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ORIGINAL ARTICLE ***Puberty, ageing and HRT***

Hormone replacement therapy and the risk of melanoma in post-menopausal women

B.M. Hicks[1,*], K.B. Kristensen[2], S.A. Pedersen[3], L.R. Hölmich[4], and A. Pottegård[2]

[1]Centre for Public Health, School of Medicine, Dentistry and Biomedical Science, Queen's University Belfast, Belfast, Northern Ireland [2]Clinical Pharmacology and Pharmacy, Department of Public Health, University of Southern Denmark, Odense, Denmark [3]Department of Clinical Biochemistry and Clinical Pharmacology, Odense University Hospital, Odense, Denmark [4]Department of Plastic Surgery, Herlev and Gentofte Hospital, Herlev, Denmark

*Correspondence address: Centre for Public Health, ICSB, Royal Victoria Hospital, Belfast, N. Ireland, BT12 6BA. Tel: +44-0-28-90-63-50-09; E-mail: B.Hicks@qub.ac.uk

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STUDY QUESTION: Is hormone replacement therapy (HRT) associated with an increased risk of melanoma skin cancer or prognostic outcomes amongst post-menopausal women?

SUMMARY ANSWER: Whilst we found evidence of an association with melanoma risk, the lack of dose-response and associations observed with recent use, localised disease and intravaginal oestrogens suggests this is a non-causal association.

WHAT IS KNOWN ALREADY: Evidence on HRT and melanoma risk remains inconclusive, with studies providing conflicting results. Furthermore, evidence on melanoma survival is sparse, with only one previous study reporting protective associations with HRT use, likely attributable to immortal time bias.

STUDY DESIGN, SIZE, DURATION: We conducted a nation-wide population-based case-control study and a retrospective cohort study utilising the Danish healthcare registries. Case-control analyses included 8279 women aged 45–85 with a first-ever diagnosis of malignant melanoma between 2000 and 2015, matched by age and calendar time to 165 580 population controls. A cohort of 6575 patients with a diagnosis of primary malignant melanoma between 2000 and 2013 and followed through 2015 was examined to determine if HRT use had an impact on melanoma survival outcomes.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Based on prescriptions dispensed since 1995, ever-use of HRT was defined as having filled at least one prescription for HRT prior to the index date. In total, 2629 cases (31.8%) and 47 026 controls (28.4%) used HRT. Conditional logistic regression was used to calculate odds ratios (ORs) for melanoma risk according to HRT use, compared with non-use, adjusting for potential confounders. For cohort analyses, Cox proportional hazards models was used to estimate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for second melanoma incidence and all-cause mortality associated with HRT.

MAIN RESULTS AND THE ROLE OF CHANCE: High use of HRT was associated with an OR of 1.21 (95% CI 1.13–1.29) for melanoma risk, with no evidence of a dose-response pattern. Results were most pronounced amongst recent high users (OR, 1.28; 95% CI 1.17–1.41), for localised disease (OR, 1.25; 95% CI 1.15–1.36) and for intravaginal oestrogen therapy (OR, 1.38; 95% CI 1.13–1.68). Compared with non-use, there was no evidence of an association for secondary melanoma for post-diagnostic new-use (fully adjusted HR, 1.56; 95% CI 0.64–3.80) or continuous HRT use (fully adjusted HR, 1.26; 95% CI 0.89–1.78). Similar associations were observed for all-cause mortality.

LIMITATIONS, REASONS FOR CAUTION: Despite the large sample size and the use of robust population-based registries with almost complete coverage, we lacked information on some important confounders including sun exposure.

WIDER IMPLICATIONS OF THE FINDINGS: Whilst we cannot rule out an association between HRT use and melanoma risk, the associations observed are also compatible with increased healthcare utilisation and thus increased melanoma detection amongst HRT users. No association between HRT use and melanoma survival outcomes was observed. This should provide some reassurance to patients and clinicians, particularly concerning the use of HRT in patients with a history of melanoma.

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Introduction

A number of risk factors for melanoma have been established including exposure to ultraviolet light (Gandini *et al.*, 2005a), fair skin (Gandini *et al.*, 2005b) and immune suppression (Olsen *et al.*, 2014a; Green and Olsen, 2015). Gender disparities in incidence have also been noted. Whilst men have a higher incidence of melanoma overall, women have the highest incidence amongst individuals ~55 years and younger, with a third of cases in women occurring during childbearing age (NORDCAN, 2019). Increases in melanoma risk during pregnancy, with oral contraceptive use and other reproductive factors (including parity, age at menarche and menopause), have been noted (Gandini *et al.*, 2011; Kvaskoff *et al.*, 2011). Furthermore, a number of epidemiological studies have identified sex as a prognostic factor amongst melanoma patients, with survival rates higher amongst females than males (Bay *et al.*, 2015; Enninga *et al.*, 2017; El Sharouni *et al.*, 2019). Therefore, there has been speculation that hormonal factors may influence melanoma incidence and survival. Indeed, preclinical studies suggest that oestrogen may play a role in melanoma carcinogenesis, with oestrogen receptors ERα and ERβ both located on melanocytes (Marzagalli *et al.*, 2016).

However, evidence surrounding hormone replacement therapy (HRT) use and melanoma risk has been inconclusive. Recent studies have reported null associations between HRT use and melanoma risk (Gandini *et al.*, 2011; Tang *et al.*, 2011; Donley *et al.*, 2019). In contrast, a recent study observed increases in melanoma risk, with stronger associations amongst past users (Cervenka *et al.*, 2019). Whilst another study also reported increases in melanoma risk with oestrogen use, reductions in risk were observed in a dose-response analysis for progestogen (Botteri *et al.*, 2017).

Studies investigating HRT and melanoma prognosis have been sparse. The only study to date investigated HRT use and melanoma disease-free survival observing protective associations; however, these results were likely influenced by immortal time bias (Mackie and Bray, 2004). Despite the lack of evidence, it has been noted that oncologists will often advise women with a history of cancer against the use of HRT, even for tumours which are not hormonal dependent (Biglia *et al.*, 2004).

Given the potential role of hormones in melanoma carcinogenesis and the conflicting and limited epidemiological evidence on HRT and melanoma risk and progression, we conducted two nationwide studies using the Danish health registries. We aimed to examine whether HRT was associated with increased risk of melanoma in a nested case-control study and with survival and risk of recurrent melanoma in a cohort of patients diagnosed with melanoma.

Materials and Methods

Data sources

We obtained data from six nationwide registry sources: the Danish Cancer Registry (Gjerstorff, 2011), the Danish Pathology Registry (Bjerregaard and Larsen, 2011), the National Prescription Registry (Wallach Kildemoes *et al.*, 2011), the National Patient Registry (Schmidt *et al.*, 2015a), Registers in Statistics Denmark on educational level (Jensen and Rasmussen, 2011) and the Civil Registration System (Schmidt *et al.*, 2014). A description of these registries is provided in Supplementary Table SI, with codes for diagnoses, drug exposure and covariates in Supplementary Table SII. All linkages were performed by Statistics Denmark.

Virtually, all medical care in Denmark is funded by the Danish National Health Service, allowing true population-based register linkage studies covering all residents of Denmark (Thygesen *et al.*, 2011). Data were linked by a unique personal identification number, assigned to all residents. Linkages were performed by Statistics Denmark.

Investigation of melanoma risk: case control study

Selection of melanoma cancer cases and population controls

From the Danish Cancer Registry, we identified cases as all women with a primary, histologically verified diagnosis of invasive cutaneous melanoma between 1 January 2000 and 31 December 2015. The date of diagnosis corresponded to the index date. We included only patients between the ages of 45–85 years at the index date and excluded patients with any residency outside of Denmark within 10 years prior to the index date. We further excluded those who had a history of primary ovarian failure, radical hysterectomy or bilateral salpingo-oophorectomy/oophorectomy, those patients with a previous history of cancer (excluding non-melanoma skin cancers) and those with xeroderma pigmentosum. Finally, we excluded those with a history of organ transplantation, HIV diagnosis or use of azathioprine, cyclosporine or mycophenolate mofetil, as immunosuppression has been associated with an increased risk of skin cancer (Dahlke *et al.*, 2014; Olsen *et al.*, 2014b; Fattouh *et al.*, 2017).

Controls were selected using risk set sampling. For each case, we selected 20 controls amongst Danish women matched by age and calendar time, applying the same selection criteria as for cases. Controls were assigned the same index date as the case to whom they were matched. Subjects were eligible for sampling as controls before they became cases. Thereby, the calculated ORs provide direct estimates of the incidence rate ratios from a cohort study utilising the source population (Rothman and Lash, 2008).

Exposure definition: systemic HRT

Based on prescriptions dispensed since 1995, ever-use of HRT was defined as having filled at least one prescription for HRT prior to the index date. HRT included all systemic agents available in Denmark during the study period, including oestrogen only, progestogen only and oestrogen and progestogen combination therapies. Hormonal intrauterine devices were not included. Intravaginal oestrogens were also not included in our HRT exposure definition as the primary indications for intravaginal oestrogens are local complaints including vaginal atrophy. Furthermore, doses administered with intravaginal therapy are markedly lower than systemic oestrogen and have minimal systemic absorption, with previous studies finding use of low-dose intravaginal oestrogens does not result in sustained serum oestrogen levels exceeding the normal menopausal range (Rigg *et al.*, 1978; Simunić *et al.*, 2003; Santen, 2015). However, intravaginal oestrogens were investigated in sensitivity analyses. High levels of HRT use were defined as filled prescriptions equivalent to ≥1000 defined daily doses (DDDs) of HRT corresponding to ∼3 years of cumulative use. This corresponded to a cumulative use of, for example, 200 mg of estriol or 5000 mg of norethisterone (World Health Organisation, 2019). For all analyses, prescriptions filled in the year prior to the index period were disregarded. This 1-year lag period was introduced to allow for a minimum latency time window and to minimise reverse causality (Rothman and Lash, 2008). The length of the lag period was varied in sensitivity analyses.

Potential confounders

We defined potential confounders as the following: (i) drugs suggested to have photosensitising properties including oral retinoids, topical retinoids, tetracycline, macrolides, flourquinolones and aminoquinolines, amiodarone, methoxypsoralene and hydrochlorothiazide (Stern *et al.*, 1984; Kaae *et al.*, 2010; Schmidt *et al.*, 2015b); (ii) oral contraceptive use; (iii) drugs suggested to potentially modify the risk of cancer including low-dose aspirin, non-steroidal anti-inflammatory drugs and statins (Jensen *et al.*, 2009; Muranushi *et al.*, 2015, 2016; Lin *et al.*, 2018); (iv) history of comorbidities (defined by diagnosis codes and related medications) including diabetes, chronic obstructive pulmonary disease (COPD), chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke (Henderson *et al.*, 2015; Dąbrowski *et al.*, 2016; Tseng *et al.*, 2016; Groothoff *et al.*, 2018); (v) Modified Charlson Comorbidity Index (CCI) score (0 low; 1–2 medium; ≥3 high) based on the prevalence of 19 chronic conditions (Charlson *et al.*, 1987) and (vi) highest achieved education (basic, medium, higher or unknown). Exposure to the drugs outlined above was defined as two or more filled prescriptions prior to the index date and hospital histories of comorbidities were defined as a primary or secondary discharge or outpatient diagnosis. For all covariates, information within 1 year prior to the index date was disregarded.

Statistical analyses

Conditional logistic regression was used to calculate ORs and 95% confidence intervals (CIs) for malignant melanoma associated with the use of HRT compared with never-use, adjusting for all potential confounders outlined above. We also performed secondary analyses to examine a potential dose-response association, stratifying cumulative HRT use by predefined categories (1–99 DDDs, 100–499 DDDs, 500–999 DDDs, 1000–2000 DDDs and >2000 DDDs). Analyses were carried out by HRT type (including oestrogen, progestogen and oestrogen/progestogen combinations, not restricted to exclusive use) and by route of HRT admission including oral HRT (oestrogen, progestogen and oestrogen/progestogen) and transdermal (oestrogen and oestrogen/progestogen). Analyses were also conducted to investigate associations with recent high use, defined as a cumulative use of ≥1000 DDDs (including the one-year lag-time) amongst users with a filled prescription in the 2 years prior to the index date. In all analyses, never use of HRT served as the reference category.

Subgroup and sensitivity analyses

A number of pre-specified subgroup and sensitivity analyses were also conducted. To examine potential effect measure modification, we stratified the main analyses according to age at index date, melanoma subtype (superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma and other melanomas) and site of melanoma (i.e. skin of head and neck, trunk, upper limb, lower limb and site unspecified) and stage at diagnosis (i.e. localised [stage I or II, depending on thickness of the tumour] or non-localised [stage III or IV]). Tests of effect measure modification was carried out by conducting a likelihood ratio test of a conditional logistic regression model without a interaction term nested in a model with an interaction term corresponding to the patient characteristic defining the subgroup of interest. To get an impression of the magnitude of the influence of individual factors on the overall risk estimate, subgroup analyses were conducted by excluding individuals with certain characteristics that have been reported as potential risk factors for melanoma, i.e. a history of diabetes, chronic renal disease and history of non-melanoma skin cancer. In addition, three sensitivity analyses were conducted. First, as a control exposure, we examined use of intravaginal oestrogens with the reference category of never-use of HRT and intravaginal oestrogens. Second, we applied a new-user design excluding prevalent users of HRT during 1995–1996. Finally, the lag time was varied between 0 and 5 years in 6-month intervals.

Investigation of melanoma prognosis: cohort analysis

Study population

We conducted a nationwide cohort study to investigate the risk of a second primary melanoma associated with the use of HRT amongst women aged 45–85 years and diagnosed with a previous melanoma. From the cases identified previously for case-control analyses, we identified those with incident melanoma between 1 January 2000 and 31 December 2013 (to ensure sufficient follow-up time). Follow-up time began 1 year after melanoma diagnosis and continued until a new melanoma diagnosis, death from any cause or end of the study period (31 December 2015), whichever occurred first. The first year of follow-up was excluded for latency purposes, to minimise detection bias due to increased contact with healthcare professionals and to ensure a true second primary melanoma diagnosis.

Exposure definition

Exposure to HRT was defined into five mutually exclusive groups (Supplementary Fig. S1); new users of HRT were those patients who had filled at least one prescription for HRT in the year post-diagnosis of melanoma but not in the 5 years prior to cohort entry.

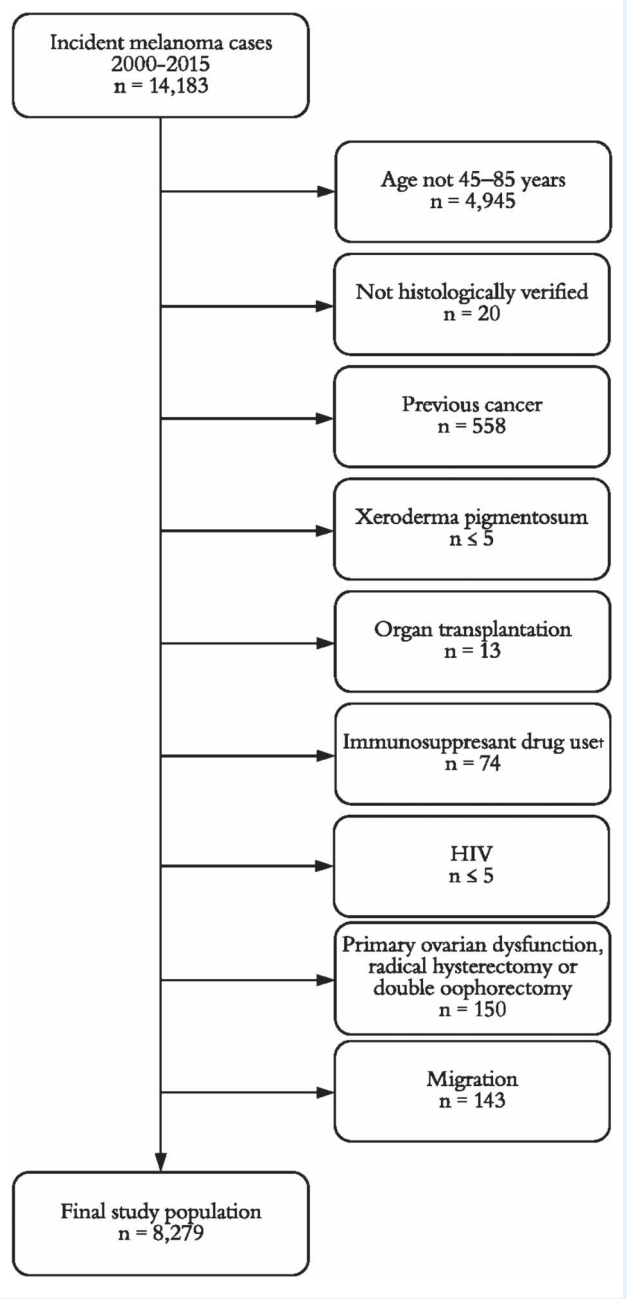

Figure 1 Flow chart of case selection. †Immunosuppressant drug use includes use of azathioprine, cyclosporine or mycophenolate mofetil.

Table 1 Characteristics of melanoma cases and matched population controls.

	Cases (n = 8279) n (%)	Controls (n = 165 580) n (%)
Age, median (IQR, years)	62 (53–71)	62 (53–71)
Use of HRT		
Never use	5650 (68.2)	118 554 (71.6)
Ever use	2629 (31.8)	47 026 (28.4)
High use[a]	1283 (15.5)	22 089 (13.3)
Use of photosensitising drugs[†]		
Topical retinoids	32 (0.4)	402 (0.2)
Oral retinoids	47 (0.6)	893 (0.5)
Tetracycline	198 (2.4)	3696 (2.2)
Macrolides	2176 (26.3)	44 976 (27.2)
Aminoquinolines	579 (7.0)	10 493 (6.3)
Amiodarone	19 (0.2)	413 (0.2)
Methoxypsoralen	(n < 5)	100 (0.1)
Hydrochlorothiazide	921 (11.1)	16 826 (10.2)
Other drug use[b]		
Oral contraceptives	1320 (15.9)	24 616 (14.9)
Aspirin	1126 (13.6)	23 097 (13.9)
Non-aspirin NSAID	4490 (54.2)	90 956 (54.9)
Statins	1256 (15.2)	26 423 (16.0)
Diagnoses		
Alcohol-related disease	132 (1.6)	4578 (2.8)
Diabetes	431 (5.2)	9952 (6.0)
COPD	276 (3.3)	7738 (4.7)
Inflammatory bowel disease	74 (0.9)	1488 (0.9)
Chronic kidney failure	63 (0.8)	1117 (0.7)
Psoriasis	212 (2.6)	4258 (2.6)
Sarcoidosis	16 (0.2)	328 (0.2)
Stroke	163 (2.0)	3428 (2.1)
CCI		
None (CCI score = 0)	6367 (76.9)	125 787 (76.0)
Low (CCI score = 1)	1055 (12.7)	24 376 (14.7)
Medium (CCI score = 2)	556 (6.7)	8976 (5.4)
High (CCI score ≥ 3)	301 (3.6)	6441 (3.9)
Highest achieved education		
Short (7–10 years)	2681 (32.4)	62 737 (37.9)
Medium (11–12 years)	3091 (37.3)	55 965 (33.8)
Long (≥13 years)	2261 (27.3)	37 568 (22.7)
Missing or unknown	246 (3.0)	9310 (5.6)

CCI = Charlson Comorbidity Index; COPD = chronic obstructive pulmonary disease; DDD = defined daily dose; HRT = hormone replacement therapy; IQR = interquartile range; NSAID = non-steroidal anti-inflammatory drug.
[a] High PPI HRT use considered filled prescriptions equivalent to ≥1000 DDDs.
[b] Having filled ≥2 prescriptions more than 2 years prior to the index date.

Continuous users were defined as those who received at least one prescription for HRT in the 2 years prior to cohort entry and in the year post cohort entry; past users were those who filled at least one prescription for HRT between 5 and 2 years pre-diagnosis but not since then, and pre-diagnostic users were defined as those patients who received at least one prescription for HRT in the 2 years prior to cohort entry but not in the year post cohort entry. Non-users were those patients who did not use HRT (excluding intravaginal oestrogens) in the 5 years prior to diagnosis and to 1 year after diagnosis and were considered the reference category for all analyses. Secondary analyses investigated HRT type and route of HRT admission. For analyses investigating associations with intravaginal oestrogen, the reference category was non-use of all HRT, including intravaginal oestrogen.

Table II Association between exposure to HRT and risk of melanoma.

Subgroups	Cases	Controls	Adjusted OR (95% CI)[c]	Adjusted OR (95% CI)[d]
Non-use	5650	118 554	1.00	1.00
Ever use	2629	47 026	1.18 (1.13–1.24)	1.18 (1.12–1.24)
High use[a]	1283	22 089	1.23 (1.15–1.32)	1.21 (1.13–1.29)
Cumulative DDDs				
1–99	557	10 690	1.08 (0.99–1.19)	1.09 (1.00–1.20)
100–499	509	9252	1.16 (1.06–1.28)	1.18 (1.07–1.30)
500–999	280	4995	1.18 (1.04–1.34)	1.18 (1.04–1.34)
1000–2000	453	7944	1.19 (1.07–1.32)	1.17 (1.06–1.30)
>2000	830	14 145	1.25 (1.16–1.36)	1.23 (1.13–1.33)
Test for trend			$P = 0.47$	$P = 0.59$
Recenct use?[b]				
Recent high use	618	10 011	1.31 (1.20–1.44)	1.28 (1.17–1.41)
Distant high use	665	12 078	1.16 (1.07–1.27)	1.14 (1.04–1.25)
Route of admission[a]				
Oral	1130	19 860	1.20 (1.12–1.28)	1.18 (1.10–1.26)
Transdermal	170	2404	1.44 (1.23–1.69)	1.37 (1.17–1.61)

CI = confidence interval; OR = odds ratio.
[a]HRT modelled as high use, corresponding to ≥1000 DDDs.
[b]Recent high use defined as a cumulative dose of ≥1000 DDDs amongst users with a filled prescription in the 2 years prior to index date.
[c]Adjusted for age and calendar time (by risk-set matching and the conditional analysis).
[d]Adjusted for drugs suggested to have photosensitizing properties, oral contraceptive use, low-dose aspirin, NSAIDs, statins, diabetes, COPD, chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke, modified Charlson Comorbidity Index (CCI) score (0 low; 1–2 medium; ≥3 high) and highest achieved education (basic, medium, higher or unknown).

Statistical analyses

Cox proportional hazard models, using time from diagnosis as the time scale, were used to estimate hazard ratios (HRs) and 95% CIs of second primary melanoma associated with the use of HRT compared with non-use. Models were adjusted for the confounders listed previously with the addition of melanoma stage (TNM Classification of Malignant Tumors). The proportional hazards assumption was assessed using Schoenfeld residuals. Analyses also investigated the association between HRT and the secondary outcome of all-cause mortality. Sensitivity analyses were conducted restricting the follow-up period to 5 years.

All analyses were performed using Stata release 15.1. According to Danish law, ethical approval is not required for registry-based studies.

Results

Investigation of melanoma risk: case control study results

We identified 14 183 cases of melanoma between 1 January 2000 and 31 December 2015. Following exclusions, 8279 cases were matched to 165 580 cancer-free controls (Fig. 1). Compared with controls, cases had a lower prevalence of alcohol-related disorders and COPD, were less likely to have a low comorbidity score and had longer durations of education. Other characteristics were similar between cases and controls (Table I).

Results for HRT use associated with secondary melanoma diagnosis are presented in Table V. Compared with non-use of HRT, the use of HRT was not associated with an increased risk of secondary melanoma for post-diagnostic new-users (fully adjusted HR, 1.56; 95% CI 0.64–3.80) or continuous HRT users (fully adjusted HR, 1.26; 95% CI 0.89–1.78). Similarly, pre-diagnostic HRT use and past use of HRT were not associated with secondary melanoma risk. Analyses by HRT type revealed null associations for oestrogen and combination therapy. New-use of progestogen post-diagnosis and continuous progestogen use were associated with increases in risk of secondary melanoma; however, these were based on a small number of events ($n \leq 6$).

Overall, 31.8% of cases and 28.4% of controls filled a prescription for HRT (Table II) yielding an adjusted OR of 1.18 (95% CI 1.12–1.24). A greater proportion of cases exhibited high use of HRT (≥1000 DDDs) than controls (15.5% vs. 13.3%) which corresponded to an adjusted OR of 1.21 (95% CI 1.13–1.29). ORs remained elevated across all cumulative DDD categories with no evidence of a dose-response (P for trend = 0.59). In recent high users, associations were more marked than in distant high users (OR, 1.28; 95% CI 1.17–1.41; OR, 1.14; 95% CI 1.04–1.25, respectively). Both oral and transdermal HRT were associated with increases in melanoma risk, which was more marked with transdermal HRT use (OR, 1.37; 95% CI 1.17–1.61).

Analyses by HRT type and melanoma risk are presented in Supplementary Table SIII. Overall, positive associations were observed with all HRT types. Similar to the primary analyses, for each HRT type, there was no evidence of dose-response relationships, and associations were more pronounced with recent high use. Results were

Table III Associations between high use of HRT (≥1000 DDDs) and the risk of melanoma by patient subgroups.

Subgroup[a]	Cases exposed/unexposed	Controls exposed/unexposed	Unadjusted OR (95% CI)[b]	*P*-value[d]	Adjusted OR (95% CI)[c]	*P*-value[d]
Age, years				0.24		0.45
<50	16/1003	308/20301	1.00 (0.60–1.66)		1.01 (0.61–1.69)	
50–60	286/1679	5043/35085	1.17 (1.02–1.34)		1.19 (1.04–1.37)	
60–75	769/1941	13 533/41365	1.22 (1.11–1.33)		1.19 (1.09–1.30)	
>75	212/1027	3205/21803	1.41 (1.21–1.65)		1.34 (1.15–1.58)	
Melanoma subtype				0.42		0.37
Superficial spreading	839/3708	14 381/78630	1.26 (1.16–1.37)		1.22 (1.13–1.33)	
Nodular	112/502	1947/9794	1.11 (0.89–1.38)		1.13 (0.91–1.42)	
Lentigo	49/166	811/3549	1.34 (0.95–1.89)		1.33 (0.93–1.90)	
Acral lentiginous	8/47	230/983	0.68 (0.31–1.50)		0.72 (0.31–1.64)	
Other	275/1227	4720/25598	1.22 (1.06–1.41)		1.20 (1.04–1.38)	
Melanoma localisation				0.09		0.09
Skin of head and neck	137/508	2195/10906	1.35 (1.11–1.66)		1.33 (1.08–1.64)	
Skin of trunk	382/1708	6347/35868	1.29 (1.14–1.45)		1.25 (1.11–1.42)	
Skin of upper limb	222/1066	4397/21870	1.04 (0.89–1.22)		0.99 (0.85–1.17)	
Skin of lower limb	438/1901	7193/40305	1.31 (1.17–1.46)		1.30 (1.16–1.46)	
Unspecified part of skin	104/467	1957/9605	1.10 (0.88–1.38)		1.08 (0.86–1.36)	
Melanoma stage[e]				<0.001		<0.001
Localised	912/3793	15 158/80311	1.29 (1.20–1.40)		1.25 (1.15–1.36)	
Non-localised	73/454	1765/8757	0.78 (0.60–1.01)		0.82 (0.63–1.08)	
Unknown	298/1403	5166/29486	1.23 (1.07–1.40)		1.22 (1.07–1.40)	
Other subgroups						
No diabetes	1220/5346	20 867/111344	1.23 (1.15–1.31)		1.21 (1.13–1.30)	
No skin cancer	1125/5219	20 871/115039	1.21 (1.13–1.30)		1.20 (1.11–1.28)	
No chronic renal failure	1272/5613	21 926/117757	1.23 (1.16–1.32)		1.21 (1.13–1.29)	

[a]High use (≥1000 DDDs).
[b]Adjusted for age and calendar time (by risk-set matching and the conditional analysis).
[c]Adjusted for drugs suggested to have photosensitizing properties, oral contraceptive use, low-dose aspirin, NSAIDs, statins, diabetes, COPD, chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke, CCI score (0 low; 1–2 medium; ≥3 high) and highest achieved education (basic, medium, higher or unknown).
[d]Derived from a likelihood ratio test of the conditional logistic regression model without interaction terms nested in a conditional logistic regression model with interaction terms.
[e]Localised disease includes stage I or II melanoma (depending on thickness of the tumour), non-localised disease includes cases with dissemination (either regionally stage III, or widespread stage IV).

similar amongst patients with exclusive use of oestrogen and oestrogen/progestogen combination therapy (Supplementary Table SIV). Likewise, associations remained largely similar across melanoma subtypes (Supplementary Table SV).

Sub-group analyses are presented in Table SIII. Overall sub-group analyses revealed similar results. However, null associations were observed for women <50 years, for nodular and acral lentiginous melanoma and for melanoma of unspecified location and of the upper limb. Additionally analysis by stage revealed associations only with localised melanoma (OR, 1.25, 95% CI 1.15 –1.36). Tests for effect measure modification showed that clinical stage modified the association ($P < 0.001$), whilst there was less evidence for effect modification by localisation ($P = 0.09$), age ($P = 0.45$) or melanoma type ($P = 0.37$). Additional analyses investigated the risk of melanoma associated with intravaginal oestrogen use, corresponding to an OR of 1.38 (95% CI 1.13–1.68) for high use (≥1000 DDDs) (Supplementary Table SVI). There was some evidence of a dose-response relationship, and associations were more marked for recent high users. In sensitivity analyses utilising a new user design (Supplementary Table SVII), estimates were attenuated, including for high HRT use (OR, 1.13; 95% CI 0.99–1.28). Similarly, null associations were observed for recent use. Finally, analyses of increasing lag periods (Supplementary Table SVIII) revealed results similar to the primary analyses.

Investigation of melanoma prognosis: cohort study results

From 8279 melanoma cases, 6575 patients with melanoma were included after excluding 1445 patients diagnosed after 2013 and 259 patients with <1-year follow-up. Patients in the cohort were followed

Table IV Characteristics of patients diagnosed with Melanoma by HRT use.

Characteristics	Non- Users (*n*= 5372), *n* (%)	Past HRT use (*n* = 381), *n* (%)	Pre-diagnostic HRT use (*n* = 257), *n* (%)	New users (*n* = 57), *n* (%)	Continuous HRT use (*n* = 508), *n* (%)
Age, median (IQR)	62 (53–71)	59 (53–66)	60 (54–66)	52 (49–56)	62 (56–69)
Use of photosensitising drugs					
Topical retinoids	9 (0.2)	—	(*n* < 5)	—	(*n* < 5)
Oral retinoids	24 (0.4)	(*n* < 5)	(*n* < 5)	—	(*n* < 5)
Tetracycline	103 (1.9)	9 (2.4)	5 (1.9)	(*n* < 5)	11 (2.2)
Macrolides	1280 (23.8)	107 (28.1)	62 (24.1)	13 (22.8)	138 (27.2)
Aminoquinolines	319 (5.9)	28 (7.3)	26 (10.1)	(*n* < 5)	39 (7.7)
Amiodarone	17 (0.3)	—	—	—	—
Methoxypsoralen	(*n* < 5)	(*n* < 5)	—	—	—
Hydrochlorothiazide	578 (10.8)	33 (8.7%)	20 (7.8)	(*n* < 5)	54 (10.6)
Other drug use					
Oral contraceptives	761 (14.2)	59 (15.5)	32 (12.5)	21 (36.8)	52 (10.2)
Aspirin	740 (13.8)	59 (15.0)	14 (5.4)	(*n* < 5)	72 (14.2)
Non-aspirin NSAID	2679 (49.9)	231 (60.6)	145 (56.4)	27 (47.4)	306 (60.2)
Statins	775 (14.4)	50 (13.1%)	25 (9.7)	(*n* < 5)	44 (8.7)
Diagnoses					
Alcohol-associated conditions	76 (1.4)	13 (3.0)	(*n* < 5)	(*n* < 5)	8 (1.6)
Diabetes	288 (5.4)	14 (3.7)	10 (3.9)	(*n* < 5)	12 (2.)
COPD	152 (2.8)	14 (3.7)	10 (3.9)	(*n* < 5)	9 (1.8)
Inflammatory bowel disease	42 (0.8)	7 (1.8)	(*n* < 5)	(*n* < 5)	6 (1.2)
Chronic renal failure	37 (0.7)	(*n* < 5)	(*n* < 5)	(*n* < 5)	5 (1.0)
Psoriasis	127 (2.4)	11 (2.9)	(*n* < 5)	(*n* < 5)	7 (1.4)
Sarcoidosis	11 (0.2)	(*n* < 5)	—	—	—
Stroke	101 (1.9)	(n < 5)	(*n* < 5)	—	10 (2.0)
CCI-score					
None (CCI Score = 0)	4194 (78.1)	306 (80.3)	202 (78.6)	51 (89.5)	399 (78.5)
Low (CCI Score = 1)	667 (12.4)	35 (9.2)	31 (12.1)	(*n* < 5)	64 (12.6)
Medium (CCI Score = 2)	319 (5.9)	27 (7.1)	17 (6.6)	(*n* < 5)	33 (6.5)
High (CCI Score ≥ 3)	192 (3.6)	13 (3.4)	7 (2.7)	(*n* < 5)	12 (2.4)
None (CCI Score = 0)					
Education					
Short	1810 (33.7)	115 (30.2)	88 (34.2)	8 (14.0)	176 (34.6)
Medium	1999 (37.2)	147 (38.6)	88 (34.2)	25 (43.9)	179 (35.2)
Long	1401 (26.1)	109 (28.6)	74 (28.8)	23 (40.4)	141 (27.8)
Unknown	162 (3.0)	10 (2.6)	7 (2.7)	(*n* < 5)	12 (2.4)

for a median (interquartile range) of 5.1 (2.6–8.6) years. Table IV presents baseline characteristics by HRT use. Overall, new users of HRT with melanoma were younger, more likely to have a history of oral contraceptive use and had a lower comorbidity score and longer education. Other characteristics remained largely similar between groups.

Results for HRT use associated with secondary melanoma diagnosis are presented in Table V. Compared with non-use of HRT, the use of HRT was not associated with an increased risk of secondary melanoma for post-diagnostic new-users (fully adjusted HR, 1.56; 95% CI 0.64–3.80) or continuous HRT users (fully adjusted HR, 1.26; 95% CI 0.89–1.78). Similarly, pre-diagnostic HRT use and past use of HRT were not associated with secondary melanoma risk. Analyses by HRT type revealed null associations for oestrogen and combination therapy. New-use of progestogen post-diagnosis and continuous progestogen use were associated with increases in risk of secondary melanoma; however, these were based on a small number of events ($n \leq 6$).

Whilst an association was observed for all-cause mortality and post-diagnostic new users (adjusted OR, 0.31; 95% CI 0.10 –0.96), this was based on a small number of events ($n < 5$) (Table VI). Associations by

Table V Crude and adjusted HRs for the association between the use of HRT and secondary melanoma.

Exposure	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR	Adjusted HR (95% CI)[b]	Adjusted HR (95% CI)[c]
Non-users	251	29 929	8.4 (7.4–5)	1.00	1.00	1.00
Past HRT use	29	2570	11.3 (7.8–16.2)	1.40 (0.95–2.06)	1.41 (0.96–2.08)	1.41 (0.96–2.08)
Pre-diagnostic HRT use	11	1789	6.2 (3.4–11.1)	0.77 (0.42–1.42)	0.77 (0.42–1.40)	0.75 (0.41–1.38)
HRT new users	5	428	11.7 (4.9–28.1)	1.49 (0.61–3.62)	1.60 (0.66–3.91)	1.56 (0.64–3.80)
Continuous HRT use	39	3967	9.8 (7.2–13.5)	1.27 (0.91–1.79)	1.26 (0.90–1.78)	1.26 (0.89–1.78)
Oestrogen						
Past HRT use	11	994	11.1 (6.1–20.0)	1.40 (0.76–2.56)	1.46 (0.79–2.68)	1.49 (0.81–2.74)
Pre-diagnostic HRT use	8	741	10.8 (5.4–21.6)	1.37 (0.68–2.76)	1.41 (0.70–2.87)	1.35 (0.66–2.74)
HRT new users	<5	236	4.2 (0.6–30.1)	0.54 (0.08–3.83)	0.57 (0.08–4.05)	0.56 (0.08–4.02)
Continuous HRT use	17	1588	10.7 (6.7–17.2)	1.39 (0.85–2.27)	1.40 (0.85–2.30)	1.40 (0.85–2.31)
Oestrogen and progestogen						
Past HRT use	15	1489	10.1 (6.1–16.7)	1.27 (0.75–2.13)	1.30 (0.77–2.19)	1.28 (0.76–2.17)
Pre-diagnostic HRT use	8	1176	6.8 (3.4–13.6)	0.88 (0.44–1.78)	0.87 (0.43–1.76)	0.86 (0.42–1.74)
HRT new users	<5	263	11.4 (3.7–35.4)	1.50 (0.46–4.69)	1.62 (0.52–5.09)	1.52 (0.48–4.78)
Continuous HRT use	29	2300	8.3 (5.3–13.0)	1.07 (0.67–1.71)	1.10 (0.69–1.76)	1.08 (0.67–1.74)
Progestogen						
Past HRT use	11	930	11.8 (6.6–21.4)	1.52 (0.83–2.79)	1.58 (0.86–2.91)	1.59 (0.86–2.91)
Pre-diagnostic HRT use	6	464	12.9 (5.8–28.8)	1.69 (0.75–3.80)	1.72 (0.76–3.89)	1.75 (0.77–3.95)
HRT new users	<5	114	35.0 (13.1–93.3)	4.17 (1.55–11.21)	4.79 (1.76–13.01)	4.39 (1.61–11.93)
Continuous HRT use	6	218	27.6 (12.4–61.4)	3.68 (1.63–8.28)	4.17 (1.84–9.47)	4.14 (1.82–9.45)

HR = hazard ratio.
[a] Per 1000 person-years.
[b] Adjusted for drugs suggested to have photosensitizing properties, oral contraceptive use, low-dose aspirin, NSAIDs, statins, diabetes, COPD, chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke, CCI score (0 low; 1–2 medium; ≥3 high) and highest achieved education (basic, medium, higher or unknown).
[c] Additionally adjusting for stage.

other HRT user groups and by HRT type revealed null associations. There was no evidence of an association with HRT use categories and secondary melanoma diagnosis or all-cause mortality in analyses by route of admission (Supplementary Tables SIX and SX) and by restricting the follow-up period to a maximum of 5 years (Supplementary Tables SXI and SXII).

Discussion

In this nationwide observational study, we found no evidence of an association between HRT use and melanoma prognostic outcomes. Whilst we observed associations for increased melanoma risk, these did not appear to follow a dose-response pattern. Further, analyses by disease stage revealed that associations were only evident for localised disease. Although we cannot rule out a causal association, taken together, these results appear to suggest that the associations observed may be a result of detection bias, likely from more intensive contact with healthcare professionals amongst HRT users.

Our results for melanoma risk correspond with that observed in a recent meta-analysis, which included a smaller number of cases than our study ($n = 2816$) and reported a relative risk [RR] of 1.16 (95% CI 0.93–1.44) for the association between ever-use of HRT and melanoma (Gandini *et al.*, 2011). In contrast, subsequent studies utilising both the Nurse's Health Initiative trial and the NIH-AARP Diet and Health study reported null associations (Tang *et al.*, 2011; Donley *et al.*, 2019). In a recent French prospective study, ever HRT use was associated with an increase in melanoma risk (HR = 1.35; 95% CI 1.07–1.71) (Cervenka *et al.*, 2019). Contrary to our results, the authors report the highest associations amongst past users (HR, 1.55; 95% CI 1.17–2.07). It is unclear why there is a discrepancy in results. Possible explanations include differing exposure definitions, with past-use defined as no HRT use within 1 year, and based on self-reported use. Furthermore, the authors failed to properly account for latency considerations and included a smaller number of melanoma cases ($n = 444$). There is also a variation in HRT types predominantly used worldwide, which could explain some of the difference in results. In our study, the most commonly used progestogen was medroxyprogesterone, and most common combination was norethisterone/oestrogen which differed from Cervenka *et al.*, (2019). In an additional study, HRT use was defined as current, non-use or past use (defined as >4 months since last prescription) with a short lag-time period (3 months), revealing associations with melanoma for current HRT use (RR 1.23; 95% CI 1.05–1.45) but not past use (RR, 1.00; 95% CI 0.80–1.25) (Botteri *et al.*, 2017). These results are similar to those observed in analyses of recent use in this study. The authors also observed associations for current oestrogen use,

Table VI Crude and adjusted HRs for the association between the use of HRT and all-cause mortality by HRT type.

Exposure	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR	Adjusted HR (95% CI)[b]	Adjusted HR (95% CI)[c]
Non-users	927	30 873	30.0 (28.2–32.0)	1.00	1.00	1.00
Past HRT use	60	2671	22.5 (17.4–28.9)	0.76 (0.58–0.98)	0.75 (0.57–0.97)	0.74 (0.57–0.97)
Pre-diagnostic HRT use	42	1844	22.8 (16.8–30.8)	0.76 (0.56–1.04)	0.83 (0.61–1.14)	0.80 (0.58–1.09)
HRT new-users	<5	452	6.6 (2.1–20.6)	0.22 (0.07–0.69)	0.33 (0.11–1.04)	0.31 (0.10–0.96)
Continuous HRT use	115	4113	28.0 (23.3–33.6)	0.94 (0.77–1.14)	1.00 (0.83–1.22)	0.97 (0.79–1.18)
Oestrogen						
Past HRT use	27	1037	26.1 (17.9–38.0)	0.87 (0.59–1.28)	0.80 (0.55–1.18)	0.86 (0.58–1.26)
Pre-diagnostic HRT use	22	778	28.3 (18.6–43.0)	0.94 (0.62–1.44)	0.98 (0.64–1.51)	0.87 (0.57–1.33)
HRT new users	5	240	20.9 (8.7–50.1)	0.69 (0.29–1.67)	0.92 (0.38–2.21)	0.88 (0.36–2.13)
Continuous HRT use	52	1660	31.3 (23.9–41.1)	1.05 (0.79–1.39)	0.95 (0.72–1.27)	0.93 (0.70–1.23)
Oestrogen and progestogen						
Past HRT use	39	1542	25.3 (18.5–34.6)	0.85 (0.62–1.17)	0.84 (0.61–1.16)	0.82 (0.60–1.14)
Pre-diagnostic HRT use	21	1211	17.3 (11.3–26.6)	0.58 (0.38–0.90)	0.68 (0.44–1.04)	0.66 (0.43–1.02)
HRT new users	<5	277	7.2 (1.8–28.9)	0.24 (0.06–0.97)	0.35 (0.09–1.42)	0.27 (0.07–1.09)
Continuous HRT use	59	2364	25.0 (19.3–32.2)	0.84 (0.64–1.09)	0.96 (0.74–1.26)	0.91 (0.69–1.19)
Progestogen						
Past HRT use	13	964	13.5 (7.8–23.2)	0.45 (0.26–0.78)	0.63 (0.36–1.10)	0.60 (0.35–1.04)
Pre-diagnostic HRT use	8	482	16.6 (8.3–33.2)	0.55 (0.28–1.11)	0.77 (0.38–1.54)	0.79 (0.39–1.59)
HRT new users	<5	131	15.3 (3.8–61.2)	0.51 (0.13–2.05)	1.05 (0.26–4.24)	0.87 (0.22–3.52)
Continuous HRT use	6	250	24.0 (10.8–53.3)	0.80 (0.36–1.79)	0.83 (0.37–1.86)	0.74 (0.33–1.67)

[a]Per 1000 person-years.
[b]Adjusted for drugs suggested to have photosensitizing properties, oral contraceptive use, low-dose aspirin, NSAIDs, statins, diabetes, COPD, chronic renal insufficiency, diseases associated with heavy alcohol consumption, inflammatory bowel disease, psoriasis, sarcoidosis and stroke, CCI score (0 low; 1–2 medium; ≥3 high) and highest achieved education (basic, medium, higher or unknown).
[c]Additionally adjusting for stage.

including for intravaginal oestrogens (RR, 1.45; 95% CI 1.12–1.88) but not combination therapy. Whilst intravaginal oestrogens may increase oestrogen levels in serum and thus may exert systemic effects (Labrie *et al.*, 2009; Wills *et al.*, 2012; Santen, 2015), the absorbed doses are considerably smaller than the doses delivered with systemic oestrogen therapy. Indeed, a number of studies have found that use of low dose vaginal oestrogens does not result in sustained serum oestrogen levels exceeding normal menopausal range (Rigg *et al.*, 1978; Santen, 2015). We observed higher estimates for intravaginal oestrogen use than systemic oestrogen therapy, with evidence of dose response ($P < 0.01$). Additionally, associations were more marked for recent users than distant users of intravaginal oestrogens. Given these findings, its likely associations with intravaginal oestrogens are also subject to detection bias.

To the best of our knowledge, only one study to date had investigated the association between HRT use and melanoma survival. In contrast to our study, Mackie and Bray (2004 reported marked increases in disease-free survival associated with HRT use in melanoma patients (adjusted HR, 0.17; 95% CI 0.05–0.62). However, this was a small study of only 206 melanoma cases, which suffered from a number of methodological short-comings including failure to use a lag period and potential immortal time bias.

A number of studies have demonstrated that HRT users tend to exhibit healthier behaviour or have more favourable socio-economic characteristics than non-users such as higher education, higher socioeconomic status and lower body mass index (BMI) (Jensen and Hilden, 1996; Li *et al.*, 2000; Lambert *et al.*, 2003). We observed higher estimates with recent use and localised disease, suggestive of increased detection of melanoma in HRT users and healthy user bias. Unfortunately, we did not have information on general practice healthcare visits or proxies for health seeking behaviour e.g. cancer screening to investigate this. However, previous studies have demonstrated that HRT users are more likely to have increased healthcare utilisation, including uptake in cancer screening services such as mammography (Li *et al.*, n.d.; MacLennan *et al.*, 1998; Cook *et al.*, 2009). Despite this, it is not possible to rule out a potential carcinogenic or tumour promoting effect of HRT on melanoma.

Whilst the association between oestrogen and melanoma is biologically plausible, the mechanisms through which sex hormones may exert their effects on melanoma remain unclear. Pre-clinical studies have suggested that oestrogens may be associated with proliferative action, whilst progesterones may exert anti-proliferation and anti-apoptosis effects (Wiedemann *et al.*, 2009; De Giorgi *et al.*, 2011). This is in contrast with the results of our study, which found elevated HRs for both oestrogen and progesterones and melanoma risk. The oestrogen receptors ERα and ERβ have both been located on melanocytes; however, pre-clinical evidence suggests that that these receptors may have

opposing effects in melanoma with ERα associated with proliferative action and ERβ with anticancer effects (Marzagalli *et al.*, 2016). The most commonly used oestrogens within our cohort were estradiol and estriol, both of which bind to both receptors with a similar binding affinity and transactivational activity (Marzagalli *et al.*, 2016). Thus, it is unclear if we would expect different formulations of oestrogen to differentially affect melanoma, rather this would likely depend on the ERα/ERβ ratio and the specific cell context.

Strengths and limitations

This study has several strengths, including a large sample size and the use of robust population-based registries with almost complete population coverage. Use of the Danish National Prescription Registry ensured the complete and high-quality assessment of prescription drug use up to a maximum of 20 years of drug exposure history (Pottegård *et al.*, 2016). Melanoma diagnoses were identified via the Danish Cancer Registry and the Danish Pathology registry and were histologically verified, thus increasing validity.

Despite these strengths, this study also had a number of limitations. Firstly, this study lacked information on a number of important risk factors for melanoma including ethnicity, skin phenotype and UV exposure. However, the majority of the Danish population are of white origin. Additionally, in a previous study conducted in Denmark, sun exposure was found to be similar between HRT users and non-users, although HRT users were more likely to use solariums (Jensen and Hilden, 1996). Whilst this study also lacked information on BMI and smoking status, we adjusted for COPD as a crude proxy for smoking. However, as smoking and obesity have been found to exert differing effects of melanoma risk, it is difficult to predict the direction in which these factors might bias estimates (Song *et al.*, 2012; Sergentanis *et al.*, 2013). We also lacked information on menopausal status and age and menopause, as well as other hormonal factors such as age at menarche and parity.

Conclusion

In this population-based study, we identified a slightly increased risk of melanoma associated with HRT use. Whilst we cannot rule out an aetiological effect of HRT on melanoma incidence, the results are compatible with increased healthcare utilisation and thus increased melanoma detection amongst HRT users.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

All authors conceived and designed the study, analysed and interpreted the data and critically revised the article for important intellectual content. A.P. acquired the data. K.B.K. analysed the data. B.M.H. wrote the article, and all authors participated in the interpretation of the results and critical revision of the article.

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Conflicts of interest

A.P. reports participation in research projects funded by Alcon, Almirall, Astellas, Astra-Zeneca, Boehringer-Ingelheim, Servier, Novo Nordisk and LEO Pharma, all with funds paid to the institution where he was employed (no personal fees) and with no relation to the work reported in this article. The other authors have no competing interests to declare.

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