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Postmenopausal hormone therapy as a risk factor for gastroesophageal reflux symptoms among female twins

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Abstract

Background & Aims—Female sex hormones have been suggested to increase the risk of gastroesophageal reflux symptoms via a relaxing effect on the lower esophageal sphincter. We investigated the relations of oral contraceptives and postmenopausal hormone therapy (HT) to risk of reflux symptoms, controlling for genetic factors and body mass.

Methods—Information on exposures and reflux symptoms was obtained by telephone interviews conducted in 1998–2002 among women in the Swedish Twin Registry. Use of oral contraceptives was also assessed in 1973 by questionnaires. Both cross-sectional and prospective nested case-control designs were used, each with external control analysis. The cross-sectional design was further submitted to monozygotic (MZ) co-twin control analysis.

Results—The cross-sectional study design comprised 4365 twins with reflux and 17,321 without. In ever users of estrogen HT the risk of reflux symptoms was increased by 32% (odds ratio [OR] 1.32; 95% confidence interval [CI] 1.18–1.47). This association remained in the nested case-control analyses and increased slightly with higher BMI. A similar pattern was observed for the use of progestin in the cross-sectional design, but no association remained in the nested case-control analysis. Use of oral contraceptives was not associated with an increased risk of reflux symptoms. Generally, the risk estimates remained virtually unchanged after adjustments for potential confounding factors, including genetic factors.

Conclusions—This population-based twin study indicates that estrogen HT is an independent risk factor for reflux symptoms, while the influence of progestin HT and oral contraceptives was less consistent.

Introduction

Gastroesophageal reflux disease (GERD) is a general health disorder, affecting about 20% of the adult population in industrialized countries at least once weekly according to community

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Conflict of interest statement

There are no conflicts of interest.

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surveys.^{1–4} Frequent symptoms of reflux, typically heartburn and regurgitation, are associated with an impaired health-related quality of life⁵, and an overall increase in mortality.^{6, 7} Moreover, GERD produces substantial economic costs to the patients and to society at large, owing to heavy prescription costs for antireflux medication⁸ and time lost from work.⁹ GERD is also one of the main risk factors for esophageal adenocarcinoma,¹⁰ a cancer with poor survival¹¹ and a rapidly increasing incidence.¹² Knowledge about the etiology of GERD, however, is limited. Several risk factors have been suggested, but currently the only ones to have been established are heredity, an increased body mass, and tobacco smoking.^{13–19} Based on twin studies, the heritability of GERD has been estimated to be 31 – 43%,^{13, 20} implying that potential confounding by genetic factors should be taken into account when investigating other risk factors for GERD. Some data suggest, including two studies by our group, that the association between obesity and reflux symptoms is stronger among women than among men^{14, 21} and that the association is strengthened by the use of postmenopausal hormone therapy (HT). We therefore hypothesized that estrogen and possibly progestin therapy might be causally linked to reflux symptoms, by having a direct effect on the lower esophageal sphincter (LES) possibly via nitric oxide. To test this hypothesis, we analyzed the association between HT and oral contraceptives and the risk of developing reflux symptoms among female twins, including evaluation of possible confounding, notably by heredity, body mass, and tobacco smoking in a population-based twin registry.

Methods

Study design

A population-based, nationwide study based on the Swedish Twin Registry was conducted, using two different study designs: cross-sectional analyses to maintain a good statistical power in all analyses and nested case-control analyses with incidence density sampling to allow a prospective estimation of the association and thereby decrease the risk of reversed causality.

Data source

A thorough description of the Swedish Twin Registry has been presented elsewhere.²² In brief, the Swedish Twin Registry was established in the late 1950s and is the largest population-based register of twin births in the world. It includes in principle all twins born in Sweden since 1886. The data collection in the current study was conducted in two ways: First, in 1973, all same-sex twin pairs in the Swedish Twin Registry born 1926–1958 were mailed a questionnaire regarding lifestyle habits, certain diseases and symptoms, and intake of selected medications, including oral contraceptives. Second, within the frames of the Screening Across the Lifespan Twin study, all surviving twins born in 1958 or earlier participated in a structured computer-assisted telephone interview, conducted by trained professional interviewers, during the period March 1998 through November 2002. The questions covered zygosity, tobacco smoking, physical exercise, education, occupation, diseases, and symptoms, including symptoms of reflux, as well as prescribed and non-prescribed medication use, and body weight and height. Data on oral contraceptives was assessed in both 1973 and 1998–2002, while all other information used was assessed in 1998–2002 only. Informed verbal consent was obtained from all study participants and the study protocol was approved by the ethics committee of the Karolinska Institutet, and by the Mayo Institutional Review Board.

Definition and assessment of the outcome: reflux symptoms

Reflux was mainly defined as troublesome symptoms of heartburn or regurgitation occurring at least once per week, a definition that has gained general consensus.²³ The 10 specific questions in the reflux symptom questionnaire that were used to determine the occurrence of reflux as part of the Screening Across the Lifespan Twin Study interview have been used and described in detail previously.^{10, 13} Briefly, all twins were first asked whether they had

regularly suffered from heartburn, pain behind the breastbone, or regurgitation of sour fluid into the mouth. If the answer to any of these three key questions was affirmative, the participants were further asked about the frequency and duration of such symptoms, as well as about radiation of pain toward the neck, nocturnal symptoms, antacid relief, and use of antireflux medication, i.e., proton-pump inhibitors or histamine-receptor antagonists. Reflux was defined, a priori, as the occurrence at least weekly of either retrosternal pain with antacid relief, or heartburn with antacid relief, or radiation toward the neck, or regurgitation of sour fluid. Twins with symptoms occurring less often than weekly or without symptoms were denoted as not having reflux. Furthermore, all participants reporting reflux symptoms were asked to report the year of onset of their reflux symptoms.

Definition and assessment of the exposure: exogenous female sex hormones

The use of oral contraceptives and the duration of such use were prospectively assessed in the questionnaire in 1973. Information on previous or current use of HT and oral contraceptives was further derived from brand names reported by the study participants during the Screening Across the Lifespan Twin Study interviews. These participants were also asked at what age they started and stopped using the HT and the total duration of the use in years. Reported preparations, used either as HT or for contraception, included oral estrogens, oral progestins, combined estrogen and progestin preparations, transdermal estradiol, and estradiol implants.

Determination of zygosity

Zygosity was determined based on questions regarding childhood similarity included in the Screening Across the Lifespan Twin Study interview. This zygosity classification method has been validated and proven to be accurate in 99% of the twin pairs when compared with analyses of DNA markers.²²

Statistical analyses

Cross-sectional analyses—To obtain good statistical power in all analyses, we analyzed the risk of reflux symptoms with the exposure (HT and oral contraceptives) and the outcome assessed simultaneously, i.e., cross-sectionally.

Nested case control analyses—The disadvantage with the cross-sectional design described above is that it does not take time into account and therefore the risk of reversed causality is considerable. To encounter this we also used a nested case-control design with incidence density sampling. In incidence density sampling, controls are sampled throughout the course of the observation period, matching each control to the time of disease onset of its matched case.²⁴ One or more controls can be selected from the set of persons who were at risk at the time that the individual was identified as a case. Information about the time of onset of reflux symptoms and start and end of exposure made this design possible. Eligible as controls were participants without reflux symptoms at the time when reflux symptoms were first experienced by the index cases. Five control participants were selected for each case of reflux symptoms. Since data regarding oral contraceptives were already available in 1973, we did not conduct any nested case-control analysis for this exposure.

The analyses within the cross-sectional and nested case-control design were both performed as external control analyses. In addition, in the cross-sectional design we also performed monozygotic co-twin control analyses. In the external control analyses, twin individuals with reflux symptoms were compared with unrelated twin individuals without symptoms, whereas in the co-twin control analyses, monozygotic twin pairs who were discordant, i.e. one twin had reflux symptoms and the other did not, were considered as matched pairs.²⁵ The additional advantage achieved in monozygotic co-twin control analyses compared with traditional matched case-control analyses is that monozygotic twins are genetically matched, since they

share 100% of their genes and they are perfectly age-matched. Furthermore, twins usually share their early life environment, including nutrition and socioeconomic background. By comparing the results from the ordinary case-control analyses with those from the monozygotic co-twin control analyses it is possible to separate genetic and shared early environmental effects from other effects.

The HT use of estrogen and combined estrogen and progestin were first categorized into two groups: ever and never use. Estrogen therapy was further classified into use of estrogen alone and then into never use, current use and past use (>1 year before the onset of reflux symptoms). Progestin therapy was dichotomized into never use and use of progestin alone. The different HT exposed groups were then categorized according to duration of the use into three groups: never users, >0–4 years, or >4 years. Type of estrogen used was classified into conjugated estrogen and estradiol. Exposure to contraceptive pills was dichotomized into never and ever use. Such exposure was analyzed separately for data collected in the 1973 questionnaire and in the Screening Across the Lifespan Twin Study questionnaire in 1998–2002. Finally, we stratified the analyses according to three categories of body mass index (BMI, measured as weight divided by height squared in kg/m²).

Odds ratios (ORs) with 95% confidence intervals (CIs), obtained from multivariable logistic regression using the Generalized Estimating Equations model,²⁶ estimated the relative risk. A basic model included adjustment for age alone as a continuous variable. In a multivariable model, we evaluated all covariates that were considered to be plausible confounders. These included birth year (five categories, adjusted for age and calendar period effects), education (four categories), tobacco smoking (ever or never), coffee use (four categories), physical activity (four categories), and BMI (six categories). In the stratified analyses, BMI was categorized into three classes (<25; 25–30; or >30 kg/m²), based on the World Health Organization definitions of overweight and obesity.²⁷ Furthermore, asthma medications such as theophylline and β -adrenergic agonists, as well as nitroglycerines, anticholinergics, calcium channel blockers, and benzodiazepines have all been shown to have a possibly relaxing effect on the LES pressure. Therefore, we adjusted for calcium channel blockers, long-acting nitroglycerines, and anticholinergics. Since data on asthma medications was not available, we used the condition asthma as a proxy for asthma medications in our analyses. Since use of calcium channel blockers and anticholinergics did not change any of the risk estimates they were left out of the final model. Possible dose-response associations were examined using a trend test in the logistic regression, based on ordinal categories of the covariate tested. For the logistic regression models the GENMOD procedure of SAS software release 9.13 (SAS Institute, Cary, NC) was used. Relative risks were estimated in the form of ORs with 95 CIs, computed by conditional logistic regression in the PHREG procedure of SAS software. The potential interaction effect between BMI and HT was tested by introducing the cross-product of the two variables into the model.

RESULTS

Study participants

A total of 24,040 female twin individuals who participated in the Screening Across the Lifespan study from 1998–2002 were included in the cross-sectional study cohort. Of these, 4365 twins were defined as cases, i.e. as having reflux symptoms, and 17,321 as controls, without reflux symptoms. Information on reflux symptoms status was missing in 2354 participants (9.8%). Among all participating twins, 597 were discordant monozygotic twin pairs regarding reflux symptoms. Some characteristics of the study participants are presented in Table 1. The overall prevalence of reflux symptoms was 20.1%. The mean age was similar in cases and controls. The median age of onset of reflux symptoms was 45 years. Case participants were more overweight (BMI 25–30) or obese (BMI >30) than control participants (46.7% and 33.4%

respectively). Ever use of estrogen was more common in the case group (30.6%) than in the control group (21.7%).

In the nested case-control design, 2678 (61.4% of all cases) case twins and 13,390 control twins were included. The general characteristics of these participants were similar to those of the cross-sectional study cohort (data not shown).

Postmenopausal hormone therapy with estrogen and risk of reflux symptoms

The results from the cross-sectional logistic regression analyses of estrogen HT in relation to reflux symptoms are presented in Table 2. The risk of reflux symptoms was 32% higher in women who had ever used estrogen than in women who had never used such therapy (OR 1.32; 95% CI 1.18–1.47). On stratification for BMI the risk estimates for reflux symptoms slightly increased with a higher BMI, but the association also remained statistically significantly increased by 21% among women of normal weight (Table 2). An increased duration of estrogen use did not further increase the risk of reflux. When dividing ever users of estrogen into past and current use, those with a history of estrogen HT use at least one year before the onset of reflux had a seemingly higher risk of reflux than those using estrogen HT at the time for reflux onset (OR 1.68; 95% CI 1.21–2.17 and OR 1.28; 95% CI 1.14–1.43 respectively). The point estimates did not differ materially in the monozygotic co-twin control analyses, suggesting that genetic factors play a limited role in the observed associations (Table 2).

The analyses of the prospective nested case-control design also revealed an increased risk of reflux symptoms among women exposed to HT with estrogen (OR 1.44; 95% CI 1.15–1.80) compared to never users, and there was a similar pattern regarding the BMI-stratified analyses (data not shown).

Postmenopausal hormone therapy with progestin and risk of reflux symptoms

Women who had ever used progestin alone (no estrogen) showed a 48% increase in the risk of reflux symptoms compared to never users (OR 1.48; 95% CI 1.06–2.06). The risk of reflux symptoms increased only slightly with higher BMI (Table 2). There was no trend with regard to the duration of progestin use (Table 2). The point estimate of ever users was decreased in the monozygotic co-twin control analysis also, but the number of cases was too small to get reliable results (OR 1.14; 95% CI 0.41–3.15).

In the nested case-control analysis, however, no increased risk of reflux symptoms was found among ever users of progestin (OR 0.96; 95% CI 0.42–2.20).

Postmenopausal hormone therapy with combined estrogen and progestin and risk of reflux symptoms

The risk of reflux symptoms in women who had ever used combined HT was not increased (OR 0.99; 95% CI 0.87–1.13), consistent with the results from the monozygotic co-twin analyses (Table 2).

The association between combined HT and reflux seemed stronger in the nested case-control analysis (OR 1.44; 95% CI 1.07–1.93) than in the cross-sectional analysis, possibly indicating an effect of reversed causality.

Type of estrogen and risk of reflux symptoms

The risk of reflux was statistically significantly increased among women who had taken preparations containing estradiol as well as in women who had used conjugated estrogen. In the monozygotic co-twin control analysis the risk estimate of reflux symptoms was higher for use of conjugated estrogen than for estradiol (data not shown).

An increased risk remained in the nested case-control analysis (OR 1.40; 95% CI 1.08–1.82).

Oral contraceptives and risk of reflux symptoms

In total, 12,669 female twins both responded to the questionnaire from 1973 and also participated in the telephone interviews in 1998–2002, out of which 8663 were defined as controls and 1993 cases. Use of contraceptive pills as assessed in 1973 did not increase the risk of reflux symptoms (OR 1.07; 95% CI 0.93–1.23), and this risk estimate was higher in the monozygotic co-twin control analysis (Table 3).

Ever use of contraceptive pills as assessed in 1998–2002 entailed a similar lack of risk (OR 1.08; 95% CI 0.93–1.26). However, in the monozygotic co-twin control analysis there was a tendency towards an increased risk of reflux, both for women using oral contraceptives in 1973 and in 1998–2002. The effect on reflux symptoms by contraceptive pills was not affected by BMI in the stratified analyses in the external case-control design, but in the monozygotic co-twin control analyses there was a tendency toward a higher risk for reflux symptoms with higher BMI (Table 3).

No interaction was seen between BMI and the use of HT (data not shown), but the adjustment for BMI in the analyses regarding past and current use attenuated the risk estimates (Table 2). Generally, the results from those in the basic model, adjusted only for birth year, did not differ materially from the multivariable model (data not shown).

DISCUSSION

This nationwide Swedish population-based twin study provides evidence that use of postmenopausal estrogen therapy is associated with an increased risk of gastro-esophageal reflux symptoms, independently of heredity, body mass, tobacco smoking. The role of progestin HT, combined HT, and oral contraceptives is more uncertain.

The validity of the study needs careful consideration. Advantages include the population-based design, the large number of participants, and the possibility to adjust for all known biologically plausible confounders, including heredity. The use of two ways of doing the analyses, i.e. external control and co-twin analyses was based on specific advantages of each of these alternatives. The external control design enabled us to test our hypothesis with good statistical power, while the co-twin analyses made it possible to evaluate possible confounding by genetic and shared early environmental factors. Furthermore, the availability of age at onset of reflux symptoms made it possible to perform the prospective nested case-control analyses, which we used as a sensitivity analysis to confirm that our results were not due to reversed causality. Information bias was reduced by the fact that the specially trained professional interviewers who asked the questions in a standardized manner were kept unaware of the study hypothesis and were blinded for zygosity and the twin partner's response. The questions regarding reflux symptoms and use of exogenous female sex hormones were part of a larger questionnaire covering several other symptoms and medications, which diminishes the risk for recall bias. These factors taken together should ensure a relatively high degree of validity. Weaknesses include the cross-sectional design used in the main analyses. In this context, it is reassuring that the association between estrogen HT and reflux symptoms remained in our nested case-control analyses, allowing evaluation of temporal relations. The results of the nested case-control analyses support our findings in the cross-sectional analyses with one exception: the risk of reflux symptoms associated with progestin observed in the cross-sectional analysis disappeared in the nested case-control analysis, leaving some doubt about the relevance of progestin in relation to reflux symptoms. Since the findings regarding progestin HT were mixed, we prefer to interpret the results conservatively, i.e. as a lack of association. The monozygotic co-twin sample supported the overall findings, but the statistical power was

limited in these analyses. Using self-reported data, through questionnaires or structured telephone interviews, always introduces the risk of misclassification for exposures as well as outcome. Concerning GERD, there are many other underlying conditions, such as pulmonary, cardiac, malignant, musculoskeletal and psychological diseases that can produce GERD-like symptoms.²⁸ The concern of misclassification of reflux when using self-administered questionnaires based on symptoms only is allayed, however, by several studies showing that the symptoms used in our questionnaire are well validated as representing true reflux disease.^{29, 30} However, any remaining misclassification of the outcome would rather dilute the observed associations and should not explain our positive findings. The use of self-reported data on oral contraceptive use has been shown to have a high level of agreement compared to using pharmacy records,³¹ and the level of misclassification of this exposure should therefore be low. Although we adjusted our results for most biologically plausible confounders, residual and unknown confounding can never be excluded, especially when the risk estimates are as weak as those identified with regard to oral contraceptives. Another potential weakness is the risk of bias in the assessment of the HT exposure. Women who take HT are leaner, more educated and tend to exercise more than non-users,^{32–34} but these factors would be more likely to decrease the risk of reflux symptoms, and thus again suggesting that our risk estimates could have been underestimated.

Our results show that the positive association between HT and reflux was seemingly stronger among past users than among current users. One interpretation of these results is that the association is not confined to current use only, but rather indicates that previous exposure might actually be involved in the true etiology of the reflux disease. For example, HT might induce weight gain, which then either remains after discontinuation of the HT, or irreversibly changes the anatomy of the gastroesophageal junction, resulting in prolonged reflux symptoms. Although no strong interaction was found between BMI and past use of HT in our study, this hypothesis gains some support from the attenuation of the relative risk estimates after our adjustment for BMI. Although the literature is sparse, our finding of a moderately increased risk of reflux symptoms among women using estrogen HT is supported by two previous studies by our research group.^{14, 21} The mechanism hypothesized to account for the relation between estrogen and reflux is relaxation of the LES via nitric oxide. Estrogen has been shown to increase nitric oxide synthesis,³⁵ and nitric oxide, in turn, is the predominant relaxing transmitter substance of the LES.³⁶ Moreover, the weak association between oral contraceptives and reflux symptoms finds some support in a study from the 1970s, where it was shown that women using oral contraceptives showed decreased LES pressures.³⁷ This effect was seen during the phase of the menstrual cycle when the women in the study ingested progestin alone or progestin and estrogen combined. Furthermore, reflux symptoms are common among pregnant women, often starting in the first trimester^{38–40}, and after delivery the symptoms usually resolve spontaneously. For many years, it was believed that reflux symptoms during pregnancy were due to an increase in abdominal pressure caused by the gravid uterus. However, in a study by Van Thiel and Wald⁴¹ it was shown that the increased abdominal pressure was counteracted by a compensatory increase in the LES pressure. Thus, it has also been proposed that the mechanism in pregnancy is not a decreased basal LES pressure per se, but rather an altered response of the LES pressure to hormonal, physiological and pharmacological stimuli.⁴² Taken together, these studies suggest that during pregnancy, the increased occurrence of reflux symptoms might be explained by raised levels of female sex hormones.^{41–43} A paradox that still remains to be explained is that while obesity and reflux symptoms are almost equally common in men and women, the complications of gastroesophageal reflux such as Barrett's esophagus and esophageal adenocarcinoma are much more common in men. One hypothesis suggests that this could be due to sex differences in fat distribution, with abdominal obesity being more common in men.⁴⁴

If our findings are confirmed in future research, the clinical implications of the findings might be considered. Our results regarding oral contraceptives and reflux symptoms indicate at most a weak effect. Thus, this study cannot be used as a basis for any clinical recommendations regarding modern oral contraceptives. In contrast, our results regarding estrogen HT and risk of reflux symptoms reveal a stronger and more consistent association, and the HT currently in use contains daily doses of estrogen that are ten times higher than the current daily dose of oral contraceptives. Therefore, if our findings are established in future research, the postmenopausal woman considered for HT should be informed that reflux symptoms is a possible side effect and if a woman who uses HT develops reflux symptoms, a treatment alternative to antireflux medication, might be an attempt to stop using HT, particularly among obese women.

In conclusion, this nationwide and population-based twin study in Sweden suggests that the use of postmenopausal estrogen therapy is an independent risk factor for reflux symptoms.

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Abbreviations

BMI	body mass index
CI	confidence interval
GERD	gastro-esophageal reflux disease
HT	postmenopausal hormone therapy
LES	lower esophageal sphincter
OR	odds ratio

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Table 1

Characteristics of study population

	Control participants		Case participants		Total	
	Number	(%)	Number	(%)	Number	(%)
Total	17,321	(79.9)	4,365	(20.1)	21,686	(100.0)
Age (years)						
Median (Range)	58	(42–104)	56	(42–96)	57	(42–104)
Body mass index (kg/m²)						
<25	10,171	(58.7)	2192	(50.2)	12,363	(57.0)
25–30	4645	(26.8)	1511	(34.6)	6156	(28.4)
>30	1144	(6.6)	529	(12.1)	1673	(7.7)
Missing	1361	(7.9)	133	(3.0)	1494	(6.9)
Postmenopausal estrogen therapy						
Never	10,670	(61.6)	2347	(53.8)	13,017	(60.0)
Ever	3757	(21.7)	1335	(30.6)	5092	(23.5)
Missing	2894	(16.7)	683	(15.6)	3577	(16.5)
Monozygotic twins	Neither reflux symptoms (pair)		Discordant reflux symptoms (pair)		Concordant for reflux symptoms(pair)	
	1703		597		157	
					Missing data for one of the twins(individual)	
					1167	

Table 2
Associations between postmenopausal hormone therapy and risk of reflux symptoms among female twins in the Swedish Twin Registry. *

	Cross-sectional: External control comparison		Cross-sectional: monozygotic control comparison	
	Controls (n)	Cases (n)	Adjusted [†] OR (95% CI)	Twin pairs (n) Univariate OR (95% CI)
Ever used estrogen				
No	10,670	2347	1.00 (reference)	423 1.00 (reference)
Yes	3757	1335	1.32 (1.18–1.47)	1.28 (0.87–1.89)
Current use	3418	1191	1.28 (1.14–1.43)	1.29 (0.87–1.92)
Past use	339	144	1.68 (1.31–2.17)	1.23 (0.51–2.94)
Never-user[‡]				
BMI <25	2420	674	1.00 (reference)	178 1.00 (reference)
BMI 25–30	1031	472	1.21 (1.04–1.41)	1.21 (0.66–2.22)
BMI >30	243	159	1.40 (1.17–1.67)	1.40 (0.44–4.41)
Duration of ever estrogen therapy				
Never-user	10,670	2347	1.00 (reference)	414 1.00 (reference)
>0–4 years	1974	710	1.33 (1.17–1.52)	1.35 (0.87–2.08)
>4 years	1690	584	1.28 (1.11–1.47)	1.33 (0.78–2.28)
Ever used progestin alone				
No	14,238	3603	1.00 (reference)	423 1.00 (reference)
Yes	189	79	1.48 (1.06–2.06)	1.14 (0.41–3.15)
Never-user[‡]				
BMI <25	129	37	1.00 (reference)	178 1.00 (reference)
BMI 25–30	44	26	1.27 (0.79–2.04)	2.00 (0.37–10.9)
BMI >30	14	15	1.44 (0.80–2.60)	2.00 (0.18–22.1)
Duration of therapy with progestin alone				
Never-user	14,238	3603	1.00 (reference)	422 1.00 (reference)
>0–4 years	125	51	1.50 (0.99–2.25)	5.00 (0.58–42.8)
>4 years	48	19	1.30 (0.65–2.61)	0.33 (0.07–1.65)
Ever used combined estrogen and progestin				
No	12,442	3031	1.00 (reference)	423 1.00 (reference)
Yes	1985	651	0.99 (0.87–1.13)	0.80 (0.50–1.27)
Never-user[‡]				
BMI <25	1324	343	1.00 (reference)	178 1.00 (reference)
BMI 25–30	538	227	0.93 (0.77–1.12)	1.07 (0.53–2.16)
BMI >30	102	68	1.01 (0.81–1.26)	1.00 (0.25–4.00)
Duration of ever combined estrogen and progestin therapy				
Never-user	12,442	3031	1.25 (0.82–1.92)	2.00 (0.18–22.1)
>0–4 years	1081	367	1.00 (reference)	420 1.00 (reference)
>4 years	859	270	1.05 (0.89–1.24)	0.85 (0.49–1.47)
			0.92 (0.76–1.11)	0.78 (0.41–1.48)

* OR denotes odds ratio and CI confidence interval.

[†] In the multivariable analyses, the covariates tobacco smoking, coffee, educational level, physical activity, asthma, long-acting nitroglycerines and BMI from the Screening Across the Lifespan Twin Study were included. BMI was excluded from the model in the BMI-stratified analyses.

[‡] The reference category for each analyzed BMI stratum was based on hormone therapy never-users in the same stratum.

Table 3
Associations between use of contraceptive pills and risk of reflux symptoms among female twins in the Swedish Twin Register*

	Cross-sectional: External control comparison			Cross-sectional: monozygotic control comparison	
	Controls (n)	Cases (n)	Adjusted OR (95%CI) [†]	Discordant pairs (n)	Univariate OR (95%CI)
Ever contraception pill use in 1973					
No	3769	911	1.00 (reference)	425	1.00 (reference)
Yes	3725	1082	1.07 (0.93–1.23)		1.33 (0.91–1.96)
Never-user [‡]			1.00 (reference)		1.00 (reference)
BMI <25	2415	582	1.16 (0.96–1.40)	202	1.56 (0.83–2.93)
BMI 25–30	1013	354	1.02 (0.81–1.28)	64	2.00 (0.68–5.85)
BMI >30	226	126	0.89 (0.59–1.36)	15	0.50 (0.05–5.51)
Ever contraception pill use in 1998–2002					
No	12,678	3177	1.00 (reference)	423	1.00 (reference)
Yes	1749	505	1.08 (0.93–1.26)		1.34 (0.83–2.17)
Never-user [‡]			1.00 (reference)		1.00 (reference)
BMI <25	1220	299	1.16 (0.96–1.41)	178	1.46 (0.72–2.96)
BMI 25–30	406	161	0.97 (0.74–1.28)	56	2.50 (0.49–12.9)
BMI >30	94	39	1.00 (0.59–1.70)	25	–

* OR denotes odds ratio and CI confidence interval.

[†] In the multivariable analyses, the covariates tobacco smoking, coffee, educational level, physical activity, asthma, long-acting nitroglycerines and BMI from the Screening Across the Lifespan Twin Study were included. BMI was excluded from the model in the BMI-stratified analyses.

[‡] The reference category for each analyzed BMI stratum was based on hormone therapy never-users in the same stratum.