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POSTMENOPAUSAL HORMONE THERAPY AND CARDIOVASCULAR RISK

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POSTMENOPAUSAL HORMONE THERAPY AND CARDIOVASCULAR RISK

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Para mi mamá, mi papá y Óscar.

ABSTRACT

Introduction and aims: Postmenopausal hormone therapy (HT) is the most effective treatment for menopausal symptoms, but its safety has been debated during the past 15 years. Previous observational studies showed benefits from HT, whereas subsequent large randomized clinical trials showed an increased risk for cardiovascular disease (CVD) and other chronic diseases; the pivotal difference between these studies was the average age at HT initiation. Subsequent re-analyses of these observational studies and the randomized clinical trials, which considered age at HT initiation and years since menopause onset, showed less dissimilar results. This phenomenon gave support to the timing hypothesis, stating that HT, if initiated early in relation to menopause onset, may exert vascular protection, whereas there may be negative consequences if initiated late. This stresses the need for further studies. There is also limited knowledge about whether oestrogen type, route of administration, and duration of HT are important for the association between HT and cardiovascular risk, while considering the timing of HT initiation. In the present thesis, the aim was to assess how HT associates with the risk of CVD in menopausal women, with a specific focus on the role of timing of HT initiation, and with consideration of oestrogen types and combinations, routes of administration, and duration of use.

Material and methods: The present thesis was based on observational data from a case-control study and pooled individual participant data from five population based cohorts. HT was categorized as early or late initiation in relation to the onset of menopause. Different groups of HT regimens and durations were considered. Coronary heart disease, stroke (composite end point), and haemorrhagic stroke end points were assessed from population registers. To assess the association between HT and CVD we performed logistic regression for the case-control study and Laplace regression for the pooled cohort data. The reference category was never use. Single and multivariable confounding control was performed for an array of factors.

Results: After multivariable-adjustment, early HT initiation was not associated with an increased risk of coronary heart disease, nor for stroke. These results held regardless of oestrogen regimen or combination (oestrogen-progestin), route of administration, and the duration. However, a specific analysis of haemorrhagic stroke revealed an increased risk if single conjugated equine oestrogens were used. Late HT initiation was associated with increased risk for coronary heart disease when the therapy was composed of equine oestrogens combined with a progestin. Risk of stroke and haemorrhagic stroke, on the other hand, were increased when single equine oestrogens were initiated late. Late initiation of combined HT was associated with an increased haemorrhagic stroke risk.

Interpretation: HT did generally not increase the risk of CVD when initiated close to the onset of menopause. The results contribute to the scientific basis that may guide clinicians who handle patients seeking treatment for climacteric symptom control. The results should however not change current practice that recommends against the use of HT for prevention of CVD.

SVENSK SAMMANFATNING

Bakgrund och syfte: Postmenopausal hormonbehandling (HT) är den mest effektiva behandlingen av klimakteriebesvär, men säkerheten har diskuterats under de senaste 15 åren. Tidigare observationsstudier visade fördelar av HT, emedan efterföljande stora randomiserade kliniska prövningar visade ökad risk för kardiovaskulär sjukdom (CVD) och andra kroniska sjukdomar. Den viktigaste skillnaden mellan dessa studier var genomsnittlig ålder vid initiering av HT. Reanalyser av dessa observationella och randomiserade studier följde och resulterade i mindre olika resultat när ålder vid initiering och antal år sedan klimakteriet togs i beaktning. Detta fenomen gav stöd till ”timinghypotesen” som stipulerar att HT har positiva effekter på kärlväggen vid tidig initiering men negativa effekter om initiering sker långt efter klimakteriet. Behovet av ytterligare studier står klart. Kunskapen är också begränsad beträffande huruvida östrogentyp, administreringsväg och duration av behandling har betydelse för kardiovaskulär risk under beaktande av tidpunkten för initiering. Föreliggande avhandling syftar bedöma hur HT associerar med risken för CVD hos menopausala kvinnor, med särskilt fokus på hur tidpunkten för initiering av HT påverkar denna risk och under beaktande av östrogentyper och kombinationer, administreringsvägar och duration av behandling.

Material och metod: Den aktuella avhandlingen baseras på observationella data från en fallkontrollstudie och från studiedeltagare i fem olika befolkningsbaserade kohorter som slagits samman. HT klassificerades som tidigt eller sent initierad i förhållande till tidpunkt för klimakteriet. Kranskärlssjukdom och stroke under en uppföljningsperiod identifierades via nationella register. Analyser av samband mellan HT och CVD genomfördes med logistisk regression för fallkontrollstudien och med Laplace-regression för de sammanslagna kohorterna. Referenskategorin utgjordes av kvinnor som aldrig använt HT. Enkel och multivariabel confounding-kontroll utfördes för en rad faktorer.

Resultat: Efter multivariabel justering var tidig initiering av HT inte associerad med ökad risk för kranskärlssjukdom och inte heller för stroke. Denna association kvarstod oavsett östrogenregim och kombination(östrogen-progestin), administreringsväg och duration av behandling. En specifik analys av hemorrhagisk stroke visade dock en ökad risk om enbart konjugerade ekvina östrogener användes. Sen initiering av HT var associerad med ökad risk för kranskärlssjukdom när konjugerade ekvina östrogener användes i kombination med ett progestin. Risk för stroke och hemorragisk stroke var däremot ökade när enbart konjugerade ekvina östrogener initierades sent. Sen initiering av kombinerat HT var associerad med ökad hemorragisk strokerisk.

Tolkning: HT ökade generellt sett inte risken för CVD när den initierades nära tidpunkten för klimakteriet. Resultaten bidrar till det vetenskapliga underlaget som kan vägleda läkare som möter kvinnor som söker behandling för klimakteriebesvär. Resultaten bör dock inte förändra nuvarande rekommendationer om att inte använda HT för att förebygga CVD.

RESUMEN EN ESPAÑOL

Introducción y objetivos: la terapia hormonal (TH) es el tratamiento más efectivo para los síntomas de la menopausia, pero su seguridad ha sido debatida en los últimos 15 años. Previos estudios observacionales mostraron beneficios de la TH, mientras que ensayos clínicos aleatorizados mostraron un mayor riesgo de enfermedad cardiovascular (ECV) y otras enfermedades crónicas. La diferencia fundamental entre estos estudios fue la edad promedio al inicio de la TH. Reanálisis de estudios observacionales y ensayos clínicos, considerando la edad al inicio de la TH y años desde la menopausia, mostraron resultados menos dispares. Este fenómeno dio soporte a la hipótesis del tiempo, sugiriendo que si la TH se inicia temprano en relación con el inicio de la menopausia ejerce protección vascular, mientras que tiene una influencia negativa si se inicia tarde. La necesidad de más estudios es clara. No es totalmente claro si el tipo de estrógenos, la vía de administración y la duración de la TH son importantes para la asociación entre la TH y el riesgo cardiovascular, en especial cuando se considera el momento del inicio de la TH. En la presente tesis, el objetivo fue evaluar cómo la TH se asocia con el riesgo de ECV en mujeres menopáusicas, con un enfoque específico en cómo el momento del inicio de la TH, los regímenes de estrógenos, las vías de administración y la duración de la TH influyen en este riesgo.

Material y métodos: la presente tesis se basa en estudios observacionales de un casos y controles y en datos agrupados de participantes de cinco cohortes basadas en la población. La TH fue categorizada como iniciación temprana o tardía en relación con el comienzo de la menopausia. Se consideraron diferentes grupos de regímenes y duraciones de la TH. La enfermedad coronaria, accidente cerebrovascular (isquémico y hemorrágico combinado) y accidente cerebrovascular hemorrágico se evaluaron a partir de los registros poblacionales. Para evaluar la asociación entre TH y ECV se realizó regresión logística para el estudio de casos y controles y regresión de Laplace para los datos de cohortes agrupadas. La categoría de referencia fueron los no usuarios. Se realizaron ajustes sencillos y multivariados para el control de factores de confusión.

Resultados: Después del ajuste multivariado, el inicio temprano de la TH no se asoció con un mayor riesgo de enfermedad coronaria ni de accidente cerebrovascular. Estos resultados se mantuvieron independientemente del régimen de estrógenos, la vía de administración y la duración. Sin embargo, un análisis específico de accidente cerebrovascular hemorrágico reveló un mayor riesgo para el uso de estrógenos equinos como terapia sencilla. El inicio tardío de la TH se asoció con un mayor riesgo de enfermedad coronaria cuando la terapia estaba compuesta de estrógenos equinos combinados con un progestágeno. El riesgo de accidente cerebrovascular y accidente cerebrovascular hemorrágico fue mayor cuando la terapia sencilla de estrógenos equinos se inició tarde. El inicio tardío de la TH combinada se asoció con un mayor riesgo de accidente cerebrovascular hemorrágico.

Interpretación: en general la TH no aumentó el riesgo de ECV cuando se inicia cerca del inicio de la menopausia. Los resultados contribuyen para guiar a los médicos que manejan pacientes que buscan tratamiento para el control de los síntomas menopaúsicos. Sin embargo, estos resultados no cambian la práctica actual que recomienda no usar la TH para la prevención de ECV.

LIST OF SCIENTIFIC PAPERS

I. CARRASQUILLA GD, Berglund A, Gigante B, Landgren BM, de Faire U, Hallqvist J and Leander K. Does menopausal hormone therapy reduce myocardial infarction risk if initiated early after menopause? A population-based case-control study. Menopause 22 (6), 598-606 (2015).

II. CARRASQUILLA GD, Chiavenna C, Bottai M, Magnusson PK, Santacatterina M, Wolk A, Hallmans G, Jansson JH, Engstrom G, Borgfeldt C, Pedersen NL, Eliasson M, Berglund A and Leander K. Timing of Postmenopausal Hormone Therapy and Risk of Coronary Heart Disease: Analysis of Combined Individual Data from Five Population-Based Cohorts. Submitted.

III. CARRASQUILLA GD, Frumento P, Berglund A, Borgfeldt C, Bottai M, Chiavenna C, Eliasson M, Engstrom G, Hallmans G, Jansson JH, Magnusson PK, Nilsson PM, Pedersen NL, Wolk A and Leander K. Postmenopausal Hormone Therapy and Risk of Stroke: A Pooled Analysis of Data from Population Based Cohort Studies. PLoS Medicine 14(11): e1002445 (2017).

CONTENTS

LIST OF ABBREVIATIONS

HT	Postmenopausal hormone therapy
RCT	Randomized controlled trial
CHD	Coronary heart disease
CVD	Cardiovascular disease
MI	Myocardial infarction
NHS	Nurses' Health Study
WHI	Women's Health Initiative
CEE	Conjugated equine oestrogen
ELITE	Early versus Late Intervention Trial with Estradiol
SHEEP	The Stockholm Heart Epidemiology Program
COMPREHEND	Combined cohorts of menopausal women—Studies of register-based health outcomes in relation to hormonal drugs
SALT	Screening Across the Lifespan Twin
MDCS	The Malmö Diet Cancer Study
ICD	International Classification of Diseases
OR	Odds ratio
CI	Confidence interval
PD	Percentile difference
BMI	Body mass index
KI	Karolinska Institutet

1 BACKGROUND

Postmenopausal hormone therapy (HT) has long been recognized as the most effective treatment for vasomotor symptoms[1] that affect more than three quarters of menopausal women.[1,2] Results from observational studies and randomized clinical trials (RCTs) have shown discrepant results concerning their impact on the risk of future chronic diseases. Observational studies, on the one hand, showed that HT is associated with a decreased risk for coronary heart disease (CHD), osteoporosis, and all-cause mortality.[3-5] On the other hand, RCTs have reported an increased risk for cardiovascular disease (CVD) and chronic diseases.[6,7]

The discrepancy between results from observational studies and RCTs may be largely explained by factors related to study design,[8] but also may be due to underlying biological circumstances.[9,10] In an observational study design, there is a risk for uncontrolled confounding. Women who used HT may have had an increased propensity to seek healthy lifestyles and preventive treatments as compared to non-users. This is usually referred to as a healthy user effect, and should be properly accounted for in observational studies. Further, under the biological circumstances, the so called timing hypothesis suggests that HT may have different vascular effects if initiated close to menopause onset compared with initiation distant from menopause.

Re-analyses of the observational studies and RCTs additionally took into account women´s age at baseline and years since menopause onset.[11-14] The previously dissimilar results then became more similar and no increased risk for CVD was found.[14] Recent RCT data showed a positive influence on subclinical atherosclerosis progression when HT was initiated within 6 years from menopause onset,[15] supporting the timing hypothesis. The relevance of timing for the risk of hard CVD clinical end points has been considered recently,[14] nonetheless unanswered questions remain concerning how the different oestrogen-progestin combinations, active ingredients, routes of administration and duration of treatment may modify the risk of CVD.[16]

This thesis was performed with the general aim to assess how HT influences CVD risk with specific focus on the influence from timing of HT initiation, HT regimens, and duration. We used observational data, along with high quality register data, to evaluate the association between HT use and CVD risk.

1.1 CARDIOVASCULAR DISEASE

CVD is an umbrella term for abnormal conditions related to the heart and blood vessels, often with atherosclerosis as the underlying aetiology. By the year 2030, it is estimated that 23.6 million people will die from CVD if the current trend continues.[17] CVD is the leading cause of death worldwide,[18] affecting not only those who suffer from the disease, but also relatives, caregivers, and society in general. In fact, mortality from CVD in women is the number 1 killer.[17] CHD and stroke are by far the most common causes of CVD, contributing to 90% of CVD deaths.[17] For the purpose of the current thesis, CHD and stroke were the only CVD causes considered.

1.1.1 Atherosclerosis

Atherosclerosis occurs when fat and cholesterol build up in the endothelium of the arteries, causing an inflammatory process that thickens and hardens the vessel walls affecting its elasticity.[19] The plaque consists of lipids, cholesterol, calcium, cell aggregations, and other substances. Over time, the plaque hardens and narrows the arteries, thereby limiting the flow of oxygenated blood. If the plaque is big enough it can narrow the lumen of the blood vessel, diminishing the blood influx and thereby creating ischemia. If the plaque ruptures, the artery may become completely blocked, which can lead to atherosclerotic CVD (e.g., CHD and stroke).[19]

1.1.2 Coronary heart disease

CHD, also known as coronary artery disease, is a condition in which an atherosclerotic plaque is deposited in the coronary arteries. These arteries supply oxygenated blood to the myocardium. As described above, the plaque may narrow the lumen of the blood vessel and this may lead to coronary ischemia. However, a plaque may also become unstable and rupture. When this happens, a blood clot is formed. The clot may grow and it can potentially block the totality of the coronary artery lumen and thereby block the blood flow to the myocardium, causing a CHD event.[20] The symptoms include pain or discomfort in the chest, usually described as pressure, although pain can also be reported in other parts of the body.[21]

Myocardial infarction (MI) follows under the umbrella term for CHD conditions, but in this case there is myocardial necrosis.[21] Over time, CHD can weaken the myocardium and lead to heart failure and arrhythmias.

The incidence of CHD in premenopausal women generally starts to increase10 to 15 years later than in men.[17,22] This age gap between men and women evens out in the seventh decade of life.[17] Main risk factors for CHD are similar between sexes. Yet, the role of menopause is unique to women. Plaque rupture is more common among

men as compared to women, but there is a lower plaque rupture incidence in younger women as compared to older.[23]

1.1.3 Stroke

A stroke, also known as cerebrovascular disease, occurs when 1) the blood supply to an area(s) of the brain tissue is suddenly interrupted, creating a blockage (ischemic stroke), or 2) when a blood vessel ruptures (haemorrhagic stroke). In the same fashion as for CHD, a person with a diminished blood flow may develop ischemia or necrosis of the brain tissue.[21] Incidence of stroke during middle age is lower among women as compared to men, but 10 years after menopause this risk increases markedly.[24]

1.2 MENOPAUSE

By 2025 it is estimated that there will be more than 1.1 billion menopausal women in the world.[25] Menopause is a physiological process defined as the cessation of menses as a consequence of diminished function of ovarian follicles. It is defined as amenorrhea for a period longer than 12 months according to the world health organization, with onset around the age of 51 years, on average. When menstruation stops due to oophorectomy or iatrogenic menopause, it is defined as induced menopause.[21]

1.2.1 Menopausal symptoms

Vasomotor and vaginal symptoms are the most common menopausal symptoms and are closely related to the hormonal changes during the menopausal transition. It is clear that vasomotor symptoms, vaginal dryness and irregular menses are caused by the menopausal transition, having an impact on sleep quality due to hot flushes and night sweats. Other symptoms suggested to be caused by menopause are mood changes, impaired memory, depression, decreased libido, and urinary incontinence.[21]

Although menopausal symptoms can be treated with and array of non-pharmacological treatments or non-hormonal pharmacological treatments,[26] without a doubt HT is the most effective treatment currently available for symptom control.[1,27]

1.3 POSTMENOPAUSAL HORMONE THERAPY

1.3.1 Historical perspective

The use of HT has varied greatly throughout its 70 years of existence.[28] HT was promoted for osteoporosis and CHD prevention, reaching a maximal prescription by the mid-1990s.[29-31] This was a time when animal research[32] and observational studies (e.g., Nurses Health Study (NHS))[5,33] suggested a benefit from HT, in

particular for the prevention of CHD. In Sweden, HT sales registered an increase in the late 1980s and reached a peak in 1999 (Figure 1), when the estimated frequency of HT use among women aged 50-69 years was 36%.[34] In the beginning of the 2000s, the RCT Heart and Estrogen/progestin Replacement Study (HERS)[35] and Women's Health Initiative (WHI)[6,7,36] showed an increased risk for CVD and other chronic diseases from HT. Consequently, a sharp decline in HT use was observed worldwide; in Sweden it went from 27% in 2002 to 9% in 2007.[34]

1.3.2 Oestrogen regimen

Data so far favour transdermal over oral oestrogen with respect to CVD risk, suggesting that the sustained release of the transdermal route provides a constant and more physiological circulatory level of oestrogen.[37] It has been suggested that conjugated equine oestrogens (CEEs) might be associated with a higher risk of incident MI than oestradiol, although authors stated that the results need replication and suggest that various oral oestrogen drugs may be associated with different levels of CVD risk.[38] CEEs are manufactured from the urine of pregnant mares and contain 10 known biologically active oestrogen compounds; the primary compounds are oestrone and equilin sulfate.[39,40] Oestradiol is a natural or bioequivalent oestrogen that contains synthetic oestradiol-17β.[38]

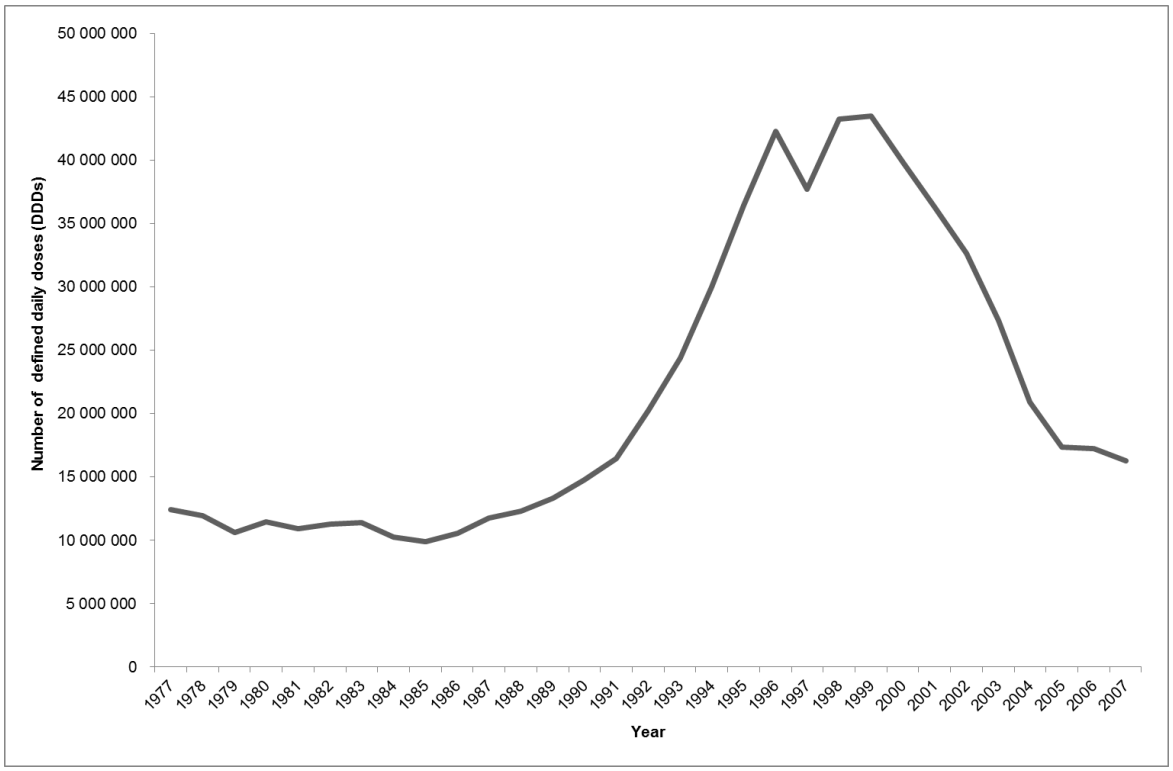

Figure 1. Postmenopausal hormone therapy sales data from the pharmacies in Stockholm County during the period 1977-2007. Postmenopausal hormone therapy (Anatomical Therapeutic Chemical (ATC) codes G03C (oestrogens), G03D

(progestogens) and G03F (progesterone and oestrogens in combination)), sold by the pharmacies in Stockholm county from 1977-2007. The dip in 1997 was due to a change in the reimbursement system and was seen for all prescription drugs. A defined daily dose (DDD) is the assumed average maintenance dose per day for a drug used for its main indication in adults.

1.3.3 Observational studies and randomized clinical trials

More than 15 years ago, a discussion was initiated about why findings from previous numerous observational studies and RCTs were discrepant with regard to cardiovascular risk. Several possible explanations have been raised, such as uncontrolled and residual confounding, different study population characteristics, different biological effects from HT depending on timing of initiation, selection bias, difference in HT regimen, and differences in the length of follow-up.[8,9,14,41,42]

In general, observational studies have consistently shown a reduction in CHD and overall mortality,[5,43-45] whereas RCTs have shown an increased risk indication when analysed without considering the age or years since menopause.[46] In 2012, the Danish Osteoporosis Prevention Study (DOPS),[41] showed a reduced risk of CVD events in women with early HT initiation, as compared to no treatment, after 10 years of follow-up. In 2016, the Early versus Late Intervention Trial with Estradiol (ELITE) concluded that initiation of HT close to menopause had a positive impact in atherosclerosis progression,[15] although there was no assessment of risk for hard clinical cardiovascular end points. The 2015 Cochrane report,[16] based on 19 RCTs, concluded that HT has little, if any, benefit overall for primary and secondary prevention of CVD events. The report also concluded that there are increased risks of stroke and venous thromboembolism.[16] The report stressed the lack of knowledge about how the different oestrogen/progestin preparations, HT durations and routes of administration affect the risk of CVD and other chronic conditions.[16] In 2017, WHI and NHS investigators published on a comparative analysis considering timing of HT initiation and concluded that the results for CVD and all-cause mortality are concordant between these two designs after all.[14]

1.3.4 The timing hypothesis

Among the explanations for discrepancies between observational studies and RCTs, one widely discussed explanation relates to the timing hypothesis, which suggests that the risks and benefits of HT depend on the time of initiation of the therapy relative to the time since menopause (Figure 2).[9,10] Early HT initiation, when the vascular endothelium would be less affected by atherosclerosis, rather than late initiation, might protect from the atherosclerotic progression.[9] Late HT initiation, on the contrary, might have a detrimental influence on the vascular tissue.[9,47]

Observational studies that investigated HT in relation to the risk of CVD and that took into account timing of initiation of the therapy, showed a reduction in CVD risk if the initiation occurred close to the onset of menopause.[11,12] In agreement with those findings, results from re-analyses of the WHI showed that the early initiation of HT was associated with a null or reduced CVD risk.[13] The recent ELITE trial also lends support to the timing hypothesis.[15] Yet, the clinical relevance for the timing hypothesis remains to be determined, especially when considering the different constellations of HT regimens.

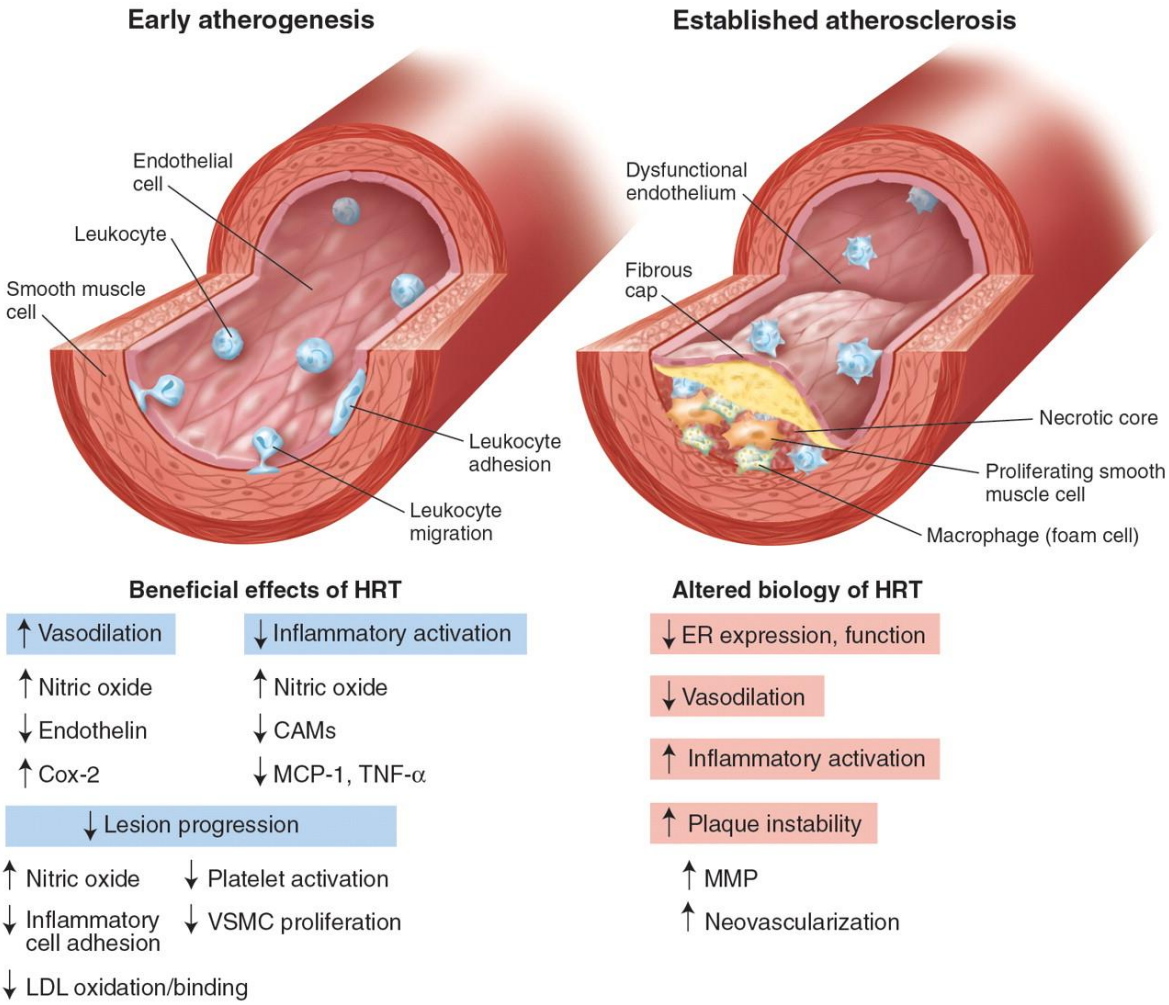

Figure 2. Graphical depiction of the timing hypothesis. Atherosclerosis, recognized as an inflammatory process, plays a big role in the vascular biology of the endothelium. Early initiation of HT improves the vascular dysfunction of the endothelium. Advanced atherosclerosis associates with an altered vascular function that in synergy with late initiation of HT make the vascular wall more prone to inflammatory disruptions. From Mendelsohn, M. E. & Karas, R. H. Science 308, 1583–1587 (2005). Reprinted with permission from The American Association for the Advancement of Science.

HRT: postmenopausal hormone therapy, ER: oestrogen receptor, CAMs: cell adhesion molecules, COX-2: cyclooxygenase 2, MCP-1: monocyte chemoattractant protein 1, TNF: tumour necrosis factor, MMP: matrix metalloproteinase, VSMC: vascular smooth muscle cell, LDL: Low-density lipoprotein.

2 AIMS

The overall aim of the thesis was to assess how HT is associated with the risk of CVD in menopausal women, with a specific focus on how timing of HT initiation influences this risk.

More in detail, we considered different spheres of the treatment:

- Type of HT (oestrogen-only, oestrogen-progestin)
- Active ingredient (oestradiol, CEEs)
- Duration of HT
- Route of administration of HT (oral, transdermal and vaginal)

To achieve these aims we used observational data: a case-control design and a cohort design, in which individual participant data from five population-based cohort studies were pooled. Two separate CVD end points were considered: CHD and stroke.

3 MATERIALS AND METHODS

3.1 THE SHEEP STUDY

The Stockholm Heart Epidemiology Program (SHEEP) is a case-control study with a study base consisting of all Swedish citizens living in Stockholm area between 1992 and 1994, who were 45 to 70 years of age without a previous clinical diagnosis of MI. The diagnosis of MI required a minimum of 2 of the following criteria: 1) certain symptoms, according to case history information; 2) specified levels of enzymes serum creatine kinase and serum lactate dehydrogenase; and 3) specified electrocardiogram changes. The SHEEP has been described in more detail elsewhere.[48,49]

3.1.1 Case ascertainment and control selection

The cases in study I are menopausal women with a first time MI. MI cases were classified using the 1991 guidelines from the Swedish Association of Cardiologists.[50] Menopausal women were identified based on questionnaire data as women who reported they were not naturally menstruating. Only non-fatal cases (i.e., patients who survived at least 28 days after their MI diagnosis) are included. At least one control individual per case was recruited in the SHEEP. Controls were randomly sampled from the study base within two days of the case incidence, and within strata of individuals of the same sex, age (± 5 years) and residential area as the case in question. Each female menopausal control candidate was checked for previous MI events since 1975 using the computerized hospital register of the Stockholm County.

3.1.2 Exposure assessment

Women who reported previous or current use of HT were considered as ever users; otherwise, they were considered as never users. Early and late HT initiation were defined using a 10-year cut-off: ≤10 and >10 years since menopause onset. If data on number of years since menopause and HT initiation were missing, age at baseline (≤60 and >60 years) was used to categorize HT users into early and late initiators. The reference category was late HT initiation. Duration of HT was defined using a 5-year cut-off: ≤5 and >5 years, with short duration as reference category. All analyses were also performed with never users as the reference category.

3.2 THE COMPREHEND STUDY

3.2.1 Study design and included cohorts

Study II and study III material were derived from the Combined cohorts of menopausal Women - Studies of register-based health outcomes in relation to hormonal drugs (COMPREHEND), which is comprised of pooled observational participant data from a number of population-based cohorts. Cohorts invited to participate in the studies included in the present thesis had available data on age at

menopause, menopausal status, HT use, and HT initiation. All agreed to participate. These cohorts are: the Swedish Mammography Cohort (SMC),[51] the Screening Across the Lifespan Twin (SALT),[52] the Northern Sweden Health and Disease Study (NSHDS),[53] the Malmö Diet Cancer (MDCS),[54] and the Women´s Health in the Lund Area study (WHILA).[55] Data were collected between 1987 and 2002, a period during which HT was frequently prescribed (Figure 1).

Women included in studies II and III were menopausal, with age at menopause being 40-59 years, and free of previous CVD.

3.2.2 Exposure assessment

All exposures were self-reported, using questionnaires, except the participants in SALT that were interviewed. Ever use of HT was defined as current or previous use of HT. Incident use of HT was defined as HT initiation within the previous year (12 months). Early and late HT initiations were defined using a 5-year cut-off (≤5 and >5 years) or a 10-year cut-off (≤10 and >10 years). The timing of HT initiation was also considered as a continuous variable.

The different regimens were categorized into oestrogen-only or combined HT. For active ingredient we considered oestradiol and CEEs. Three different routes of administration were considered: oral, transdermal, and vaginal. The duration of HT was classified as short or long (≤5 and >5 years).

3.2.3 Outcome assessment

CHD and stroke incident cases were identified from the Swedish National Patient Register and the Swedish Cause of Death Register. We considered main diagnoses and underlying causes of death. The end of follow-up varied between the cohorts, from 2010/12/31 to 2013/12/31. International Classification of Diseases (ICD) codes were used to identify MI (ICD-9 410/411; ICD-10 I21/I22), CHD death (ICD-9 410-414; ICD-10 I20-I25), stroke (ICD-9 430–434/436–438, ICD-10 I60–I69), and haemorrhagic stroke (ICD-9 430–432, ICD-10 I60–I62).

3.3 STATISTICAL ANALYSIS

Regression methods permit assessing the effect of one or more exposures of interest, adjusting for other predictors. This is essential in epidemiology when we are interested in controlling for the impact of various potential confounders or assessing the role of mediators.

For study I unconditional logistic regression was performed to obtain crude and multivariable adjusted odds ratios (OR) of MI associated with HT with corresponding 95% confidence intervals (CI). For study II and study III Laplace regression was implemented to assess the association between HT and CHD or stroke risk, respectively; crude and multivariable adjusted results were reported as percentile differences (PD) with 95% CI. Kaplan-Meier estimator was also used while controlling for confounding in studies II and III.

3.3.1 Logistic regression

Logistic regression[56] is used to describe how the probability of a binary outcome depends on covariates and allows to calculate the OR associated with one or more exposures of interest, adjusting for potential confounders. Estimation is carried out by maximum likelihood.

The specific maximum likelihood approach depends on whether the data are matched or unmatched. Usually unconditional logistic regression analysis overestimates the OR when the data is matched.[57] Unconditional logistic regression is appropriate when the number of parameters is small relative to the number of subjects, whereas the conditional approach is appropriate when the number of parameters is large.[57] For study I, conditional and unconditional logistic regression were performed, if results between these two approaches did not differ, unconditional logistic regression was usually preferred given the better precision.

3.3.2 Kaplan-Meier product-limit estimator

There are many situations in which one may want to examine the distribution of time-to-event, which is typically a censored response variable. Censoring occurs when the follow-up does not end with the event of interest. Causes of censoring include drop-out, death by another cause, and administrative censoring. The Kaplan-Meier estimator[58] permits estimating survival curves in the presence of censored data. In the present project, we used the Kaplan-Meier estimator to make cross-stratified comparisons of the HT groups (early initiation, late initiation, and never use), describing the cumulative incidence of CVD across levels of different covariates.

3.3.3 Quantile regression and censored quantile regression

Quantile regression [59,60] estimates conditional quantiles of a continuous variable given a set of covariates, providing a complete description of the outcome distribution. Quantile regression is a common and useful tool in other fields like economics and finances.

A number of methods[61-67] have been studied to extend quantile regression to censored data. Some methods[61-63] are implemented in the R package "quantreg". However, they require complicated recursive algorithms and become slow with large sample sizes, which make them unsuitable for our data. Wang and Wang's estimator[66] uses local Kaplan-Meier to obtain a preliminary estimate of the conditional distribution; however, this method is suggested to be quite unstable and rather slow. Leng and Tong's[65] approach is similar, but estimates the conditional distribution of the censoring variable instead. This simplifies the problem when the censored variable can be assumed to not depend on covariates.

For studies II and III, we decided to use Laplace regression[64] which is undoubtedly the most computationally efficient among the mentioned approaches. Although this estimator has been shown to be biased,[68] it has been demonstrated that the bias is negligible and the mean squared error is usually smaller than that of other methods.[68] Given the available sample size, Laplace regression appeared as the most natural choice, thanks to its computation speed and ease of implementation. Censored Laplace regression is implemented in the STATA command "laplace".

Censored quantile regression allows for assessing the association between HT and CVD by estimating survival PD between two or more exposure groups. PD along with 95% CI can be expressed in any time units (e.g. hours, days, months, years). We calculated the 5th PD (for CHD and stroke) and the 1st PD (for haemorrhagic stroke). The PD is interpreted as the number of event-free years among exposed women as compared to unexposed (reference category). Here, we will generically refer to disease-free years when describing the PD.

The proportion of women who developed the event was 5% for CHD, 7% for stroke and 1% for haemorrhagic stroke. Due to the large proportion of censoring, calculation of PD beyond the 5th for CHD and stroke, or the 1st PD for haemorrhagic strokey, would involve data extrapolation and therefore provide misleading inference.

3.4 CONFOUNDING CONTROL

3.4.1 Study I

For study I, potential confounding factors considered were age, residential area, surgical menopause, smoking status, physical inactivity, alcohol consumption, body mass index (BMI), and socioeconomic status (occupation and educational level). The crude associations (adjusted for age only) were subsequently adjusted for single and multiple potential confounding factors. For intermediate factors, we grouped

different factors into categories of normal or abnormal: 1) lipid profile, 2) glucose metabolism, 3) coagulation/fibrinolysis, 4) inflammation, and 5) homocysteine levels. Then we added these categories one by one to the final multivariable-adjusted model to assess any possible influence on the point estimates from the potentially intermediate factors.

3.4.2 Study II and III

Based on previous literature, we considered the following factors as potential confounders: age at baseline, age at menopause, level of education, smoking status, BMI, level of physical activity, type of menopause, parity, use of oral contraceptives, alcohol consumption, family history of CVD, cohort, and the presence of hypertension, dyslipidaemia, or diabetes.

Each one of the variables listed above were graphically displayed into Kaplan–Meier curves describing the cumulative incidence of the event of interest, cross-stratifying by timing of HT initiation (5-year cut-off). Further, after visually assessing these survival curves the variables were incorporated one at a time in the regression model that was adjusted only for age at baseline. This was performed in order to assess if there was a ≥10% change of the point estimates.

The variables included in the final regression models for studies II and III were age at baseline, level of education, smoking status, BMI, level of physical activity, and age at menopause onset. For study II, hypertension, dyslipidaemia, and diabetes were then added into the final model.

3.5 ETHICAL CONSIDERATIONS

The studies in the present thesis were conducted in accordance with the Helsinki Declaration and approved by the Regional Ethical Committee at Karolinska Institutet (KI), Sweden or the Regional Ethical Review Board in Stockholm. The ethical permit for Study I was obtained in 1991, reference number 911259. For studies II and III, ethical permits were obtained in 2012 (reference number 2012/2027-31/4), with supplementary amendment in 2014 (reference number 2014/1971-32) and 2017 (reference number 2017/485-32).

This thesis project only utilized data that have already been collected. Informed consent from participants were retrieved according to the Helsinki declaration. To prevent a person’s identity from being connected with information, the SHEEP work data sets were de-identified. In order to protect data integrity and privacy, the

datasets are stored locally in a highly secure server with restricted access at the Institute of Environmental Medicine (IMM), KI, Stockholm. The COMPREHEND database does not include personal identity information.

4 RESULTS

4.1 STUDY I

An inverse association between HT ever use as compared to never use and MI in the crude analysis was attenuated after multivariable adjustments. Current use of HT as compared to never use was associated with an inverse association, which attenuated after adjustment. Incident initiation of HT, defined as initiation within the previous year, as compared to never use, was associated with an inverse association that was attenuated after adjustments (Figure 3).

An inverse association between early HT initiation, as compared to never use, and MI was observed in the crude analysis, but was attenuated after adjustments. The corresponding result for late HT initiation, as compared to never use, shows no association. An inverse association between long duration of HT (as compared to never use) and MI was found in the crude analysis, but it was attenuated after adjustments. The corresponding analyses for short HT duration showed no association (Figure 3).

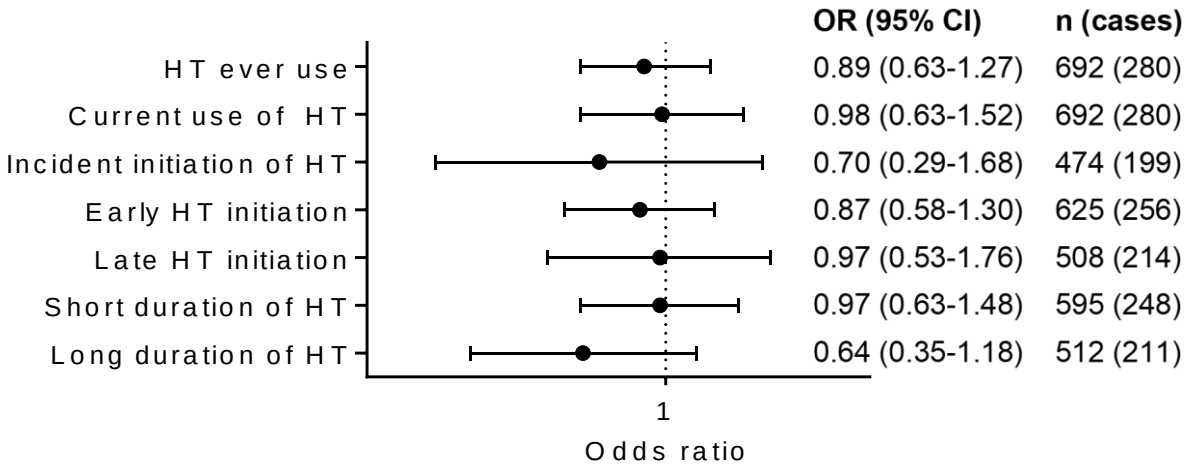

Figure 3. Associations between menopausal HT and myocardial infarction. The reference group is never use. Results are reported as odds ratios along with 95% CI. Multivariable adjustments were performed for age, residential area, hysterectomy, oophorectomy, current smoking, ex-smoking, physical inactivity, alcohol consumption, BMI, and socioeconomic status.

HT: postmenopausal hormone therapy, OR: odds ratio, CI: confidence intervals.

4.2 STUDY II AND III

Early initiation of HT was associated with longer CHD- and stroke-free periods than never use. Late HT initiation was associated with CHD- and stroke-free periods similar to that of never users (Figure 4). Oestrogen-only and combined HT were associated with longer CHD- and stroke-free periods regardless of the timing of HT initiation (Figure 5). The corresponding results for late initiation were less precise (Figure 5). Oestradiol and CEEs, when initiated early, were associated with longer CHD- and stroke-free periods, respectively. CEEs used as combined HT, initiated late, was associated with a shorter CHD-free period. CEEs used as single HT, initiated late, was associated with a shorter stroke-free period. Late initiation of oestradiol used as combined HT, but not as a single, tended to be associated with a shorter period free from CHD than never use (Figure 5).

Oral route of administration, when initiated early, was associated with longer CHD-free periods; for late initiation results were inconclusive. With transdermal and vaginal administration, the results were inconclusive (Figure 4). When initiated early, but not when initiated late, HT of both short and long duration was associated with a longer CHD-free period than never use (Figure 4).

The overall findings for haemorrhagic stroke were similar as those presented for stroke, with the difference that use of single CEEs was associated with an increased risk regardless of timing of HT initiation. Further, late initiation of combined HT was associated with an increased risk of haemorrhagic stroke (study III).

In general, when HT was initiated early, the disease-free period was longer for CHD as compared to stroke (Figure 4 and 5).

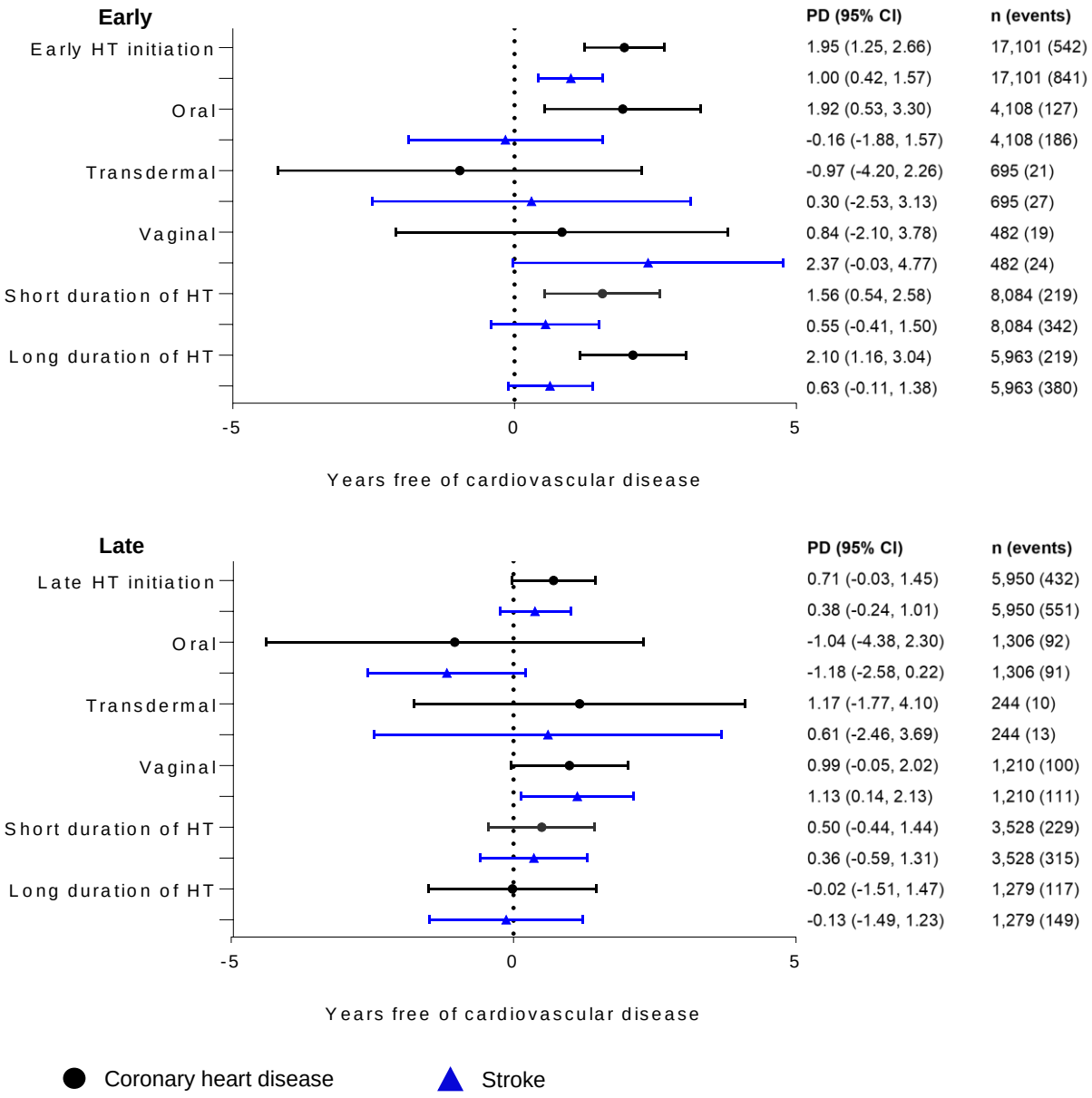

Figure 4. Cardiovascular disease (CHD and stroke)-free years in relation to route of administrations and duration of HT by timing of initiation using the 5-year cut-off. The reference group is never use. For stroke (blue triangle), the adjusted models include age at baseline, level of education, smoking status, BMI, level of physical activity, and age at menopause onset. For CHD (black circles), the adjusted models include the same factors as for stroke and diabetes, dyslipidaemia, and hypertension. The 5th PD with 95% CI were calculated.

CHD: Coronary heart disease, HT: postmenopausal hormone therapy, PD: percentile differences, CI: confidence intervals.

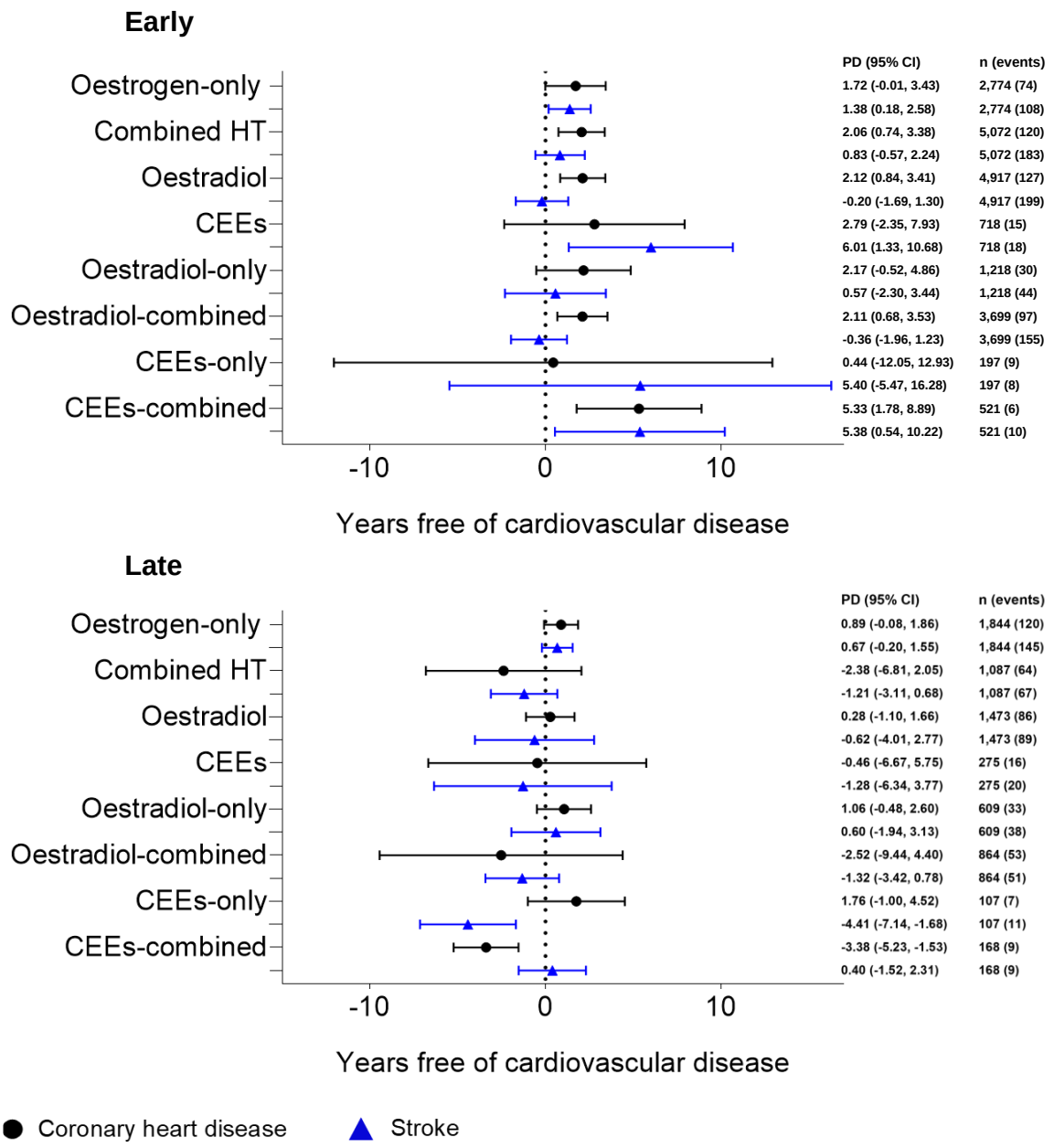

Figure 5. Cardiovascular disease (CHD or stroke)-free years in relation to type and active ingredient of HT by timing of initiation using the 5-year cut-off. The reference group is never use. For stroke (blue triangle), the adjusted models included age at baseline, level of education, smoking status, BMI, level of physical activity, and age at menopause onset. For CHD (black circles), the adjusted models include the same factors as for stroke and diabetes, dyslipidaemia, and hypertension. The 5th PD with 95% CI were calculated.

CHD: Coronary heart disease, HT: postmenopausal hormone therapy, PD: percentile differences, CI: confidence intervals, CEEs: conjugated equine oestrogens.

5 DISCUSSION

5.1 SUMMARY FINDINGS

Early HT initiation was not associated with an increased risk for CHD, nor for stroke. This held regardless of the regimen and duration. However, if single CEEs were used, an increased risk of haemorrhagic stroke was found. Late HT initiation was associated with an increased risk of CHD when the therapy was composed of CEEs and combined with a progestin. Risks of stroke (composite end point) and haemorrhagic stroke were increased when single CEE therapy was initiated late. Late initiation of combined HT was associated with an increased haemorrhagic stroke risk.

5.2 STRENGTHS AND LIMITATIONS

The strengths of the present project are several. The datasets are large with population-based design; and the women included come from real world settings, thus using different kinds of HT preparations, routes of administrations and durations. This allowed assessing different HT exposure categories. The data available were detailed, which enabled us to perform thorough analyses by timing of HT initiation. The extensive detailed data on several covariates permitted thorough confounding control for a substantial number of factors. Further, the use of register based data to define outcomes with high validity[69] and negligible loss to follow-up is an asset for the present project. Another advantage is that the cohorts included are rather homogeneous, as they are comprised of women with comparable access to health care; and the Swedish health care system is universal. Finally, an important strength is that we were able to utilize advanced methods in epidemiology and biostatistics to answer our research questions.

One of the limitations is that we have no repeated investigations of use of HT among study participants. This limits the possibility to evaluate immediate effects from starting HT and also possible effects from discontinuing HT. In the same line, we could not assess time-varying exposures; HT users may have discontinued HT during the follow-up period, and women classified as never users may have initiated HT. Data on routes of administration were not possible to extract from all the cohorts and data availability was not sufficient to produce results with good precision. Further, we did not have information on the specific types of progestin used and therefore analyses considering progestin type and CVD risk were not possible.

Uncontrolled confounding and residual confounding are limitations inherent to the observational study design applied in the present studies. However, data on a large

number of potential confounders were available and thoroughly accounted for in the analyses.

A potential limitation of the present project relates to external validity. Although the timing hypothesis should be relevant for women of different ethnic backgrounds, extrapolation of the results should be done cautiously, because women of other ethnicities may have other risk profiles.

5.3 METHODOLOGICAL CONSIDERATIONS

The way subjects are selected into an epidemiological study, how variables of interest are measured and whether potential confounders are considered, are crucial in determining different sources of selection bias, information bias, and confounding.

5.3.1 Selection bias

Selection bias occurs when the association between the exposure and outcome differs between those who participate and those who were potentially eligible, including non-participants.[70] In short, it arises when the studied individuals are not representative of the population under study from which conclusions will be drawn.

In the studies forming the basis for the present thesis project, not all subjects returned and completed the questionnaires or participated in the interview; non-responsive subjects may differ in exposure to HT compared to responders, and they may at the same time have a different cardiovascular risk profile compared to participants. In the SHEEP study the response rate was 76.8% for cases and 68.9% for controls. Given this fairly high response rate, this may not substantially have affected the associations. Further, a previous Danish case-control study found no differences between responders and non-responders in the report of use of HT between stroke cases or controls.[71] Thus, we have no reasons to believe that non-responding cases and controls had different exposure levels as compared to responders. The response rate in the COMPREHEND study material varied between 40% (MDCS) and 74% (SALT). Again, we have no reason to believe that non-responders had a different HT use than responders.

The inclusion of prevalent HT users may introduce selection bias, because prevalent users is a group enriched with treatment adherent individuals as compared to the incident user group.[72] On the other hand, it has been argued that the inclusion of a mixture of incident and prevalent users in epidemiological studies may do more benefit than harm to epidemiology.[73] In the present thesis, we included a mixture of

incident and prevalent users, but also performed analyses considering incident users only.

Observational studies have been criticized for not being efficient when capturing early events as compared to RCTs,[74,75] since prevalent users at enrolment will have been using HT for variable and potentially lengthy periods before baseline investigations. Therefore, most of the information is from long-term users, reflecting women who have survived a potential short-term risk immediately after HT initiation.[8,74] We performed analysis among women who recently started HT and we found no evidence of increased short-term risk with HT.

Immortal time bias occurs when there is inappropriate consideration of the follow-up time and the exposure status during the design of the study. It has been defined as "a period of follow-up during which, by design, death or the study outcome cannot occur".[76] During the design phase of studies II and III, with the purpose to avoid immortal time bias, we considered different possibilities for when to start the follow-up and when to assess the exposure status. We decided to start the follow-up at baseline investigations and follow-up until incident CVD.

5.3.2 Misclassification of exposure

Systematic errors from measuring the exposure are referred to as misclassification that can be differential and non-differential. Differential misclassification of the exposure is related to the outcome occurrence; otherwise it is non-differential misclassification.[70] The magnitude and the direction of the exposure measurement errors have an impact on study results.

In study I, HT exposure could be misclassified in cases and controls, especially since HT was self-reported. As data were collected backwards in time, the MI event in cases might theoretically have influenced the manner they recalled. The prevailing idea in society concerning HT was that it provided more protection than harm. Possibly women who suffered a MI, therefore may have been prone to underestimate their use of HT more than controls. This would lead to that the inverse associations observed being overestimated. Another possibility is that the recall was not affected by the case status and that the information was of equal quality in cases and controls. Women in the SHEEP study were shown pictures of the HT packages which should have supported an accurate recall. Still, a slight non-differential misclassification seems possible, biasing results towards the null.

For studies II and III, misclassification of the exposures is most likely non-differential; we do not have strong reasons to believe that reports of HT use were different in those who later were to develop CVD as compared to those who were not.

5.3.3 Confounding

A confounder is associated with the exposure and outcome of interest, but at the same time it is not an intermediate factor in the pathway between exposure and outcome.[70] We considered an array of potential confounding factors in the present thesis and performed a thorough confounding control strategy.

A common type of confounding encountered in pharmacoepidemiology is confounding by indication. This is a bias that occurs when the exposure of interest is selectively taken or not taken among those who develop the event of interest.[77] During the time when the studies were performed, HT was broadly prescribed to middle aged women, mostly for osteoporosis prevention.[78]

Even when a confounder is controlled for in a study, it may be that it was not adequately done; this phenomenon is referred to as residual confounding. Residual confounding in observational analysis may be unavoidable. However, in the present project we reasoned on the best variables to be included in the analyses and whether categorical or continuous variables should be considered. For instance, in some situations, considering a continuous variable may assume linearity although categorical variables, allowing for more flexibility, were preferred. We often considered both, continuous and categorical, and results did not change.

The healthy user effect, as defined above, is the tendency for individuals who have a healthy lifestyle or who receive a preventive treatment to seek further preventive advice, treatments, or engage in healthy behaviours.[79] Women who used HT in the observational studies were often wealthier, leaner, more physically active, health conscious, and visited their physicians more regularly. Therefore, in the present thesis, we adjusted for BMI and different lifestyle factors to control for this healthy user effect.

5.3.4 Missing data

There are different types of missing data usually referred to as: 1) missing completely at random (when the probability of missing is unrelated to the observed and unobserved data); 2) missing at random (when the missing is related to some of

the observed data); and 3) missing not at random (when the probability of missing is related to the unobserved data).

A common manner to handle missing data with an easy implementation and interpretation is performing a complete-case analysis; this is done through deleting from the analysis those subjects that have missing values. This method is valid under two circumstances: 1) when the subjects have been randomly sampled and the missing is completely at random, and 2) when subjects have been randomly sampled within levels of covariates used for stratification.[70] We performed sensitivity analysis for studies II and III, utilizing the complete-case approach and found no differences between the overall results and the results from complete-case analysis.

There are other methods for handling missing data. One of those is imputation that predict and complete the values that are missing using the observed data and the pattern of data missing. Imputation is computationally slow and time demanding when applied in censored quantile regression, especially in the presence of large datasets. Another method is inverse probability weighting using complete records assigning specific weights based on the estimated probability from the complete records. These methods assume that data is missing at random.[70] We did not use imputation given that is computationally slow and no package using Laplace has been implemented for STATA so far.

5.4 FUTURE PERSPECTIVES

Results from previous RCTs are primarily based on one oestrogen and route (i.e. oral CEEs) whereas HT risks and benefits have been reported to be influenced by a constellation of pharmacoepidemiological factors like dosing, formulation type, route of administration, and timing of HT initiation. Therefore, future studies should try to elucidate how these constellations of factors influence the risk of CVD and other chronic diseases. For instance, an RCT assessing different routes (e.g., transdermal or vaginal), durations, and doses of oestradiol is warranted. From an ethical perspective, such a study would perhaps be feasible given the fact that the risk of chronic disease associated with this type of oestrogen has not been observed.

Although CHD and stroke are the main causes of CVD, a relevant end point that remains to be explored is the risk of venous thromboembolism. Different HT regimens and durations should be considered.

The potential harm from HT is not only limited to CVD, therefore, it is important to continue further research assessing risks of other diseases. One crucial research question relates to whether early HT initiation may affect the risk of dementia and other cognitive impairments in elderly women. Although results are inconsistent, concern remains on HT safety for cancer, thus further research is needed assessing malignant tumours as end points. The COMPREHEND study will be a useful platform to further study these research gaps.

Although not mentioned in the current thesis, there is a neglected group of women that has menopause earlier (usually <45 years of age), often referred to as primary ovarian insufficiency or premature menopause. These women have higher risk for osteoporosis, CHD, and dementia compared to women who have their menopause at a normal age.[80] Future research considering these women, evaluating hormone related factors, such as HT and oral contraceptives in relation to CVD risk and other diseases, is warranted.

5.5 CLINICAL AND PUBLIC HEALTH IMPLICATIONS

Current clinical practice recommends against the use of HT for the purpose of primary or secondary prevention of any chronic disease, including CHD and stroke.[81] When using HT in postmenopausal women the purpose should be the treatment of menopausal symptoms[82-84] taking into consideration age, time since menopause, and CVD risk.[82]

The present work may aid menopausal women considering the use of HT for symptom relief, and aid clinicians in guiding women in making the best treatment choice that will improve their menopause symptomatology, quality of life and well-being. This research will help in identifying suitable candidates for HT while considering type of oestrogen, active ingredient, and starting time of HT in relation to menopause onset from a CVD perspective.

5.6 CONCLUDING REMARKS

Early HT initiation in relation to menopause onset was not associated with an increased risk of CHD or stroke, with one exception – single CEE therapy was associated with an increased risk for haemorrhagic stroke.

Late HT initiation was associated with an increased risk of CHD if the regimen was combined CEE therapy. For stroke and haemorrhagic stroke, single CEE initiated late was associated with an increased risk. For haemorrhagic stroke, combined therapy initiated late was associated with an increased risk.

The present thesis lends support to the clinical relevance of the timing hypothesis for CVD.

The studies included in this thesis may aid clinicians in guiding women who consider HT for menopausal symptom control. However, further research assessing the safety of HT considering other chronic diseases is needed.

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