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Department of Public Health

Postmenopausal hormone therapy and dementia, cognition, and mortality

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PhD thesis

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Laura Løkkegaard Johansen
Odense, July 2022

Papers in thesis

Paper 1
Løkkegaard LE, Thinggaard M, Nygaard M, Hallas J, Osler M, Christensen K. Systemic hormone therapy and dementia: A nested case-control and co-twin control study. Maturitas 2022 [ePub ahead of print]

Paper 2
Løkkegaard LE, Christensen K, Hallas J, Osler M, Thinggaard M. Postmenopausal hormone therapy and cognition in twins. 2022 [manuscript]

Paper 3
Løkkegaard LE, Thinggaard M, Hallas J, Osler M, Christensen K. Postmenopausal hormone therapy and mortality: User profile differences before and after the Women's Health Initiative study. 2022 [Submitted manuscript]

Abbreviations

AD	Alzheimer's Disease
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CCS	Cognitive Composite Score
CI	Confidence Interval
CPR	Personal identification number
CRS	Danish Civil Registration System
DNPR	Danish National Patient Registry
DTR	Danish Twin Registry
DZ	Dizygotic
HR	Hazard Ratio
HT	Hormone therapy
ICD	International Classification of Diseases
IUD	Intrauterine Device
LSADT	Longitudinal Study of Aging Danish Twins
MADT	The Study of Middle-Aged Danish Twins
MZ	Monozygotic
NPR	Danish National Prescription Registry
OR	Odds Ratios
PPV	Positive Predictive Value
SD	Standard deviation
UZ	Unknown zygosity
WHI	Women's Health Initiative
WHIMS	Women's Health Initiative Memory Study
WHIMSY	Women's Health Initiative Memory Study of Younger Women

Table of content

1 Summary

1.1 English

As life expectancy increases, so does the concerns for the health and well-being at advanced ages. Especially, loss of cognitive function and risk of dementia are two concerns that pose great personal and societal consequences. So, prevention of such diseases is of increasing relevance, and one major focus has been health in mid-life, as this may play an essential role in aging.
A pivotal mid-life event for women is menopause, which is a phase potentially influenced by e.g., vasomotor, genitourinary, and musculoskeletal symptoms that can last well into the postmenopausal years. A way to relieve these symptoms is by using hormone therapy (HT) consisting of oestrogen with or without a progestogen – a treatment that has been known and used for decades. HT was, due to findings from observational studies, generally believed beneficial in the relief of menopausal and postmenopausal symptoms and disease prevention. However, this perception changed when findings from a large clinical, randomized trial, the Women's Health Initiative (WHI) study, were published in 2002 concluding that the risk of using systemic HT outweighed the benefits of disease prevention. These findings prompted widespread media attention, and they led to alterations in HT guidelines, which caused a sharp decline in the prevalence of systemic HT.
Several explanations for the discrepant findings on HT and disease prevention between observational studies and clinical trials have subsequently been posed. Both an introduction of a healthy user bias in observational studies prior to the 2002 WHI publication and the timing of HT in relation to menopause have been suggested as possible reasons. Environmental and genetic factors, not controlled for in the observational studies, may also play a part.

Twin studies could help illuminate especially the latter hypothesis as twins present a unique study population due to shared genetic factors and early childhood environment. This thesis aimed to investigate the association between HT and the outcomes dementia, cognition, and mortality in a study population of Danish female twins from the Danish Twin Registry and a 5% random sample of female singletons from the Danish background population.
Both study populations had to meet the age criteria of being born before 1950 and being alive by 1995 to ensure a minimum age of 45 years and thus being close to or past the average menopausal age when information on HT became available from a Danish nationwide register on medicine. Information on dementia diagnoses and mortality was also obtained through Danish nationwide health registries with observation time beginning in 1995. Cognitive function was registered in the Danish Twin Registry and assessed as a part of The Longitudinal Study of Aging Danish Twins beginning in 1995 and the Middle-Aged Danish Twin Study beginning in 1998 using a cognitive composite score.

Paper 1 examined the association between systemic HT and dementia and found an overall borderline statistically significant tendency towards an increased risk of dementia for systemic HT users in both the study samples of twins and singletons. A statistically significant increase in risk of dementia for systemic HT users was observed before the 2002 WHI publication in both study populations. This finding could

indicate confounding by indication as alterations in guidelines after 2002 may have led to an exclusion of the most fragile women. It indicates a potential change in the HT user profile before and after the 2002 WHI publication.
As persistent mild cognitive impairment has shown a high risk of progressing to dementia, Paper 2 investigated the association between HT and cognitive function in Danish female twins and found that systemic HT users aged 70+ had a lower cognitive function than non-users in analyses adjusted for age, education, social class, and unobserved familial confounding. Longitudinal data on systemic HT users aged 70+ showed that the lower cognitive function was most explicit before 2002, whereas after 2002 the cognitive function was closer to that of non-users. This finding aligned with the tendency observed in Paper 1. However, longitudinal data in younger twins (aged 50-69) showed a lower cognitive function in systemic HT users after 2002 compared to non-users, and a change in the HT user profile seemed to have occurred as those who changed from systemic HT to local HT after 2002, or dropped it altogether, performed cognitively better within this age group. A likely explanation for the observed tendencies in this study is the introduction of a selection bias in the wake of the 2002 WHI publication.
To further elaborate on these findings, Paper 3 investigated the association between HT and mortality in different age groups before and after the 2002 WHI publication in both a study population of twins and singletons. Analyses were adjusted for education and unobserved familial confounding, respectively. In both study populations, the prevalence of systemic HT decreased markedly following the 2002 WHI publication, while local HT use increased. Among twins aged 56-60, the mortality risk changed from lower before 2002 to similar to that of the background population for systemic HT users following the 2002 WHI publication – a tendency also observed in singleton females aged 56-75. These findings suggest an altogether different HT user profile after 2002, perhaps driven by the healthiest users deciding to either drop systemic HT or switch to local HT as recommendations changed following the WHI publication.

Overall, we found it likely that selection rather than causality underlies the observed associations between HT and dementia, cognition, and mortality in Danish twins and singletons, based on studies spanning across year 2002, as the risk of the various outcomes for HT users appeared to differ before and after 2002. This implied that systemic HT users had undergone a selection following the 2002 WHI publication, potentially due to the alterations made in the guidelines on HT prescription after 2002. Our findings highlight the importance of examining not only the differences between HT users and non-user, but also the differences in HT users before and after 2002, and it emphasizes the importance of adequate confounder control in future observational studies when examining HT use, as confounders may vary markedly over time and may be related to initiation, regimen, and dose of HT. Alternatively, as we are now 20 years from the 2002 WHI publication, observational studies could focus solely on HT users after 2002, as they may provide a more stable base for generalisation of results to current HT users.

1.2 Danish (dansk resumé)

Samtidig med at den forventede levealder stiger, øges bekymringen for sundhedstilstanden hos mennesker i de højeste aldre. Specielt er tab af kognitiv funktion og risiko for demens to tilstande, der kan have store personlige og samfundsmæssige konsekvenser, og derfor er forebyggelse af disse af stigende vigtighed. Der har været et særligt fokus på at undersøge sundheden hos mennesker midt i livet, da sundhedstilstanden i denne del af livet kan have betydning for, hvordan man ældes.
For kvinder er overgangsalderen en central begivenhed midt i livet; en fase, som potentielt er påvirket af vasomotoriske-, urogenitale- og muskuloskeletale symptomer, som kan fortsætte et godt stykke tid ind i de postmenopausale år. En måde at mindske disse symptomer på er gennem hormonbehandling, enten med østrogen alene eller i kombination med et gestagen - en behandling som har været kendt i årtier. På baggrund af resultater fra observationsstudier blev hormonbehandling generelt betragtet som værende gavnlig både som symptombehandling og som sygdomsforebyggelse. Den opfattelse ændrede sig dog, da resultaterne fra et stort klinisk, randomiseret studie, the Women's Health Initiative (WHI), blev publiceret i 2002. Studiet konkluderede, at risikoen ved brug af systemisk hormonbehandling var større end fordelene ved hormonbehandlingen. Disse resultater fik stor mediebevågenhed og gav anledning til, at man ændrede hormonbehandlingsvejledningerne, hvilket medførte et fald i brugen af systemisk hormonterapi.
Der er blevet fremsat flere forklaringer på de modsatrettede fund fra hhv. observationsstudierne og de kliniske forsøg vedrørende hormonbehandling og sygdomsforebyggelse. Både indførelsen af et et såkaldt "healthy user bias" i observationsstudier udført før udgivelsen af resultaterne fra WHI-studiet i 2002 og timingen af hormonbehandling i forbindelse med overgangsalderen er blevet foreslået som mulige årsager. Miljømæssige og genetiske faktorer, der ellers ikke kontrolleres for i observationsstudier, kan også have spillet en rolle.

Tvillingestudier kan hjælpe med at belyse især sidstnævnte forklaring, da tvillinger udgør en unik studiepopulation grundet deres fælles opvækst og genetiske ophav. Denne afhandling har til formål at undersøge sammenhængen mellem hormonbehandling og udfaldene demens, kognition og mortalitet hos kvindelige danske tvillinger og en tilfældig 5% stikprøve af enkeltfødte danske kvinder fra baggrundsbefolkningen.
Begge studiepopulationer var underlagt et alderskriterie, nemlig at kvinderne skulle være født før 1950 og i live i 1995, da information vedrørende brugen af hormonbehandling blev tilgængelig i 1995 via et nationalt lægemiddelregister, samt for at sikre at kvinderne havde en minimumsalder på 45 år og dermed var tæt på den gennemsnitlige overgangsalder. Oplysninger om demens diagnoser og mortalitet blev også indhentet gennem danske, nationale sundhedsregistre med begyndende observationstid i 1995. Kognitiv funktion blev registreret i det Danske Tvilling Register, da den var blevet vurderet ved hjælp af en cognitive composite score som led i The Longitudinal Study of Aging Danish Twins begyndende i 1995 og The Middle-Aged Danish Twin Study begyndende i 1998.

Det første studie, der indgår i denne afhandling, undersøgte sammenhængen mellem systemisk brug af hormonterapi og demensudvikling og fandt en let øget risiko for demens hos brugere af systemisk hormonbehandling hos både tvillinger og enkeltfødte. En statistisk signifikant øget risiko for demens hos brugere af systemisk hormonterapi blev observeret før WHI-publikationen i 2002 hos begge studiepopulationer. Disse fund kan været forekommet på baggrund af confounding by indication, da ændringer i hor-

monbehandlingsvejledninger, indført efter 2002, kan have medført en eksklusion af de skrøbeligste kvinder. Dette indikerer en potentiel ændring i hormonterapi-brugerprofilen før og efter WHI-publikationen fra 2002.
Da persisterende mild kognitiv svækkelse har vist høj risiko for at udvikle sig til demens, var fokus for det andet studie i denne afhandling en undersøgelse af sammenhængen mellem hormonbehandling og kognitiv funktion hos danske kvindelige tvillinger. Undersøgelsen fandt, at brugere af systemisk hormonterapi, der var ældre end 70 år, havde en lavere kognitiv funktion end ikke-brugere i analyser justeret for alder, uddannelse og socialklasse, samt "unobserved familial confounding". Longitudinelle data viste, at den lavere kognitive funktion observeret hos brugere af systemisk hormonterapi, der var ældre end 70 år, var tydeligere før 2002, hvorimod den kognitive funktion efter 2002 var mere lig ikke-brugernes. Dette stemte overens med den observerede tendens i demensstudiet. Longitudinelle data hos yngre tvillinger i alderen 50-69 viste derimod en lavere kognitiv funktion hos brugere af systemisk hormonterapi efter 2002 sammenlignet med ikke-brugere. Desuden skete der tilsyneladende en ændring i hormonterapi-brugerprofilen efter 2002, da de kvinder, som skiftede fra systemisk til lokal brug af hormonterapi efter 2002, eller helt droppede behandlingen, havde en bedre kognitiv funktion i denne aldersgruppe. En sandsynlig forklaring på tendenserne i dette studie er indførelsen af en selektionsbias som følge af udgivelsen af 2002 WHI-publikationen.
For yderligere at forklare disse fund undersøgte det tredje studie sammenhængen mellem hormonbehandling og mortalitet i forskellige aldersgrupper før og efter WHI-publikationen i 2002 hos både tvillinger og enkeltfødte. Analyserne blev justeret for henholdsvis uddannelse og "unobserved familial confounding". I begge studiepopulationer faldt prævalensen af systemisk hormonterapi markant efter WHI-publikationen i 2002, mens prævalensen af lokal hormonterapi steg. Blandt tvillinger i alderen 56-60 år, som brugte systemisk hormonterapi, ændrede mortaliteten sig fra lavere før 2002 til svarende til baggrundsbefolkningens risiko efter 2002. En tendens der også sås hos enkeltfødte i alderen 56-75. Disse resultater tyder på en ændring i hormonterapibrugerprofilen efter udgivelsen af WHI-publikationen i 2002, måske forårsaget af at de sundeste brugere besluttede enten at droppe systemisk hormonterapi eller skiftede til lokal hormonterapi, da hormonterapivejledningerne ændredes som følge af WHI-publikationen.

Samlet set finder vi det sandsynligt, at de observerede associationer mellem hormonterapi og demens, kognition og mortalitet hos danske tvillinger og enkeltfødte skyldes en underliggende selektion snarere end kausalitet, da risikoen ændredes for hormonterapibrugere før og efter 2002, hvor studierne blev gennemført. Dette tyder på, at især brugere af systemisk hormonterapi efter 2002 gennemgik en selektion, formentlig på baggrund af de ændrede vejledninger om brug af hormonterapi som følge af 2002 WHI-publikationen. Resultaterne understreger vigtigheden af ikke kun at undersøge forskelle mellem hormonterapibrugere og ikke-brugere, men også forskellen mellem brugere før og efter 2002. Det understreger også vigtigheden af grundig konfounder-kontrol i fremtidige observationsstudier af hormonterapibrug, da konfoundere kan variere markant over tid og potentielt være relateret til hormonterapi-initiering, dosis og behandlingsregime. Alternativt, da vi nu er 20 år fra 2002 WHI-publikationen, kunne fremtidige observationsstudier udelukkende fokusere på hormonterapi-brugere efter 2002, da de kan give et mere stabilt grundlag for generalisering af resultater til nuværende hormonterapi-brugere.

2 Introduction

Life expectancy has drastically increased throughout the 20th century and continue to do so, particularly in high income countries.[1] The demographic development in Denmark from 1950 to 2020 has shown an increase in individuals older than 65 years, which is expected to rise even further in the future, as illustrated in Figure 1, due to various mortality improvements, e.g., higher living standards, better education and nutrition, and access to medicine.[2, 3]

As life expectancy increases, so does the concerns for the quality of life at advanced ages. Loss of cognitive function and risk of dementia are two great concerns, as they pose great personal and societal consequences.[4-6] So, prevention of such diseases is of increasing relevance, and one major focus has been mid-life, as it may play an essential role in health and aging.[7]

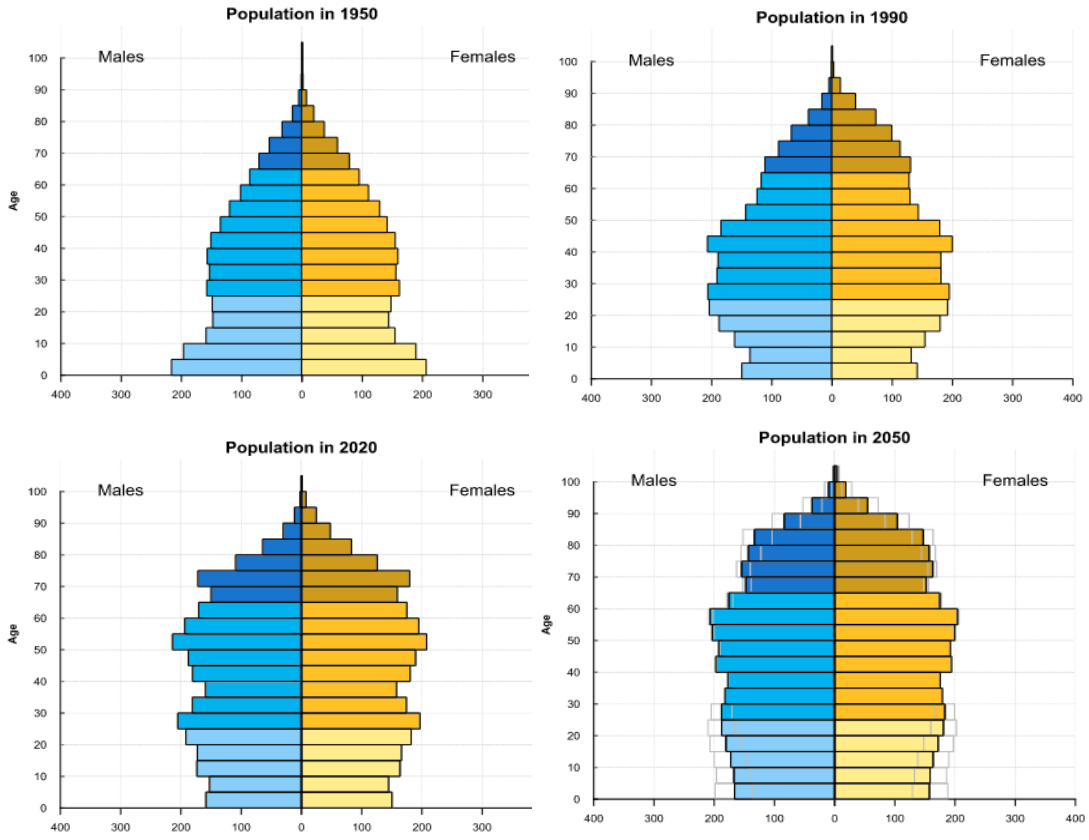

Figure 1. Danish population pyramids in 1950, 1990, 2020, and a 2050 forecast with women illustrated in yellow and men in blue. The horizontal axis indicate population in thousands and the vertical axis indicates 10-year age groups.[2]

2.1 Menopause

A pivotal mid-life event for women is menopause. Menopause is a normal, biological, and inevitable transition, which typically happens around the age of 50-53 for European women if occurring naturally.[8] However, for some women menopause can be chemically or surgically induced.
Naturally occurring menopause occurs when the ovarian egg reserve is exhausted, which causes changes to the otherwise meticulously regulated hormonal feedback system between the hypothalamus, pituitary gland, and the gonadal glands. It disrupts secretion of the gonadal hormones, oestrogen and progestogen, and causes the menstrual period to fluctuate. Eventually the hormonal production will cease all together, stopping the menstrual cycle, and marking the end of the reproductive years.[9] This phase of oscillating hormonal levels can last several years, but when a woman has not had a menstrual period for 12 months, she is considered postmenopausal.[10]
The menopausal hormonal changes can result in a wide range of symptoms affecting approximately 80% of all women, and 20% in a severe degree.[10-12] The decreasing level of oestrogen during menopause is associated with changes in the central nervous system, e.g., vasomotor symptoms (hot flashes and night sweats) and changes in mood, cognitive function, and sleep.[12] The genitourinary tract may also be affected by the hormonal changes leading to vulvovaginal atrophy, stress incontinence, and sexual dysfunction.[12] Furthermore, the musculoskeletal system may also be influenced causing joint pain and loss of muscle and bone mass.[13] Vasomotor symptoms last on average 6-10 years, and thus possibly well into the postmenopausal years, while genitourinary symptoms may persist throughout life if left untreated.[9] These bothersome symptoms may have a great impact on the quality of life in menopausal and postmenopausal women.[14, 15]

2.1.1 Management and treatment

Menopausal or postmenopausal symptoms can be managed using hormone therapy (HT) which consists of either oestrogen alone or oestrogen in combination with a progestogen. Which HT regimen is recommended depends on whether the woman has had a hysterectomy. The combined oestrogen-progestogen therapy is used in women with an intact uterus, as oestrogen alone increase the risk of endometrial cancer.[16, 17] HT consisting of oestrogen alone is only used in women who has undergone a hysterectomy.
The principle behind the current guidelines on HT as treatment for menopausal or postmenopausal symptoms is: Lowest possible HT dosage for the shortest amount of time for effective treatment to be achieved.[18] However, in Denmark, the first line of action in menopausal or postmenopausal symptom management, for women with moderate to severe vasomotor symptoms, is three months of lifestyle intervention which include avoiding nicotine, caffeine, alcohol, and spicy food. If lifestyle interventions do not provide an effect, then HT is recommended. Systemic HT, typically consisting of either oral or transdermal oestrogen, can be initiated in women younger than 60 or within 10 years of menopause onset and is used mainly to relieve vasomotor symptoms, while local HT, consisting of vaginal oestrogen, is recommended for genitourinary symptoms without resctrictions.[19] After a maximum of five years HT should be discontinued by either stopping all together or gradual tapering over 3-6 months.[18] The use of HT is contraindicated in women with high risk of cardiovascular disease, breast cancer, venous thromboembolism, or liver disease.[20]

2.1.2 History of hormone therapy

The use of HT as a treatment for menopausal and postmenopausal symptoms has been known for decades, during which time its reputation has fluctuated, so HT is still a topic of debate.[21-25]
In the 1960s, HT was branded as a way for women to stay feminine forever.[26] However, some cracks in

the HT image began to appear in the 1970s, as unopposed oestrogen was found to be associated with increased risk of endometrial cancer.[17, 27] This particular problem could be solved by combining oestrogen with a progestogen, which reduced the risk of endometrial cancer.[28]
In the 1990s, the reputation of HT was restored and it was now believed not only beneficial for treating menopausal and postmenopausal symptoms but also beneficial in the prevention of chronic diseases based on findings from numerous observational studies.[29-31] And so, it was rebranded as a way to stay healthy forever.[26]
At this time, systemic HT was widely used as 52% of American women aged 48-57 in 1992 reported ever using systemic HT.[32] In Britain, the systemic HT prevalence was 50% in women aged 50-64 between 1996 and 2000,[33] while systemic ever use had a prevalence of 39% in Danish women aged 50-57 in 2002.[34]
In 2002, the Women's Health Initiative (WHI) study published their findings from a seminal randomized controlled trial. The WHI study found evidence that the risks associated with systemic HT outweighed the benefits for disease prevention.[35] This finding prompted an early arrestation of the trial, widespread media attention, and caused new recommendations for HT use to be implemented. It was now suggested that systemic HT was not to be used by asymptomatic women, and if usage was necessary, it had to be kept in the lowest dosage possible.[26, 36, 37] The media attention and alterations in recommendations after the 2002 WHI publication are considered the main reasons for the decline in the prevalence of HT use, which was observed worldwide after 2002.[34, 36, 38-40]
Over time, findings from observational studies have stood in contrast to findings from the WHI study. Both designs are not without its flaws and pitfalls, as observational studies present the possibility of several biases and uncontrolled confounders, whereas the WHI study was flawed by design as it included asymptomatic women on average 63.2 years of age, thus potentially a decade from menopause.[26, 41] A hypothesis was formed embracing and aligning the discrepant findings between observational studies and the WHI study: the critical window or the timing hypothesis. It suggested that HT initiated closer to menopause may have beneficial effects, whereas later may increase the risk of various outcomes.[42, 43] The theory being that if HT was initiated before extensive tissue damage due to aging, it might be beneficial.[42] Another hypothesis for the discrepant findings between observational and clinical studies was that HT users provided a better health profile than non-users prior to the 2002 WHI publication, and thus the beneficial effects of HT found in observational studies are in fact due to a healthy user bias.[44, 45] Furthermore, observational studies may be influenced by environmental and genetic factors providing residual confounding, which could potentially play a role in the discrepant findings in the research field of HT.[46]

2.2 Dementia and hormone therapy

Prior to 2002, dementia was considered one of the chronical diseases from which HT could potentially protect.[47] Dementia is a group of neurocognitive disorders characterized by abnormal brain changes, which can lead to cognitive decline, changes in behaviour and loss of function. In 2015, about 47 million were living with dementia globally, and by 2050 this number is expected to triple.[6] Approximately 70% of dementia cases worldwide is caused by Alzheimer's Disease (AD).[48]
There seems to be a sex difference in the risk of developing AD as women have an increased risk compared to men.[49] Additionally, men with AD outperform women with AD on several cognitive domains including visuospatial abilities and verbal processing.[50] The mechanism behind this sex difference is not fully understood, however the menopausal decrease of oestrogen is hypothesized to contribute to the lower performance in women.[51-53]
The association between systemic HT and dementia has been studied for decades with conflicting results. Many observational studies have reported a decreased risk of dementia for systemic HT users.[54-57] Yet, an ancillary study to the WHI study, the Women's Health Initiative Memory Study (WHIMS), found an in-

creased risk of dementia in postmenopausal women using systemic HT, concluding that it should not be used to prevent dementia or mild cognitive impairment.[58, 59] As in the 2002 WHI study, the WHIMS included older women more than a decade from menopause, and as a result limited their recommendation to women older than 65. However, the WHIMS findings were recently supported by a large, nation-wide, Finnish case-control study, which found an over-all increased risk of AD for systemic HT users, regardless of age at initation.[60]
This is a research area with contradictory findings for decades, but with continued relevance, as underlying mechanisms of dementia are still unknown, and both environmental and genetic factors influence the risk of developing dementia.[6, 61]

2.3 Cognition and hormone therapy

Gonadal hormones have been shown to influence several areas connected to cognition and memory besides regulating the uterine and ovarian cycles.[51] The emphasis in most literature is on oestrogen, but progestogen may also act on neurones to some extent.[51] Basic science has found enhanced spatial memory, which is associated with the hippocampus in gonadectomized rats provided with oestrogen-based HT.[62-64] Oestrogen receptors have further been located in the hippocampal and basal forebrain and the occipital, parietal, and frontal cortex, all involved in cognitive function and memory, and proved to not only enhance cellular markers of memory, but also provide a neuroprotective effect on toxic damage associated with AD.[65]
Persistent mild cognitive impairment, defined as a cognitive decline not hindering activities of daily life but still greater than the expected when accounting for age and educational level, has shown a high risk of progressing to dementia.[66] It has recently been suggested that pathophysiological changes, causing such cognitive impairment and potentially dementia, can occur up to two decades before diagnosis.[67] Oestrogen has however been hypothesized to protect against such pathophysiological changes.[67]
As for the dementia research, the research on HT and cognition has showed conflicting findings for decades. The abovementioned WHIMS study also examined the risk of mild cognitive impairment in women 65 or older using systemic HT, and found that the risk of using HT did not prevent mild cognitive impairment, but on the contrary increased the risk, and concluded that HT was not recommended for prevention against cognitive decline.[58, 59]
However, as the timing hypothesis suggested, it could be problematic making such recommendations based on studies on women potentially more than a decade from menopause as the risk estimates may change with age. So, studies on recently postmenopausal women were conducted, including the Women's Health Initiative Memory Study of Younger Women (WHIMSY) which included women who joined the WHI study when aged 50-55, and no effect of HT on cognitive function was found. [68-71]

2.4 Mortality and hormone therapy

The evidence gathered through decades on the risks and benefits of HT has proven it to be a complex balance. The net effect of this health balance is all-cause mortality.
Observational studies on HT and mortality, published before 2002, indicated that systemic HT initiated during early menopause could potentially reduce all-cause mortality rates in late life by up to 40%.[72] These findings supported the notion that systemic HT was beneficial in the prevention of chronical diseases and associated mortality.
However, this view changed following the 2002 WHI publication, as the initial WHI report found no beneficial effect of systemic HT on all-cause mortality.[73] It actually showed a 15% greater risk of all-cause mortality when using combined oestrogen-progestogen therapy, although not statistically significant. The 13-

year follow-up report from WHI found that neither oestrogen alone nor combined with a progestogen affected the all-cause mortality.[74]
Findings from the initial report and the 13- year follow-up report were supported by the 18-year follow-up report from WHI, and a conclusion of systemic HT neither increasing nor decreasing risk of all-cause mortality was made.[75] The most recent Cochrane review supported this and concluded that all-cause mortality did not differ between HT exposed and placebo.[8]
Yet, when stratifying according to age in the 13 and 18-year follow-up reports, a lower all-cause mortality was seen in women aged 50-59 using systemic HT compared to non-users, while women aged 70-79 had an increased mortality risk compared to non-users.[74, 75] A meta-analysis supported this finding by investigating the association between HT and mortality in younger postmenopausal women, pooling the results from 8 observational studies and 19 randomized trials including the WHI study, and showed a relative risk of 0.72 (credible interval 0.62-0.82).[76]
The complex balance of risk and benefits concerning HT use is further complicated by risk estimates varying over time.[77] In the wake of the 2002 WHI publication alterations were made in guidelines on HT use, thus making analyses of time varying associations especially relevant as the health profile of HT users could have changed over time.

2.5 Twin research setting

Twins provide a unique population and design due to their common upbringing and genetic composition. Monozygotic (MZ) twins share 100% of their genes as they come from the same fertilized egg, while dizygotic (DZ) twins come from two separate, fertilized eggs and share 50% of their genes like other siblings.[78] Twins further share an intrauterine environment during pregnancy, and a childhood and adolescence environment once born. The unique setting of a co-twin control design, in which a twin pair is discordant for a given exposure, enables control for potential unobserved familial confounding through shared genetic factors and childhood environment, as twins provide the best matched case and control, and can possibly help in the disentanglement of selection from causality.[79, 80]
Combining this with information on health recorded through nationwide registries provide a suitable setting for research on HT and dementia, cognition, and mortality, which could potentially shed new light on a research area with decades of conflicting findings.

3 Aim

The overall aim of this thesis was to investigate the association between HT and dementia, cognition, and mortality in postmenopausal women using a random 5% sample of the general Danish population (singletons) and Danish twins and linkage of Danish nationwide registries on healthcare from 1995 and onwards. Additionally, we aimed to investigate if the associations differed before and after the 2002 WHI publication and subsequent change in HT guidelines, as it could have caused an alteration in the HT user profile. The specific aims of the three papers were:

Paper 1
To investigate the association between systemic HT and dementia in postmenopausal female singletons and twins. We used Danish registries on healthcare services and controlled for education and, additionally, for shared childhood and genetic factors in the co-twin control study.

Paper 2
To assess the association between HT and cognition in a population of middle-aged and older Danish twins using cross-sectional and longitudinal analyses which were controlled for age, education, and social class.

Paper 3
To examine the associations between HT and all-cause mortality in both postmenopausal singletons and twins within different age groups, while controlling analyses for education and potential unobserved familial confounding, respectively.

4 Material and methods

This section provides information on the data used in this thesis and the registries from which data was retrieved.

4.1 Data and registries

The Danish healthcare system is based on the principle of providing equal and universal access to healthcare for the Danish population and is mainly tax-financed.[81] The healthcare system collects information, for reimbursement purposes, which is registered in Danish nationwide registries using an individual, unique identification number (CPR-number). This setup enables register linkage and provides valuable resources for epidemiological research.[82] All three studies in this thesis are based on register linkage performed on Statistics Denmark's secure server, which gave access to deidentified, individual-level data. This PhD-project was registered in the University of Southern Denmark's internal list and complied with the rules in the General Data Protection Regulation. Following registries were used in the papers included in this thesis and an overview of the registries is provided in Figure 2:

Danish Civil Registration System
The Danish Civil Registration System (CRS) was established in 1968 and assigns all Danish residents with a CPR-number enabling register linkage.[83] The CRS provides information on date of birth, sex, date of death, and place of residence, and it is continuously updated. Data from the CRS is generally considered complete and of high accuracy.[82]

The Danish Twin Registry
The Danish Twin Registry (DTR) is a population-based registry established in 1953. It contains information on more than 175,000 twins born since 1870 from 140 birth cohorts.[84] Information on zygosity in same-sex twin pairs has been obtained through a questionnaire with four questions on similarity, which led to a misclassification of less than 5%.[85] The DTR contains several surveys of which two are used in this thesis:

> *The Longitudinal Study of Aging Danish Twins* (LSADT) was initiated in 1995 and included living, Danish-resident twins aged 75 or older. Additional cohorts of twins aged 70+ were added in 1997, 1999, and 2001 with follow-up every second year until 2005. A total of 4731 participants completed the intake assessment throughout all waves. The LSADT surveys were interview-based examinations with comprehensive questionnaires.[84] The assessment included information on demographic background, health, and cognitive function, and complete twin pairs were assessed by different interviewers to minimize bias.[86]
>
> *The Study of Middle-Aged Danish Twins* (MADT) was initiated to investigate how mid-life health, lifestyle, and functioning affects health and mortality later in life, as a parallel to the LSADT.[84, 87] From each birth cohort between 1931-1952, 120 intact twin pairs, where both were alive and living in Denmark, were randomly selected. In 1998-1999, 4314 twins completed an in-person intake assessment like that of the LSADT. A follow-up assessment of the MADT cohort was conducted in 2008-2011 and included a questionnaire, in-person inter-

view, and examination, as a part of a national collaboration, and was completed by 2380 twins.[84]

The Danish National Prescription Registry

The Danish National Prescription Registry (NPR) has since 1995 gathered information on redeemed prescriptions in Denmark in both pharmacies and nursing-homes, and thus provides more than 20 years of nationwide coverage of Danish drug use. It contains information on the prescriber (e.g., prescriber practice code), the drug user (e.g., CPR number, age at dispensing date), dispensing (e.g., date of dispensing, Anatomical Therapeutic Chemical (ATC) code, and quantity measured in defined daily doses), and pharmacy (e.g., dispensing pharmacy code).[88] The NPR does not include information on medication administered during hospital admissions, medication used by institutionalized persons, or medication supplied by treatment centres.[89] Furthermore, until 1996 prescriptions to children under the age of 16 were registered under the mother's name. The data from NPR is considered of high quality due to the universal reimbursement system, which provides a strong economic incentive for record-keeping, and the electronically scanned barcode, with which drug-packages are labelled and links to registries containing information on the drug, thus minimising observer and information bias.[88]

The Danish National Patient Registry

The Danish National Patient Registry (DNPR) was established in 1977 and contains information on hospital admission. As of 1995, it also included information from contact with outpatient clinics and emergency departments. Information on diagnoses were coded according to the WHO International Classification of Diseases (ICD) 8th revision from 1977 to 1993 (ICD-8) and 10th revision from 1994 onwards (ICD-10).[90] The DNPR also contains variables on personal data (e.g. CPR number) and admission data (e.g. admission type, date of admission). The registry provides nationwide coverage and low risk of selection bias due to the Danish universal health care system. However, validity studies on diagnose-coding from the DNPR have found varying positive predictive values (PPV) ranging from 66% to 83%, depending on the clinical specialty.[90]

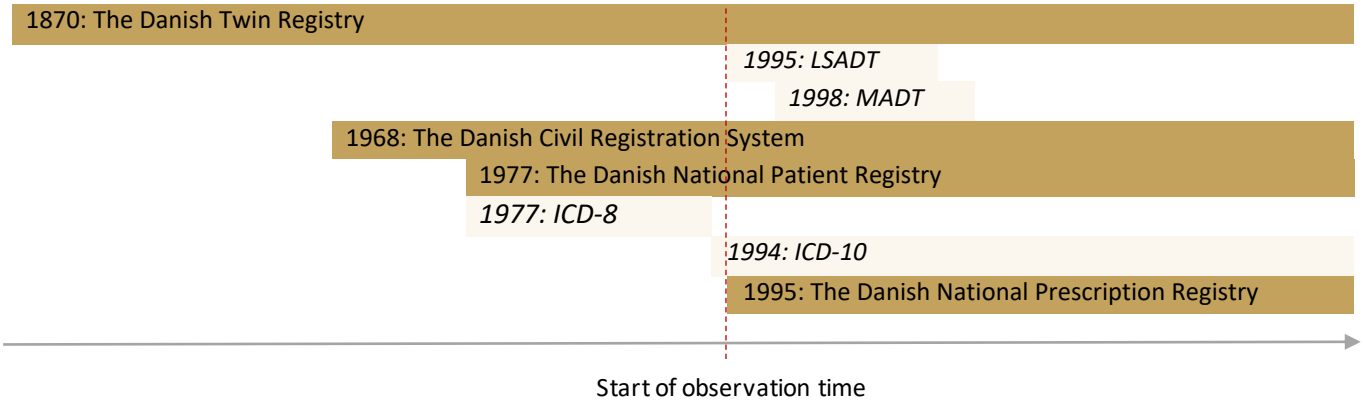

Figure 2. Overview of Danish nationwide registries

4.2 Hormone therapy exposure

The NPR has collected information on oestrogen and progestogen prescriptions since 1995.
The HT exposure definition was based on the HT regimens: Continuous oestrogen, continuous combined oestrogen and progestogen, and cyclic combined oestrogen and progestogen. The ATC codes listed in Table 1 were used to identify the combination of prescriptions forming the HT regimens, e.g., redeemed prescriptions on continuous oestrogen and a hormonal intrauterine device (IUD) containing continuous progestogen would be defined as a continuous combined oestrogen and progestogen regimen. Women who only had prescriptions on a hormonal IUD were excluded in all three papers, as it could have been prescribed for contraceptive purposes.
Users with no other prescriptions than cyclic progestogen were excluded from the study populations in Papers 2 and 3 as use of cyclic progestogen only may have been prescribed on other indications than menopausal or postmenopausal symptoms, e.g., irregular menstrual bleeding. They were, however, included in the HT user group in Paper 1 to increase the sample size, although consisted of less than 1% of the HT users.
If one or more HT prescriptions were redeemed within the defined observation times in the three papers, described in detail in section 5-7, the woman was categorized as a HT user.
Once the HT regimen was identified, the HT exposure was defined as either systemic HT or local HT depending on route of administration. Systemic HT included oral and transdermal routes of administration, while local HT was use of vaginal oestrogen exclusively. If a woman had redeemed prescriptions on both a systemic HT and a hormonal IUD with continuous progestogen or vaginal oestrogen, the woman was considered a systemic HT user.

Table 1. Anatomical Therapeutic Chemical (ATC) codes and the corresponding hormone therapy (HT) regimen used in the three papers

HT regimen:	ATC codes:
Continuous oestrogen	G03C A03, G03C A04, G03C A53, G03C A57, G03C B01
Continuous combined oestrogen and progestogen	G03F A01, G03F A12, G03F A15, G03F A17
Cyclic combined oestrogen and progestogen	G03F B01, G03F B05, G03F B06, G03F B09, G03F B11, G03H B01
Continuous progestogen[a]	G02BA03
Cyclic progestogen[b]	G03D A02, G03D A04, G03D C02, G03D C03

[a] Women with no other prescriptions than continuous progestogen through a hormonal IUD were excluded from analyses in all three papers. If a woman had prescriptions on both continuous progestogen and oestrogen, the woman was considered a systemic HT user.
[b] In Papers 2 and 3, cyclic progestogen was included in the exposure if combined with oestrogen, but in Paper 1 use of cyclic progestogen only was also included in the exposure, though consisting of less than 1%.

In Paper 1 only systemic HT exposure was included, as the outcome was dementia and local HT use was expected to have limited systemic effect.[91] A latency period of one year was also included in Paper 1, where systemic HT prescriptions redeemed less than a year before diagnosis or thereafter was disregarded. Paper 1 further included cumulative defined daily doses to investigate the potential presence of a dose-response effect.
In Paper 2 and 3 both systemic and local HT exposure was included, as the aim of the studies was broadened to further investigate changes in HT use over time.

Through Danish registries, no information on menopausal age or indication for HT was available. So, to estimate if the HT indication was related to menopause, we investigated the age of the first redeemed systemic HT prescription within the youngest birth cohort included in this thesis. In women born 1940-1944, the percentage of individuals with at least one prescription of systemic HT from 1995–2011 was 47.7%. The vast majority of systemic HT users appeared to have initiated HT around the time of menopause, as 98.3% of the beforementioned individuals had redeemed a systemic HT prescription before age 60, and 82.9% before age 55, and are thus expected to be recently postmenopausal, as the average age for menopause in European women is 50-53.[8] In Paper 2, information on indication for HT was available through the LSADT survey included in the DTR, as the twins were asked if they received HT for other reasons than menopause.

4.3 Outcome measures

4.3.1 Assessment of dementia

The DNPR contains information on dementia and has registered diagnoses given according to ICD-8 and ICD-10 codes (Table 2). A validity study on dementia diagnoses from the DNPR showed a PPV of 85.8% for overall dementia, whereas AD had a PPV of 81.0% and vascular dementia had a PPV of 18.5.[92] Thus, an overall dementia diagnosis retrieved from the DNPR is considered of high validity, whereas dividing according to dementia subtype is concluded to be less reliable. Dementia diagnoses are used as outcome in Paper 1 as a binary variable.

Table 2. Dementia classification according to ICD-8 and ICD-10 used in Paper 2

	ICD-8:	ICD-10:
Dementia	290.00–290.99	F00.0–F03.9, G30.0–G30.9

Abbreviation: ICD, International Classification of Diseases

4.3.2 Assessment of cognition

The participants from the MADT and the LSADT survey were subjected to a five-component test assessing cognitive function. The tasks included in the test battery were:

1. A test of fluency in which the participants were asked to name as many animals as possible in one minute. The score in this test was the number of correctly named animals minus errors.

2. A forward digit span test, which consisted of two trials. In each trial, the participants were asked to report back increasingly larger number sequences with increasing degree of difficulty. There were seven degrees of difficulty in each of the trials, and each correctly named number sequence resulted in one point, thus the combined maximum score for the forward digit span test was 14. The test was stopped if the participant failed the same degree of difficulty in both trials.
3. A backward digit span test, which also consisted of two trials, was performed in a way similar to the forward digit span test but with participants reporting back the number sequence in reverse order. The maximum score for this test was also 14.
4. An immediate recall test which included 12 unrelated words. The 12 words were read to the participant, who was then asked to recall as many of the words as possible. Each correctly recalled word resulted in one point, causing a maximum score of 12.
5. A delayed recall test in which the participant was asked to recall as many of the same 12 words as in the immediate recall test, but after a 10-minute delay, also resulting in a maximum score of 12.

The scores from the above-mentioned tests were standardized, firstly, by calculating mean and standard deviation (SD) for the five tests for those aged 46-49 in the MADT survey. Secondly, those values were then used to standardize all measures from all the surveys. These measures were then summed to generate the 5-variable cognitive composite score (CCS).
The CCS has been widely used and has been shown to be age sensitive in Danish women aged 45-90 as a difference of 2.5 SD was observed.[93] It has a high internal consistency reliability (0.75) and is moderately stable over 2-year intervals (0.60).[94] To compare age-related differences, a t-score was calculated for the CCS. The cognitive scores were linearly transformed to a mean of 50 and a SD of 10 within the youngest age group, meaning that a difference in t-score of 10 between exposed and unexposed equals that exposed are one SD higher than unexposed.[93]
In Paper 2, the CCS was included in the analyses as a continuous variable, and changes in CCS was included on an individual level by calculating the difference in CCS from the first assessment to the second, thus a negative change equals cognitive decline.

4.3.3 Assessment of mortality

The CRS holds information on vital status and date of the event. It is continuously updated and linked to Statistics Denmark from where data on all-cause mortality was retrieved. Death was only recorded and registered in the CRS if it occurred in Denmark or if the Danish authorities were informed of the death.[83]
In Paper 3, information on all-cause mortality was retrieved from 1995-2020 and used as a continuous variable.

4.4 Study design

This PhD thesis consists of three observational studies.
Paper 1 on HT and dementia included both a nested case-control and a co-twin control study design. Paper 2 on HT and cognition included a cross-sectional and longitudinal study design, while Paper 3 on HT and mortality used a cohort study design.

4.5 Confounders

Difference in socioeconomic status, which includes education, between HT users and non-users has previously been suggested as a confounder responsible for the opposite findings on HT and various outcomes from clinical trials and observational studies.[46] Information on education was included in all three

papers in this thesis as the Danish research setting provided information on education from Statistics Denmark's Demographic Database, and was defined as number of school years in 1980.[95]
In Papers 1 and 3 education was used as proxy for socioeconomic status, while in Paper 2 information on social class, based on vocational education, employment, and number of subordinates, was available through the surveys included in the DTR,[96] and used in addition to education. In Paper 1, education was used as a binary variable, divided according to length of education ($\leq$ 7 years or $>$7 years). In Paper 2, education was categorized into elementary, vocational, and higher education. Lastly, in Paper 3, education was used a continuous variable as it was defined as completed number of school years in 1980.
All three papers reported on results from twin studies, which per design controlled for potential unobserved familial confounding through shared early childhood environment and genetic factors.

5 Hormone therapy and dementia (Paper 1)

This section provides information on the study population, the statistical analyses and considerations, and the main results from Paper 1: Systemic hormone therapy and dementia: A nested case-control and a co-twin control study.

5.1 Study population

The study populations included in Paper 1 consisted of female singletons from a random 5% sample of the Danish background population retrieved through the CRS and a study population of Danish female twins from the DTR. Emigrants and those with missing or annulled CPR-number were excluded (Table 3). Additionally, both study populations were restricted to individuals born before 1950 and alive by 1995. These age criteria were set to ensure that they were at least 45 years of age, when information on hormone therapy became available in 1995.

Table 3. Exclusion criteria and number of individuals excluded within each category in both the study population of singletons from a random 5% sample of the Danish background population and the study population of twins

	SINGLETONS (n=445,662)	TWINS (n=151,091)
EXCLUSION CRITERIA		
Males	227,855	77,385
Born after 1949 and/or dead before 1995	160,475	57,726
Triplets/Quadruplets	-	124
Unknown sex of co-twin	-	22
Inadequate information on CPR[a]	1017	34
Emigration	2360	228
No dementia	50,695	14,710
Diagnosis before 1997	521	110
Diagnosis before age 60	39	14
Co-twin dead at time of dementia diagnosis	-	450
Vaginal oestrogen users or hormonal IUD only	465	84
Total number of cases	**2235**	**204**

Abbreviations: CPR, personal identification number; IUD, intrauterine device

[a] CPR information missing, annulled or tax address.

To be considered a dementia, a diagnosis of dementia had to be given after 1997, and the individual had to be at least 60 years old at the time of the diagnosis. This two-year lag period (from 1995–1997) was included to minimize inclusion of prevalent dementia cases. The age limit of 60 years was introduced as a try to exclude the heritable forms of early onset dementia. Those who used vaginal oestrogen or a hor-

monal IUD only were excluded, as they were considered an intermediary group possibly experiencing limited systemic effect of HT.[91]
Thus, the nested case-control study consisted of 2235 eligible dementia cases. All except two cases were matched with five female controls born the same year as the case and living without dementia at the time of the case's dementia diagnosis, resulting in 11,028 controls. The co-twin control study consisted of 204 twin cases, who all had a living co-twin without dementia at the time of their diagnosis.
In this study, the end of case inclusion was 31/12-2011, so the observation time was from 1/1-1995 until 31/12-2011.

5.2 Statistical analyses

The statistical analyses were divided into two, as Paper 1 included two different study populations: Singletons and twins.
In the singleton study population, the association between HT and dementia was examined using conditional logistic regression.
In the co-twin control study, the association was investigated as an intrapair analysis using both conditional logistic regression and McNemar's χ^2-test. This analysis was further split according to zygosity to examine if any difference in risk of dementia existed between MZ and DZ twins.
The association between systemic HT and dementia was examined and Odds Ratios (OR) and 95% Confidence Interval (CI) were presented in both study populations.
In both the nested case-control study and the co-twin control study, a sub-analysis was performed splitting cases into age at first diagnosis (before or after age 80) and adjusted for education. This was done to examine the influence of individuals with missing information on education, as they were not included in the education registry due to advanced age.
Another analysis was included in Paper 1 to examine the influence of the alterations in HT guidelines made following the 2002 WHI publication. HT users were divided into two groups according to time of exposure (i.e., HT received before 1/1-2003 or HT continued/initiated after 1/1-2003). Systemic HT users before 2003 had a follow-up time from 1997–2011. After 2003, the follow-up time was 2005–2011. As in the overall analysis, this sub-analysis also included the two-year lag time for dementia diagnoses and the one-year latency period between HT initiation and dementia diagnosis.
The user status in this analysis was defined as never user, previous user (before 2003), or current user (2003–2011). It included 2305 singletons and 218 twins, which differs from number of individuals in the primary analysis (1995–2011). This is due to some singletons (N=70) and twins (N=14), who were originally considered non-users, but were excluded from the analysis after 2003 due to use of vaginal oestrogen or a hormonal IUD.

5.3 Statistical considerations

The nested case-control study design used random sampling of five controls for each case. So, to test the influence of the selection of the five controls on the association a time-dependent Cox regression was also implemented.[97] The results from the Cox regression were similar to the estimates from the conditional logistic regression, which led to the conclusion that the randomness of control selection did not influence the reported results from the conditional logistic regression.
Two sensitivity analyses were performed in both the singleton and twin study population. The first categorized women who received 1–2 HT prescriptions as non-users. The intent was to limit the risk of misclassification as users of 1–2 HT prescriptions may have received too small a HT dosage to properly be considered HT users and were possibly more like non-users. However, women who had 1–2 HT prescrip-

tions in 1995 were not categorized as non-users, as they may have received HT before the NPR was established in 1995.
The second sensitivity included all dementia diagnoses regardless of age at diagnosis. This was done to test the soundness of the defined age criteria for dementia diagnoses (i.e., excluding those with dementia diagnosis before age 60), which was an attempt to exclude the heritable forms of dementia. None of the sensitivity analyses altered the risk estimates notably.
We controlled for education using a binary variable, which was divided according to length of education (≤ 7 years or >7 years). Using a binary variable could potentially increase the risk of residual confounding if the categorisation was insufficient. To check this, we also controlled for education using years of education as a continuous variable and found the adjusted associations did not differ markedly compared to controlling for education with the binary variable.

5.4 Results

An increased risk of dementia was found in the singleton study population with an OR of 1.05, 95% CI [0.93–1.19], however not statistically significant. This tendency towards a slightly increased risk was still seen when stratifying according to age at dementia diagnosis and adjusting for education.
In the twin study population, a borderline statistically significant association between systemic HT and dementia was found with an OR = 2.10, 95% CI = [0.99–4.46]. When stratifying according to age at dementia diagnosis and adjusting for education the tendency of an increased risk attenuated.

In both study populations, a statistically significantly increased risk of dementia was observed before 2003, when dividing according to HT use before or after 2003 (Table 4). The singleton study showed an OR of 1.14 (95% CI = [1.01-1.28]) before 2003, while the intrapair twin analysis showed an OR of 2.20 (95% CI = [1.04-4.65]).

Table 4. Conditional logistic regression providing Odds Ratios and 95% Confidence Intervals showing the association between dementia and systemic hormone therapy in the singleton and twin study population before the 2002 WHI publication (before 1/1-2003) and after the 2002 WHI publication (after 1/1-2003) in which the exposure was divided according to previous, current, and never use after 2002.

	BEFORE WHI[a]				AFTER WHI[b]		
SINGLETONS	Case (n)	Control (n)	OR (95% CI)		Case (n)	Control (n)	OR (95% CI)
Systemic HT	403	1823	1.14 [1.01-1.28]*	Previous HT[c]	132	562	1.20 [0.98-1.47]
No HT	1902	9694	1 (ref)	Current HT[d]	142	728	1.00 [0.82-1.21]
				Never-use	1016	5160	1 (ref)
TWINS	Case (n)	Control (n)	OR (95% CI)		Case (n)	Control (n)	OR (95% CI)
Systemic HT	42	30	2.20 [1.04-4.65]*	Previous HT[c]	21	6	10.65 [2.08-54.54]*
No HT	176	188	1 (ref)	Current HT[d]	12	17	1.55 [0.43-5.56]
				Never-use	78	88	1 (ref)

Abbreviations: WHI, Women's Health Initiative; HT, hormone therapy; OR, odds ratio; CI, confidence interval
[a] Systemic HT use (1995–2002) and dementia (1997–2011)
[b] Systemic HT use (1995–2003 or 2003–2011) and dementia (2005–2011)
[c] Systemic HT use (1995–2002) and dementia (2005–2011)
[d] Systemic HT use (2003–2011) and dementia (2005–2011)
* p-value < 0.05

Further division according to user status: never user, previous user (before 2003), or current user (2003–2011) indicated that the increased risk estimates derived from the previous user group when comparing the risk estimate (OR of 1.20, 95% CI = [0.98-1.47]) to that of the current user group (OR = 1.00, 95% CI = [0.82-1.21]) in the singleton study population. The same tendency was observed in the twin population when comparing the risk estimate of the previous users (OR of 10.65, 95% CI = [2.08-54.54]) to that of the current user group (OR = 1.55, 95% CI = [0.43-5.56]).
An intrapair analysis of complete twin pairs discordant for systemic HT exposure was also performed to control for potential unobserved familial confounding. A total of 31 twin pairs were included in the intra-pair analysis. In 21 (67.7%) of the pairs, the twin receiving systemic HT also developed dementia, resulting in an OR of 2.10 (95% CI = [0.95-4.99]) (Table 5). A zygosity stratification was also performed and showed a larger effect in MZ twins compared to DZ twins. However, the sample sizes were small and showed no significant difference in ORs.

Table 5. McNemar's χ^2-test showing Intrapair difference in risk of dementia in twin pairs discordant for systemic hormone therapy use and stratified according to zygosity showing Odds Ratios and 95% Confidence Intervals.

		Co-twin		
Dementia case		Systemic HT	No HT use	
	Systemic HT	20	21	41
	No HT use	10	153	163
		30	174	204
All twins[a]				
OR [95% CI]	2.10 [0.95–4.99], p-value = 0.071			
MZ twins				
OR [95% CI]	6.00 [0.73–275.99], p-value = 0.125			
DZ twins				
OR [95% CI]	2.50 [0.92–7.86], p-value = 0.078			

[a] MZ, DZ, UZ

Abbreviations: HT, hormone therapy; UZ, unknown zygosity; MZ, monozygotic; DZ, dizygotic; OR, odds ratio; CI, confidence interval

Overall, the twin study population and the singleton study population showed the same tendencies of increased dementia risk for systemic HT users, especially before 2003. However, the association appeared stronger in the twin study population, as dementia has shown to be a disease with a high heritability[98], so the signal is expected to be more accurate when controlling for genetic factors.[99]

6 Hormone therapy and cognition (Paper 2)

This section provides information on the study population, the statistical analyses and considerations, and the main results from Paper 2: Postmenopausal hormone therapy and cognition in twins.

6.1 Study population

The study population included in Paper 2 comprised of Danish female twins from the LSADT and the MADT surveys, born before 1945 and alive by 1995. As in Paper 1, the age restriction was incorporated to ensure that the population was close to or past the average menopausal age, when information on HT became available in 1995.
Paper 2 included a cross-sectional and a longitudinal study design. The study population included in the cross-sectional study consisted of MADT twins aged 50+ at intake in 1998 (n=1722) and LSADT twins aged 70+ at intake into the LSADT surveys from 1995–2001 (n=2788). The longitudinal study consisted of the 1722 MADT twins with cognitive assessment in 1998. Of these, 864 had a cognitive assessment in both 1998 (baseline) and in 2008 (follow-up). Additionally, 1225 twins from the 2001 LSADT survey were included in the longitudinal study. Of these, 666 twins had a cognitive assessment in both 2001 (baseline) and in 2005 (follow-up). This resulted in an observational time of 4–10 years.

6.2 Statistical analyses

Paper 2 on HT and cognition included both cross-sectional and longitudinal analyses, in which the cognitive functions of local HT and systemic HT users were compared to non-users. A fractional polynomial regression was performed, as age was not linearly associated with cognition, and adjusted for age, education, and social class. The LSADT twins (aged 70+) had provided information on the reason for taking HT, and those who reported using HT for other reasons than menopause were excluded from the analyses. A cluster option was included to consider within pair dependency, and residual plots were used to check model assumptions. An additional intrapair analysis was performed using exposure discordant twin pairs to control for potential unobserved familial confounding. The intrapair analyses were further adjusted for education and social class and divided according to zygosity.

6.3 Statistical considerations

In addition to the fractional polynomial regression models included in Paper 2, an extension of the regression model was performed which further included Body Mass Index (BMI), as it could be a potential confounder. However, including BMI in the model did not change the estimates, and thus BMI was not included in the final analysis, as the variable provided no additional information.
Furthermore, the registration of HT exposure and cognition was measured within the same calendar year, which could potentially provide issues, so a sub-analysis was performed using HT exposure the year prior to the cognitive assessment to include a lag-time. It did not alter the estimates.
In the longitudinal analyses, individuals with missing cognitive assessment at the time of follow-up could potentially influence the results causing a selection bias. This bias was attempted minimized by including

a statistical inverse probability weight to the individuals with one cognitive assessment at baseline and adjusting for death between baseline and follow-up.

6.4 Results

Cross-sectionally, the MADT twins aged 50–69 showed no difference in cognitive function when comparing systemic HT users and local HT users to non-users, respectively, before the 2002 WHI publication (Table 6). The twins from LSADT aged 70+ using local HT had a statistically significantly higher cognitive function than non-users, while systemic HT users had a statistically significantly lower cognitive function than non-users illustrated by the negative difference in cognitive score (Table 6) with the cognitive differences corresponding to around one quarter of a SD. Intrapair analyses generally supported the findings from the cross-sectional analyses.

Table 6. Cross-sectional differences in cognitive composite score between hormone therapy users (local or systemic) and non-users for the age groups 50-69 and 70+ before the 2002 WHI publication. Fractional polynomial regressions were adjusted for age, education, and social class, and twins aged 70+ who reported taking HT for other reasons than menopause were excluded from the analysis.

	Age 50-69		Age 70+		All	
	Coeff. (95% CI)	p-value	Coeff. (95% CI)	p-value	Coeff. (95% CI)	p-value
HT use						
No	0 (ref)		0 (ref)		0 (ref)	
Local	1.43 [-0.19; 3.05]	0.083	2.58 [0.74; 4.41]	0.006	2.06 [0.84; 3.28]	0.001
Systemic	0.72 [-0.47; 1.91]	0.237	-2.98 [-5.12; -0.84]	0.006	0.00 [-1.04; 1.05]	0.996

Abbreviation: HT, hormone therapy; CI, confidence interval; coeff., coefficient

Longitudinally, a change in cognitive function for systemic HT users compared to non-users was observed in MADT twins aged 50–69 over a 10-year period with a difference in cognitive function of 0.72 (95% CI = [-0.47; 1.91]) in 1998 and -2.57 (95% CI = [-5.73; 0.58] in 2008 (Figure 3). The LSADT twins aged 70+ showed the opposite tendency over a 4-year period, as those using systemic HT before 2002 had a lower cognitive function than non-users (coeff. -2.06 (95% CI = [-4.33; 0.20]), but after 2002, no significant difference between systemic HT users and non-users was found (coeff. -0.74 (95% CI = [-4.87; 3.38) (Figure 3).

The MADT twins who used systemic HT in 1998 and either switched to local HT or dropped it altogether at time of the follow-up assessment (2008–2011) had a smaller cognitive decline than those who continued to use systemic HT, as seen by the smaller difference in cognition in these groups shown in Table 7A. The LSADT twins showed that those systemic HT users who changed to local HT or dropped HT altogether by the time of the follow-up assessment had a larger decline in cognitive function than those who continued systemic HT use (Table 7B). Thus, the older twins showed the opposite tendency compared to that observed in the younger twins.

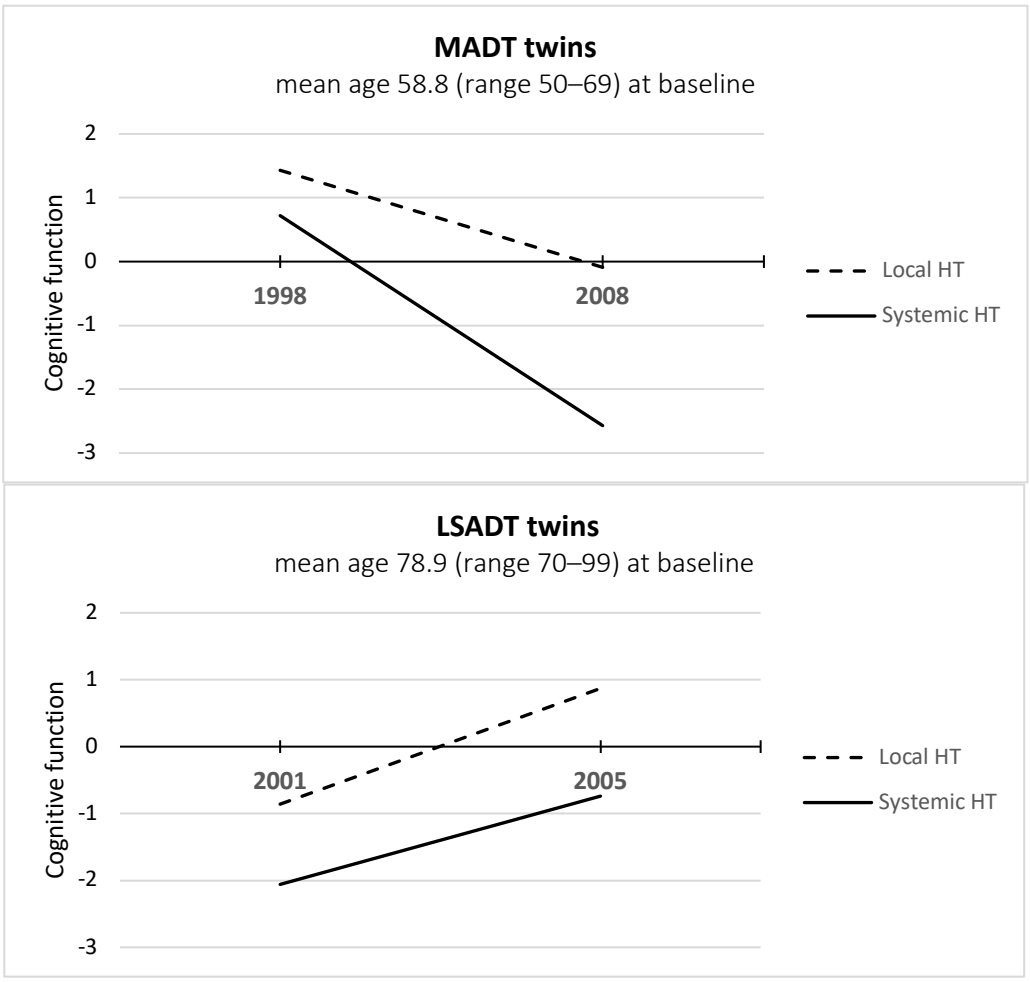

Figure 3. Longitudinal analysis showing changes in cognitive function for systemic and local hormone therapy (HT) users compared to non-users in twins from Middle-Aged Danish Twins study (MADT) and Longitudinal Study of Aging Danish Twins (LSADT) before and after the 2002 WHI publication. Fractional polynomial regressions were adjusted for age, education, and social class and inverse probability weights applied.

Table 7. Longitudinal analysis using fractional polynomial regressions adjusted for age, education, and social class showing difference in cognitive function for the different hormone therapy (HT) user groups (systemic, local, or no use) in Middle-Aged Danish Twins (MADT) between 1998 and 2008–2011 (Table 7A), and in Longitudinal Study of Aging Danish Twins (LSADT) between 2001 and 2005 (Table 7B).

A)	**MADT (2008-11 vs 1998)**					
			Follow-up			
			HT use			
		No		Local		Systemic
	n[a]	Diff. (95% CI)	N	Diff. (95% CI)	N	Diff. (95% CI)
Intake						
HT use						
No	512	-2.9 [-3.6; -2.1]	75	-4.1 [-6.0; -2.2]	5	-5.9 [-15.5; 3.8]
Local	35	-4.3 [-8.4; -0.3]	26	-2.3 [-5.9; 1.4]	<5	-
Systemic	134	-2.9 [-4.2; -1.6]*	44	-3.6 [-6.2; -1.0]	27	-6.4 [-10.1; -2.7]
B)	**LSADT (2005 vs 2001)**					
			Follow-up			
			HT use			
		No		Local		Systemic
	n[a]	Diff. (95% CI)	N	Diff. (95% CI)	N	Diff. (95% CI)
Intake						
HT use						
No	509	-2.8 [-3.6; -2.1]	35	-2.1 [-4.4; 0.3]	0	-
Local	28	-4.9 [-9.2; -0.7]	23	-1.3 [-4.0; 1.4]	0	-
Systemic	22	-1.2 [-4.5; 2.1]	<5	-	31	-0.5 [-2.9; 1.9]

Abbreviations: MADT, study of Middle-Aged and Danish Twins; LSADT, Longitudinal Study of Aging Danish Twins; HT, hormone therapy; diff., difference

[a] Indicating numbers with cognitive measures in both surveys.

*Statistically significantly different from systemic hormone therapy users at both intake and follow-up.

Our finding included in Paper 2 that older LSADT twins using systemic HT had a lower cognitive function than non-users before 2002 but systemic HT users after 2002 had a cognitive function near that of non-users align with the tendency observed in Paper 1 on HT and dementia. However, in this study, we observed the opposite tendency in the younger twins using systemic HT as they showed a similar cognitive function to non-users before 2002, but after 2002 systemic HT users had a lower cognitive function than non-users. Although sample sizes were small, we further observed a difference in cognitive function related to whether it was systemic HT, local HT, or no HT at all that was used before and after 2002. These findings combined cautiously suggest that a selection bias underlie the association between HT and cognition rather than causal effects, potentially due to the 2002 WHI publication and subsequent alterations in HT guidelines.

7 Hormone therapy and mortality (Paper 3)

This section provides information on the study population, the statistical analyses and considerations, and the main results from Paper 3: Postmenopausal hormone therapy and mortality: User profile differences before and after the Women's Health Initiative study.

7.1 Study population

The study populations included in Paper 3 consisted of female singletons from a random 5% sample of the Danish background population retrieved from the CRS and a study population of Danish female twins retrieved from the DTR. All included individuals had to be born before 1950 and alive by 1995, where information of HT became available. This ensured that the study population was within or past the menopausal age, as the youngest birth cohort included in this study would be at least 45 years of age at the start of observation time.
Individuals with inadequate CPR information were excluded as were emigrants since information on death was only recorded if it occurred in Denmark or if the Danish authorities were alerted of the fact. Triplets, quadruplets, and twins with unknown sex of co-twin were also excluded from the twin study population.
HT exposure status was examined in 1995, 2000, 2005 and 2010 in which at least one HT prescription had to be redeemed to be classified as exposed, and both exposed and unexposed in those respective years had to survive until 31st of December that year to be included in the study. This resulted in 52,388 female singletons in the singleton study population and 15,261 female twins in the twin study population.
The study populations were followed from 1/1-1995 until 31/12-2020. The follow-up time was, for comparison purposes, set to 15 years from the exposure years 1995, 2000 and 2005. Follow-up time was 10 years from 2010, as end of this study's observation time was in 2020.

7.2 Statistical analyses

The association between HT and mortality was investigated using Cox proportional hazards models adjusted for age and education which provided Hazard Ratios (HR). Proportional hazards assumptions were tested with no violation found. Exposure to HT was examined in different age groups in 1995, 2000, 2005, and 2010 to illustrate potential variation in risk over time. Different HT user categories were examined in the singleton study population in 2000 and 2005, and analyses were adjusted for education. Educational levels in systemic and local HT users were compared to non-users by a quantile regression estimating median differences.
In the twin study population, an intrapair Cox proportional hazards model adjusted for education was performed examining risk for different age groups if exposed in 1995, 2000, 2005, and 2010, respectively, in complete twin pairs who were HT exposure discordant.

7.3 Statistical considerations

Emigrants were excluded from the study population, which could potentially provide some informative censoring, as there lies information in them not dying while residing in Denmark. However, the percentages of emigrants within the singleton and twin study population were approximately 4.5% and 1.5%, respectively. The area of interest, investigated in this study, was differences in the HT user profiles over time more than the causal effects of HT on mortality, and thus the relatively small percentage of emigrants were not expected to influence the reported findings significantly.

7.4 Results

In both study populations, a decrease in systemic HT prevalence was observed following the 2002 WHI publication from 25% in 2000 to 10% in 2005. Additionally, we found an increased prevalence of local HT users from 5% to 10% between 2000 and 2005.

In the twin study population, a lower mortality was observed in systemic HT users aged 56–60 in 2000 in intrapair analyses adjusted for education, which increased in 2005 close to that of the background population (Figure 4). Findings from the singleton study population supported this tendency in those aged 56–60. The observed tendency was further expanded in the singleton analyses, as a lower mortality was found in women aged 56–75 in 2000 when adjusting for education, and the mortality rose in 2005 to that of the background population.
In the singleton study population, we were also able to examine potential changes in the HT user profile between 2000 and 2005 across age groups, due to the larger sample size. We found that systemic HT users either dropped systemic HT or changed to local HT following the 2002 WHI publication (Table 8).

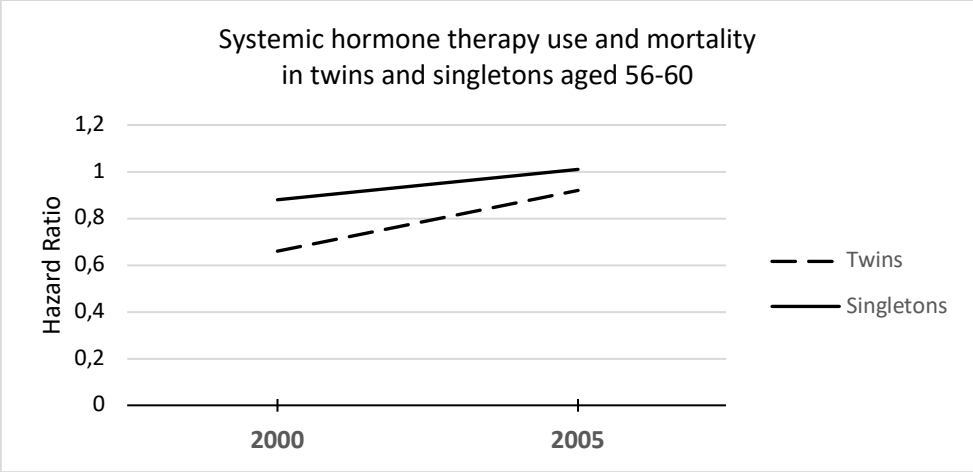

Figure 4. Cox proportional hazard models adjusted for age and education showing changes in mortality for systemic HT users aged 56-60 before and after the 2002 WHI publication in the singleton study population (full line). Intrapair analysis adjusted for education and potential unobserved familial confounding showing changes in mortality for systemic HT users in the age group 56–60 before and after the 2002 WHI publication in the twin study population (dotted line).

Table 8. Cox proportional hazard models adjusted for age and education showing the association between different hormone therapy user categories and mortality before and after the 2002 WHI publication in singletons aged 56-60.

	BEFORE WHI[a]		AFTER WHI[b]	
SINGLETONS	n (%)	HR (95% CI)	n (%)	HR (95% CI)
No hormone therapy in 5 years	4562 (56.5)	1 (ref)	5700 (60.8)	1 (ref)
Systemic hormone therapy, continuous	1797 (22.3)	0.88 [0.76 ; 1.01]	917 (9.8)	0.98 [0.82 ; 1.19]
Systemic hormone therapy, initiated	153 (1.9)	0.77 [0.48 ; 1.25]	37 (0.4)	1.44 [0.64 ; 3.21]
Systemic hormone therapy, previous	687 (8.5)	0.90 [0.73 ; 1.11]	1333 (14.2)	0.98 [0.83 ; 1.15]
Local hormone therapy, changed from systemic	86 (1.1)	0.80 [0.45 ; 1.42]	269 (2.9)	0.42 [0.26 ; 0.69]

Abbreviations: WHI, Women's Health Initiative; HR, Hazard Ratio

[a] Hormone therapy exposure in 2000

[b] Hormone therapy exposure in 2005

Combining our findings of a decreased prevalence of systemic HT between 2000 and 2005 and a mortality which rose in 2005 to that of the background population suggest a change in the HT user profile following the 2002 WHI publication. This is supported by our findings of a changed pattern of use between 2000 and 2005 where potential healthy systemic HT users appear to either drop systemic HT or change to local HT. It emphasizes the importance of sufficient confounder control when examining HT use as they may vary markedly over time.

8 Discussion

In this section the main findings from the three papers, which have been reported in this thesis, will be discussed, compared to existing literature, and methodological issues considered.

8.1 Summary of findings

The underlying mechanisms of dementia development are still unknown, but there is a well-documented sex difference in dementia prevalence, which suggests that the menopausal decrease of oestrogen could play a part in the disease development.[49, 51-53] The findings reported in Paper 1 of no overall association between HT and dementia between 1995–2011, but an increased risk of dementia for systemic HT users before 2003 in both twins and singletons, drew the attention to the potential impact the 2002 WHI publication[35] has had on subsequent observational studies. The findings in Paper 1 indicated that the 2002 WHI publication may have caused an exclusion of the most fragile women from HT use after the 2002. A notion which deserved further exploration.

So, we examined the association between HT and cognition in Danish female twins in Paper 2, as persistent mild cognitive impairment has shown a high risk of progressing to dementia.[66] It has further been suggested that pathophysiological changes, causing cognitive impairment, and potentially dementia can occur up to two decades before diagnosis, for which oestrogen has been hypothesized to protect against.[67, 100] In line with findings from Paper 1 on dementia, older female twins using systemic HT showed a lower cognitive function before 2002, but cognition was near that of non-users after 2002 for systemic HT users. The reverse tendency was observed in twins closer to the average age of menopause or recently postmenopausal with a lower cognitive function observed in the wake of the 2002 WHI study in systemic HT users. Additionally, a different pattern of HT use was found in the younger twins after 2002, as those changing from systemic HT to local HT or dropping it altogether performed better cognitively. This finding suggested a change in the HT user profile following the 2002 WHI publication and thus an introduction of a selection bias which may underlie the association.

To further explore the repercussion of the 2002 WHI publication, we aimed to investigate the influence of HT on the net effect of health, mortality, before and after 2002 in both twins and singletons. First, we observed a decrease in systemic HT prevalence between 2000 and 2005 in both study populations, but an increase in the prevalence of local HT. Secondly, systemic HT users showed a lower mortality prior to 2002 in both twins and singletons close to the average menopausal age, but the mortality rose to that of the background population after 2002 for the same age group. It appeared that the healthiest users decided to either drop systemic HT or change to local HT in the wake of the 2002 WHI publication, in line with reported findings on the HT user profile in younger twins in Paper 2. When combining findings from the studies included in this thesis, an indication of an alteration in HT user profile following the 2002 WHI publication appeared, likely due the introduction of a selection bias underlying associations in studies spanning across 2002, rather than causal effects.

8.2 Comparison to other studies

The use of HT has a long and complex history in which the research field of HT and dementia, cognition, and mortality has provided contradictory findings for decades.[35, 54, 59, 60, 70, 72, 101-104] To explain these discrepant findings several hypotheses have been proposed.[105, 106]

The timing hypothesis

A timing hypothesis, or a window of opportunity, has been suggested in which systemic HT initiated closer to menopause would provide beneficial health effects, but initiated later, systemic HT would increase the risk of various outcomes.[42, 107] Previous studies examining HT and cognition have supported this hypothesis as findings indicated that systemic HT initiated in younger, recently menopausal women (aged 50–54) had no effect on the cognitive function, whereas for postmenopausal women (aged 65–79) a decrease in cognitive function was observed.[68, 108] The hypothesis was further supported by a reanalysis of the WHI study examining the timing hypothesis showing that younger, menopausal women initiating HT tended to have a decreased risk of coronary heart disease and mortality compared to women further from menopause.[109] Our findings reported in this thesis, appeared not to support the timing hypothesis, as we observed the contrary tendency. Albeit the studies included in this thesis were not designed specifically to investigate the timing hypothesis, as information of age at menopause is not available through Danish registries. The studies were however designed to control for shared environmental and genetic factors, which could potentially underlie the association between HT and various outcomes, as the use of the co-twin control study design is considered a helpful tool in examining whether an association is more likely to be attributed to selection or causality.[80]

Bias and residual confounding

One specific selection bias hypothesized to influence the research area of HT was the healthy user bias. It was introduced to align the historically conflicting findings between clinical and observational studies and was suggested to be present in observational studies prior to 2002, as healthier, better educated, women were suggested to favour systemic HT use.[44, 110] We did not find that education influenced the associations nor that systemic HT users had lower risk of dementia or better cognitive function than non-users prior to 2002, but we did find that systemic HT users had a lower mortality than non-users before 2002.
A Danish cross-sectional study investigated the association between socioeconomic status and HT use prior to the 2002 WHI publication in more than 120,000 postmenopausal women and found no significant socioeconomic gradient in HT use and suggested that Danish observational studies may have been less susceptible to socioeconomic confounding, perhaps due to the structure of the healthcare system.[111] The studies included in this thesis did, however, suggest the presence of another selection bias in the wake of the 2002 WHI publication as an alteration may have occurred in HT user profile after 2002. This alteration could potentially have affected the association between HT and various outcomes in observational studies if the observational time span across 2002.

Change in HT user profile

We observed a decrease in systemic HT prevalence following the 2002 WHI publication, as the prevalence changed from 25% in 2000 to 10 % in 2005. This aligns with findings reported in studies from other countries.[38-40] A large American cohort study and The Study of Women Across the Nation found a decrease in initiation and continuation of systemic HT after 2002,[112, 113] and the latter concluded that the alteration in the recommendations on HT use following the 2002 WHI publication were implemented across America.[112]

Few studies have examined the differences in the HT user profile before and after the 2002 WHI publication. A German study examined the difference in HT use in two surveys conducted before and after 2002.[114] A decline in self-reported HT use was observed especially among women with high social status. Additionally, use of HT was associated with higher social status, lower BMI, and healthier lifestyle before 2002, but not after. A Canadian study conducted between 1998 and 2003 described the trends in HT use a year after the 2002 WHI publication.[115] They observed a significant decline in HT prescriptions after 2002 but also found it less likely that women with risk factors of cardiovascular disease or coronary artery disease ceased HT after 2002.
Our findings support these studies, as we observed that women, who performed cognitively better, either dropped HT or switched to local HT after 2002. We further observed a decrease in the prevalence of systemic HT and a rise in mortality after 2002 indicating that healthier HT users changed their pattern of HT use in the wake of the 2002 WHI publication. Thus, due to the following abrupt discontinuation of systemic HT in the wake of the 2002 WHI publication, the healthy user bias appeared to have changed into another type of selection bias underlying the associations on various outcomes. [44, 110, 114, 116, 117]

8.3 Methodological considerations

8.3.1 Strengths

Strength of twin studies
A major strength was the use of the co-twin control study design. The design enabled control for potential unobserved familial confounding due to shared genetic factors and rearing environment e.g., childhood socioeconomic factors,[79] which could potentially influence the associations between HT and cognition, dementia, and mortality. Exposure discordant MZ twin pairs provide the optimal match on unobserved familial confounding, such as personality and risk taking, which have substantial genetic components to its variations and provide a helpful tool in the disentanglement of selection from causal effects.[79, 80]

Singleton study population
The 5% random sample of singletons from the Danish background population, included in Papers 1 and 3, was retrieved from the CRS and provided a strength in the size of the sample and the practically complete follow-up. Retrieving the singleton study population from Danish registries further minimizes selection bias, compared to other methods of recruitment.

Danish research setting
The structure of the Danish healthcare system and storage of information in Danish registries offer a unique research setting, as registries can be linked through the CPR number. This linkage of registries is help diminish certain biases as the registries have nationwide coverage and rely on a healthcare system with equal and universal access., e.g., the use of the NPR minimizes recall-bias regarding exposure to HT.

8.3.2 Limitations

Exposure definition
The hormone, oestrogen, is considered the main hormone of interest when investigating HT and the risk of various outcomes. However, it has been hypothesized that progestogen could potentiate the effect of oestrogen,[60] and so the studies in this thesis could have benefitted from dividing analyses according to HT regimen, as the risk may vary according to type of HT regimen. Additionally, investigating the cumulative

dosage of HT over a lifetime could also have been of relevance but as registries with information on HT began in 1995, so did our observation time. Thus, associations reported in this thesis were not divided according to HT regimen and dosage due to lack of statistical power and limited observational time.
Additionally, it would have been preferable to include the ATC code G03FA11 (continuous combined oestrogen and progestogen) in the HT exposure, but this was unfortunately left off the application to the NPR and once discovered, alteration in the application was not accessible within the timeframe of the PhD-project due to an extraordinary long waiting time on approvals from the relevant authorities, caused by COVID-19. This may underestimate the number of HT users, although this specific ATC code appeared to only have been sold from 1997–1999, and thus only available for a limited time within our observational time.[118]
In Paper 1 on dementia, users of cyclic progestogen only were included in the exposure group to increase the sample size, although indication may not have been relief of menopausal symptoms, but they were excluded in Papers 2 and 3. The inclusion of this HT user group in Paper 1 could potentially overestimate the dementia risk for HT users, as cyclic progestogen may have been prescribed to pre-menopausal women for irregular bleedings, albeit they comprised of less than 1% in the study population, and thus were not expected to affect the risk estimates noticeably as the vast majority of the HT users used oestrogen alone or in combination with a progestogen.
It must also be mentioned that the NPR registered children's prescriptions in their mother's name until 1996. Local HT can be prescribed to girls with labial adhesions and could therefore slightly overestimate the use of local HT in Papers 2 and 3 if such prescriptions were registered in their mother's name. However, this mother-child registration was only applicable the first year of the observation time in Papers 2 and 3 and was not expected to alter the associations with local HT considerably.

Follow-up
Paper 1 on dementia had a limited follow-up from 1995–2011, which was the maximal observation time available when this PhD-project was initiated. The association found between HT and dementia could be explained by confounding by indication or by the short follow-up. Thus, expanding the follow-up time would have been preferable, but due to an extraordinary long waiting time on approvals from the relevant authorities, caused by COVID-19, this was not accessible within the timeframe of the PhD-project.

Bias
There is a possibility of left truncation bias, as the NPR began in 1995 and individuals included in the study populations had to be alive by then. Paper 1 was sensitive to left truncation bias and so a two-year lag period was included in which dementia cases were only considered from 1997 and onwards to minimize inclusion of prevalent dementia cases. Additionally, a sensitivity analysis was performed in which those with 1–2 HT prescriptions were considered non-users to limit misclassification, but those receiving 1–2 HT prescriptions in 1995 were still included in the analyses, as they could have received HT before the observational time began.
As of 2020, 39,778 individuals were living in Denmark with a registered dementia diagnosis but based on population surveys, the actual number of individuals living with the disease, is expected to be much higher, approximately 87,000.[119] Although the validity of dementia diagnoses is considered high, the association between HT and dementia in Paper 1 may be influenced by a potentially large number of individuals living with cognitive impairment, possibly dementia, but managing and thus not seeking a diagnosis, causing a detection bias. Paper 2 on cognitive function was performed in part to shed light on this otherwise overlooked group.
Even though it was previously argued that Danish nationwide registries, including the DTR, provided min-

imal selection bias, a potential selection did occur in Paper 2 later in the process due to non-attendance in the follow-up cognitive assessment or mortality. This selection bias was attempted minimized by the statistical use of an inverse probability weight and analyses were adjusted for death between assessments.
It must also be mentioned that NPR does only include information on prescriptions redeemed, and thus contain no information on HT user compliance, which could overestimate the use of HT in all three papers included in this thesis and include a compliance bias.[106]
Confounding bias could affect the association between HT and dementia, cognition, and mortality. Studies have shown that women who smoke go into natural menopause earlier than non-smokers[120] and may experience vasomotor symptoms more frequently,[121] so it would have been preferable to include information on various lifestyle factors like smoking as it could potentially be a confounder, especially in Paper 3 on mortality. Information on lifestyle factors was not available through the Danish registries, but we adjusted for the potential confounders for which we did have information, e.g., education, however findings from the studies included in this thesis suggest that confounders may also change over time, especially if studies span across 2002, and are thus difficult to control for.

Generalisability
The generalisability of results from the twin study populations to the general Danish population has been questioned, as twins could potentially be a select group. However, large studies on health, survival and cognitive function have shown that twins can be considered representative for the general population.[122-125] Also, similar tendencies between singletons and twins was found in Papers 1 and 3, which add to the notion that twins can be compared to the general population.

9 Conclusion

Findings on Danish twins and singletons reported in this PhD thesis provide support to the hypothesis that a selection bias caused by the 2002 WHI study underlie the association between HT and the outcomes dementia, cognition, and mortality rather than causal effects. The twins provided a unique study population as they shared childhood environment and genetic factors, and so the co-twin control study design allowed us to control for possible unobserved familial confounding.

9.1 Implication of findings

Our findings suggest that the HT user profile is very different before and after 2002, when the findings from the seminal WHI study were published. Systemic HT was more commonly prescribed prior to 2002, but the prevalence drastically declined thereafter. This decline is most likely attributed to the alterations made in HT recommendations following the 2002 WHI publication, as it was then suggested that HT be kept to a minimum dosage for the shortest amount of time. Furthermore, asymptomatic women or women presenting with a history of risk factors were unable to receive HT, which potentially changed the HT user profile and underlying confounders.
Findings included in this thesis highlights the importance of applying adequate confounder control in observational studies spanning across 2002 when examining the association between HT and various outcomes as confounders may alter with time, due to the change in HT user profile in the wake of the 2002 WHI publication.

9.2 Future research

Based on findings included in this thesis, we suggest that future observational studies provide adequate confounder control if spanning across 2002, or provide stratified risk estimates before and after 2002, as confounders may vary markedly over time and can possibly be related to initiation, regimen, and dose of HT. Alternatively, as we are now 20 years from the 2002 WHI publication, observational studies could focus solely on HT users after 2002, as they may provide a more stable base for generalisation of results to current HT users.

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