 (8.4)	92 (10.2)
Ever HRT use	203 (0.5)[a]	275 (3.2)	1315 (9.1)	253 (28.4)
	Outcome			
BMI (kg/m^2)	25.2 ± 4.6	26.0 ± 4.9	26.2 ± 4.5	27.3 ± 5.0
SBP (mm Hg)	119 ± 13	121 ± 14	125 ± 16	126 ± 16
DBP (mm Hg)	71 ± 9	72 ± 9	73 ± 9	72 ± 9
TC (mmol/L)	4.7 ± 0.8	5.0 ± 0.9	5.6 ± 1.0	5.5 ± 1.0
LDL-c (mmol/L)	2.9 ± 0.8	3.1 ± 0.8	3.6 ± 0.9	3.5 ± 0.9
HDL-c (mmol/L)	1.6 ± 0.4	1.6 ± 0.4	1.7 ± 0.4	1.6 ± 0.4
TG (mmol/L)	1.0 ± 0.5	1.0 ± 0.6	1.1 ± 0.6	1.2 ± 0.7
Glucose (mmol/L)	4.8 ± 0.6	4.9 ± 0.7	5.0 ± 0.8	5.1 ± 1.0

Values given in mean ± SD or n (%)
[a]All reported a currently regular cycle
HT hormone replacement therapy; BMI body mass index; SBP systolic blood pressure; DBP diastolic blood pressure; TC total cholesterol; LDL-c low-density lipoprotein cholesterol; HDL-c high-density lipoprotein cholesterol; TG triglycerides

Fully adjusted mean TC and LDL-c levels were 0.1 mmol/L higher in the perimenopausal group compared to the premenopausal group, and 0.2-0.4 mmol/L higher in the naturally postmenopausal group compared to the premenopausal group across the range of 45-55 years, which reached statistical significance (Figure 1C). Between 37 and 49 years, adjusted TC levels were 0.2-0.4 mmol/L higher in the surgically postmenopausal group compared to women in the premenopausal group, and significantly higher than all three other groups (Figure 1C). Between 46 and 55 years, adjusted LDL-c levels in the peri- and naturally postmenopausal groups were 0.1 and 0.3 mmol/L, respectively. Surgically postmenopausal women had significantly higher adjusted LDL-c levels than all other women between the ages of 38 and 49. Linear regression analyses within the age strata echoed these results (appendix 1: Tables S3 and S4). With respect to chronological aging, the average adjusted difference in TC and LDL-c levels between 45 and 50 years ranged from 0.2 to 0.5 and 0.2 to 0.4 mmol/L, respectively (Table 3).

No clear differences were observed in mean adjusted HDL-c or glucose levels between the menopausal status groups at all ages (Figure 1E and F, Appendix 1: Tables S5 and S6). Compared to women aged 45 years, mean adjusted HDL-c and glucose levels were 0.0-0.1 mmol/L higher at age 50, dependent on menopausal status group (Table 3).

Fully adjusted mean TG levels were up to 12% higher in surgically postmenopausal women compared to premenopausal women between the ages 42 and 53. Between the ages 32 and 52, BMI levels were up to 3.2 kg/m^2 higher in surgically postmenopausal compared to premenopausal women. In these age ranges, TG and BMI levels were significantly higher in surgically postmenopausal women compared to women in all other menopausal status groups (Figure 1G and H). In contrast, compared to premenopausal women, TG levels were 5-22% lower in postmenopausal women between the ages of 30 and 48. Similar results were found in the linear regression analyses in each age stratum, although the differences with the surgically postmenopausal group were not significant, possibly due to lack of power (Appendix 1: Tables S5 and S6). At age 50, TG levels were 0.1 mmol/L higher in all menopausal status groups compared to age 45 (Table 3). Adjusted BMI levels were either the same or between 0.1-0.4 kg/m^2 lower at age 50 compared to age 45, depending on the menopausal status group (Table 3).

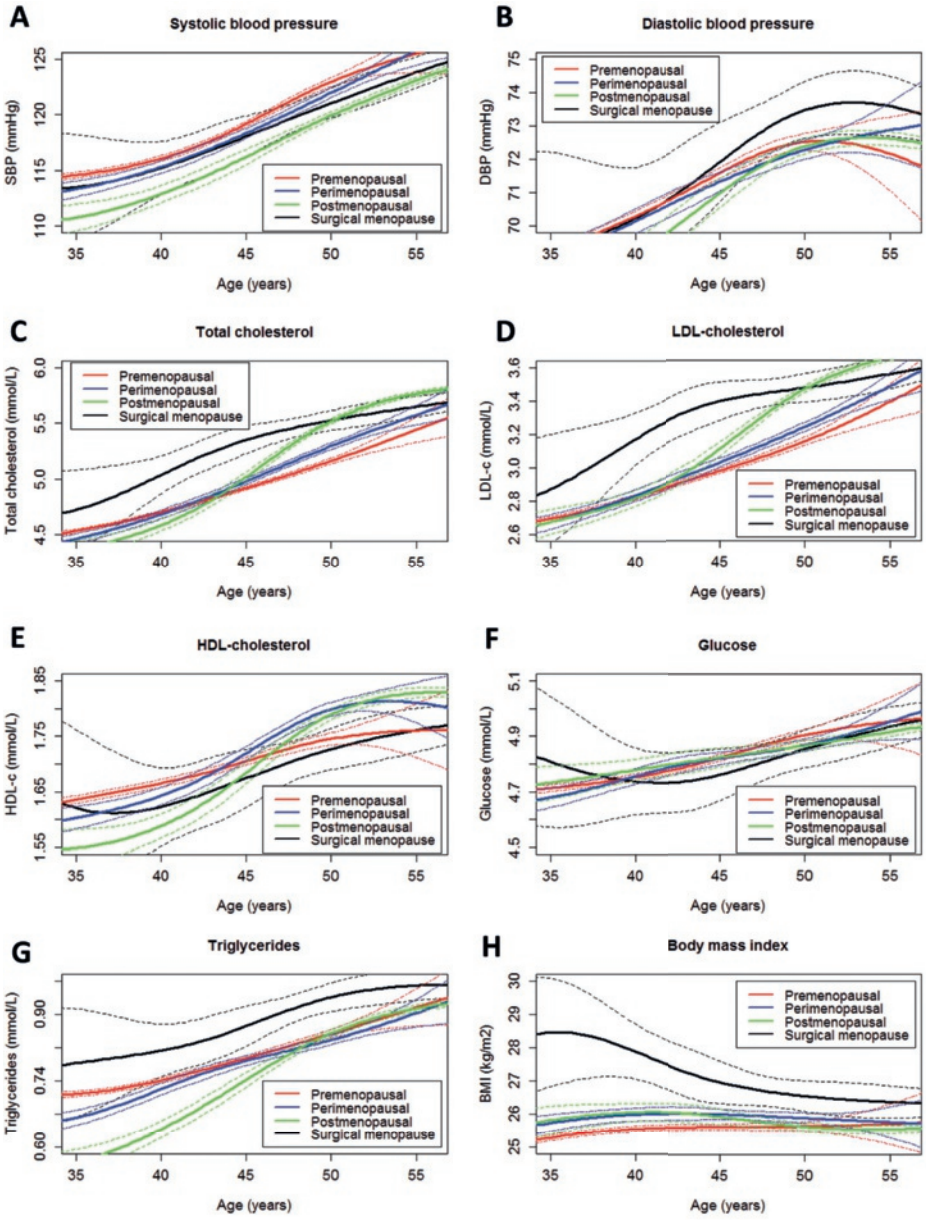

Figure 1 A-H. Associations of adjusted cardiovascular risk factors with age per menopausal status group. Cardiovascular risk factor levels were adjusted for age, hormonal contraceptive use, smoking status and body mass index. The premenopausal status group comprised a total of 39,379 women, whereas the perimenopausal group 8669 women, the naturally postmenopausal group 14,514 women, and the surgically postmenopausal group 904 women. ***a*** *Systolic blood pressure,* ***b*** *Diastolic blood pressure,* ***c*** *Total cholesterol,* ***d*** *LDL-cholesterol,* ***e*** *HDL-cholesterol,* ***f*** *Glucose,* ***g*** *Triglycerides and* ***h*** *Body mass index*

Sensitivity analyses

The sensitivity analyses are summarized for each outcome in appendices 2, 3, 4, 5, 6, 7, 8, and 9: Figures S1-S8. First, inclusion of the 5293 women with an age-based reproductive status did not alter the results. Second, the exclusion of women who used cholesterol- or blood pressure-lowering medication (n=1880 and n=4705, respectively) also did not alter the results, although the confidence interval of the surgical menopause group became wider. Third, excluding 1260 women in the perimenopausal group with an irregular cycle since several years additionally did not alter the nature of the results for the perimenopausal group. Fourth, exclusion of women using hormonal contraception (n=23,076) and HT (n=2056) caused an expected widening of the confidence intervals due to the reduced power. This did not affect the overall results, with the exception of a more marked difference in TC, LDL-c and TG levels between young pre- and postmenopausal women (Appendices 4, 5 and 7: Figures S3, S4 and S6).

Table 3. Absolute differences in adjusted risk factors for women aged between 45-50 and 35-55 years per menopausal status group

	Premenopausal	Perimenopausal	Naturally Post-menopausal	Surgically meno-pausal
Difference in adjusted risk factor levels (95% CI) between 45-50 years				
SBP (mm Hg)	3.8 (3.6 to 3.9)	3.6 (3.6 to 3.7)	3.7 (3.4 to 4.1)	3.0 (2.5 to 3.5)
DBP (mm Hg)	0.9 (0.8 to 1.0)	1.0 (1.0 to 1.0)	1.4 (1.2 to 1.6)	1.6 (1.2 to 1.9)
TC (mmol/L)	0.2 (0.2 to 0.3)	0.3 (0.3 to 0.3)	0.5 (0.5 to 0.5)	0.2 (0.1 to 0.2)
LDL-c (mmol/L)	0.2 (0.2 to 0.2)	0.2 (0.2 to 0.2)	0.4 (0.3 to 0.4)	0.1 (0.0 to 0.1)
HDL-c (mmol/L)	0.0 (0.0 to 0.0)	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.1)	0.1 (0.0 to 0.1)
Glucose (mmol/L)	0.1 (0.1 to 0.1)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.1)	0.1 (0.1 to 0.1)
TG (mmol/L)	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.1)
BMI (kg/m^2)	0.0 (-0.0 to 0.1)	-0.1 (-0.1 to -0.1)	-0.3 (-0.4 to -0.2)	-0.4 (-0.6 to -0.2)

SBP systolic blood pressure; DBP diastolic blood pressure; TC total cholesterol; LDL-c low-density lipoprotein cholesterol; HDL-c high-density lipoprotein cholesterol; TG triglycerides; BMI body mass index

Discussion

This study presents a unique view of reproductive aging, independently of biological aging. We observed an association of CVD risk factors with menopausal status within several clusters of annual age strata, indicating that this relationship cannot be explained by the effects of chronological aging alone. The magnitude of differences in CVD risk factors between menopausal status groups did vary with age, highlighting the added role of chronological aging. Based on these results, it seems likely that both chronological aging and menopausal status contribute to the CVD risk profile of aging women.

Naturally postmenopausal women had lower adjusted SBP levels across a large age range than pre-, peri or surgically postmenopausal women. Prior reports found a later reproductive stage to be associated with increased blood pressure[9,16,26], while others reported a lack of any association after adjustment for age[6,7,13,27,28]. A longitudinal study in 193 women was the first to detect a decreased SBP level in post- compared to premenopausal women[29], hypothesizing that a diminishing ovarian reserve exhibits a protective effect on increasing SBP levels. By design we cannot confirm this hypothesis, but our results do contest previous reports of an adverse blood pressure milieu in a peri- and postmenopausal state[9,16,26].

Where lipid levels are concerned, previous findings are less ambiguous and correspond well to our results. LDL-c and TC levels are widely thought to be influenced by the menopausal transition[6] or associated with menopausal status[4,5,7,10,30–33]. In fact, the approximate difference in LDL-c levels of 11 mg/dL (0.28 mmol/L) observed by Matthews *et al.* [6] between the year preceding and following the final menstrual period fits well within the range of our observations. The decrease of estradiol throughout the menopausal transition may not play a role in this regard, as TC and LDL-c levels did not correlate with total or free estradiol in 99 postmenopausal women[34]. On the other hand, postmenopausal hormone therapy was associated with a better lipid profile compared to placebo in a meta-analysis of 28 trials[35]. Another explanation is the reduced activity of LDL-c receptors or lipoprotein lipase in a postmenopausal state[36,37].

In our population, differences in LDL-c and TC levels between menopausal status groups only became evident after the age of 45, after which LDL-c and TC levels more sharply increased in the peri- and postmenopausal groups. While a rapid increase in lipid levels was previously linked to the menopausal transition[4,7], our results do suggest that chronological aging is equally involved. Indeed, the adjusted difference in TC and LDL-c values in the interval of 45-50 years was equal to the maximum observed differences between the menopausal status groups. It may be possible that, with increasing age, the availability of compensatory mechanisms to neutralize hyperlipidaemia diminishes.

Surgically postmenopausal women, having undergone a bilateral oophorectomy, had consistently higher BMI and TG levels than the remaining women in the same age stratum, the latter even after adjusting for BMI. Others observed similar results[13,38–42], with the odds of becoming obese specifically increasing after bilateral oophorectomy[41]. Interestingly, the adjusted BMI of pre-, peri- and naturally postmenopausal women hardly differed throughout the study population, which is in line with previous findings[38], but at odds with the observation that the menopausal transition influences fat distribution[15,32].

For the past two decades, the relationship of menopause with CVD risk factors has been studied extensively through a myriad of ways. As most previous research was based on smaller study populations, often with significantly differing ages between pre- and postmenopausal

groups, we hope to provide a substantial contribution to this age-old question with our study. Its strengths are the use of a large study population, with the ability to compare menopausal status groups and CVD risk factors within yearly age strata, over a wide age range. The protocolled assessment of study parameters and relative lack of missing information limit the chance of bias. Unfortunately, this was not quite the case for the classification of menopausal status. It is likely that some postmenopausal women using hormonal contraception or HT were classified as premenopausal due to the report of a regular cycle, and that some premenopausal women with an irregular cycle were wrongly classified as peri- or postmenopausal[43]. The exclusion of women using exogenous hormones did not have an obvious impact of the overall results, with the exception that the lipid profile of young postmenopausal women appeared notably more unfavourable than the other groups in this analysis. It is possible that this difference is due to the putative benefits of hormone supplementation in young women in particular[44], or incorrect classification of premenopausal women using hormonal contraception as postmenopausal. In order to be considered postmenopausal, women had to report in the questionnaire that they had entered menopause in addition to reporting an amenorrhea of at least a year, which makes large-scale misclassification in this category less likely. Moreover, the finding of very young women with non-iatrogenic menopause corresponds to our observations in clinical practice and other Dutch cohort studies and could therefore well be a realistic representation. Finally, due to the small number of women with surgical menopause, there is insufficient power to separately compare this group in all yearly strata. However, as this group of women represents a different clinical entity than natural menopause, we chose to maintain this classification.

Because the results did not differ with the sensitivity analyses, we do not believe this strongly influences our findings. As this was a cross-sectional study, our observations are limited to associations without drawing conclusions on causality, where it would have been preferable to follow the included women longitudinally. Lastly, due to the small number of women with surgical menopause, there is insufficient power to separately compare this group in all yearly strata. However, as this group of women represents a different clinical entity than natural menopause, we chose to maintain this classification.

As this was a cross-sectional study, our observations are limited to associations without drawing conclusions on causality. A previous study was able to longitudinally follow CVD risk factors[6], providing important information on the changes surrounding the menopausal transition. It is by definition impossible to distinguish these changes from general aging throughout the menopausal transition in the same participant, however, which is why our current study provides an important contribution from a different perspective. Although we are able to confirm previous reports of differences in lipid parameters based on menopausal status, the clinical implications of the observed differences may be limited. A reduction of LDL-c of 1.0 mmol/L was

associated with a 22% decreased rate of major vascular events in an extensive meta-analysis of individual patient data[45], but this difference is 2.5 to 10 times larger than menopause-related differences in this study or the study by Matthews et al.[6], and in fact more approximate to the differences found with 20 years of chronological age. It may be that the increased risk of cardiovascular events observed in postmenopausal women, the causality of which is a matter of debate in itself[11,12,46], is mediated through other pathways such as oxidative stress and inflammation[47]. A previous proposal of lipid screening of women entering the menopausal transition[6] may therefore not prove beneficial. That being said, vigilance of changing lipid parameters in high-risk women as they pass both biological and reproductive aging thresholds may be worthwhile.

Conclusions

In conclusion, we observed independent associations of both age and menopausal status with selected CVD risk factors, mainly at the level of lipid metabolism, in a large population-based cohort. The clinical ramifications of a more unfavourable CVD risk factor profile with the transition to menopause may be limited, however.

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References

1 van der Schouw YT, van der Graaf Y, Steyerberg EW, *et al.* Age at menopause as a risk factor for cardiovascular mortality. *Lancet* 1996;**347**:714–8.

2 Lisabeth LD, Beiser AS, Brown DL, *et al.* Age at natural menopause and risk of ischemic stroke the framingham heart study. *Stroke* 2009;**40**:1044–9. doi:10.1161/STROKEAHA.108.542993

3 El Khoudary SR, Santoro N, Chen HY, *et al.* Trajectories of estradiol and follicle-stimulating hormone over the menopause transition and early markers of atherosclerosis after menopause. *Eur J Prev Cardiol* 2015;**23**:694–703. doi:10.1177/2047487315607044

4 Auro K, Joensuu A, Fischer K, *et al.* A metabolic view on menopause and ageing. *Nat Commun* 2014;**5**:1–11. doi:10.1038/ncomms5708

5 Peters HW, Westendorp IC, Hak a E, *et al.* Menopausal status and risk factors for cardiovascular disease. *J Intern Med* 1999;**246**:521–8. doi:10.1046/j.1365-2796.1999.00547.x

6 Matthews KA, Crawford SL, Chae CU, *et al.* Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? *JAmCollCardiol* 2009;**54**:2366–73.

7 Kuh D, Langenberg C, Hardy R, *et al.* Cardiovascular risk at age 53 years in relation to the menopause transition and use of hormone replacement therapy: A prospective British birth cohort study. *BJOG An Int J Obstet Gynaecol* 2005;**112**:476–85. doi:10.1111/j.1471-0528.2005.00416.x

8 Casiglia E, Tikhonoff V, Boschetti G, *et al.* Arterial Stiffness and Related Variables Across Menopausal Status: An Epidemiologic Study. *J Women's Heal* 2013;**22**:75–84. doi:10.1089/jwh.2012.3666

9 Son MK, Lim NK, Lim JY, *et al.* Difference in blood pressure between early and late menopausal transition was significant in healthy Korean women. *BMC Womens Health* 2015;**15**:1–7. doi:10.1186/s12905-015-0219-9

10 Anagnostis P, Stevenson JC, Crook D, *et al.* Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions. *Maturitas* 2015;**81**:62–8. doi:10.1016/j.maturitas.2015.02.262

11 Atsma F, Bartelink ML, Grobbee DE, *et al.* Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. *Menopause* 2006;**13**:265–79.

12 Vaidya D, Becker DM, Bittner V, *et al.* Ageing, menopause, and ischaemic heart disease mortality in England, Wales, and the United States: modelling study of national mortality data. *Bmj* 2011;**343**:d5170–d5170. doi:10.1136/bmj.d5170

13 Appiah D, Schreiner PJ, Bower JK, *et al.* Is Surgical Menopause Associated With Future Levels of Cardiovascular Risk Factor Independent of Antecedent Levels? The CARDIA Study. *Am J Epidemiol* 2015;**182**:991–9. doi:10.1093/aje/kwv162

14 Bittner V. Menopause, Age, and Cardiovascular Risk. A Complex Relationship. *J Am Coll Cardiol* 2009;**54**:2374–5. doi:10.1016/j.jacc.2009.10.008

15 Sowers MF, Zheng H, Tomey K, *et al.* Changes in body composition in women over six years at midlife: Ovarian and chronological aging. *J Clin Endocrinol Metab* 2007;**92**:895–901. doi:10.1210/jc.2006-1393

16 Zanchetti A, Facchetti R, Cesana GC, *et al.* Menopause-related blood pressure increase and its relationship to age and body mass index: The SIMONA epidemiological study. *J Hypertens* 2005;**23**:2269–76. doi:10.1097/01.hjh.0000194118.35098.43

17 Stolk RP, Rosmalen JGM, Postma DS, *et al.* Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. *Eur J Epidemiol* 2008;**23**:67–74. doi:10.1007/s10654-007-9204-4

18 Scholtens S, Smidt N, Swertz MA, *et al.* Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol* 2015;**44**:1172–80. doi:10.1093/ije/dyu229

19 Freeman EW, Sammel MD, Lin H, *et al.* Contribution of the rate of change of antimüllerian hormone in estimating time to menopause for late reproductive-age women. *Fertil Steril* 2012;**98**:1254–1259.e2. doi:10.1016/j.fertnstert.2012.07.1139

20 Soules MR, Sherman S, Ph D, *et al.* Executive Summary. 2001;**10**:843–8.

21 Slagter SN, Van Vliet-Ostaptchouk J V., Vonk JM, *et al.* Combined effects of smoking and alcohol on metabolic syndrome: The lifelines cohort study. *PLoS One* 2014;**9**. doi:10.1371/journal.pone.0096406

22 Gurrin LC, Scurrah KJ, Hazelton ML. Tutorial in biostatistics: Spline smoothing with linear mixed models. *Stat Med* 2005;**24**:3361–81. doi:10.1002/sim.2193

23 Roshani D, Ghaderi E. Comparing smoothing techniques for fitting the nonlinear effect of covariate in cox models. *Acta Inform Medica* 2016;**24**:38–41. doi:10.5455/aim.2016.24.38-41

24 Zhang Z. Multiple imputation with multivariate imputation by chained equation (MICE) package. *Ann Transl Med* 2016;**4**:30. doi:10.3978/j.issn.2305-5839.2015.12.63

25 Clavel-Chapelon F. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. *Br J Cancer* 2002;**86**:723–7. doi:10.1038/sj.bjc.6600124

26 Staessen J, Bulpitt CJ, Fagard R, *et al.* The influence of menopause on blood pressure. *J Hum Hypertens* 1989;**3**:427–33. doi:10.1007/978-3-642-79077-5

27 Casiglia E, D'Este D, Ginocchio G, *et al.* Lack of influence of menopause on blood pressure and cardiovascular risk profile: A 16-year longitudinal study concerning a cohort of 568 women. J. Hypertens. 1996;**14**:729–36. doi:10.1097/00004872-199606000-00008

28 Portaluppi F, Pansini F, Manfredini R, *et al.* Relative influence of menopausal status, age, and body mass index on blood pressure. *Hypertension* 1997;**29**:976–9. doi:10.1161/01.HYP.29.4.976

29 Van Beresteyn ECH, Van 't Hof MA, De Waard H. Contributions of ovarian failure and aging to blood pressure in normotensive perimenopausal women: A mixed longitudinal study. *Am J Epidemiol* 1989;**129**:947–55. doi:10.1093/oxfordjournals.aje.a115227

30 Bonithon-kopp C, Scarabin PY, Darne B, *et al.* Menopause-related changes in lipoproteins and some other cardiovascular risk factors. *Int J Epidemiol* 1990;**19**:42–8. doi:10.1093/ije/19.1.42

31 De Aloysio D, Gambacciani M, Meschia M, *et al.* The effect of menopause on blood lipid and lipoprotein levels. *Atherosclerosis* 1999;**147**:147–53. doi:10.1016/S0021-9150(99)00315-9

32 Abdulnour J, Doucet É, Brochu M, *et al.* The effect of the menopausal transition on body composition and cardiometabolic risk factors: A Montreal-Ottawa New Emerging Team group study. *Menopause* 2012;**19**:760–7. doi:10.1097/gme.0b013e318240f6f3

33 He L, Tang X, Li N, *et al.* Menopause with cardiovascular disease and its risk factors among rural Chinese women in Beijing: a population-based study. *Maturitas* 2012;**72**:132–8. doi:10.1016/j.maturitas.2012.02.013

34 Chen Y, Zeleniuch-Jacquotte A, Arslan AA, *et al.* Endogenous hormones and coronary heart disease in postmenopausal women. *Atherosclerosis* 2011;**216**:414–9. doi:10.1016/j.atherosclerosis.2011.01.053

35 Casanova G, Bossardi Ramos R, Ziegelmann P, *et al.* Effects of Low-Dose Versus Placebo or Conventional-Dose Postmenopausal Hormone Therapy on Variables Related to Cardiovascular Risk: A Systematic Review and Meta-Analyses of Randomized Clinical Trials. *J Clin Endocrinol Metab* 2015;**100**:1028–37. doi:10.1210/jc.2014-3301

36 Arca M, Vega GL, Grundy SM. Hypercholesterolemia in Postmenopausal Women: Metabolic Defects and Response to Low-Dose Lovastatin. *JAMA J Am Med Assoc* 1994;**271**:453–9. doi:10.1001/jama.1994.03510300059039

37 Ferrara CM, Lynch NA, Nicklas BJ, *et al.* Differences in adipose tissue metabolism between postmenopausal and perimenopausal women. *J Clin Endocrinol Metab* 2002;**87**:4166–70. doi:10.1210/jc.2001-012034

38 Matthews KA, Abrams B, Crawford S, *et al.* Body mass index in mid-life women: Relative influence of menopause, hormone use, and ethnicity. *Int J Obes* 2001;**25**:863–73. doi:10.1038/sj.ijo.0801618

39 Dørum A, Tonstad S, Liavaag AH, *et al.* Bilateral oophorectomy before 50 years of age is significantly associated with the metabolic syndrome and Framingham risk score: A controlled, population-based study (HUNT-2). *Gynecol Oncol* 2008;**109**:377–83. doi:10.1016/j.ygyno.2008.02.025

40 Sutton-Tyrrell K, Zhao X, Santoro N, *et al.* Reproductive hormones and obesity: 9 years of observation from the study of women's health across the nation. *Am J Epidemiol* 2010;**171**:1203–13. doi:10.1093/aje/kwq049

41 Gibson CJ, Thurston RC, El Khoudary SR, *et al.* Body mass index following natural menopause and hysterectomy with and without bilateral oophorectomy. *Int J Obes* 2013;**37**:809–13. doi:10.1038/ijo.2012.164

42 McCarthy AM, Menke A, Visvanathan K. Association of bilateral oophorectomy and body fatness in a representative sample of US women. *Gynecol Oncol* 2013;**129**:559–64. doi:10.1016/j.ygyno.2013.02.016

43 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**:722–33. doi:10.1016/S0895-4356(99)00202-4

44 Hodis HN, Mack WJ. The timing hypothesis and hormone replacement therapy: A paradigm shift in the primary prevention of coronary heart disease in women. Part 1: Comparison of therapeutic efficacy. *J Am Geriatr Soc* 2013;**61**:1005–10. doi:10.1111/jgs.12140

45 Baigent C, Blackwell L, Emberson J, *et al.* Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170 000 participants in 26 randomised trials. *Lancet* 2010;**376**:1670–81. doi:10.1016/S0140-6736(10)61350-5

46 Kim C, Cushman M, Khodneva Y, *et al.* Risk of Incident Coronary Heart Disease Events in Men Compared to Women by Menopause Type and Race. *J Am Heart Assoc* 2015;**4**:1–10. doi:10.1161/JAHA.115.001881

47 Moreau KL, Hildreth KL. Vascular Aging across the Menopause Transition in Healthy Women. *Adv Vasc Med* 2014;**2014**:1–12. doi:10.1155/2014/204390

Appendix 1

Table S1. Differences (95% CI) in adjusted **Systolic blood pressure** *levels (mm Hg) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-1.5 (-2.0 to -0.9)	-3.6 (-4.7 to -2.4)	-0.16 (-6.4 to 5.9)
35	0.0 (Ref)	-1.5 (-3.5 to 0.5)	-3.0 (-5.9 to -0.1)	8.5 (-12.0 to 28.9)
36	0.0 (Ref)	-2.3 (-4.2 to -0.5)	-2.7 (-5.3 to -0.1)	2.3 (-6.8 to 11.5)
37	0.0 (Ref)	-2.3 (-4.2 to -0.4)	-4.5 (-7.0 to -2.1)	2.3 (-5.1 to 10.0)
38	0.0 (Ref)	0.6 (-1.3 to 2.6)	-5.0 (-7.9 to -2.0)	-2.2 (-11.4 to 7.0)
39	0.0 (Ref)	-0.4 (-2.4 to 1.5)	-2.5 (-4.9 to -0.1)	-3.6 (-9.8 to 2.6)
40	0.0 (Ref)	-0.7 (-2.5 to 1.1)	-2.6 (-5.2 to -0.1)	-6.2 (-15.2 to 2.7)
41	0.0 (Ref)	-1.1 (-2.7 to 0.5)	-4.6 (-6.9 to -2.3)	-5.1 (-12.2 to 2.0)
42	0.0 (Ref)	-1.2 (-2.8 to 0.5)	-2.7 (-5.1 to 0.4)	0.5 (-5.7 to 6.7)
43	0.0 (Ref)	-0.1 (-1.6 to 1.3)	-3.8 (-6.1 to -1.6)	1.7 (-3.7 to 7.0)
44	0.0 (Ref)	-1.0 (-2.5 to 0.5)	-3.1 (-5.5 to -0.8)	1.2 (-4.9 to 7.2)
45	0.0 (Ref)	0.1 (-1.3 to 1.5)	-3.0 (-5.3 to -0.7)	-1.7 (-6.9 to 3.5)
46	0.0 (Ref)	-1.0 (-2.4 to 0.3)	-3.7 (-5.7 to -1.7)	-3.2 (-7.5 to 1.1)
47	0.0 (Ref)	-1.3 (-2.6 to -0.1)	-2.7 (-4.4 to -0.9)	-0.0 (-4.4 to 4.4)
48	0.0 (Ref)	-2.2 (-3.6 to -0.9)	-2.5 (-4.1 to -0.9)	-0.9 (-5.2 to 3.5)
49	0.0 (Ref)	-1.0 (-2.4 to 0.3)	-4.2 (-5.8 to -2.7)	-3.6 (-7.4 to 0.1)
50	0.0 (Ref)	0.2 (-1.2 to 1.7)	-1.6 (-3.1 to -0.1)	-1.6 (-5.8 to 2.5)
51	0.0 (Ref)	-0.5 (-2.4 to 1.4)	-2.6 (-4.5 to -0.8)	-1.0 (-6.1 to 4.1)
52	0.0 (Ref)	0.6 (-2.7 to 3.9)	-2.2 (-5.2 to 0.8)	2.6 (-4.9 to 10.2)
53	0.0 (Ref)	-1.8 (-6.1 to 2.6)	-4.5 (-8.5 to -0.4)	-7.1 (-14.3 to 0.1)
54	0.0 (Ref)	1.3 (-3.5 to 6.2)	-1.7 (-6.0 to 2.7)	1.7 (-4.8 to 8.1)
55	0.0 (Ref)	-4.2 (-11.2 to 2.9)	-6.3 (-12.4 to -0.2)	-6.1 (-14.8 to 2.5)
≥56	0.0 (Ref)	-1.5 (-9.7 to 6.8)	-1.9 (-9.1 to 5.2)	-0.5 (-7.8 to 6.8)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Red cells indicate p-value <0.0001, green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S2. Differences (95% CI) in adjusted **Diastolic blood pressure** *levels (mm Hg) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-0.4 (-0.8 to -0.0)	-1.2 (-2.0 to -0.3)	2.1 (-2.1 to 6.3)
35	0.0 (Ref)	-0.8 (-2.3 to 0.6)	-1.4 (-3.5 to 0.7)	2.9 (-11.7 to 17.6)
36	0.0 (Ref)	-0.0 (-1.4 to 1.3)	-1.0 (-2.9 to -0.8)	0.2 (-6.4 to 6.8)
37	0.0 (Ref)	-0.9 (-2.3 to 0.5)	-2.3 (-4.1 to -0.5)	0.2 (-5.1 to 5.6)
38	0.0 (Ref)	0.9 (-0.4 to 2.4)	-2.5 (-4.6 to -0.3)	0.2 (-6.3 to 6.8)
39	0.0 (Ref)	0.7 (-0.6 to 2.1)	-1.5 (-3.1 to 0.2)	-2.0 (-6.3 to 2.3)
40	0.0 (Ref)	-0.5 (-1.7 to 0.7)	-1.5 (-3.2 to 0.2)	-3.1 (-9.1 to 2.8)
41	0.0 (Ref)	0.1 (-1.0 to 1.3)	-2.4 (-4.1 to -0.8)	-4.0 (-9.0 to 0.5)
42	0.0 (Ref)	-0.8 (-1.9 to 0.3)	-0.9 (-2.5 to 0.7)	3.6 (-0.6 to 7.7)
43	0.0 (Ref)	0.0 (-1.0 to 1.0)	-0.4 (-1.9 to 1.1)	3.0 (-0.6 to 6.6)
44	0.0 (Ref)	-0.4 (-1.3 to 1.6)	-1.2 (-2.8 to 0.3)	2.9 (-1.0 to 6.8)
45	0.0 (Ref)	0.2 (-0.7 to 1.1)	-0.7 (-2.2 to 0.7)	-0.3 (-3.6 to 3.0)
46	0.0 (Ref)	-0.7 (-1.6 to 0.1)	-0.5 (-1.8 to 0.7)	-1.5 (-4.2 to 1.3)
47	0.0 (Ref)	-0.8 (-1.6 to 0.0)	-0.7 (-1.8 to 1.4)	0.6 (-2.2 to 3.5)
48	0.0 (Ref)	-0.4 (-1.2 to 0.5)	0.4 (-0.7 to 1.4)	-0.6 (-3.3 to 2.2)
49	0.0 (Ref)	-0.8 (-1.6 to 0.1)	-0.9 (-1.9 to 0.0)	0.3 (-2.1 to 2.7)
50	0.0 (Ref)	0.6 (-0.3 to 1.5)	0.6 (-0.4 to 1.5)	0.3 (-2.3 to 2.9)
51	0.0 (Ref)	-0.8 (-1.9 to 0.4)	0.0 (-1.1 to 1.2)	2.0 (-1.0 to 5.1)
52	0.0 (Ref)	2.2 (0.2 to 4.2)	1.5 (-0.3 to 3.3)	3.2 (-1.3 to 7.7)
53	0.0 (Ref)	-0.3 (-3.0 to 2.4)	-0.5 (-3.0 to 1.9)	-1.5 (-5.6 to 2.9)
54	0.0 (Ref)	1.4 (-1.6 to 4.4)	0.5 (-2.2 to 3.2)	3.0 (-1.0 to 7.0)
55	0.0 (Ref)	-4.2 (-8.4 to 0.1)	-3.9 (-7.5 to -0.2)	-3.9 (-9.0 to 1.3)
≥56	0.0 (Ref)	-0.3 (-4.8 to 4.2)	-0.2 (-4.2 to 3.7)	0.6 (-3.4 to 4.6)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Green cells indicate p-value <0.001.

Table S3. Differences (95% CI) in adjusted **Total cholesterol** *levels (mmol/L) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-0.1 (-0.1 to -0.1)	-0.2 (-0.3 to -0.1)	0.1 (-0.4 to 0.7)
35	0.0 (Ref)	-0.3 (-0.4 to -0.1)	-0.2 (-0.4 to 0.0)	-0.0 (-1.5 to 1.5)
36	0.0 (Ref)	-0.1 (-0.2 to 0.1)	-0.2 (-0.4 to 0.0)	0.6 (-0.1 to 1.2)
37	0.0 (Ref)	0.0 (-0.1 to 0.2)	-0.3 (-0.5 to -0.1)	0.5 (-0.1 to 1.2)
38	0.0 (Ref)	-0.1 (-0.2 to 0.1)	-0.3 (-0.5 to 0.1)	-0.2 (-0.8 to 0.5)
39	0.0 (Ref)	0.0 (-0.1 to 0.2)	-0.2 (-0.3 to -0.0)	0.3 (-0.3 to 0.8)
40	0.0 (Ref)	0.0 (-0.1 to 0.2)	-0.1 (-0.3 to 0.1)	-0.2 (-0.8 to 0.4)
41	0.0 (Ref)	0.0 (-0.1 to 0.1)	-0.0 (-0.2 to 0.1)	0.5 (0.0 to 1.0)
42	0.0 (Ref)	-0.1 (-0.2 to 0.0)	-0.1 (-0.2 to 0.1)	0.7 (0.3 to 1.1)
43	0.0 (Ref)	0.0 (-0.1 to 0.1)	0.1 (-0.1 to 0.2)	0.5 (0.1 to 0.8)
44	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.0 (-0.1 to 0.2)	0.5 (0.1 to 0.8)
45	0.0 (Ref)	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.3)	0.6 (0.3 to 1.0)
46	0.0 (Ref)	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.2)	0.1 (-0.2 to 0.3)
47	0.0 (Ref)	0.0 (-0.0 to 0.1)	0.3 (0.2 to 0.4)	0.6 (0.3 to 0.9)
48	0.0 (Ref)	0.1 (0.1 to 0.2)	0.3 (0.2 to 0.4)	0.1 (-0.1 to 0.4)
49	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.3 (0.2 to 0.4)	0.3 (0.0 to 0.5)
50	0.0 (Ref)	0.1 (-0.0 to 0.2)	0.3 (0.2 to 0.4)	0.3 (0.1 to 0.6)
51	0.0 (Ref)	0.1 (-0.1 to 0.2)	0.3 (0.2 to 0.4)	0.1 (-0.3 to 0.4)
52	0.0 (Ref)	0.2 (0.0 to 0.4)	0.4 (0.2 to 0.6)	0.2 (-0.2 to 0.7)
53	0.0 (Ref)	0.2 (-0.1 to 0.5)	0.4 (0.1 to 0.6)	0.5 (0.0 to 0.9)
54	0.0 (Ref)	0.0 (-0.3 to 0.3)	0.2 (-0.1 to 0.5)	0.3 (-0.1 to 0.8)
55	0.0 (Ref)	-0.2 (-0.6 to 0.2)	0.0 (-0.4 to 0.4)	-0.0 (-0.6 to 0.5)
≥56	0.0 (Ref)	0.2 (-0.3 to 0.7)	0.5 (0.1 to 1.0)	0.5 (0.1 to 0.9)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Red cells indicate p-value <0.0001, green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S4. Differences (95% CI) in adjusted **LDL-cholesterol** *levels (mmol/L) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-0.1 (-0.1 to -0.0)	-0.1 (-0.2 to -0.0)	0.2 (-0.3 to 0.7)
35	0.0 (Ref)	-0.2 (-0.3 to -0.1)	-0.1 (-0.3 to 0.1)	-0.1 (-1.5 to 1.3)
36	0.0 (Ref)	-0.0 (-0.2 to 0.1)	-0.0 (-0.2 to 0.1)	0.4 (-0.2 to 1.0)
37	0.0 (Ref)	0.1 (-0.1 to 0.2)	-0.1 (-0.3 to 0.0)	0.4 (-0.2 to 1.0)
38	0.0 (Ref)	-0.0 (-0.2 to 0.1)	-0.1 (-0.3 to 0.1)	-0.1 (-0.7 to 0.5)
39	0.0 (Ref)	0.0 (-0.1 to 0.2)	-0.0 (-0.2 to 0.1)	0.3 (-0.2 to 0.8)
40	0.0 (Ref)	0.0 (-0.1 to 0.1)	-0.1 (-0.2 to 0.1)	-0.3 (-0.9 to 0.3)
41	0.0 (Ref)	0.1 (-0.0 to 0.2)	0.1 (-0.0 to 0.3)	0.6 (0.1 to 1.0)
42	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.0 (-0.1 to 0.2)	0.5 (0.1 to 0.9)
43	0.0 (Ref)	0.0 (-0.1 to 0.1)	0.1 (-0.0 to 0.3)	0.5 (0.2 to 0.8)
44	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.0 (-0.1 to 0.2)	0.4 (0.1 to 0.8)
45	0.0 (Ref)	0.1 (0.0 to 0.2)	0.2 (0.0 to 0.3)	0.5 (0.3 to 0.8)
46	0.0 (Ref)	0.1 (0.0 to 0.2)	0.1 (0.0 to 0.2)	0.1 (-0.1 to 0.4)
47	0.0 (Ref)	-0.0 (-0.1 to 0.7)	0.2 (0.1 to 0.4)	0.6 (0.3 to 0.9)
48	0.0 (Ref)	0.1 (0.0 to 0.2)	0.2 (0.1 to 0.3)	0.1 (-0.2 to 0.3)
49	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.3 (0.2 to 0.3)	0.2 (0.0 to 0.5)
50	0.0 (Ref)	0.1 (-0.0 to 0.1)	0.3 (0.2 to 0.4)	0.3 (0.1 to 0.6)
51	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.3 (0.1 to 0.4)	0.2 (-0.1 to 0.5)
52	0.0 (Ref)	0.2 (0.0 to 0.4)	0.3 (0.1 to 0.5)	0.1 (-0.3 to 0.6)
53	0.0 (Ref)	0.2 (-0.0 to 0.5)	0.4 (0.1 to 0.6)	0.5 (0.1 to 0.9)
54	0.0 (Ref)	-0.0 (-0.3 to 0.3)	0.1 (-0.2 to 0.4)	0.3 (-0.2 to 0.7)
55	0.0 (Ref)	-0.1 (-0.6 to 0.3)	0.0 (-0.3 to 0.4)	-0.0 (-0.5 to 0.5)
≥56	0.0 (Ref)	0.2 (-0.3 to 0.7)	0.5 (0.1 to 0.9)	0.5 (0.0 to 0.9)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Red cells indicate p-value <0.0001, green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S5. Differences (95% CI) in adjusted **HDL-cholesterol** *levels (mmol/L) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-0.0 (-0.0 to -0.0)	-0.0 (-0.1 to 0.0)	-0.0 (-0.2 to 0.2)
35	0.0 (Ref)	-0.1 (-0.1 to 0.0)	-0.1 (-0.2 to 0.0)	-0.0 (-0.7 to 0.6)
36	0.0 (Ref)	-0.0 (-0.1 to 0.1)	-0.1 (-0.2 to 0.0)	0.1 (-0.2 to 0.4)
37	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.1 (-0.2 to -0.0)	0.1 (-0.2 to 0.3)
38	0.0 (Ref)	0.0 (-0.1 to 0.1)	-0.1 (-0.2 to -0.1)	-0.1 (-0.4 to 0.2)
39	0.0 (Ref)	-0.0 (-0.1 to 0.1)	-0.1 (-0.2 to -0.1)	0.0 (-0.2 to 0.2)
40	0.0 (Ref)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.0)	-0.1 (-0.4 to 0.1)
41	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.1 (-0.2 to -0.0)	-0.1 (-0.3 to 0.1)
42	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.1 (-0.1 to 0.0)	0.2 (0.0 to 0.3)
43	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.0 (-0.1 to 0.0)	-0.1 (-0.2 to 0.1)
44	0.0 (Ref)	-0.0 (-0.0 to 0.0)	-0.0 (-0.1 to 0.1)	-0.0 (-0.2 to 0.1)
45	0.0 (Ref)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.0)	0.0 (-0.1 to 0.2)
46	0.0 (Ref)	0.0 (-0.0 to 0.0)	0.0 (-0.0 to 0.1)	-0.1 (-0.2 to 0.0)
47	0.0 (Ref)	0.0 (0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.0 (-0.2 to 0.1)
48	0.0 (Ref)	0.0 (0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.1)
49	0.0 (Ref)	0.0 (-0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.1 (-0.2 to 0.0)
50	0.0 (Ref)	0.0 (0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.1 (-0.2 to 0.0)
51	0.0 (Ref)	0.1 (0.0 to 0.1)	0.0 (-0.0 to 0.1)	-0.1 (-0.3 to 0.0)
52	0.0 (Ref)	0.0 (-0.1 to 0.1)	0.0 (-0.0 to 0.1)	-0.0 (-0.2 to 0.2)
53	0.0 (Ref)	0.0 (-0.1 to 0.1)	0.1 (-0.1 to 0.2)	0.1 (-0.1 to 0.3)
54	0.0 (Ref)	0.1 (-0.1 to 0.2)	0.1 (-0.0 to 0.2)	0.1 (-0.1 to 0.3)
55	0.0 (Ref)	-0.1 (-0.2 to 0.1)	0.0 (-0.1 to 0.2)	-0.0 (-0.3 to 0.2)
≥56	0.0 (Ref)	0.1 (-0.1 to 0.3)	0.1 (-0.1 to 0.3)	0.1 (-0.1 to 0.3)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S6. Differences (95% CI) in adjusted **Glucose** *levels (mmol/L) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	-0.0 (-0.0 to 0.0)	0.1 (-0.0 to 0.1)	0.6 (0.3 to 0.9)
35	0.0 (Ref)	0.1 (-0.0 to 0.2)	0.0 (-0.1 to 0.2)	-0.4 (-1.6 to 0.7)
36	0.0 (Ref)	-0.1 (-0.2 to 0.1)	0.1 (-0.1 to 0.2)	-0.0 (-0.7 to 0.6)
37	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.1 (-0.1 to 0.2)	-0.2 (-0.5 to 0.1)
38	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.0 (-0.2 to 0.1)	0.0 (-0.4 to 0.4)
39	0.0 (Ref)	-0.1 (-0.2 to 0.0)	0.0 (-0.1 to 0.2)	0.0 (-0.3 to 0.3)
40	0.0 (Ref)	0.0 (-0.1 to 0.1)	-0.0 (-0.2 to 0.1)	-0.3 (-0.8 to 1.3)
41	0.0 (Ref)	-0.0 (-0.1 to 0.1)	0.0 (-0.1 to 0.2)	-0.1 (-0.5 to 0.4)
42	0.0 (Ref)	-0.0 (-0.1 to 0.0)	0.1 (-0.0 to 0.2)	-0.2 (-0.4 to 0.1)
43	0.0 (Ref)	0.1 (-0.0 to 0.1)	0.1 (-0.0 to 0.2)	-0.1 (-0.3 to 0.2)
44	0.0 (Ref)	0.0 (-0.1 to 0.1)	0.1 (-0.0 to 0.2)	-0.1 (-0.5 to 0.2)
45	0.0 (Ref)	-0.1 (-0.1 to 0.0)	0.0 (-0.1 to 0.1)	-0.1 (-0.4 to 0.2)
46	0.0 (Ref)	-0.0 (-0.1 to 0.0)	0.1 (-0.0 to 0.1)	-0.1 (-0.3 to 0.1)
47	0.0 (Ref)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.1)	0.3 (0.1 to 0.6)
48	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.1 (-0.2 to -0.0)	-0.0 (-0.2 to 0.2)
49	0.0 (Ref)	-0.0 (-0.1 to 0.1)	-0.0 (-0.1 to 0.1)	-0.1 (-0.3 to 0.1)
50	0.0 (Ref)	-0.0 (-0.1 to 0.0)	-0.0 (-0.1 to 0.1)	-0.1 (-0.3 to 0.1)
51	0.0 (Ref)	-0.1 (-0.2 to 0.0)	-0.1 (-0.1 to 0.0)	0.1 (-0.1 to 0.4)
52	0.0 (Ref)	-0.0 (-0.2 to 0.1)	-0.0 (-0.2 to 0.1)	-0.0 (-0.3 to 0.3)
53	0.0 (Ref)	-0.1 (-0.3 to 0.2)	0.1 (-0.1 to 0.2)	-0.1 (-0.5 to 0.2)
54	0.0 (Ref)	0.0 (-0.2 to 0.2)	-0.0 (-0.2 to 0.2)	-0.1 (-0.4 to 0.1)
55	0.0 (Ref)	0.1 (-0.3 to 0.4)	-0.0 (-0.3 to 0.3)	-0.1 (-0.5 to 0.3)
≥56	0.0 (Ref)	0.1 (-0.3 to 0.5)	0.0 (-0.3 to 0.4)	0.1 (-0.3 to 0.5)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S7. **Proportional** *differences (95% CI) in adjusted* $_{log}$**Triglyceride** *levels (mmol/L) per age stratum, in reference to premenopausal participants.*

Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	0.9 (0.9 to 0.9)	0.8 (0.7 to 0.8)	1.0 (0.8 to 1.3)
35	0.0 (Ref)	0.9 (0.8 to 1.0)	0.8 (0.7 to 0.9)	1.5 (0.7 to 3.4)
36	0.0 (Ref)	0.9 (0.9 to 1.0)	0.8 (0.8 to 0.9)	1.0 (0.7 to 1.4)
37	0.0 (Ref)	1.0 (0.9 to 1.1)	0.8 (0.7 to 0.8)	1.4 (1.0 to 1.9)
38	0.0 (Ref)	0.9 (0.9 to 1.0)	0.7 (0.7 to 0.8)	0.9 (0.7 to 1.3)
39	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.8 to 0.9)	1.0 (0.8 to 1.3)
40	0.0 (Ref)	1.0 (0.9 to 1.0)	0.8 (0.7 to 0.9)	1.3 (0.9 to 1.7)
41	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.8 to 1.0)	1.1 (0.9 to 1.4)
42	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.8 to 1.0)	1.1 (0.9 to 1.4)
43	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.8 to 1.0)	1.1 (0.9 to 1.3)
44	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.8 to 1.0)	1.1 (0.9 to 1.3)
45	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.9 to 1.0)	1.1 (0.9 to 1.3)
46	0.0 (Ref)	1.0 (0.9 to 1.0)	0.9 (0.9 to 1.0)	1.1 (0.9 to 1.2)
47	0.0 (Ref)	1.0 (1.0 to 1.0)	1.0 (0.9 to 1.0)	1.1 (0.9 to 1.2)
48	0.0 (Ref)	1.0 (0.9 to 1.0)	1.0 (1.0 to 1.0)	1.1 (0.9 to 1.2)
49	0.0 (Ref)	0.9 (0.9 to 1.0)	1.0 (0.9 to 1.0)	1.1 (1.0 to 1.2)
50	0.0 (Ref)	1.0 (0.9 to 1.0)	1.0 (1.0 to 1.1)	1.1 (1.0 to 1.3)
51	0.0 (Ref)	0.9 (0.9 to 1.0)	1.0 (0.9 to 1.0)	1.1 (0.9 to 1.2)
52	0.0 (Ref)	1.1 (1.0 to 1.2)	1.0 (0.9 to 1.1)	1.2 (1.0 to 1.5)
53	0.0 (Ref)	0.8 (0.7 to 1.0)	0.9 (0.8 to 1.0)	0.9 (0.7 to 1.1)
54	0.0 (Ref)	1.0 (0.8 to 1.1)	1.0 (0.8 to 1.1)	1.0 (0.8 to 1.2)
55	0.0 (Ref)	1.0 (0.8 to 0.2)	0.9 (0.8 to 1.1)	1.0 (0.8 to 1.3)
≥56	0.0 (Ref)	0.98 (0.6 to 1.0)	0.8 (0.7 to 1.0)	0.8 (0.7 to 1.0)

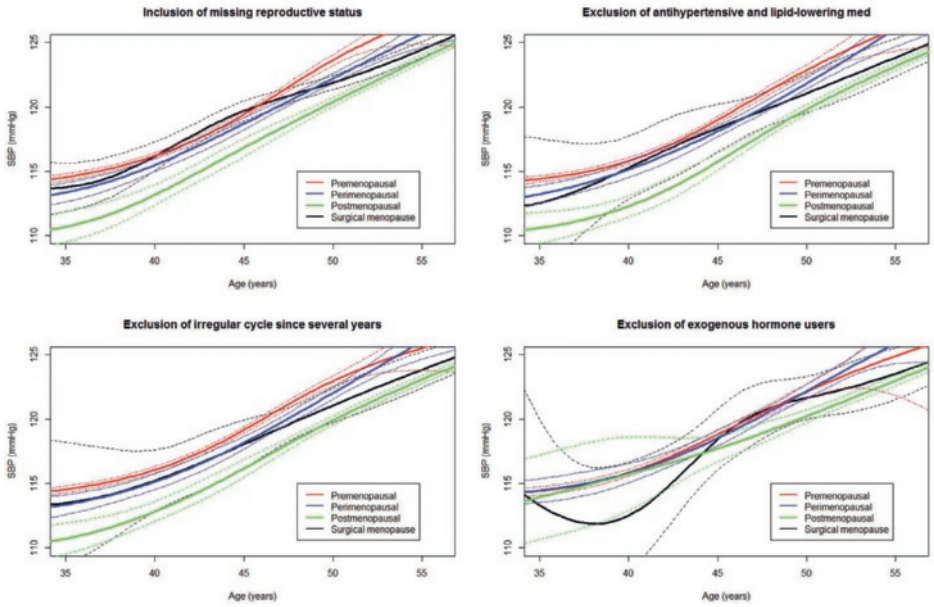
Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use, smoking status and BMI. Premenopausal women are the reference category. Red cells indicate p-value <0.0001, green cells indicate p-value <0.001, blue cells indicate p-value <0.05.

Table S8. Differences (95% CI) in adjusted **BMI** *levels (kg/m²) per age stratum, in reference to premenopausal participants.*

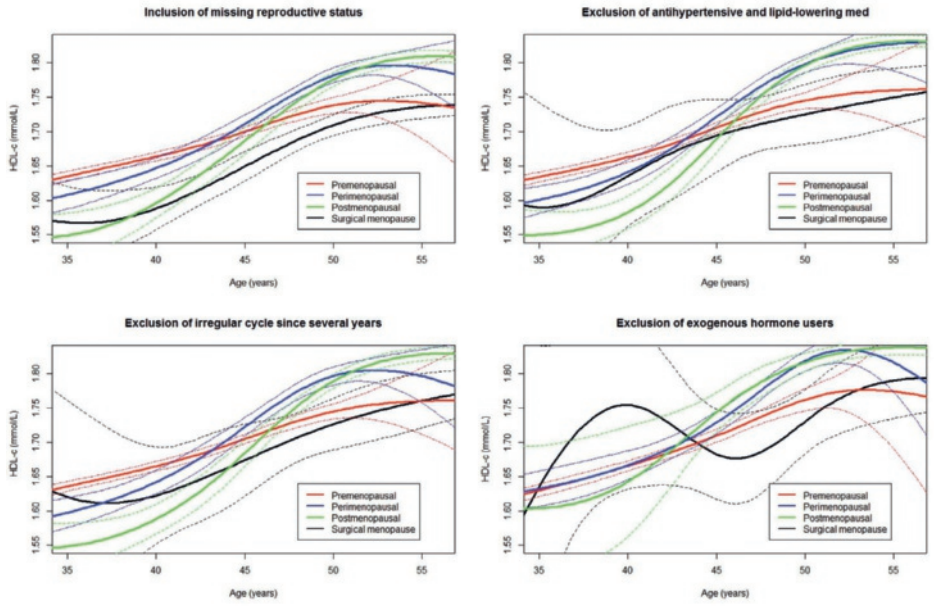
Age (years)	Premenopausal (reference)	Perimenopausal	Naturally postmenopausal	Surgically menopausal
≤34	0.0 (Ref)	0.5 (0.3 to 0.8)	1.1 (0.6 to 1.6)	2.9 (0.3 to 5.5)
35	0.0 (Ref)	-0.1 (-1.0 to 0.8)	0.5 (-0.8 to 1.8)	1.9 (-7.1 to 10.9)
36	0.0 (Ref)	0.8 (-0.0 to 1.6)	0.3 (-0.8 to 1.5)	1.1 (-3.0 to 5.2)
37	0.0 (Ref)	0.4 (-0.4 to 1.3)	0.9 (-0.2 to 2.0)	3.7 (0.4 to 7.0)
38	0.0 (Ref)	0.2 (-0.6 to 1.0)	1.2 (-0.1 to 2.4)	0.7 (-3.2 to 4.5)
39	0.0 (Ref)	0.6 (-0.2 to 1.4)	-0.2 (-1.2 to 0.7)	3.7 (1.3 to 6.2)
40	0.0 (Ref)	0.5 (-0.2 to 1.2)	0.7 (-0.3 to 1.7)	7.8 (4.3 to 11.3)
41	0.0 (Ref)	0.4 (-0.2 to 1.1)	0.2 (-0.7 to 1.2)	0.4 (-2.4 to 3.2)
42	0.0 (Ref)	0.6 (0.0 to 1.3)	-0.1 (-1.0 to 0.7)	0.7 (-1.6 to 2.9)
43	0.0 (Ref)	0.5 (-0.0 to 1.0)	0.2 (-0.6 to 1.1)	1.2 (-0.8 to 3.2)
44	0.0 (Ref)	0.2 (-0.3 to 0.7)	0.4 (-0.4 to 1.2)	2.4 (0.3 to 4.5)
45	0.0 (Ref)	0.0 (-0.4 to 0.5)	0.5 (-0.3 to 1.3)	2.1 (0.3 to 0.9)
46	0.0 (Ref)	0.9 (0.4 to 1.3)	0.7 (0.1 to 1.4)	0.8 (-0.6 to 2.2)
47	0.0 (Ref)	0.5 (0.0 to 0.9)	0.2 (-0.4 to 0.8)	1.2 (-0.3 to 2.6)
48	0.0 (Ref)	0.0 (-0.4 to 0.4)	0.1 (-0.5 to 0.6)	1.2 (-0.2 to 2.6)
49	0.0 (Ref)	0.6 (0.1 to 1.0)	0.2 (-0.3 to 0.7)	1.0 (-0.1 to 2.2)
50	0.0 (Ref)	0.4 (-0.1 to 0.8)	0.0 (-0.4 to 0.5)	1.0 (-0.2 to 2.2)
51	0.0 (Ref)	0.1 (-0.5 to 0.6)	-0.0 (-0.6 to 0.5)	0.2 (-1.3 to 1.7)
52	0.0 (Ref)	0.7 (-0.3 to 1.6)	0.3 (-0.6 to 1.2)	2.2 (0.1 to 4.4)
53	0.0 (Ref)	0.3 (-1.0 to 1.6)	-0.1 (-1.3 to 1.1)	-0.1 (-2.2 to 2.0)
54	0.0 (Ref)	-0.2 (-1.6 to 1.2)	-0.2 (-1.5 to 1.1)	0.4 (-1.5 to 2.3)
55	0.0 (Ref)	0.6 (-1.4 to 2.5)	0.3 (-1.3 to 2.0)	1.1 (-1.3 to 3.4)
≥56	0.0 (Ref)	-1.4 (-3.6 to 0.7)	-1.2 (-3.1 to 0.6)	-0.5 (-2.4 to 1.5)

Values reflect coefficients of linear regression analyses in each age stratum, adjusted for OC use and smoking status. Premenopausal women are the reference category. Red cells indicate p-value <0.0001, green cells indicate p-value <0.001.

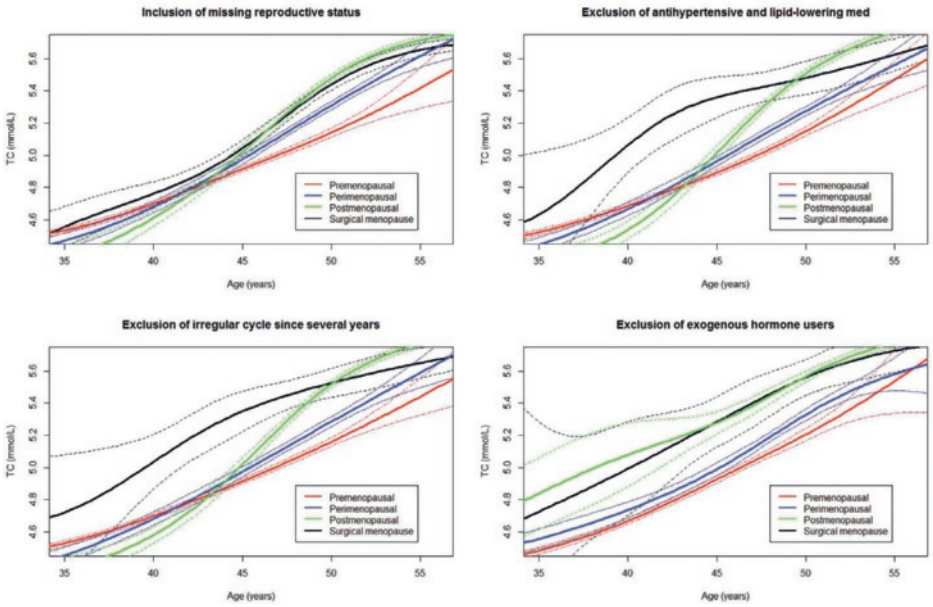
Appendix 2: Figure S1



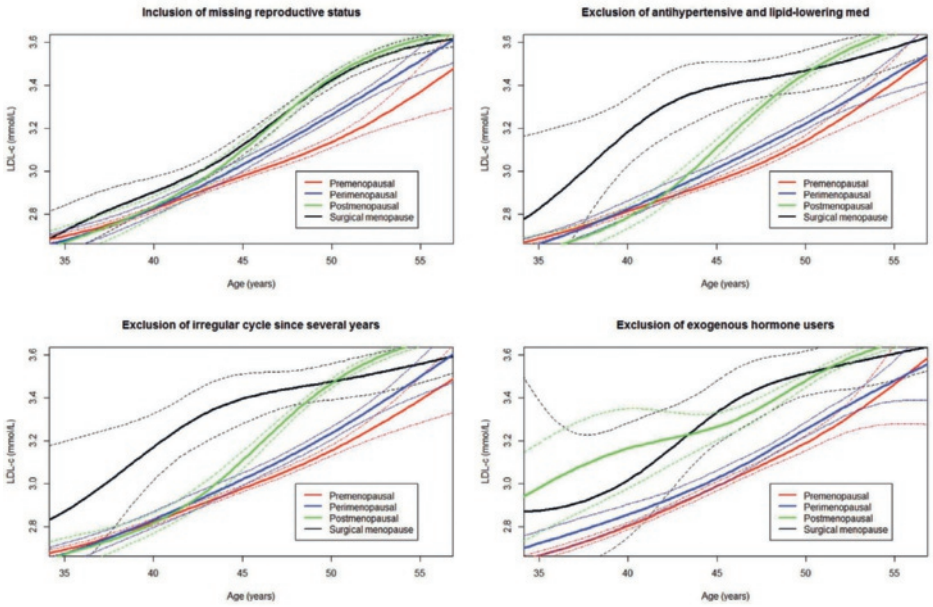
Appendix 3: Figure S2



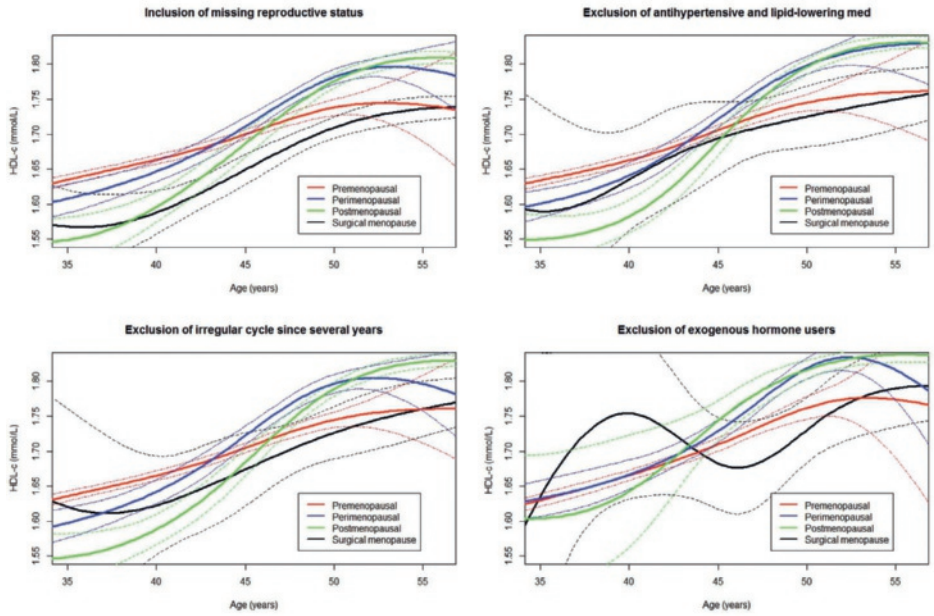
Appendix 4: Figure S3



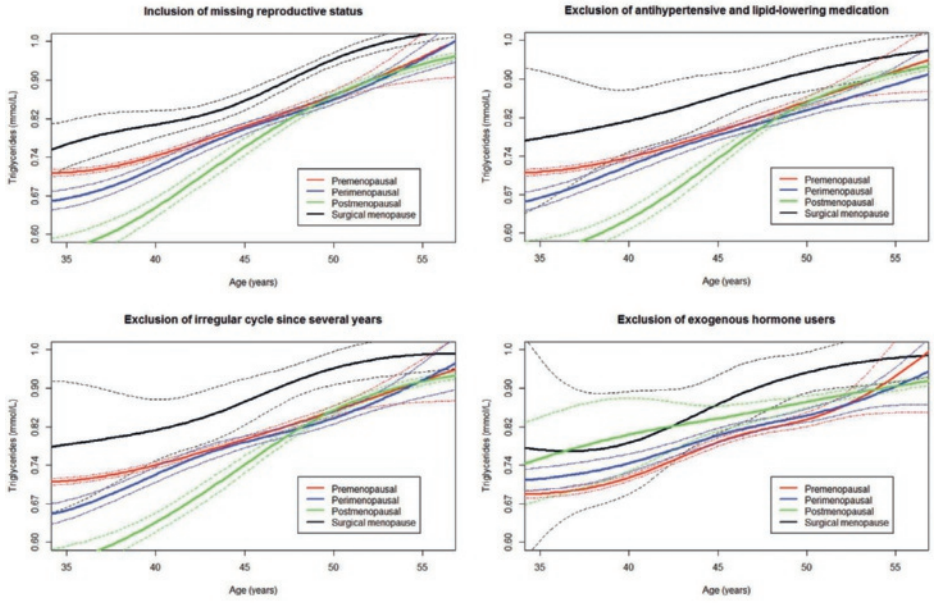
Appendix 5: Figure S4



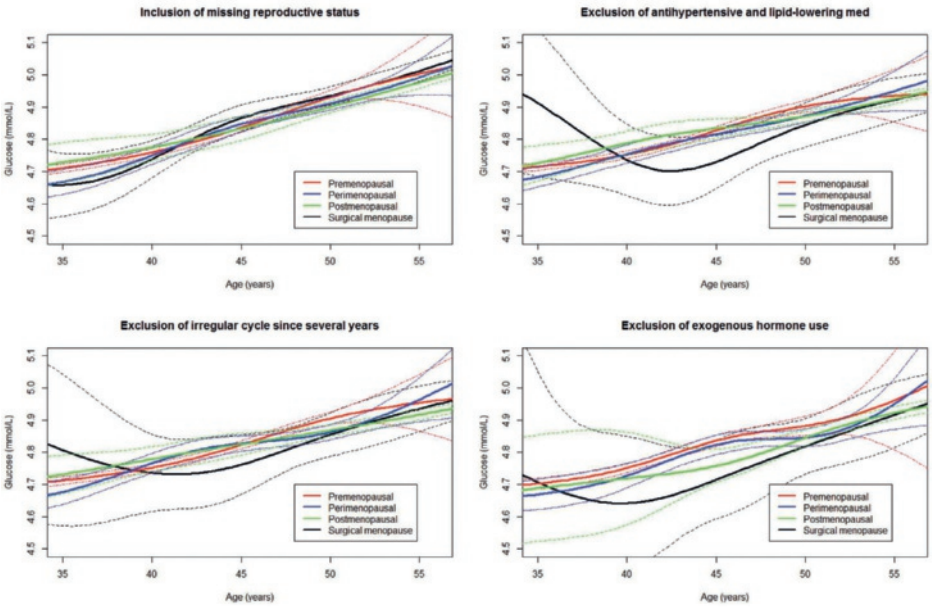
Appendix 6: Figure S5



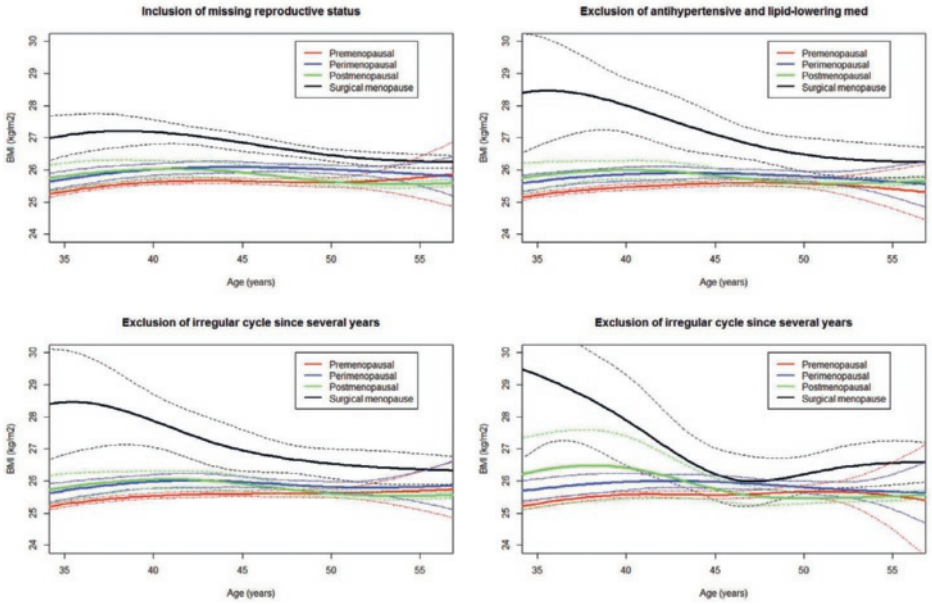
Appendix 7: Figure S6



Appendix 8: Figure S7



Appendix 9: Figure S8



CHAPTER 2.2

Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis

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Abstract

Background: Earlier age at menopause has been associated with increased risk of coronary heart disease (CHD), but the shape of association and role of established cardiovascular risk factors remain unclear. Therefore, we examined the associations between menopausal characteristics and CHD risk; the shape of the association between age at menopause and CHD risk; and the extent to which these associations are explained by established cardiovascular risk factors.

Methods: We used data from EPIC-CVD, a case-cohort study, which includes data from 23 centers from 10 European countries. We included only women, of whom 10 880 comprise the randomly selected subcohort, supplemented with 4522 cases outside the subcohort. We conducted Prentice-weighted Cox proportional hazards regressions with age as underlying time scale, stratified by country, and adjusted for relevant confounders.

Results: Postmenopausal women were at higher CHD risk compared to premenopausal women (HR=1.23, 95%CI=1.08-1.40, p=0.002) but the association attenuated after adjustment (HR=1.13, 95%CI=0.98-1.30, p=0.09). Among postmenopausal women, earlier menopause was linearly associated with higher CHD risk (HR per-year decrease=1.02, 95%CI=1.01-1.03, p<0.001). After adjustment, women with a surgical menopause were at higher risk of CHD compared to those with natural menopause (HR=1.25, 95%CI=1.10-1.42, p=<0.001), but this attenuated after additional adjustment for age at menopause (HR=1.15, 95%CI=1.00-1.33, p=0.05). A proportion of the association was explained by cardiovascular risk factors.

Conclusions: Earlier and surgical menopause were associated with higher CHD risk. These associations could partially, but not totally, be explained by differences in conventional cardiovascular risk factors. These women might benefit from close monitoring of cardiovascular risk factors and disease.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in men and women from Western countries with 17.5 million deaths worldwide in 2012, representing 31% of all global deaths[1]. Approximately 7.4 million of these were due to coronary heart disease (CHD). CHD risk increases in women after the age of 50, leading to suggestions that menopause may be a contributing factor[2–4]. A recent meta-analysis suggested that women who had early menopause (before age 45) are at 50% higher CHD risk compared to those with later menopause[5]. However, that analysis was not able to include nine studies out of the 14 studies they found examining the association between age at menopause and CHD, nor was it able to examine whether there is a (non-)linear dose-response relationship or threshold effect or whether type of menopause (surgical or natural) was associated with CHD risk.

The biological mechanisms through which menopause might influence CHD risk are postulated to include reductions in estrogen levels, but rises in conventional cardiovascular risk factors (e.g. major lipids and blood pressure) around the time of menopause may also play a role[6–8]. However, the extent to which the association between menopausal characteristics and CHD can be explained by such factors remains unclear.

We conducted a large pan-European prospective case-cohort study (EPIC-CVD) with an average of 11 years of follow-up to quantify 1) the associations of menopausal status, age at menopause and type of menopause with risk of CHD, 2) we also examined the shape of the relationship between age at menopause (as a continuous exposure) and risk of CHD, and 3) we assessed the extent to which the associations of menopausal characteristics with risk of CHD could be explained by established cardiovascular risk factors.

Subjects and Methods

Subjects

We used data from female participants in the EPIC-CVD study, a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study[9]. EPIC consists of 519 978 adults (366 521 women), aged between 35 and 70 at baseline, and recruited from 23 centers across 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2000. Baseline questionnaires included questions on diet, lifestyle, reproductive and medical factors. Blood samples were collected for approximately 70% of the participants and stored in liquid nitrogen at -196°C. For EPIC-CVD, a representative subcohort of 18 249 participants was selected by simple random sampling, stratified by center, from participants who had available stored blood and buffy coat samples[10,11]. After exclusion of 609 participants with a prior history of myocardial infarction or stroke at baseline, 17 640 subcohort members remained. After subsequent exclusion of the

6760 men, a subcohort of 10 880 women remained of whom 231 had a CHD event. Subsequently, incident CHD cases in women outside the subcohort were added to the study sample using the same exclusion criteria (N=4522).

EPIC complies with the Declaration of Helsinki, and all participants gave written informed consent before participating in this study. The study was approved by the local ethics committees of the participating centers and the Institutional Review Board of the International Agency for Research on Cancer (IARC, Lyon).

Menopausal status, timing and type of menopause

Menopause was assessed by questionnaire at baseline. Women were categorized as *premenopausal* if they had experienced menses over the past 12 months before recruitment, and by design, for women with missing or incomplete questionnaires, if they were 54 years or younger at recruitment. The premenopausal group also includes the perimenopausal women, since numbers were too small to analyze them as a separate group. Women were categorized as *postmenopausal* if they had experienced no menses for 12 months or longer due to natural or surgical menopause, and by design, for women with missing or incomplete questionnaire data, if they were 55 years or older at recruitment[12].

Postmenopausal women were classified as having had a natural or surgical menopause, where surgical menopause was defined as having had a hysterectomy, unilateral or bilateral ovariectomy, only when age at surgery preceded or was equal to age at menopause. In the Malmö centre, since the age at removal of a woman's womb and/or one or both ovaries was not recorded, women were classified as having had a surgical menopause regardless of age at surgery and age at menopause was then imputed (see below). For naturally postmenopausal women, age at menopause was defined as the age at which they had their last menstruation. For surgically postmenopausal women, their age at surgery was used instead. Since most other studies compare early menopause with late menopause, we present risk associations for decreases (rather than increases) in age at menopause, by multiplying age at menopause with -1.

Covariate measurement

Baseline questionnaires included questions on age, smoking status (current, former, never), highest education level (no schooling/primary school, secondary school, vocational education/university), age at menarche (≤10, 11, 12, 13, 14, 15, 16, ≥17), full term pregnancy (yes/no) and whether participants had ever used postmenopausal hormones (yes/no). All centres used trained professionals to measure height and weight except the French centre, for which self-reported measures were used for a subset of participants, and Oxford, for which recalibrated self-reported measures were used based on a comparison between self-reported and measured data in a subset

of participants. Both height and weight were adjusted for clothing worn[9,13]. Body mass index (BMI) was calculated as weight divided by the square of height in metres and was categorised (≤20, >20-<25, ≥25-<30, ≥30 kg/m^2). Physical activity was categorized using the Cambridge Physical Activity Index into inactive, moderately inactive, moderately active and active[14]. Baseline systolic and diastolic blood pressure measurements were available in 62% of participants[11]. Therefore, to maximize the availability of information, we used a composite variable ("high blood pressure", available in 98% of participants) defined as any of self-reported hypertension, self-reported use of anti-hypertensive medication, systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.

Serum biomarkers were measured in baseline non-fasted samples at Stichting Huisartsen Laboratorium (Etten-Leur, the Netherlands), and included high-sensitivity C-reactive protein (hsCRP), total cholesterol, high density lipoprotein cholesterol (HDL-c) and triglycerides. Erythrocyte haemoglobin A1c (HbA1c) was measured using the Tosoh-G8 HPLC analyzer (Tosoh Bioscience, Japan); all other biomarkers were measured using a Cobas enzymatic assay (Roche Diagnostics, Mannheim, Germany) on a Roche HitachiModular P analyzer.

First fatal or non-fatal coronary heart disease event

First fatal or non-fatal CHD events were defined by codes 410-414 of the International classification of diseases Ninth Edition (ICD-9), and codes I20-I25 of the Tenth Edition (ICD-10). Methods used in the recruitment centers to determine first non-fatal CHD events included self-report and linkage with morbidity or hospital registries. Non-fatal CHD events were further validated by a review of medical records and/or linkage with registries. Fatal CHD events were generally determined through mortality registries[11]. The final year of follow-up for CHD events varied between centers from 2003 to 2010 and median follow-up time was 11 years.

Statistical analyses

Missing values in the exposures and covariates were imputed with multiple imputation using the package MICE in R[15] with 10 imputations and 50 iterations (Appendix 1). Women from Norway were excluded prior to imputation due to high levels of missing data. Hazard ratios were estimated using Prentice-weighted Cox proportional hazards regression, with age as the underlying time scale and with country-stratified baseline hazards[16]. Robust standard errors were used to construct 95% confidence intervals. In order to study the association between menopausal characteristics and CHD three levels of covariate adjustment were applied: adjustment for age at baseline only (Model 1), further adjustment for CHD risk factors: smoking status, BMI, HbA1c, education level and physical activity (Model 2), and further adjustment for reproductive factors: age at menarche, full term pregnancy and ever hormone use (Model 3), which will be presented

as the fully adjusted model. Model 4 includes the established cardiovascular risk factors that might mediate the association between menopausal characteristics and CHD (total cholesterol, HDL-c, triglycerides, high blood pressure and C-reactive protein). Since the association between menopausal age and CHD may vary depending on smoking and obesity status[17], we also assessed effect-modification by including interaction terms between menopausal age and smoking status and between menopausal age and obesity status, respectively, in Model 3 (the fully adjusted model). Surgically postmenopausal women tend to have an earlier age at menopause[18–20]. Thus, the analysis of type of menopause was also adjusted for age at menopause (Model 3b).

To verify the expected linear relationship between age at menopause and CHD, we used floating absolute risks to display the hazard ratios (HRs) for age at menopause categories (<40, 40-44, 45-49, 50-54, >=55 [reference]) and CHD risk in Model 3. Floating absolute risks redistribute the overall variance across the groups[21].

To estimate the proportion of the association between menopause and CHD risk that could be explained by potential mediators that were also CVD risk factors, we used the difference method[22,23] for which two regression coefficients of the exposure-outcome association are required; the direct effect (i.e., with adjustment for the possible mediators or established CVD risk factors) and the total effect (without adjustment). First, the total effect of each menopausal characteristic on CHD was estimated based on Model 1 (adjusted for age). Subsequently, for each model of adjustment separately we estimated the direct effect when removing the indirect via the added risk factors. The proportion of the effect explained (PE) by the mediators was then calculated as: PE=(total effect-direct effect)/total effect, where effects were considered on the logarithmic scale, i.e., log(HR). Thereafter, we performed the same analyses for each separate risk factor. Bootstrap resampling (1000 bootstrap samples) was used to obtain 95% confidence intervals (CI) around the PE (Appendix 1).

We performed three sensitivity analyses: (1) restricting to women who had never used hormone therapy, since age at menopause may be difficult to determine under hormone use and the effects of surgical menopause on CHD are attenuated in women using hormone therapy (HT)[24–26]; (2) excluding the first two-years of follow-up to reduce the likelihood of reverse causality; (3) excluding women with unilateral ovariectomy or hysterectomy from the surgical menopause category to reflect alternative definitions of surgical menopause used previously. We also conducted a complete case analysis and compared results with the multiple imputation approach. All analyses were performed on each imputed dataset separately and the estimates were pooled using Rubin's rules[27], with R version 3.2.0[28].

Results

After exclusions, there were 10 880 women in the subcohort and 4753 incident CHD cases (231 of whom were also in the subcohort) comprising a total of 15 402 participants, of whom 5486 were premenopausal and 9916 were postmenopausal. Compared to premenopausal women, postmenopausal women in the subcohort were older, less likely to be smokers, less educated, more likely to have a history of high blood pressure and had higher total cholesterol levels and BMI (Table 1). Mean age at menopause was 49.2 years (standard deviation [SD] 4.5) for women with a natural menopause and 45.1 (SD 5.8) for women with a surgical menopause.

Table 1. Baseline characteristics of women in the subcohort of the EPIC-CVD case-cohort study

	Premenopausal N = 5486	**Postmenopausal N = 9916**
	CHD risk factors	
Age at baseline	44.8 ± 6.5	59.7 ± 6.8
Smoking status	2788 (51.7%)	5386 (55.0%)
Never	1133 (21.0%)	2141 (21.9%)
Former	1474 (27.3%)	2266 (23.1%)
Current		
Body Mass Index (kg/m²)[a]	390 (7.2%)	418 (4.2%)
≤20	2543 (46.7%)	3653 (37.0%)
>20-<25	1679 (30.9%)	3739 (37.9%)
≥25-<30	828 (15.2%)	2057 (20.8%)
≥30		
Physical activity	1448 (26.8%)	3155 (32.2%)
Inactive	1871 (34.6%)	3313 (33.9%)
Moderately inactive	1188 (22.0%)	1798 (18.4%)
Moderately active	894 (16.6%)	1517 (15.5%)
Active		
Education level	1926 (35.9%)	4926 (52.0%)
No schooling/Primary school	1065 (19.8%)	1174 (12.4%)
Secondary school	2376 (44.3%)	3380 (35.7%)
Vocational/University		
High blood pressure (History)	1249 (23.0%)	5201 (53.4%)
HbA1c (%)[b]	5.4 (5.1-5.5)	5.6 (5.4-5.8)
hsCRP (mg/L)[b]	1.0 (0.4-2.2)	1.5 (0.7-3.3)
Total cholesterol (mmol/L)	5.6 ± 1.0	6.4 ± 1.2
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.5 ± 0.4
Triglycerides (mmol/L)[b]	0.9 (0.7-1.3)	1.2 (0.9-1.8)
	Reproductive factors	
Age at menopause (years)[c]	/	47.6 ± 5.6
Full term pregnancy (Yes)	4415 (87.0%)	8562 (88.5%)
Ever hormone use (Yes)	444 (9.4%)	3006 (33.8%)

Table 1. Continued

	Premenopausal N = 5486	Postmenopausal N = 9916
Reproductive factors		
Age at menarche	209 (4.1%)	271 (2.8%)
≤10	698 (13.6%)	1006 (10.4%)
11	1270 (24.7%)	1733 (18.0%)
12	1330 (25.8%)	2228 (23.1%)
13	1051 (20.4%)	2235 (23.2%)
14	375 (7.3%)	1208 (12.5%)
15	156 (3.0%)	595 (6.2%)
16	57 (1.1%)	365 (3.8%)
≥17		

Mean ± Standard Deviation
[a]Adjusted for clothing
[b]Median (Q1-Q3)
[c]Only postmenopausal women

Postmenopausal women had a higher CHD risk compared with premenopausal women (age-adjusted, Model 1, HR=1.23, 95%CI: 1.08-1.40, p-value=0.002) (Table 2), but this attenuated in the fully adjusted model (Model 3 HR=1.13, 95%CI: 0.98-1.30, p-value=0.09). In the association between age at menopause and CHD, BMI was an effect modifier (p=0.003) where smoking was not (p=0.56). The BMI stratified results (Appendix Table 1) showed that in women with a BMI of 25 or higher, each one-year decrease in age at menopause resulted in a 3% higher CHD risk (fully adjusted Model 3 HR[BMI≥25-<30] 1.03, 95%CI: 1.01-1.04, p-value=0.001; HR[BMI≥30] 1.03, 95%CI: 1.01-1.05, p-value=0.001). Women with a BMI below 25 had only a 1% increase in CHD risk for each one-year decrease in age at menopause (fully adjusted Model 3 HR[BMI>20-<25] 1.01, 95%CI: 0.99-1.02, p-value=0.40; HR[BMI≤20] 1.01, 95%CI: 0.97-1.06, p-value=0.55). Postmenopausal women with a surgical menopause had a higher risk of CHD compared with women with a natural menopause (fully adjusted Model 3 HR=1.25, 95%CI: 1.10-1.42, p<0.001), which attenuated on further adjustment for age at menopause (Model 3b HR=1.15, 95%CI: 1.00-1.33, p=0.05) (Table 3).

Age at menopause had an approximately linear association with CHD risk, with women in the lowest category (menopausal age <40) having a 51% (fully adjusted model 3 HR=1.51, 95%CI: 1.15-1.98), p=0.003) higher risk than those in the highest category (menopausal age ≥55) (Figure 1). In addition, Table 4 showed that for each one year decrease in age at menopause, CHD risk was 2% higher (fully adjusted Model 3 HR=1.02, 95%CI: 1.01-1.03, p<0.001) and for each SD decrease (7.9 years) in age at menopause, risk was 16% higher (fully adjusted Model 3 HR=1.16, 95%CI: 1.08-1.25, p<0.001).

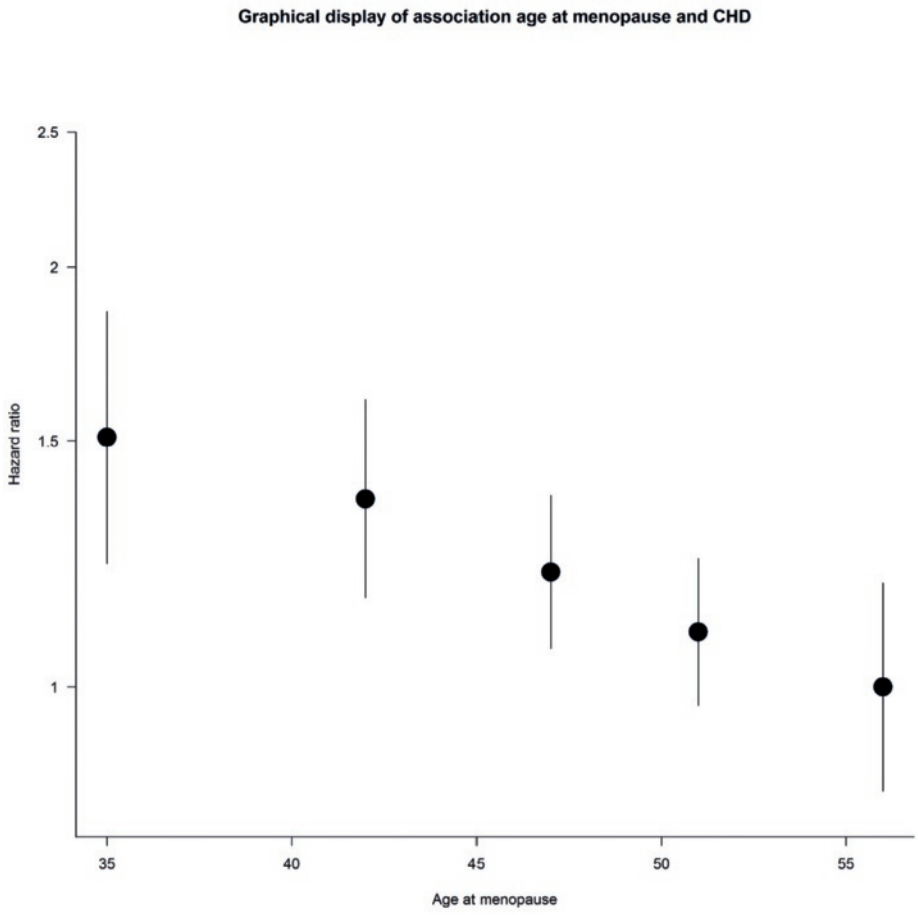

Figure 1. Graphical display of the linear relationship between age at menopause and CHD using floating absolute risks to display the hazard ratios (HRs) for age at menopause categories (<40, 40-44, 45-49, 50-54, ≥55 [reference]).

Finally, in all analyses, we added possible mediators for the associations in model 4. For postmenopausal compared to premenopausal women we found that adding the established risk factors in Model 4 explains an additional 20% of the association compared with the fully adjusted model 3 (Table 2). In the association with age at menopause, the possible mediators or established risk factors explained an additional 10% of the association compared with model 3, although the HR only slightly changes (Table 4). Finally, for type of menopause the possible mediators explained an additional part of the association of approximately 10% compared to model 3b. However in this case, it seemed that age at menopause explained the largest part of the association (Table 3). Furthermore, Appendix Table 2 shows the proportion explained of all the risk factors separately.

Table 2. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between menopausal status and any first CHD event

Postmenopausal vs. premenopausal			
Model	*HR (95%CI)*	*p-value*	*PE% (95%CI)[a]*
Model 1[b]	1.23 (1.08-1.40)	0.002	/
Model 2[c]	1.15 (1.00-1.32)	0.05	33.3 (24.6-44.9)
Model 3[d]	1.13 (0.98-1.30)	0.09	40.5 (30.4-54.6)
Model 4[e]	1.08 (0.93-1.26)	0.29	60.7 (54.4-80.6)

Mean N(events); Postmenopausal 9916 (4074), premenopausal 5486 (679)
[a]PE = Proportion Explained
[b]Model 1: Adjusted for age.
[c]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[d]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use
[e]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure

Table 3. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between type of menopause and any first CHD event in postmenopausal women

Surgical vs. natural menopause			
Model	***HR (95%CI)***	***p-value***	***PE% (95%CI)[a]***
Model 1[b]	1.31 (1.16-1.47)	<0.001	/
Model 2[c]	1.26 (1.11-1.44)	<0.001	12.7 (6.6-19.2)
Model 3[d]	1.25 (1.10-1.42)	<0.001	17.6 (10.4-25.4)
Model 3b[e]	1.15 (1.00-1.33)	0.05	47.2 (37.3-59.1)
Model 4[f]	1.12 (0.96-1.29)	0.15	59.2 (46.6-73.5)

Mean N(events); Surgical 2206 (935), natural 7710 (3139)
[a]PE = Proportion Explained
[b]Model 1: Adjusted for age.
[c]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[d]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[e]Model 3b: Additionally adjusted for age at menopause.
[f]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure

Table 4. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between age at menopause and any first CHD event in postmenopausal women

	HR per year decrease in age at menopause		HR per SD decrease in age at menopause		
Model	***HR (95%CI)***	***p-value***	***HR (95%CI)***	***p-value***	***PE% (95%CI)[a]***
Model 1[b]	1.03 (1.02-1.04)	<0.001	1.23 (1.15-1.33)	<0.001	/
Model 2[c]	1.02 (1.01-1.03)	<0.001	1.17 (1.08-1.26)	<0.001	25.7 (21.0-31.4)
Model 3[d]	1.02 (1.01-1.03)	<0.001	1.16 (1.08-1.25)	<0.001	28.6 (23.2-34.5)
Model 4[e]	1.02 (1.01-1.03)	0.001	1.14 (1.05-1.23)	0.001	38.7 (30.4-44.6)

Mean N(events); 9916(4074)
[a]PE = Proportion Explained
[b]Model 1: Adjusted for age.
[c]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[d]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[e]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure

Sensitivity analyses

Similar results were obtained in analyses that were restricted to women who never used HT (Appendix Tables 3-5) and that excluded the first two years of follow-up (Appendix Tables 6-8). When surgical menopause was defined as bilateral ovariectomy only, the risk estimates for menopausal status attenuated compared with the main analyses (fully adjusted Model 3 HR=0.99, 95%CI: 0.86-1.14, p=0.92) as did the results for type of menopause (HR=1.00, 95%CI=0.81-1.24 for model 3b) (Appendix Tables 9, 10). The complete case analysis (data not shown) gave similar results to those from the multiple imputation approach.

Discussion

Our study has shown that age at menopause has an inverse dose-response relationship with risk of CHD. Surgical menopause is also associated with an increased CHD risk, even once the earlier age at menopause is accounted for. A proportion of the risk appears to be explained by cardiovascular risk factors that have been postulated to mediate the associations of menopausal characteristics with risk of CHD.

Our finding that the higher risk of CHD in postmenopausal women attenuated upon adjustment for conventional cardiovascular risk factors and reproductive factors is in line with a previous meta-analysis[29] that also found an increased risk for postmenopausal women. Similarly, our finding that earlier menopause is associated with a higher CHD risk is also consistent with a recent meta-analysis[5] that showed a higher CHD risk for women with an age at menopause before 45. However, our access to individual participant data (rather than literature-based summary results) meant that we were able to amplify previous findings by showing that the relationship

is continuous and approximately linear across the range in age at menopause; hence, there is no clear age threshold below which early menopause appears to be of intrinsic concern. We identified BMI as effect modifier, and the stratified results appeared similar to the findings of a smaller study[18], which suggested that age of menopause has a stronger association with CHD in obese women compared to non-obese women.

Previous evidence on the associations of surgical and natural menopause with CHD is conflicting[2,3,24,30,31]. Comparison of these studies is difficult, since the definition of surgical menopause and inclusion of women using HT differs by study. Notably, none of the studies on surgical menopause adjusted for age at menopause in their analysis, notwithstanding the fact that a surgical menopause occurs consistently earlier than a natural menopause. Our study shows that the association between surgical menopause and CHD risk is largely explained by the earlier age at menopause, but residual risk remains.

As conventional cardiovascular risk factors such as blood pressure, lipids and CRP, rise around the age of menopause, we specifically examined the extent to which these potential mediators explained the associations we observed in Model 4. Our analyses suggested that these factors can explain part of the association between menopausal characteristics and higher risk of CHD, because the greatest difference in percentage of proportion explained was found between Model 3 and Model 4 in each association. This concurs with the findings of several other studies, which showed the greatest changes in lipid levels around the time of menopausal transition[7,8,32–36]. As EPIC-CVD has only a single measure of these risk factors at baseline (i.e. after the menopause in postmenopausal women), it is not possible to reliably distinguish whether the attenuations seen are due to mediation or confounding[37].

Our study has several strengths. We used data from a large prospective study encompassing diverse European populations with a long duration of follow-up and a substantial number of validated incident CHD events. The availability of a wide range of cardiovascular and reproductive risk factors allowed us to systematically examine the effects of accounting for these factors. We were also able to examine the impact of hormone therapy use, which has not been possible in many previous studies. Potential limitations include missing or incomplete menopause data, which may have led to non-differential misclassification resulting in underestimation of the true associations[38], and the possibility of residual confounding. As EPIC-CVD did not have measures of sex hormones, we were not able to evaluate the contribution of estrogen. Finally, a substantial number of the premenopausal women would likely have become postmenopausal during the follow-up period. Therefore, our associations may have been slightly underestimated.

In conclusion, earlier age at menopause and surgical menopause are both associated with higher risk of CHD, which might suggest that these women need close monitoring in clinical practice. The excess risk is, in part, explained by conventional cardiovascular risk factors.

Therefore, these risk factors should play an important role in the monitoring of these women. However, there is still a residual association between menopausal characteristics and CHD of which the mechanism is not fully understood, and which merits further research.

References

1. WHO. Cardiovascular diseases (CVDs) Fact sheet number 137. 2015.
2. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. AnnInternMed. 1976;85(4):447-52.
3. Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. Annals of internal medicine. 1978;89(2):157-61.
4. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. NEnglJMed. 1987;316(18):1105-10.
5. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R, 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(7):767-76.
6. Lindquist O. Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. Preventive medicine. 1982;11(2):162-72.
7. 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? JAmCollCardiol. 2009;54(25):2366-73.
8. Peters HW, Westendorp IC, Hak AE, Grobbee DE, Stehouwer CD, Hofman A, et al. Menopausal status and risk factors for cardiovascular disease. J Intern Med. 1999;246(6):521-8.
9. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5(6B):1113-24.
10. Langenberg C, Sharp S, Forouhi NG, Franks PW, Schulze MB, Kerrison N, et al. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. Diabetologia. 2011;54(9):2272-82.
11. Danesh J, Saracci R, Berglund G, Feskens E, Overvad K, Panico S, et al. EPIC-Heart: the cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. European journal of epidemiology. 2007;22(2):129-41.
12. Clavel-Chapelon F. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. British journal of cancer. 2002;86(5):723-7.

13. Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). Public health nutrition. 2002;5(6B):1147-62.
14. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public health nutrition. 2003;6(4):407-13.
15. van Buuren SG-O, K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software. 2011;45(3).
16. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika. 1986;73:1-11.
17. Morris DH, Jones ME, Schoemaker MJ, McFadden E, Ashworth A, Swerdlow AJ. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. AmJEpidemiol. 2012;175(10):998-1005.
18. Ossewaarde ME, Bots ML, Verbeek AL, Peeters PH, van der Graaf Y, Grobbee DE, et al. Age at menopause, cause-specific mortality and total life expectancy. Epidemiology. 2005;16(4):556-62.
19. Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. Menopause. 2012;19(10):1081-7.
20. Matthews KA, Gibson CJ, El Khoudary SR, Thurston RC. Changes in cardiovascular risk factors by hysterectomy status with and without oophorectomy: Study of Women's Health Across the Nation. JAmCollCardiol. 2013;62(3):191-200.
21. Easton DFP, J.; Babiker A.G. Floating absolute risk: an alternative to relative risk in survival and case-control analysis avoiding an arbitrary reference group. Statistics in Medicine. 1991;10(7):1025-35.
22. Judd CMK, D.A. Process analysis: estimating mediation in treatment evaluations. . Eval Rev 1981;5(5):602-19.
23. Baron RMK, D.A. . The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. . J Pers Soc Psychol. 1986;51(6):1173-82.
24. Rivera CM, Grossardt BR, Rhodes DJ, Brown RD, Jr., Roger VL, Melton LJ, 3rd, et al. Increased cardiovascular mortality after early bilateral oophorectomy. Menopause. 2009;16(1):15-23.
25. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Preventive medicine. 1991;20(1):47-63.

26. Grodstein F, Manson JE, Stampfer MJ. Postmenopausal hormone use and secondary prevention of coronary events in the nurses' health study. a prospective, observational study. Annals of internal medicine. 2001;135(1):1-8.
27. Rubin DB. Multiple Imputation for Non Response in Surveys: Wiley; 1987.
28. Team RC. R: A language and environment for statistical computing. 3.2.0 ed. Vienna, Austria: R Foundation for Statistical computing; 2015.
29. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006;13(2):265-79.
30. Falkeborn M, Schairer C, Naessen T, Persson I. Risk of myocardial infarction after oophorectomy and hysterectomy. Journal of clinical epidemiology. 2000;53(8):832-7.
31. Peters SA, Woodward M. Women's reproductive factors and incident cardiovascular disease in the UK Biobank. Heart. 2018.
32. Do KA, Green A, Guthrie JR, Dudley EC, Burger HG, Dennerstein L. Longitudinal study of risk factors for coronary heart disease across the menopausal transition. American journal of epidemiology. 2000;151(6):584-93.
33. Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF. Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke; a journal of cerebral circulation. 2001;32(5):1104-11.
34. Torng PL, Su TC, Sung FC, Chien KL, Huang SC, Chow SN, et al. Effects of menopause on intraindividual changes in serum lipids, blood pressure, and body weight--the Chin-Shan Community Cardiovascular Cohort study. Atherosclerosis. 2002;161(2):409-15.
35. Schubert CM, Rogers NL, Remsberg KE, Sun SS, Chumlea WC, Demerath EW, et al. Lipids, lipoproteins, lifestyle, adiposity and fat-free mass during middle age: the Fels Longitudinal Study. Int J Obes (Lond). 2006;30(2):251-60.
36. de Kat AC, Dam V, Onland-Moret NC, Eijkemans MJ, Broekmans FJ, van der Schouw YT. Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. BMC Med. 2017;15(1):2.
37. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. Journal of the American College of Cardiology. 2006;47(10):1976-83.
38. Rothman KJ. Epidemiology, an introduction: Oxford University Press; 2012.

Appendix 1: Additional details of methods

Imputation

Missing values in the exposures, covariates and mediators were imputed with multiple imputation using the package MICE in R with 10 imputations and 50 iterations. Predictive mean matching was used for continuous variables (total cholesterol, HDL-cholesterol, triglycerides, hsCRP, HbA1c, age at ovariectomy, age at hysterectomy, age at menopause and time in study (the latter was not used as a predictor)), logistic regression for categorical variables with two levels (high blood pressure, full term pregnancy, oral contraceptive use, hysterectomy and ever hormone use), multinomial logit models for categorical variables with more than two levels (ovariectomy and smoking status) and ordered logit models for ordered categorical variables (physical activity, number of full term pregnancies, education level and BMI). In addition, we included age at baseline and age at event since age was the underlying time scale of the Prentice weighted Cox proportional hazards analysis; country or center, whichever had the highest correlation with the imputed variable, an indicator for CHD status and the country specific Prentice-weighted Nelson Aalen estimators, which were further weighted by the country-specific sampling fraction. Furthermore, we also included the interaction terms that were used in the model to account for effect modification.

We checked all the predictors for correlation, whenever two predictors were too highly correlated (≤ -0.7 or ≥ 0.7) only one was chosen as a predictor for each imputed variable. The predictor that had the highest correlation with the imputed variable was chosen as predictor in the imputation model for that variable.

Bootstrapping

Bootstrap sampling (1000 bootstrap samples) was used to obtain 95% confidence intervals (CI) around the PE. We took bootstrap samples from each imputed dataset and in each bootstrap sample we calculated the coefficients with and without the mediator. Next we pooled the total effects and direct effects of imputation one to ten for each first bootstrap with Rubin's Rule, and repeated this for the second until the 1000th bootstrap. Finally, we calculated 1000 PEs of which we took the 0.025th and the 0.975th percentile as the 95% CI.

Appendix 2: Additional tables showing effect modification and sensitivity analyses

Appendix Table 1. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between age at menopause and any first CHD event stratified by BMI

Model	*≤20 HR (95% CI), p-value*	*>20-<25 HR (95% CI), p-value*	*≥25-<30 HR (95% CI), p-value*	*≥30 HR (95% CI), p-value*
Model 1[a]	1.03 (0.99-1.07), 0.19	1.01 (1.00-1.03), 0.08	1.03 (1.02-1.05), <0.001	1.04 (1.02-1.06), <0.001
Model 2[b]	1.01 (0,97-1.06), 0.54	1.01 (0.99-1.02), 0.43	1.03 (1.01-1.04), 0.001	1.04 (1.02-1.05), <0.001
Model 3[c]	1.01 (0.97-1.06), 0.55	1.01 (0.99-1.02), 0.40	1.03 (1.01-1.04), 0.001	1.03 (1.01-1.05), 0.001
Model 4[d]	1.01 (0.97-1.07), 0.56	1.00 (0.99-1.02), 0.66	1.02 (1.01-1.04), 0.01	1.04 (1.02-1.06), <0.001

[a]*Model 1: Adjusted for age.*

[b]*Model 2: Additionally adjusted for smoking status, HbA1c, education level and physical activity.*

[c]*Model 3: Additionally adjusted for full term pregnancy (yes/no), age at menarche and ever hormone use.*

[d]*Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.*

Appendix Table 2. Proportion of association explained by covariates

	Cox regression for model 1 + covariate HR (95%CI), p-value	Proportion explained by covariate PE% (95%CI(%))
	Menopausal status (post vs pre)	
Total effect	*1.23 (1.08-1.40), 0.002*	/
Smoking status	1.18 (1.03-1.35), 0.01	18.7 (14.0-24.8)
BMI	1.21 (1.06-1.39), 0.004	6.1 (3.4-9.7)
HbA1c	1.21 (1.06-1.39), 0.005	5.9 (1.3-10.6)
Education level	1.20 (1.05-1.37), 0.01	10.9 (8.4-14.4)
Physical activity	1.23 (1.08-1.41), 0.002	-0.7 (-2.8-1.1)
Full term pregnancy	1.23 (1.08-1.40), 0.002	0.1 (-0.6-0.9)
Age at menarche	1.21 (1.06-1.38, 0.005	7.3 (5.2-10.2)
Hormone use	1.24 (1.09-1.42), 0.002	-5.3 (-9.4- -1.4)
hsCRP	1.22 (1.06-1.39), 0.004	4.9 (2.4-8.1)
Total cholesterol	1.17 (1.02-1.34), 0.02	23.6 (19.0-30.4)
HDL	1.22 (1.07-1.40), 0.003	2.3 (-1.9-7.2)
Triglycerides	1.18 (1.03-1.34), 0.02	21.3 (13.6-25.5)
High blood pressure	1.22 (1.07-1.40), 0.003	2.7 (-1.5-7.2)
	Age at menopause	
Total effect	*1.027 (1.018-1.036), <0.001*	/
Smoking status	1.022 (1.013-1.031), <0.001	18.8 (15.9-22.0)
BMI	1.028 (1.019-1.037), <0.001	-2.3 (-4.3- -0.4)
HbA1c	1.027 (1.017-1.036), <0.001	1.8 (-0.7-4.7)
Education level	1.026 (1.017-1.035), <0.001	3.9 (2.7-5.3)
Physical activity	1.027 (1.018-1.036), <0.001	1.1 (-0.2-2.5)
Full term pregnancy	1.027 (1.018-1.037), <0.001	-0.9 (-1.6- -0.5)
Age at menarche	1.026 (1.017-1.035), <0.001	4.8 (3.1-6.0)
Hormone use	1.027 (1.018-1.036), <0.001	-0.7 (-1.2- -0.3)
hsCRP	1.026 (1.017-1.036), <0.001	2.7 (1.1-4.7)
Total cholesterol	1.026 (1.017-1.036), <0.001	2.4 (0.2-4.3)
HDL	1.025 (1.016-1.035), <0.001	5.9 (3.0-9.0)
Triglycerides	1.026 (1.017-1.035), <0.001	3.8 (2.7-9.2)
High blood pressure	1.028 (1.018-1.037), <0.001	-2.4 (-5.1-0.2)
	Type of menopause (surgical vs natural)	
Total effect	*1.31 (1.16-1.47), <0.001*	/
Smoking status	1.29 (1.15-1.46), <0.001	4.8 (1.5-8.0)
BMI	1.30 (1.15-1.46), <0.001	3.2 (0.7-5.6)
HbA1c	1.32 (1.17-1.49), <0.001	-3.7 (-7.7-0.3)
Education level	1.30 (1.15-1.46), <0.001	2.7 (0.8-4.7)

Appendix Table 2. Continued

	Cox regression for model 1 + covariate HR (95%CI), p-value	Proportion explained by covariate PE% (95%CI(%))
Physical activity	1.30 (1.15-1.47), <0.001	2.0 (0.2-3.9)
Full term pregnancy	1.31 (1.16-1.47), <0.001	0.01 (-0.8-0.7)
Age at menarche	1.29 (1.14-1.45), <0.001	5.5 (3.3-7.2)
Hormone use	1.32 (1.17-1.48), <0.001	-2.5 (-3.9- -1.4)
hsCRP	1.29 (1.14-1.45), <0.001	6.2 (2.8-9.6)
Total cholesterol	1.29 (1.14-1.45), <0.001	6.7 (3.3-9.5)
HDL	1.29 (1.14-1.46), <0.001	4.8 (0.7-8.8)
Triglycerides	1.27 (1.12-1.44), <0.001	11.1 (9.4-22.9)
High blood pressure	1.26 (1.12-1.43), <0.001	13.0 (9.3-16.8)
Age at menopause	1.16 (1.02-1.33), 0.02	43.6 (35.3-52.4)

Appendix Table 3. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between menopausal status and any first CHD event, HT users excluded

	Postmenopausal vs. premenopausal	
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.26 (1.06-1.48)	0.01
Model 2[b]	1.14 (0.96-1.36)	0.13
Model 3[c]	1.12 (0.94-1.33)	0.21
Model 4[d]	1.06 (0.88-1.28)	0.53

Mean N(events); Postmenopausal 6505 (2785), premenopausal 4912 (565)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche.
[d]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 4. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between age at menopause and any first CHD event, HT users excluded

	HR per year decrease in age at menopause	
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.02 (1.01-1.04)	<0.001
Model 2[b]	1.02 (1.00-1.03)	0.01
Model 3[c]	1.02 (1.00-1.03)	0.01
Model 4[d]	1.01 (1.00-1.03)	0.09

Mean N (events); 6505 (2785)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche.

[d]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 5. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between type of menopause and any first CHD event, HT users excluded

Surgical vs. natural menopause		
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.25 (1.07-1.46)	0.01
Model 2[b]	1.24 (1.05-1.47)	0.01
Model 3[c]	1.22 (1.03-1.45)	0.02
Model 3b[d]	1.14 (0.95-1.38)	0.16
Model 4[e]	1.12 (0.92-1.37)	0.27

Mean N (events); Surgical 1259 (543), natural 5246 (2243)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche.
[d]Model 3b: Additionally adjusted for age at menopause
[e]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 6. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between menopausal status and any first CHD event, first 2 years of follow-up excluded

Postmenopausal vs. premenopausal		
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.20 (1.05-1.37)	0.01
Model 2[b]	1.12 (0.97-1.29)	0.12
Model 3[c]	1.11 (0.96-1.28)	0.16
Model 4[d]	1.06 (0.91-1.24)	0.43

Mean N (events); Postmenopausal 9514 (3696), premenopausal 5418 (626)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[d]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 7. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between age at menopause and any first CHD event, first 2 years of follow-up excluded

HR per year decrease in age at menopause		
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.03 (1.02-1.04)	<0.001
Model 2[b]	1.02 (1.01-1.03)	<0.001
Model 3[c]	1.02 (1.01-1.03)	<0.001
Model 4[d]	1.02 (1.01-1.03)	0.002

Mean N (events): 9514 (3696)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[d]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 8. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between type of menopause and any first CHD event, first 2 years of follow-up excluded

	Surgical vs. natural menopause	
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.31 (1.16-1.49)	<0.001
Model 2[b]	1.27 (1.12-1.45)	<0.001
Model 3[c]	1.26 (1.10-1.44)	0.001
Model 3b[d]	1.17 (1.01-1.35)	0.04
Model 4[e]	1.13 (0.97-1.31)	0.11

Mean N (events): Surgical 2105 (837), natural 7409 (2859)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[d]Model 3b: Additionally adjusted for age at menopause
[e]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 9. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between menopausal status and any first CHD event, surgical menopause defined as bilateral ovariectomy only.

	Postmenopausal vs. premenopausal	
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.04 (0.92-1.19)	0.52
Model 2[b]	1.00 (0.87-1.15)	0.99
Model 3[c]	0.99 (0.86-1.14)	0.92
Model 4[d]	0.95 (0.82-1.10)	0.50

Mean N (events); Postmenopausal 9251 (3882), premenopausal 6151 (871)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[d]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

Appendix Table 10. Hazard Ratio (HR) and 95% confidence intervals (CI) for the association between type of menopause and any first CHD event among postmenopausal women, surgical menopause defined as bilateral ovariectomy only

Surgical vs. natural menopause		
Model	***HR (95%CI)***	***p-value***
Model 1[a]	1.22 (1.010-1.47)	0.04
Model 2[b]	1.10 (0.89-1.34)	0.38
Model 3[c]	1.08 (0.88-1.33)	0.47
Model 3b[d]	1.00 (0.81-1.24)	1.00
Model 4[e]	0.92 (0.74-1.15)	0.47

Mean N (events); Surgical 856 (332), natural 9152 (3747)
[a]Model 1: Adjusted for age.
[b]Model 2: Additionally adjusted for smoking status, BMI, HbA1c, education level, physical activity.
[c]Model 3: Additionally adjusted for full term pregnancy, age at menarche, ever hormone use.
[d]Model 3b: Additionally adjusted for age at menopause
[e]Model 4: Additionally adjusted for high sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, triglycerides and high blood pressure.

CHAPTER 2.3

Age at natural menopause and coronary heart disease risk and cardiovascular risk factors: a two-sample Mendelian Randomization study

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In preparation

Abstract

Background: Accelerated reproductive aging, as indicated by early menopause, has been associated with increased risk of cardiovascular disease (CVD) in women. However, the underlying mechanisms remain unclear. Observational studies are prone to confounding and reversed causality can play a role. Mendelian randomization (MR) studies can help to establish causality. Genome wide association studies (GWAS) identified 56 genomic variants associated with age at natural menopause (ANM) which are mainly implicated in genome stability, immune function and mitochondrial biogenesis. These processes are not sex-specific and might underlie reproductive ageing in men as well. We aimed to establish the causal association between reproductive aging and fatal or non-fatal coronary heart disease (CHD), and between reproductive aging and cardiovascular risk factors.

Methods: An MR analysis was conducted using summarized data from three studies (UK Biobank, a modified version of CARDIoGRAMplusC4D and EPIC-CVD) with together 417,579 participants from European descent (including 49,150 CHD cases). For the analyses between ANM and CVD risk factors, we used data from publicly available GWAS data of the Global Lipids Genetics Consortium (total cholesterol, high density lipoprotein cholesterol, triglycerides) and MAGIC (HbA1c, fasting glucose) and EPIC-CVD. We performed a 2-sample MR using four methods to estimate causal effects: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression.

Results: Our MR analyses show no association between ANM and CHD in women (Relative Risk Estimate $(RRE)_{IVW}$: 0.99, 95% confidence interval (CI): 0.97;1.01), men (RRE_{IVW}: 1.00, 95%CI: 0.97;1.02), or in the sex-combined analysis (IVW: 1.00, 95%CI: 0.98;1.01). Similarly, no association was found between ANM and any of the cardiovascular risk factors.

Conclusion: Reproductive aging is not causally associated with CHD and cardiovascular risk factors in women, nor in men. The association between early menopause and CHD risk in observational studies might be the result of residual confounding, reversed causation, or reflect a shared aetiology that results in both earlier menopause and higher CHD risk.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in both men and women[1]. Accelerated reproductive aging, as indicated by early menopause, has been associated with increased risk of CVD in women[2–5]. The mechanisms underlying this association are not fully understood yet; deterioration of traditional CVD risk factors, in particular cholesterol, have been suggested to play a role[6,7].

In observational studies, it is difficult to disentangle the potential independent effect of accelerated reproductive aging on CVD risk from the effect of general aging, as residual confounding can still be present. Furthermore, reversed causality can also play a role here, as women with an unfavourable CVD risk profile have been reported to experience accelerated reproductive aging[8]. Mendelian Randomization (MR) designs, exploiting the principle of random independent segregation of alleles at meiosis, are a means to establish causality in situations where randomized clinical trials are impossible[9,10]. In MR studies, single nucleotide polymorphisms (SNPs) associated with the exposure as found in genome-wide association studies (GWAS) are used as instrumental variables.

To date, GWAS on age at natural menopause (ANM) have reported 56 SNPs, that are mainly implicated in genome stability (DNA repair), immune function and mitochondrial biogenesis[11]. As these mechanisms are not specific for women, it could be hypothesized that this mechanism underlies reproductive aging in men as well.

Indeed, in men reproductive functions also decline with aging[12–14], and some of these, e.g. erectile dysfunction and decreasing testosterone levels, sometimes referred to as andropause, have been associated with increased CVD risk[15,16]. However, male reproductive aging is a gradual process into old age, and therefore, it is more complicated to study health effects of accelerated reproductive aging in males.

A recent study in three cohorts showed that a weighted genetic risk score (wGRS) of the 56 GWAS variants for earlier ANM was associated with increased CVD risk and increased coronary heart disease (CHD) death in women, but not in men[17]. However, in a formal MR analysis of publicly available data, which was conducted in women only, the increased risk could not be confirmed.

The aims of the present study are to establish the causal association between reproductive aging and fatal or non-fatal CHD, and to gain more insight in possible mechanisms between reproductive aging and cardiovascular risk factors in women, using 56 SNPs associated with a younger ANM. Furthermore, we aim to establish whether the same mechanisms are associated with CHD and traditional cardiovascular risk factors in men as well, using the same set of SNPs.

Methods

Study populations and outcomes

Fatal or non-fatal CHD

We used data from 417,579 participants of European descent (including 49,150 CHD cases) from three studies: the UK Biobank[18], a modified version of the CARDIoGRAMplusC4D consortium (m-CARDIoGRAMplusC4D) since we could only include those studies that provided us with sex-specific summary data (Cardiogenics, Thiseas, AMC-PAS, Duke 2, CCGB 2, ITH 2, OHGS A2, OHGS B2, OHGS C2, Germifs I, Germifs II, Germifs III, Germifs IV, Life-Heart and Luric[19]), and the EPIC-CVD case-cohort study[20]. Details of the three studies (UK Biobank, m-CARDIoGRAMplusC4D and EPIC-CVD), including definitions of fatal or non-fatal CHD in each study, can be found in appendix 1.

Traditional CVD risk factors

For the associations between ANM and CVD risk factors, we again used data from EPIC-CVD and combined these with publicly available GWAS summary statistics of the Global Lipids Genetics Consortium[21] (total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides) and MAGIC[22,23] (HbA1c, fasting glucose). Details on these consortia can be found in appendix 1.We did not have access to sex-specific data for these risk factors; therefore we could only perform the pooled MR analyses for men and women combined.

Genotyping and SNP selection

Genotyping in the UK Biobank was performed using the Affymetrix UK BiLEVE Axiom array and the Affymetrix UK Biobank Axiom Array[18,24]. The m-CARDIoGRAMplusC4D studies are used various genotyping methods as described previously[19]. EPIC-CVD participants were genotyped with the Human Core Exome array, Illumina 660 Quad array, and Omni Exome Express array. The Global Lipids Genetics Consortium and MAGIC also used different assays as described previously[21–23].

A recent genome-wide meta-analysis identified 56 SNPs associated with younger ANM among European descents, 54 common HapMap SNPs and two Exome chip SNPs[11]. All SNPs passed the threshold of p<5e-6, but not all the threshold of p<5e-8. No linkage disequilibrium (LD) at R^2>0.9 was present among these 57 SNPs. Pleiotropic effects were investigated by searching GWAS catalog[25] and Phenoscanner[26] for the SNPs or its proxies (R^2>0.8).

Statistical analyses

For CHD, odds ratios and standard errors for the SNP outcome relations were derived through contact persons of UK Biobank and m-CARDIoGRAMplusC4D. Secondly, we derived effect estimates and standard errors for the cardiovascular risk factors (Global Lipids Genetics Consortium[21] for total cholesterol, HDL cholesterol and triglycerides, and MAGIC[22,23] for HbA1c and fasting

glucose) using Phenoscanner[26]. Finally, for both CHD and cardiovascular risk factors, estimates and standard errors were calculated by the authors in EPIC-CVD. For CHD, we used Prentice-weighted Cox proportional hazards regression adjusted for age, country, the first two principal components and array to calculate hazard ratios. For the CVD risk factors, we first had to impute the observational data of EPIC-CVD (not the genetic data) using multiple imputation with the MICE package in R[27] with 10 imputations and 50 iterations, including the CVD risk factors, SNPs and other baseline characteristics as predictors. Subsequently, we derived regression coefficients with linear regression in the subcohort and the cases, separately in each imputation, using the same adjustments as for CHD. Thereafter we pooled the results with Rubin's Rule[28].

We performed a 2-sample MR using four separate methods to estimate causal effects for binary (CHD) and continuous (total cholesterol, HDL cholesterol, triglycerides, apolipoprotein A (apoA1), apolipoprotein B (apoB), C-reactive protein (CRP), glucose and HbA1c) outcomes: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R[29]. The IVW provides a consistent estimate and assumes that all assumptions of the instrumental variable are met, the median based and MR-Egger methods provide estimates under weaker assumptions, with the MR-Egger additionally providing an intercept that represents the average pleiotropic effect[30,31]. When unbalanced horizontal pleiotropy is absent, results of all methods are expected to be consistent[32]. We first conducted sex-specific and sex-combined MR analyses for CHD in all three studies (UK Biobank, m-CARDIoGRAMplusC4D, EPIC-CVD) separately. Subsequently, we pooled the estimates with a fixed effect model as is standard in MR studies. Similarly, MR analyses were performed for reproductive aging and each cardiovascular risk factor in each study separately (EPIC-CVD, Global Lipids Genetics Consortium, MAGIC) and then pooled using a fixed effects model. All analyses were conducted with R version 3.2.0[33].

Results

Table 1 provides an overview of the numbers of cases and non-cases in UK Biobank, m-CARDIoGRAMplusC4D, and EPIC-CVD.

Table 2 shows the results for the association between reproductive aging and CHD for MR method stratified by sex and by study (UKBiobank, m-CARDIoGRAMplusC4D, and EPIC-CVD). In women, the IVW analyses in each study separately showed no causal association between reproductive aging and CHD, nor when studies were pooled together (RRE_{IVW}=0.99; 95% confidence interval [CI]=0.96;1.02). The MR-Egger method indicated no pleiotropic effects (intercept = 0.004, p=0.318) and resulted in an estimate of the relative risk of 0.97 (95%CI=0.94;1.02) in the pooled data. Similar results were found for men with a pooled RRE_{IVW} of 1.00 (95%CI=0.97;1.02), also indicating no pleiotropic effects (intercept = 0.000, p=0.948; $RRE_{MR\text{-}Egger}$=1.00 (95%CI=0.95;1.05),

and for the sex-combined results (pooled RRE_{IVW}=1.00, 95%CI=0.98;1.01; $RRE_{MR\text{-}egger}$=1.00 (95%CI=1.00;1.01), intercept=0.002 (p=0.537)).

Table 1. Number of cases and controls by study, stratified by sex.

	Women		**Men**		**Total**	
Study	Cases	Controls	Cases	Controls	Cases	Controls
UK Biobank	13,671	185,202	11,681	157,089	25,352	342,291
m-CARDIoGRAMplusC4D	2,970	5,282	10,215	6,218	13,185	11,500
EPIC-CVD*	3,528	8,637	5,501	5,388	9,029	14,025
Total	20,169	199,121	27,391	168,695	47,566	367,815

Table 2. Relative risk estimates of the association between reproductive aging and coronary heart disease for individual studies and the pooled cohorts

	UK Biobank OR (95%CI)	**m-CARDIoGRAM plusC4D** OR (95%CI)	**EPIC-CVD** HR (95%CI)	**Pooled relative risk estimates**
		Women		
Simple median	0.99 (0.96;1.02)	0.97 (0.89;1.06)	0.97 (0.78;1.21)	0.99 (0.96;1.02)
Weighted median	0.99 (0.96;1.02)	0.97 (0.89;1.06)	1.08 (0.88;1.33)	0.99 (0.96;1.02)
IVW*	0.99 (0.97;1.02)	0.98 (0.91;1.05)	1.02 (0.89;1.17)	0.99 (0.97;1.01)
MR-Egger	0.98 (0.94;1.02)	0.88 (0.76;1.02)	1.29 (0.91;1.83)	0.97 (0.94;1.02)
		Men		
Simple median	0.99 (0.95;1.02)	1.05 (0.99;1.12)	0.98 (0.81;1.18)	1.01 (0.98;1.04)
Weighted median	0.99 (0.96;1.03)	1.05 (0.99;1.12)	0.84 (0.71;1.01)	1.01 (0.98;1.04)
IVW*	0.99 (0.96;1.01)	1.03 (0.99;1.08)	0.93 (0.82;1.05)	1.00 (0.97;1.02)
MR-Egger	0.98 (0.93;1.03)	1.02 (0.92;1.12)	0.85 (0.62;1.16)	1.00 (0.95;1.05)
		Sex-combined		
Simple median	0.99 (0.96;1.01)	1.00 (0.95;1.05)	1.01 (0.88;1.16)	1.00 (0.98;1.03)
Weighted median	0.99 (0.96;1.01)	1.04 (0.99;1.10)	1.01 (0.87;1.16)	1.00 (0.98;1.03)
IVW*	0.99 (0.98;1.01)	1.02 (0.98;1.06)	0.99 (0.90;1.08)	1.00 (0.98;1.01)
MR-Egger	0.98 (0.95;1.01)	0.99 (1.08;0.86)	1.07 (0.85;1.33)	1.00 (1.00;1.01)

**Inverse-variance weighted*

Table 3 shows the IVW results for the association between reproductive aging and cardiovascular risk factors, with sex-specific estimates only from EPIC-CVD and sex-combined estimates from both publicly available data and EPIC-CVD, as well as pooled estimates. For each one-year decrease in genetic age at menopause, total cholesterol decreased with 0.027 mmol/L in women

in IVW-analysis, however this was not statistically significant (95%CI= -0.055;0.000). Similarly, reproductive aging was also not causally associated with total cholesterol in men ($beta_{IVW}$=0.024 mmol/L, 95%CI= -0.002;0.050), nor in the sex-combined results (pooled $beta_{IVW}$=0.004 mmol/L, 95%CI= -0.008;0.016). Again, no pleiotropic effects were detected (Appendix 2). Similarly, no causal association was found for HDL cholesterol (women: $beta_{IVW}$= -0.006 mmol/L, 95%CI= -0015;0.003; men: $beta_{IVW}$=0.004 mmol/L, 95%CI= -0.004;0.012; pooled sex-combined $beta_{IVW}$=0.002 mmol/L, 95%CI= -0.007;0.010), and triglycerides (women: $beta_{IVW}$=0.004 mmol/L, 95%CI= -0.014;0.022; men: $beta_{IVW}$=0.035 mmol/L, 95%CI= 0.007;0.064; pooled sex-combined $beta_{IVW}$=0.002 mmol/L, 95%CI= -0.014;0.018). Furthermore, we found no causal association between reproductive aging and ApoA1, ApoB, CRP, glucose, and HbA1c (table 3).

Table 3. Estimates of IVW analyses for the association between age at natural menopause and cardiovascular risk factors. Results for individual studies and the pooled cohorts.

	Women	**Men**	**Sex-combined**		
	EPIC-CVD beta (95%CI)	**EPIC-CVD** beta (95%CI)	**EPIC-CVD** beta (95%CI)	**Publicly available data*** beta (95%CI)	**Pooled estimate** beta (95%CI)
Total cholesterol (mmol/L)	-0.027 (-0.055;0.000)	0.024 (-0.002;0.050)	-0.008 (-0.030;0.014)	0.007 (-0.006;0.020)	0.004 (-0.008;0.016)
HDL cholesterol (mmol/L)	-0.006 (-0.015;0.003)	0.004 (-0.004;0.012)	-0.002 (-0.010;0.005)	0.008 (-0.006;0.022)	0.002 (-0.007;0.010)
Triglycerides (mmol/L)	0.004 (-0.014;0.022)	0.035 (0.007;0.064)	0.018 (0.000;0.037)	0.007 (-0.006;0.020)	0.002 (-0.014;0.018)
Apolipoprotein A1 (g/L)	-0.006 (-0.013;0.002)	0.007 (0.001;0.014)	-0.001 (-0.006;0.005)	/	/
Apolipoprotein B (g/L)	-0.003 (-0.009;0.003)	0.004 (-0.002;0.011)	0.000 (-0.005;0.005)	/	/
C-reactive protein (mg/L)	0.023 (-0.079;0.126)	0.075 (-0.044;0.194)	0.046 (-0.036;0.127)	/	/
Glucose (mmol/L)	0.018 (-0.015;0.052)	0.027 (-0.018;0.072)	0.023 (-0.008;0.054)	0.002 (-0.004;0.008)	0.003 (-0.003;0.008)
HbA1c (%)	-0.002 (-0.015;0.011)	0.015 (-0.001;0.031)	0.005 (-0.006;0.016)	0.000 (-0.007;0.007)	0.001 (-0.005;0.008)

**Global Lipids Genetics Consortium for total cholesterol, HDL cholesterol and triglycerides; MAGIC consortium for glucose and HbA1c*

***EPIC-CVD non-fasting glucose pooled with summary statistics of fasting glucose.*

Discussion

This study did not find a causal association between reproductive aging and CHD risk in women, nor in men. Furthermore, this study shows that there is no causal association between reproductive aging and CVD risk factors, including cholesterol levels, in women and men.

Our findings are partly in contrast with one previous study investigating the association between ANM SNPs and CHD death, which reported a significantly increased risk of CHD death with a wGRS in women, but not in men[17]. However, our findings are in line with those of the MR analysis they presented in the same paper, using CARDIoGRAMplusC4D data, which was also null. The discrepancy between the wGRS and MR findings is likely due to the fact that the wGRS analysis was adjusted for several known CVD risk factors (current smoking, body mass index, hypertension, type 2 diabetes, total cholesterol, and lipid treatment). This might induce a biased association between the genetic variant and the outcome through the confounder(s), also known as collider bias[34,35]. In addition, the number of cases used for the wGRS analyses was small (only 541 CHD deaths in women), so we cannot exclude a chance finding.

Strengths of this study are that, to the best of our knowledge, this is the largest MR study of associations of reproductive aging and CHD to date with 20,169 CHD events in women and 27,391 in men; we used several methods for MR analyses all yielding consistent results for the tested hypotheses; and all SNPs had a genome-wide significant association with ANM[11]. Some limitations need to be acknowledged. First, our analyses with glucose were based on both fasting (MAGIC) and non-fasting estimates (EPIC-CVD). Although both are associated with an increased CVD risk[36,37] it might not be appropriate to combine them, since different SNPs might drive the association and underlying mechanisms could be different. Third, most of the observational studies include women with an extremely early menopause or premature ovary insufficiency (POI), and two recent systematic reviews and meta-analyses of observational studies showed that POI is associated with both fatal and non-fatal CHD and CVD[38,39]. The GWAS on ANM included only women with an ANM between 40 and 60 years and therefore did not include women with an extremely early menopause (<40) or POI, so we could not properly study this association in our MR study. However, a recent GWAS on early menopause revealed no new genetic variants for early menopause and showed that the genetic aetiology of early menopause overlaps with that of ANM. Thus early menopause is at least partly explained by the same polygenic variants as ANM[40].

Our MR-study suggested that the association between reproductive aging and CHD may not be causal. However, most observational studies do find an association between early age at menopause and CHD in women. We have several explanations for this finding. First, observational studies are susceptible to residual confounding and reversed causation. It is possible that residual confounding is still present. Postmenopausal women are by definition older than premenopausal women, making it challenging to separate the effects of biological aging from the various phases

of the reproductive aging process. Hence, residual confounding due to age may still be present in observational studies. Second, reverse causation is another potential problem in observational studies. Although most studies assume that an early ANM increases CHD risk, it might be possible that an unfavourable cardiovascular risk profile, or accelerated vascular aging, causes an early ANM. One previous study showed indeed that higher cholesterol levels prior to menopause were associated with earlier menopause[8]. However, another study found no association between CVD and subsequent age at menopause[41]. If anything, women with CVD had a lower risk of becoming postmenopausal (HR=0.96 for CVD and HR=0.87 for MI), suggesting that menopause occurred later in these women[41], but none of these results were statistically significant due to the small number of cases. Finally, even though our sample size is large, the genetic variants included only explain a small part of the variation in age at natural menopause. This reduces power, and even though we have included a large number of cases and this is the largest MR study on menopause and CHD risk to date, we still cannot exclude the possibility that even larger sample sizes are needed.

MR uses SNPs, that are randomly assigned by birth, as instrumental variables, and as such provides a method to assess causality[42]. However, an MR study makes several assumptions, that have to be taken into account[43]. The first assumption is that the genetic marker is associated with the exposure. The SNPs used in our study were all statistically significantly associated with ANM in the latest and largest GWAS[11]. The second and third assumptions are that the association between the genetic marker and the outcome is explained exclusively through the exposure of interest and is unconfounded. This is often referred to as the absence of pleiotropy, which means that the genetic variant is not associated with other phenotypes. Although our Phenoscanner search showed that a few of the SNPs are associated with age at menarche or sex hormone levels, and thus that some pleiotropy may be present, our MR-Egger analysis showed no indication of pleiotropy, since all intercepts were zero or very close to zero and non-significant[31]. We therefore assume that our results are not biased due to pleiotropy.

In summary, reproductive aging is not causally associated with CHD and cardiovascular risk factors in women, nor in men. The association between early menopause and CHD risk in observational studies might be the result of residual confounding, reversed causation, or reflect a shared aetiology that results in both earlier menopause and higher CHD risk.

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References

1 Timmis A, Townsend N, Gale C, *et al.* European Society of Cardiology: Cardiovascular disease statistics 2017. *Eur Heart J* 2018;**39**:508–77. doi:10.1093/eurheartj/ehx628

2 van der Schouw YT, van der Graaf Y, Steyerberg EW, *et al.* Age at menopause as a risk factor for cardiovascular mortality. *Lancet* 1996;**347**:714–8.

3 Ossewaarde ME, Bots ML, Verbeek AL, *et al.* Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology* 2005;**16**:556–62.http://www.ncbi.nlm.nih.gov/pubmed/15951675

4 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**:767–76. doi:10.1001/jamacardio.2016.2415

5 Dam V, van der Schouw YT, Onland-Moret NC, *et al.* Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis (submitted). 2018.

6 Matthews KA, Crawford SL, Chae CU, *et al.* Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? *JAmCollCardiol* 2009;**54**:2366–73.

7 de Kat AC, Dam V, Onland-Moret NC, *et al.* Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. *BMC Med* 2017;**15**:2. doi:10.1186/s12916-016-0762-8

8 Kok HS, van Asselt KM, van der Schouw YT, *et al.* Heart Disease Risk Determines Menopausal Age Rather Than the Reverse. *J Am Coll Cardiol* 2006;**47**:1976–83. doi:10.1016/j.jacc.2005.12.066

9 Smith GD, Ebrahim S. 'Mendelian randomization': Can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;**32**:1–22. doi:10.1093/ije/dyg070

10 de Haan HGG, Siegerink B, van Hylckama Vlieg A. [Mendelian randomisation]. *Ned Tijdschr Geneeskd* 2014;**158**:A7547.http://www.ncbi.nlm.nih.gov/pubmed/25322353

11 Day FR. Europe PMC Funders Group Large-scale genomic analyses link reproductive ageing to hypothalamic signaling , breast cancer susceptibility and BRCA1-mediated DNA repair. *Nat Genet* 2016;**47**:1294–303. doi:10.1038/ng.3412.Large-scale

12 Kühnert B, Nieschlag E. Reproductive functions of the ageing male. *Hum Reprod Update* 2004;**10**:327–39. doi:10.1093/humupd/dmh030

13 Johnson SL, Dunleavy J, Gemmell NJ, *et al.* Consistent age-dependent declines in human semen quality: A systematic review and meta-analysis. *Ageing Res Rev* 2015;**19**:22–33. doi:10.1016/j.arr.2014.10.007

14 Ramasamy R, Chiba K, Butler P, *et al.* Male biological clock: A critical analysis of advanced paternal age. *Fertil Steril* 2015;**103**:1402–6. doi:10.1016/j.fertnstert.2015.03.011

15 Lewis BH, Legato M, Fisch H. Medical implications of the male biological clock. *J Am Med Assoc* 2006;**296**:2369–71. doi:10.1001/jama.296.19.2369

16 Vainionpää KJ, Topo P. The making of an ageing disease: The representation of the male menopause in Finnish medical literature. *Ageing Soc* 2005;**25**:841–61. doi:10.1017/S0144686X05003946

17 Sarnowski C, Kavousi M, Isaacs S, *et al.* Genetic variants associated with earlier age at menopause increase the risk of cardiovascular events in women. *Menopause* 2017;**25**:1. doi:10.1097/GME.0000000000001017

18 Sudlow C, Gallacher J, Allen N, *et al.* UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLoS Med* 2015;**12**:1–10. doi:10.1371/journal.pmed.1001779

19 Nikpay M, Goel A, Won H, *et al.* A comprehensive 1000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2016;**47**:1121–30. doi:10.1038/ng.3396.A

20 Danesh J, Saracci R, Berglund G, *et al.* EPIC-Heart: the cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. *Eur J Epidemiol* 2007;**22**:129–41. doi:10.1007/s10654-006-9096-8

21 Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, *et al.* Discovery and Refinement of Loci Associated with Lipid Levels. *Nat Genet* 2013;**45**:1–24. doi:10.1038/ng.2797. Discovery

22 Dupuis J, Bouatia-naji N, Langenberg C. Common variants at ten genomic loci influence hemoglobin A. 2010;**59**:1–27. doi:10.2337/db10-0502.N.S.

23 Dupuis J, Langenberg C, Prokopenko I, *et al.* New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet* 2010;**42**:105–16. doi:10.1038/ng.520.New

24 Collins R. What makes UK Biobank special? *Lancet* 2012;**379**:1173–4. doi:10.1016/S0140-6736(12)60404-8

25 Welter D, MacArthur J, Morales J, *et al.* The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014;**42**:1001–6. doi:10.1093/nar/gkt1229

26 Staley JR, Blackshaw J, Kamat MA, *et al.* PhenoScanner: A database of human genotype-phenotype associations. *Bioinformatics* 2016;**32**:3207–9. doi:10.1093/bioinformatics/btw373

27 Buuren S, Groothuis-Oudshoorn K. mice: Multivariate imputation by chained equations in R. *J Stat Softw* 2011;**45**. doi:10.18637/jss.v045.i03

28 Rubin DB. *Multiple Imputation for Non Response in Surveys.* Wiley 1987.

29 Yavorska O, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. 2017.

30 Bowden J, Davey Smith G, Haycock PC, *et al.* Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016;**40**:304–14. doi:10.1002/gepi.21965

31 Burgess S, Thompson SG. Erratum to: Interpreting findings from Mendelian randomization using the MR-Egger method (Eur J Epidemiol, 10.1007/s10654-017-0255-x). *Eur J Epidemiol* 2017;**32**:391–2. doi:10.1007/s10654-017-0276-5

32 Dale CE, Fatemifar G, Palmer TM, *et al.* Causal Associations of Adiposity and Body Fat Distribution with Coronary Heart Disease, Stroke Subtypes, and Type 2 Diabetes Mellitus: A Mendelian Randomization Analysis. *Circulation* 2017;**135**:2373–88. doi:10.1161/CIRCULATIONAHA.116.026560

33 Team RC. R: A language and environment for statistical computing. 2016.https://www.r-project.org/

34 Haycock PC, Burgess S, Wade KH, *et al.* Statistical Commentary Best (but oft-forgotten) practices : the design , analysis , and interpretation of Mendelian randomization studies 1. *Am J Clin Nutr* 2016;**103**:965–78. doi:10.3945/ajcn.115.118216.INTRODUCTION

35 Day FR, Loh PR, Scott RA, *et al.* A Robust Example of Collider Bias in a Genetic Association Study. *Am J Hum Genet* 2016;**98**:392–3. doi:10.1016/j.ajhg.2015.12.019

36 Levitzky YS, Pencina MJ, D'Agostino RB, *et al.* Impact of Impaired Fasting Glucose on Cardiovascular Disease. The Framingham Heart Study. *J Am Coll Cardiol* 2008;**51**:264–70. doi:10.1016/j.jacc.2007.09.038

37 Benn M, Tybjærg-Hansen A, McCarthy MI, *et al.* Nonfasting glucose, ischemic heart disease, and myocardial infarction: A mendelian randomization study. *J Am Coll Cardiol* 2012;**59**:2356–65. doi:10.1016/j.jacc.2012.02.043

38 Tao XY, Zuo AZ, Wang JQ, *et al.* Effect of primary ovarian insufficiency and early natural menopause on mortality: A meta-analysis. *Climacteric* 2016;**19**:27–36. doi:10.3109/13697137.2015.1094784

39 Roeters Van Lennep JE, Heida KY, Bots ML, *et al.* Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. *Eur J Prev Cardiol* 2016;**23**:178–86. doi:10.1177/2047487314556004

40 Perry JRB, Corre T, Esko T, *et al.* A genome-wide association study of early menopause and the combined impact of identified variants. *Hum Mol Genet* 2013;**22**:1465–72. doi:10.1093/hmg/dds551

41 Li J, Eriksson M, Czene K, *et al.* Common diseases as determinants of menopausal age. *Hum Reprod* 2016;**31**:2856–64. doi:10.1093/humrep/dew264

42 Burgess S, Thompson SG. *Mendelian Randomization: Methods for Using Genetic Variants in Causal Estimation*. CRC Press 2015. http://search.proquest.com/docview/1685406996?accountid=9851%5Cnhttp://tf5lu9ym5n.search.serialssolutions.com/?ctx_ver=Z39.88-2004&ctx_enc=info:ofi/enc:UTF-8&rfr_id=info:sid/ProQ%3Aagriculturejournals&rft_val_fmt=info:ofi/fmt:kev:mtx:journal&rft.genre=unk

43 Vanderweele TJ, Tchetgen EJT, Kraft P. Methodological challenges in Mendelian randomization. *Epidemiology* 2015;**25**:427–35. doi:10.1097/EDE.0000000000000081.Methodological

44 Riboli E, Hunt KJ, Slimani N, *et al.* European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;**5**:1113–24. doi:10.1079/PHN2002394

45 Langenberg C, Sharp S, Forouhi NG, *et al.* Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. *Diabetologia* 2011;**54**:2272–82.

46 Anderson GL, Limacher M, Assaf AR, *et al.* EPIC-Heart: the cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. *Menopause* 2002;**5**:129–41. doi:10.1007/s10654-006-9096-8

Appendix 1 Description of studies included

UK Biobank

UK Biobank is a large, population-based cohort study established to study the interrelationships between environment, lifestyle and genes. The UK Biobank (www.ukbiobank.ac.uk) recruited over 500,000 men and women between 2006 and 2010[24], aged between 37 and 73 years at baseline. For the analyses with CHD we could use 367,643 participants of which 25,352 CHD cases. The UK Biobank was approved by the North West Multi-Centre Research Ethics Committee and all participants provided written informed consent. Fatal or non-fatal CHD was defined as myocardial infarction (International Classification of diseases Tenth Edition [ICD-10] I21.0, I21.1, I21.2, I21.4, I21.9), coronary artery bypass grafting (Office of Population Censuses and Surveys four [OPCS-4] K40.1-40.4, K41.1-41.4, K45.1-45.5), and coronary angioplasty with or without stenting (OPCS-4 K49.1-49.2, K49-8-49.9, K50.2, K75.1-75.4, K75.8-75.9).

EPIC-CVD

EPIC-CVD is a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study[44]. Briefly, EPIC consists of 519,978 participants (366,521 women and 153,457 men) recruited from 23 centers across 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2000, and aged between 35 and 70 years at baseline. For EPIC-CVD, a representative subcohort of 18,249 participants was selected by simple random sampling, stratified by center, from participants who had available stored blood and buffy coat samples[45,46]. After exclusion of 615 participants with a prior history of myocardial infarction or stroke at baseline, 17,634 subcohort members remained. Subsequently, incident CHD cases outside the subcohort were added to the study sample using the same exclusion criteria (N=17,821). Genetic data was available for 14,025 subcohort members and 9,029 CHD cases outside the subcohort, resulting in a study sample of 23,054 people. EPIC complies with the Declaration of Helsinki, and all participants gave written informed consent before participating in this study. The study was approved by the local ethics committees of the participating centers and the Institutional Review Board of the International Agency for Research on Cancer (IARC, Lyon). Fatal and non-fatal CHD was defined by codes 410-414 of International Classification of Disease Ninth Edition (ICD-9), and codes I20-I25 of ICD-10.

m-CARDIoGRAMplusC4D

Cardiogenics

Cases from Germany and England were under the age of 65 with a confirmed primary MI within the preceding 3-36 months. Exclusion criteria were 1) a history of diabetes mellitus based on plasma glucose >7.0 mmol/l or HbA1c >0.7, 2) renal insufficiency, 3) patients not on statin therapy, 4) current smokers. The Paris cohort comprised patients aged 33 to 87, recruited within the BAAAC (Banque d'ADN et d'ARN de patients présentant une Athérosclérose Coronarienne) study with symptoms of acute coronary syndrome who had one stenosis >50% diagnosed in at least one major coronary artery. Controls were healthy individuals (aged 32 to 65 years) recruited in Cambridge who were blood donors recruited as part of the Cambridge Bioresource[19].

CCGB 2

Cases had at least one of myocardial infarction, coronary artery bypass graft, percutaneous intervention or a stenosis of at least 50% in at least one epicardial vessel. Diabetic cases and cases aged greater than 55 for men or 65 for women were excluded. Controls had a CTA or angiogram demonstrating no stenosis of greater than 50%. Controls were required to be at least 65 years old for men and 70 years old for women at the time of recruitment[19].

DUKE 2

The Duke Cathgen study recruited individuals though the cardiac catheterization laboratories at Duke University Medical Centre (Durham, NC, USA). Clinical data were provided by the Duke Database for Cardiovascular Disease (DDCD). Cases had at least one epicardial coronary vessel with at least 50% blockage. Age of onset was no older than 65 for women and 55 for men. Subjects (case and control), were excluded if they had severe pulmonary hypertension or congenital heart disease or were diabetic. Controls with a history of ICC/PCI, CABG, MI or transplant were excluded. Controls were required to be at least 50 years old[19].

GerMIFS I-IV

Cases from the GerMIFS I study had a strong positive family history for CAD and an early onset of disease, i.e. were enriched for a strong genetic component. Population-based subjects were entered as controls. GerMIFS II: patients had a validated MI with a strong genetic component as documented by an early age of onset (prior to the age of 60 years) and a positive family history for CAD in 59.4% of patients. Patients were identified following their admission for acute treatment of MI or in cardiac rehabilitation clinics. Population-based controls were derived from the MONICA/KORA Augsburg survey F4 and the PopGen blood donor sample 2 (PopGen-BSP). GerMIFS III (KORA): cases of non-fatal MI were identified in the KORA registry with DNA available. Hospitalized

survivors of MI who are 26-74 years of age are routinely entered into this registry. The diagnosis of a MI was made with the use of the algorithm of the World Health Organization's Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) projects. Controls were from the population based Augsburg KORA S4/F4 study and PopGen. The GerMIFS IV cases consist of consecutive patients referred for coronary angiography, classified as CAD or MI cases based on the coronary angiogram (at least a 50% stenosis in one major coronary vessel) and age of onset (<65 years in males, and <70 years in females). Control samples were recruited as part of the Berlin Aging Study II (BASE-II), a multidisciplinary study investigating factors related to human ageing. All subjects were recruited from the Berlin metropolitan area and underwent an extensive phenotypic assessment, including a two-day medical examination. None of the BASE-II subjects included here reported a history of CAD or MI, nor showed any current signs of acute cardiovascular disease[19].

LURIC

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study consists of 3,316 Caucasian patients hospitalized for coronary angiography between 1997 and 2000 at a tertiary care center in Southwestern Germany. Clinical indications for angiography were chest pain or a positive non-invasive stress test suggestive of myocardial ischemia. To limit clinical heterogeneity, individuals suffering from acture illnesses other than acute coronary syndrome, chronic non-cardiac diseases and a history of malignancy within the past 5 years were excluded. In LURIC, CAD was defined as the presence of a visible luminal narrowing (>50% stenosis) in at least one of 15 coronary segments according to a classification of the American Heart Association. The study was approved by the ethics committee at the "Ärztekammer Rheinland-Pfalz" and was conducted in accordance with the "Declaration of Helsinki". Informed written consent was obtained from all participants[19].

OHGS A2, B2, C2

The Ottawa Heart Genomics study. Cases had at least one of myocardial infarction, coronary artery bypass graft, percutaneous intervention or a stenosis of at least 50% in at least one epicardial vessel. Diabetic cases and cases aged greater than 55 for men or 65 for women were excluded. Controls were either asymptomatic for cardiovascular disease or had had a CTA or angiogram demonstrating no stenosis of greater than 50%. Controls were required to be at least 65 years old for men and 70 years old for women at the time of recruitment[19].

THISEAS

The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility recruited from three hospitals in the area of Athens (Greece). Cases were subjects with a first-ever

MI before age of 70 years presenting with either ACS or stable CAD defined as >50% stenosis in at least one of the three main coronary vessels assessed by coronary angiography. ACS was defined as acute MI or unstable angina corresponding to class III of the Braunwald classification. ACS patients have also undergone coronary angiography examination that verified the presence of significant stenosis. Controls were subjects age matched without MI/CAD history with negative coronary angiography findings (<30% stenosis), or negative stress test, or subjects without symptoms of disease that were admitted at the same hospitals as cases and were free of any cardiovascular disease, cancer, or inflammatory diseases. Subjects with renal or hepatic disease were excluded from both study groups[19].

LIFE-Heart

LIFE-Heart is an observational study that recruits patients undergoing first-time diagnostic coronary angiography due to suspected stable CAD with previously untreated coronary arteries, patients with stable left main coronary artery disease and patients with acute myocardial infarction. For the present study, we defined CAD cases as luminal reduction of >50% in any vessel. Normal angiograms were considered as controls. Cases of myocardial infarction were defined as the subset of CAD cases with either anamnestic MI, acute MI (requiring revascularization via percutaneous coronary intervention) or elevated troponine levels. MI controls were defined as normal angiograms with neither the above mentioned criteria of MI. The study meets the ethical standards or the Declaration of Helsinki. It has been approved by the Ethics Committee of the Medical Faculty of the University of Leipzig, Germany (Reg. No 276-2005) and is registered at ClinicalTrials.gov (NCT00497887). Written informed consent including agreement with genetic analyses was obtained from all participants[19].

ITH 2

The INTERHEART study uses worldwide cases and controls of European ethnicity. Incident acute MI, presenting to a hospital within 24 hours of symptom onset. Age and sex matched hospital and community based, with no previous diagnosis of heart disease or history of exertional chest pain[19].

Appendix 2

Table S1. Estimates of the association between age at natural menopause and cardiovascular risk factors. Results for individual studies and the pooled cohorts.

	Women	Men	Sex-combined		
	EPIC-CVD	EPIC-CVD	EPIC-CVD	Publicly available data*	Pooled
			Total cholesterol (mmol/L)		
Simple median	-0.022 (-0.061;0.017)	0.029 (-0.011;0.068)	0.017 (-0.013;0.047)	0.000 (-0.014;0.013)	0.005 (-0.007;0.017)
Weighted median	-0.059 (-0.098;-0.021)	0.029 (-0.010;0.069)	-0.020 (-0.051;0.010)	0.001 (-0.012;0.015)	0.003 (-0.010;0.015)
IVW	-0.027 (-0.055;0.000)	0.024 (-0.002;0.050)	-0.008 (-0.030;0.014)	0.007 (-0.006;0.020)	0.004 (-0.008;0.016)
MR-Egger	-0.066 (-0.122;0.009)	0.013 (-0.041;0.066)	-0.035 (-0.081;0.010)	0.008 (-0.019;0.036)	0.001 (-0.025;0.026)
			HDL cholesterol (mmol/L)		
Simple median	0.004 (-0.010;0.018)	0.006 (-0.006;0.018)	0.002 (-0.008;0.012)	-0.002 (-0.016;0.012)	-0.004 (-0.012;0.004)
Weighted median	-0.010 (-0.024;0.004)	0.005 (-0.007;0.017)	-0.006 (-0.016;0.004)	0.010 (-0.004;0.024)	-0.003 (-0.010;0.005)
IVW	-0.006 (-0.015;0.003)	0.004 (-0.004;0.012)	-0.002 (-0.010;0.005)	0.008 (-0.006;0.022)	0.002 (-0.007;0.010)
MR-Egger	-0.024 (-0.042;-0.005)	-0.002 (-0.019;0.015)	-0.015 (-0.030;0.001)	0.008 (-0.023;0.039)	-0.006 (-0.023;0.011)
			Triglycerides (mmol/L)		
Simple median	-0.003 (-0.030;0.023)	0.024 (-0.018;0.066)	0.015 (-0.010;0.039)	0.000 (-0.014;0.013)	0.007 (-0.004;0.019)
Weighted median	-0.004 (-0.030;0.023)	0.035 (-0.008;0.078)	0.024 (0.000;0.048)	0.001 (-0.012;0.015)	0.005 (-0.006;0.016)

Table S1. Continued

	Women	Men	Sex-combined		
	EPIC-CVD	EPIC-CVD	EPIC-CVD	Publicly available data*	Pooled
IVW	0.004 (-0.014;0.022)	0.035 (0.007;0.064)	0.018 (0.000;0.037)	0.007 (-0.006;0.020)	0.002 (-0.014;0.018)
MR-Egger	0.003 (-0.034;0.040)	0.053 (-0.007;0.112)	0.024 (-0.014;0.063)	0.008 (-0.019;0.036)	0.007 (-0.028;0.041)
			Apolipoprotein A1 (g/L)		
Simple median	0.000 (-0.011;0.011)	0.007 (-0.003;0.018)	0.001 (-0.007;0.009)	/	/
Weighted median	-0.008 (-0.019;0.002)	0.010 (0.000;0.020)	-0.004 (-0.011;0.004)	/	/
IVW	-0.006 (-0.013;0.002)	0.007 (0.001;0.014)	-0.001 (-0.006;0.005)	/	/
MR-Egger	-0.022 (-0.036;-0.007)	0.007 (-0.007;0.021)	-0.009 (-0.021;0.002)	/	/
			Apolipoprotein B (g/L)		
Simple median	-0.001 (-0.010;0.008)	0.007 (-0.003;0.017)	0.003 (-0.005;0.010)	/	/
Weighted median	-0.009 (-0.017;0.000)	0.000 (-0.009;0.010)	-0.004 (-0.011;0.003)	/	/
IVW	-0.003 (-0.009;0.003)	0.004 (-0.002;0.011)	0.000 (-0.005;0.005)	/	/
MR-Egger	-0.012 (-0.024;0.001)	0.002 (-0.012;0.016)	-0.006 (-0.016;0.005)	/	/
			C-reactive protein (mg/L)		

Table S1. Continued

Simple median	0.074 (-0.077;0.226)	0.132 (-0.044;0.308)	0.058 (-0.057;0.173)	/	/
Weighted median	0.069 (-0.082;0.219)	0.006 (-0.168;0.179)	0.055 (-0.058;0.168)	/	/
IVW	0.023 (-0.079;0.126)	0.075 (-0.044;0.194)	0.046 (-0.036;0.127)	/	/
MR-Egger	0.052 (-0.163;0.268)	0.006 (-0.241;0.254)	0.034 (-0.136;0.205)	/	/
			Glucose (mmol/L)		
Simple median	0.024 (-0.030;0.077)	0.059 (-0.011;0.129)	0.024 (-0.021;0.070)	-0.002 (-0.011;0.007)	0.001 (-0.008;0.010)
Weighted median	0.005 (-0.048;0.059)	0.014 (-0.054;0.083)	0.012 (-0.034;0.057)	-0.003 (-0.011;0.005)	-0.002 (-0.010;0.005)
IVW	0.018 (-0.015;0.052)	0.027 (-0.018;0.072)	0.023 (-0.008;0.054)	0.002 (-0.004;0.008)	0.003 (-0.003;0.008)
MR-Egger	-0.022 (-0.091;0.048)	-0.012 (-0.105;0.081)	-0.017 (-0.081;0.047)	-0.001 (-0.012;0.010)	-0.002 (-0.012;0.009)
			HbA1c (%)		
Simple median	-0.004 (-0.024;0.018)	0.014 (-0.011;0.040)	0.008 (-0.008;0.023)	-0.001 (-0.011;0.009)	0.005 (-0.004;0.013)
Weighted median	-0.003 (-0.023;0.018)	0.014 (-0.011;0.039)	0.003 (-0.013;0.018)	-0.003 (-0.012;0.007)	0.003 (-0.006;0.011)
IVW	-0.002 (-0.015;0.011)	0.015 (-0.001;0.031)	0.005 (-0.006;0.016)	0.000 (-0.007;0.007)	0.001 (-0.005;0.008)
MR-Egger	0.000 (-0.028;0.029)	0.006 (-0.027;0.040)	0.002 (-0.021;0.026)	0.004 (-0.012;0.020)	0.004 (-0.011;0.018)

CHAPTER 2.4

Vasomotor menopausal symptoms and cardiovascular disease risk in midlife: a longitudinal study

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Submitted

Abstract

Background: Vasomotor menopausal symptoms, i.e. hot flushes and night sweats, have been associated with cardiovascular risk factors and intermediate cardiovascular outcomes. However, the limited studies on clinical cardiovascular endpoints show contradictory results.

Objective: To examine the association between vasomotor menopausal symptoms, hot flushes and night sweats and cardiovascular disease, coronary heart disease and cerebrovascular disease

Study Design: We used data from the 1946-51 cohort from the Australian Longitudinal Study on Women's Health, a national prospective cohort study. These women were 45-50 years at baseline in 1996 and 7,112 with available hospital separation data were included in this analysis. First fatal or non-fatal cardiovascular disease, coronary heart disease, and cerebrovascular disease events were obtained through linkage with hospital admission data, the National Death Index, and Medicare Benefits Schedule. Hot flushes and night sweats were assessed via questionnaires at each main survey and a vasomotor menopausal symptoms variable was created from these responses. Additionally, we calculated the duration of symptoms based on whether or not women reported vasomotor menopausal symptoms in each survey.

Results: There were 946 cardiovascular disease, 502 coronary heart disease and 152 cerebrovascular disease events. There was no consistent evidence of any association with vasomotor menopausal symptoms, hot flushes and night sweats. We did find marginally statistically significant associations between presence of night sweats and cardiovascular disease (Hazard Ratio = 1.16, 95% Confidence Interval: 1.00-1.35), and between the duration of vasomotor menopausal symptoms [years] and coronary heart disease (Hazard Ratio$_{\text{per year}}$ = 1.03, 95%Confidence Interval: 1.00-1.05).

Conclusion: In this large longitudinal study with 20 years of follow-up and clinical outcomes we did not find a convincing association between vasomotor menopausal symptoms, hot flushes, night sweats and cardiovascular disease, coronary heart disease and cerebrovascular disease.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in women from high income countries[1]. CVD accounted for almost 30% of all deaths in Australian women in 2015[2]. Recently, research on CVD has shifted towards a more sex-specific focus since there are marked sex-differences in the development and progression of CVD and its risk factors[3].

A recent review showed an association between hot flushes (HF) and night sweats (NS) during menopausal transition and CVD risk factors: higher systolic and diastolic blood pressure, higher circulating total cholesterol levels and higher Body Mass Index (BMI) compared to women without these vasomotor menopausal symptoms (VMS)[4]. Furthermore, several studies also found associations between VMS and intermediate CVD outcomes: lower flow mediated dilation, higher aortic calcification, higher carotid intima media thickness and plaque[5–8], which in turn are prospectively associated with incident CVD. These results indicate that HF and NS might increase CVD risk. However, only a few studies investigated the association between HF, NS or VMS with hard CVD endpoints[9–13]. These studies show contradictory results, varying between less CVD risk, more CVD risk and no association at all. Furthermore, most studies only study VMS as a whole and they all suggest that duration of symptoms should be further investigated in relation to CVD.

Although an association between VMS with cardiovascular risk factors and intermediate endpoints seems clear, the association with clinical outcomes needs further investigation, in particular with respect duration of menopausal symptoms and individual types of symptoms (i.e. HF and NS). Therefore, the aim of the current study was to examine the association between VMS (and HF and NS separately) and the occurrence and duration of each type of symptom with fatal and non-fatal CVD, CHD, and cerebrovascular disease.

Materials and Methods

Study population

Details of the Australian Longitudinal Study on Women's Health (ALSWH) were described previously[14,15] and are available at www.alswh.org.au. In brief, ALSWH is national prospective cohort study that recruited a nationally representative sample of over 40,000 women in 1996. Three age groups were sampled: 18-23 (1973-78 cohort), 45-50 (1946-51 cohort), and 70-75 (1921-26 cohort). All women were sampled randomly from the national health insurance scheme (Medicare) that includes all Australian citizens and permanent residents. Women from rural and remote areas were intentionally oversampled[14,15].

For this study we used data from the 1946-51 cohort who have been surveyed every 2-3 years since the start of ALSWH. At baseline (1996) 13,715 women agreed to participate. At Survey 2 13,499 women were still eligible (i.e., they could be located, and had not withdrawn or died) and 12,338 responded (91.7%). The corresponding numbers for successive surveys were: at Survey

3 13,149 women were eligible and 11,226 responded (85.4%); at Survey 4 12,843 women were eligible and 10,905 responded (84.9%); at Survey 5 12,466 women were eligible and 10,638 responded (85.3%); at Survey 6 12,063 women were eligible and 10,011 responded (83.0%); at Survey 7 11,291 women were eligible and 9,151 responded (81.0%); and at Survey 8 (2016) 10,719 women were eligible and 8,622 responded (80.4%).

Of the 13,715 women at baseline, we included 7,112 women in the final analysis sample, because we only had hospital data for women living in the Australia Capital Territory, New South Wales, Queensland, South Australia and Western Australia. Furthermore, 601 women opted out from linkage in the Australia Capital Territory and New South Wales, 804 in Queensland and 753 in West Australia. In South Australia an explicit consent was used, which resulted in a higher rate of 'opt out' of 4460. All participants gave written informed consent and the study was approved by the Human Research Ethics Committee of the University of Newcastle and the University of Queensland.

Cardiovascular disease, coronary heart disease and cerebrovascular disease

First fatal or non-fatal CVD, CHD and cerebrovascular disease events were obtained through linkage with hospital separations data, the National Death Index (NDI) and Medicare Benefits Schedule (MBS) databases. If participants withdrew from the ALSWH study, linked data remained available unless the participant had opted out of linkage. Time to event was calculated from the return date of Survey 1 (baseline) until the first event, and death from any other cause or the end of availability of hospital data in the last known State of residence was used as the censoring date. Hospital data were available from May 2001 until March 2017 for New South Wales, from March 2002 until December 2011 for Queensland, from January 1970 until December 2015 for West Australia, from July 2004 until June 2013 for Australian Capital Territory and from January 2002 until December 2010 for South Australia. Causes of death data were available until October 2015. If a woman had an event or if her observation was censored, survey data collected after this event was not used in the analysis.

The hospital data were coded by the Australian version of the International Classification of Diseases (ICD-10-AM) for the diagnoses and the Australian Classification of Health Interventions (ACHI) for procedures. Causes of death were also coded according to ICD-10-AM. From the MBS database we used MBS-specific codes for the following procedures: percutaneous transluminal balloon angioplasty or bypass of coronary arteries (to define CHD events), endarterectomy of neck arteries (to define cerebrovascular disease) and procedures performed for peripheral arterial occlusive disease or abdominal aneurysm for CVD. All CHD and cerebrovascular disease events were also counted as CVD events. All relevant ICD, ACHI and MBS codes can be found in Appendix 1.

Vasomotor symptoms of menopause

Both HF and NS were assessed via a self-reported questionnaire at each ALSWH survey. Women responded to the questions: 'In the last 12 months, have you had any of the following: 1) hot flushes, 2) night sweats?' Response options were 'never', 'rarely', 'sometimes', or 'often'. For the occurrence of symptoms we dichotomized the responses into 'no', including 'never' and 'rarely', and 'yes, including 'sometimes' and 'often'. Additionally, we created a VMS variable, combining HF and NS, which was defined 'yes' when either HF or NS was 'yes'.

We also calculated a crude measure of duration of symptoms from the responses to the survey questionnaires that went out every 2-3 years. For example, the duration for HF was based on the dichotomous variables. Duration at Survey 1: if women reported presence of HF at Survey 1, we assumed the duration to be one year; while for women who did not report HF at Survey 1 the duration of symptoms was set to zero years. Duration at Survey 2: if women answered 'No' for HF at Survey 2, we defined the duration as that of Survey 1. If women answered 'Yes' at Survey 2 and duration at Survey 1 was 0 years, we defined the duration as being 1year. If women answered 'Yes' at Survey 2 and duration at Survey 1 was 1 year, we defined the duration as being 3 years (addition of 2 years, since there were 2 years between Survey 1 and Survey 2). Duration at Survey 3: if women answered 'No' for HF at Survey 3, we defined the duration as that of Survey 2. If women answered 'Yes' at Survey 3 and duration at Survey 2 was 0 years, we defined the duration as being 1 year. If women answered 'Yes' at Survey 3 and duration at Survey 2 was 1 year, we defined the duration as being 4 years (addition of 3 years, since there were 3 years between Survey 2 and Survey 3). If women answered 'Yes' at Survey 3 and duration at Survey 2 was 3 years, we defined the duration as being 6 years. This was continued until Survey 8 and was calculated in the same way for NS and VMS.

Covariates

Education level was determined at Survey 1 and categorised as: low (no formal qualifications or school certificate), middle (higher school certificate, trade/apprenticeships, or certificate or diploma), or high (university degree or higher degree). Other covariates were ascertained at each ALSWH survey. BMI was defined as self-reported weight (kg) divided by the square of self-reported height (m^2) and was categorised as underweight (<18.5 kg/m^2), healthy weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) or obese (≥30 kg/m^2). Physical activity data were only consistently measured from Survey 3 onwards and categorised as nil/sedentary (<0-10 minutes/week), low (11-150 minutes/week), moderate (151-300 minutes/week), or high (>300 minutes/week). Smoking was categorised as never smoker, former smoker or current smoker. Alcohol consumption was categorised in line with the Australian National Health and Medical Research Council (NHMRC) guidelines as non-drinker, rarely drinks (alcohol consumption less than once a month), low risk

(up to two drinks a day), or risky drinker (three or more drinks a day)[16]. Finally, the women were asked if they were currently using menopausal hormone therapy (MHT) in each survey.

Statistical analysis

Baseline characteristics were described by percentage (number, N) or mean (standard deviation, SD) across categories of HF and NS. We used two sets of Cox regression models, stratified by State of residence (due to different follow-up times), to investigate the associations between: (i) time varying occurrence of VMS, HF, NS, and (ii) duration of these symptoms, and the times to CVD, CHD and cerebrovascular disease events, or censoring. Robust standard errors were used to construct 95% confidence intervals (CI). We applied four levels of covariate adjustment: adjustment for baseline age (Model 1), further adjustment for time-varying MHT use (Model 2), further adjustment for well-known cardiovascular risk factors: education level, time-varying physical activity, time-varying smoking status and time-varying alcohol status (Model 3), and further adjusted for time-varying BMI (Model 4). As a sensitivity analysis we also investigated the association between HF and NS with four response categories (never [reference category], rarely, sometimes, often) with the various outcomes. These analyses were performed with R version 3.4.2[17]. The main results are based on an available case analysis. We performed a sensitivity analyses with multiple imputed data, methods and results can be found in Appendix 2.

Results

At baseline 4440 women never had HF or NS, 861 women only had HF, 250 only had NS and 1498 had both HF and NS (at this survey, data on VMS were missing for 63 women who provided data a later surveys). Baseline characteristics showed that women with HF had less education, were more likely to use MHT, to be current smokers, risky drinkers and obese, compared to women without HF (Table 1). The same differences were found between women with and without NS. The baseline characteristics were comparable between women with HF or NS or without HF or NS.

Table 1. Baseline (1996) characteristics of ALSWH participants born in 1946-51who had hospital data available, stratified by reporting of hot flushes and night sweats at baseline

Characteristics	Hot flushes (N) N = 4693	Hot flushes (Y) N = 2372	Night Sweats (N) N = 5310	Night Sweats (Y) N = 1752
Age[a]	47.5 (1.5)	47.8 (1.4)	47.5 (1.5)	47.9 (1.4)
MHT use (yes)	15.5% (727)	31.1% (736)	17.2% (912)	31.2% (545)
Education level	49.0% (2284)	62.4% (1460)	51.2% (2694)	60.5% (1047)
Low	36.2% (1685)	29.7% (696)	35.0% (1845)	30.9% (535)
Middle	14.8% (690)	7.9% (185)	13.8% (726)	8.7% (150)
High				

Table 1. Continued

Characteristics	Hot flushes (N) N = 4693	Hot flushes (Y) N = 2372	Night Sweats (N) N = 5310	Night Sweats (Y) N = 1752
Physical activity[b]	17.2% (661)	22.0% (403)	17.8% (771)	21.9% (291)
Nil/Sedentary	37.6% (1442)	37.3% (683)	38.0% (1645)	36.3% (482)
Low	20.3% (780)	17.4% (318)	20.0% (866)	17.3% (230)
Moderate	24.8% (950)	23.3% (427)	24.3% (1051)	24.4% (324)
High				
Smoking status	54.8% (2498)	48.3% (1109)	55.2% (2846)	44.7% (759)
Never smoker	29.4 % (1341)	28.6% (655)	28.7% (1477)	30.3% (514)
Former smoker	15.8% (721)	23.1% (530)	16.1% (831)	25.0% (425)
Current smoker				
Alcohol status	14.6% (681)	16.8% (395)	15.1% (799)	16.1% (278)
Non-drinker	31.3% (1461)	32.0% (752)	31.7% (1674)	30.9% (535)
Rarely drinks	49.2% (2291)	44.8% (1053)	48.4% (2556)	45.5% (786)
Low risk drinker	4.9% (228)	6.4% (151)	4.7% (250)	7.5% (130)
Risky drinker				
BMI	2.1% (94)	1.2% (28)	1.8% (93)	1.7% (28)
Underweight	53.7% (2426)	42.2% (959)	51.8% (2648)	44.0% (739)
Healthy weight	27.3% (1232)	32.6% (741)	28.4% (1451)	31.0% (521)
Overweight	17.0% (767)	24.0% (546)	18.0% (919)	23.3% (391)
Obese				

[a]*Mean (SD)*
[b]*Based on survey 3*

There were 946 CVD events, including 502 CHD events and 152 cerebrovascular events, over the 20 years of follow-up. Overall, we did not find an association between time-varying VMS (Hazard Ratio [HR]=1.05, 95%CI: 0.91-1.21, Table 2) or HF (HR=1.05, 95%CI: 0.91-1.22, Table 2) and CVD events. For NS we only found a marginally statistically significant association with CVD risk (HR=1.16, 95%CI: 1.00-1.35, Table 2). Also, the duration of symptoms was not associated with CVD events as shown in Table 3 ($HR_{per\ year}$ for HF duration = 1.00, 95%CI: 0.97-1.02; $HR_{per\ year}$ for NS duration = 1.00, 95%CI: 0.97-1.02; $HR_{per\ year}$ for VMS duration = 1.01, 95%CI: 0.99-1.03). Finally, the sensitivity analyses with four response categories for the symptoms showed no association with HF and CVD events and only a significant association for the 'often' category in the association between NS and CVD events (HR = 1.26, 95%CI: 1.02-1.55, Appendix 3, Table S3.1 and S3.2).

Table 2. Hazard Ratios for the association between time-varying vasomotor symptoms, hot flushes and night sweats and cardiovascular disease
N=7112 (events=946)

Model	VMS (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	1.12 (0.99-1.28)	1.12 (0.99-1.28)	1.23 (1.08-1.41)
Model 2**	1.12 (0.98-1.27)	1.12 (0.98-1.28)	1.23 (1.07-1.41)
Model 3***	1.06 (0.92-1.22)	1.06 (0.92-1.23)	1.19 (1.03-1.38)
Model 4****	1.05 (0.91-1.21)	1.05 (0.91-1.22)	1.16 (1.00-1.35)

For each symptom the 'No' group is the reference group.
**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for MHT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table 3. Hazard Ratios for the association between time-varying duration of symptoms and cardiovascular disease
N=7112 (events=946)

Model	VMS duration	HF duration	NS duration
Model 1*	1.01 (0.99-1.03)	1.00 (0.98-1.02)	0.99 (0.97-1.02)
Model 2**	1.01 (0.99-1.03)	1.00 (0.98-1.02)	0.99 (0.97-1.02)
Model 3***	1.01 (0.99-1.03)	1.00 (0.97-1.02)	1.00 (0.97-1.02)
Model 4****	1.01 (0.99-1.03)	1.00 (0.97-1.02)	1.00 (0.97-1.02)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for MHT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

We also did not find an association between VMS (HR=1.02, 95%CI: 0.84-1.24, Table 4), HF (HR=1.05, 95%CI: 0.86-1.28, Table 4) and NS (HR=1.03, 95%CI: 0.84-1.27, Table 4) and CHD events. The duration of VMS was marginally statistically significantly associated with CHD risk ($HR_{per\ year}$=1.03, 95%CI: 1.00-1.05, Table 5). However, the duration of neither HF ($HR_{per\ year}$=1.02, 95%CI: 0.99-1.06, Table 9) nor NS ($HR_{per\ year}$=1.02, 95%CI: 0.98-1.05) were statistically significantly associated with CHD risk. Lastly, the sensitivity analyses of HF and NS with four response categories showed no association with CHD risk (Appendix 3, Table S3.3 and S3.4).

Table 4. Hazard Ratios for the association between time-varying vasomotor symptoms, hot flushes and night sweats and coronary heart disease
N=7112 (events=502)

Model	VMS (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	1.15 (0.96-1.37)	1.15 (0.96-1.37)	1.18 (0.98-1.42)
Model 2**	1.15 (0.96-1.37)	1.15 (0.96-1.37)	1.18 (0.98-1.42)
Model 3***	1.04 (0.86-1.26)	1.06 (0.87-1.29)	1.07 (0.88-1.32)
Model 4****	1.02 (0.84-1.24)	1.05 (0.86-1.28)	1.03 (0.84-1.27)

For each symptom the 'No' group is the reference group
**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for MHT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table 5. Hazard Ratios for the association between time-varying duration of symptoms and coronary heart disease
N=7112 (events=502)

Model	VMS duration	HF duration	NS duration
Model 1*	1.03 (1.00-1.05)	1.02 (0.98-1.05)	1.02 (0.98-1.05)
Model 2**	1.03 (1.00-1.05)	1.02 (0.98-1.05)	1.02 (0.98-1.05)
Model 3***	1.03 (1.00-1.05)	1.02 (0.99-1.06)	1.02 (0.98-1.05)
Model 4****	1.03 (1.00-1.05)	1.02 (0.99-1.06)	1.02 (0.98-1.05)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for MHT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Finally, we studied the association between VMS, HF, NS and cerebrovascular disease, but we did not find an association (HR for VMS=0.96, 95%CI: 0.67-1.39, Table 6; HR for HF=0.93, 95%CI: 0.64-1.36, Table 6; HR for NS=1.34, 95%CI: 0.93-1.04, Table 6). In addition, duration of symptoms showed no association ($HR_{per\ year}$ for VMS duration=0.99, 95%CI: 0.96-1.03; $HR_{per\ year}$ for HF duration=0.98, 95%CI: 0.93-1.04; $HR_{per\ year}$ for NS duration=1.00, 95%CI: 0.95-1.06; Table 7). Lastly, the sensitivity analyses with HF in categories showed no association with cerebrovascular disease and the analyses with NS showed only a marginally statistically significant results for the 'often' group with a HR of 1.67 (95%CI: 1.00-2.80, Appendix 3, Table S3.5 and S3.6).

Table 6. Hazard Ratios for the association between time-varying vasomotor symptoms and cerebrovascular disease
N=7112 (events=152)

Model	VMS (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	0.96 (0.69-1.33)	0.95 (0.68-1.33)	1.31 (0.94-1.84)
Model 2**	0.95 (0.69-1.33)	0.95 (0.68-1.33)	1.31 (0.93-1.83)
Model 3***	0.96 (0.67-1.38)	0.93 (0.64-1.36)	1.34 (0.92-1.94)
Model 4****	0.96 (0.67-1.39)	0.93 (0.64-1.36)	1.34 (0.92-1.94)

For each symptom the 'No' group is the reference group
**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table 7. Hazard Ratios for the association between time-varying duration of symptoms and cerebrovascular disease
N=7112 (events=152)

Model	VMS duration	HF duration	NS duration
Model 1*	0.99 (0.95-1.03)	0.97 (0.92-1.03)	0.98 (0.93-1.04)
Model 2**	0.99 (0.95-1.03)	0.97 (0.92-1.03)	0.98 (0.93-1.04)
Model 3***	0.99 (0.95-1.03)	0.98 (0.93-1.04)	1.00 (0.95-1.06)
Model 4****	0.99 (0.96-1.03)	0.98 (0.93-1.04)	1.00 (0.95-1.06)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

As a sensitivity analyses we also performed all analyses, in the imputed data. This yielded approximately the same results (Appendix 2).

Discussion

In this longitudinal study with 20 years of follow-up and clinical outcomes we did not find an association between VMS, HF, NS and CVD, CHD or cerebrovascular disease. We did find a 16% increased CVD risk in women with NS compared to women without NS and a 3% increased CHD risk for every additional year a women experiences VMS. However, effect sizes are small, not clinically relevant, and only marginally statistically significant (lower 95% CIs of 1.00). Moreover, given the number of associations tested these findings could also very well be chance findings.

Only a few studies investigated the association between VMS, HF and/or NS and CVD, CHD and/or cerebrovascular diseases[9–13] and one study included a meta-analysis[18]. These studies found very divergent results, with some studies showing a decreased risk of CVD, CHD or stroke

for women experiencing VMS[11], some an increased risk[11–13], but often not statistically significant[9–12]. This may be unexpected, since studies that investigated the association between VMS and cardiovascular risk factors[4] or intermediate outcomes[6,7] convincingly show increased risk in women with VMS, suggesting a likely association with clinical endpoints. There could be several explanations for this discrepancy. For instance, the studies that investigated CVD risk factors or intermediate outcomes found only small effect sizes for their outcomes, which were almost always continuous. For studies with clinical outcomes, which are often dichotomous, the sample size would need to be much larger to have enough power to show a difference between women with and without VMS. The largest study comparable to our study in design, included 10,787 women of whom 303 developed CHD, and did not find any statistically significant associations with either HF or NS in the fully adjusted models[10]. The largest study on VMS included 60,027 women of whom 2,812 developed CVD, 1,726 developed CHD and 1226 developed stroke. In this study, in the fully adjusted models, a statistically significant association was only found between 'late VMS' and CVD and CHD, but not for 'early' or 'persistent (early and late)' VMS[11]. However, the definitions of 'early', 'late', and 'persistent' VMS were based on recall of VMS between menopause onset and enrolment in the study. Enrolment in the study was after menopause, which makes it very hard to compare the findings with our results.

Another explanation could be the large number of cross-sectional studies that investigated the association between VMS and cardiovascular risk factors. Since VMS have a temporary nature, it might be possible that the elevations in CVD risk factors are also temporary, making the association with CVD less obvious. In our study, we had repeated measurements of the VMS, which we modelled as time-varying variables, taking the temporary nature of VMS into account.

In addition to the inconsistent associations found in empirical studies, the physiological pathways are not fully understood. A possible mechanism suggested to underlie the putative association between VMS and CVD is the oestrogen decline during the menopausal transition. Oestrogen decline is often accompanied by the development of VMS and women experiencing severe VMS often use MHT to reduce the symptoms. The decline in oestrogen is also suggested to be associated with an increased CVD risk[19,20], which might be counteracted by MHT use. However, recent meta-analyses of clinical trials with MHT found no associations for cardiac or stroke mortality[21], or even an increased CHD risk[22].

Strengths of this study are the long follow-up, the repeated measurements of a large number of covariates and the multiple-registry approach to identify cases. However, some limitations need to be acknowledged. First, we had to make some assumptions to calculate the duration of symptoms. We assumed that, if a woman had HF or NS at one survey and again on the next survey, she had a duration of two or three years, depending on the time between surveys. However, it is possible that women had symptoms that went away and came back again. This might have led

to some misclassification, because some women might be classified with a longer duration than they really had. Therefore, the results with duration might entail an underrepresentation of the real effect. Second, for some States hospital data were not available before baseline, so we could not exclude prevalent cases. However, as this number would be expected to be very small since women were only 45-50 years old at baseline we do not think that this has influenced the results. Third, we did not exclude women using MHT. However, women using MHT probably reflect the group with the most severe symptoms; therefore, excluding them might give a false representation of symptom variety. On the other hand, including them could introduce misclassification. Women with severe symptoms often use MHT to reduce their symptoms, which might result in them filling in 'No' on the VMS question. We tried to avoid this by asking for symptoms in the past 12 months, but still an average of 16.4% of women in each survey reported not having VMS while using MHT. This could result in an underestimation of the effect size.

In conclusion, in this longitudinal study with long follow-up and clinical outcomes we did not find a convincing association between VMS, HF, NS and CVD, CHD and cerebrovascular disease.

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References

1 Murray CJL, Barber RM, Foreman KJ, *et al.* Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: Quantifying the epidemiological transition. *Lancet* 2015;**386**:2145–91. doi:10.1016/S0140-6736(15)61340-X

2 Statistics AB of. Causes of Death, Australia, 2016. Canberra: 2016.

3 Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovascular Disease. *Physiol Rev* 2017;**97**:1–37. doi:10.1152/physrev.00021.2015

4 Franco OH, Muka T, Colpani V, *et al.* Vasomotor symptoms in women and cardiovascular risk markers: Systematic review and meta-analysis. *Maturitas* 2015;**81**:353–61. doi:10.1016/j.maturitas.2015.04.016

5 Thurston RC, Sutton-Tyrrell K, Everson-Rose SA, *et al.* Hot flashes and subclinical cardiovascular disease: Findings from the Study of Women's Health Across the Nation Heart Study. *Circulation* 2008;**118**:1234–40. doi:10.1161/CIRCULATIONAHA.108.776823

6 Thurston RC, Kuller LH, Edmundowicz D, *et al.* History of hot flashes and aortic calcification among postmenopausal women. *Menopause* 2010;**17**:256–61. doi:10.1097/gme.0b013e3181c1ad3d

7 Thurston RC, Chang Y, Barinas-Mitchell E, *et al.* Menopausal Hot Flashes and Carotid Intima Media Thickness among Midlife Women. *Stroke* 2016;**47**:2910–5. doi:10.1161/STROKEAHA.116.014674

8 Thurston RC, El Khoudary SR, Tepper PG, *et al.* Trajectories of Vasomotor Symptoms and Carotid Intima Media Thickness in the Study of Women's Health Across the Nation. *Stroke* 2016;**47**:12–7. doi:10.1161/STROKEAHA.115.010600

9 Svartberg J, von Mühlen D, Kritz-Silverstein D, *et al.* Vasomotor symptoms and mortality. *Menopause* 2009;**16**:888–91. doi:10.1097/gme.0b013e3181a4866b

10 Gast G-CM, Pop VJM, Samsioe GN, *et al.* Vasomotor menopausal symptoms are associated with increased risk of coronary heart disease. *Menopause* 2011;**18**:146–51. doi:10.1097/gme.0b013e3181f464fb

11 Szmuilowicz ED, Manson JE, Rossouw JE, *et al.* VAsomotor symptoms and vascular events in postmenopausal women. *Menopause* 2012;**18**:603–10. doi:10.1097/gme.0b013e3182014849. Vasomotor

12 Herber-Gast GCM, Brown WJ, Mishra GD. Hot flushes and night sweats are associated with coronary heart disease risk in midlife: A longitudinal study. *BJOG An Int J Obstet Gynaecol* 2015;**122**:1560–7. doi:10.1111/1471-0528.13163

13 Thurston RC, Johnson BD, Shufelt CL, *et al.* Menopausal symptoms and cardiovascular disease mortality in the Women's Ischemia Syndrome Evaluation (WISE). *Menopause* 2017;**24**:126–32. doi:10.1097/GME.0000000000000731

14 Lee C, Dobson AJ, Brown WJ, *et al.* Cohort Profile: The Australian longitudinal study on Women's Health. *Int J Epidemiol* 2005;**34**:987–91. doi:10.1093/ije/dyi098

15 Dobson AJ, Hockey R, Brown WJ, *et al.* Cohort profile update: Australian longitudinal study on women's health. *Int J Epidemiol* 2015;**44**:1547. doi:10.1093/ije/dyv110

16 Council AGNH and MR. Australina Alcohol Guidelines: Health Risks and Benefits. 2001.

17 Team RC. R: A language and environment for statistical computing. 2016.https://www.r-project.org/

18 Muka T, Oliver-Williams C, Colpani V, *et al.* Association of vasomotor and other menopausal symptoms with risk of cardiovascular disease: A systematic review and meta-analysis. *PLoS One* 2016;**11**:1–15. doi:10.1371/journal.pone.0157417

19 Guthrie JR, Dennerstein L, Hopper JL, *et al.* Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstet Gynecol* 1996;**88**:437–42. doi:10.1016/0029-7844(96)00196-2

20 Rossouw JE, Prentice RL, Manson JE, *et al.* and Risk of Cardiovascular Disease by Age and Years Since Menopause. *J Am Med Assoc* 2007;**297**:1465–78.

21 Benkhadra K, Mohammed K, Al Nofal A, *et al.* Menopausal Hormone Therapy and Mortality: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab* 2015;**100**:4021–8. doi:10.1210/jc.2015-2238

22 Beral V, Banks E, Reeves G. Evidence from randomised trials on the long-term effects of hormone replacement therapy. *Lancet* 2002;**360**:942–4. doi:10.1016/s0140-6736(02)11032-4

23 Welch C, Bartlett J, Petersen I. Application of multiple imputation using the two-fold fully conditional specification algorithm in longitudinal clinical data. *Stata J* 2014;**14**:418–31. doi:The Stata Journal

24 Welch CA, Petersen I, Bartlett JW, *et al.* Evaluation of two-fold fully conditional specification multiple imputation for longitudinal electronic health record data. *Stat Med* 2014;**33**:3725–37. doi:10.1002/sim.6184

25 StataCorp. Stata Statistical Software: Release 14. 2015.

Appendix 1

Table A.1. Codes used for ascertainment of outcomes

Outcome	Coding	
CVD	ICD-10-AM	I11, I12, I13, I12 to I27, I46.1, I46.9, I48 to I50, I51.6, I51.9, I60, I61, I62.1, I62.9, I63 to I72,
		I73.9, I74, I77.9
	ICD-10-ACHI	as for IHD and cerebrovascular disease + 32739-00, 32742-00, 33115-00, 33118-00, 35303-06/07, 35307-00/01, 35309-06/07
	MBS codes	as for IHD and cerebrovascular disease + 32712, 32739, 32742, 32748, 32751, 32754, 32757, 32703, 32721, 32724, 32730, 32733, 32736, 32708, 32710, 32711, 32715, 32718, 32760, 32763, 32766, 3276933506, 33509, 33512 , 33515, 33518, 33521, 33524, 33527, 33530, 33533, 33536, 33539, 33543, 33545, 33548, 33551, 33554, 33800, 33806, 35100, 34103, 33080, 33112, 33115, 33116, 33118, 33119, 33121, 33151, 33154, 33157, 33160, 35303, 35306, 35307, 35309
CHD	ICD-10-AM	I20 to I25
	ICD-10-ACHI	38300-00, 38303-00, 38306-00/01/02, 38497-00/01/02/03, 38500-00/01/02, 38503-00/01/02
	MBS codes	38300, 38303, 38306, 38315, 38315, 38318, 38497, 38498, 38500, 38501, 38503, 38504
Cerebro-vascular	ICD-10-AM	I60 to I69
Disease	ICD-10-ACHI	33500-00
	MBS codes	33500, 32700, 32703

ICD-10-ACHI codes are described according to ICD-10-ACHI edition 8. MBS coding can be found at http://www.mbsonline.gov.au.

Appendix 2

Methods for multiple imputation

Missing values in the survey data ranged from 0.2% to 40.5%, mainly due to Survey 3, in which questions were slightly differently asked compared to the other surveys. As a sensitivity analysis we also performed multiple imputation using the two-fold fully conditional specification algorithm [23,24], with 10 imputations, 20 among-time iterations and 10 within-time iterations. We used a time window of two, so measurements recorded at t-2 until t+2 were included in the imputations for time t. If a woman was lost to follow-up, the two-fold algorithm replaces imputed values falling outside her follow-up period with missing values. In order to reach convergence in the imputation, we had to dichotomize all the time-varying covariates. For physical activity we combined 'nil/ sedentary' with 'low' and 'moderate' with 'high', for alcohol use we combined 'non-drinker' with 'rarely drinks' and 'low risk' with 'risky drinker', for smoking we combined 'never' with 'former' and for BMI we combined 'underweight' with 'normal weight' and 'overweight' with 'obese'. Additionally, we had to include indicator variables for 'missing' categories for physical activity and alcohol. These missing value indicator categories were not used in the available case analysis. Imputation was performed with Stata version 14.0 [25].

Results for multiple imputation

Table S2.1. Hazard Ratio's for the association between time-varying hot flushes and cardiovascular disease, **all states combined, strata(state) as variable in model**
No = Never/Rarely, Yes = Sometimes/Often

Model	VSM (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	1.10 (0.96-1.26)	1.11 (0.97-1.27)	1.20 (1.04-1.38)
Model 2**	1.10 (0.96-1.25)	1.11 (0.97-1.27)	1.20 (1.04-1.38)
Model 3***	1.04 (0.91-1.19)	1.06 (0.92-1.21)	1.14 (0.99-1.31)
Model 4****	1.03 (0.90-1.18)	1.04 (0.91-1.19)	1.13 (0.98-1.30)

For each symptom the 'No' group is the reference group
**Model 1 adjusted for age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S2.2. Hazard Ratio's for the association between time-varying duration of symptoms and cardiovascular disease, **all states combined, strata(state) as variable in model**

Model	VSM duration	HF duration	NS duration
Model 1*	1.01 (1.00-1.03)	1.00 (0.98-1.02)	1.00 (0.97-1.02)
Model 2**	1.01 (1.00-1.03)	1.00 (0.98-1.02)	1.00 (0.97-1.02)
Model 3***	1.02 (1.00-1.03)	1.01 (0.98-1.03)	1.00 (0.98-1.03)
Model 4****	1.01 (1.00-1.03)	1.00 (0.98-1.03)	1.00 (0.98-1.03)

**Model 1 adjusted for age (time-varying)*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S2.3. Hazard Ratio's for the association between time-varying hot flushes and coronary heart disease, **all states combined, strata(state) as variable in model**
No = Never/Rarely, Yes = Sometimes/Often

Model	VSM (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	1.09 (0.91-1.31)	1.11 (0.92-1.33)	1.11 (0.92-1.34)
Model 2**	1.09 (0.91-1.31)	1.11 (0.92-1.33)	1.11 (0.92-1.34)
Model 3***	1.04 (0.87-1.25)	1.06 (0.88-1.28)	1.07 (0.88-1.29)
Model 4****	1.03 (0.85-1.23)	1.05 (0.87-1.26)	1.05 (0.87-1.27)

For each symptom the 'No' group is the reference group
**Model 1 adjusted for age (time-varying)*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S2.4. Hazard Ratio's for the association between time-varying duration of symptoms and coronary heart disease, **all states combined, strata(state) as variable in model**

Model	HF duration	NS duration	VSM duration
Model 1*	1.02 (0.99-1.05)	1.00 (0.97-1.04)	1.03 (1.01-1.05)
Model 2**	1.02 (0.99-1.05)	1.00 (0.97-1.04)	1.03 (1.01-1.05)
Model 3***	1.03 (0.99-1.06)	1.01 (0.98-1.05)	1.03 (1.00-1.06)
Model 4****	1.02 (0.99-1.06)	1.01 (0.98-1.04)	1.03 (1.01-1.05)

**Model 1 adjusted for age (time-varying)*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S2.5. Hazard Ratio's for the association between time-varying hot flushes and cerebrovascular disease, **all states combined, strata(state) as variable in model**
No = Never/Rarely, Yes = Sometimes/Often

Model	VSM (Yes)	Hot flushes (Yes)	Night sweats (Yes)
Model 1*	1.01 (0.73-1.40)	0.99 (0.71-1.39)	1.31 (0.94-1.84)
Model 2**	1.00 (0.72-1.39)	0.99 (0.71-1.38)	1.30 (0.93-1.83)
Model 3***	0.94 (0.68-1.32)	0.93 (0.66-1.31)	1.23 (0.87-1.73)
Model 4****	0.95 (0.68-1.33)	0.94 (0.67-1.32)	1.24 (0.88-1.75)

For each symptom the 'No' group is the reference group
**Model 1 adjusted for age (time-varying)*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S2.6. Hazard Ratio's for the association between time-varying duration of symptoms and cerebrovascular disease, **all states combined, strata(state) as variable in model**

Model	VSM duration	HF duration	NS duration
Model 1*	0.99 (0.96-1.03)	0.98 (0.92-1.04)	0.98 (0.93-1.04)
Model 2**	0.99 (0.96-1.03)	0.98 (0.92-1.04)	0.98 (0.93-1.04)
Model 3***	1.00 (0.96-1.04)	0.98 (0.93-1.04)	0.99 (0.93-1.05)
Model 4****	1.00 (0.96-1.04)	0.98 (0.93-1.05)	0.99 (0.93-1.05)

**Model 1 adjusted for age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Appendix 3

Table S3.1. Hazard Ratios for the association between time-varying hot flushes and cardiovascular disease N=7112 (events=946)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.87 (0.70-1.06)	1.01 (0.85-1.19)	1.20 (1.00-1.43)
Model 2**	Ref.	0.86 (0.71-1.06)	1.01 (0.85-1.18)	1.20 (1.00-1.43)
Model 3***	Ref.	0.84 (0.67-1.04)	0.96 (0.80-1.14)	1.11 (0.91-1.35)
Model 4****	Ref.	0.84 (0.67-1.04)	0.95 (0.79-1.13)	1.10 (0.90-1.34)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S3.2. Hazard Ratios for the association between time-varying night sweats and cardiovascular disease N=7112 (events=946)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.94 (0.77-1.15)	1.13 (0.95-1.33)	1.36 (1.13-1.65)
Model 2**	Ref.	0.94 (0.77-1.15)	1.13 (0.95-1.33)	1.36 (1.13-1.65)
Model 3***	Ref.	0.87 (0.71-1.09)	1.08 (0.90-1.30)	1.29 (1.05-1.59)
Model 4****	Ref.	0.88 (0.70-1.09)	1.06 (0.88-1.27)	1.26 (1.02-1.55)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S3.3. Hazard Ratios for the association between time-varying hot flushes and coronary heart disease N=7112 (events=502)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.92 (0.70-1.21)	1.10 (0.88-1.37)	1.15 (0.90-1.48)
Model 2**	Ref.	0.93 (0.71-1.22)	1.10 (0.89-1.37)	1.16 (0.90-1.48)
Model 3***	Ref.	0.88 (0.65-1.18)	1.02 (0.80-1.30)	1.03 (0.79-1.36)
Model 4****	Ref.	0.87 (0.64-1.18)	1.01 (0.79-1.29)	1.01 (0.77-1.34)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S3.4. Hazard Ratios for the association between time-varying night sweats and coronary heart disease N=7112 (events=502)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.99 (0.76-1.28)	1.04 (0.83-1.32)	1.39 (1.08-1.80)
Model 2**	Ref.	0.99 (0.76-1.29)	1.05 (0.83-1.32)	1.40 (1.09-1.80)
Model 3***	Ref.	0.88 (0.66-1.18)	0.94 (0.72-1.22)	1.22 (0.92-1.62)
Model 4****	Ref.	0.88 (0.65-1.18)	0.90 (0.69-1.18)	1.18 (0.89-1.57)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S3.5. Hazard Ratios for the association between time-varying hot flushes and cerebrovascular disease N=7114 (events=152)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.58 (0.34-1.00)	0.73 (0.48-1.11)	1.04 (0.65-1.63)
Model 2**	Ref.	0.58 (0.34-0.99)	0.72 (0.47-1.10)	1.02 (0.65-1.61)
Model 3***	Ref.	0.57 (0.31-1.05)	0.67 (0.42-1.08)	1.07 (0.65-1.76)
Model 4****	Ref.	0.58 (0.32-1.06)	0.67 (0.42-1.08)	1.08 (0.66-1.78)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

Table S3.6. Hazard Ratios for the association between time-varying night sweats and cerebrovascular disease N=7114 (events=152)

Model	Never	Rarely	Sometimes	Often
Model 1*	Ref.	0.91 (0.55-1.50)	1.14 (0.75-1.74)	1.53 (0.96-2.44)
Model 2**	Ref.	0.90 (0.55-1.49)	1.14 (0.75-1.73)	1.52 (0.95-2.43)
Model 3***	Ref.	0.96 (0.55-1.67)	1.14 (0.72-1.81)	1.66 (0.99-2.79)
Model 4****	Ref.	0.97 (0.56-1.68)	1.13 (0.71-1.81)	1.67 (1.00-2.80)

**Model 1 adjusted for baseline age*
***Model 2 additionally adjusted for HRT use (time-varying)*
****Model 3 additionally adjusted for education level and time-varying physical activity, smoking status, and alcohol status*
*****Model 4 additionally adjusted for time-varying BMI*

CHAPTER 2.5

Association of polycystic ovary syndrome and risk or cardiovascular disease, coronary heart disease and stroke

V Dam, N C Onland-Moret, J M A Boer, W M M Verschuren, Y T van der Schouw

Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder with a prevalence of 6-15%. A large part of women with PCOS is overweight or obese, has a higher impaired glucose tolerance and more often type 2 diabetes. Meta-analyses suggest that women with PCOS have an increased risk for cardiovascular disease (CVD), coronary heart disease (CHD), and stroke. However, pooled risk estimates were not adjusted for BMI, and two meta-analyses included a large study on cycle irregularity instead of PCOS. This study aimed to examine the independent association between PCOS and CVD, CHD and stroke.

Methods: We used data from EPIC-NL, including 29,751 women at baseline. PCOS was assessed at the fourth follow-up questionnaire using a previously developed and validated questionnaire. For women that did not fill in this questionnaire we imputed PCOS. Subsequently, we used Cox proportional hazards analysis to calculate hazard ratios (HR) for CVD, CHD and stroke, adjusted for potential confounders. We further adjusted for BMI and lipid levels to investigate the independent effect of PCOS.

Results: PCOS was associated with slightly but non-significantly increased risks of CVD (HR=1.22, 95% confidence interval (CI): 0.79-1.89), CHD (HR=1.24, 95%CI: 0.85-1.80) and stroke (HR=1.21, 95%CI: 0.73-1.99). Both BMI as well as lipid levels further attenuated the HRs towards the null.

Conclusions: This study did not find an independent association between PCOS and CVD, CHD or stroke. BMI, HDL and non-HDL seem to play an important role in determining CVD risk in women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence of 6-15%, depending on the criteria used for defining PCOS[1,2]. Approximately 35% to 50% of women with PCOS are overweight (Body Mass Index [BMI] >25 kg/m^2) or obese (BMI >27 kg/m^2)[3]. Furthermore, women with PCOS have a higher prevalence of impaired glucose tolerance and type 2 diabetes, independent of and additive to obesity[4]. Also dyslipidemia, characterized by low high density lipoprotein (HDL) cholesterol and increased triglycerides, low density lipoprotein (LDL) cholesterol and non-HDL cholesterol, is more common in women with PCOS compared to those without[5]. These findings suggest that women with PCOS might also be of increased risk for cardiovascular diseases (CVD).

Three recent systematic reviews and meta-analyses summarized the evidence for the association of PCOS with CVD, coronary heart disease (CHD) and stroke[6–8]. Overall, they suggest that women with PCOS have a 1.3-2.0 times increased risk for CVD, CHD and stroke, although not consistently significant for the latter two. The level of covariate adjustment in the individual studies included in the meta-analyses differs substantially, while in particular BMI, diabetes and lipids as confounders or, possibly, intermediates in this association are not always accounted for. Furthermore, all three meta-analyses reported mostly on case-control studies and rather small cohort studies. The only large cohort study included in two of the meta-analyses[6,7], did not actually examine PCOS, but menstrual irregularity[9]. Two more recent registry-based studies reported a significantly increased CHD[10] and CVD risk[11] for women with PCOS. However, in the first study only a small number of events occurred during a rather short follow-up period. Moreover, they did not adjust for BMI. The latter of the two did adjust for obesity, but only investigated CVD and not CHD and stroke separately. Overall, it remains unclear whether there is an independent effect of PCOS on CVD, CHD and stroke.

We aimed to examine the independent association between PCOS and CVD, CHD and stroke in a large (N=29,751) prospective cohort study with approximately 20 years of follow-up, and a large number of possible confounders and intermediate risk factors measured.

Methods

Subjects

For this study we used data from the EPIC-NL cohort which included 40,011 participants. EPIC-NL is a combination of the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) cohort and the Prospect cohort. The MORGEN cohort consists of both men and women aged 20-59 from three Dutch cities (Amsterdam, Doetinchem and Maastricht), who were enrolled between 1993 and 1997. The Prospect cohort consists of women aged 49-70 years who participated in a breast cancer screening program in Utrecht, the Netherlands, between 1993 and 1997[12]. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of

the University Medical Center Utrecht and the Medical Ethical Committee of TNO Nutrition and Food Research (MORGEN-EPIC).

For this study we included only women (N=29,751) and excluded women who did not give consent for linkage with disease- and vital status registries for retrieving the CVD outcomes (N=1,253) and the women from Doetinchem, since they were not invited for the fourth follow-up survey (N=3,854). Subsequently, we excluded prevalent cases for each outcome, resulting in 24,314 women for the analysis with CVD (N=330 prevalent cases), 24,434 women for the analysis with CHD (N=210 prevalent cases) and 24,583 women for the analysis with stroke (N=61 prevalent cases).

Polycystic ovary syndrome

PCOS was assessed in the fourth survey round of EPIC-NL in 2015, in which 6,719 women participated. In order to define PCOS we used the questionnaire developed and validated by Pedersen et al[13]. This questionnaire included four questions on irregular/long lasting menstrual cycles, serious extra hair growth, obesity or overweight, and milky nipple discharge between age 16 and 45. Answers were scored according to Pedersen with ≥ 2 points qualifying for a PCOS diagnosis. Through this questionnaire we ascertained 178 women with PCOS at follow-up round four. Additionally, we included the question "Were you ever diagnosed with PCOS by a doctor?" which resulted in 65 women with PCOS. After combining the two and removing duplicates, there were 218 women with PCOS.

Due to various reasons like death, moving away or opting out for participation, only 6,719 participants filled in this fourth survey. In the remainder of the women, we used the information on PCOS of follow-up four from the women that did complete this survey, and the baseline data and the outcomes to impute a diagnosis of PCOS. We used chained equations with multiple imputations using the MICE package[14] in R[15] with 10 imputations and 100 iterations. This resulted in an average of 1124 (4.6%) women with PCOS in each imputation.

Covariate measurement

At baseline, information on demographic characteristics, lifestyle characteristics, the presence of chronic diseases, and risk factors of chronic diseases was obtained with a questionnaire, and a physical examination was performed for anthropometrics and blood pressure according to standard operating procedures. The baseline questionnaire contained questions including age, smoking (current, former, never), education level (low: primary education, lower vocational education, advanced elementary education; middle: intermediate vocational education, higher general secondary education 3rd year with success, higher general secondary education completed; high: higher vocational education, university to bachelor exam, university completed), diabetes (yes/no), and physical activity (measured with a validated questionnaire, from which the Cambridge

Physical Activity Index was calculated and categorized as inactive, moderately inactive, moderately active, active[16]). Furthermore, the questionnaire also contained questions about reproductive factors including age at menarche, ever been pregnant (yes/no), full term pregnancy (yes/no), menopausal status (premenopausal, perimenopausal, natural postmenopausal and surgical postmenopausal, with surgical postmenopausal defined as hysterectomy, uni- and bilateral ovariectomy) and oral contraceptive use (ever/never).

BMI was calculated as weight divided by height squared (kg/m^2). Systolic and diastolic blood pressures were obtained as the mean of two measurements in the supine position on the left arm using a random zero sphygmomanometer (MORGEN), or on the right arm using a BOSO Oscillomat (Bosch & Son, Jungingen, Germany) (Prospect). Hypertension was defined when one or more of the following criteria were fulfilled: diastolic blood pressure > 90 mmHg, systolic blood pressure > 140 mmHg, self-reported presence of hypertension or use of hypertensive medication. Total and High Density Lipoprotein (HDL) cholesterol concentrations were measured using standardized enzymatic methods. Non-HDL cholesterol was calculated by subtracting HDL-cholesterol levels from total cholesterol levels.

Outcome definition

We studied three different outcomes: CVD (N=2951), CHD (N=1602) and stroke (N=996). CVD was defined by codes 410-414, 427.5, 428, 415.1, 443.9, 430-438, 440-442, 444, 798.1, 798.2, 798.9 of the International classification of diseases Ninth Edition (ICD-9), and codes I20-I26, I46, R96, G45, I60-I67, I69-I74, I50 of the Tenth Edition (ICD-10). CHD was defined by codes 410-414, 427.5, 798.1, 798.2, 798.9 of ICD-9, and I20-I25, I46, R96 of ICD-10. Stroke was defined by codes 430-438 of ICD-9 and I60-I67, I69, G45 of ICD-10.

Statistical analysis

Baseline characteristics were presented stratified by PCOS for women that filled in the fourth survey, as mean ± SD or median (interquartile range [IQR]) for continuous variables and % (N) for categorical variables. Missing values in the covariates were imputed using multiple imputations as described above. All analyses were conducted within each imputed dataset and estimates were pooled with Rubin's Rule[17].

Hazard ratios were estimated using Cox proportional hazards regression, stratified by cohort. We used several levels of covariate adjustment: adjustment for age at baseline (Model 1), further adjustment for CVD risk factors: smoking status, education level, physical activity, and hypertension (Model 2), further adjustment for reproductive factors: age at menarche, pregnancy, menopausal status, oral contraceptives use and breastfeeding (Model 3). Subsequently, we individually added BMI, diabetes and HDL and non-HDL cholesterol to model 3 to investigate whether PCOS in itself is

associated with disease or whether a potential increased risk is due to the fact that these women often have higher BMI, diabetes and hyperlipidemia. This resulted in Model 4 (Model 3 + BMI), Model 5 (Model 3 + diabetes), and Model 6 (Model 3 + HDL + non-HDL).

As a sensitivity analysis, we conducted the same analyses including only the women that filled in follow-up round four. The rationale for conducting this analysis was to show the necessity of data imputation. By imputing the missing information on PCOS, we attempted to prevent bias that might be induced by selective drop-out, i.e. mainly when women with PCOS who also developed CVD have dropped out. If selective drop-out occurred, the estimates of this analysis will deviate from the analysis with the imputed data.

Results

Table 1 shows the baseline characteristics of women who participated in follow-up survey four (n=6719). The prevalence of PCOS was 3.2% (N=218) and the median age was comparable between women with and without PCOS (median 52.6 years, IQR 49.8-57.6 and 51.5, IQR 48.1-57.3, respectively). Furthermore, women with PCOS were more often inactive, more often had hypertension and diabetes, had a higher BMI, more often had irregular menstrual cycles and less often breastfed their children, while the percentage of full-term pregnancies was equal.

Table 1. Baseline characteristics by PCOS, only for women that completed the fourth follow-up survey (N=6719)

	No PCOS ***96.8% (6501)***	**PCOS** ***3.2% (218)***
Age (y)	52.6 (49.8-57.6)	51.5 (48.1-57.3)
Smoking ***Never*** ***Former*** ***Current***	43.2% (2806) 36.0% (2338) 20.5% (1333)	37.6% (82) 38.5% (84) 23.9% (52)
Education level ***Low*** ***Middle*** ***High***	51.0% (3316) 20.1% (1307) 28.5% (1850)	56.4% (123) 18.8% (41) 24.8% (54)
Physical activity ***Inactive*** ***Moderately inactive*** ***Moderately active*** ***Active***	3.9% (253) 24.1% (1564) 27.1% (1764) 2920 (44.9%)	6.0% (13) 29.8% (65) 24.3% (53) 39.9% (87)
Hypertension	32.8% (2132)	41.3% (90)
Diabetes	1.3% (82)	2.8% (6)
BMI	24.3 (22.4-26.8)	26.4 (23.7-30.1)
HDL cholesterol	1.6 ± 0.4	1.5 ± 0.4
Non-HDL cholesterol	4.0 ± 1.1	4.0 ± 1.1

Table 1. Continued

	No PCOS ***96.8% (6501)***	**PCOS** ***3.2% (218)***
Age at menarche	13.3 ± 1.5	13.2 ± 1.6
Irregular cycle	13.1% (852)	27.1% (59)
Ever been pregnant	80.5% (5231)	78.9% (172)
Ever had a full term pregnancy	78.3% (5092)	78.4% (171)
Menopausal status	28.0% (1818)	27.5% (60)
Premenopausal	14.9% (968)	11.9% (26)
Perimenopausal	41.6% (2703)	32.6% (71)
Postmenopausal	15.6%% (1012)	28.0% (61)
Surgical postmenopausal		
Ever used pill	76.2% (4955)	79.8% (174)
Ever breastfed	63.0% (4095)	55.5% (121)

PCOS was associated with a slightly increased risk of CVD, although not statistically significant (Model 3: HR=1.22, 95% Confidence Interval (CI): 0.79-1.89, table 2). In particular, BMI and lipid levels seem to explain a large part of the association, with BMI attenuating the HR to 1.15 (95%CI: 0.73-1.81) when added to model 3, and HDL and non-HDL attenuating the HR to 1.17 (95%CI: 0.71-1.82).

Furthermore, PCOS was also associated with a small, non-significant increased risk of CHD (table 2: HR=1.24, 95%CI: 0.85-1.80 in Model 3). And again, BMI and the lipid levels decreased the HR (Model 3 and BMI: HR=1.16, 95%CI: 0.79-1.71; Model 3 and HDL and non-HDL cholesterol: HR=1.18, 95%CI=0.80-1.73).

Table 2. Pooled Hazard Ratios for the association between PCOS and CVD, CHD and stroke

	CVD ***HR (95% CI)***	**CHD** ***HR (95% CI)***	**Stroke** ***HR (95% CI)***
Model 1*	1.39 (0.92-2.11)	1.44 (1.00-2.07)	1.33 (0.84-2.12)
Model 2**	1.25 (0.81-1.94)	1.28 (0.88-1.87)	1.21 (0.74-1.99)
Model 3***	1.22 (0.79-1.89)	1.24 (0.85-1.80)	1.21 (0.73-1.99)
Model 4†	1.15 (0.73-1.81)	1.16 (0.79-1.71)	1.18 (0.71-1.96)
Model 5‡	1.21 (0.76-1.91)	1.22 (0.81-1.82)	1.20 (0.72-1.99)
Model 6††	1.17 (0.75-1.82)	1.18 (0.80-1.73)	1.18 (0.71-1.95)

**Adjusted for age at baseline*
***Additionally adjusted for smoking status, education level, physical activity, and hypertension*
****Additionally adjusted for age at menarche, ever pregnant, menopausal status, oral contraceptives use and ever breastfed*
† Model 3 additionally adjusted for BMI
‡ Model 3 additionally adjusted for diabetes
†† Model 3 additionally adjusted for HDL cholesterol and non-HDL cholesterol

Finally, PCOS was also associated with a small and non-significant increased risk of stroke (table 2: HR=1.21, 95%CI: 0.73-1.99). Again, BMI and the lipid levels decreased the HR, although less pronounced compared to CVD and CHD (Model 3 and BMI: HR=1.18, 95%CI: 0.71-1.96; Model 3 and HDL and non-HDL: HR=1.18, 95%CI: 0.71-1.95).

In the sensitivity analyses, restricting our analyses to women who completed the questionnaire in follow-up round 4, the HRs decreased for all outcomes (table 3), resulting in a HR of 1.15 (95%CI: 0.74-1.78, Model 3) for CVD, a HR of 1.04 for CHD (95%CI: 0.58-1.87, Model 3), and a HR of 1.18 (95%CI: 0.52-2.70 Model 3) for stroke.

Table 3. Pooled Hazard Ratios for the association between PCOS and CVD, CHD and stroke (only women that filled in the fourth follow-up survey)

	CVD *HR (95% CI)*	**CHD** *HR (95% CI)*	**Stroke** *HR (95% CI)*
Model 1*	1.34 (0.86-2.07)	1.26 (0.71-2.25)	1.35 (0.60-3.07)
Model 2**	1.20 (0.77-1.86)	1.11 (0.62-1.98)	1.27 (0.56-2.88)
Model 3***	1.15 (0.74-1.78)	1.04 (0.58-1.87)	1.18 (0.52-2.70)
Model 4†	1.10 (0.71-1.72)	0.99 (0.55-1.79)	1.27 (0.55-2.90)
Model 5‡	1.14 (0.73-1.77)	1.03 (0.58-1.85)	1.19 (0.52-2.70)
Model 6††	1.10 (0.71-1.72)	1.00 (0.56-1.79)	1.17 (0.51-2.68)

**Adjusted for age at baseline*
***Additionally adjusted for smoking status, education level, physical activity, and hypertension*
****Additionally adjusted for age at menarche, ever pregnant, menopausal status, oral contraceptives use and ever breastfed*
† Model 3 additionally adjusted for BMI
‡ Model 3 additionally adjusted for diabetes
†† Model 3 additionally adjusted for HDL cholesterol and non-HDL cholesterol

Discussion

In this large study with approximately 20 years of follow-up we found a small, but not statistically significant increased risk for CVD, CHD and stroke in women with PCOS when adjusting for confounders. Adjustment for BMI and the lipid levels further reduced the risk estimates towards null, suggesting that these explain most of the association between PCOS and CVD risk.

This study has several strengths. First, we have a long follow-up with a median of 14 (IQR: 13-16) years, compared to a median follow-up of 11 years (IQR 7-16)[11] and a mean follow-up of 5.9 years (SD=4.0)[10] in the two most recent registry-based studies. Second, we were able to study CVD, CHD and stroke separately because of the large number of events (CVD=2951, CHD=1602, stroke=996) in our study, while the studies included in the meta-analyses generally had only few events. Third, this study included a large number of covariates we could adjust for in the

analyses, while most studies in the meta-analyses did not adjust for covariates or only for a rather small number. Particularly, we were able to adjust for BMI, diabetes and lipid levels on top of the fully adjusted model, enabling us to examine the independent effect of PCOS on the outcomes.

Some limitations need to be acknowledged. First, we had to assess PCOS retrospectively. It was not included in the data collection until the fourth follow-up round of the study, when our participants were approximately 40-90 years of age. Therefore, we were unable to use the Rotterdam or other generally-known diagnostic criteria[18,19]. Moreover, physical examination including intra-vaginal ultrasound is not feasible in large population-based (cohort) studies. The criteria defined by Pedersen et al[13] are easily used on a large scale in a questionnaire and do not require physical examination. In addition, in a validation study the questionnaire had a sensitivity of 85.4% and a specificity of 93.4%. Second, because we did not collect data on PCOS until follow-up round four, our study had a large percentage of missing data on PCOS. Using multiple imputation to overcome this, we prevented bias that might have been induced by selective drop-out, and at the same time increased the power of our analyses when compared to complete case analysis. Our sensitivity analysis restricted to women that filled in the fourth follow-up survey (i.e. with complete case information on PCOS) resulted in lower HR estimates, which indeed suggests that especially women with PCOS who also developed CVD have dropped out. Third, we found a relatively low prevalence of PCOS in our study compared to the generally reported prevalence of 6-15%[1,2] which might be explained by the retrospective use of this questionnaire. Also recall bias cannot be excluded, as women had to answer questions about, amongst other things, period regularity between ages 16 and 45.

Our study did not find a significant association between PCOS and CVD, CHD and stroke in contrast to two recent registry-based studies[10,11] and the meta-analyses. Our estimates in model 3 for CVD, CHD and stroke were generally somewhat lower compared to previous studies[6–8,10,11]. However, two of the meta-analyses[6,7] included a large cohort study on cycle irregularity instead of PCOS which had the highest weight in both meta-analyses. In EPIC-NL cycle irregularity also increased the risk for CHD[20]. Although cycle irregularity is among the diagnostic criteria of PCOS, it is only one criterion, and not a sufficient one. In our study cycle irregularities were indeed more common in PCOS (27%) but 13% of women without PCOS still reported cycle irregularities, indicating that cycle irregularity is not synonymous for PCOS. Therefore, it might be inappropriate to include this study in the meta-analysis.

Furthermore, the level of covariate adjustment in the studies included in the three meta-analyses varied substantially between studies. Part of the studies did not adjust for confounding, some only adjusted for age or age and BMI, and only one study also adjusted for diabetes and lipid levels. Our study showed that additional adjustment for BMI or lipid levels attenuated the estimates towards null, suggesting that most of the association between PCOS and CVD risk is due

to differences in BMI and lipid levels. Therefore, BMI and lipid levels are potentially intermediate factors in the association, which biologically makes sense as women with PCOS often have higher BMI[3] and non-HDL levels, and lower HDL levels[5]. This is confirmed by other studies that do not find a significant association anymore when taking BMI into account[8]. Moreover, a recent Mendelian Randomization study showed that genetic variants associated with PCOS are also causally associated with an increased BMI[21]. On the other hand, adjustment for obesity did not seem to impact the effect estimate in the Danish registry-based study which is interesting, since the number of women with obesity differed substantially between women with and without PCOS (11% and 1%, respectively)[11].

In conclusion, this large study with long follow-up did not find independent associations between PCOS and CVD, CHD and stroke. BMI, HDL and non-HDL seem to play an important role in determining cardiovascular risk in women with PCOS.

References

1 Group TREP consensus workshop. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41–7. doi:10.1093/humrep/deh098

2 March WA, Moore VM, Willson KJ, *et al.* The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010;**25**:544–51. doi:10.1093/humrep/dep399

3 Hart R, Hickey M, Franks S. Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol* 2004;**18**:671–83. doi:10.1016/j.bpobgyn.2004.05.001

4 Moran LJ, Misso ML, Wild RA, *et al.* Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. *Hum Reprod Update* 2010;**16**:347–63. doi:10.1093/humupd/dmq001

5 Wild RA, Rizzo M, Clifton S, *et al.* Lipid levels in polycystic ovary syndrome: Systematic review and meta-analysis. *Fertil Steril* 2011;**95**:1073–1079.e11. doi:10.1016/j.fertnstert.2010.12.027

6 Zhao L, Zhu Z, Lou H, *et al.* Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. *Oncotarget* 2016;**7**:33715–21. doi:10.18632/oncotarget.9553

7 de Groot PCM, Dekkers OM, Romijn JA, *et al.* PCOS, coronary heart disease, stroke and the influence of obesity: A systematic review and meta-analysis. *Hum Reprod Update* 2011;**17**:495–500. doi:10.1093/humupd/dmr001

8 Anderson SA, Barry JA, Hardiman PJ. Risk of coronary heart disease and risk of stroke in women with polycystic ovary syndrome: A systematic review and meta-analysis. *Int J Cardiol* 2014;**176**:486. doi:10.1016/j.ijcard.2014.06.079

9 Solomon CG, Hu F, Dunaif A, *et al.* Menstrual Cycle Irregularity and Risk for Future Cardiovascular Disease. *J Clin Endocrinol Metab* 2002;**87**:2013–7.

10 Ding D-C, Tsai I-J, Wang J-H, *et al.* Coronary artery disease risk in young women with polycystic ovary syndrome. *Oncotarget* 2018;**9**:8756–64. doi:10.18632/oncotarget.23985

11 Glintborg D, Rubin KH, Nybo M, *et al.* Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. *Cardiovasc Diabetol* 2018;**17**:1–12. doi:10.1186/s12933-018-0680-5

12 Beulens JWJ, Monninkhof EM, Monique Verschuren WM, *et al.* Cohort profile: The EPIC-NL study. *Int J Epidemiol* 2010;**39**:1170–8. doi:10.1093/ije/dyp217

13 Pedersen SD, Brar S, Faris P, *et al.* Polycystic ovary syndrome: validated questionnaire for use in diagnosis. *Can Fam physician Médecin Fam Can* 2007;**53**:1042–7, 1041. doi:53/6/1041 [pii]

14 Buuren S, Groothuis-Oudshoorn K. mice: Multivariate imputation by chained equations in R. *J Stat Softw* 2011;**45**. doi:10.18637/jss.v045.i03

15 Team RC. R: A language and environment for statistical computing. 2016.https://www.r-project.org/

16 Wareham NJ, Jakes RW, Rennie KL, *et al.* Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Heal Nutr* 2003;**6**:407–13. doi:10.1079/PHN2002439

17 Rubin DB. *Multiple Imputation for Non Response in Surveys.* Wiley 1987.

18 Fauser BCJM, Tarlatzis BC, Rebar RW, *et al.* Consensus on women's health aspects of polycystic ovary syndrome (PCOS): The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril* 2012;**97**:28–38.e25. doi:10.1016/j.fertnstert.2011.09.024

19 Azziz R, Carmina E, Dewailly D, *et al.* Position statement: Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: An androgen excess society guideline. *J Clin Endocrinol Metab* 2006;**91**:4237–45. doi:10.1210/jc.2006-0178

20 Gast GCM, Grobbee DE, Smit HA, *et al.* Menstrual cycle characteristics and risk of coronary heart disease and type 2 diabetes. *Fertil Steril* 2010;**94**:2379–81. doi:10.1016/j.fertnstert.2010.03.044

21 Day FR, Hinds DA, Tung JY, *et al.* Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun* 2015;**6**:1–7. doi:10.1038/ncomms9464

PART TWO

Prediction of cardiovascular disease risk in women

CHAPTER 3.1

Cardiovascular risk prediction models for women in the general population: a systematic review

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Submitted

Abstract

Background: To provide a comprehensive overview of cardiovascular disease (CVD) risk prediction models for women and models that include female-specific predictors.

Methods: We performed a systematic review of CVD risk prediction models for women in the general population by updating a previous review. We searched Medline and Embase up to July 2017 and included studies in which; (a) a new model was developed, (b) an existing model was validated, or (c) a predictor was added to an existing model.

Results: A total of 285 prediction models for women have been developed, of these 160 (56%) were female-specific models, in which a separate model was developed solely in women and 125 (44%) were sex-predictor models. Out of the 160 female-specific models, 2 (1.3%) included one or more female-specific predictors. A total of 591 validations of sex-predictor or female-specific models were identified in 206 papers. Of these, 333 (56%) validations concerned nine models (five versions of Framingham, SCORE, Pooled Cohort Equations and QRISK). The median C-were comparable for sex-predictor and female-specific models. In 260 articles the added value of new predictors to an existing model was described, however in only 3 of these female-specific predictors were added.

Conclusions: There is an abundance of models for women in the general population. Female-specific and sex-predictor models have similar predictors and performance. Female-specific predictors are rarely included. Further research is needed to assess the added value of female-specific predictors to CVD models for women and provide physicians with a well-performing prediction model for women.

Introduction

Differences between women and men in cardiovascular disease (CVD) have been recognized decades ago[1], pertaining to clinical presentation, pathophysiological mechanisms, course of disease and prognosis[2–5]. As symptoms of CVD are more subtle in women, there is often delayed diagnosis, and thus treatment and consequently poorer prognosis and outcomes compared with men[6]. It is crucial to identify sex differences to optimize diagnostic and management strategies for both women and men[7]. Although women and men share many CVD risk factors, which are often used in prediction models for the general population, there are also female-specific risk factors. Well known examples are early menarche and menopause, primary ovarian insufficiency, pregnancy complications, polycystic ovary syndrome, and use of hormones[8–10].

Preventive measures are available to reduce the cardiovascular disease burden. Numerous strategies to reduce the CVD burden have been implemented to identify persons at high risk. As seen in a systematic review published in 2016, over 350 prediction models have been developed in recent years aiming to identify individuals at high CVD risk in the general population[11]. Guidelines in Europe and the Unites States currently recommend the use of Systematic COronary Risk Evaluation (SCORE) or the Pooled Cohort Equations in the general population, both for women and men[12,13].

Although several female-specific CVD risk factors have been identified, predictors in most implemented CVD prediction models seem generally similar for women and men. As clinical presentation, pathophysiological mechanisms, course of disease and prognosis differ between women and men; risk prediction likely differs between as well. Therefore, we aimed to provide an overview of available CVD risk prediction models for women and of models that include female-specific predictors.

Methods

Systematic literature search

For this review we used the results of the review by Damen *et al* on all future CVD prediction models for the general population, both men and women[11]. As shown by this review, the number of newly developed CVD prediction models grew excessively in recent years. For this reason, we complemented the results of Damen *et al,* by performing an update of their search. Details of the review by Damen *et al* were published previously[11]. In the original search, Medline and Embase were searched until June 1st 2013 in order to identify articles on prediction models for the occurrence of CVD in the general population, published after 2004. Articles which dated before 2004 were subtracted from the review by Beswick *et al*[14]. Articles were included when they reported one or more multivariable (i.e. including at least 2 predictors) prediction models, tools or scores to predict future CVD in the general population (development papers), articles that

investigated the added value of certain predictors (incremental value papers) and articles that validated existing models (validation papers). Table 1 provides an overview of the key terminology.

Table 1. Key definitions

Model developed for women	A model developed for women, either separately for women (female-specific model) or where sex is incorporated as a predictor (sex-predictor model)
Female-specific model	A model developed in a dataset of women only, with a separate regression model or risk chart for women
Sex-predictor model	A model developed in a dataset of women and men, which uses sex as a predictor in the model
Development	When a new model is derived from a dataset
Incremental value paper	When one or more predictors are added to an existing model to study whether the performance of the model improves after adding the predictor(s)
Validation paper	When the performance of an existing model is verified in a different population
Female-specific predictor	A risk factor that is very clearly female specific such as: early menarche and menopause, primary ovarian insufficiency, pregnancy complications, and polycystic ovary syndrome
Discrimination	Indicates how well the model distinguishes between persons with an outcome event and persons without an outcome event, often depicted as the C statistic
C statistic	Measure of discrimination of the model and quantifies the area under the receiver operator curve (ROC). Ranges from 0.5 to 1.0, where 0.5 resembles a coin-toss and 1.0 is a perfect discrimination.

For the present systematic review, we updated the search of Damen *et al* until 26th of July 2017. Title and abstract screening were conducted using the same in- and exclusion criteria as Damen *et al*. However, in the full text screening we included only models specifically developed to predict CVD in women. We defined 'model developed for women' as 1) female-specific models, in which a separate model was developed in women only and 2) sex-predictor models, in which sex was included as a predictor (e.g. covariate) in the model (Table 1). Models that were developed on men only or models that did not include sex as a predictor were excluded. For the validation papers, only studies that validated a prediction model developed for women were included. Studies in which a predictor was added to an existing model (incremental value papers) were also included. Incremental value or validation studies in men only were excluded.

Screening and data extraction

The titles and abstracts retrieved by the search were divided randomly among the researchers (SJB, VD or LJJS) and screened independently. Studies were not screened in duplicate, but to guarantee uniformity in screening, 30 abstracts were screened by all three researchers and discussed afterwards. In the screening stage, all papers that were labeled as 'any doubt' were included for full text screening.

For full text screening the papers were divided in three different subsets for independent screening by one of the three researchers (SJB, VD or LJJS). Again, full text screening was not performed in duplicate; a subset of 20 papers from each researcher was screened by all three researchers to achieve uniformity. Articles labeled as "any doubt" were resolved by discussion among the three reviewers to reach consensus. Hand searching based on included articles and 'snowballing' were used to search for additional studies.

Finally, data extraction was performed in a pre-specified data-extraction format based on the CHecklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies (CHARMS)[15]. All three reviewers read the papers and subsequently filled in the data-extraction format together to guarantee agreement on the extracted information. In this stage, disagreements were settled by an additional reviewer (JAAGD or YTvdS). For papers in which a model was developed we extracted the same information as Damen *et al*. and additionally determined whether the model was a female-specific or sex-predictor model. All developed models were then assessed for quality based on reliability defined as 1) model externally validated 2) model externally validated in a separate investigation/paper and 3) C statistic > 0.7. If the development model did not report a C statistic, we used the mean C statistic of the external validations. Reliable models, which met these criteria were assessed for clinical usability for 1) 10 predictors or fewer, 2) full regression model or chart reporte and 3) availability of an online calculator

For every included incremental value paper we extracted author, year, journal, the model that was used to calculate incremental value and whether this model was female-specific or sex-predictor and which predictors actually had incremental value. In addition, predictors considered for incremental value were also extracted.

Finally, for the validation papers we extracted author, year, journal and which model was validated. For the models that were validated >5 times and at least in once in an external study, we subsequently extracted additional information: characteristics of the validation cohort (country, number of participants, age range, number of events), and performance measures (Table 1). We also extracted whether the validation cohort existed of men and women or women only (studies with men only were previously excluded). When studies used a cohort consisting of both men and

women, the model could be validated on men and women together or separately. When validated in men and women separately we only included the validation on women.

Descriptive analyses

Results are presented as descriptive statistics and no formal synthesis of results was performed, since this was beyond the main goal of the current study. Results are presented as counts or percentages where indicated. Combined summary measures of studies and models (e.g. C statistics and number of participants in a cohort) are presented as medians and/or ranges. Proportions were compared with the Chi-square test. Analyses were performed using SPSS 24 (IBM, Armonk, New York).

Results

Figure 1 depicts the study flow diagram. From the study by Damen and colleagues, 249 articles were included that described models developed for women. The updated search, after removing duplicates, resulted in 9348 new references. After title and abstract screening, 2290 articles were eligible for full text assessment. Full text screening resulted in 244 included articles from the updated search and two additional references identified through snowballing. These 246 papers were added to the 249 papers from Damen *et al* and in total, this review includes 495 papers on models for women (Figure 1).

In 133 papers prediction models for women were developed. In 206 papers a model was validated and 260 papers concerned incremental value studies. Since papers can develop a model, validate a model and calculate the incremental value of a predictor on an existing model in the same paper, these numbers do not add up to the total of 495 papers.

Development of new prediction models

In 133 distinct papers, 285 cardiovascular risk prediction models were developed. Of these, 160 (56%) were developed solely on women and are henceforth denoted as female-specific models. The remaining 125 (44%) were sex-predictor models (Table 2). Table 2 shows the year in which the models were published. Clearly, new models are still being developed in large numbers, with the majority of the models developed in the last decade (on average 16 new models developed each year). Before 1990, 62% of the developed models were sex-predictor models. Between 1991 and 2010 female-specific models were developed more often than sex-predictor models, since 2010 these proportions are equally divided.

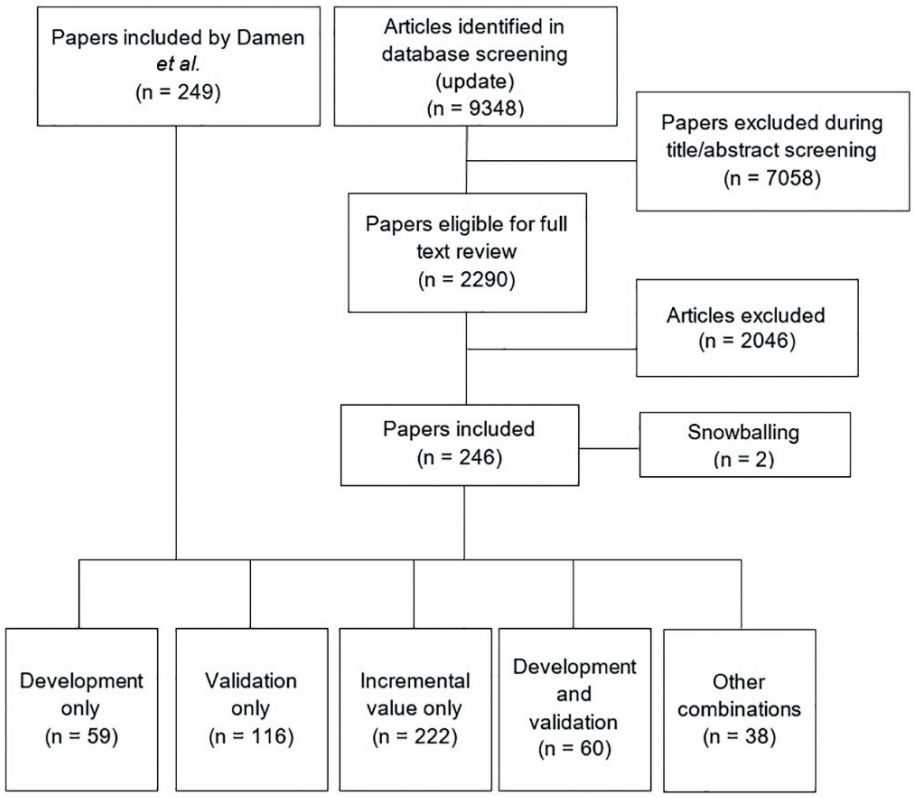

Figure 1. Study flow diagram. The papers that were identified by the updated search were added to the papers from the study by Damen and colleagues, resulting in a total of 495 papers.

Table 2. Number of developed models over time

Year	1967 – 1990	1991 - 2000	2001 – 2010	2011 - 2017	Total
Sex predictor	21 (62%)	21 (35%)	28 (35%)	55 (50%)	125 (44%)
Female specific	13 (38%)	39 (65%)	52 (65%)	56 (50%)	160 (56%)
Total	34 (100%)	60 (100%)	80 (100%)	111 (100%)	285 (100%)

Predictors in the development papers

For the models that were specifically developed for women, it was of particular interest whether female-specific predictors were included in the model. Only 2 out of the 160 developed female-specific models (1.3%) included a female-specific risk factor. In the first, D'Agostino and colleagues developed a model including menopause (yes/no) and an interaction with menopause and age as predictors[16]. In the second, Parikh and colleagues considered the predictors pregnancy status, number of live births, age at menarche, menstrual irregularity, age at first birth, stillbirths,

miscarriages, infertility ≥1 year, infertility cause and breastfeeding for inclusion in a model with established risk factors. The final model presented included in addition to age the female-specific risk factors: menstrual irregularity, age at first birth, still births, miscarriages and breastfeeding and had a C-statistic of 0.675 in the derivation cohort[17].

The median number of predictors for the female-specific models was 6 [IQR: 5 - 8] and for the sex-predictor models was 8 [IQR: 7 - 10], including the predictor for sex. Figure 2 shows the percentage of sex-predictor and female-specific models that included the nine most often-used predictors. By definition sex was not a predictor in any of the female-specific models. Total cholesterol was used more frequently in female-specific models (58% vs. 36%, difference 22% 95%CI 10%-33%). For the remaining eight predictors most frequently identified in the models (age, smoking, diabetes mellitus, systolic blood pressure, HDL, hypertension, diastolic blood pressure, and LDL), the frequency of predictors used was similar for the both model types.

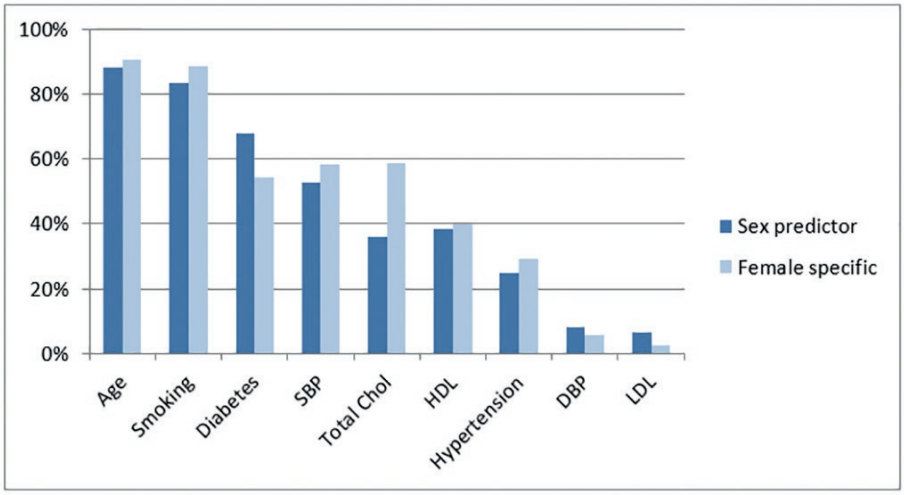

Figure 2. Most frequently used predictors for the sex predictor and female-specific models. HDL; High-density lipoprotein. Total Chol; total cholesterol. LDL; Low-density lipoprotein. SBP; systolic blood pressure. DBP; Diastolic blood pressure.

The apparent C-statistic (i.e. the C-statistic in the development models) was reported in 66 (53%) of the sex-predictor models and in 59 (37%) of the female-specific models. The median of the C-statistics were similar (0.797 for the sex-predictor models [range: 0.610 - 1.000] and 0.787 for the female-specific models [range: 0.660 - 0.918]). The full list of identified development papers in the updated search is available as S1 Table.

Validation of prediction models

A total of 206 articles described 591 validations of sex-predictor or female-specific models. The models that were validated more than five times and at least once in a separate paper, were; SCORE Conroy 2003 (n=63), Framingham Wilson 1998 (n=61 validations), Pooled Cohort Equations Goff 2013 (n=52), Framingham D'Agostino 2008 (n=48), Framingham Anderson 1991a (n=40), Framingham ATP III 2002 (n=29), Framingham Wolf 1991 (n=20), Framingham Anderson 1991b (n=14), and QRISK Hippisley-Cox 2007 (n=6) (Table 3). The 333 validations of these nine models will be discussed further. The only model that is a sex-predictor model is Framingham Anderson 1991a, which was validated 15 (37%) times in men and women and 25 (63%) times in women only. The eight female-specific models were validated 119 (41%) times in men and women together. The other 174 validations (59%) were performed in women only. A C-statistic was reported in 70% of these validation studies and ranged from 0.449 to 0.993. For the models validated in women only the median C-statistic was slightly higher than for the models validated in both women and men (0.750 [range: 0.449 – 0.920] vs 0.737 [range: 0.480 – 0.993]). The full list of validated models identified in the updated search is available as S2 Table.

Table 3. Most frequently validated models

	1	2	3	4	5	6	7	8	9
	SCORE Conroy 2003 n = 63	Framing-ham Wilson 1998 n = 61	Pooled Cohort Equations Goff 2013 n = 52	Framing-ham D'Agostino 2008 n = 48	Framing-ham Anderson 1991 n = 40	Framing-ham ATP III 2002 n =29	Framing-ham Wolf 1991 n = 20	Framing-ham Anderson 1991 n = 14	QRISK Hippisley-Cox 2007 n = 6
Validated on:									
Men and Women	26	27	16	28	15	15	6	1	0
Women Separately	37	34	36	20	25	14	14	13	6
Location									
Asia	8	7	8	10	1	1	1	1	0
Australia	4	0	1	1	10	1	0	1	0
Europe	43	20	7	22	28	3	9	8	6
North America	8	32	34	13	1	24	10	4	0
Age									
Min, median	40	40	40	40	35	45	55	35	35
Max, median	65	74	79	79	74	82	99	64	74
Median # of:									
Participants [range]	7573 [203-44649]	3554 [246-163627]	4218 [392-307591]	2613 [136-542987]	2105 [302-797373]	3716 [613-436517]	3507 [401-23983]	3014 [331-542783]	542987 [306111-797373]
Events [range]	157 [10-4842]	213 [8-24659]	150 [9-4658]	146 [15-18173]	86 [1-29057]	384 [35-2343]	160 [24-939]	158 [5-18173]	29057 [18027-29057]

Table 3. Continued

Performance									
Median C statistic [range]	0.762 [0.610-0.910]	0.724 [0.449-0.920]	0.740 [0.580-0.850]	0.768 [0.576-0.841]	0.770 [0.530-0.993]	0.691 [0.630-0.844]	0.710 [0.480-0.780]	0.771 [0.765-0.777]	0.794 [0.788-0.814]

Incremental value

In 260 articles the added value of a predictor to an existing female-specific or sex-predictor model was described. In 3 (1.1%) papers female-specific risk factors were added to an existing model, all of which were recently published (2016 n=2 and 2017 n=1)[18–20]. In the previously discussed paper by Parikh and colleagues, female-specific predictors were added to established risk factors, resulting in a final model including age at first birth, still births, miscarriages and breastfeeding. This slightly improved the model, C-statistic of 0.730, where the model with only established risk factors had a C-statistic of 0.726[17]. In a study by van der Meer and colleagues, the female-specific predictors age at menarche, menopausal status/age, hormone use, gestational hypertension and diabetes, number of children, miscarriages/stillbirths were added to established risk factors. The addition of these predictors did non apparently improve the discrimination or calibration of the model beyond the established risk factors[21]. In the third paper, Zhou and colleagues added amongst other predictors (African American ethnicity, physical exercise level, BMI, waist circumference, height, HDL cholesterol), use of hormone replacement therapy in postmenopausal women to the Framingham Stroke Risk Score (Wolf 1991). The addition of this predictor set improved discrimination and calibration of the model in women; however, the separate performance of hormone use was not reported[22].

The full list of incremental value papers identified by the updated search is available as S3 Table.

Reliability and clinical usability of available models

All 285 models developed for women were first assessed for reliability and were regarded so if they met the following criteria: 1) model externally validated 2) externally validated in a separate investigation/paper and 3) a C statistic >0.7. Of the 285 models, 40 (14%) met these criteria and were considered reliable (Table 4). Of these 40, 25 (63%) were female-specific and 15 (37%) were sex-predictor models. Following, these models were assessed for clinical usability based on the presence of 1) 10 predictors or fewer, 2) full regression model or chart reported and 3) online calculator available (Table 4). The SCORE and Framingham 2008 model had the highest usability score as they met all criteria. Other models with high usability are the Pooled Cohort Equations (African American), Framingham 30 year and the Framingham stroke models as they have 10 or fewer predictors and an online calculator available. The remaining models either had more than 10 predictors or no calculator available, rendering them less appealing for clinical practice.

Table 4. Clinical usability of models that met the reliability criteria

Model – study name	Author - Year	Number of separate models	< 10 predictors	Full regression formula	Risk Chart	Online calculator
Framingham	Anderson 1991a	12	✓	✓	×	×
Framingham	Anderson 1991b	2	✓	✓	✓	×
--	Assmann 2007	2	✓	×	✓	×
ARIC	Chambless 2003	2	×	✓	×	✓
SCORE	Conroy 2003	6	✓	✓	✓	✓
Framingham	D'Agostino 2008	2	✓	✓	✓	✓
Framingham	ATP II	1	✓	✓	✓	×
--	Gaziano 2008	2	✓	×	✓	×
Pooled Cohort Equations (African American)	Goff 2013	1	✓	✓	×	✓
Pooled Cohort Equations (White)	Goff 2013	1	×	✓	×	✓
QRISK	Hippisley-Cox 2007	1	✓	×	×	×
QRISK2	Hippisley-Cox 2008	2	×	×	×	×
QRISK lifetime	Hippisley-Cox 2010	1	×	×	×	✓
--	Lumley 2002	1	✓	×	✓	×
Framingham (30 years)	Pencina 2009	1	✓	×	×	✓
--	Schnabel 2009	1	✓	×	✓	×
Framingham	Wilson 1998	1	✓	✓	✓	×
Framingham (Stroke)	Wolf 1991	1	✓	×	✓	✓

Clinical usability was scored for the models which met all criteria for reliability: 1) model externally validated 2) externally validated in a separate investigation/paper, and 3) a C statistic >0.7.

Discussion

In this study we provided an overview of the available CVD risk assessment models for women in the general population. We identified a wide range of models that have been developed over the past decades, including 160 female-specific models (e.g. models that are developed for use in women only) and 125 sex-predictor models (e.g. models that include sex as a predictor). Despite this large quantity, only two of the 160 (1.3%) female-specific models included female-specific

predictors[16,17]. Of the 260 studies in which the added value of a predictor was assessed, only three (1.1%) investigated the added value of a female-specific predictor[17,21,22].

Our study has several major strengths. We performed an extensive search up to July 2017 and systematically selected studies for inclusion. Detailed and thorough data extraction of essential information such as type of models, predictors, population and model discrimination, was performed by means of standardized forms and was done by three investigators together for the development models to ensure uniformity. Limitations of our study should be mentioned. First, we did not include models specifically made for men and thus could not compare differences in performance and predictors between men and women. Second, in some validation studies it was not clear which models were validated when the original development article reported on more than one model. We assumed that all models in the article were validated, but this may have led to an overestimation of the actual number of times prediction models were validated. Third, we did not include articles written in a language different than English and articles of which the full text could not be retrieved. Also, as this exceeded the purpose of the review and such a large number of models were identified, we did not perform formal critical appraisal of the included studies. Finally, as calibration was reported in a heterogeneous manner, conclusions for this performance measure could not be drawn. Furthermore, in papers the measure for calibration was often not reported. In order to guarantee uniformity, new studies reporting on prediction models should adhere to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement[23,24].

The models described in this review often comprise several variations of established, sex-independent predictors such as age, blood pressure, lipid levels and smoking indicating that these predictors attribute most to the current performance of the models. Interestingly, the results showed that both the female-specific models as well as the sex-predictor models often comprise these same established predictors and do not differ substantially in estimated C-statistic. This might imply that using sex as predictor in a model is just as effective as developing a female-specific model. Of the nine most frequently validated models in women the C-statistic as a measure of performance was reported in 59% of the validation studies. The median of the reported C-statistics indicated good performance for most models (C-statistic >0.70), although the range varied from 0.48 to 0.99. This indicates that although these models generally perform well, they can definitely be improved. Of all 285 developed models, only 40 (14%) met the quality criteria for reliability. When these models were further assessed for clinical usability only 2/40 (5%), the SCORE and Framingham 2008 model, met all criteria. Other models which met most criteria and had a risk calculator available were the Pooled cohort equations, Framingham 30 years and Framingham stroke model. Based on both these reliability and clinical usability criteria, these models seem best suitable for implementation in clinical practice. Models without an online calculator are likely

less attractive for use in clinical practice.

Our findings are in line with a previous study by Goh and colleagues, in which the utility of CVD prediction models for women was appraised[18]. They also concluded that there is room for improvement in CVD prediction models for women and this could be achieved by adding predictors which may perform well in women. Remarkably is that none of the predictors suggested by Goh, such as obesity, physical activity and coronary artery calcium, are female-specific. It must be noted that in the study by Goh and colleagues the search was limited to five years before publication (2008-2013). The study was restricted to six models, where we in our study considered any model identified by the search strategy. The 2011 guidelines for the prevention of CVD in women[25] categorize women as 'at risk' when having one or more major risk factors. Aside from the established risk factors found in most prediction models, they explicitly include the female-specific risk factors of a history of preeclampsia, gestational diabetes, or pregnancy-induced hypertension. However, none of these disorders are used in any of the prediction models for women in this review.

Although many models have been developed in women only, it seems that differences between men and women in CVD risk assessment are still not fully recognized. Many female specific risk factors for CVD have been identified in recent years, but their predictive potential has not been tested or even considered in risk prediction models within the scope of our review. Our search only identified two development studies that included a female-specific risk factor in the model[16,17]. Improvement of the existing models might be achieved in adding female-specific predictors. However, in most of the incremental value studies we found, female-specific predictors were not even considered as potential predictors for added value. Of the 260 incremental value studies, three added a female-specific predictor. Of these, one reported no improvement in performance and one observed a slight improvement in discrimination. The third did not report on improvement of individual predictors. A reason for not finding any substantial improvement could be that studies missed information on several important female-specific risk factors like preeclampsia, polycystic ovary syndrome and infant birth weight. Therefore, it is important to further investigate the potential added value of female-specific predictors. Most female-specific predictors become apparent at an early stage in life whereas CVD events mostly occur after the age of 50. An additional benefit is that these predictors can be easily obtained from the medical history. This underlines the potential of these predictors, as risk assessment is ideally performed decades before the anticipated event, in order to implement and optimize effect of preventive strategies. Although we identified a total of 495 papers on CVD prediction models for women, it is still uncertain whether these can be improved by female-specific predictors. However, it should be mentioned that finding new predictors that improve model performance on top of the well-known predictors seems challenging[20]. It is possible that current models, which often aim to estimate

the 10-year risk based on a single assessment, have reached their maximal predictive potential and cannot be further improved. A new type of model, for example the dynamic model, in which an individual's risk is continuously updated over time, could further advance preventive strategies.

Conclusions

In conclusion, the results of our study urge further research in order to provide physicians with a well-performing and properly validated prediction model for women. As suggested, future studies should consider female-specific predictors and ideally assess their added value to models which already perform well instead of developing completely new models[11]. Furthermore, both female-specific as well as sex-predictor models overlay in predictors and performance. This suggests that for CVD prediction models without female-specific predictors, development in women only is not necessary when sex is used as a predictor.

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References

1 Healy B. The Yentl Syndrome. *N Engl J Med* 1991;**325**:274–6.

2 Dean J, Cruz S Dela, Mehta PK, *et al.* Coronary microvascular dysfunction: sex-specific risk, diagnosis, and therapy. *Nat Rev Cardiol* 2015;**12**:406–14. doi:10.1038/nrcardio.2015.72

3 Gillis AM. Atrial Fibrillation and Ventricular Arrhythmias: Sex Differences in Electrophysiology, Epidemiology, Clinical Presentation, and Clinical Outcomes. *Circulation* 2017;**135**:593–608. doi:10.1161/CIRCULATIONAHA.116.025312

4 Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovascular Disease. *Physiol Rev* 2017;**97**:1–37. doi:10.1152/physrev.00021.2015

5 Ventura-Clapier R, Dworatzek E, Seeland U, *et al.* Sex in basic research: concepts in the cardiovascular field. *Cardiovasc Res* 2017;**113**:711–24. doi:10.1093/cvr/cvx066

6 Mosca L, Linfante AH, Benjamin EJ, *et al.* National study of physician awareness and adherence to cardiovascular disease prevention guidelines. *Circulation* 2005;**111**:499–510. doi:10.1161/01.CIR.0000154568.43333.82

7 Baggio G, Corsini A, Floreani A, *et al.* Gender medicine: A task for the third millennium. *Clin Chem Lab Med* 2013;**51**:713–27. doi:10.1515/cclm-2012-0849

8 Tan YY, Gast G-CM, van der Schouw YT. Gender differences in risk factors for coronary heart disease. *Maturitas* 2010;**65**:149–60. doi:10.1016/j.maturitas.2009.09.023

9 Appelman Y, van Rijn BB, Ten Haaf ME, *et al.* Sex differences in cardiovascular risk factors and disease prevention. *Atherosclerosis* 2015;**241**:211–8. doi:10.1016/j.atherosclerosis.2015.01.027

10 Zoet GA, Benschop L, Boersma E, *et al.* Prevalence of Subclinical Coronary Artery Disease Assessed by Coronary Computed Tomography Angiography in 45- to 55-Year-Old Women With a History of Preeclampsia. *Circulation* 2018;**137**:877–9. doi:10.1161/CIRCULATIONAHA.117.032695

11 Damen JAAG, Hooft L, Schuit E, *et al.* Prediction models for cardiovascular disease risk in the general population: systematic review. *BMJ* 2016;**353**:i2416. doi:10.1136/bmj.i2416

12 Goff DC, Lloyd-Jones DM, Bennett G, *et al.* 2013 ACC/AHA guideline on the assessment of cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. *J Am Coll Cardiol* 2014;**63**:2935–59. doi:10.1016/j.jacc.2013.11.005

13 Piepoli MF, Hoes AW, Agewall S, *et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* 2016;**37**:2315–81. doi:10.1093/eurheartj/ehw106

14 Beswick A, Brindle P, Fahey T, *et al.* A Systematic Review of Risk Scoring Methods and Clinical Decision Aids Used in the Primary Prevention of Coronary Heart Disease. 2008;:1–361.

15 Moons KGM, de Groot J a H, Bouwmeester W, *et al.* Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies: The CHARMS Checklist. *PLoS Med* 2014;**11**. doi:10.1371/journal.pmed.1001744

16 D'agostino RB, Russell MW, Huse DM, *et al.* Primary and subsequent coronary risk appraisal: New results from The Framingham Study. *Am Heart J* 2000;**139**:272–81. doi:10.1016/S0002-8703(00)90236-9

17 Parikh NI, Jeppson RP, Berger JS, *et al.* Reproductive risk factors and coronary heart disease in the women's health initiative observational study. *Circulation* 2016;**133**:2149–58. doi:10.1161/CIRCULATIONAHA.115.017854

18 Goh LG, Dhaliwal SS, Lee a H, *et al.* Utility of established cardiovascular disease risk score models for the 10-year prediction of disease outcomes in women. *Expert Rev Cardiovasc Ther* 2013;**11**:425–35. doi:10.1586/erc.13.26

19 Mosca L, Benjamin EJ, Berra K, *et al.* Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update. *J Am Coll Cardiol* 2011;**57**:1404–23. doi:10.1016/j.jacc.2011.02.005

20 Wang TJ, Gona P, Larson MG, *et al.* Multiple Biomarkers for the Prediction of First Major Cardiovascular Events and Death. *N Engl J Med* 2006;**355**:2631–9. doi:10.1056/NEJMoa055373

21 Van Der Meer MG, Van Der Graaf Y, Schuit E, *et al.* Added Value of Female-Specific Factors beyond Traditional Predictors for Future Cardiovascular Disease. *J Am Coll Cardiol* 2016;**67**:2084–6. doi:10.1016/j.jacc.2016.02.031

22 Zhou XH, Wang X, Duncan A, *et al.* Statistical evaluation of adding multiple risk factors improves Framingham stroke risk score. *BMC Med Res Methodol* 2017;**17**:1–13. doi:10.1186/s12874-017-0330-8

23 Collins GS, Reitsma JB, Altman DG, *et al.* Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD Statement. *Eur Urol* 2015;**67**:1142–51. doi:10.1016/j.eururo.2014.11.025

24 Moons KGM, Altman DG, Reitsma JB, *et al.* Transparent Reporting of a multivariable prediction model for Individual Prognosis or Disagnosis (TRIPOD): Explanantion and Elaboration. *Ann Intern Med* 2015;**162**.

25 Mosca L, Benjamin EJ, Berra K, *et al.* Effectiveness-based guidelines for the prevention of cardiovascular disease in women-2011 update: A Guideline from the American Heart Association. *Circulation* 2011;**123**:1243–62. doi:10.1161/CIR.0b013e31820faaf8

Appendix 1

S1 Table. Articles that developed a new model in the updated search and their external validation

First author, publication year	Number of models developed	Female-specific or sex-predictor	Number of articles in which model is validated
Artigao-Rodenas, 2015	1	Sex-predictor	1 (Artigao-Rodenas, 2015)
Backholer, 2017	3	Sex-predictor	1 (Backholer, 2017)
Bali, 2016	1	Sex-predictor	1 (Bali, 2016)
Borglykke, 2010	5	Female-specific	1 (Borglykke, 2010)
Chahal, 2015	7	Sex-predictor	-
Chiuve, 2014	1	Female-specific	-
Cooney, 2012	1	Female-specific	1 (Cooney, 2012)
Cross, 2013	1	Sex-predictor	-
Deo, 2016	1	Sex-predictor	1 (Deo, 2016)
Dhoble, 2014	4	Sex-predictor	-
Fox, 2016	6	Sex-predictor	1 (Fox, 2016)
Goff, 2013	2 (PCE)	Female-specific	20 (Muntner, 2014; Lee, 2015; Khalili, 2015; Kavousi, 2014; Jung, 2015; DeFilippis, 2015; Chia, 2014; Andersson, 2015; Yang, 2016; Cook, 2014; De Las Heras Gala, 2016; DeFilippis, 2017; Emdin, 2017; Foraker, 2016; Goff, 2013; Karmali, 2015; Mortensen, 2017; Qureshi, 2016; Rana, 2016; Zhang, 2017)
Hajifathalian, 2015	2	Female-specific	1 (Hajifathalian, 2015)
Hensley, 1998	1	Female-specific	-
Hippisley-Cox, 2013	1 (QSTROKE)	Female-specific	2 (Hippisley-Cox, 2014; Parmar, 2015)
Hippisley-Cox, 2017	3	Female-specific	1 (Hippisley-Cox, 2017)
Ho, 2016	2	Sex-predictor	1 (Ho, 2016)
Howard, 2017	1	Sex-predictor	1 (Howard, 2017)
Hu, 2014	1	Sex-predictor	-
Jairam, 2015	1	Sex-predictor	
Jee, 2014	4	Female-specific	-
Johansson	1	Sex-predictor	
Jung, 2015	1	Female-specific	-
Kovalchik, 2013	1	Female-specific	1 (Kovalchik, 2013)
Kusmana, 2002	1	Sex-predictor	-
Liu, 2016	1	Female-specific	
Manuel, 2015	1	Female-specific	

S1 Table. Continued

First author, publication year	Number of models developed	Female-specific or sex-predictor	Number of articles in which model is validated
Marino, 2014	2	Female-specific	-
Marrugat, 2014	6	Female-specific	1 (Marrugat, 2014)
McClelland, 2015	2	Sex-predictor	1 (McClelland, 2015)
McNeil, 2001	2	Female-specific	-
Nishimura, 2014	4	Sex-predictor	-
Nobel, 2014	1	Sex-predictor	-
Onat, 2017	1	Female-specific	
Parikh, 2016	1	Female-specific	-
Parmar, 2014	1	Sex-predictor	1 (Parmar, 2014)
Paynter, 2014	3	Female-specific	-
Piotrowski, 2016	2	Female-specific	
Selmer, 2017	1	Female-specific	
Stam-Slob, 2017	1	Sex-predictor	
Vartiainen, 2016	3	Female-specific	-
Wang, 2016	1	Female-specific	
Wickramasinghe, 2014	1	Female-specific	-
Woodward, 2007	1	Female-specific	
Woodward, 2006	2	Female-specific	-
Würtz, 2015	2	Sex-predictor	1 (Würtz, 2015)
Yang, 2016	1	Female-specific	-
Yatsuya, 2016	2	Sex-predictor	-
Yudkin, 1999	1	Female-specific	-

S2 Table. Models validated in the update

Model Validated	Author, year model developed	Number of articles in which model is validated
Framingham	Anderson - 1991	2 (Goh, 2014; Tilin, 2014)
Framingham	ATP III - 2002	5 (DeFilippis, 2015; Dhoble, 2014; Hu, 2014; Qureshi, 2016; Kavousi, 2014)
YDR	Colditz - 2000	1 (De Vito, 2015)
SCORE	Conroy - 2003	15 (Goh, 2014; Jdanov, 2014; Jorstad 2014; Kavousi, 2014; Mortsensen, 2015; Selvarajah, 2014; Vikhireva, 2014-a; Vikhireva, 2014-b, Baena-Diez, 2017; Mortensen, 2017; De Las Heras Gala, 2016; Qureshi, 2016; Berard, 2016; Sawano, 2016; Piotrowski, 2016)
SSVMod	Counsell - 2002	1 (Sim, 2016)
Framingham	D'Agostino - 2008	9 (Artigao-Rodenas, 2013; Chia, 2015; DeFilipis, 2015; Marino, 2014; Selvarajah, 2014, Fatema, 2016, Qureshi, 2016; Chamnan, 2016, Sepanlou, 2015)
CHADS2	Gage - 2001	1 (Yuan, 2017)
QRISK2	Hippisley-Cox - 2008	2 (Hippisley-Cox, 2014; Tilin, 2014)
CBC Score	Horne - 2009	1 (Horne, 2015)
SCORE – Germany	Keil – 2005	1 (Rucker, 2016)
CHA2DS2 - VASC	Lip - 2010	1 (Yuan, 2017)
Framingham - Regicor	Marrugat - 2003	1 (Marrugat, 2014)
HellenicSCORE	Panagiotakos - 2007	1 (Panagiotakos, 2015)
Framingham	Pencina - 2009	1 (van Kempen, 2014)
Reynolds Risk	Ridker - 2007	1 (DeFilippis, 2015)
Dubbo	Simons - 2003	1 (Weatherley, 2011)
SCORE-NL	Van Dis - 2010	1 (Van Dis, 2014)
NIHSSMod	Weimar – 2004	1 (Sim, 2016)
WHO/ISH	WHO - 2007	2 (Raghu, 2015; Selvarajah, 2014)
SCORE – Sweden	Wilhelmsen - 2004	1 (Karjalainen, 2017)
Framingham	Wilson - 1998	4 (DeFilippis, 2015; Hu, 2914; Nishimura, 2014; Fowkes, 2014)
Framingham	Wolf - 1991	6 (Hippisley-Cox, 2013; McClure, 2014; Parmar, 2014; Sabayan, 2013; Dufouil, 2017; Howard, 2017)

S3 Table. Models used for incremental value in the update

Model used for incremental value	Author, year model developed	Number of articles used for IV
ARIC HF	Agarwal - 2012	1 (Nambi, 2013)
Framingham	Anderson - 1991	1 (Wassertheil-Smel, 2014)
Framingham	ATP III - 2002	2 (Hadamitzky, 2013; Valentini, 2015)
SCORE	Conroy - 2003	7 (Faeh, 2013; Ferrario, 2014; Groot, 2015; Schnohr, 2015; Sehestedt, 2011; Vikhireva, 2014; Woznicka-Leskiew, 2015)
Framingham	Cupples - 1988	1 (Lluis-Ganella, 2012)
Framingham	D'Agostino - 1994	2 (Gibson, 2014; Ziegelbauer)
Framingham	D'Agostino - 2000	1 (Aljaroudi, 2013)
Framingham	D'Agostino - 2001	1 (Yeboah, 2014)
Framingham	D'Agostino - 2008	5 (Armstrong, 2014; Criqui, 2013; Goh, 2014; Kunutsor, 2015; Lopez-Suarez, 2014)
AGLA	Eckardstein - 2012	1 (Romanens, 2014)
--	Ferrario -2005	1 (Veronesi, 2014)
Framingham	Unspecified	8 (Badheka, 2013-a; Badheka, 2013-b; Brouwers, 2014; Gaibazzi, 2014; Lindberg, 2014; Okwuosa, 2014; Willeit, 2014; Woznicka-Leskiew, 2015)
Pooled Cohort Equations	Goff - 2013	3 (Everett, 2015; Kim, 2014; Okwuosa, 2014)
QRISK2	Hippisley-Cox - 2008	1 (Weng, 2015)
REGICOR	Marrugat - 2003	2 (Velescu, 2015; Llius-Ganella, 2012)
Laboratory Report Model	Nambi - 2013	Nambi - 2013
HellenicSCORE	Panagiotakos - 2007	1 (Georgousopoulou, 2015)
Reynolds Risk	Ridker - 2007	4 (Everett, 2015; Everett, 2014; Kim, 2014; Shah, 2014)
NL-SCORE	Smulders - 2008	1 (Van Dis, 2012)
Traditional Risk Factors	-	7 (Baber, 2015; Candell-Riera, 2013; Funke-Kaiser, 2014; Gardin, 2014; Kunutsor, 2014; Nielson, 2014; Nimomiya, 2013)
Framingham	Wilson - 1998	11 (Bérard, 2013; Britton, 2013; Fowkes, 2014; Gronewold, 2014; Kalsch, 2014; Lyngbaek, 2012; Mahabadi, 2015; Polak, 2015; Valentini, 2015; Weng, 2015; Zalawadiya, 2015)

S1 Text. Full list of included papers from the update

1. Abbasi A, Kieneker LM, Corpeleijn E, Gansevoort RT, Gans RO, Struck J, de Boer RA, Hillege HL, Stolk RP, Navis G and Bakker SJ. Plasma N-terminal Prosomatostatin and Risk of Incident Cardiovascular Disease and All-Cause Mortality in a Prospective Observational Cohort: the PREVEND Study. *Clin Chem*. 2017;63:278-287.
2. Abraham G, Havulinna AS, Bhalala OG, Byars SG, De Livera AM, Yetukuri L, Tikkanen E, Perola M, Schunkert H, Sijbrands EJ, Palotie A, Samani NJ, Salomaa V, Ripatti S and Inouye M. Genomic prediction of coronary heart disease. *Eur Heart J*. 2016;37:3267-3278.
3. Aijala M, Ronkainen J, Huusko T, Malo E, Savolainen ER, Savolainen MJ, Salonurmi T, Bloigu R, Kesaniemi YA and Ukkola O. The fat mass and obesity-associated (FTO) gene variant rs9939609 predicts long-term incidence of cardiovascular disease and related death independent of the traditional risk factors. *Ann Med*. 2015;47:655-63.
4. AlJaroudi WA, Alraies MC, Halley C, Menon V, Rodriguez LL, Grimm RA, Thomas JD and Jaber WA. Incremental prognostic value of diastolic dysfunction in low risk patients undergoing echocardiography: beyond Framingham score. *Int J Cardiovas Imag*. 2013;29:1441-1450.
5. Amato M, Veglia F, de Faire U, Giral P, Rauramaa R, Smit AJ, Kurl S, Ravani A, Frigerio B, Sansaro D, Bonomi A, Tedesco CC, Castelnuovo S, Mannarino E, Humphries SE, Hamsten A, Tremoli E, Baldassarre D and group Is. Carotid plaque-thickness and common carotid IMT show additive value in cardiovascular risk prediction and reclassification. *Atherosclerosis*. 2017;263:412-419.
6. Ambale-Venkatesh B, Yoneyama K, Sharma RK, Ohyama Y, Wu CO, Burke GL, Shea S, Gomes AS, Young AA, Bluemke DA and Lima JAC. Left ventricular shape predicts different types of cardiovascular events in the general population. *Heart*. 2017;103:507-515.
7. Andersson C, Enserro D, Larson MG, Xanthakis V and Vasan RS. Implications of the US cholesterol guidelines on eligibility for statin therapy in the community: comparison of observed and predicted risks in the Framingham Heart Study Offspring Cohort. *Journal of the American Heart Association*. 2015;4(4).
8. Armstrong AC, Liu K, Lewis CE, Sidney S, Colangelo LA, Kishi S, Ambale-Venkatesh B, Arynchyn A, Jacobs DR, Jr., Correia LC, Gidding SS and Lima JA. Left atrial dimension and traditional cardiovascular risk factors predict 20-year clinical cardiovascular events in young healthy adults: the CARDIA study. *Eur Heart J Cardiovasc Imaging*. 2014;15:893-9.
9. Artigao-Rodenas LM, Carbayo-Herencia JA, Divison-Garrote JA, Gil-Guillen VF, Masso-Orozco J, Simarro-Rueda M, Molina-Escribano F, Sanchis C, Carrion-Valero L, Lopez de Coca E, Caldevilla D, Lopez-Abril J, Carratala-Munuera C, Lopez-Pineda A and Grupo de Enfermedades Vasculares de A. Framingham risk score for prediction of cardiovascular diseases: a population-based study from southern Europe. *PLoS ONE*. 2013;8:e73529.

10. Artigao-Rodenas LM, Carbayo-Herencia JA, Palazon-Bru A, Divison-Garrote JA, Sanchis-Domenech C, Vigo-Aguiar I and Gil-Guillen VF. Construction and Validation of a 14-Year Cardiovascular Risk Score for Use in the General Population: The Puras-GEVA Chart. *Medicine*. 2015;94:e1980.
11. Astor BC, Shafi T, Hoogeveen RC, Matsushita K, Ballantyne CM, Inker LA and Coresh J. Novel Markers of Kidney Function as Predictors of ESRD, Cardiovascular Disease, and Mortality in the General Population. *Am J Kidney Dis*. 2012;59:653-662.
12. Baber U, Mehran R, Sartori S, Schoos MM, Sillesen H, Muntendam P, Garcia MJ, Gregson J, Pocock S, Falk E and Fuster V. Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: the BioImage study. *J Am Coll Cardiol*. 2015;65:1065-74.
13. Backholer K, Hirakawa Y, Tonkin A, Giles G, Magliano DJ, Colagiuri S, Harris M, Mitchell P, Nelson M, Shaw JE, Simmons D, Simons L, Taylor A, Harding J, Gopinath B and Woodward M. Development of an Australian cardiovascular disease mortality risk score using multiple imputation and recalibration from national statistics. *BMC Cardiovascular Disorders*. 2017;17:17.
14. Badheka AO, Patel NJ, Grover PM, Shah N, Singh V, Deshmukh A, Mehta K, Chothani A, Hoosien M, Rathod A, Savani GT, Marzouka GR, Gupta S, Mitrani RD, Moscucci M and Cohen MG. ST-T wave abnormality in lead aVR and reclassification of cardiovascular risk (from the National Health and Nutrition Examination Survey-III). *American Journal of Cardiology*. 2013;112:805-10.
15. Badheka AO, Singh V, Patel NJ, Deshmukh A, Shah N, Chothani A, Mehta K, Grover P, Savani GT, Gupta S, Rathod A, Marzouka GR, Mitrani RD, Moscucci M and Cohen MG. QRS duration on electrocardiography and cardiovascular mortality (from the National Health and Nutrition Examination Survey-III). *American Journal of Cardiology*. 2013;112:671-7.
16. Baena-Diez JM, Subirana I, Ramos R, Gomez de la Camara A, Elosua R, Vila J, Marin-Ibanez A, Guembe MJ, Rigo F, Tormo-Diaz MJ, Moreno-Iribas C, Cabre JJ, Segura A, Lapetra J, Quesada M, Medrano MJ, Gonzalez-Diego P, Frontera G, Gavrila D, Ardanaz E, Basora J, Garcia JM, Garcia-Lareo M, Gutierrez-Fuentes JA, Mayoral E, Sala J, I RD, Frances A, Castell C, Grau M and Marrugat J. Validity Assessment of Low-risk SCORE Function and SCORE Function Calibrated to the Spanish Population in the FRESCO Cohorts. *Rev Esp Cardiol (Engl Ed)*. 2017.
17. Bali V, Yermilov I, Coutts K and Legorreta AP. Novel screening metric for the identification of at-risk peripheral artery disease patients using administrative claims data. *Vascular Medicine*. 2016;21:33-40.

18. Barr EL, Reutens A, Magliano DJ, Wolfe R, Lu ZX, Sikaris KA, Tanamas SK, Atkins R, Chadban S, Shaw JE and Polkinghorne KR. Cystatin C estimated glomerular filtration rate and all-cause and cardiovascular disease mortality risk in the general population: AusDiab study. *Nephrology (Carlton)*. 2017;22:243-250.
19. Bellinazzi VR, Cipolli JA, Pimenta MV, Guimaraes PV, Pio-Magalhaes JA, Coelho-Filho OR, Biering-Sorensen T, Matos-Souza JR, Sposito AC and Nadruz W, Jr. Carotid flow velocity/diameter ratio is a predictor of cardiovascular events in hypertensive patients. *J Hypertens*. 2015;33:2054-60.
20. Berard E, Bongard V, Ruidavets JB, Amar J and Ferrieres J. Pulse wave velocity, pulse pressure and number of carotid or femoral plaques improve prediction of cardiovascular death in a population at low risk. *J Hum Hypertens*. 2013;27:529-534.
21. Berard E, Seguro F, Bongard V, Dallongeville J, Arveiler D, Amouyel P, Wagner A, Ruidavets JB and Ferrieres J. Predictive Accuracy of the European Society of Cardiology SCORE Among French People. *Journal of Cardiopulmonary Rehabilitation & Prevention*. 2016;36:38-48.
22. Biering-Sorensen T, Biering-Sorensen SR, Olsen FJ, Sengelov M, Jorgensen PG, Mogelvang R, Shah AM and Jensen JS. Global Longitudinal Strain by Echocardiography Predicts Long-Term Risk of Cardiovascular Morbidity and Mortality in a Low-Risk General Population: The Copenhagen City Heart Study. *Circ Cardiovasc Imaging*. 2017;10.
23. Biering-Sorensen T, Mogelvang R, Schnohr P and Jensen JS. Cardiac Time Intervals Measured by Tissue Doppler Imaging M-mode: Association With Hypertension, Left Ventricular Geometry, and Future Ischemic Cardiovascular Diseases. *Journal of the American Heart Association*. 2016;5.
24. Blaha MJ, Budoff MJ, Tota-Maharaj R, Dardari ZA, Wong ND, Kronmal RA, Eng J, Post WS, Blumenthal RS and Nasir K. Improving the CAC Score by Addition of Regional Measures of Calcium Distribution: Multi-Ethnic Study of Atherosclerosis. *JACC Cardiovasc Imaging*. 2016;9:1407-1416.
25. Blankenberg S, Salomaa V, Makarova N, Ojeda F, Wild P, Lackner KJ, Jorgensen T, Thorand B, Peters A, Nauck M, Petersmann A, Vartiainen E, Veronesi G, Brambilla P, Costanzo S, Iacoviello L, Linden G, Yarnell J, Patterson CC, Everett BM, Ridker PM, Kontto J, Schnabel RB, Koenig W, Kee F, Zeller T, Kuulasmaa K and Investigators B. Troponin I and cardiovascular risk prediction in the general population: the BiomarCaRE consortium. *Eur Heart J*. 2016;37:2428-+.
26. Borglykke A, Andreasen AH, Kuulasmaa K, Sans S, Ducimetiere P, Vanuzzo D, Ferrario MM, Palmieri L, Karvanen J, Tunstall-Pedoe H, Jorgensen T and Project M. Stroke risk estimation across nine European countries in the MORGAM project. *Heart*. 2010;96:1997-2004.
27. Bos D, Ikram MA, Leening MJG and Ikram MK. The Revised Framingham Stroke Risk Profile in a Primary Prevention Population: The Rotterdam Study. *Circulation*. 2017;135:2207-2209.

28. Bozorgmanesh M, Sardarinia M, Hajsheikholeslami F, Azizi F and Hadaegh F. CVD-predictive performances of "a body shape index" versus simple anthropometric measures: Tehran lipid and glucose study. *Eur J Nutr*. 2016;55:147-157.
29. Britton KA, Massaro JM, Murabito JM, Kreger BE, Hoffmann U and Fox CS. Body fat distribution, incident cardiovascular disease, cancer, and all-cause mortality. *J Am Coll Cardiol*. 2013;62:921-5.
30. Brouwers FP, Asselbergs FW, Hillege HL, Gansevoort RT, de Boer RA and van Gilst WH. Elevated urinary albumin excretion complements the Framingham Risk Score for the prediction of cardiovascular risk - response to treatment in the PREVEND IT trial. *IJC Heart and Vessels*. 2014;4:193-197.
31. Brownrigg JR, Hughes CO, Burleigh D, Karthikesalingam A, Patterson BO, Holt PJ, Thompson MM, de Lusignan S, Ray KK and Hinchliffe RJ. Microvascular disease and risk of cardiovascular events among individuals with type 2 diabetes: a population-level cohort study. *Lancet Diabetes Endocrinol*. 2016;4:588-97.
32. Brutsaert EF, Shitole S, Biggs ML, Mukamal KJ, deBoer IH, Thacker EL, Barzilay JI, Djousse L, Ix JH, Smith NL, Kaplan RC, Siscovick DS, Psaty BM and Kizer JR. Relations of Postload and Fasting Glucose With Incident Cardiovascular Disease and Mortality Late in Life: The Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci*. 2016;71:370-7.
33. Bye A, Rosjo H, Nauman J, Silva GJ, Follestad T, Omland T and Wisloff U. Circulating microRNAs predict future fatal myocardial infarction in healthy individuals - The HUNT study. *J Mol Cell Cardiol*. 2016;97:162-8.
34. Candell-Riera J, Ferreira-Gonzalez I, Marsal JR, Aguade-Bruix S, Cuberas-Borros G, Pujol P, Romero-Farina G, Nazarena-Pizzi M, de Leon G, Castell-Conesa J and Garcia-Dorado D. Usefulness of exercise test and myocardial perfusion-gated single photon emission computed tomography to improve the prediction of major events. *Circ Cardiovasc Imaging*. 2013;6:531-41.
35. Chahal H, Bluemke DA, Wu CO, McClelland R, Liu K, Shea SJ, Burke G, Balfour P, Herrington D, Shi P, Post W, Olson J, Watson KE, Folsom AR and Lima JA. Heart failure risk prediction in the Multi-Ethnic Study of Atherosclerosis. *Heart*. 2015;101:58-64.
36. Chambless LE, Folsom AR, Sharrett AR, Sorlie P, Couper D, Szklo M and Nieto FJ. Coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study. *J Clin Epidemiol*. 2003;56:880-90.
37. Chamnan P, Simmons RK, Sharp SJ, Khaw KT, Wareham NJ and Griffin SJ. Repeat Cardiovascular Risk Assessment after Four Years: Is There Improvement in Risk Prediction? *PLoS One*. 2016;11:e0147417.

38. Chang X, Salim A, Dorajoo R, Han Y, Khor CC, van Dam RM, Yuan JM, Koh WP, Liu J, Goh DY, Wang X, Teo YY, Friedlander Y and Heng CK. Utility of genetic and non-genetic risk factors in predicting coronary heart disease in Singaporean Chinese. *Eur J Prev Cardiol*. 2017;24:153-160.
39. Chia YC, Gray SYW, Ching SM, Lim HM and Chinna K. Validation of the Framingham general cardiovascular risk score in a multiethnic Asian population: a retrospective cohort study. *Bmj Open*. 2015;5.
40. Chia YC, Lim HM and Ching SM. Validation of the pooled cohort risk score in an Asian population - a retrospective cohort study. *BMC Cardiovascular Disorders*. 2014;14.
41. Chiuve SE, Cook NR, Shay CM, Rexrode KM, Albert CM, Manson JE, Willett WC and Rimm EB. Lifestyle-based prediction model for the prevention of CVD: the Healthy Heart Score. *Journal of the American Heart Association*. 2014;3:e000954.
42. Cho I, Chang HJ, B OH, Shin S, Sung JM, Lin FY, Achenbach S, Heo R, Berman DS, Budoff MJ, Callister TQ, Al-Mallah MH, Cademartiri F, Chinnaiyan K, Chow BJ, Dunning AM, DeLago A, Villines TC, Hadamitzky M, Hausleiter J, Leipsic J, Shaw LJ, Kaufmann PA, Cury RC, Feuchtner G, Kim YJ, Maffei E, Raff G, Pontone G, Andreini D and Min JK. Incremental prognostic utility of coronary CT angiography for asymptomatic patients based upon extent and severity of coronary artery calcium: results from the COronary CT Angiography EvaluatioN For Clinical Outcomes InteRnational Multicenter (CONFIRM) study. *Eur Heart J*. 2015;36:501-8.
43. Christophersen IE, Yin X, Larson MG, Lubitz SA, Magnani JW, McManus DD, Ellinor PT and Benjamin EJ. A comparison of the CHARGE-AF and the CHA2DS2-VASc risk scores for prediction of atrial fibrillation in the Framingham Heart Study. *American Heart Journal*. 2016;178:45-54.
44. Colantonio LD, Richman JS, Carson AP, Lloyd-Jones DM, Howard G, Deng L, Howard VJ, Safford MM, Muntner P and Goff DC, Jr. Performance of the Atherosclerotic Cardiovascular Disease Pooled Cohort Risk Equations by Social Deprivation Status. *Journal of the American Heart Association*. 2017;6.
45. Cook NR and Ridker PM. Further Insight Into the Cardiovascular Risk Calculator The Roles of Statins, Revascularizations, and Underascertainment in the Women's Health Study. *Jama Intern Med*. 2014;174:1964-1971.
46. Cooney MT, Vartiainen E, Laatikainen T, De Bacquer D, McGorrian C, Dudina A, Graham I, Score and investigators F. Cardiovascular risk age: concepts and practicalities. *Heart*. 2012;98:941-6.
47. Criqui MH, Denenberg JO, Ix JH, McClelland RL, Wassel CL, Rifkin DE, Carr JJ, Budoff MJ and Allison MA. Calcium density of coronary artery plaque and risk of incident cardiovascular events. *JAMA*. 2014;311:271-8.

48. Cross DS, McCarty CA, Steinhubl SR, Carey DJ and Erlich PM. Development of a multi-institutional cohort to facilitate cardiovascular disease biomarker validation using existing biorepository samples linked to electronic health records. *Clinical Cardiology*. 2013;36:486-91.
49. Daniels LB, Clopton P, deFilippi CR, Sanchez OA, Bahrami H, Lima JA, Tracy RP, Siscovick D, Bertoni AG, Greenland P, Cushman M, Maisel AS and Criqui MH. Serial measurement of N-terminal pro-B-type natriuretic peptide and cardiac troponin T for cardiovascular disease risk assessment in the Multi-Ethnic Study of Atherosclerosis (MESA). *American Heart Journal*. 2015;170:1170-1183.
50. De Las Heras Gala T, Geisel MH, Peters A, Thorand B, Baumert J, Lehmann N, Jockel KH, Moebus S, Erbel R, Mahabadi AA, Koenig W, Heinrich J, Holle R, Leidl R, Meisinger C, Strauch K, Roggenbuck U, Slomiany U, Beck EM, Offner A, Munkel S, Bauer M, Schrader S, Peter R and Hirche H. Recalibration of the ACC/AHA risk score in two population-based German cohorts. *PLoS ONE*. 2016;11:e0164688.
51. de Lemos JA, Ayers CR, Levine B, deFilippi CR, Wang TJ, Hundley WG, Berry JD, Seliger SL, McGuire DK, Ouyang P, Drazner MH, Budoff M, Greenland P, Ballantyne CM and Khera A. Multimodality Strategy for Cardiovascular Risk Assessment: Performance in 2 Population-Based Cohorts. *Circulation*. 2017;135:2119-2132.
52. De Vito KM, Baer HJ, Dart H, Chiuve SE, Rimm EB and Colditz GA. Validation of a risk prediction tool for coronary heart disease in middle-aged women. *BMC Womens Health*. 2015;15:101.
53. DeFilippis AP, Young R and Blaha MJ. Calibration and Discrimination Among Multiple Cardiovascular Risk Scores in a Modern Multiethnic Cohort RESPONSE. *Ann Intern Med*. 2015;163:68-69.
54. DeFilippis AP, Young R, McEvoy JW, Michos ED, Sandfort V, Kronmal RA, McClelland RL and Blaha MJ. Risk score overestimation: the impact of individual cardiovascular risk factors and preventive therapies on the performance of the American Heart Association-American College of Cardiology-Atherosclerotic Cardiovascular Disease risk score in a modern multi-ethnic cohort. *Eur Heart J*. 2017;38:598-608.
55. Deo R, Norby FL, Katz R, Sotoodehnia N, Adabag S, DeFilippi CR, Kestenbaum B, Chen LY, Heckbert SR, Folsom AR, Kronmal RA, Konety S, Patton KK, Siscovick D, Shlipak MG and Alonso A. Development and Validation of a Sudden Cardiac Death Prediction Model for the General Population. *Circulation*. 2016;134:806-16.
56. Desai CS, Bartz TM, Gottdiener JS, Lloyd-Jones DM and Gardin JM. Usefulness of Left Ventricular Mass and Geometry for Determining 10-Year Prediction of Cardiovascular Disease in Adults Aged >65 Years (from the Cardiovascular Health Study). *American Journal of Cardiology*. 2016;118:684-90.

57. Dhoble A, Lahr BD, Allison TG, Bailey KR, Thomas RJ, Lopez-Jimenez F, Kullo IJ, Gupta B and Kopecky SL. Predicting long-term cardiovascular risk using the mayo clinic cardiovascular risk score in a referral population. *American Journal of Cardiology*. 2014;114:704-10.

58. Di Angelantonio E, Gao P, Khan H, Butterworth AS, Wormser D, Kaptoge S, Kondapally Seshasai SR, Thompson A, Sarwar N, Willeit P, Ridker PM, Barr ELM, Khaw KT, Psaty BM, Brenner H, Balkau B, Dekker JM, Lawlor DA, Daimon M, Willeit J, Njolstad I, Nissinen A, Brunner EJ, Kuller LH, Price JF, Sundstrom J, Knuiman MW, Feskens EJM, Verschuren WMM, Wald N, Bakker SJL, Whincup PH, Ford I, Goldbourt U, Gomez-de-la-Camara A, Gallacher J, Simons LA, Rosengren A, Sutherland SE, Bjorkelund C, Blazer DG, Wassertheil-Smoller S, Onat A, Marin Ibanez A, Casiglia E, Jukema JW, Simpson LM, Giampaoli S, Nordestgaard BG, Selmer R, Wennberg P, Kauhanen J, Salonen JT, Dankner R, Barrett-Connor E, Kavousi M, Gudnason V, Evans D, Wallace RB, Cushman M, D'Agostino RB, Umans JG, Kiyohara Y, Nakagawa H, Sato S, Gillum RF, Folsom AR, van der Schouw YT, Moons KG, Griffin SJ, Sattar N, Wareham NJ, Selvin E, Thompson SG and Danesh J. Glycated hemoglobin measurement and prediction of cardiovascular disease. *JAMA - Journal of the American Medical Association*. 2014;311:1225-1233.

59. Dufouil C, Beiser A, McLure LA, Wolf PA, Tzourio C, Howard VJ, Westwood AJ, Himali JJ, Sullivan L, Aparicio HJ, Kelly-Hayes M, Ritchie K, Kase CS, Pikula A, Romero JR, D'Agostino RB, Samieri C, Vasan RS, Chene G, Howard G and Seshadri S. Revised Framingham Stroke Risk Profile to Reflect Temporal Trends. *Circulation*. 2017;135:1145-1159.

60. Eggers KM, Kempf T, Larsson A, Lindahl B, Venge P, Wallentin L, Wollert KC and Lind L. Evaluation of Temporal Changes in Cardiovascular Biomarker Concentrations Improves Risk Prediction in an Elderly Population from the Community. *Clin Chem*. 2016;62:485-93.

61. Emdin CA, Khera AV, Natarajan P, Klarin D, Baber U, Mehran R, Rader DJ, Fuster V and Kathiresan S. Evaluation of the Pooled Cohort Equations for Prediction of Cardiovascular Risk in a Contemporary Prospective Cohort. *American Journal of Cardiology*. 2017;119:881-885.

62. Evans TE, O'Sullivan MJ, de Groot M, Niessen WJ, Hofman A, Krestin GP, van der Lugt A, Portegies ML, Koudstaal PJ, Bos D, Vernooij MW and Ikram MA. White Matter Microstructure Improves Stroke Risk Prediction in the General Population. *Stroke*. 2016;47:2756-2762.

63. Everett BM, Berger JS, Manson JE, Ridker PM and Cook NR. B-type natriuretic peptides improve cardiovascular disease risk prediction in a cohort of women. *J Am Coll Cardiol*. 2014;64:1789-97.

64. Everett BM, Ridker PM, Cook NR and Pradhan AD. Usefulness of B-type Natriuretic Peptides to Predict Cardiovascular Events in Women (from the Women's Health Study). *American Journal of Cardiology*. 2015;116:532-7.

65. Faeh D, Rohrmann S and Braun J. Better risk assessment with glycated hemoglobin instead of cholesterol in CVD risk prediction charts. *Eur J Epidemiol*. 2013;28:551-5.
66. Fatema K, Rahman B, Zwar NA, Milton AH and Ali L. Short-term predictive ability of selected cardiovascular risk prediction models in a rural Bangladeshi population: a case-cohort study. *BMC Cardiovascular Disorders*. 2016;16:105.
67. Ferrario MM, Veronesi G, Chambless LE, Tunstall-Pedoe H, Kuulasmaa K, Salomaa V, Borglykke A, Hart N, Soderberg S, Cesana G and Project M. The contribution of educational class in improving accuracy of cardiovascular risk prediction across European regions: The MORGAM Project Cohort Component. *Heart*. 2014;100:1179-87.
68. Fogacci F, Cicero AF, D'Addato S, D'Agostini L, Rosticci M, Giovannini M, Bertagnin E, Borghi C and Brisighella Heart Study G. Serum lipoprotein(a) level as long-term predictor of cardiovascular mortality in a large sample of subjects in primary cardiovascular prevention: data from the Brisighella Heart Study. *Eur J Intern Med*. 2017;37:49-55.
69. Foraker RE, Greiner M, Sims M, Tucker KL, Towfighi A, Bidulescu A, Shoben AB, Smith S, Talegawkar S, Blackshear C, Wang W, Hardy NC and O'Brien E. Comparison of risk scores for the prediction of stroke in African Americans: Findings from the Jackson Heart Study. *Am Heart J*. 2016;177:25-32.
70. Fowkes FG, Murray GD, Butcher I, Folsom AR, Hirsch AT, Couper DJ, Debacker G, Kornitzer M, Newman AB, Sutton-Tyrrell KC, Cushman M, Lee AJ, Price JF, D'Agostino RB, Sr., Murabito JM, Norman P, Masaki KH, Bouter LM, Heine RJ, Stehouwer CD, McDermott MM, Stoffers HE, Knottnerus JA, Ogren M, Hedblad B, Koenig W, Meisinger C, Cauley JA, Franco O, Hunink MG, Hofman A, Witteman JC, Criqui MH, Langer RD, Hiatt WR, Hamman RF and Ankle Brachial Index C. Development and validation of an ankle brachial index risk model for the prediction of cardiovascular events. *Eur J Prev Cardiolog*. 2014;21:310-20.
71. Fox ER, Samdarshi TE, Musani SK, Pencina MJ, Sung JH, Bertoni AG, Xanthakis V, Balfour PC, Jr., Shreenivas SS, Covington C, Liebson PR, Sarpong DF, Butler KR, Mosley TH, Rosamond WD, Folsom AR, Herrington DM, Vasan RS and Taylor HA. Development and Validation of Risk Prediction Models for Cardiovascular Events in Black Adults: The Jackson Heart Study Cohort. *JAMA Cardiol*. 2016;1:15-25.
72. Funke-Kaiser A, Havulinna AS, Zeller T, Appelbaum S, Jousilahti P, Vartiainen E, Blankenberg S, Sydow K and Salomaa V. Predictive value of midregional pro-adrenomedullin compared to natriuretic peptides for incident cardiovascular disease and heart failure in the population-based FINRISK 1997 cohort. *Annals of Medicine*. 2014;46:155-162.

73. Gaibazzi N, Rigo F, Facchetti R, Carerj S, Giannattasio C, Moreo A, Mureddu G, Paini A, Grolla E, Faden G, Cesana F and Faggiano P. Ultrasound carotid intima-media thickness, carotid plaque and cardiac calcium incrementally add to the Framingham Risk Score for the prediction of angiographic coronary artery disease: A multicenter prospective study. *International Journal of Cardiology*. 2014;177:708-710.

74. Garcia-Gil M, Parramon D, Comas-Cufi M, Marti R, Ponjoan A, Alves-Cabratosa L, Blanch J, Petersen I, Elosua R, Grau M, Salvador B and Ramos R. Role of renal function in cardiovascular risk assessment: A retrospective cohort study in a population with low incidence of coronary heart disease. *Prev Med*. 2016;89:200-6.

75. Gardin JM, Bartz TM, Polak JF, O'Leary DH and Wong ND. What do carotid intima-media thickness and plaque add to the prediction of stroke and cardiovascular disease risk in older adults? The cardiovascular health study. *J Am Soc Echocardiogr*. 2014;27:998-1005 e2.

76. Georgousopoulou EN, Panagiotakos DB, Bougatsas D, Chatzigeorgiou M, Kavouras SA, Chrysohoou C, Skoumas I, Tousoulis D, Stefanadis C and Pitsavos C. Physical Activity Level Improves the Predictive Accuracy of Cardiovascular Disease Risk Score: The ATTICA Study (2002-2012). *Int J Prev Med*. 2016;7:52.

77. Georgousopoulou EN, Panagiotakos DB, Pitsavos C, Stefanadis C and group As. Assessment of diet quality improves the classification ability of cardiovascular risk score in predicting future events: The 10-year follow-up of the ATTICA study (2002-2012). *Eur J Prev Cardiol*. 2015;22:1488-98.

78. Gibson AO, Blaha MJ, Arnan MK, Sacco RL, Szklo M, Herrington DM and Yeboah J. Coronary artery calcium and incident cerebrovascular events in an asymptomatic cohort. The MESA Study. *JACC Cardiovasc Imaging*. 2014;7:1108-15.

79. Goff DC, Jr., Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Sr., Gibbons R, Greenland P, Lackland DT, Levy D, O'Donnell CJ, Robinson JG, Schwartz JS, Shero ST, Smith SC, Jr., Sorlie P, Stone NJ, Wilson PW and American College of Cardiology/American Heart Association Task Force on Practice G. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2935-59.

80. Goh LG, Dhaliwal SS, Welborn TA, Lee AH and Della PR. Anthropometric measurements of general and central obesity and the prediction of cardiovascular disease risk in women: a cross-sectional study. *BMJ Open*. 2014;4:e004138.

81. Goh LG, Welborn TA and Dhaliwal SS. Independent external validation of cardiovascular disease mortality in women utilising Framingham and SCORE risk models: a mortality follow-up study. *BMC Womens Health*. 2014;14:118.

82. Grau M, Subirana I, Vila J, Elosua R, Ramos R, Sala J, Degano IR, Tresserras R, Bielsa O and Marrugat J. Validation of a population coronary disease predictive system: the CASSANDRA model. *J Epidemiol Community Health*. 2014;68:1012-9.

83. Graversen P, Abildstrom SZ, Jespersen L, Borglykke A and Prescott E. Cardiovascular risk prediction: Can Systematic Coronary Risk Evaluation (SCORE) be improved by adding simple risk markers? Results from the Copenhagen City Heart Study. *Eur J Prev Cardiol*. 2016;23:1546-56.

84. Greve SV, Blicher MK, Kruger R, Sehestedt T, Gram-Kampmann E, Rasmussen S, Vishram JKK, Boutouyrie P, Laurent S and Olsen MH. Estimated carotid-femoral pulse wave velocity has similar predictive value as measured carotid-femoral pulse wave velocity. *J Hypertens*. 2016;34:1279-1289.

85. Gronewold J, Hermann DM, Lehmann N, Kroger K, Lauterbach K, Berger K, Weimar C, Kalsch HI, Moebus S, Jockel KH, Bauer M, Erbel R and Heinz Nixdorf Recall Study Investigative G. Ankle-brachial index predicts stroke in the general population in addition to classical risk factors. *Atherosclerosis*. 2014;233:545-50.

86. Groot A, Bots ML, Rutten FH, den Ruijter HM, Numans ME and Vaartjes I. Measurement of ECG abnormalities and cardiovascular risk classification: a cohort study of primary care patients in the Netherlands. *Br J Gen Pract*. 2015;65:e1-8.

87. Guarrera S, Fiorito G, Onland-Moret NC, Russo A, Agnoli C, Allione A, Di Gaetano C, Mattiello A, Ricceri F, Chiodini P, Polidoro S, Frasca G, Verschuren MWM, Boer JMA, Iacoviello L, van der Schouw YT, Tumino R, Vineis P, Krogh V, Panico S, Sacerdote C and Matullo G. Gene-specific DNA methylation profiles and LINE-1 hypomethylation are associated with myocardial infarction risk. *Clin Epigenetics*. 2015;7:133.

88. Gullu O, Tekindal MA, Ates C, Ekici B and Yavuz Y. Assessing the classification performance of the mean platelet volume (MPV) in a cardiovascular risk prediction model. *Biomedical Research (India)*. 2017;28:705-710.

89. Hadamitzky M, Achenbach S, Al-Mallah M, Berman D, Budoff M, Cademartiri F, Callister T, Chang HJ, Cheng V, Chinnaiyan K, Chow BJW, Cury R, Delago A, Dunning A, Feuchtner G, Gomez M, Kaufmann P, Kim YJ, Leipsic J, Lin FY, Maffei E, Min JK, Raff G, Shaw LJ, Villines TC, Hausleiter J and Investigators C. Optimized Prognostic Score for Coronary Computed Tomographic Angiography Results From the CONFIRM Registry (COronary CT Angiography EvaluatioN For Clinical Outcomes: An InteRnational Multicenter Registry). *Journal of the American College of Cardiology*. 2013;62:468-476.

90. Hajifathalian K, Ueda P, Lu Y, Woodward M, Ahmadvand A, Aguilar-Salinas CA, Azizi F, Cifkova R, Di Cesare M, Eriksen L, Farzadfar F, Ikeda N, Khalili D, Khang YH, Lanska V, Leon-Munoz L, Magliano D, Msyamboza KP, Oh K, Rodriguez-Artalejo F, Rojas-Martinez R, Shaw JE, Stevens GA, Tolstrup J, Zhou B, Salomon JA, Ezzati M and Danaei G. A novel risk score to predict cardiovascular disease risk in national populations (Globorisk): a pooled analysis of prospective cohorts and health examination surveys. *Lancet Diabetes Endocrinol*. 2015;3:339-55.
91. Hartaigh BO, Valenti V, Cho I, Schulman-Marcus J, Gransar H, Knapper J, Kelkar AA, Xie JX, Chang HJ, Shaw LJ, Callister TQ and Min JK. 15-Year prognostic utility of coronary artery calcium scoring for all-cause mortality in the elderly. *Atherosclerosis*. 2016;246:361-6.
92. Hensley WJ and Mansfield CH. Lipoproteins, atherogenicity, age and risk of myocardial infarction. *Australian and New Zealand Journal of Public Health*. 1999;23:174-178.
93. Hippisley-Cox J, Coupland C and Brindle P. Derivation and validation of QStroke score for predicting risk of ischaemic stroke in primary care and comparison with other risk scores: a prospective open cohort study. *BMJ*. 2013;346:f2573.
94. Hippisley-Cox J, Coupland C and Brindle P. The performance of seven QPrediction risk scores in an independent external sample of patients from general practice: a validation study. *BMJ Open*. 2014;4:e005809.
95. Hippisley-Cox J, Coupland C and Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *BMJ*. 2017;357:j2099.
96. Ho JE, Enserro D, Brouwers FP, Kizer JR, Shah SJ, Psaty BM, Bartz TM, Santhanakrishnan R, Lee DS, Chan C, Liu K, Blaha MJ, Hillege HL, van der Harst P, van Gilst WH, Kop WJ, Gansevoort RT, Vasan RS, Gardin JM, Levy D, Gottdiener JS, de Boer RA and Larson MG. Predicting Heart Failure With Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Subtypes. *Circulation: Heart Failure*. 2016;9(6).
97. Hoffmann U, Massaro JM, D'Agostino RB, Sr., Kathiresan S, Fox CS and O'Donnell CJ. Cardiovascular Event Prediction and Risk Reclassification by Coronary, Aortic, and Valvular Calcification in the Framingham Heart Study. *Journal of the American Heart Association*. 2016;5.
98. Horne BD, Anderson JL, Muhlestein JB, Ridker PM and Paynter NP. Complete blood count risk score and its components, including RDW, are associated with mortality in the JUPITER trial. *Eur J Prev Cardiol*. 2015;22:519-526.

99. Hoshide S, Yano Y, Haimoto H, Yamagiwa K, Uchiba K, Nagasaka S, Matsui Y, Nakamura A, Fukutomi M, Eguchi K, Ishikawa J, Kario K and Group JHS. Morning and Evening Home Blood Pressure and Risks of Incident Stroke and Coronary Artery Disease in the Japanese General Practice Population: The Japan Morning Surge-Home Blood Pressure Study. *Hypertension*. 2016;68:54-61.

100. Howard G, McClure LA, Moy CS, Howard VJ, Judd SE, Yuan Y, Long DL, Muntner P, Safford MM and Kleindorfer DO. Self-Reported Stroke Risk Stratification: Reasons for Geographic and Racial Differences in Stroke Study. *Stroke*. 2017;48:1737-1743.

101. Htun NM, Magliano DJ, Zhang ZY, Lyons J, Petit T, Nkuipou-Kenfack E, Ramirez-Torres A, von Zur Muhlen C, Maahs D, Schanstra JP, Pontillo C, Pejchinovski M, Snell-Bergeon JK, Delles C, Mischak H, Staessen JA, Shaw JE, Koeck T and Peter K. Prediction of acute coronary syndromes by urinary proteome analysis. *PLoS One*. 2017;12:e0172036.

102. Hu G, Root M and Duncan AW. Adding multiple risk factors improves Framingham coronary heart disease risk scores. *Vasc Health Risk Manag*. 2014;10:557-62.

103. Hwang YC, Park CY, Ahn HY and Cho NH. Prediction of future development of cardiovascular disease with an equation to estimate apolipoprotein B: A community-based cohort study. *Medicine*. 2016;95:e3644.

104. Inzhakova G, Zhou H, Morris M, Early MI, Xiang AH, Jacobsen SJ and Derose SF. Potential of Risk-Based Population Guidelines to Reduce Cardiovascular Risk in a Large Integrated Health System. *Am J Manag Care*. 2016;22:E161-+.

105. Iribarren C, Chandra M, Rana JS, Hlatky MA, Fortmann SP, Quertermous T and Go AS. High-sensitivity cardiac troponin I and incident coronary heart disease among asymptomatic older adults. *Heart*. 2016;102:1177-82.

106. Iribarren C, Lu M, Jorgenson E, Martinez M, Lluis-Ganella C, Subirana I, Salas E and Elosua R. Clinical Utility of Multimarker Genetic Risk Scores for Prediction of Incident Coronary Heart Disease: A Cohort Study Among Over 51 Thousand Individuals of European Ancestry. *Circ Cardiovasc Genet*. 2016;9:531-540.

107. Israel A, Kivity S, Sidi Y, Segev S, Berkovitch A, Klempfner R, Lavi B, Goldenberg I and Maor E. Use of exercise capacity to improve SCORE risk prediction model in asymptomatic adults. *Eur Heart J*. 2016;37:2300-6.

108. Jairam PM, de Jong PA, Mali WP, Isgum I, van der Graaf Y and study-group P. Cardiovascular disease prediction: do pulmonary disease-related chest CT features have added value? *Eur Radiol*. 2015;25:1646-54.

109. Jdanov DA, Deev AD, Jasilionis D, Shalnova SA, Shkolnikova MA and Shkolnikov VM. Recalibration of the SCORE risk chart for the Russian population. *Eur J Epidemiol*. 2014;29:621-628.

110. Jee SH, Jang Y, Oh DJ, Oh BH, Lee SH, Park SW, Seung KB, Mok Y, Jung KJ, Kimm H, Yun YD, Baek SJ, Lee DC, Choi SH, Kim MJ, Sung J, Cho B, Kim ES, Yu BY, Lee TY, Kim JS, Lee YJ, Oh JK, Kim SH, Park JK, Koh SB, Park SB, Lee SY, Yoo CI, Kim MC, Kim HK, Park JS, Kim HC, Lee GJ and Woodward M. A coronary heart disease prediction model: the Korean Heart Study. *BMJ Open*. 2014;4:e005025.

111. Johansson JK, Puukka PJ, Niiranen TJ, Varis J, Peltonen M, Salomaa V and Jula AM. Health 2000 score - development and validation of a novel cardiovascular risk score. *Ann Med*. 2016;48:403-409.

112. Jorstad HT, Boekholdt SM, Wareham NJ, Khaw KT and Peters RJ. The Dutch SCORE-based risk charts seriously underestimate the risk of cardiovascular disease. *Netherlands Heart Journal*. 2017;25:173-180.

113. Jorstad HT, Colkesen EB, Minneboo M, Peters RJ, Boekholdt SM, Tijssen JG, Wareham NJ and Khaw KT. The Systematic COronary Risk Evaluation (SCORE) in a large UK population: 10-year follow-up in the EPIC-Norfolk prospective population study. *Eur J Prev Cardiol*. 2015;22:119-26.

114. Jung CH, Lee MJ, Kang YM, Yang DH, Kang JW, Kim EH, Park DW, Park JY, Kim HK and Lee WJ. 2013 ACC/AHA versus 2004 NECP ATP III Guidelines in the Assignment of Statin Treatment in a Korean Population with Subclinical Coronary Atherosclerosis. *PLoS ONE [Electronic Resource]*. 2015;10:e0137478.

115. Jung KJ, Jang Y, Oh DJ, Oh BH, Lee SH, Park SW, Seung KB, Kim HK, Yun YD, Choi SH, Sung J, Lee TY, Kim SH, Koh SB, Kim MC, Chang Kim H, Kimm H, Nam C, Park S and Jee SH. The ACC/AHA 2013 pooled cohort equations compared to a Korean Risk Prediction Model for atherosclerotic cardiovascular disease. *Atherosclerosis*. 2015;242:367-75.

116. Kaess BM, de Las Heras Gala T, Zierer A, Meisinger C, Wahl S, Peters A, Todd J, Herder C, Huth C, Thorand B and Koenig W. Ultra-sensitive troponin I is an independent predictor of incident coronary heart disease in the general population. *Eur J Epidemiol*. 2017;32:583-591.

117. Kalsch H, Lehmann N, Mahabadi AA, Bauer M, Kara K, Huppe P, Moebus S, Mohlenkamp S, Dragano N, Schmermund A, Stang A, Jockel KH, Erbel R and Investigator Group of the Heinz Nixdorf Recall S. Beyond Framingham risk factors and coronary calcification: does aortic valve calcification improve risk prediction? The Heinz Nixdorf Recall Study. *Heart*. 2014;100:930-7.

118. Kanjilal S, Rao VS, Mukherjee M, Natesha BK, Renuka KS, Sibi K, Iyengar SS and Kakkar VV. Application of cardiovascular disease risk prediction models and the relevance of novel biomarkers to risk stratification in Asian Indians. *Vasc Health Risk Manag*. 2008;4:199-211.

119. Karas MG, Devereux RB, Wiebers DO, Whisnant JP, Best LG, Lee ET, Howard BV, Roman MJ, Umans JG and Kizer JR. Incremental value of biochemical and echocardiographic measures in prediction of ischemic stroke: the Strong Heart Study. *Stroke*. 2012;43:720-6.

120. Karjalainen T, Adiels M, Bjorck L, Cooney MT, Graham I, Perk J, Rosengren A, Soderberg S and Eliasson M. An evaluation of the performance of SCORE Sweden 2015 in estimating cardiovascular risk: The Northern Sweden MONICA Study 1999-2014. *Eur J Prev Cardiol*. 2017;24:103-110.

121. Karmali KN, Ning H, Goff DC and Lloyd-Jones DM. Identifying Individuals at Risk for Cardiovascular Events Across the Spectrum of Blood Pressure Levels. *Journal of the American Heart Association*. 2015;4:e002126.

122. Kavousi M, Desai CS, Ayers C, Blumenthal RS, Budoff MJ, Mahabadi AA, Ikram MA, van der Lugt A, Hofman A, Erbel R, Khera A, Geisel MH, Jockel KH, Lehmann N, Hoffmann U, O'Donnell CJ, Massaro JM, Liu K, Mohlenkamp S, Ning H, Franco OH and Greenland P. Prevalence and Prognostic Implications of Coronary Artery Calcification in Low-Risk Women: A Meta-analysis. *JAMA*. 2016;316:2126-2134.

123. Kavousi M, Leening MJ, Nanchen D, Greenland P, Graham IM, Steyerberg EW, Ikram MA, Stricker BH, Hofman A and Franco OH. Comparison of application of the ACC/AHA guidelines, Adult Treatment Panel III guidelines, and European Society of Cardiology guidelines for cardiovascular disease prevention in a European cohort. *JAMA*. 2014;311:1416-23.

124. Khalili D, Asgari S, Hadaegh F, Steyerberg EW, Rahimi K, Fahimfar N and Azizi F. A new approach to test validity and clinical usefulness of the 2013 ACC/AHA guideline on statin therapy: A population-based study. *Int J Cardiol*. 2015;184:587-94.

125. Kim TH, Choi HS, Bae JC, Moon JH, Kim HK, Choi SH, Lim S, Park DJ, Park KS, Jang HC, Lee MK, Cho NH and Park YJ. Subclinical hypothyroidism in addition to common risk scores for prediction of cardiovascular disease: a 10-year community-based cohort study. *Eur J Endocrinol*. 2014;171:649-57.

126. Konety SH, Koene RJ, Norby FL, Wilsdon T, Alonso A, Siscovick D, Sotoodehnia N, Gottdiener J, Fox ER, Chen LY, Adabag S and Folsom AR. Echocardiographic Predictors of Sudden Cardiac Death: The Atherosclerosis Risk in Communities Study and Cardiovascular Health Study. *Circ Cardiovasc Imaging*. 2016;9.

127. Konfino J, Fernandez A, Penko J, Mason A, Martinez E, Coxson P, Heller D, Moran A, Bibbins-Domingo K, Perez-Stable EJ and Mejia R. Comparing Strategies for Lipid Lowering in Argentina: An Analysis from the CVD Policy Model-Argentina. *J Gen Intern Med*. 2017;32:524-533.

128. Kovalchik SA and Pfeiffer RM. Population-based absolute risk estimation with survey data. *Lifetime Data Anal*. 2014;20:252-75.

129. Kreger BE, Cupples LA and Kannel WB. The electrocardiogram in prediction of sudden death: Framingham Study experience. *Am Heart J*. 1987;113:377-82.
130. Kumarathurai P, Mouridsen MR, Mattsson N, Larsen BS, Nielsen OW, Gerds TA and Sajadieh A. Atrial ectopy and N-terminal pro-B-type natriuretic peptide as predictors of atrial fibrillation: a population-based cohort study. *Europace*. 2017;19:364-370.
131. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Gansevoort RT and Dullaart RP. Circulating gamma glutamyltransferase and prediction of cardiovascular disease. *Atherosclerosis*. 2015;238:356-64.
132. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Gansevoort RT, Gregson J and Dullaart RP. Serum Alkaline Phosphatase and Risk of Incident Cardiovascular Disease: Interrelationship with High Sensitivity C-Reactive Protein. *PLoS One*. 2015;10:e0132822.
133. Kusmana D. The influence of smoking cessation, regular physical exercise and/or physical activity on survival: A 13 years cohort study of the Indonesian population in Jakarta. *Medical Journal of Indonesia*. 2002;11:230-242.
134. Langley SR, Willeit K, Didangelos A, Matic LP, Skroblin P, Barallobre-Barreiro J, Lengquist M, Rungger G, Kapustin A, Kedenko L, Molenaar C, Lu R, Barwari T, Suna G, Yin X, Iglseder B, Paulweber B, Willeit P, Shalhoub J, Pasterkamp G, Davies AH, Monaco C, Hedin U, Shanahan CM, Willeit J, Kiechl S and Mayr M. Extracellular matrix proteomics identifies molecular signature of symptomatic carotid plaques. *J Clin Invest*. 2017;127:1546-1560.
135. Lee CH, Woo YC, Lam JK, Fong CH, Cheung BM, Lam KS and Tan KC. Validation of the Pooled Cohort equations in a long-term cohort study of Hong Kong Chinese. *J Clin Lipidol*. 2015;9:640-6 e2.
136. Lee JW, Hur J, Choi SI, Chun EJ, Kang JW, Jin GY, Kim EY, Yong HS, Kang EJ, Han K, Lee HS and Choi BW. Incremental prognostic value of computed tomography in stroke: rationale and design of the IMPACTS study. *Int J Cardiovasc Imaging*. 2016;32 Suppl 1:83-9.
137. Lin YH, Glei D, Weinstein M, Wu SI and Chien KL. Additive value of interleukin-6 and C-reactive protein in risk prediction for all-cause and cardiovascular mortality among a representative adult cohort in Taiwan. *J Formos Med Assoc*. 2017;116:982-992.
138. Lindberg S, Jensen JS, Mogelvang R, Pedersen SH, Galatius S, Flyvbjerg A and Magnusson NE. Plasma Neutrophil Gelatinase-Associated Lipocalinin in the General Population Association With Inflammation and Prognosis. *Arterioscl Throm Vas*. 2014;34:2135-2142.
139. Liu L, Tang Z, Li X, Luo Y, Guo J, Li H, Liu X, Tao L, Yan A and Guo X. A novel risk score to the prediction of 10-year risk for coronary artery disease among the elderly in Beijing based on competing risk model. *Medicine (United States)*. 2016;95.
140. Lluis-Ganella C, Subirana I, Lucas G, Tomas M, Munoz D, Senti M, Salas E, Sala J, Ramos R, Ordovas JM, Marrugat J and Elosua R. Assessment of the value of a genetic risk score in improving the estimation of coronary risk. *Atherosclerosis*. 2012;222:456-63.

141. Lopez-Suarez A, Bascunana-Quirell A, Beltran-Robles M, Elvira-Gonzalez J, Fernandez-Palacin F, Barroso-Casamitjana E and Solino-Ocana I. Metabolic syndrome does not improve the prediction of 5-year cardiovascular disease and total mortality over standard risk markers. Prospective population based study. *Medicine*. 2014;93:e212.

142. Lyngbaek S, Marott JL, Sehestedt T, Hansen TW, Olsen MH, Andersen O, Linneberg A, Haugaard SB, Eugen-Olsen J, Hansen PR and Jeppesen J. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol*. 2013;167:2904-11.

143. Mahabadi AA, Berg MH, Lehmann N, Kalsch H, Bauer M, Kara K, Dragano N, Moebus S, Jockel KH, Erbel R and Mohlenkamp S. Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: the Heinz Nixdorf Recall Study. *J Am Coll Cardiol*. 2013;61:1388-95.

144. Mahabadi AA, Lehmann N, Mohlenkamp S, Pundt N, Dykun I, Roggenbuck U, Moebus S, Jockel KH, Erbel R, Kalsch H and Heinz Nixdorf Investigative G. Noncoronary Measures Enhance the Predictive Value of Cardiac CT Above Traditional Risk Factors and CAC Score in the General Population. *JACC Cardiovasc Imaging*. 2016;9:1177-1185.

145. Manuel DG, Tuna M, Perez R, Tanuseputro P, Hennessy D, Bennett C, Rosella L, Sanmartin C, van Walraven C and Tu JV. Predicting Stroke Risk Based on Health Behaviours: Development of the Stroke Population Risk Tool (SPoRT). *PLoS One*. 2015;10:e0143342.

146. Marino M, Li Y, Pencina MJ, D'Agostino RB, Sr., Berkman LF and Buxton OM. Quantifying cardiometabolic risk using modifiable non-self-reported risk factors. *Am J Prev Med*. 2014;47:131-40.

147. Marrugat J, Subirana I, Ramos R, Vila J, Marin-Ibanez A, Guembe MJ, Rigo F, Tormo Diaz MJ, Moreno-Iribas C, Cabre JJ, Segura A, Baena-Diez JM, de la Camara AG, Lapetra J, Grau M, Quesada M, Medrano MJ, Gonzalez Diego P, Frontera G, Gavrila D, Aicua EA, Basora J, Garcia JM, Garcia-Lareo M, Gutierrez JA, Mayoral E, Sala J, D'Agostino R, Elosua R and Investigators F. Derivation and validation of a set of 10-year cardiovascular risk predictive functions in Spain: the FRESCO Study. *Prev Med*. 2014;61:66-74.

148. Matsushita K, Sang Y, Ballew SH, Astor BC, Hoogeveen RC, Solomon SD, Ballantyne CM, Woodward M and Coresh J. Cardiac and kidney markers for cardiovascular prediction in individuals with chronic kidney disease: the Atherosclerosis Risk in Communities study. *Arterioscler Thromb Vasc Biol*. 2014;34:1770-7.

149. McClelland RL, Jorgensen NW, Budoff M, Blaha MJ, Post WS, Kronmal RA, Bild DE, Shea S, Liu K, Watson KE, Folsom AR, Khera A, Ayers C, Mahabadi AA, Lehmann N, Jockel KH, Moebus S, Carr JJ, Erbel R and Burke GL. 10-Year Coronary Heart Disease Risk Prediction Using Coronary Artery Calcium and Traditional Risk Factors: Derivation in the MESA (Multi-Ethnic Study of Atherosclerosis) With Validation in the HNR (Heinz Nixdorf Recall) Study and the DHS (Dallas Heart Study). *J Am Coll Cardiol*. 2015;66:1643-53.

150. McClure LA, Kleindorfer DO, Kissela BM, Cushman M, Soliman EZ and Howard G. Assessing the performance of the Framingham Stroke Risk Score in the reasons for geographic and racial differences in stroke cohort. *Stroke*. 2014;45:1716-20.

151. McNeil JJ, Peeters A, Liew D, Lim S and Vos T. A model for predicting the future incidence of coronary heart disease within percentiles of coronary heart disease risk. *J Cardiovasc Risk*. 2001;8:31-7.

152. Mody P, Joshi PH, Khera A, Ayers CR and Rohatgi A. Beyond Coronary Calcification, Family History, and C-Reactive Protein: Cholesterol Efflux Capacity and Cardiovascular Risk Prediction. *J Am Coll Cardiol*. 2016;67:2480-7.

153. Morris RW, Cooper JA, Shah T, Wong A, Drenos F, Engmann J, McLachlan S, Jefferis B, Dale C, Hardy R, Kuh D, Ben-Shlomo Y, Wannamethee SG, Whincup PH, Casas JP, Kivimaki M, Kumari M, Talmud PJ, Price JF, Dudbridge F, Hingorani AD, Humphries SE and Consortium U. Marginal role for 53 common genetic variants in cardiovascular disease prediction. *Heart*. 2016;102:1640-7.

154. Mortensen MB, Afzal S, Nordestgaard BG and Falk E. The high-density lipoprotein-adjusted SCORE model worsens SCORE-based risk classification in a contemporary population of 30,824 Europeans: the Copenhagen General Population Study. *Eur Heart J*. 2015;36:2446-53.

155. Mortensen MB, Nordestgaard BG, Afzal S and Falk E. ACC/AHA guidelines superior to ESC/EAS guidelines for primary prevention with statins in non-diabetic Europeans: the Copenhagen General Population Study. *Eur Heart J*. 2017;38:586-594.

156. Muntner P, Colantonio LD, Cushman M, Goff DC, Jr., Howard G, Howard VJ, Kissela B, Levitan EB, Lloyd-Jones DM and Safford MM. Validation of the atherosclerotic cardiovascular disease Pooled Cohort risk equations. *JAMA*. 2014;311:1406-15.

157. Nambi V, Liu X, Chambless LE, de Lemos JA, Virani SS, Agarwal S, Boerwinkle E, Hoogeveen RC, Aguilar D, Astor BC, Srinivas PR, Deswal A, Mosley TH, Coresh J, Folsom AR, Heiss G and Ballantyne CM. Troponin T and N-terminal pro-B-type natriuretic peptide: a biomarker approach to predict heart failure risk--the atherosclerosis risk in communities study. *Clin Chem*. 2013;59:1802-10.

158. Nargesi AA, Heidari B, Esteghamati S, Hafezi-Nejad N, Sheikhbahaei S, Pajouhi A, Nakhjavani M and Esteghamati A. Contribution of vitamin D deficiency to the risk of coronary heart disease in subjects with essential hypertension. *Atherosclerosis*. 2016;244:165-71.

159. Nauman J, Nes BM, Lavie CJ, Jackson AS, Sui X, Coombes JS, Blair SN and Wisloff U. Prediction of Cardiovascular Mortality by Estimated Cardiorespiratory Fitness Independent of Traditional Risk Factors: The HUNT Study. *Mayo Clin Proc*. 2017;92:218-227.

160. Ndumele CE, Matsushita K, Sang Y, Lazo M, Agarwal SK, Nambi V, Deswal A, Blumenthal RS, Ballantyne CM, Coresh J and Selvin E. N-Terminal Pro-Brain Natriuretic Peptide and Heart Failure Risk Among Individuals With and Without Obesity: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 2016;133:631-8.

161. Nielsen JB, Graff C, Rasmussen PV, Pietersen A, Lind B, Olesen MS, Struijk JJ, Haunso S, Svendsen JH, Kober L, Gerds TA and Holst AG. Risk prediction of cardiovascular death based on the QTc interval: evaluating age and gender differences in a large primary care population. *Eur Heart J*. 2014;35:1335-44.

162. Ninomiya T, Kojima I, Doi Y, Fukuhara M, Hirakawa Y, Hata J, Kitazono T and Kiyohara Y. Brachial-ankle pulse wave velocity predicts the development of cardiovascular disease in a general Japanese population: the Hisayama Study. *J Hypertens*. 2013;31:477-83; discussion 483.

163. Nishimura K, Okamura T, Watanabe M, Nakai M, Takegami M, Higashiyama A, Kokubo Y, Okayama A and Miyamoto Y. Predicting coronary heart disease using risk factor categories for a Japanese urban population, and comparison with the framingham risk score: the suita study. *J Atheroscler Thromb*. 2014;21:784-98.

164. Nobel L, Mayo NE, Hanley J, Nadeau L and Daskalopoulou SS. MyRisk_Stroke Calculator: A Personalized Stroke Risk Assessment Tool for the General Population. *J Clin Neurol*. 2014;10:1-9.

165. Ogunwale AN, Morrison AC, Sun W, Dodge RC, Virani SS, Taylor A, Gottesman RF, Yang E, Wei P, McEvoy JW, Heiss G, Boerwinkle E, Ballantyne CM and Nambi V. The impact of multiple single day blood pressure readings on cardiovascular risk estimation: The Atherosclerosis Risk in Communities study. *Eur J Prev Cardiol*. 2016;23:1529-36.

166. Okwuosa TM, Soliman EZ, Lopez F, Williams KA, Alonso A and Ferdinand KC. Left ventricular hypertrophy and cardiovascular disease risk prediction and reclassification in blacks and whites: the Atherosclerosis Risk in Communities Study. *Am Heart J*. 2015;169:155-61 e5.

167. Onat A, Can G, Kaya A, Keskin M, Hayiroglu MI and Yuksel H. Algorithm for predicting CHD death risk in Turkish adults: conventional factors contribute only moderately in women. *Anatol J Cardiol*. 2017;17:436-444.

168. Paixao AR, Ayers CR, El Sabbagh A, Sanghavi M, Berry JD, Rohatgi A, Kumbhani DJ, McGuire DK, Das SR, de Lemos JA and Khera A. Coronary Artery Calcium Improves Risk Classification in Younger Populations. *JACC Cardiovasc Imaging*. 2015;8:1285-93.

169. Panagiotakos DB, Georgousopoulou EN, Fitzgerald AP, Pitsavos C and Stefanadis C. Validation of the HellenicSCORE (a Calibration of the ESC SCORE Project) Regarding 10-Year Risk of Fatal Cardiovascular Disease in Greece. *Hell J Cardiol*. 2015;56:302-308.

170. Parikh NI, Jeppson RP, Berger JS, Eaton CB, Kroenke CH, LeBlanc ES, Lewis CE, Loucks EB, Parker DR, Rillamas-Sun E, Ryckman KK, Waring ME, Schenken RS, Johnson KC, Edstedt-Bonamy AK, Allison MA and Howard BV. Reproductive Risk Factors and Coronary Heart Disease in the Women's Health Initiative Observational Study. *Circulation*. 2016;133:2149-58.

171. Park GM, Han S, Kim SH, Jo MW, Her SH, Lee JB, Lee MS, Kim HC, Ahn JM, Lee SW, Kim YH, Kim BJ, Koh JM, Kim HK, Choe J, Park SW and Park SJ. Model for assessing cardiovascular risk in a Korean population. *Circ Cardiovasc Qual Outcomes*. 2014;7:944-51.

172. Parmar P, Krishnamurthi R, Ikram MA, Hofman A, Mirza SS, Varakin Y, Kravchenko M, Piradov M, Thrift AG, Norrving B, Wang W, Mandal DK, Barker-Collo S, Sahathevan R, Davis S, Saposnik G, Kivipelto M, Sindi S, Bornstein NM, Giroud M, Bejot Y, Brainin M, Poulton R, Narayan KM, Correia M, Freire A, Kokubo Y, Wiebers D, Mensah G, BinDhim NF, Barber PA, Pandian JD, Hankey GJ, Mehndiratta MM, Azhagammal S, Ibrahim NM, Abbott M, Rush E, Hume P, Hussein T, Bhattacharjee R, Purohit M, Feigin VL and Stroke Riskometer TMCWG. The Stroke Riskometer(TM) App: validation of a data collection tool and stroke risk predictor. *Int J Stroke*. 2015;10:231-44.

173. Paynter NP, Crainiceanu CM, Sharrett AR, Chambless LE and Coresh J. Effect of correcting for long-term variation in major coronary heart disease risk factors: relative hazard estimation and risk prediction in the Atherosclerosis Risk in Communities Study. *Ann Epidemiol*. 2012;22:191-7.

174. Paynter NP, LaMonte MJ, Manson JE, Martin LW, Phillips LS, Ridker PM, Robinson JG and Cook NR. Comparison of lifestyle-based and traditional cardiovascular disease prediction in a multiethnic cohort of nonsmoking women. *Circulation*. 2014;130:1466-73.

175. Pencina MJ, D'Agostino RB, Zdrojewski T, Williams K, Thanassoulis G, Furberg CD, Peterson ED, Vasan RS and Sniderman AD. Apolipoprotein B improves risk assessment of future coronary heart disease in the Framingham Heart Study beyond LDL-C and non-HDL-C. *Eur J Prev Cardiol*. 2015;22:1321-7.

176. Perez HA, Garcia NH, Spence JD and Armando LJ. Adding carotid total plaque area to the Framingham risk score improves cardiovascular risk classification. *Arch Med Sci*. 2016;12:513-20.

177. Piotrowski W, Waskiewicz A and Cicha-Mikolajczyk A. Global cardiovascular mortality risk in the adult Polish population: prospective assessment of the cohorts studied in multicentre national WOBASZ and WOBASZ Senior studies. *Kardiologia Polska*. 2016;74:262-73.

178. Polak JF, Szklo M and O'Leary DH. Associations of Coronary Heart Disease with Common Carotid Artery Near and Far Wall Intima-Media Thickness: The Multi-Ethnic Study of Atherosclerosis. *J Am Soc Echocardiogr.* 2015;28:1114-21.

179. Polenz GF, Leiria TL, Essebag V, Kruse ML, Pires LM, Nogueira TB, Guimaraes RB, Santanna RT and GG DEL. CHA2 DS2 VASc Score as a Predictor of Cardiovascular Events in Ambulatory Patients without Atrial Fibrillation. *Pacing Clin Electrophysiol*. 2015;38:1412-7.

180. Qureshi WT, Michos ED, Flueckiger P, Blaha M, Sandfort V, Herrington DM, Burke G and Yeboah J. Impact of Replacing the Pooled Cohort Equation With Other Cardiovascular Disease Risk Scores on Atherosclerotic Cardiovascular Disease Risk Assessment (from the Multi-Ethnic Study of Atherosclerosis [MESA]). *American Journal of Cardiology*. 2016;118:691-6.

181. Raggi P, Cooil B and Callister TQ. Use of electron beam tomography data to develop models for prediction of hard coronary events. *Am Heart J*. 2001;141:375-82.

182. Raghu A, Praveen D, Peiris D, Tarassenko L and Clifford G. Implications of Cardiovascular Disease Risk Assessment Using the WHO/ISH Risk Prediction Charts in Rural India. *PLoS One*. 2015;10:e0133618.

183. Rana JS, Tabada GH, Solomon MD, Lo JC, Jaffe MG, Sung SH, Ballantyne CM and Go AS. Accuracy of the Atherosclerotic Cardiovascular Risk Equation in a Large Contemporary, Multiethnic Population. *J Am Coll Cardiol*. 2016;67:2118-2130.

184. Romanens M, Ackermann F, Sudano I, Szucs T and Spence JD. Arterial age as a substitute for chronological age in the AGLA risk function could improve coronary risk prediction. *Swiss Med Wkly*. 2014;144:w13967.

185. Rucker V, Keil U, Fitzgerald AP, Malzahn U, Prugger C, Ertl G, Heuschmann PU and Neuhauser H. Predicting 10-Year Risk of Fatal Cardiovascular Disease in Germany: An Update Based on the SCORE-Deutschland Risk Charts. *PLoS One*. 2016;11:e0162188.

186. Sabayan B, Gussekloo J, de Ruijter W, Westendorp RG and de Craen AJ. Framingham stroke risk score and cognitive impairment for predicting first-time stroke in the oldest old. *Stroke*. 2013;44:1866-71.

187. Salim A, Tai ES, Tan VY, Welsh AH, Liew R, Naidoo N, Wu Y, Yuan JM, Koh WP and van Dam RM. C-reactive protein and serum creatinine, but not haemoglobin A1c, are independent predictors of coronary heart disease risk in non-diabetic Chinese. *Eur J Prev Cardiolog*. 2016;23:1339-49.

188. Sanchez-Inigo L, Navarro-Gonzalez D, Fernandez-Montero A, Pastrana-Delgado J and Martinez JA. The TyG index may predict the development of cardiovascular events. *European Journal of Clinical Investigation*. 2016;46:189-97.

189. Sardarinia M, Ansari R, Azizi F, Hadaegh F and Bozorgmanesh M. Mortality prediction of a body shape index versus traditional anthropometric measures in an Iranian population: Tehran Lipid and Glucose Study. *Nutrition*. 2017;33:105-112.

190. Sawano M, Kohsaka S, Okamura T, Inohara T, Sugiyama D, Watanabe M, Nakamura Y, Higashiyama A, Kadota A, Okud N, Murakami Y, Ohkubo T, Fujiyoshi A, Miura K, Okayama A, Ueshima H, National Integrated Project for Prospective Observation of Non-Communicable D and its Trends in the Aged Research G. Validation of the european SCORE risk chart in the healthy middle-aged Japanese. *Atherosclerosis*. 2016;252:116-21.

191. Schiopu A, Bengtsson E, Goncalves I, Nilsson J, Fredrikson GN and Bjorkbacka H. Associations Between Macrophage Colony-Stimulating Factor and Monocyte Chemotactic Protein 1 in Plasma and First-Time Coronary Events: A Nested Case-Control Study. *Journal of the American Heart Association*. 2016;5.

192. Schnohr P, Marott JL, Kristensen TS, Gyntelberg F, Gronbaek M, Lange P, Jensen MT, Jensen GB and Prescott E. Ranking of psychosocial and traditional risk factors by importance for coronary heart disease: the Copenhagen City Heart Study. *Eur Heart J*. 2015;36:1385-93.

193. Sehestedt T, Jeppesen J, Hansen TW, Rasmussen S, Wachtell K, Ibsen H, Torp-Pedersen C and Olsen MH. Can ambulatory blood pressure measurements substitute assessment of subclinical cardiovascular damage? *J Hypertens*. 2012;30:513-21.

194. Seidelmann SB, Claggett B, Bravo PE, Gupta A, Farhad H, Klein BE, Klein R, Di Carli M and Solomon SD. Retinal Vessel Calibers in Predicting Long-Term Cardiovascular Outcomes: The Atherosclerosis Risk in Communities Study. *Circulation*. 2016;134:1328-1338.

195. Selmer R, Igland J, Ariansen I, Tverdal A, Njolstad I, Furu K, Tell GS and Klemsdal TO. NORRISK 2: A Norwegian risk model for acute cerebral stroke and myocardial infarction. *Eur J Prev Cardiol*. 2017;24:773-782.

196. Selvarajah S, Kaur G, Haniff J, Cheong KC, Hiong TG, van der Graaf Y and Bots ML. Comparison of the Framingham Risk Score, SCORE and WHO/ISH cardiovascular risk prediction models in an Asian population. *Int J Cardiol*. 2014;176:211-8.

197. Sepanlou SG, Malekzadeh R, Poustchi H, Sharafkhah M, Ghodsi S, Malekzadeh F, Etemadi A, Pourshams A, Pharoah PD, Abnet CC, Brennan P, Boffetta P, Dawsey SM and Kamangar F. The clinical performance of an office-based risk scoring system for fatal cardiovascular diseases in North-East of Iran. *PLoS One*. 2015;10:e0126779.

198. Shah N, Pahuja M, Pant S, Handa A, Agarwal V, Patel N and Dusaj R. Red cell distribution width and risk of cardiovascular mortality: Insights from National Health and Nutrition Examination Survey (NHANES)-III. *Int J Cardiol*. 2017;232:105-110.

199. Shah N, Parikh V, Patel N, Patel N, Badheka A, Deshmukh A, Rathod A and Lafferty J. Neutrophil lymphocyte ratio significantly improves the Framingham risk score in prediction of coronary heart disease mortality: insights from the National Health and Nutrition Examination Survey-III. *Int J Cardiol*. 2014;171:390-7.
200. Sharma A, Ghatge M, Mundkur L and Vangala RK. Translational informatics approach for identifying the functional molecular communicators linking coronary artery disease, infection and inflammation. *Mol Med Rep*. 2016;13:3904-12.
201. Shoamanesh A, Preis SR, Beiser AS, Kase CS, Wolf PA, Vasan RS, Benjamin EJ, Seshadri S and Romero JR. Circulating biomarkers and incident ischemic stroke in the Framingham Offspring Study. *Neurology*. 2016;87:1206-11.
202. Sim J, Teece L, Dennis MS, Roffe C and Team SSS. Validation and Recalibration of Two Multivariable Prognostic Models for Survival and Independence in Acute Stroke. *Plos One*. 2016;11.
203. Solbu MD, Toft I, Lochen ML, Mathiesen EB, Eriksen BO, Melsom T, Njolstad I, Wilsgaard T and Jenssen TG. N-Acetyl-beta-D-Glucosaminidase Does Not Enhance Prediction of Cardiovascular or All-Cause Mortality by Albuminuria in a Low-Risk Population. *J Am Soc Nephrol*. 2016;27:533-42.
204. Sonneveld MA, de Maat MP, Portegies ML, Kavousi M, Hofman A, Turecek PL, Rottensteiner H, Scheiflinger F, Koudstaal PJ, Ikram MA and Leebeek FW. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. *Blood*. 2015;126:2739-46.
205. Stam-Slob MC, Visseren FLJ, Jukema JW, van der Graaf Y, Poulter NR, Gupta A, Sattar N, Macfarlane PW, Kearney PM, de Craen AJM and Trompet S. Personalized absolute benefit of statin treatment for primary or secondary prevention of vascular disease in individual elderly patients. *Clin Res Cardiol*. 2017;106:58-68.
206. Tada H, Melander O, Louie JZ, Catanese JJ, Rowland CM, Devlin JJ, Kathiresan S and Shiffman D. Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. *Eur Heart J*. 2016;37:561-7.
207. Tang WH, Topol EJ, Fan Y, Wu Y, Cho L, Stevenson C, Ellis SG and Hazen SL. Prognostic value of estimated functional capacity incremental to cardiac biomarkers in stable cardiac patients. *Journal of the American Heart Association*. 2014;3:e000960.
208. Thompson SG, Pyke SD and Wood DA. Using a coronary risk score for screening and intervention in general practice. British Family Heart Study. *J Cardiovasc Risk*. 1996;3:301-6.
209. Tillin T, Hughes AD, Whincup P, Mayet J, Sattar N, McKeigue PM, Chaturvedi N and Group SS. Ethnicity and prediction of cardiovascular disease: performance of QRISK2 and Framingham scores in a U.K. tri-ethnic prospective cohort study (SABRE--Southall And Brent REvisited). *Heart*. 2014;100:60-7.

210. Valenti V, B OH, Heo R, Cho I, Schulman-Marcus J, Gransar H, Truong QA, Shaw LJ, Knapper J, Kelkar AA, Sandesara P, Lin FY, Sciarretta S, Chang HJ, Callister TQ and Min JK. A 15-Year Warranty Period for Asymptomatic Individuals Without Coronary Artery Calcium: A Prospective Follow-Up of 9,715 Individuals. *JACC Cardiovasc Imaging*. 2015;8:900-9.

211. van der Meer MG, van der Graaf Y, Schuit E, Peelen LM, Verschuren WM, Boer JM, Moons KG, Nathoe HM, Appelman Y and van der Schouw YT. Added Value of Female-Specific Factors Beyond Traditional Predictors for Future Cardiovascular Disease. *J Am Coll Cardiol*. 2016;67:2084-6.

212. van Dis I, Geleijnse JM, Boer JM, Kromhout D, Boshuizen H, Grobbee DE, van der Schouw YT and Verschuren WM. Effect of including nonfatal events in cardiovascular risk estimation, illustrated with data from The Netherlands. *Eur J Prev Cardiol*. 2014;21:377-83.

213. van Dis I, Geleijnse JM, Kromhout D, Boer J, Boshuizen H and Verschuren WM. Do obesity and parental history of myocardial infarction improve cardiovascular risk prediction? *Eur J Prev Cardiol*. 2013;20:793-9.

214. van Kempen BJ, Ferket BS, Kavousi M, Leening MJ, Steyerberg EW, Ikram MA, Witteman JC, Hofman A, Franco OH and Hunink MG. Performance of Framingham cardiovascular disease (CVD) predictions in the Rotterdam Study taking into account competing risks and disentangling CVD into coronary heart disease (CHD) and stroke. *Int J Cardiol*. 2014;171:413-8.

215. Vartiainen E, Laatikainen T, Peltonen M and Puska P. Predicting Coronary Heart Disease and Stroke The FINRISK Calculator. *Glob Heart*. 2016;11:213-216.

216. Velescu A, Clara A, Penafiel J, Ramos R, Marti R, Grau M, Degano IR, Marrugat J, Elosua R and Group RS. Adding low ankle brachial index to classical risk factors improves the prediction of major cardiovascular events. The REGICOR study. *Atherosclerosis*. 2015;241:357-63.

217. Venkatesh S, O'Neal WT, Broughton ST, Shah AJ and Soliman EZ. Utility of Normal Findings on Electrocardiogram and Echocardiogram in Subjects >= 65 Years. *American Journal of Cardiology*. 2017;119:856-861.

218. Verbeek R, Sandhu MS, Hovingh GK, Sjouke B, Wareham NJ, Zwinderman AH, Kastelein JJ, Khaw KT, Tsimikas S and Boekholdt SM. Lipoprotein(a) Improves Cardiovascular Risk Prediction Based on Established Risk Algorithms. *J Am Coll Cardiol*. 2017;69:1513-1515.

219. Veronesi G, Giampaoli S, Vanuzzo D, Gianfagna F, Palmieri L, Grassi G, Cesana G and Ferrario MM. Combined use of short-term and long-term cardiovascular risk scores in primary prevention: An assessment of clinical utility. *Journal of Cardiovascular Medicine*. 2017;18:318-324.

220. Veronesi G, Gianfagna F, Giampaoli S, Chambless LE, Grassi G, Cesana G and Ferrario MM. Validity of a long-term cardiovascular disease risk prediction equation for low-incidence populations: the CAMUNI-MATISS Cohorts Collaboration study. *Eur J Prev Cardiol*. 2015;22:1618-25.

221. Veronesi G, Gianfagna F, Giampaoli S, Chambless LE, Mancia G, Cesana G and Ferrario MM. Improving long-term prediction of first cardiovascular event: The contribution of family history of coronary heart disease and social status. *Preventive Medicine*. 2014;64:75-80.

222. Vikhireva O, Broda G, Kubinova R, Malyutina S, Pajak A, Tamosiunas A, Skodova Z, Simonova G, Bobak M and Pikhart H. Does Inclusion of Education and Marital Status Improve SCORE Performance in Central and Eastern Europe and Former Soviet Union? Findings from MONICA and HAPIEE Cohorts. *Plos One*. 2014;9.

223. Vikhireva O, Pajak A, Broda G, Malyutina S, Tamosiunas A, Kubinova R, Simonova G, Skodova Z, Bobak M and Pikhart H. SCORE performance in Central and Eastern Europe and former Soviet Union: MONICA and HAPIEE results. *Eur Heart J*. 2014;35:571-7.

224. Waks JW, Sitlani CM, Soliman EZ, Kabir M, Ghafoori E, Biggs ML, Henrikson CA, Sotoodehnia N, Biering-Sorensen T, Agarwal SK, Siscovick DS, Post WS, Solomon SD, Buxton AE, Josephson ME and Tereshchenko LG. Global Electric Heterogeneity Risk Score for Prediction of Sudden Cardiac Death in the General Population: The Atherosclerosis Risk in Communities (ARIC) and Cardiovascular Health (CHS) Studies. *Circulation*. 2016;133:2222-34.

225. Wang Y, Liu J, Wang W, Wang M, Qi Y, Xie WX, Li Y, Sun JY, Liu J and Zhao D. Lifetime risk of stroke in young-aged andmiddle-aged Chinese population: the Chinese Multi-Provincial Cohort Study. *J Hypertens*. 2016;34:2434-2440.

226. Wassertheil-Smoller S, McGinn A, Allison M, Ca T, Curb D, Eaton C, Hendrix S, Kaplan R, Ko M, Martin LW and Xue X. Improvement in stroke risk prediction: role of C-reactive protein and lipoprotein-associated phospholipase A2 in the women's health initiative. *Int J Stroke*. 2014;9:902-9.

227. Weatherley ND and Jackson PR. The new Sheffield risk and benefit tables for the elderly. *QJM*. 2011;104:3-12.

228. Welsh P, Hart C, Papacosta O, Preiss D, McConnachie A, Murray H, Ramsay S, Upton M, Watt G, Whincup P, Wannamethee G and Sattar N. Prediction of Cardiovascular Disease Risk by Cardiac Biomarkers in 2 United Kingdom Cohort Studies: Does Utility Depend on Risk Thresholds For Treatment? *Hypertension*. 2016;67:309-15.

229. Weng SF, Kai J, Guha IN and Qureshi N. The value of aspartate aminotransferase and alanine aminotransferase in cardiovascular disease risk assessment. *Open Heart*. 2015;2:e000272.

230. Wickramasinghe CD, Ayers CR, Das S, de Lemos JA, Willis BL and Berry JD. Prediction of 30-Year Risk for Cardiovascular Mortality by Fitness and Risk Factor Levels The Cooper Center Longitudinal Study. *Circ-Cardiovasc Qual*. 2014;7:597-602.

231. Willeit P, Kiechl S, Kronenberg F, Witztum JL, Santer P, Mayr M, Xu Q, Mayr A, Willeit J and Tsimikas S. Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. *J Am Coll Cardiol*. 2014;64:851-60.

232. Woodward M. Coronary risk prediction for those with and without diabetes. *European Journal of Cardiovascular Prevention and Rehabilitation*. 2006;13:30-36.

233. Woodward M, Brindle P, Tunstall-Pedoe H and estimation Sgor. Adding social deprivation and family history to cardiovascular risk assessment: the ASSIGN score from the Scottish Heart Health Extended Cohort (SHHEC). *Heart*. 2007;93:172-6.

234. Woznicka-Leskiewicz L, Posadzy-Malaczynska A and Juszkat R. The impact of ankle brachial index and pulse wave velocity on cardiovascular risk according to SCORE and Framingham scales and sex differences. *J Hum Hypertens*. 2015;29:502-10.

235. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkila V, Jula A, Kahonen M, Lehtimaki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasan RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M and Salomaa V. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation*. 2015;131:774-85.

236. Yang X, Li J, Hu D, Chen J, Li Y, Huang J, Liu X, Liu F, Cao J, Shen C, Yu L, Lu F, Wu X, Zhao L, Wu X and Gu D. Predicting the 10-Year Risks of Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China). *Circulation*. 2016;134:1430-1440.

237. Yatsuya H, Iso H, Li Y, Yamagishi K, Kokubo Y, Saito I, Sawada N, Inoue M and Tsugane S. Development of a Risk Equation for the Incidence of Coronary Artery Disease and Ischemic Stroke for Middle-Aged Japanese- Japan Public Health Center-Based Prospective Study. *Circulation Journal*. 2016;80:1386-95.

238. Yeboah J, Carr JJ, Terry JG, Ding J, Zeb I, Liu S, Nasir K, Post W, Blumenthal RS and Budoff MJ. Computed tomography-derived cardiovascular risk markers, incident cardiovascular events, and all-cause mortality in nondiabetics: the Multi-Ethnic Study of Atherosclerosis. *Eur J Prev Cardiol*. 2014;21:1233-41.

239. Yeboah J, Young R, McClelland RL, Delaney JC, Polonsky TS, Dawood FZ, Blaha MJ, Miedema MD, Sibley CT, Carr JJ, Burke GL, Goff DC, Jr., Psaty BM, Greenland P and Herrington DM. Utility of Nontraditional Risk Markers in Atherosclerotic Cardiovascular Disease Risk Assessment. *J Am Coll Cardiol*. 2016;67:139-147.

240. Yuan Z, Voss EA, DeFalco FJ, Pan G, Ryan PB, Yannicelli D and Nessel C. Risk Prediction for Ischemic Stroke and Transient Ischemic Attack in Patients Without Atrial Fibrillation: A Retrospective Cohort Study. *J Stroke Cerebrovasc Dis*. 2017;26:1721-1731.
241. Yudkin J. Beta-blockers in heart failure. *Diabetic Medicine*. 1999;16:785-7.
242. Zalawadiya SK, Veeranna V, Mallikethi-Reddy S, Bavishi C, Lunagaria A, Kottam A and Afonso L. Uric acid and cardiovascular disease risk reclassification: findings from NHANES III. *Eur J Prev Cardiol*. 2015;22:513-8.
243. Zhang Y, Schottker B, Florath I, Stock C, Butterbach K, Holleczek B, Mons U and Brenner H. Smoking-Associated DNA Methylation Biomarkers and Their Predictive Value for All-Cause and Cardiovascular Mortality. *Environ Health Perspect*. 2016;124:67-74.
244. Zhang Z, Gillespie C, Bowman B and Yang Q. Prediction of atherosclerotic cardiovascular disease mortality in a nationally representative cohort using a set of risk factors from pooled cohort risk equations. *PLoS ONE*. 2017;12.
245. Zhou XH, Wang X, Duncan A, Hu G and Zheng J. Statistical evaluation of adding multiple risk factors improves Framingham stroke risk score. *BMC Med Res Methodol*. 2017;17:58.
246. Ziegelbauer K, Schaefer C, Steinmetz H, Sitzer M and Lorenz MW. Clinical usefulness of carotid ultrasound to improve stroke risk assessment: ten-year results from the Carotid Atherosclerosis Progression Study (CAPS). *Eur J Prev Cardiol*. 2013;20:837-43.

CHAPTER 3.2

Cardiovascular risk model performance in women with and without hypertensive disorders of pregnancy

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Abstract

Objectives: Compare the predictive performance of Framingham Risk Score (FRS), Pooled Cohort Equations (PCEs) and Systematic COronary Risk Evaluation (SCORE) model between women with and without a history of hypertensive disorders of pregnancy (hHDP), and determine effects of recalibration and refitting on predictive performance.

Methods: We included 29 751 women, 6302 with hHDP and 17 369 without. We assessed whether models accurately predicted observed 10-year cardiovascular disease (CVD) risk (calibration) and whether they accurately distinguished between women developing CVD during follow-up and not (discrimination), separately for women with and without hHDP. We also recalibrated (updating intercept and slope) and refitted (recalculating coefficients) the models.

Results: Original FRS and PCEs overpredicted 10-year CVD risks, with expected:observed (E:O) ratios ranging from 1.51 (for FRS in women with hHDP) to 2.29 (for PCEs in women without hHDP), while E:O ratios were close to 1 for SCORE. Overprediction attenuated slightly after recalibration for FRS and PCEs in both hHDP groups. Discrimination was reasonable for all models, with C-statistics ranging from 0.70-0.81 (women with hHDP) and 0.72-0.74 (women without hHDP). C-statistics improved slightly after refitting 0.71-0.83 (with hHDP) and 0.73-0.80 (without hHDP). The E:O ratio of the original PCEs was statistically significantly better in women with hHDP compared with women without hHDP.

Conclusions: SCORE performed best in terms of both calibration and discrimination, while FRS and PCEs overpredicted risk in women with and without hHDP, but improved after recalibrating and refitting the models. No separate model for women with hHDP seems necessary, despite their higher baseline risk.

Introduction

Hypertensive disorders of pregnancy (HDP), defined by the International Society for the Study of Hypertension in Pregnancy as chronic hypertension, gestational hypertension, pre-eclampsia (de novo or superimposed on chronic hypertension), and white coat hypertension[1], are associated with cardiovascular disease (CVD) in women, with a relative risk (RR) as high as 7.7 after a pregnancy complicated by early onset pre-eclampsia[2,3]. Less severe forms of HDP are also associated with an increased risk for coronary heart disease with RRs ranging from 1.3 to 2.2[3,4], an increased risk for stroke ranging from 1.3 to 1.9[3–5] and an increased risk for overall CVD with ranging from 1.3 to 2.8[6].

Recently, the Dutch guideline on cardiovascular risk management after reproductive and pregnancy-related disorders was introduced and HDP have been added to the American guidelines for the prevention of cardiovascular disease in women[6,7]. These guidelines describe HDP as 'failed stress test', which might unmask subclinical vascular or metabolic disease. Therefore, pregnancy might be a 'window of opportunity' for physicians to identify women with increased risk of CVD and improve their cardiovascular health[8]. Furthermore, two recent papers reported that up to 30% of women with HDP develop chronic hypertension after pregnancy and advocated close after-pregnancy surveillance by their physician[9,10] in line with the Dutch recommendation[6].

An abundance of CVD risk prediction models for the general population is available[11]. Several studies have found that women with a history of HDP (hHDP) or pre-eclampsia have a higher predicted CVD risk compared with women with a normotensive pregnancy[12,13]. However, the validity of these risk prediction models in women with hHDP is not established, although these women have a higher baseline risk compared with women without hHDP. Hence, the aim of this study was to externally validate the Framingham Risk Score (FRS)[14], the Pooled Cohort Equations (PCEs)[15], and the Systematic COronary Risk Evaluation (SCORE) model[16], in women with and without hHDP. Second, we aimed to recalibrate and/or refit these models. All three models are often used in clinical practice and have a separate risk equation for women. The results are reported according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) reporting guideline[17].

Materials and Methods

Subjects

For this study, we used data from the Dutch contribution to the European Prospective Investigation into Cancer and nutrition (EPIC-NL) cohort which included 40 011 participants. EPIC-NL is a combination of the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) cohort and the Prospect cohort. MORGEN consists of both men and women aged 20-59 from three Dutch cities (Amsterdam, Doetinchem, and Maastricht), who were enrolled between 1993 and

1997. Prospect consists of women aged 49-70 years who participated in a breast cancer screening programme in Utrecht, the Netherlands, between 1993 and 1997[18]. The study complies with the Declaration of Helsinki.

For this study, only women were included (n=29 751). Of these women, 6302 had answered the question: 'Did you suffer from high blood pressure during pregnancy?' with 'Yes'; 17 369 answered 'No'; 5754 had never been pregnant and 326 women were missing on this question.

Models

FRS, PCEs and SCORE include classical CVD risk factors as predictors, with some variation between models. They have a prediction horizon of 10 years, and each model predicts a different outcome, that is, composite CVD for FRS, hard atherosclerotic cardiovascular disease (ASCVD) for PCEs, and fatal CVD for SCORE. Details on the three risk models can be found in appendix 1.

Cardiovascular Outcomes

In EPIC-NL CVD events in participants were followed up by regular linkage to several disease registries. Information on deaths was obtained through linkage with municipal population registries and causes of death through linkage with 'Statistics Netherlands'. Non-fatal CVD events were obtained from a register of hospital discharge diagnoses from the Dutch Hospital Association and Order of Medical Specialists. Follow-up was complete until 1 January 2011, but we only used the first 10 years of follow-up since all three models had a prediction horizon of 10 years. Prevalent cases were also identified through linkage with the register of hospital discharge diagnoses and by self-reports in the baseline general questionnaire[18] and were excluded from the study population. The models we validated predicted different outcomes, so for each validation we used the outcome as predicted by the original model, that is, composite CVD for FRS, hard ASCVD for PCEs and fatal CVD for SCORE (appendix 1).

Predictors

The predictors of the three models were all available in EPIC-NL. A general baseline questionnaire contained questions about age, smoking, the presence of chronic diseases and medication prescription. In Prospect, systolic and diastolic blood pressures were measured in supine position twice using a Boso Oscillomat. In MORGEN, blood pressure was measured using a random zero sphygmomanometer. Body weight and waist and hip circumference were measured in light indoor clothing without shoes in both cohorts. Body weight was measured to the nearest 0.5 kg with a floor scale (Seca). In addition, a single 30 mL non-fasting blood sample was obtained and stored at -196ºC[18].

Statistical analysis

Baseline characteristics were presented as mean ± SD or median (IQR) for continuous variables and %(N) for categorical variables. No sex-based or race/ethnicity-based differences were presented, since the study population consisted of only Caucasian women.

Data on predictors used in the three risk prediction models were available for almost all (94%) participants in EPIC-NL. Data were not missing completely at random, but we assumed they were missing at random, so we chose to impute the data with multiple imputation by chained equations using the Multivariate Imputation by Chained Equations (MICE) package in R[19,20]. After 10 imputations with 50 iterations, we had very good convergence, so we used these numbers for our final imputation. All model validations were done within each imputed data set and thereafter the model performance estimates were pooled with Rubin's Rule.

After imputation, we first excluded women who did not give consent for linkage with disease and vital status registries for retrieving the CVD outcomes, leaving 28 498 women in each imputed dataset. Subsequently, we excluded women who had never been pregnant, since this group had too few events to adequately validate the models, leaving 23 010 women in each imputed dataset. Then we selected women using the same inclusion and exclusion criteria that were used in the respective development populations[14–16], which led to three subsets: for validation of FRS, we included participants who were free of prevalent CVD and between 30 and 74 years old (22 328 women in each imputed dataset); for validation of PCEs, we included participants between 40 and 79 years of age, who were apparently healthy and free of a history of nonfatal myocardial infarction, stroke, heart failure, percutaneous coronary intervention, coronary artery bypass surgery or atrial fibrillation (20 090 women in each imputed dataset); for validation of SCORE, we included participants between 19 and 81 years old, who were free of a history of non-fatal myocardial infarction (22 941 women in each imputed dataset).

We calculated the absolute observed risk for each predicted outcome in percentage within 10 years. Next, we fitted the models in our data with the coefficients as published in the original papers and calculated the absolute predicted risks (percentage within 10 years). The performance of the models was evaluated by means of calibration and discrimination. Calibration reflects the agreement between the observed and predicted risks; discrimination reflects how well the model distinguishes people with the outcome from people without the outcome. Calibration plots were constructed by plotting deciles of predicted probabilities against the Kaplan-Meier estimate for that group. Since a method for pooling calibration plots across multiply imputed data sets is not available yet, we decided to stack the calibration plots of the different imputation sets on top of each other[21]. A perfect calibration plot would show a straight 45º line. Additionally, using the predicted and observed risks mentioned above, we calculated the expected(predicted):observed (E:O) ratio with its 95% confidence interval (CI); if expected and observed risks are in agreement,

the E:O ratio is 1. The discriminative performance was assessed by calculating the C-statistic with a 95% CI; a C-statistic of 0.5 indicates that the model does not discriminate between women who develop the outcome during follow-up and women who do not and a C-statistic of 1 would be perfect discrimination.

Subsequently, we examined whether recalibrating, that is, recalculating the intercept and slope based on our own data, improved the performance of the models. Lastly, for benchmarking or reference purposes, we refitted the models (ie, recalculating the regression coefficients of all original predictors in our own data) to see what the optimal performance of the three models was.

After recalibrating and refitting the models, we tested whether the models performed statistically significantly different in women with and without hHDP. Therefore, we calculated the difference in C-statistics and the ratio of E:O ratios in 1000 bootstraps in each imputed dataset (n=10). When the 1000 bootstraps formed a normal distribution, we subtracted the 0.025^{th} and 0.975^{th} percentiles to form the 95% CI. In case of a non-normal distribution, we would take the logit of the difference in C-statistic and the log of the ratio of E:O ratios. Subsequently we pooled the results over the 10 imputations with Rubin's Rule.

Results

Table 1 shows the baseline characteristics by presence or absence of hHDP. Women with hHDP in general had a worse cardiovascular risk profile. Women with hHDP also developed more cardiovascular events for all three definitions.

First, we checked the performance of the original models. As shown in table 2, the observed CVD incidence was higher in women with hHDP compared to women without hHDP for all three models. The predicted risk of FRS and PCEs showed an overprediction compared with the observed risk, while the SCORE model predicted risk much better with only a slight overprediction in women with hHDP and a slight under prediction in women without hHDP. Comparable with the predicted risks, calibration, as shown with the calibration plots in figures 1-6, also showed an overprediction by FRS and PCEs and good prediction by SCORE, as did the E:O ratios presented in table 2. Discrimination for the original models in women with and without hHDP was reasonable for FRS and PCEs ranging from 0.70 to 0.72 and quite good for SCORE ranging from 0.74 to 0.81 (table 2). The E:O ratio of the original PCE model was statistically significantly better in women with hHDP compared with women without hHDP.

Table 1. Baseline characteristics of EPIC-NL by hypertensive disorders of pregnancy

EPIC-NL (N=22,753)			
	HDP[b] N=6,083 (26.7%)	**No HDP N=16,670 (73.3%)**	**p-value**
Age[a]	54.4 [50.2; 60.4]	53.6 [49.5; 59.3]	<0.001
Systolic blood pressure (mmHg)[a]	131.5 [119.0; 145.5]	124.0 [112.0; 136.5]	<0.001
Hypertension medication (Yes)	21.3% (1298)	10.5% (1744)	<0.001
Total cholesterol (mg/dl)	222.0 ± 40.5	220.3 ± 41.4	0.007
HDL cholesterol (mg/dl)	60.2 ± 15.7	60.2 ± 15.8	0.993
Diabetes (Yes)	3.4% (205)	2.1% (353)	<0.001
Smoking (Yes)	21.7% (1,318)	28.9% (4,808)	<0.001
Events at 10-year follow-up according to definition:			
FRS[c]	7.2% (437)	5.6% (935)	<0.001
PCE[d]	2.8% (168)	1.9% (323)	<0.001
SCORE[e]	0.8% (46)	0.7% (124)	0.993

Results based on non-imputed data
[a]Median [IQR]
[b]Hypertensive Disorders of Pregnancy
[c]Framingham Risk Score
[d]Pooled Cohort Equations
[e]Systematic COronary Risk Evaluation model

Table 2. Performance of the original, recalibrated and refitted FRS, PCE and SCORE.

	Framingham Risk Score			Pooled Cohort Equations			SCORE		
	Women with HDP[b]	*Women without HDP*	*Comparison women with and without[d]*	*Women with HDP*	*Women without HDP*	*Comparison women with and without[d]*	*Women with HDP*	*Women without HDP*	*Comparison women with and without[d]*
Observed absolute risk[a] (within 10 years)	6.7%	5.3%	1.4%	2.7%	1.9%	0.8%	0.74%	0.73%	0.01%
Predicted risk (within 10 years)									
Original	10.1%	8.3%	1.8%	5.0%	4.4%	0.6%	0.75%	0.70%	0.05%
Recalibrated	9.2%	7.4%	1.8%	4.2%	3.0%	1.2%	0.83%	0.75%	0.08%
Refit	8.9%	7.6%	1.3%	3.9%	2.8%	1.1%	0.82%	0.77%	0.05%
E:O ratio[c] (95% CI)									
Original	1.51 (1.48; 1.55)	1.55 (1.53; 1.58)	0.98 (0.86; 1.08)	1.85 (1.80; 1.91)	2.29 (2.24; 2.33)	0.82 (0.66; 0.98)	0.99 (0.94; 1.03)	0.95 (0.94; 0.97)	1.06 (0.69; 1.43)
Recalibrated	1.37 (1.34; 1.40)	1.39 (1.37; 1.41)	0.98 (0.97; 1.00)	1.55 (1.51; 1.59)	1.57 (1.54; 1.60)	0.99 (0.97; 1.01)	1.10 (1.04; 1.16)	1.02 (0.99; 1.04)	1.09 (0.82; 1.36)
Refit	1.33 (1.30; 1.36)	1.43 (1.41; 1.45)	0.93 (0.85; 1.02)	1.42 (1.38; 1.46)	1.44 (1.42; 1.47)	1.00 (0.82; 1.19)	1.08 (1.01; 1.14)	1.05 (1.02; 1.08)	1.04 (0.78; 1.31)
C-statistic (95% CI)									
Original	0.70 (0.68; 0.73)	0.72 (0.71; 0.74)	-0.02 (-0.05; 0.01)	0.72 (0.68; 0.76)	0.72 (0.69; 0.75)	-0.005 (-0.06; 0.05)	0.81 (0.75; 0.86)	0.74 (0.70; 0.78)	0.07 (-0.01; 0.14)
Recalibrated	0.70 (0.68; 0.73)	0.72 (0.71; 0.74)	-0.02 (-0.05; 0.01)	0.72 (0.68; 0.76)	0.72 (0.69; 0.75)	-0.005 (-0.06; 0.05)	0.81 (0.76; 0.87)	0.75 (0.71; 0.79)	0.06 (-0.004;0.13)
Refit	0.71 (0.68; 0.73)	0.73 (0.71; 0.75)	-0.02 (-0.05; 0.01)	0.72 (0.68; 0.76)	0.73 (0.70; 0.76)	-0.001 (-0.05; 0.05)	0.83 (0.78; 0.89)	0.80 (0.76; 0.84)	0.03 (-0.05; 0.10)

[a] *Non-imputed data, only events within first ten years.*

[b] *Hypertensive Disorders of Pregnancy*

[c] *Expected:Observed ratio*

[d] *Difference between C-statistic of women with HDP and without HDP; and ratio of E:O ratios between women with HDP and without HDP. Difference in C-statistic is significant if 0 is not included in the 95% CI; ratio of E:O ratios is significant if 1 is not included in the 95% CI*

Next, recalibration of the models improved predicted risks for FRS and PCEs; compared with the original model, the predicted risks were more in line with the observed risks. For SCORE the predicted risks worsened in women with hHDP but improved in women without hHDP as shown in Table 2. All three calibration plots (figures 1-6) still show an overprediction, but also a slight improvement compared to the original model for RS and PCEs. Calibration plots of SCORE show a slight overprediction for women with hHDP and stay approximately the same for women without hHDP. The E:O ratios were in line with these findings as well. We did not find a statistically significant difference between the C-statistics of women with and without hHDP or E:O ratios of women with or without hHDP of the recalibrated models.

As a final step, we refitted all coefficients of the models in our own data for benchmarking purposes and to improve discrimination. This only slightly improved discrimination for FRS and PCEs in both women with hHDP and women without hHDP (shown in table 2). For SCORE, discrimination improved in women with hHDP and the largest improvement in discrimination was seen in women without hHDP. Calibration also slightly improved in all three models as shown by the calibration plots, except for the refitted FRS in women without hHDP (figures 1-6). The same tendency is shown by the E:O ratios. For SCORE, refitting the model improved the E:O ratio for women with hHDP compared with the recalibrated, but not the original model. The E:O ratio for women without hHDP got worse compared with both the original and recalibrated model, also shown in table 2. Furthermore, also for the refitted models, we did not find a statistically significant difference between the C-statistics of women with and without hHDP and the E:O ratios of women with and without hHDP.

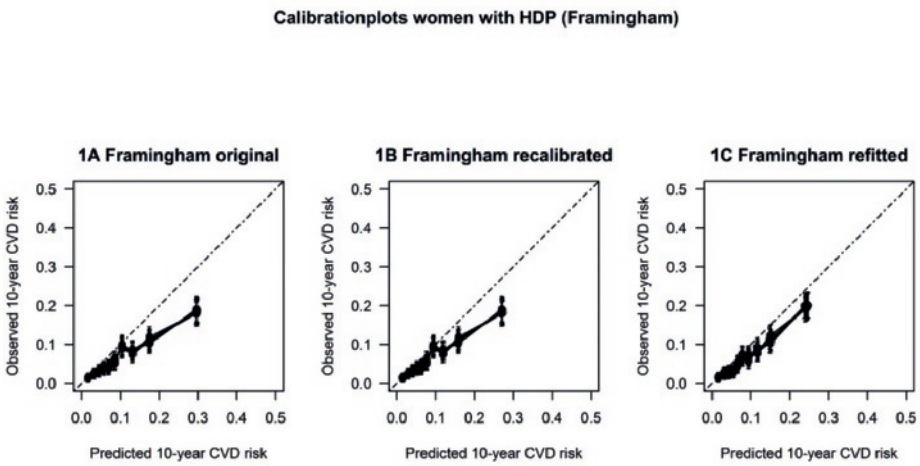

Figure. 1 *Calibration plots FRS (women with hHDP). Plot 1A, 1B, and 1C respectively show the calibration of the original FRS model, the recalibrated FRS model, and the refitted FRS model in women with hHDP. The dotted 45 degree line shows a perfect calibration.*

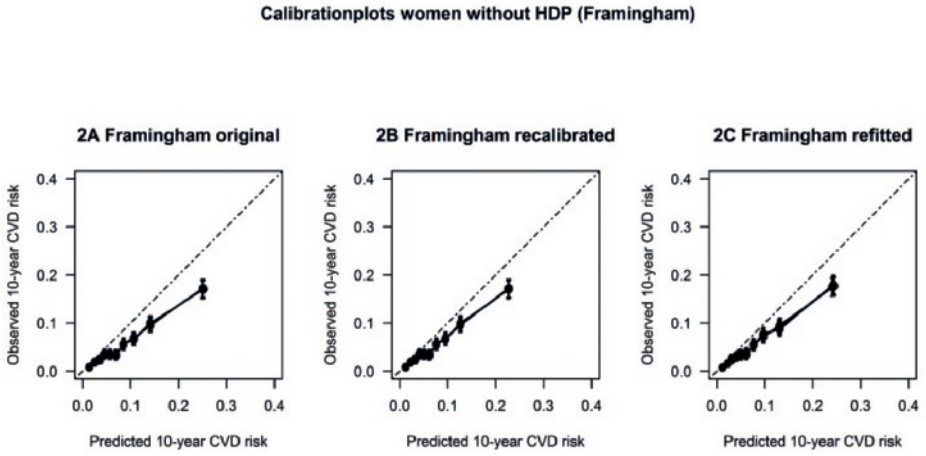

Figure. 2 *Calibration plots FRS (women without hHDP). Plot 2A, 2B, and 2C respectively show the calibration of the original FRS model, the recalibrated FRS model, and the refitted FRS model in women without hHDP. The dotted 45 degree line shows a perfect calibration.*

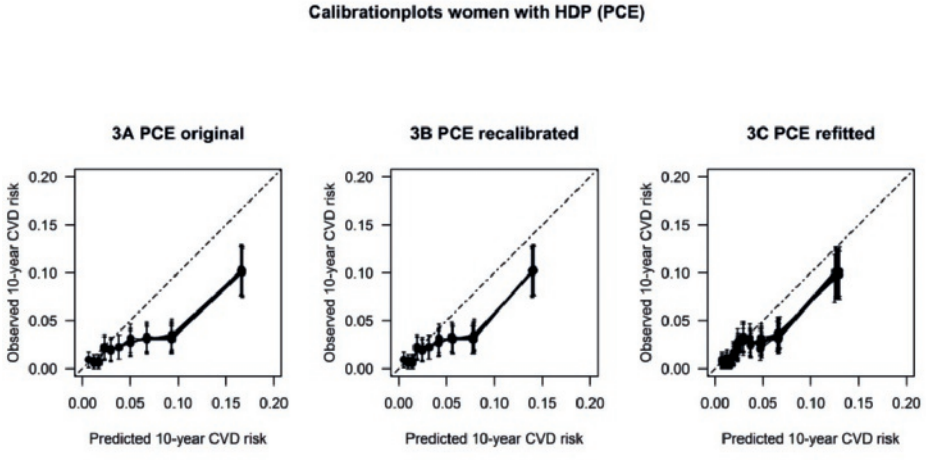

Figure. 3 *Calibration plots PCE (women with hHDP). Plot 3A, 3B, and 3C respectively show the calibration of the original PCE model, the recalibrated PCE model, and the refitted PCE model in women with hHDP. The dotted 45 degree line shows a perfect calibration.*

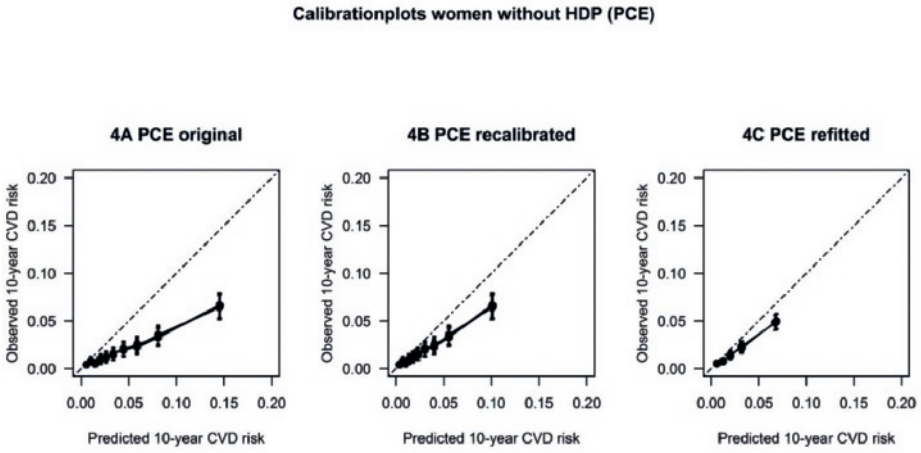

Figure. 4 *Calibration plots PCE (women without hHDP). Plot 4A, 4B, and 4C respectively show the calibration of the original PCE model, the recalibrated PCE model, and the refitted PCE model in women without hHDP. The dotted 45 degree line shows a perfect calibration.*

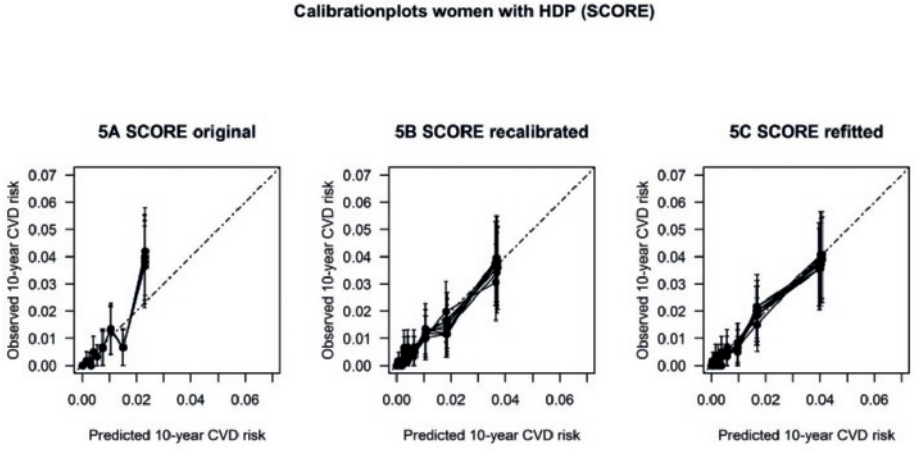

Figure. 5 *Calibration plots SCORE (women with hHDP). Plot 5A, 5B, and 5C respectively show the calibration of the original SCORE model, the recalibrated SCORE model, and the refitted SCORE model in women with hHDP. The dotted 45 degree line shows a perfect calibration.*

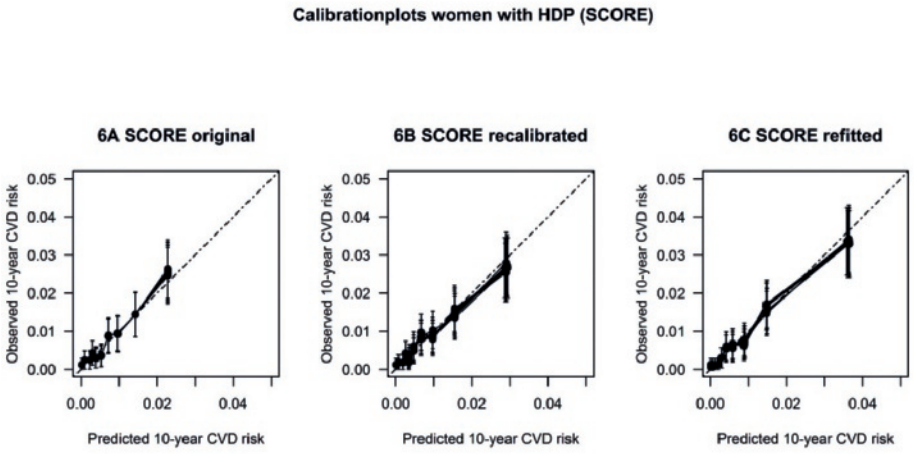

Figure. 6 *Calibration plots SCORE (women without hHDP). Plot 6A, 6B, and 6C respectively show the calibration of the original SCORE model, the recalibrated SCORE model, and the refitted SCORE model in women without hHDP. The dotted 45 degree line shows a perfect calibration.*

Discussion

This study showed that the observed absolute risk of CVD for women with hHDP is 1.0-1.4 times higher compared to women without hHDP. The FRS and PCE models overpredicted risk in all women, either with or without hHDP. The SCORE model only slightly overpredicted risk in women with hHDP and underpredicted risk in women without hHDP. Recalibrating FRS and PCEs improved calibration, but refitting the models, as expected, gave the best calibration. The SCORE model performed best in its original form in women with hHDP in terms of calibration, while the recalibrated form performs best in women without hHDP. Discrimination of the models was similar as in the development studies and slightly improved after refitting the models. There was no significant difference in performance between the models. These results show that no separate model for women with HDP seems necessary.

Limitations and strengths

This study was limited by the assessment of HDP history which was based on the simple, self-reported question 'Did you suffer from high blood pressure during pregnancy?' This question does not provide information on the most severe HDP, like pre-eclampsia, and milder variants of blood pressure elevation are probably also included. This might explain the high prevalence of hHDP (21.3%) in our study. Furthermore, sensitivity of maternal recall of HDP varies between studies

ranging from 31% to 100%, but specificity is generally high (over 90%)[22]. Another limitation is the definition of the FRS outcome which includes heart failure. In our study the diagnosis heart failure is obtained from the main diagnosis in the hospital discharge registry. However, many people are not in the first place admitted to the hospital for heart failure, but heart failure might be a secondary diagnosis. These cases are missing in our definition. Next, it would have been interesting to validate the Reynolds Risk Score[23], since this model is specifically made for women. However, several of the predictors used in this model were not available in EPIC-NL. Finally, with interpreting the results, one should keep in mind that these models all predict a different outcome. Moreover, FRS and PCEs both state they predict CVD, but they use a different definition of CVD. We took this into account by creating a corresponding outcome for each model in our own data, but clinicians should decide what outcome they want to predict in their patients. Strengths of this study are the large sample size and long follow-up with a median of 15 years (IQR = 14-16), which made it possible to validate the original models with their original prediction horizons. Furthermore, the cohort had all predictors and outcomes available that were used in the original models.

Previous research

As far as we know, no other studies have validated or updated conventional cardiovascular risk scores in women with hHDP. There are some studies that used these risk scores in women with hHDP and found that women with hHDP have higher mean predicted risks than women without hHDP, which is similar to our findings[12,13,24]. However, these studies do not formally validate or recalibrate the models and therefore probably yield overpredicted risks. It has been shown that 15-20 years after HDP, women with hHDP still have a worse cardiovascular risk profile compared with women without hHDP, including a higher body mass index (BMI), greater waist circumference and more unfavourable lipid levels[25,26], which underpins the importance of well-validated prediction models in these women.

The original SCORE model seemed to perform best in women with hHDP; however, this model only predicts CVD mortality. Both FRS and PCEs overpredicted risk in women with and without HDP, like previous external validations of these models in the general population showed[27,28]. After recalibration, both models performed equally well in predicting fatal and non-fatal CVDs irrespective of HDP. Recently, a study from our group showed that, already at a young age, women with early-onset pre-eclampsia have higher systolic and diastolic blood pressures compared with women with a previous normotensive pregnancy[24]. Furthermore, women with hHDP have over three times more chance of developing hypertension in the first decade after their hypertensive pregnancy[29]. Since systolic blood pressure and antihypertensive medication are important predictors in all three models, this might explain the good performance of the models in women with hHDP. Therefore, our study implies that there is no need for a new prediction

model for women with hHDP and physicians can use the best validated model available to predict risk in this subgroup of women. The fact that the models work very well in women with HDP might explain why adding HDP to existing CVD risk prediction models does not seem to improve performance[30].

Conclusions

Most importantly, we found that no separate model for women with hHDP seems needed, because existing risk prediction models perform equally well in women with and without hHDP. Moreover, the original SCORE model performed best in both groups of women, with good discrimination as well as very good calibration. The FRS and PCE models performed worse compared to SCORE, but their calibration and discrimination improved substantially after recalibrating and refitting the models. Besides, SCORE only predicts CVD mortality, while FRS and PCEs predict a composite of CVD morbidity and mortality, making them more generally applicable. These findings show that physicians can use these models in women of the general population and also for after-pregnancy surveillance in women with hHDP, although FRS and PCEs need some updating before use.

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References

1. Tranquilli a. L, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4:97–104.
2. Brown MC, Best KE, Pearce MS, Waugh J, Robson SC, Bell R. Cardiovascular disease risk in women with pre-eclampsia: Systematic review and meta-analysis. Eur J Epidemiol. 2013;28:1–19.
3. Bellamy L, Casas J-P, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. Bmj. 2007;335:974.
4. Männistö T, Mendola P, Vääräsmäki M, Järvelin M-R, Hartikainen A-L, Pouta A, et al. Elevated blood pressure in pregnancy and subsequent chronic disease risk. Circulation. 2013;127:681–90.
5. Canoy D, Cairns BJ, Balkwill A, Wright FL, Khalil A, Beral V, et al. Hypertension in pregnancy and risk of coronary heart disease and stroke: A prospective study in a large UK cohort. Int J Cardiol. The Authors; 2016;222:1012–8.
6. Heida KY, Bots ML, de Groot CJ, van Dunné FM, Hammoud NM, Hoek A, et al. Cardiovascular risk management after reproductive and pregnancy-related disorders: A Dutch multidisciplinary evidence-based guideline. Eur J Prev Cardiol. 2016;23:1863–79.
7. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women—2011 Update. J Am Coll Cardiol. Elsevier Inc.; 2011;57:1404–23.
8. Paauw ND, van Rijn BB, Lely a. T, Joles J a. Pregnancy as a critical window for blood pressure regulation in mother and child: programming and reprogramming. Acta Physiol. 2016;
9. Barrett HL. Hypertensive disorders of pregnancy. BMJ. 2017;
10. Parikh NI, Gonzalez J. Preeclampsia and Hypertension Courting a long while: time to make it official. JAMA Intern Med. 2017;177.
11. Damen J a a G, Hooft L, Schuit E, Debray TP a, Collins GS, Tzoulaki I, et al. Prediction models for cardiovascular disease risk in the general population: systematic review. Bmj. 2016;i2416.
12. Smith GN, Pudwell J, Walker M, Wen S-W. Ten-Year, Thirty-Year, and Lifetime Cardiovascular Disease Risk Estimates Following a Pregnancy Complicated by Preeclampsia ; for the Pre-Eclampsia New Emerging Team. J Obs Gynaecol Can. Elsevier Masson SAS; 2012;34:830–5.
13. Hermes W, Tamsma JT, Grootendorst DC, Franx A, van der Post J, van Pampus MG, et al. Cardiovascular risk estimation in women with a history of hypertensive pregnancy disorders at term: a longitudinal follow-up study. BMC Pregnancy Childbirth. 2013;13:126.
14. D'Agostino RB, Vasan RS, Pencina MJ, Wolf P a., Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: The Framingham heart study. Circulation. 2008;117:743–53.

15. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. J Am Coll Cardiol. 2014;63:2935–59.
16. Conroy RM, Pyörälä K, Fitzgerald a. P, Sans S, Menotti a., De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: The SCORE project. Eur Heart J. 2003;24:987–1003.
17. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): the TRIPOD Statement. Ann Intern Med. 2015;162.
18. Beulens JWJ, Monninkhof EM, Verschuren WMM, van der Schouw YT, Smit J, Ocke MC, et al. Cohort profile: the EPIC-NL study. Int J Epidemiol [Internet]. 2010 [cited 2014 Nov 12];39:1170–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19483199
19. van Buuren K. S. G-O. mice: Multivariate Imputation by Chained Equations in R. J Stat Softw. 2011;45.
20. Team RC. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical computing; 2016. Available from: https://www.r-project.org/
21. Wood AM, Royston P, White IR. The estimation and use of predictions for the assessment of model performance using large samples with multiply imputed data. Biometrical J. 2015;57:614–32.
22. Stuart JJ, Bairey Merz CN, Berga SL, Miller VM, Ouyang P, Shufelt CL, et al. Maternal recall of hypertensive disorders in pregnancy: a systematic review. J Women's Heal. 2013;22:37–47.
23. Ridker PM, Buring JE, Cook NR. Algorithms for the Assessment of Global Cardiovascular Risk in Women. Jama. 2007;297:611–20.
24. Van Rijn BB, Nijdam ME, Bruinse HW, Roest M, Uiterwaal CS, Grobbee DE, et al. Cardiovascular disease risk factors in women with a history of early-onset preeclampsia. Obstet Gynecol. 2013;121:1040–8.
25. Veerbeek JHW, Hermes W, Breimer AY, Van Rijn BB, Koenen S V., Mol BW, et al. Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension. Hypertension. 2015;65:600–6.
26. Zoet G a., Koster MPH, Velthuis BK, De Groot CJM, Maas AHEM, Fauser BCJM, et al. Determinants of future cardiovascular health in women with a history of preeclampsia. Maturitas. Elsevier Ireland Ltd; 2015;82:153–61.
27. Majed B, Tafflet M, Kee F, Haas B, Ferrieres J, Montaye M, et al. External validation of the 2008 Framingham cardiovascular risk equation for CHD and stroke events in a European population of middle-aged men. The PRIME study. Prev Med (Baltim). 2013;57:49–54.

28. Cook NR, Ridker PM. Calibration of the pooled cohort equations for atherosclerotic cardiovascular disease: An update. Ann Intern Med. 2016;165:786–94.
29. Behrens I, Basit S, Melbye M, Lykke JA, Wohlfahrt JA, Bundgaard H, et al. Risk of post-pregnancy hypertension in women with a history of hypertensive disorders of pregnancy: nationwide cohort study. BMJ. 2017;
30. Van Der Meer MG, Van Der Graaf Y, Schuit E, Peelen LM, Verschuren WMM, Boer JM a, et al. Added Value of Female-Specific Factors beyond Traditional Predictors for Future Cardiovascular Disease. J Am Coll Cardiol. 2016;67:2084–6.

CHAPTER 4

General discussion

Cardiovascular disease (CVD) is the leading cause of death and disability in women worldwide[1]. In 2015, CVD accounted for 22% of all disability adjusted life-years (DALYs) lost in women[2]. Women and men share traditional CVD risk factors, but female-specific risk factors seem to play a role in women as well[3–5]. Our first aim was to establish whether female-specific risk factors (menopausal status, age at menopause, vasomotor menopausal symptoms (VMS) and polycystic ovary syndrome (PCOS)) were (causally) associated with CVD, coronary heart disease (CHD) and stroke. Additionally, we aimed to establish whether changes in cardiovascular risk factors are a mechanism underlying these associations.

Our observational findings on menopause support the general believe that women have an unfavourable cardiovascular risk profile around and after menopause and that CHD risk in women increases after menopause (Chapter 2.1 and Chapter 2.2). However, our Mendelian Randomization (MR) study showed that the association between menopause and CHD risk is likely not causal (Chapter 2.3). Additionally, we did not find associations for VMS and PCOS, and thus literature on these risk factors remains inconclusive. Therefore, it will be worthwhile to also conduct MR studies on these and other female-specific risk factors to establish causality. This is already feasible for age at menarche[6], PCOS[7,8], preeclampsia[9] and gestational diabetes[10,11], since Genome Wide Association Studies (GWAS) are already available for these determinants. Also for VMS a first GWAS was conducted, but in a mixed ancestry population, which may limit its applicability in Caucasian populations[12].

Besides unravelling the association between female-specific risk factors and CVD with MR studies, future research could also take a different approach towards these female-specific risk factors. Menopause marks the end of the reproductive lifespan, beginning from menarche, sometimes including the development of PCOS, and often including pregnancies, with or without complications like preeclampsia or gestational diabetes. Moreover, this reproductive lifespan can also end prematurely when a woman develops premature ovarian insufficiency. Hence, it will be interesting to study the joint impact of more than one female-specific risk factor and take the whole reproductive lifespan into account. One way to do this is with a life-course epidemiology approach, a method that examines how biological (including genetics), behavioural and social factors throughout life and across generations, act independently, cumulatively and interactively to influence health[13]. Within the reproductive lifespan there might be an opportunity to identify certain trajectories or subpopulations, e.g. women with an early menarche and early menopause, women with an early menarche but late menopause etc., in whom we could describe longitudinal changes in cardiovascular risk factors or even link this to CVD events. One of the main problems with this approach is the need for longitudinal data across the whole life course, like birth cohorts with extremely long follow-up. Some suggestions to overcome this problem are using cross-sectional data from a single study with a very wide age range, or creating a large

cohort by combining data of several studies with different age groups[14]. This first suggestion has similarities with our study on menopausal status and CVD risk factors in Chapter 2.1, where we investigate the association between menopausal status and CVD risk factors in 1-year age strata. This enabled us to study the association between menopausal status and CVD risk factors within and between age groups. A similar approach can be taken using reproductive lifespan trajectories. However, most cross-sectional studies do not have data on CVD events and using this approach it is not possible to take survival time into account. The second approach is more comparable to a longitudinal study, but the challenge is how to combine studies in different generations with different subjects and how to follow them up. If we can do this properly, without introducing too much bias, this approach would generate a treasure chest of data. The InterLACE study is an example of such a study, which combines individual participant data of over 200,000 women from 13 studies from seven countries and still increasing[15]. Other studies that come close to a life-course approach, are the registry-based cohorts used in the Scandinavian countries. However, these studies often do not have much covariate information, which makes it difficult to properly adjust for confounders.

Within this life-course approach or the reproductive lifespan it is also possible to look for critical or sensitive periods that might influence outcomes in the future. A critical period is a period in which an exposure can have adverse or protective effects on future disease outcomes, but outside this period there is no excess risk associated with the exposure (e.g. poor growth in utero)[13,14,16]. Throughout a sensitive period an exposure has a stronger effect on development and disease risk than it would have during other periods, and thus might still be associated with the outcome, but less strong than during the sensitive period (e.g. current smokers had an earlier age at menopause than former smokers, suggesting perimenopause to be a sensitive period when the effect of smoking may be more important than smoking history in explaining an early menopause)[13,14,16]. Possible critical or sensitive periods in a woman her reproductive lifespan might be pregnancies, whether or not complicated with hypertension or gestational diabetes, and the menopausal transition. Another step further would be to combine these methods. If there are certain trajectories or subpopulations we could identify within the reproductive lifespan, how do sensitive or critical periods influence these trajectories? And how are genetics and the environmental factors involved?

A concept that relates closely to combining all these factors is the exposome. This concept was first described by Wild, and covers the totality of human environment (i.e. non-genetic), exposures from conception onwards, complementing the genome[17]. It generally includes three domains that overlap and complement each other: a general external domain including macro-level factors such as climate, social and economic context and psychosocial factors; a specific external domain including agents such as environmental pollutants, diet and drugs; and

a specific internal domain including inflammation, metabolism and gut microflora[17,18]. Studies that tried to study the exposome have shown the complexity of comprehensively measuring and analysing the exposome, but they all share a similar holistic approach, rather than a single exposure approach[18]. Recently, investigators proposed to define the 'pregnancy exposome', because pregnancy is a specific period in life with potential lifelong consequences of internal and general and specific external exposures [18]. A recent exposome study on obesity showed that, by modelling collections of highly-correlated variables, variables concentrated around social deprivation, community infrastructure and climate were connected to obesity. The authors suggest that interventions to decrease obesity should be planned around community organisation and structure, rather than the individual level[19]. Similarly, it might be interesting to identify a 'female exposome' in which we could combine a cluster of exposures that are specific for women which takes their reproductive lifespan into account. We could study the influence of menarche, pregnancies, fertility, menopause and the environmental stressors on these determinants and their association with cardiovascular disease.

Although the causes and mechanisms underlying CVD risk in women are not fully understood yet, it is evident that also in women CVD is an important cause of morbidity and mortality. Therefore, it is important that we identify women at high risk and implement preventive measures when necessary. Our third aim was to assess the availability and performance of existing CVD risk prediction models for women in the general population and to establish whether the performance of a subset of commonly used models differs between women with and without a history of hypertensive disorders of pregnancy (HDP). In our review (Chapter 3.1) we showed that an abundance of cardiovascular risk prediction models is available for the general population and many of them are developed specifically for women or include sex as a predictor. Most of these models have a moderately to good performance and it has been thought that these models could improve by adding female-specific risk factors. However, several studies that implemented this method, could not confirm this hypothesis[20,21]. Since we do not find a causal association between female-specific risk factors and CVD, it is in fact not surprising that these risk factors do not improve cardiovascular risk prediction. When applying the well-known CVD risk prediction models in women with HDP or preeclampsia, they do predict higher absolute cardiovascular risks compared to women with normal pregnancies[22], something we also observed in our validation study. Thus, these women might benefit from close monitoring or screening for CVD as is also advised in the American guideline for the prevention of cardiovascular disease in women. This thesis showed that the well-known models used in the general population can also be used in women with HDP (Chapter 3.2), and no separate model for women with HDP is necessary. A recent Dutch study modelled the effect of a yearly blood pressure screening, starting one year postpartum, in women with preeclampsia. Women lived on average 12 days longer in perfect

health (a 0.03 increase in Quality Adjusted Life Years (QALYs)), which is a rather small effect, but it did save 1071 euros per person[23]. When GPs would screen for other risk factors and CVD in these and other women with pregnancy complications as well, this increase in QALYs might further increase to a more clinically relevant gain and save even more money. A complication is that these women are often young when pregnancy-related disorders occur and it is currently unknown what the best age is to start screening. A recent study does show that 30% of women with a history of preeclampsia show signs of coronary atherosclerosis already at age 45 to 55 years, compared to only 18% in the reference group[24]. This suggests that it might be beneficial to start screening these women already at an early age, before they present with any complaints. Establishing the optimal age to start screening could be done with modelling studies that model different starting ages and subsequently calculate the decreased CVD risk, gained QALYs and/or reduced costs[25]. Or we could implement a newly developed selection method[26], based on the expected burden of CVD instead as well as the predicted risk. The expected burden is calculated by multiplying the predicted risk by the consequences of experiencing CVD. By selecting high risk women for preventive treatment based on both the expected burden as well as the predicted risk, one treats both older women with high CVD risk, as well as younger women with a low absolute risk but potentially a high health loss[26].

Conclusion

The benefits of studying sex-differences, the focus on female-specific risk factors and the monitoring of women with pregnancy related disorders, all in relation to CVD, becomes increasingly evident. However, research that focusses on one female-specific risk factor only might not be sufficient anymore. Therefore, a more life-course epidemiology or exposome approach that takes the whole reproductive lifespan and all its influences into account might be an interesting new focus for future research. Aiming at identifying certain trajectories or subpopulations within the general female population that might further explain the mechanisms underlying the increased CVD risk in women.. These subpopulations or trajectories might also be a starting point to improve risk prediction in women, since we would be able to better identify women at the highest risk or improve CVD risk prediction models. For now, general practitioners should be aware of the fact that some groups of women, e.g. women with pregnancy complications like preeclampsia, are at increased risk and might benefit from closer monitoring and screening for CVD or CVD risk factors.

References

1 Murray CJL, Barber RM, Foreman KJ, *et al.* Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: Quantifying the epidemiological transition. *Lancet* 2015;**386**:2145–91. doi:10.1016/S0140-6736(15)61340-X

2 Timmis A, Townsend N, Gale C, *et al.* European Society of Cardiology: Cardiovascular disease statistics 2017. *Eur Heart J* 2018;**39**:508–77. doi:10.1093/eurheartj/ehx628

3 Muka T, Oliver-Williams C, Kunutsor S, *et al.* Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: A systematic review and meta-analysis. *JAMA Cardiol* 2016;**1**:767–76. doi:10.1001/jamacardio.2016.2415

4 Franco OH, Muka T, Colpani V, *et al.* Vasomotor symptoms in women and cardiovascular risk markers: Systematic review and meta-analysis. *Maturitas* 2015;**81**:353–61. doi:10.1016/j.maturitas.2015.04.016

5 Brown MC, Best KE, Pearce MS, *et al.* Cardiovascular disease risk in women with pre-eclampsia: Systematic review and meta-analysis. *Eur J Epidemiol* 2013;**28**:1–19. doi:10.1007/s10654-013-9762-6

6 Perry JRB, Day F, Elks CE, *et al.* Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature* 2014;**514**:92–7. doi:10.1038/nature13545

7 Day FR, Hinds DA, Tung JY, *et al.* Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun* 2015;**6**:1–7. doi:10.1038/ncomms9464

8 Hayes MG, Urbanek M, Ehrmann DA, *et al.* Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun* 2015;**6**:1–12. doi:10.1038/ncomms8502

9 Johnson MP, Brennecke SP, East CE, *et al.* Genome-wide association scan identifies a risk locus for preeclampsia on 2q14, near the inhibin, beta B gene. *PLoS One* 2012;**7**. doi:10.1371/journal.pone.0033666

10 Hayes MG, Urbanek M, Hivert MF, *et al.* Identification of HKDC1 and BACE2 as genes influencing glycemic traits during pregnancy through genome-wide association studies. *Diabetes* 2013;**62**:3282–91. doi:10.2337/db12-1692

11 Ding M, Chavarro J, Olsen S, *et al.* Genetic variants of gestational diabetes mellitus: a study of 112 SNPs among 8722 women in two independent populations. *Diabetologia* 2018;**61**:1758–68. doi:10.1007/s00125-018-4637-8

12 Crandall C, Manson J, Hohensee C, *et al.* Association of genetic variation in the tachykinin receptor 3 locus with hot flashes and night sweats in the Women's Health Initiative Study. *Menopause* 2017;**24**:252–61. doi:10.1097/GME.0000000000000763

13 Mishra GD, Cooper R, Kuh D. A life course approach to reproductive health: Theory and methods. *Maturitas* 2010;**65**:92–7. doi:10.1016/j.maturitas.2009.12.009

14 Ben-Shlomo Y, Cooper R, Kuh D. The last two decades of life course epidemiology, and its relevance for research on ageing. *Int J Epidemiol* 2016;**45**:973–88. doi:10.1093/ije/dyw096

15 Mishra GD, Anderson D, Schoenaker DAJM, *et al.* InterLACE: A new international collaboration for a life course approach to women's reproductive health and chronic disease events. *Maturitas* 2013;**74**:235–40. doi:10.1016/j.maturitas.2012.12.011

16 Ben-Shlomo Y, Kuh D. What is a Life Course Approach to Chronic Disease Epidemiology? Conceptual Models in Life Course Epidemiology. *Int J Epidemiol* 2002;**31**:285–93. doi:10.1093/ije/31.2.285

17 Wild CP. Complementing the genome with an 'exposome': The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:1847–50. doi:10.1158/1055-9965.EPI-05-0456

18 Robinson O, Vrijheid M. The Pregnancy Exposome. *Curr Environ Heal reports* 2015;**2**:204–13. doi:10.1007/s40572-015-0043-2

19 Gittner LAS, Kilbourne BJ, Vadapalli R, *et al.* A multifactorial obesity model developed from nationwide public health exposome data and modern computational analyses. *Obes Res Clin Pract* 2017;**11**:522–33. doi:10.1016/j.orcp.2017.05.001

20 Van Der Meer MG, Van Der Graaf Y, Schuit E, *et al.* Added Value of Female-Specific Factors beyond Traditional Predictors for Future Cardiovascular Disease. *J Am Coll Cardiol* 2016;**67**:2084–6. doi:10.1016/j.jacc.2016.02.031

21 Parikh NI, Jeppson RP, Berger JS, *et al.* Reproductive risk factors and coronary heart disease in the women's health initiative observational study. *Circulation* 2016;**133**:2149–58. doi:10.1161/CIRCULATIONAHA.115.017854

22 van Rijn BB, Nijdam M-E, Bruinse HW, *et al.* Cardiovascular Disease Risk Factors in Women With a History of Early-Onset Preeclampsia. *Obstet Gynecol* 2013;**121**:1040–8. doi:10.1097/AOG.0b013e31828ea3b5

23 Drost JT, Grutters JPC, van der Wilt GJ, *et al.* Yearly hypertension screening in women with a history of pre-eclampsia: A cost-effectiveness analysis. *Netherlands Hear J* 2015;**23**:585–91. doi:10.1007/s12471-015-0760-z

24 Zoet GA, Benschop L, Boersma E, *et al.* Prevalence of Subclinical Coronary Artery Disease Assessed by Coronary Computed Tomography Angiography in 45- to 55-Year-Old Women With a History of Preeclampsia. *Circulation* 2018;**137**:877–9. doi:10.1161/CIRCULATIONAHA.117.032695

25 Kahn R, Alperin P, Eddy D, *et al.* Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. *Lancet* 2010;**375**:1365–74. doi:10.1016/S0140-6736(09)62162-0

26 Lagerweij GR, de Wit GA, Moons KG, *et al.* A new selection method to increase the health benefits of CVD prevention strategies. *Eur J Prev Cardiol* 2018;:204748731775294. doi:10.1177/2047487317752948

CHAPTER 5

Final chapters

Summary

The overall aims of this thesis were to study whether female-specific risk factors (menopausal status, age at menopause, vasomotor menopausal symptoms (VMS) and polycystic ovary syndrome (PCOS)) are (causally) associated with cardiovascular disease (CVD), coronary heart disease (CHD) and stroke; to investigate the possible of cardiovascular risk factors as mechanisms underlying this association; and to review CVD risk prediction models for women in the general population and validate a selection of these models (Framingham Risk Score (FRS), Pooled Cohort Equations (PCE) and SCORE) in women with and without a history of hypertensive disorders of pregnancy (HDP).

Chapter 2.1 aimed to disentangle the relationships of menopausal status and chronological aging with CVD risk factors in the largest study population to date, the LifeLines cohort study (N=63,466). We cross-sectionally compared CVD risk factors between women with a different menopausal status within the same yearly age strata. Chronological age and menopausal status are both independently associated with CVD risk factors. Specifically, after the age of 45, peri- and postmenopausal women had more unfavourable lipid levels compared to premenopausal women in their same age stratum. Based on the comparatively smaller observed differences associated with menopausal status than chronological aging, the biological significance of a more unfavourable lipid profile in later reproductive stage may be less obvious than previously thought.

In **Chapter 2.2** we examined the associations between menopausal characteristics and CHD risk; the shape of the association between age at menopause and CHD risk; and the extent to which these associations are explained by established CVD risk factors. We used data from EPIC-CVD, a case-cohort study including data from 23 centres from 10 European countries. We included only women, of whom 10,880 comprised the randomly selected subcohort, supplemented with 4522 CHD cases outside the subcohort. Postmenopausal women were at higher CHD risk compared to premenopausal women, although not statistically significant (HR=1.13, 95% confidence interval (CI): 0.98-1.30). Among postmenopausal women, earlier menopause was linearly associated with higher CHD risk (HR per-year decrease=1.02, 95%CI: 1.01-1.03). After adjustment, women with a surgical menopause were at higher risk of CHD compared to those with natural menopause (HR=1.25, 95%CI: 1.10-1.42), but this attenuated after additional adjustment for age at menopause (HR=1.15, 95%CI: 1.00-1.33). A proportion of the association was explained by CVD risk factors, in particular lipid levels. Women with an earlier and surgical menopause might benefit from close monitoring of cardiovascular risk factors and disease.

In order to strengthen the results from Chapter 2.2, we conducted a Mendelian Randomization (MR) study in **Chapter 2.3**, aiming to establish a causal association between reproductive aging

and fatal or non-fatal CHD, and between reproductive aging and CVD risk factors. Since the 56 genomic variants associated with age at natural menopause (ANM) in GWAS are mainly implicated in genome stability, immune function and mitochondrial biogenesis, which are not sex-specific processes, we studied women as well as men. We conducted an MR analysis using summarized data from three studies (UK Biobank, a modified version of CARDIoGRAMplusC4D and EPIC-CVD) with together 417,579 from European descent (including 49,150 CHD cases). For the analyses between ANM and CVD risk factors, we used data from publicly available GWAS data of the Global Lipids Genetics Consortium (total cholesterol, high density lipoprotein cholesterol, triglycerides) and MAGIC (HbA1c, fasting glucose) and EPIC-CVD. Reproductive aging was not associated with CHD in women (Inversed Variance Weighted method (IVW): 0.99, 95%CI: 0.97-1.01), men (IVW: 1.00, 95%CI: 0.97-1.02), or in the sex-combined analysis (IVW: 1.00, 95%CI: 0.98-1.01). Similarly, no association was found between ANM and any of the cardiovascular risk factors.

Chapter 2.4 examined the association between VMS, hot flushes and night sweats, and CVD, CHD and cerebrovascular disease among 7,112 women from the 1946-51 cohort from the Australian Longitudinal Study on Women's Health, aged 45-50 years at baseline in 1996. There was no consistent evidence of any association with VMS, hot flushes and night sweats. We did find marginally statistically significant associations between presence of night sweats and CVD (HR=1.16, 95%CI: 1.00-1.35), and between duration of VMS [years] and CHD ($HR_{per\ year}$=1.03, 95%CI: 1.00-1.05). Overall, this study did not convincingly show an association between VMS, hot flushes, night sweats and CVD, CHD and cerebrovascular disease.

The association between PCOS and CVD, CHD and stroke was studied in **Chapter 2.5**, using data from the EPIC-NL (N=29,751 women at baseline) cohort. PCOS was associated with a modestly, non-significantly increased CVD risk (HR=1.22, 95%CI: 0.79-1.89), CHD risk (HR=1.24, 95%CI: 0.85-1.80) or stroke risk (HR=1.21, 95%CI=0.73-1.99). Both BMI as well as lipid levels substantially decreased the HRs in addition to adjustment of CVD and reproductive risk factors.

After these etiologic studies, the following two chapters are focussing on the prognosis of CVD in women. First, **Chapter 3.1** provides a comprehensive overview of CVD risk prediction models for women and models that included female-specific predictors published until July 2017. A total of 285 prediction models for women have been developed, of these 160 (56% were female-specific models, in which a separate model was developed solely in women and 125 (44%) were sex-predictor models (including sex as predictor). A total of 591 validations were identified, of these 333 (56%) concerned nine models (five versions of Framingham, SCORE, PCE and QRISK). The median C-statistics were comparable for sex-predictor and female-specific models. In 260 articles

the added value of new predictors to an existing model was described, however, in only 3 of these female-specific predictors were added. There is an abundance of models for women in the general population. Female-specific and sex-predictor models have similar predictors and performance.

Chapter 3.2 compares the predictive performance of FRS, PCE and SCORE between women with and without a history of HDP (hHDP) and determines effects of recalibration and refitting on predictive performance. We used data from EPIC-NL, including 29,751 women (6302 with hHDP and 17,369 without). SCORE performed best in terms of both calibration and discrimination, while FRS and PCE overpredicted risk in women with and without hHDP, but improved after recalibrating and refitting the models. No separate model for women with hHDP seems necessary, despite their higher baseline risk.

In **Chapter 4**, the main findings presented in this thesis are discussed and implications for further research are given. Future research should establish causality for other female-specific risk factors using MR analysis as well. Furthermore, it would be interesting to focus on a more life-course epidemiology approach taking the whole reproductive lifespan into account instead of examining each female-specific risk factor separately. Women with an increased CVD risk, like women with HDP, preeclampsia or gestational diabetes, might benefit from close monitoring or screening for CVD or elevated CVD risk factor levels.

Samenvatting

In dit proefschrift is onderzocht of vrouwspecifieke risicofactoren (menopauzale status, menopauze leeftijd, vasomotorische menopauzale symptomen (VMS) en polycysteus ovarium syndroom (PCOS)), al dan niet causaal, geassocieerd zijn met hart- en vaatziekten (HVZ) en beroerte en welke mogelijke cardiovasculaire risicofactoren het mechanisme van deze associatie kunnen verklaren. Verder is een overzicht gegeven van risicovoorspellingsmodellen voor HVZ in vrouwen van de algemene bevolking en is een selectie van deze modellen (Framingham Risk Score (FRS), Pooled Cohort Equations (PCE) en SCORE) gevalideerd in vrouwen met en zonder een geschiedenis van zwangerschapshypertensie (hHDP).

Hoofdstuk 2.1 was bedoeld om de relatie tussen de menopauze status en chronologische veroudering met cardiovasculaire risicofactoren te ontrafelen in de grootste studiepopulatie tot nu toe, de LifeLines cohortstudie (N=63.466). We vergeleken cardiovasculaire risicofactoren tussen vrouwen met verschillende menopauzale statussen, binnen dezelfde jaarlijkse leeftijdsgroepen. Chronologische leeftijd en menopauzale status zijn beide onafhankelijk van elkaar geassocieerd met cardiovasculaire risicofactoren. Vooral na de leeftijd van 45 jaar hadden peri- en postmenopauzale vrouwen meer ongunstige lipide niveaus vergeleken met premenopauzale vrouwen van dezelfde leeftijd. Op basis van de relatief kleinere verschillende geassocieerd met menopauzale status dan met chronologische veroudering, kan de biologische betekenis van een meer ongunstig lipidenprofiel in een latere reproductieve fase minder voor de hand liggend zijn dan eerder werd gedacht.

Hoofdstuk 2.2 onderzocht de associaties tussen menopauzale karakteristieken en het risico op HVZ; de vorm van de associatie tussen menopauze leeftijd en het risico op HVZ; en de mate waarin deze associaties verklaard worden door bekende cardiovasculaire risicofactoren. We gebruikten gegevens van EPIC-CVD, een case-cohortstudie met gegevens van 23 centra uit 10 Europese landen. We includeerden alleen vrouwen, van wie 10.880 het willekeurig geselecteerde subcohort vormden, aangevuld met 4522 gevallen van HVZ buiten dit subcohort. Postmenopauzale vrouwen hadden een hoger risico op HVZ in vergelijking met premenopausale vrouwen, hoewel niet statistisch significant (Hazard Ratio (HR)=1,13, 95% betrouwbaarheidsinterval (95%BI): 0,98-1,30). Bij postmenopauzale vrouwen was een vroegere menopauze lineair geassocieerd met een hoger risico op HVZ (HR-daling per jaar=1,02, 95%BI: 1,01-1,03). Na correctie hadden vrouwen met een chirurgische menopauze een hoger risico op HVZ in vergelijking met vrouwen met een natuurlijke menopauze (HR=1,25, 95%BI: 1,10-1,42), maar dit verzwakte na aanvullende correctie voor menopauze leeftijd (HR=1,15, 95%BI: 1,00-1,33). Een deel van de associatie werd verklaard door cardiovasculaire risicofactoren, voornamelijk lipiden. Vrouwen met een vroegere

en vrouwen met een chirurgische menopauze kunnen baat hebben bij nauwgezette controle van cardiovasculaire risicofactoren en HVZ.

Om de resultaten van hoofdstuk 2.2 te versterken hebben we in **hoofdstuk 2.3** een Mendeliaanse Randomisatie (MR)-studie uitgevoerd, gericht op het vaststellen van een causaal verband tussen reproductieve veroudering en fatale of niet-fatale HVZ, en tussen reproductieve veroudering en cardiovasculaire risicofactoren. De 56 genetische varianten geassocieerd met leeftijd van natuurlijke menopauze (ANM) zijn voornamelijk betrokken bij genoomstabiliteit, immuunfunctie en mitochondriale biogenese. Dit zijn geen sekse specifieke processen, daarom hebben we zowel vrouwen als mannen bestudeerd. We hebben een MR-analyse uitgevoerd met behulp van gegevens uit drie onderzoeken (UK Biobank, een aangepaste versie van CARDIoGRAMplusC4D en EPIC-CVD) met samen 417.579 personen van Europese afkomst (inclusief 49.150 gevallen van HVZ). Voor de analyses tussen ANM en cardiovasculaire risicofactoren hebben we gegevens gebruikt van openbaar beschikbare gegevens van het Global Lipids Genetics Consortium (totaal cholesterol, HDL-cholesterol, triglyceriden) en MAGIC (HbA1c, nuchter glucose) en EPIC-CVD. Reproductieve veroudering was niet geassocieerd met HVZ bij vrouwen (Inversed Variance Weighted Method (IVW): 0,99, 95%BI: 0,97-1,01), mannen (IVW: 1,00, 95%BI: 0,97-1,02), of in de geslacht gecombineerde analyse (IVW: 1,00, 95%BI: 0,98-1,01). Evenzo werd geen associatie gevonden tussen ANM en een van de cardiovasculaire risicofactoren.

Hoofdstuk 2.4 onderzocht de associatie tussen vasomotorische menopauzale symptomen (VMS), opvliegers en nachtelijk zweten, en HVZ en beroerte bij 7112 vrouwen die meededen aan de Australische longitudinale studie over de gezondheid van vrouwen, met een leeftijd van 45-50 jaar bij de start van de studie in 1996. Er was geen consistent bewijs van enige associatie met VMS, opvliegers en nachtelijk zweten. We vonden marginaal statistisch significante associaties tussen de aanwezigheid van nachtelijk zweten en HVZ (HR=1,16, 95%BI: 1,00-1,35) en tussen de duur van VMS [jaren] en HVZ (HR per jaar=1,03, 95%BI: 1,00-1,05). Dit onderzoek vond geen overtuigend bewijs voor een verband tussen VMS, opvliegers en nachtelijk zweten en HVZ en beroerte.

De associatie tussen PCOS en HVZ en beroerte werd bestudeerd in **Hoofdstuk 2.5** met behulp van gegevens uit het EPIC-NL cohort (N=29.751 vrouwen bij baseline). PCOS was geassocieerd met een bescheiden, niet-significant, verhoogd risico op HVZ (HR=1,22, 95%BI: 0,79-1,89), coronaire HVZ (HR=1,24, 95%BI: 0,85-1,80) en beroerte (HR=1,21, 95%BI: 0,73-1,99). Zowel BMI als de lipide niveaus verminderden de HRs aanzienlijk, naast de correctie van cardiovasculaire en reproductieve risicofactoren.

Na deze etiologische studies concentreerden de volgende twee hoofdstukken zich op de prognose van HVZ bij vrouwen. **Hoofdstuk 3.1** gaf een uitgebreid overzicht van cardiovasculaire risicovoorspellingsmodellen voor vrouwen en modellen die vrouwspecifieke voorspellers bevatten, gepubliceerd tot juli 2017. In totaal zijn er 285 voorspellingsmodellen voor vrouwen ontwikkeld, van deze waren 160 (56%) vrouwspecifieke modellen, waarbij een apart model uitsluitend bij vrouwen werd ontwikkeld en 125 (44%) modellen met geslacht als voorspeller. Er werden in totaal 591 validaties geïdentificeerd, van deze betroffen er 333 (56%) dezelfde negen modellen (vijf versies van Framingham, SCORE, PCE en QRISK.) De mediane C-statistieken waren vergelijkbaar voor modellen met geslacht als voorspeller en de vrouwspecifieke modellen. In 260 artikelen werd de toegevoegde waarde van nieuwe voorspellers in een bestaand model beschreven, echter in slechts 3 van de deze zijn vrouwspecifieke voorspellers toegevoegd. Er is een overvloed aan modellen voor vrouwen in de algemene populatie. Modellen met geslacht als voorspeller en vrouwspecifieke modellen hebben vergelijkbare voorspellers en prestaties.

Hoofdstuk 3.2 vergelijkt de voorspellende prestaties van FRS, PCE en SCORE in vrouwen met en zonder hHDP en bepaalt de effecten van herkalibratie en hermodelleren voor de voorspellende prestaties. We gebruikten gegevens van 29.751 vrouwen (6302 met hHDP en 17.369 zonder) uit het EPIC-NL cohort. SCORE presteerde het best in termen van zowel kalibratie als discriminatie. FRS en PCE voorspelden het risico bij vrouwen met en zonder hHDP te hoog, maar verbeterden na het opnieuw kalibreren en opnieuw modelleren van de modellen. Een nieuw model voor vrouwen met hHDP lijkt niet nodig, ondanks hun hogere basisrisico.

In **Hoofdstuk 4** worden de belangrijkste bevindingen uit dit proefschrift besproken en worden implicaties voor verder onderzoek gegeven. Toekomstig onderzoek zou ook causaliteit moeten vaststellen voor andere vrouwspecifieke risicofactoren met behulp van MR-analyses. Verder zou het interessant zijn om de aandacht te vestigen op een meer levensloopgerichte epidemiologische benadering waarbij de hele reproductieve levensduur in aanmerking wordt genomen in plaats van elke vrouwspecifieke risicofactor apart onderzoeken. Vrouwen met een verhoogd cardiovasculair risico, zoals vrouwen met HDP, pre-eclampsie of zwangerschapsdiabetes, kunnen baat hebben bij nauwlettende monitoring of screening op HVZ en cardiovasculaire risicofactoren.

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About the author

Veerle Dam was born on the 14th of October 1989 in Roosendaal, the Netherlands. After graduation from the Markland College in Oudenbosch, she started the Bachelor program Nutrition and Dietetics at the HAN University of applied Sciences in Nijmegen in 2008. In 2012, she started the Master program Health Sciences at VU university Amsterdam. During her Master, she worked on a research project on the association between vitamin K and the metabolic syndrome at the Julius Center for Primary Care and Health Sciences of the University Medical Center Utrecht. In 2014, she started her PhD project leading to this thesis, also at the Julius Center, under supervision of prof. dr. ir. Yvonne van der Schouw and dr. Charlotte Onland-Moret. In 2017, she received a Master of Science in Clinical Epidemiology from Utrecht University. Currently, Veerle works at the Erasmus Medical Center Rotterdam and Sanquin Research Amsterdam as a post-doctoral researcher.

Publications of this thesis

Chapter 2.1

AC de Kat, **V Dam**, NC Onland-Moret, MJ Eijkemans, FJ Broekmans, YT van der Schouw. Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. *BMC Medicine* 2017;15:2

Chapter 2.2

V Dam, YT van der Schouw, NC Onland-Moret, RHH Groenwold, SAE Peters, S Burgess, AM Wood, M Chirlaque, KGM Moons, C Oliver-Williams, E Schuit, K Tikk, E Weiderpass, M Holm, A Tjønneland, T Kühn, RT Fortner, A Trichopoulou, A Karakatsani, C La Vecchia, P Ferrari, M Gunter, G Masala, S Sieri, R Tumino, S Panico, JMA Boer, WMM Verschuren, E Salamanca-Fernández, L Arriola, C Moreno-Iribas, G Engström, O Melander, M Nordendahl, P Wennberg, TJ Key, S Colorado-Yohar, G Matullo, K Overvad, F Clavel-Chapelon, H Boeing, JR Quiros, E di Angelantonio, C Langenberg, MJ Sweeting, E Riboli, NJ Wareham, J Danesh, A Butterworth. Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis. *Submitted*

Chapter 2.3

V Dam, NC Onland-Moret, S Burgess, M Chirlaque, KGM Moons, SAE Peters, K Tikk, E Weiderpass, C Oliver-Williams, AM Wood, YT van der Schouw. Age at natural menopause and coronary heart disease risk and cardiovascular risk factors: a two-sample Mendelian Randomization study. *In preparation*

Chapter 2.4

V Dam, AJ Dobson, NC Onland-Moret, YT van der Schouw, GD Mishra. Vasomotor menopausal symptoms and cardiovascular disease risk in midlife: a longitudinal study. *Submitted*

Chapter 2.5

V Dam, NC Onland-Moret, JMA Boer, WMM Verschuren, YT van der Schouw. Association of polycystic ovary syndrome and risk of cardiovascular disease, coronary heart disease and stroke. *In preparation*

Chapter 3.1

V Dam*, SJ Baart*, LJJ Scheres*, JAAG Damen, R Spijker, E Schuit, TPA Debray, BCJM Fauser, E Boersma, KGM Moons, YT van der Schouw, on behalf of the CREW consortium. Cardiovascular risk

prediction models for women in the general population: a systematic review. *Authors contributed equally. *Submitted*

Chapter 3.2

V Dam, NC Onland-Moret, WMM Verschuren, JMA Boer, L Benschop, A Franx, KGM Moons, E Boersma, YT van der Schouw, on behalf of the CREW-consortium. Cardiovascular risk model performance in women with and without hypertensive disorders of pregnancy. *Heart* 2018

Other publications

V Dam, GW Dalmeijer, C Vermeer, NE Drummen, MH Knapen, YT van der Schouw, JW Beulens. Association between vitamin K and the metabolic syndrome: a 10-year follow-up study in adults. *The Journal of Clinical Endocrinology & Metabolism*, 2015;100:6