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Hormone replacement therapy and risk of severe asthma exacerbation in peri-menopausal and post-menopausal women: 17-year national cohort study

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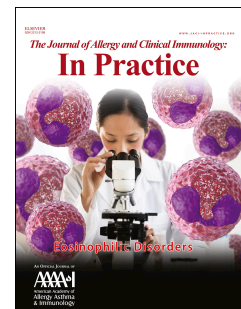
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Hormone replacement therapy and risk of severe asthma exacerbation in peri-menopausal and post-menopausal women: 17-year national cohort study

Running head: HRT and asthma exacerbation in women

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50

51 **CONFLICT OF INTERESTS**

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ABSTRACT

Background

The impact of hormone replacement therapy (HRT) on clinical outcomes in menopausal women is uncertain.

Objective

To investigate the association between use of HRT and severe asthma exacerbation in peri-menopausal and post-menopausal women with asthma.

Methods

We used the Optimum Patient Care Research Database, a population-based longitudinal primary care database in the UK, to construct a 17-year (1/1/2000–12/31/2016) cohort of peri-menopausal and post-menopausal (46-70 years, $N=31,656$) women. We defined use, subtypes, and duration of HRT use. Severe asthma exacerbation was defined as an asthma-related hospitalization, emergency department visits due to asthma and/or prescription of oral corticosteroids. Analyses were undertaken using multilevel mixed-effects Poisson regression.

Results

At baseline, 22% of women were using any HRT, 11% combined HRT, and 11% estrogen-only HRT. Previous, but not current, use of any (incidence rate ratio [IRR] 1.24, 95%CI 1.22-1.26), combined (IRR 1.28, 95%CI 1.25-1.31), estrogen-only (IRR 1.18, 95%CI 1.14-1.21), and longer duration (1-2 years: IRR 1.16; 95%CI 1.13-1.19; 3-4 years: IRR 1.43; 95%CI 1.38-1.48; 5+ years: IRR 1.32; 95%CI 1.28-1.36) of HRT use were associated with increased risk of severe asthma exacerbation compared to non-use. The risk estimates were greater among lean women ($BMI < 25 \text{ kg/m}^2$) than among heavier women ($BMI 25-29.9 \text{ kg/m}^2$ and $\geq 30 \text{ kg/m}^2$) and higher among smokers than non-smokers.

Conclusion

Use of HRT and subtypes, particularly previous, but not current, use and use for more than two years, is associated with an increased risk of severe asthma exacerbation in peri-menopausal/post-menopausal women with established asthma. Lean women and smokers are at greater risk than heavier women and non-smokers, respectively.

Protocol registration: European Union electronic Register of Post-Authorisation Studies (EUPAS22967)

Keywords

asthma exacerbation, combined hormone therapy, estrogen-only therapy, hormone replacement therapy, menopause, women, cohort study

LIST OF ABBREVIATIONS

Hormonal replacement therapy (HRT)

Body mass index (BMI)

Anonymized Data Ethics and Protocol Transparency Committee (ADEPT)

Optimum Patient Care Research Database (OPCRD)

Directed acyclic graph (DAG)

Bilateral salpingo-oophorectomy [BSO]

Index of Multiple Deprivation (IMD)

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

Reporting of studies Conducted using Observational Routinely-collected Data (RECORD)

General practitioner (GP)

Quality and Outcomes Framework (QOF)

Estrogen receptors (ERs)

Optimum Patient Care (OPC)

Observational and Pragmatic Research Institute Pte Ltd (OPRI)

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HIGHLIGHT BOX

- *What is already known about the topic?*

Evidence is lacking on the impact of use of HRT on clinical outcomes of asthma in peri-menopausal and post-menopausal women with asthma

- *What does this article add to our knowledge?*

Use of HRT and in the long-term may increase risk of severe asthma exacerbation in peri-menopausal and post-menopausal women.

- *How does this study impact current management guidelines?*

Although current findings do not suggest changes in management of asthma in peri-menopausal and post-menopausal women, studies uncovering the mechanisms through which HRT impact on clinical outcomes of asthma are required.

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174

175 INTRODUCTION

176 Hormone replacement therapy (HRT), an umbrella term used for both estrogen-only and combined estrogen-
177 progestogen hormone treatments, has long been used to mitigate the symptoms caused by menopause, a
178 period of decline in the production of endogenous estrogen and progesterone hormones in peri-menopausal
179 and post-menopausal women.¹ Given the sex-related differences in asthma pathogenesis and clinical
180 manifestation, largely attributed to the role of sex steroid hormones,²⁻⁴ HRT has long been suspected to play
181 a role in asthma in women.⁵ For several decades, several studies have attempted to address this question, but
182 the underlying evidence base is uncertain as findings from previous studies are conflicting.⁶ Findings from
183 our recent systematic review and meta-analysis showed that to date, there are no prospective cohort studies
184 investigating the impact of use of HRT on clinical outcomes of asthma in peri-menopausal and post-
185 menopausal women with established asthma.⁶ Previous studies, which have been cross-sectional and
186 retrospective case-control studies, are hampered by their inability to establish temporality of association
187 between HRT and clinical outcomes of asthma, self-selection bias, and ascertainment bias.⁶ To overcome
188 these limitations, there is need to undertake long-term longitudinal cohort studies to investigate and clarify
189 the impact of use of HRT on subsequent clinical outcomes of asthma in peri-menopausal and post-
190 menopausal women with established asthma.

191

192 The aim of the current study was to investigate the association of use of HRT, its subtypes and duration of
193 use with severe asthma exacerbation in peri-menopausal and post-menopausal women with asthma. We also
194 examined whether body mass index (BMI), cigarette smoking, and menopausal status (peri- and post-
195 menopause) modified these associations.

196

197 METHODS

198 Ethics approvals and permissions

199 The Anonymised Data Ethics and Protocol Transparency Committee, responsible for approval of research
200 using the Optimum Patient Care Research Database (OPCRD), gave approval for the current study (reference
201 number: ADEPT1317). In addition, Optimum Patient Care has an existing ethics approval for the use of

OPCRD for research purposes from the NHS Health Research Authority (REC Ref: 15/EM/150). All researchers involved in data analysis completed required information governance courses.

Protocol registration and publication

The study protocol was registered with the European Union electronic Register of Post-Authorization Studies (EUPAS22967) and also published in a peer-reviewed journal prior to undertaking the analyses.⁷

Study design and population

OPCRD is a longitudinal anonymized primary care database, comprising 630 primary care practices across the UK with over six million patients at the time of extracting data for the current study. The database is used to conduct epidemiological, clinical, and pharmaceutical studies ([www.http://optimumpatientcare.org/opcrd/](http://www.optimumpatientcare.org/opcrd/)).^{8,9} We established an open (meaning that cohort members can enter or leave the cohort anytime during follow-up) retrospective cohort of peri-menopausal and post-menopausal women (46-70 years) who had any asthma event (including diagnosis, hospitalization, medication prescription) using previously established Read codes (Online Supplementary File) starting from baseline on January 1, 2000. Participants entered the cohort from baseline or date of registration to a general practice or the year of turning age 46 years and were followed-up until December 31, 2016. Participants exited the cohort on the date of death, deregistration from a practice, year of turning 70 years, or December 31, 2016, whichever came first. In total, 31,656 peri-menopausal and post-menopausal women were included in the study (Figure 1).

Ascertainment of hormonal contraceptives and HRT

We used the Read Clinical Classification System¹⁰ (Read codes; Online Supplementary File) to extract information on prescription of HRT. Based on the extracted information, we defined the following exposure categories for the use of HRT for each year of follow-up:

1. Previous (anytime in the past) and current (during that year) use versus non-use
2. Previous and current use of subtypes of HRT (combined estrogen/progestogen, estrogen-only)
3. Duration of use: 1-2 years; 3-4 years; 5 years or more versus non-use.

Patient's use of HRT was individually ascertained for every year. In cases where a patient experienced severe asthma exacerbation, HRT use was only counted if the prescription for HRT preceded the asthma exacerbation event in that year.

Potential confounding variables

We selected potential confounding factors based on evidence from previous literature and demonstrated these using a directed acyclic graph (Figure E1 in the Online Repository File). Using relevant Read codes (Online Repository File), we extracted information from the database and defined the following variables: gravidity, BMI, smoking, Charlson Comorbidity Index,¹¹ gynecological conditions that are potentially subject to hormonal manipulation – either through supplementation or inhibition - and those that potentially result from estrogen deficiency (endometriosis, polycystic ovary syndrome, acne, bilateral salpingo-oophorectomy (BSO), hysterectomy with BSO, hysterectomy without BSO, fibroids, and menstrual bleeding complaints identified under coding terms: menorrhagia, metrorrhagia; menometrorrhagia),¹² and Index of Multiple Deprivation (IMD).¹³ The IMD is a multidimensional measure used to categorize neighborhoods across the UK on the basis of material living conditions in order to determine resource allocation and service provision. Thus, while the IMD provides information on neighborhood relative deprivation, it gives an indirect indication of socioeconomic status of the neighborhood.¹³ Age was derived using the date of birth. Baseline asthma severity was classified based on the Global Initiative for Asthma (GINA) severity steps.

Study outcome

Our study outcome was occurrence of severe asthma exacerbation (Read codes in Online Supplementary File), which was defined according to the recommendation of the European Respiratory Society/American Thoracic Society¹⁴ as asthma-related hospitalization, emergency department visits due to asthma and/or prescription of oral corticosteroids. We counted the yearly number of asthma exacerbations, measured longitudinally during the 17-year follow-up on each patient. We considered severe asthma exacerbation occurring within a 14-day period as one event. This definition has been validated and used in previous studies using the OPCR database.¹⁵

258 Statistical analyses

259 We used the Pearson's Chi-square test to describe the distribution of the baseline study characteristics in
 260 relation to the use of HRT and severe asthma exacerbation (Table 1). To study the association between HRT
 261 use and severe asthma exacerbation, we used multilevel mixed-effects Poisson regression. As the mean
 262 number of annual severe asthma exacerbation (0.26) and its variance (0.52) were not exactly the same, we
 263 initially implemented multilevel mixed-effects negative binomial regression to the data, but the models did
 264 not converge. For this reason, we used multilevel mixed effects Poisson regression with QR decomposition,
 265 which provides an alternative to fitting models for Poisson distributed data. By using the QR decomposition
 266 of the variance-components matrix., this analysis approach aids improvement of the convergence of models
 267 when the variance components is close to the border of the parameter space.¹⁶ In estimating the association
 268 between HRT and severe asthma exacerbation (but not in the descriptive analyses), women who experienced
 269 any asthma exacerbation at baseline (prevalent cases, $n=5,172$, see Figure 1 and Table 1) were excluded from
 270 the analysis, allowing us to estimate a temporal association between baseline HRT and post-baseline severe
 271 asthma exacerbation (i.e. new severe asthma exacerbation). The models, while accounting for variations
 272 across general practices, also took into account the nesting of patients within general practices; that is, the
 273 models included random effects for both patients and practices. Furthermore, the models accounted for the
 274 longitudinal nature of the data, so that each variable was allowed to take different values at each year of
 275 follow-up. We adjusted for the above-described potential confounding variables, which were included in the
 276 models using the categories given in Table 1. Interactions of BMI and smoking with HRT were examined by
 277 including interaction terms in each adjusted model; the analyses were subsequently stratified by BMI or
 278 smoking if the interaction terms achieved p-values of <0.20 . The analyses were in addition stratified by
 279 menopausal status (peri- and post-menopausal women). We evaluated the potential for residual confounding
 280 after the adjusted regression analyses by calculating the E-values for the observed estimates of association
 281 between HRT use and severe asthma exacerbation.¹⁷ The estimates of the E-value indicate the minimum
 282 magnitude of association that an unmeasured confounder should have both in relation to respective
 283 exposures and the outcome in order to negate the observed estimate of association between the respective
 284 exposures and the outcome.¹⁷ Data management and editing were undertaken using R version 3.5.1 (R
 285 Foundation for Statistical Computing, Vienna, Austria). Statistical analyses were undertaken using and Stata
 286 14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

287

288 **Reporting**

289 We followed the recommendations of STROBE (Strengthening the Reporting of Observational Studies in
 290 Epidemiology)¹⁸ and RECORD (Reporting of studies Conducted using Observational Routinely-collected
 291 Data)¹⁹ in reporting the study results.

292 **RESULTS**293 *Characteristics of the study populations*

294 The 17-year follow-up of the 31,656 women amounted to 366,678 person-years of follow-up time (Figure 1).
 295 At baseline (i.e., the year a woman joined the study cohort), 22% of women were using any HRT, 11%
 296 combined HRT, and 11% estrogen-only HRT. Use of any HRT, combined estrogen/progestogen HRT, and
 297 estrogen-only HRT was highest among women 51-55 years of age and then decreased afterward; higher in
 298 ex/current smokers than non-smokers; in those with BMI <25kg/m² than in those with greater BMI; in
 299 women who had never been pregnant than those who had been; less in women with co-morbidity score of 1-
 300 3 than those with lower and higher scores; and relatively equally distributed between IMD quintiles (Table
 301 1). Use of any HRT and estrogen-only HRT was higher in women with a prior gynecological condition (i.e.,
 302 situations resulting in estrogen-deficiency: BSO, hysterectomy with BSO; and also, management of peri-
 303 menopausal menstrual bleeding complaints) than those without, but use of combined estrogen/progestogen
 304 HRT was higher in those without prior gynecological condition than those with. Use of any HRT and
 305 combined estrogen/progestogen HRT was more common among peri-menopausal women than among post-
 306 menopausal women, while use of combined estrogen-only HRT was more common among post-menopausal
 307 women than among peri-menopausal women.

308

309 A total of 5,172 (16%) of the women had experienced severe asthma exacerbation at baseline (Figure 1 and
 310 Table 1). The annual mean number of severe asthma exacerbation at baseline was 0.26 (standard deviation
 311 0.71). Severe asthma exacerbation at baseline were highest in women age 46-50 years than in other age
 312 groups; higher in ex-/current smokers than in non-smokers; highest in obese than in non-obese women;
 313 highest in women who had three or more pregnancies than women who had fewer pregnancies; higher in
 314 women with a prior gynecological condition than those without; highest in women with co-morbidity score
 315 of four and above than those with lower scores; higher in peri- than in post-menopausal women; and

relatively equally distributed between IMD quintiles and between baseline use and non-use of HRT (Table 1). Post-baseline severe asthma exacerbation was 11%, 13%, and 19% in women with BMI of <25, 25-29.9, and ≥ 30 kg/m², respectively, among women who had not experienced any severe asthma exacerbation at baseline (Data not shown).

Association between use of HRT and severe asthma exacerbation

Previous use of any HRT, combined estrogen/progestogen HRT, and estrogen-only HRT, compared to non-use, was associated with an increased risk of severe asthma exacerbation (Figure 2). Whilst current use of any HRT, compared to non-use, was associated with a small increased risk of severe asthma exacerbation, current use of combined estrogen/progestogen and estrogen-only HRT were not significantly associated with severe asthma exacerbation. Compared to non-HRT use, any duration of use was associated with an increased risk of exacerbation (Figure 2), but the risk was lowest for 1-2 years of use and highest for 3-4 years of use. While the results were not substantially different between peri-menopausal and post-menopausal women, previous use of estrogen-only HRT was associated with up to 24% (95%CI 16-29) but only 7% (95%CI 3-12) increased risk of severe asthma exacerbation in peri-menopausal and post-menopausal women, respectively (Figure 3). The estimated E-values for the association between use of any HRT and severe asthma exacerbation ranged from 1.00 (for current use of any HRT) to 2.21 (for 3-4 years use of HRT), signifying that an unmeasured confounder requires a minimum risk ratio of 1.00 and maximum of 2.21 in order to negate the observed association between HRT and severe asthma exacerbation after adjusting for all other confounding factors (data not shown). The least E-values were observed for current HRT use and subtypes, indicating that while these results may be influenced by residual confounding, the results for previous HRT use and duration of HRT are less likely to be negated by residual confounding.

In stratified analyses by BMI, while there was an increased risk of severe asthma exacerbation with HRT use across the BMI groups, in most cases the risk estimates were highest for women with BMI <25 kg/m², followed by those for women with BMI 25-29.9 kg/m², and less in obese women (BMI ≥ 30 kg/m²) (Table 2). In the stratified analyses by smoking status, only previous and current use of any HRT and estrogen-only HRT, but not combined estrogen/progestogen and duration of HRT use, were associated with an increased

risk of severe asthma exacerbation among non-smokers. However, among ex/current smokers, use of any HRT, combined estrogen/progestogen, and estrogen-only HRT, previously and currently, and long-term HRT use, was clearly associated with an increased risk of severe asthma exacerbation (Table 2).

DISCUSSION

In this national longitudinal cohort of peri-menopausal and post-menopausal women with asthma who were followed for 17 years, we found that previous but not current use of any HRT, combined estrogen/progestogen HRT, and estrogen-only HRT and long-term use were associated with an increased risk of severe asthma exacerbation. These results were overall similar for peri- and post-menopausal women, but the risk estimates were greater for lean women than heavier women and greater for smokers than non-smokers. Sensitivity analyses examining the impact of residual confounding on these results showed that, while the observations made with regards to current HRT use may be influenced by residual confounding, the results regarding previous and duration of HRT use were generally unlikely to be influenced of residual confounding.

OPCRD is one of the leading primary care databases in the UK dedicated to clinical and pharmaco-epidemiologic studies with well-characterized patient cohort that is largely representative of the UK primary care population. At the time of undertaking the current study, there were 6.3 million patients in the OPCR database covering 630 general practices from across the UK. With the large sample size in the current study, we could address the question of impact of HRT on severe asthma exacerbation by considering the role of both duration and subtypes of HRT, thus providing precise estimates of effect sizes of the associations. With a 17-year follow-up and in a longitudinal perspective, we were able to study the long-term impact of HRT on severe asthma exacerbation. The longitudinal framework also allowed us the opportunity to evaluate the time-dependence of the impact of HRT on the outcome. Based on this, our analysis took into account the possibility that participants' use of HRT could change over the study follow-up period. Therefore, participants' HRT use status changed from non-users in the years they did not use HRT to users in the years they used HRT. Given the challenge of immortal time bias, a common bias in pharmaco-epidemiologic

374 studies evaluating the effect of medical therapies on disease outcomes, the approach of allowing time-
375 dependencies in the use of HRT is useful in minimizing the impact of immortal time bias.²⁰ All study
376 variables were defined using the Read Clinical Classification System, which is a standardized system for
377 documenting primary care events in the UK, thereby ensuring consistent recording of the study variables.¹⁰

378

379 While it may be considered that the Quality and Outcomes Framework (QOF), a national initiative
380 established in 2004 incentivizing primary care centers to maintain a register of asthma patients for
381 improvements in recording of clinical events, may have led to differential diagnosis of asthma during the
382 study follow-up, 13 of the 17 years of follow-up of our study occurred post-adoption of QOF.²¹ Therefore,
383 given that the majority of the study period occurred during the QOF program, any bias due to under-
384 diagnosis of asthma would have been minimal. We defined severe asthma exacerbation following
385 recommendation of the European Respiratory Society/American Thoracic Society¹⁴ and this definition has
386 been previously defined within the OPCR database.¹⁵ However, provision of asthma action plans with oral
387 corticosteroids scripts may possibly lead to over estimates in those having one severe asthma exacerbation.
388 There is a possibility that some asthma cases might have been a result of misdiagnosis of COPD. However,
389 the READ codes we used for defining asthma have been previously validated in another comparable GP
390 database, which resulted in high accuracy.²² Moreover, the concomitance between asthma and COPD based
391 on READ codes has been estimated to be less than 15%.²³ Therefore, any potential misclassification of
392 asthma in study as COPD will be minimal and is unlikely to change our conclusions from our study. Several
393 other conditions, e.g. endometriosis and fibroids, may be subject to hormonal manipulation – either through
394 supplementation or inhibition – or may potentially result from estrogen deficiency, thus may introduce
395 confounding by indication. In order to minimize this, we identified a list of these conditions, including those
396 that HRT may be used as a therapy and adjusted for these in our analyses. We defined peri-menopausal and
397 post-menopausal age, including cut-off for peri- and post-menopausal women, based only on the age of the
398 women, because the age of menopause is not recorded in the database as an event and there was no
399 additional information from the database to enhance these definitions. However, the chosen ages reflect the
400 average ages of the menopausal events in the UK. Information on HRT was based only on medication
401 prescription and thus we could not establish whether the women actually used the prescribed medications,
402 because we did not have information on medication dispensation.

403

404 Prior to embarking on the data analysis, we registered the analysis protocol for the current study, which was
 405 also published in a peer-reviewed journal, providing details of our analysis strategies.⁷ This ensured the
 406 transparency of the current work and any deviations from the protocol have been explained in the current
 407 manuscript. Registering and publishing the protocol before undertaking the analysis enhanced the
 408 transparency of our work. The protocol included plan for propensity score analysis for adjusting for potential
 409 confounding factors,⁴ but this could not be implemented as planned because we found no appropriate
 410 algorithm for propensity score analyses for multi-level models with time-varying covariates. In the absence
 411 of propensity score analysis, we performed conventional confounder adjustment by simultaneously adjusting
 412 for all the confounding factors in the statistical models. To minimize the impact of residual confounding on
 413 the effect estimates, we calculated the E-value, which provides evidence that our results regarding previous
 414 HRT use were not substantially influenced by residual confounding, while results for current HRT use
 415 seemed to have been influence by residual confounding. Our plan to investigate the role of routes of
 416 administration of HRT (oral, transdermal, subcutaneous, intramuscular, or local intrauterine) was not
 417 possible, because this information was not consistently recorded in the database. In the context of coronary
 418 heart disease and overall mortality, the timing hypothesis has been highlighted, indicating that the timing of
 419 use of HRT in proximity to the timing of onset of menopause seems to be critical and thus provides a
 420 window of opportunity for primary prevention.²⁴ However, while to our knowledge no previous study has
 421 evaluated this hypothesis in the context of asthma, we did not have the required data to define this aspect in
 422 the current study.

423

424 Of the seven studies²⁵⁻³¹ identified in our previous systematic review⁶ to have investigated the association
 425 between HRT and clinical outcomes of asthma in peri-menopausal and post-menopausal women, four were
 426 cross-sectional studies²⁵⁻²⁸ while three were very small retrospective case-control studies.²⁹⁻³¹ The design of
 427 the studies, which was graded as weak in that systematic review, does not allow establishment of temporality
 428 in the association between HRT and clinical outcomes of asthma, thus the previous studies are of poorer
 429 quality and not comparable to the current study.⁶ Assessment of HRT and study outcomes, based on self-
 430 report in most of the previous studies, exposed the studies to greater risk of ascertainment bias. To date, the
 431 current study, with long-term follow-up and longitudinal design, is the most robustly undertaken to address

the question of the role of HRT in clinical outcomes of asthma in peri-menopausal and post-menopausal women. Our results indicate that use of HRT and subtypes, particularly previous rather than current use was associated with an increased risk of severe asthma exacerbation, both in peri- and post-menopausal women. Furthermore, we found that long term rather than short-term HRT use was associated with an increased risk of severe asthma exacerbation. The increased risk of HRT use on severe asthma exacerbation was greatest in women with BMI $<25 \text{ kg/m}^2$ and least in those with BMI $\geq 30 \text{ kg/m}^2$, as well greater in ex/current smokers than in non-smokers.

The proposed impact of HRT on asthma is complex, as HRT has been shown to exert both anti-inflammatory and pro-inflammatory effects on innate^{32,33} and adaptive immunity.³⁴ The role of HRT in inflammation is attributed primarily to the effects of estrogen through the activities of estrogen receptors (ERs), ER α and ER β , both of which are expressed on immune cells, but are also present in the lung; they regulate cells and pathways in both the innate and adaptive immune system.³⁵ Monocytes in premenopausal women are reported to have lower amounts of ER α RNA than monocytes isolated from males and post-menopausal women, suggesting that higher estradiol levels correlate with reduced ER α expression.³⁶ ER α and ER β RNA levels however did not differ in male and female B and T lymphocytes, or in lymphocytes of peri- and post-menopausal women.³⁶ A preponderance of one or other ER is suggested to modify the effects of estrogen³⁷ and an imbalance of their expression has been shown between asthmatic and healthy airways.³⁷ However, the molecular mechanisms that may result in sex differences in ER expression in particular immune cells remain to be defined. Further mechanistic studies are required to better understand the biology underpinning the influence of HRT on clinical outcomes in peri-menopausal and post-menopausal women with established asthma and also to understand whether the route of steroid replacement delivery plays a role. Overall, our results indicate that previous and long-term, but not current, HRT use are crucial, perhaps indicating that withdrawal of HRT use among those with long-term exposures may constitute a preventative measure for severe asthma exacerbations. It is however, unclear whether the increased number of exacerbations given previous or long-term HRT use is a one-off risk, possibly coincident with a sudden decline in estrogen, or represent a continuing effect.

Interaction between HRT and BMI in relation to asthma has been hypothesized to perhaps be an indication of a common pathway for the impact of HRT and BMI on asthma.³⁷ While BMI is closely linked to insulin resistance,^{39,40} there is evidence that insulin resistance is also closely involved in local estrogen production.^{41,42} In women with low BMI, insulin resistance is low and this may portend a pro-inflammatory effect of HRT, but in women with high BMI, there appears a counterbalance between estrogen and insulin resistance, whereby the effect of HRT on asthma may be minimal.³⁸ These propositions may explain our current observations in which the impact of HRT on risk of severe asthma exacerbation was greatest for lean women and lowest for heavier women. A possible explanation for our findings may be that low levels of estrogen (and symptoms thereof) at onset of menopause prompts the use of HRT. During the period of use of HRT the woman is somewhat protected from asthma exacerbations. However, when discontinuing treatment, which is recommended after 1-5 years, and being non-obese, the levels of estrogen drop, potentially increasing women's risk of asthma exacerbations. Concerning the differential effect of HRT on severe asthma exacerbation between non-smokers and smokers, the potential underlying mechanism is unclear, thus require further studies for confirmation as well as mechanistic investigations to highlight the biological pathways through which these results are tenable.

CONCLUSIONS

Our results demonstrate that the use of HRT and subtypes by peri-menopausal and post-menopausal women with established asthma, particularly previous HRT use and long-term use but not current use is associated with an increased risk of severe asthma exacerbation. Lean women and smokers were at greatest risk than heavier women and smokers, respectively. There is a need to confirm the current findings by further longitudinal studies. In such studies, identification of new users of HRT will enhance establishment of casual relationship between HRT and clinical outcomes of asthma. Furthermore, mechanistic studies investigating the biologic processes underlying the impact of HRT on clinical outcomes of asthma in peri-menopausal and post-menopausal women are required.

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AUTHORS' CONTRIBUTION

Conceived idea for the study (BN, AS); designed the study (BN, AS, CRS, CMH); data collection (BN, AS, RP, DP, INS, FA); data analyses (BN, RP, HT, AS, SAS); clinical/primary care expertise on asthma and OPCRd-related expertise (DR, DP); primary care coding expertise (SM); expertise on sex steroids (HC); drafted the manuscript (BN, AS); all authors critically revised the manuscript for intellectual content and approved the final version.

REFERENCES

1. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med* 1992; 117: 1016-1037.
2. Kynnyk JA, Mastronarde JG, McCallister JW. Asthma, the sex difference. *Curr Opin Pulm Med* 2011; 17: 6-11.
3. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. *Curr Opin Allergy Clin Immunol* 2013; 13: 92-99.
4. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; 16: 626-638.
5. Tattersfield AE. Is postmenopausal HRT a risk factor for adult-onset asthma? *Thorax* 65: 282-284.
6. McCleary N, Nwaru BI, Nurmatov UB, Critchley H, Sheikh A. Endogenous and exogenous sex steroid hormones in asthma and allergy in females: a systematic review and meta-analysis. *J Allergy Clin Immunol* 2018; 141: 1510-1513.e8.
7. Nwaru BI, Simpson CR, Soyiri IN, Pillinger R, Appiagyei F, Ryan D, et al. Exogenous sex steroid hormones and asthma in females: protocol for a population-based retrospective cohort study using a UK primary care database. *BMJ Open* 2018; 8: e020075.

8. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Med* 2015; 3: 849-858.
9. Jones RC, Price D, Ryan D, Sims EJ, von Ziegenweidt J, Mascarenhas L, et al. Opportunities to diagnose chronic obstructive pulmonary disease in routine care in the UK: a retrospective study of a clinical cohort. *Lancet Respir Med* 2014; 2: 267-276.
10. Williams T, van Staa T, Puri S, Eaton S. Recent advances and use of the General Practice Research Database as an example of a UK Primary Care Data resource. *Ther Adv Drug Saf* 2012; 3: 89-99.
11. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chron Dis* 1987; 40: 373-383.
12. Kvaskoff M, Mu F, Terry KL, Harris HR, Poole EM, Farland L, et al. Endometriosis: a high-risk population for major chronic diseases? *Hum Reprod Update* 2015; 21: 500-506.
13. McLennan D, Noble S, Noble M, Plunkett E, Wright G, Gutacker N. The English Indices of Deprivation 2019. London: Ministry of Housing, Communities and Local Government.
14. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343-373.
15. Colice G, Chisholm A, Dima AL, Reddel HK, Burden A, Martin RJ, et al. Performance of database-derived severe exacerbation and asthma control measures in asthma: responsiveness and predictive utility in a UK primary care database with linked questionnaire data. *Pragmat Obs Res* 2018; 9: 29-42.
16. Garson D. Multilevel modelling: applications in STATA®, IBM® SPSS®, SAS®, R & HLM. 2019. Sage Publications, Inc. California, USA.
17. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. *Ann Intern Med* 2017; 167: 268-274.
18. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *I Clin Epidemiol* 2008; 61: 344-349.

19. Benchimol EI, Smeeth L, Guttman A, Harron K, Moher D, Petersen I, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *Plos Med* 2015; 12: e1001885.
20. Lévesque LE, Hanley JA, Kezouh A, Suissa S. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. *Bmj* 2010;340:b5087.
21. Simpson CR, Sheikh A. Trends in the epidemiology of asthma in England: a national study of 333,294 patients. *J R Soc Med* 2010; 103: 98–106.
22. Nissen F, Morales DR, Mullerova H, Smeeth L, Douglas IJ, Quint JK. Validation of asthma recording in the Clinical Practice Research Datalink (CPRD). *BMJ Open*. 2017;7(8):e017474.
23. Nissen F, Morales DR, Mullerova H, Smeeth L, Douglas IJ, Quint JK. Concomitant diagnosis of asthma and COPD: a quantitative study in UK primary care. *Br J Gen Pr*. 2018;68(676):e775–82.
24. Hodis HN, Collins P, Mack WJ, Schierbeck LL. The timing hypothesis for coronary heart disease prevention with hormone therapy: past, present and future in perspective. *Climacteric* 2012; 15: 217-228.
25. Carlson CL, Cushman M, Enright PL, Cauley JA, Newman AB, Cardiovascular Health Study Research Group. Hormone replacement therapy is associated with higher FEV1 in elderly women. *Am J Respir Crit Care Med* 2001; 163: 423-428.
26. Khatibi A, Agardh CD, Lidfeldt J, Samsioe G. Nonhormonal drug use and its relation to androgens in perimenopausal women: a population-based study of Swedish women. The Women's Health in the Lund Area Study. *Menopause* 2009; 16: 315-319.
27. Mueller JE, Frye C, Brasche S, Heinrich J. Association of hormone replacement therapy with bronchial hyper-responsiveness. *Respir Med* 2003; 97: 990-992.
28. Songur N, Aydin ZD, Ozturk O, Sahin U, Khayri U, Bircan A, et al. Respiratory symptoms, pulmonary function, and reproductive history: Isparta Menopause and Health Study. *J Women's Health* 2010; 19: 1145-1154.
29. Kos-Kudla B, Ostrowska Z, Marek B, Ciesielska-Kopacz N, Kudla M. Effects of hormone replacement therapy on endocrine and spirometric parameters in asthmatic postmenopausal women. *Gynecol Endocrinol* 2001; 15(4): 304-11.

30. Paleev NP, Shabalin VN, Chereiskaia NK, Iurina TM, Slivets ON, Shapovalenko SA. Specific aspects of the course of bronchial asthma therapy in perimenopausal period. *Klinicheskaia Meditsina* 1999; 77: 17-20.
31. Paleev NR, Chereiskaya NK, Slivets ON, Shapovalenko SA, Podrezova LA. Integrative study of bronchial asthma in perimenopausal women. *Vestnik Rossiiskoi akademii meditsinskikh nauk* 2002; (2): 16-20.
32. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev* 2007;28:521–574.
33. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol* 2015;294:63–69.
34. Moulton VR. Sex hormones in acquired immunity and autoimmune disease. *Front Immunol* 2018;9:2279.
35. Kovats S. Estrogen Receptors Regulate Innate Immune Cells and Signaling Pathways. *Cell Immunol* 2015; 294:63-69.
36. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett* 2005; 97:107–113.22.
37. Aravamudan B, Goorhouse KJ, Unnikrishnan G, Thompson MA, Pabelick CM, Hawse JR, et al. Differential expression of estrogen receptor variants in response to inflammation signals in human airway smooth muscle. *J Cell Physiol* 2017;232:1754–1760.
38. Gomez Real F, Svanes C, Björnsson EH, Franklin KA, Gislason D, Gislason T, et al. Hormone replacement therapy, body mass index and asthma in perimenopausal women: a cross-sectional survey. *Thorax* 2006; 61: 34-40.
39. Barrett-Connor E, Frette C. NIDDM, impaired glucose tolerance, and pulmonary function in older adults. The Rancho Bernardo Study. *Diabetes Care* 1996; 19: 1441–1444
40. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; 254–257.
41. Kalish G M, Barrett-Connor E, Laughlin G A, Gulanski BI, Postmenopausal Estrogen/Progestin Intervention Trial. Association of endogenous sex hormones and insulin resistance among postmenopausal women: results from the Postmenopausal Estrogen/Progestin Intervention Trial. *J Clin Endocrinol Metab* 2003; 88: 1646–1652.

42. Simpson E, Rubin G, Clyne C. Robertson K, O'Donnell L, Jones M, et al. The role of local estrogen biosynthesis in males and females. *Trends Endocrinol Metab* 2000; 11: 184–188.

FIGURE LEGENDS

Figure 1. Flowchart of study cohort and baseline severe asthma exacerbation and HRT use

Figure 2. Unadjusted and adjusted associations between use of hormone replacement therapy (HRT) and severe asthma exacerbation in all women. Women with severe asthma exacerbation at baseline were excluded in these analyses in order to estimate the impact of HRT on new asthma exacerbation

Figure 3. Adjusted associations between use of hormone replacement therapy (HRT) and severe asthma exacerbation by menopausal status. Women with severe asthma exacerbation at baseline ($n=5,172$) were excluded in these analyses in order to estimate the impact of HRT on new asthma exacerbation

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Table 1. Baseline characteristics by use of hormone replacement therapy (HRT) and severe asthma exacerbation at baseline

	Frequency <i>N</i> = 31,656 <i>n</i> (%)	Used any HRT <i>n</i> =7,061 (22.3%) <i>n</i> (%)	No HRT use <i>n</i> =24,554 (77.6%) <i>n</i> (%)	Used combined HRT <i>n</i> =3,472 (11%) <i>n</i> (%)	Used estrogen- only HRT <i>n</i> =3,589 (11.3%) <i>n</i> (%)	Mean number of severe asthma exacerbation (standard deviation)	Had severe asthma exacerbation ³ <i>n</i> =5,172 (16%) <i>n</i> (%)
Age, years							
46-50	14,677 (46.4)	2,875 (19.6)	11,802 (80.4)	1,649 (11.2)	1,226 (8.3)	0.29 (0.75)	2,763 (18.8)
51-55	6,087 (19.2)	1,818 (29.9)	4,269 (70.1)	872 (14.3)	946 (15.5)	0.22 (0.66)	840 (13.8)
56-60	4,722 (14.9)	1,290 (27.3)	3,432 (72.7)	558 (11.8)	732 (15.5)	0.24 (0.74)	679 (14.4)
61-65	3,599 (11.4)	745 (20.7)	2,854 (79.3)	278 (7.7)	467 (13.0)	0.22 (0.66)	508 (14.1)
66-70	2,571 (8.1)	333 (12.9)	2,238 (87.0)	115 (4.5)	218 (8.5)	0.23 (0.69)	382 (14.9)
Smoking status							
Non-smoker	13,395 (42.3)	2,701 (20.2)	10,694 (79.8)	1,321 (9.9)	1,380 (10.30)	0.21 (0.63)	1,875 (14.0)
Ex/current smoker	18,261 (57.7)	4,360 (23.9)	13,901 (76.1)	2,151 (11.8)	2,209 (12.1)	0.29 (0.78)	3,297 (18.0)
Body mass index, kg/m ²							
<25	9,210 (29.1)	2,323 (25.2)	6,887 (74.8)	1,246 (13.5)	1,077 (11.7)	0.22 (0.67)	1,311 (14.2)

25.29.9	10,265 (32.4)	2,410 (23.5)	7,855 (76.5)	1,102 (10.7)	1,308 (12.7)	0.23 (0.67)	1,517 (14.8)
≥30	12,181 (38.5)	2,328 (19.1)	9,853 (80.9)	1,124 (9.2)	1,204 (9.9)	0.31 (0.79)	2,344 (19.2)
Gravidity							
None	19,805 (62.6)	4,665 (23.5)	15,140 (76.4)	2,290 (11.6)	2,375 (12.0)	0.23 (0.69)	2,891 (14.6)
One	4,219 (13.3)	843 (20.0)	3,376 (80.0)	412 (9.8)	431 (10.2)	0.29 (0.73)	794 (18.8)
Two	4,142 (13.1)	842 (20.3)	3,300 (79.7)	418 (10.1)	424 (10.2)	0.28 (0.73)	752 (18.2)
Three or more	3,490 (11.0)	711 (20.4)	2,779 (79.6)	352 (10.1)	359 (10.3)	0.34 (0.82)	735 (21.1)
Any gynaecological condition ¹							
No	12,049 (38.1)	2,323 (19.3)	9,726 (80.7)	1,612 (13.4)	711 (5.9)	0.22 (0.69)	1,671 (13.9)
Yes	19,607 (61.9)	4,738 (24.2)	14,869 (75.8)	1,860 (9.5)	2,878 (14.7)	0.28 (0.73)	3,501 (17.9)
Charlson Comorbidity Index							
0	24,592 (77.7)	5,653 (23.0)	18,939 (77.0)	2,871 (11.7)	2,782 (11.3)	0.23 (0.66)	3,658 (14.9)
1-3	1,189 (3.8)	201 (16.90)	988 (83.1)	87 (7.3)	114 (9.6)	0.31 (0.81)	230 (19.3)
4+	5,875 (18.6)	1,207 (20.5)	4,668 (79.5)	514 (8.7)	693 (11.8)	0.37 (0.89)	1,284 (21.9)
Index of Multiple Deprivation quintiles							
1 st quintile (least deprived)	6,126 (19.3)	1,299 (21.2)	4,827 (78.8)	667 (10.9)	632 (10.3)	0.26 (0.73)	1,006 (16.4)

2 nd quintile	5,688 (18.0)	1,132 (19.9)	4,556 (80.1)	533 (9.4)	599 (10.5)	0.28 (0.77)	975 (17.1)
3 rd quintile	6,259 (19.8)	1,507 (24.1)	4,752 (75.9)	727 (11.6)	780 (12.5)	0.24 (0.66)	986 (15.7)
4 th quintile	7,060 (22.3)	1,665 (23.6)	5,395 (76.4)	817 (11.6)	848 (12.0)	0.25 (0.71)	1,162 (16.5)
5 th quintile (most deprived)	6,523 (20.6)	1,458 (22.3)	5,065 (77.6)	728 (11.2)	730 (11.2)	0.25 (0.72)	1,043 (16.0)
Menopausal status							
Peri-menopause	18,366 (58.0)	4,243 (23.1)	14,123 (76.9)	2,413 (13.1)	1,830 (10.0)	0.27 (0.73)	3,230 (17.6)
Post-menopause	13,290 (42.0)	2,818 (21.2)	10,472 (78.8)	1,059 (8.0)	1,759 (13.2)	0.23 (0.69)	1,942 (14.6)
Used any HRT at baseline							
No	24,595 (77.7)	----	----	----	----	0.25 (0.71)	4,017 (16.3)
Yes	7,061 (22.3)					0.26 (0.75)	1,155 (16.4)

¹Defined as having any of the following conditions: endometriosis, polycystic ovary syndrome, menorrhagia, acne, metrorrhagia, bilateral salpingo-oophorectomy (BSO), hysterectomy BSO, hysterectomy, fibroids, and menometrorrhagia

³Those with severe asthma exacerbation ($n=5,172$) at baseline were excluded in subsequent analyses investigating the association between HRT use and severe asthma exacerbations in order to estimate only new asthma exacerbation

Table 2. Association between use of hormone replacement therapy (HRT) and severe asthma exacerbation in peri-menopausal and menopausal women, stratified by BMI and smoking¹

HRT use	Stratified analyses by BMI Incidence Rate Ratio (95% CI) ^{2,3}			Stratified analyses by smoking status Hazard Ratio (95% CI) ^{4,5}	
	<25 kg/m ²	25-29.9 kg/m ²	≥30 kg/m ²	Non-smokers	Smokers
Previous use of any HRT					
None	1	1	1	1	1
Yes	1.37 (1.32-1.42)	1.25 (1.21-1.30)	1.18 (1.15-1.22)	1.08 (1.04-1.11)	1.32 (1.29-1.35)
Current use of any HRT					
None	1	1	1	1	1
Yes	1.20 (1.15-1.25)	1.11 (1.06-1.16)	0.98 (0.94-1.02)	1.13 (1.08-1.18)	1.10 (1.07-1.14)
Type of HRT (previous use)					
None	1	1	1	1	1
Estrogen only	1.49 (1.41-1.58)	1.25 (1.18-1.32)	1.08 (1.03-1.14)	1.19 (1.13-1.26)	1.22 (1.17-1.26)
Combined estrogen/progestogen	1.34 (1.29-1.40)	1.25 (1.20-1.31)	1.34 (1.28-1.38)	1.03 (0.99-1.07)	1.43 (1.39-1.46)
Type of HRT (current use)					
None	1	1	1	1	1

Estrogen only	1.34 (1.27-1.41)	1.16 (1.10-1.22)	1.01 (0.96-1.06)	1.19 (1.13-1.25)	1.08 (1.05-1.13)
Combined estrogen/progestogen	1.06 (1.00-1.12)	1.04 (0.97-1.11)	0.94 (0.88-1.00)	1.05 (0.98-1.12)	1.14 (1.09-1.18)
Duration of use of any HRT, years					
None	1	1	1	1	1
1-2	1.25 (1.20-1.31)	1.25 (1.20-1.30)	0.98 (0.95-1.01)	0.97 (0.94-1.01)	1.09 (1.07-1.12)
3-4	2.18 (2.06-2.30)	1.02 (0.96-1.08)	1.24 (1.19-1.29)	0.95 (0.91-1.00)	1.31 (1.27-1.36)
5+	1.53 (1.44-1.62)	1.42 (1.34-1.49)	1.35 (1.30-1.41)	1.04 (0.99-1.10)	1.39 (1.34-1.43)

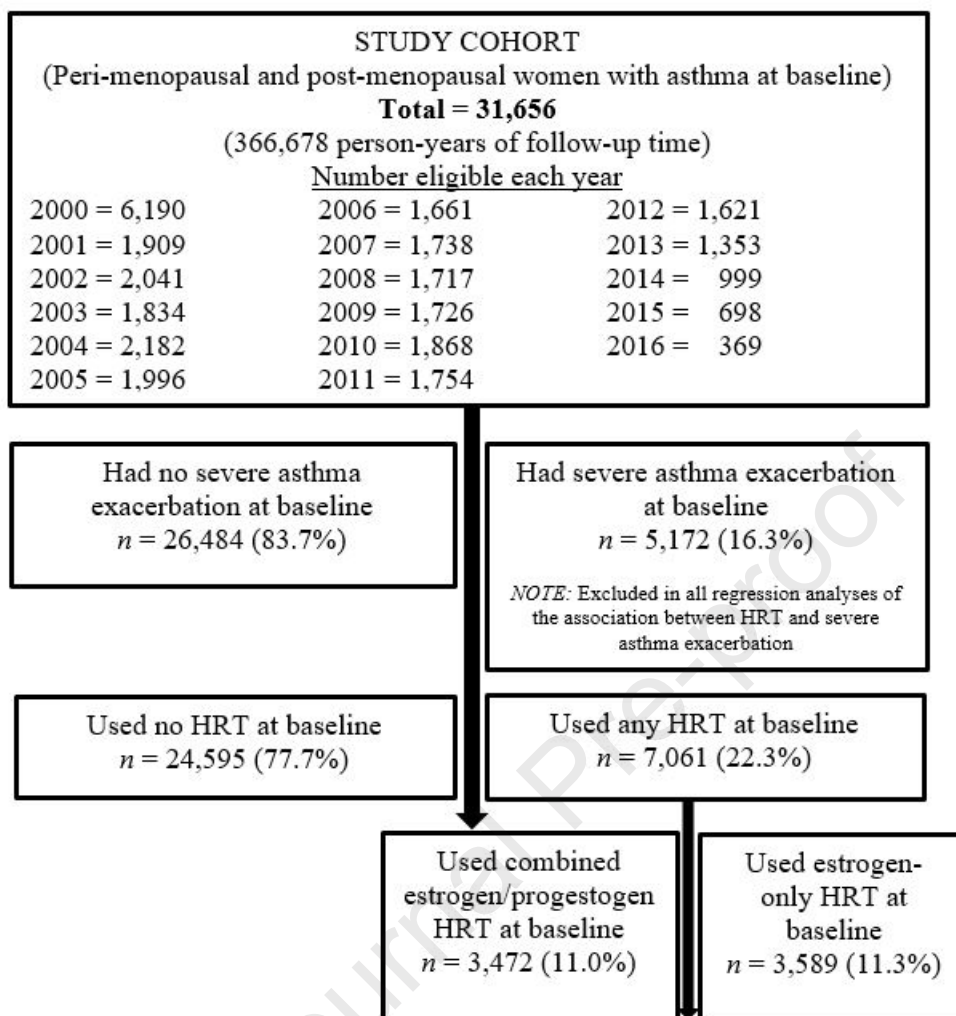
¹All analysis were based on multilevel mixed-effects Poisson regression that accounted for clustering of patients within GP practices. Women with severe asthma exacerbation at baseline ($n=5,172$) were excluded in these analyses in order to estimate the impact of HRT on new asthma exacerbation

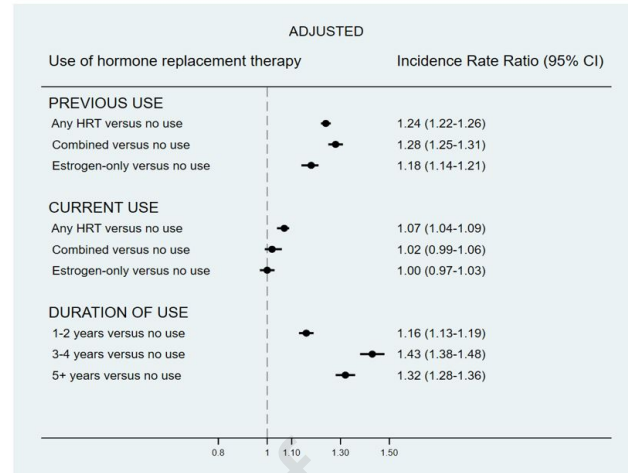
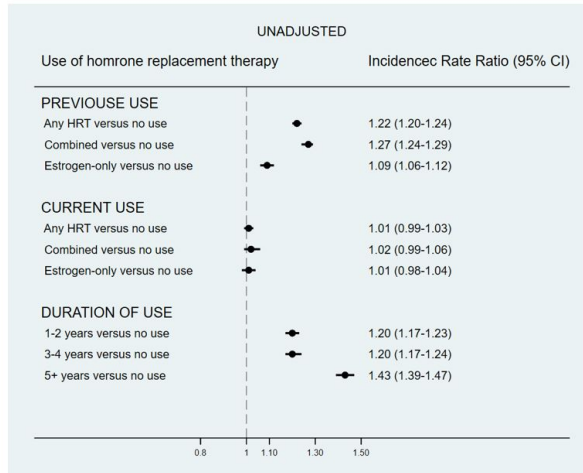
²Adjusted for asthma severity level at baseline, age, smoking, Charlson co-morbidity index, gravidity, any gynecological condition, and index of multiple deprivation

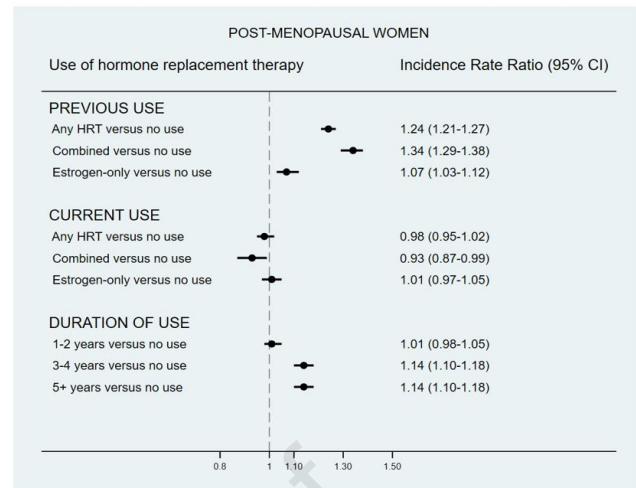
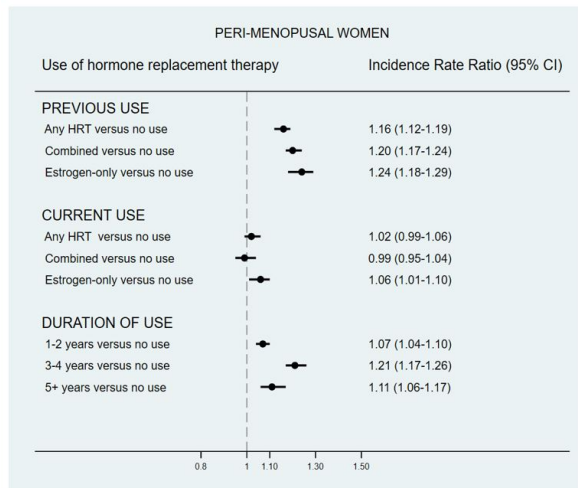
³Stratified analyses performed after interaction term between use of HRT and BMI gave $p<0.20$

⁴Adjusted for asthma severity level at baseline, age, Charlson co-morbidity index, BMI, gravidity, any gynecological condition, and index of multiple deprivation

⁵Stratified analyses performed after interaction term between use of HRT and smoking gave $p<0.20$







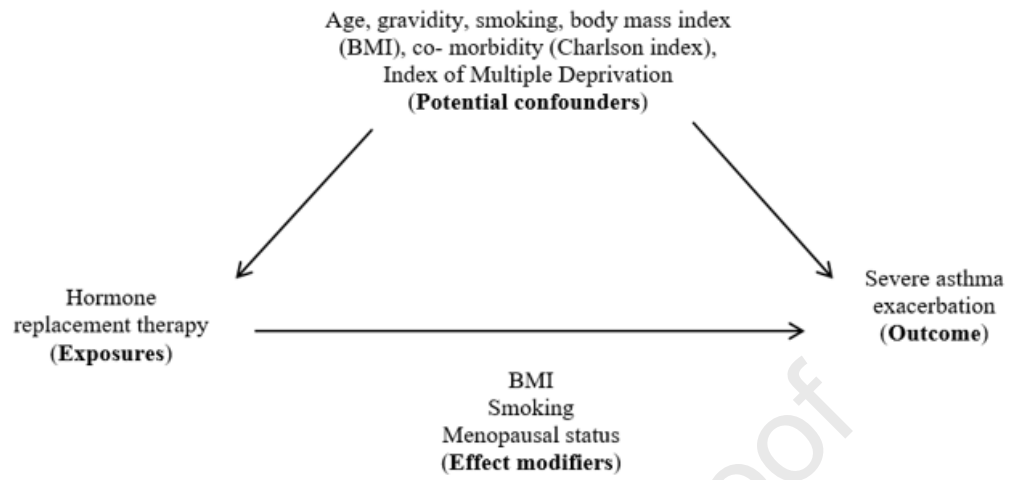


Figure E1