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ORIGINAL RESEARCH ARTICLE

# Postmenopausal Estrogen Therapy and Risk of Gallstone Disease: A Population-Based Case–Control Study

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**Abstract**
*Background* Female gender and increasing age are key risk factors for gallstone disease; therefore, postmenopausal women are at high risk. Estrogen increases cholesterol saturation of bile and may further increase gallstone risk, but population-based evidence is sparse.
*Objective* Our objective was to examine the association between postmenopausal estrogen therapy and risk of gallstone disease and the impact of duration of treatment and use of opposing progestin.
*Study Design* We conducted a population-based case–control study. Cases were postmenopausal women (defined as aged ≥45 years) with gallstone disease identified in the period 1996–2010. For each case, we selected ten population controls matched to cases by age and sex. We defined exposure as any use of estrogen (opposed and unopposed by progestin). Cases/controls were categorized as current estrogen users if their last prescription was redeemed <90 days before gallstone diagnosis (or corresponding date for controls); all other users were categorized as former users. The reference group consisted of cases/controls with no/rare estrogen use.
*Setting* Medical databases covering the population of Northern Denmark (2.4 million inhabitants through the period 1996–2010).
*Main Outcome Measure* We used conditional logistic regression to compute adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) of gallstone disease in women treated with estrogen. The ORs were adjusted for relevant comorbidity, other drugs known to influence gallstone risk, and parity.
*Results* We identified 16,386 cases with gallstone disease and 163,860 controls. A total of 1,425 cases (8.7 %) and 8,930 controls (5.4 %) were current estrogen users, yielding an adjusted OR for gallstone disease of 1.74 (95 % CI 1.64–1.85) compared with non-users. The corresponding adjusted OR for former users was 1.35 (95 % CI 1.28–1.42). The results suggested a duration response for current users. Use of unopposed estrogen was associated with higher adjusted ORs than estrogen opposed by progestin.
*Conclusion* Postmenopausal estrogen therapy was associated with increased risk of gallstone disease in current and former estrogen users. Use of unopposed estrogen was associated with higher risk than use of estrogen opposed by progestin; this finding needs to be confirmed and explored further in future studies.

**Electronic supplementary material** The online version of this article (doi:10.1007/s40264-013-0118-7) contains supplementary material, which is available to authorized users.

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## 1 Introduction

The gender difference in the prevalence of gallstones is presumed to be caused by endogenous female sex hormones, particularly estrogen, which increase cholesterol secretion and diminish bile salt secretion, and thereby promote the development of gallstone disease [1–3]. Exogenous estrogen, as provided in postmenopausal

replacement therapy, also increases the cholesterol saturation of bile [4–6]. Exogenous estrogen therefore may further increase the risk of gallstone disease in postmenopausal women, a population group already at high risk of this disease due to gender and age [2, 3, 7].

Previous trials and observational studies found a 30–100 % increase in risk of gallstone disease in women receiving postmenopausal estrogen therapy [8–13]. However, these studies primarily focused on the association between estrogen and severe gallstone disease requiring gallbladder surgery. In addition, in the majority of studies, information on exposure, gallstone disease, and covariates was obtained only through baseline and follow-up questionnaires [11, 12, 14], thus introducing potential biases (recall bias, detection bias, and loss to follow-up) [15]. Studies also lacked details on important covariates such as cardiovascular disease and use of relevant co-medication [1, 11, 12, 16]. Finally, only two previous studies have explored the influence of concomitant use of progestin [11, 14], although progestin may be assumed to lead to increased risk of gallstone disease due to reduced bile salt secretion and impaired gallbladder emptying [2, 17].

Thus, in order to provide evidence to evaluate the association between postmenopausal estrogen use and occurrence of gallstone disease in a large general population, we conducted a study using Danish population-based health registries. The study also evaluated the impact of duration of treatment and use of opposing progestin.

## 2 Materials and Methods

### 2.1 Source Population and Setting

This population-based case–control study utilized information from health registries covering the population of Northern Denmark. Our source population consisted of approximately 2.4 million inhabitants of Northern Denmark during the period 1996–2010 [18]. We used the civil registration number, a personal identifier assigned to each Danish citizen at birth and to residents at immigration, to link the health registries [19]. For each hospital admission since 1977, the Danish National Registry of Patients (DNRP) has recorded the patient's civil registration number, dates of admission and discharge (including outpatient and emergency admissions since 1995), surgical procedures, and up to 20 discharge diagnoses coded by doctors according to the *International Classification of Diseases* (ICD) (eighth revision until the end of 1993 and tenth revision thereafter) [20].

### 2.2 Cases Diagnosed with Gallstone Disease

We identified the first hospitalization or outpatient clinic visit for postmenopausal women (defined as age $\geq$45 years) with gallstone disease between 1 January 1996 and 31 December 2010, as recorded in the DNRP. 'Gallstone disease' was defined as either an ICD code for gallstone disease, cholecystitis (Supplementary Table 1), or a procedure code for gallbladder surgery (Supplementary Table 2) registered in the DNRP. The earliest recorded date for each case was considered the index date. We excluded cases with pre-existing gallstone disease or a history of liver, bile duct, or pancreatic cancer by searching the DNRP back to 1977. To ensure at least 2 years of prescription history in the prescription database, we excluded cases who had resided in Northern Denmark for $<$2 years.

### 2.3 Population-Based Controls

For each case, we selected ten population controls with no record of gallstone disease before the index date of the cases, matched to cases by age and sex. Controls were subjected to the same exclusion criteria as cases. The controls were selected from the Danish Civil Registration System, which is updated daily and has maintained records on vital statistics, date of death, and place of residence for all Danish citizens and residents since 1 April 1968 [19]. Controls were selected using risk-set sampling [15]. Cases and their matched controls were both assigned an index date identical to the date on which the case was diagnosed with gallstone disease.

### 2.4 Use of Postmenopausal Estrogen Therapy

To secure reimbursement from the National Health Service, pharmacies in Northern Denmark transmit each prescription transaction, including the patient's civil registration number, the type and amount of drug prescribed according to the Anatomical Therapeutic Chemical classification system (Supplementary Table 3), and the date on which the drug was dispensed, to the Aarhus University Prescription Database (AUPD) [21–23].

From the AUPD, we ascertained any use of estrogen before the index date, both opposed and unopposed by progestin. Because of minimal systemic absorption, vaginally administered estrogen was excluded [24, 25]. We classified patients as estrogen users if they had redeemed at least two prescriptions prior to the index date or as rare/never users (reference group) if they had no or only one redeemed prescription. Based on clinical experience and the most likely prescription pattern, users were categorized

as current users if their last prescription for estrogen was redeemed ≤90 days before the index date, or as former users if their last prescription was >90 days before the index date. Both current and former users were also categorized according to duration of treatment, calculated as the time between the first and last prescription prior to the index date (<1, 1–4, and >4 years), and intensity of treatment, calculated as the total number of redeemed prescriptions (2–4 prescriptions, 5–19 prescriptions, and ≥20 prescriptions).

### 2.5 Covariates

We collected data on potential confounding factors [3, 26–28] from the DNRP, the AUPD, and the Danish Medical Birth Registry (DMBR). We searched the DNRP for any hospital records with diagnoses/procedure codes denoting alcoholism, cancer, cardiovascular disease (cardiac disease, apoplexia cerebri, and transient ischemic attack), chronic obstructive pulmonary disease, diabetes, hyperlipidemia, hypothyroidism, hysterectomy, inflammatory bowel disease, liver cirrhosis, obesity, or renal failure occurring before the index date (Supplementary Tables 1 and 2). We searched patient records in the AUPD for prescriptions for thiazides or statins and other lipid-lowering drugs (Supplementary Table 3). Finally, we searched the DMBR, which contains data on all childbirths in Denmark since 1973 (including maternal civil registration numbers), and the Danish Civil Registration System to determine the parity of the study participants [29]. We included each of these covariates individually as a dichotomous variable in our adjusted analyses.

### 2.6 Statistical Analyses

We calculated the frequency of cases and controls within the categories demographic characteristics, drug exposures, and covariates. In the main analysis, we calculated adjusted odds ratios (ORs) and 95 % confidence intervals (CI) associating undifferentiated estrogen use with occurrence of gallstone disease, using conditional logistic regression. Because we used risk-set sampling to select controls, these ORs approximate incidence rate ratios [15]. We calculated ORs associating current and former estrogen use with the occurrence of gallstone disease, adjusted for the covariates mentioned above. We categorized exposure by the duration and intensity of treatment and according to presence or absence of opposing progestin.

We performed additional analyses to explore the observed associations. First, to explore the impact of comorbidities, the ORs were stratified by history of diabetes, cardiovascular disease, and obesity. For this analysis, we used conventional logistic regression models, because the stratification required that we dissolve the matched sets, allowing us to control for the matched factors as well as the other covariates. Second, to examine the possible prolonged effect of estrogen after discontinuation, we conducted an analysis in which we grouped former users according to time since discontinuation of estrogen therapy as of the index date (≤24 or >24 months before the index date). Third, to explore the development of the association throughout the study period, we stratified the ORs according to time of index date (index date ≤2002 or >2002).

Fourth, we conducted separate analyses in which we changed the cutoffs for current and former use to ≤30 and >30 and ≤180 and >180 days, respectively, from the date of the last prescription to the index date. Fifth, to explore if categorization of cases/controls with only one redeemed prescription as non-exposed had any influence on the overall results, we conducted an analyses in which they were excluded. Sixth, to avoid misclassifying the duration of use, we performed an analysis restricted to women who started their treatment course during the study period (1996–2010), with a minimum of 180 days of non-use prior to therapy initiation [30]. The risk of underestimating the risk of gallstone disease early in the treatment period was thereby reduced. Finally, to reduce the potential for misclassification of gallstone disease, we restricted the analysis to cases who underwent gallbladder surgery.

All analyses were carried out using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

The study was approved by the Danish Data Protection Board (record no. 2009-41-3866).

## 3 Results

We identified 16,386 women with gallstone disease and 163,860 population controls. The median age at diagnosis (index date) was 63.2 years. Comorbid diseases and previous hysterectomy were more frequent in cases than in controls, and cases were more likely to have given birth to more than two children than were controls (Table 1).

A total of 3,533 cases (21.6 %) with gallstone disease and 25,729 population controls (15.7 %) had been prescribed estrogen, yielding an overall adjusted OR of 1.48 (95 % CI 1.42–1.55) for gallstone disease compared with never/rare users (reference). A total of 1,425 cases (8.7 %) and 8,930 controls (5.4 %) were current estrogen users, yielding an adjusted OR of 1.74 (95 % CI 1.64–1.85). Less than 1 year of treatment was associated with an adjusted OR of 1.47 (95 % CI 1.17–1.85) (Table 2). A total of 2,108 cases (12.9 %) with gallstone disease and 16,799 controls (10.3 %) were former estrogen users, corresponding to an

**Table 1** Characteristics of cases with gallstone disease and population controls in Northern Denmark, 1996–2010

| | Cases with gallstone disease | | Population controls | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Total no. | 16,386 | 100 | 163,860 | 100 |
| Age (years) | | | | |
| 45–59 | 6,810 | 41.6 | 68,093 | 41.6 |
| 60–79 | 7,033 | 42.9 | 70,337 | 42.9 |
| ≥80 | 2,543 | 15.5 | 25,430 | 15.5 |
| Time period | | | | |
| 1996–2000 | 3,553 | 21.7 | 35,530 | 21.7 |
| 2001–2004 | 4,957 | 30.3 | 49,570 | 30.3 |
| 2005–2010 | 7,876 | 48.1 | 78,760 | 48.1 |
| Parity | | | | |
| 0 or unknown | 3,059 | 18.7 | 32,854 | 20.1 |
| 1 and 2 | 8,447 | 51.6 | 87,293 | 53.3 |
| >2 | 4,880 | 29.8 | 43,713 | 26.7 |
| Previous hospital diagnoses | | | | |
| Alcoholism | 300 | 1.8 | 2,668 | 1.6 |
| Cancer[a] | 1,858 | 11.3 | 14,229 | 8.7 |
| Cardiovascular disease | 3,547 | 21.6 | 25,188 | 15.4 |
| COPD | 958 | 5.8 | 6,509 | 4.0 |
| Diabetes | 1,151 | 7.0 | 8,606 | 5.3 |
| Hyperlipidemia | 487 | 3.0 | 3,340 | 2.0 |
| Hypothyroidism | 341 | 2.1 | 2,321 | 1.4 |
| Hysterectomy | 739 | 4.5 | 5,231 | 3.2 |
| IBD | 184 | 1.1 | 1,355 | 0.8 |
| Liver cirrhosis | 46 | 0.3 | 295 | 0.2 |
| Obesity | 881 | 5.4 | 4,067 | 2.5 |
| Renal failure | 141 | 0.9 | 763 | 0.5 |
| Stroke | 624 | 3.8 | 5,404 | 3.3 |
| Transient ischemic attack | 331 | 2.0 | 2,877 | 1.8 |
| Previous treatment | | | | |
| Other lipid-lowering[b] | 115 | 0.7 | 726 | 0.4 |
| Statins | 2,052 | 12.5 | 17,717 | 10.8 |
| Thiazides | 220 | 1.3 | 1,764 | 1.1 |

*COPD* chronic obstructive pulmonary disease, *IBD* inflammatory bowel disease

[a] All cancers except liver, bile duct, and pancreatic cancer, which were excluded from the study

[b] Fibrates, niacins, and resins

adjusted OR of 1.35 (95 % CI 1.28–1.42) compared with never/rare users (Table 2). In the main analysis, the adjusted ORs for current users increased with duration of use, although the CI of the specific estimates overlapped. There was no relationship between increasing number of prescriptions (i.e. intensity of treatment) and risk of gallstone disease for either current or former estrogen users (Supplementary Table 4).

Among cases and controls using estrogen therapy, 62.0 % (2,191) and 67.1 % (17,266) used progestin concomitantly. Compared with never/rare users of estrogen, use of opposed estrogen was associated with an adjusted OR of 1.40 (95 % CI 1.33–1.47) for gallstone disease (Table 3). The adjusted OR was 1.65 (95 % CI 1.55–1.75) for use of unopposed estrogen. The associations for both opposed and unopposed estrogen were strongest among current users.

The association between gallstone disease and current/former estrogen use was less pronounced in strata of subjects with a diagnosis of diabetes, obesity, and cardiovascular disease than in strata of subjects without these diseases (Table 4).

Formers users who discontinued treatment ≤24 months before the index date had an adjusted OR for gallstone disease of 1.51 (95 % CI 1.39–1.64) compared with the reference group of never/rare users, while former users who discontinued treatment >24 months prior to the index date had an adjusted OR of 1.28 (95 % CI 1.20–1.36).

The association between gallstone disease and estrogen use was present throughout the study period, and was more

**Table 2** Use of estrogen and risk of gallstone disease, stratified according to duration of treatment

| Estrogen use | Cases with gallstone disease | | Population controls | | Crude OR[a] | 95 % CI | Adjusted OR[b] | 95 % CI |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | | |
| None | 12,853 | 78.4 | 138,131 | 84.3 | 1.00 | Reference | 1.00 | Reference |
| User | 3,533 | 21.6 | 25,729 | 15.7 | 1.52 | 1.46–1.58 | 1.48 | 1.42–1.55 |
| Current use | | | | | | | | |
| Overall | 1,425 | 8.7 | 8,930 | 5.4 | 1.76 | 1.66–1.87 | 1.74 | 1.64–1.85 |
| <1 year | 85 | 0.5 | 616 | 0.4 | 1.50 | 1.20–1.89 | 1.47 | 1.17–1.85 |
| 1–4 years | 449 | 2.7 | 2,872 | 1.8 | 1.73 | 1.56–1.91 | 1.71 | 1.54–1.90 |
| >4 years | 891 | 5.4 | 5,442 | 3.3 | 1.81 | 1.68–1.95 | 1.78 | 1.65–1.92 |
| Former use | | | | | | | | |
| Overall | 2,108 | 12.9 | 16,999 | 10.3 | 1.38 | 1.31–1.45 | 1.35 | 1.28–1.42 |
| <1 year | 381 | 2.3 | 3,327 | 2.0 | 1.26 | 1.13–1.40 | 1.22 | 1.09–1.36 |
| 1–4 years | 693 | 4.2 | 5,419 | 3.3 | 1.41 | 1.30–1.53 | 1.38 | 1.28–1.50 |
| >4 years | 1,034 | 6.3 | 8,053 | 4.9 | 1.42 | 1.32–1.52 | 1.38 | 1.29–1.48 |

Northern Denmark, 1996–2010

*CI* confidence interval, *OR* odds ratio

[a] Matched by age, sex and county

[b] Adjusted for the previous hospital discharge diagnoses and treatments mentioned in Table 1, parity and period of diagnosis. Matched by age, sex, and county

**Table 3** Use of estrogen and risk of gallstone disease, stratified according to concomitant use of progestin

| Estrogen use | Cases with gallstone disease | | Population controls | | Crude OR[a] | 95 % CI | Adjusted OR[b] | 95 % CI |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | | |
| None | 12,853 | 78.4 | 138,131 | 84.3 | 1.00 | Reference | 1.00 | Reference |
| Estrogen only | | | | | | | | |
| Overall | 1,342 | 8.2 | 8,463 | 5.2 | 1.73 | 1.63–1.84 | 1.65 | 1.55–1.75 |
| Current | 565 | 3.4 | 3,071 | 1.9 | 2.01 | 1.83–2.20 | 1.92 | 1.75–2.10 |
| Former | 777 | 4.7 | 5,392 | 3.3 | 1.57 | 1.45–1.70 | 1.49 | 1.38–1.61 |
| Estrogen and progestin | | | | | | | | |
| Overall | 2,191 | 13.4 | 17,266 | 10.5 | 1.40 | 1.33–1.47 | 1.40 | 1.33–1.47 |
| Current | 777 | 4.7 | 5,270 | 3.2 | 1.63 | 1.51–1.77 | 1.66 | 1.53–1.80 |
| Former | 1.414 | 8.6 | 11.996 | 7.3 | 1.30 | 1.22–1.38 | 1.28 | 1.21–1.36 |

Northern Denmark, 1996–2010

*CI* confidence interval, *OR* odds ratio

[a] Matched by age, sex and county

[b] Adjusted for the previous hospital discharge diagnoses and treatments mentioned in Table 1, parity, and period of diagnosis. Matched by age, sex, and county

pronounced among cases/controls with an index date ≤2002 than in subjects with a later index date (Supplementary Table 5).

Our results were not affected when the cut-off date for current and former users was changed from 90 to 30 or 180 days (Supplementary Table 6). Nor were they changed when cases/controls with only one redeemed prescription were excluded from the analysis (Supplementary Table 7). Furthermore, when the analysis was restricted to cases ($n = 15{,}566$) and controls ($n = 150{,}100$) with a minimum of 180 days of non-use prior to therapy initiation, the adjusted ORs for current and former estrogen users were 1.65 (95 % CI 1.54–1.78) and 1.31 (95 % CI 1.23–1.39), respectively (Table 5). The associations did not change with increasing duration of treatment. Finally, among cases with gallbladder surgery ($n = 377$) and their controls ($n = 3{,}770$), current estrogen use was associated with an adjusted OR of 1.10 (95 % CI 0.67–1.80), while the adjusted OR was 0.99 (95 % CI 0.70–1.41) for former users.

**Table 4** Use of estrogen and risk of gallstone disease in the presence or absence of diabetes, cardiovascular disease, and obesity in Northern Denmark, 1996–2010

| | Crude OR[a] | 95 % CI | Adjusted OR[b] | 95 % CI |
|---|---|---|---|---|
| Previous hospital diagnosis of diabetes | | | | |
| Current user | 1.41 | 1.05–1.89 | 1.37 | 1.02–1.84 |
| Former user | 1.21 | 0.99–1.48 | 1.19 | 0.97–1.46 |
| No previous hospital diagnosis of diabetes | | | | |
| Current user | 1.76 | 1.65–1.87 | 1.73 | 1.62–1.83 |
| Former user | 1.37 | 1.30–1.44 | 1.34 | 1.27–1.41 |
| Previous hospital diagnosis of cardiovascular disease | | | | |
| Current user | 1.63 | 1.42–1.87 | 1.65 | 1.44–1.90 |
| Former user | 1.14 | 1.02–1.27 | 1.15 | 1.03–1.28 |
| No previous hospital diagnosis of cardiovascular disease | | | | |
| Current user | 1.73 | 1.62–1.85 | 1.71 | 1.60–1.83 |
| Former user | 1.40 | 1.32–1.48 | 1.38 | 1.30–1.45 |
| Previous hospital diagnosis of obesity | | | | |
| Current user | 1.46 | 1.05–2.01 | 1.43 | 1.03–1.99 |
| Former user | 0.99 | 0.78–1.26 | 0.99 | 0.77–1.26 |
| No previous hospital diagnosis of obesity | | | | |
| Current user | 1.76 | 1.65–1.87 | 1.72 | 1.62–1.83 |
| Former user | 1.38 | 1.31–1.45 | 1.35 | 1.28–1.42 |

*CI* confidence interval, *OR* odds ratio

[a] Matched by age, sex, and county

[b] Adjusted for the previous hospital discharge diagnoses and treatments mentioned in Table 1, parity, and period of diagnosis. Matched by age, sex, and county

## 4 Discussion

This large population-based case–control study showed that current and former users of postmenopausal estrogen therapy were at increased risk of gallstone disease compared with never/rare users. ORs were increased as early as during the first year of treatment and remained increased for more than 2 years after discontinuation of treatment, indicating a rapid onset of risk and a long-standing effect. Current use of estrogen and use of unopposed estrogen had the strongest association with gallstone risk. In patients with known risk factors for gallstone disease, such as diabetes or obesity, the association between estrogen and gallstone disease was less pronounced than in patients without such risk factors, probably reflecting that the addition of estrogen had limited influence in patients with an a priori high risk of gallstone disease.

In the setting of a large general population and using a wide definition of gallstone disease, our main findings support the overall association between estrogen therapy and risk of gallstone disease found in previous studies [3, 11–14, 31]. Other studies have suggested a lower risk of estrogen-associated gallstone disease when estrogen was opposed by progestin [11, 14]. To our knowledge, we are the first to report an overall lower risk. Cirillo et al. [11] reported data from the Women's Health Initiative postmenopausal hormone trial (WHI), in which gallbladder diseases and gallbladder-related procedures were included as secondary outcomes among 22,579 women [32]. Hazard ratios (HRs) comparing treatment and placebo indicated increased gallstone risk among women using estrogen (HR 1.67, 95 % CI 1.35–2.06) and estrogen plus progestin (HR 1.59, 95 % CI 1.28–1.97). However, women in the estrogen/placebo group (hysterectomized women) had a higher prevalence of gallstone risk factors (comorbidity, previous hormone replacement therapy [HRT] use and high body mass index [BMI]) than women in the estrogen plus progestin/placebo group (non-hysterectomized women), which could explain the different gallstone risks.

In a questionnaire-based cohort study Hart et al. [12] found an increased risk of symptomatic gallstone disease among women aged 45–74 years who were ever users of HRT (relative risk [RR] 1.94, 95 % CI 1.17–3.22). The findings were supported by Grodstein et al. [14], who explored the association among postmenopausal women included in The Nurses' Health Study. He reported a RR of 2.2 (95 % CI 2.0–2.6) for cholecystectomy in current users of unopposed estrogen, while the RR for current users of estrogen combined with progestin was 1.8 (95 % CI 1.4–2.4). However, the analyses were not adjusted for hysterectomy, and the number of women using combined therapy in the study was relatively low.

As we adjusted our analyses for previous hysterectomy, our finding of a protective effect of progestin on estrogen-associated gallstone disease cannot be explained by confounding due to differences between hysterectomized and non-hysterectomized women. However, no previous clinical or biological studies have confirmed this. Thus, the nature of the protective effect of progestin is yet to be clarified.

Our finding of a prompt effect of estrogen on risk of gallstone disease is similar to that of other studies [10, 11, 13, 14, 33]. This effect is most likely explained by a rapid increase in the biliary concentration of lipids and change in the composition of bile [5]. However, the increased risk early in the treatment course could also be due to detection bias. The study by Grodstein et al. [14] indicated an increased risk of gallstone disease with increasing length of estrogen therapy. Overall, our results do not confirm such duration response, which is in accordance with the findings by Gonzalez-Perez and Garcia Rodriguez [13], who, in a nested case–control study, found no difference in the risk of gallstone-disease among current users who had received $<1$ or $\geq 1$ year of estrogen therapy, respectively.

### 4.1 Strengths and Limitations

The strengths of our study include its population-based design within a universal healthcare system with complete

**Table 5** Use of estrogen and the risk of gallstone disease, stratified according to duration of treatment

| Estrogen use | Cases with gallstone disease | | Population Controls | | Crude OR[a] | 95 % CI | Adjusted OR[b] | 95 % CI |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | | |
| None | 13,273 | 85.3 | 134,140 | 89.4 | 1.00 | Reference | 1.00 | Reference |
| User | 2,293 | 14.7 | 15,960 | 10.6 | 1.46 | 1.39–1.54 | 1.43 | 1.36–1.50 |
| Current use | | | | | | | | |
| Overall | 942 | 6.1 | 5,711 | 3.8 | 1.68 | 1.56–1.81 | 1.65 | 1.54–1.78 |
| <1 year | 236 | 1.5 | 1,405 | 0.9 | 1.71 | 1.49–1.97 | 1.68 | 1.46–1.93 |
| 1–4 years | 398 | 2.6 | 2,468 | 1.6 | 1.65 | 1.48–1.84 | 1.63 | 1.46–1.81 |
| >4 years | 308 | 2.0 | 1,838 | 1.2 | 1.70 | 1.50–1.92 | 1.67 | 1.48–1.89 |
| Former use | | | | | | | | |
| Overall | 1,351 | 8.7 | 10,249 | 6.8 | 1.34 | 1.26–1.42 | 1.31 | 1.23–1.39 |
| <1 year | 593 | 3.8 | 4,446 | 3.0 | 1.36 | 1.24–1.48 | 1.32 | 1.21–1.44 |
| 1–4 years | 510 | 3.3 | 3,850 | 2.6 | 1.35 | 1.23–1.49 | 1.33 | 1.21–1.46 |
| >4 years | 248 | 1.6 | 1,953 | 1.3 | 1.28 | 1.12–1.47 | 1.24 | 1.08–1.42 |

The analysis was restricted to cases and controls with a minimum of 180 days of nonuse prior to the time of therapy initiation. Northern Denmark, 1996–2010

*CI* confidence interval, *OR* odds ratio

[a] Matched by age, sex, and county

[b] Adjusted for the previous hospital discharge diagnoses and treatments mentioned in Table 1, parity, and period of diagnosis. Matched by age, sex, and county

hospital and prescription history and access to appropriate population controls. Our data were collected from well validated databases, thereby diminishing the risk of measurement errors [34, 35]. Finally, our analyses were adjusted for several important risk factors for gallstone disease. Our study also has limitations. We used redeemed prescriptions as a surrogate for actual estrogen consumption, which may be inaccurate. However, Løkkegaard et al. [37] found the positive predictive value of one redeemed prescription to be 74.8 % for estimating actual use. Because we defined estrogen users as women with at least two redeemed prescriptions, the exposure misclassification is likely to be minor.

The categorization of current and former users and duration of treatment also may not be entirely accurate. There might be current users in the group of former users and vice versa. Fortunately, the analysis in which we changed the cut-off date for current and former users supported the results of our main analysis. The potential inaccuracy of measurement of duration of treatment could stem from inclusion of cases and controls who started their current course of treatment before the beginning of the study period, thus introducing prevalent user bias [30]. The analysis restricted to cases and controls with a minimum of 180 days of non-use prior to therapy initiation supported our main findings. However, the results did not support an association between risk of gallstone disease and duration of treatment, which therefore could be a sign of duration misclassification in the main analysis.

Another concern is misclassification of gallstone disease in the DNRP. Although the diagnoses used in our definition of gallstone disease have not been validated in previous studies, the overall quality and accuracy of data in the DNRP has been estimated to be high [35, 36]. Moreover, it seems unlikely that misclassification of gallstone disease would be unequally distributed between estrogen users and non-users, and such misclassification would therefore bias ORs towards 1.0.

Only 20–25 % of patients with gallstones will develop symptoms [2, 3]. Detection of asymptomatic or mild gallstone disease might be more likely among estrogen users than among non-users due to increased clinical surveillance, thus being a potential source of bias. This bias would cause an overestimation of the association between gallstone disease and estrogen. Although the analysis we restricted to cases with gallbladder surgery (which seems less likely to be impacted by detection bias) was limited by imprecise estimates, it might in fact indicate that the association found in our study could be explained by detection bias.

Women receiving postmenopausal HRT might be healthier and have higher socioeconomic status than non-users, which could lead to healthy user bias [16, 38, 39]. Any healthy user bias would cause an underestimation of the gallstone risk and therefore cannot explain our overall findings. However, our finding of a weakening of the association through the study period could indicate healthy user bias in the recent decade.

Another concern is that we were unable to adjust for BMI, which could be a confounder in our study. However, studies have shown that women using estrogen replacement therapy generally have a lower BMI than non-users, and confounding by BMI would therefore conservatively bias our ORs [16, 38, 40, 41]. In addition, our stratified analysis confirmed an association between estrogen therapy and gallstone disease among obese individuals.

## 5 Conclusion

This population-based case–control study showed that short- and long-term use of postmenopausal estrogen opposed or unopposed by progestin is associated with elevated risk of gallstone disease in both current and former estrogen users. The association is strongest in current users and in users of unopposed estrogen. The latter needs to be confirmed in future studies, which should also explore the association between progestin and estrogen-associated gallstone disease. Future research is also needed to clarify the effect—if any—of route of administration on gallstone risk.

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**Conflict of interest** Maja Hellfritzsch Simonsen, Rune Erichsen, Tine Frøslev, Jørgen Rungby, and Henrik Toft Sørensen have no conflict of interests that are directly relevant to the content of this manuscript.

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