









ORIGINAL ARTICLE

Endogenous sex hormones and risk of venous thromboembolism in young women

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Abstract

Background: The risk of venous thromboembolism (VTE) in young women can predominantly be attributed to exogenous hormone use. The influence of (abnormalities in) endogenous sex hormones, as in polycystic ovary syndrome (PCOS) or primary ovarian insufficiency (POI), on VTE risk is uncertain.

Objectives: To assess the association between endogenous sex hormone levels and VTE risk.

Methods: Women aged ≤ 45 years from the MEGA case-control study who provided a blood sample in the absence of exogenous hormone exposure or pregnancy were included. Sex hormone-binding globulin (SHBG), estradiol, follicle-stimulating hormone (FSH) and testosterone were measured. The free androgen index (FAI) and estradiol to testosterone ratio (E:T) were calculated. VTE risk was assessed according to quartiles (Qs) of levels and clinical cut-offs as proxies for PCOS (FAI > 4.5) and POI (FSH > 40 U/L). Logistic regression models were used to estimate adjusted odds ratios (ORs) with 95% confidence intervals (CIs).

Results: Six hundred and sixty-five women (369 cases; 296 controls) were eligible for the analyses. Testosterone and FSH levels, E:T and POI (FSH > 40 U/L vs FSH \leq 40 U/L) were not associated with VTE risk. For estradiol, VTE risk was increased with levels in Q4 vs Q1 (OR 1.6; 95% CI 1.0-2.5). There was a dose-response relationship between SHBG levels and VTE risk, with the highest OR at Q4 vs Q1: 2.0 (95% CI 1.2-3.3). FAI > 4.5 (PCOS proxy) vs FAI \leq 4.5 was associated with increased VTE risk (OR 3.3; 95% CI 0.9-11.8).

Conclusions: Estradiol, SHBG and FAI were associated with VTE risk, suggesting a role for endogenous sex hormones in the pathophysiology of VTE in young women.

KEYWORDS

hormones, polycystic ovary syndrome, premature ovarian failure, venous thromboembolism, women

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1 | BACKGROUND

Venous thromboembolism (VTE) includes both deep vein thrombosis and pulmonary embolism, and is a potentially life-threatening disease. After myocardial infarction and stroke, it is the most frequent cardiovascular disease worldwide.¹ The incidence of VTE is higher in women than in men up until the age of 50 years.² This can mainly be attributed to oral contraceptive use and pregnancy, which strongly increase VTE risk.³⁻⁵ In men, there are reports implicating testosterone therapy with an increased VTE risk.⁶ Endogenous hormones also seem to play an important role in VTE risk, as is exemplified by the increased VTE risk observed during pregnancy.^{7,8} In addition, female-specific disorders involving abnormalities in endogenous sex hormone levels, such as primary ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS) have been associated with (arterial) cardiovascular diseases.⁹⁻¹³ In a Danish study, women with PCOS had a 2-fold increased VTE risk as compared with healthy controls.¹¹ In another Danish cohort study, there no association was observed between overall estrogen and testosterone levels and VTE risk; however, there were only a few events in premenopausal women, and time from the blood draw to the event was several years.¹⁴ Further studies on endogenous sex hormone levels and VTE risk are scarce, and often concern men or postmenopausal women.¹⁵⁻¹⁷ Therefore, we set out to explore the association between endogenous sex hormone levels, both overall and as proxies for POI and PCOS, and VTE risk in women aged up to the age of 45 years.

2 | METHODS

2.1 | Design and study population

For this study we used data from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study. The MEGA study is a large population based case-control study, the design of which has been described in detail previously.^{18,19} In brief, between March 1999 and September 2004, consecutive patients with a first VTE aged <70 years were recruited at one of six anticoagulation clinics in the Netherlands. All VTE diagnoses were confirmed by objective imaging tests, e.g., Doppler ultrasonography for deep vein thrombosis, and ventilation perfusion scan, computed tomography or pulmonary angiogram for pulmonary embolism. All diagnoses were cross-validated on the basis of medical records or hospital discharge letters, which were obtained from general practitioners or hospitals. Partners of patients were asked to participate as controls. An additional control group was recruited by random digit dialing. All participants were asked to complete a thorough questionnaire on VTE risk factors. Furthermore, participants were asked to donate a blood sample and DNA. At the time of blood sampling, information on current pregnancy or oral contraceptive use was also collected. The time of participation or index date was the date of diagnosis for cases (VTE patients) and for their respective partners participating as controls. For random digit dial controls,

Essentials

- The influence of endogenous sex-hormones on venous thromboembolism (VTE) risk is largely unknown.
- We assessed the association between several sex hormones and VTE risk in young women.
- In a case-control study VTE risk was assessed for quartiles of hormone levels and cut-off values.
- Estradiol, sex-hormone binding globulin and free androgen index were associated with VTE risk.

the index date was the date of completion of the questionnaire. All participants of the MEGA study gave written informed consent. The MEGA study was approved by the Medical Ethics Committee of the Leiden University Medical Center in the Netherlands.

For the present study, we included women up to the age of 45 years who had not been pregnant in the year before the index date. Of these, we included only participants who provided a blood sample, were not pregnant, and did not use any hormones (e.g., oral contraceptives or hormone replacement therapy) at the time of blood draw.

2.2 | Blood samples and laboratory measurements

Cases were asked to donate a blood sample at least 3 months after cessation of anticoagulant therapy. For patients who were receiving long-term anticoagulant therapy, blood was drawn after 1 year. The partner controls were invited to donate a blood sample at the same moment as their partners (i.e., the cases with a VTE). The random digit dial control group was invited for blood sampling around the moment of completion of the questionnaire. All collected blood samples were drawn into standard sodium citrate-containing vacuum tubes, and were centrifuged for 10 minutes at 4°C. Aliquoted plasma was then stored at -80°C.

Estradiol and follicle-stimulating hormone (FSH) were analyzed on a Modular E170 immunoanalyzer (Roche Diagnostics), and sex hormone-binding globulin (SHBG) levels were measured on an Immulite 2000 XPi immunoanalyser (Siemens Diagnostics). Total testosterone levels were measured by the use of liquid chromatography-mass spectrometry (LC-MS). All laboratory measurements were performed without knowledge of the case-control status of the samples.

2.3 | Free androgen index

The free androgen index (FAI) is commonly used in clinical practice to assess androgen status, and is calculated on the basis of based on total testosterone and SHBG levels.²⁰

$$\text{Free androgen index} = 100 * \frac{\text{Total testosterone (nmol/L)}}{\text{Sex hormone binding globulin (nmol/L)}}$$

The FAI is the ratio between total testosterone and SHBG concentration, and thus has no units.²⁰

2.4 | Proxy for POI and PCOS

As data for the full clinical criteria were not available, a proxy for POI was assigned on the basis of FSH levels of >40 U/L.²¹ A proxy for PCOS was assigned on the basis of an FAI of >4.5.^{10,22,23}

2.5 | Estradiol to testosterone ratio

The magnitude of VTE risk in oral contraceptive users is dependent on the dose of estrogen and the type of progestogen. As the estrogen and the progestogen in the are considered to reflect the “estrogenicity” and the “androgenicity” of the preparation,²⁴ we investigated the association between the endogenous estradiol to testosterone ratio (E:T) and VTE risk. E:T was calculated as follows:

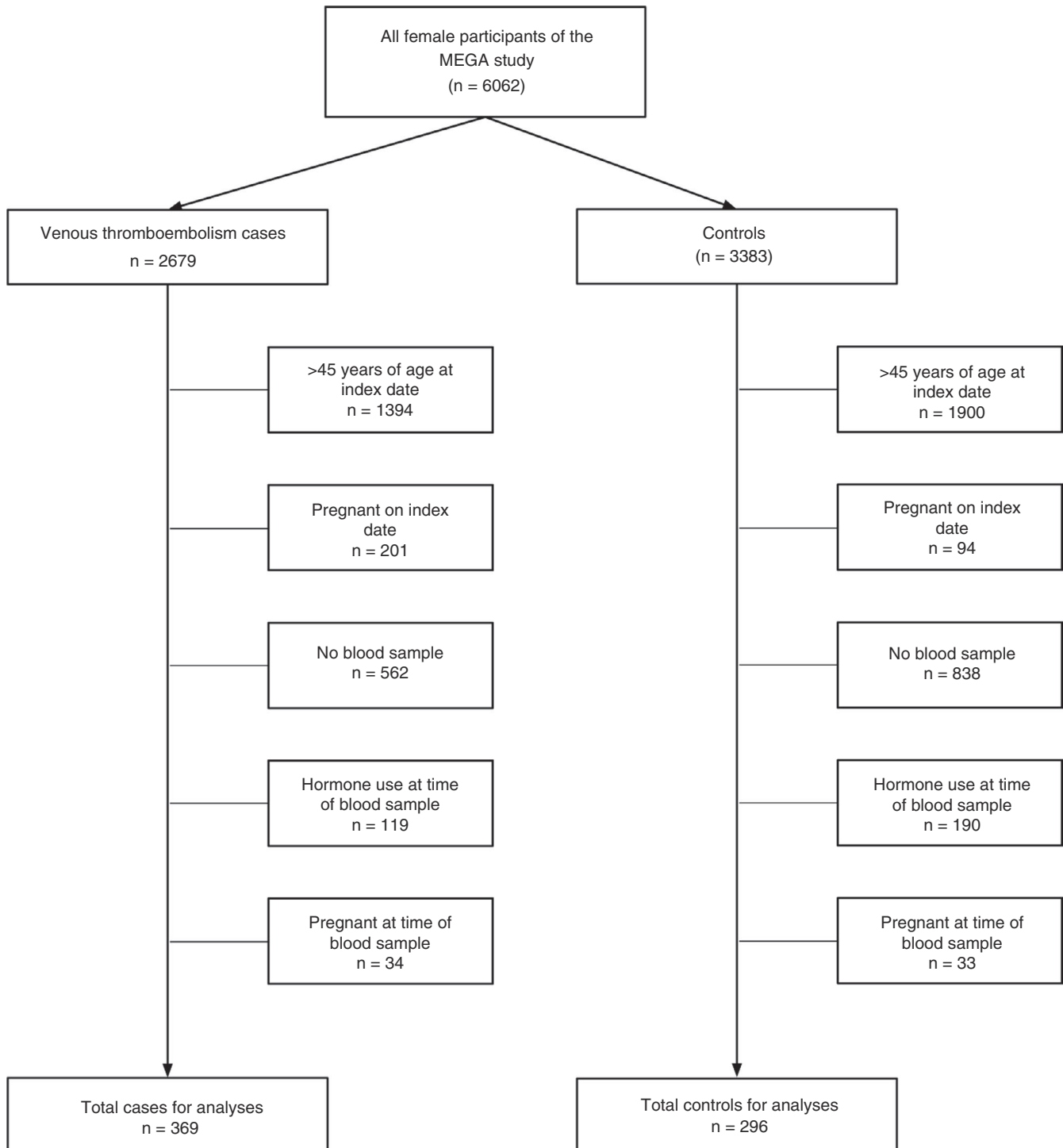


FIGURE 1 Flowchart of the study population

$$\text{Estradiol to testosterone ratio} = \frac{\text{Estradiol (pmol/L)}}{\text{Total testosterone (nmol/L)}}$$

2.6 | Statistical analyses

First, logistic regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for quartiles of hormone levels (based on levels in the controls) and VTE risk. This was performed in a similar manner for quartiles of the free androgen index. Second, ORs with 95% CIs were estimated for the proxies for POI (vs levels in the first quartile and vs no POI) and PCOS (vs values in the first quartile and vs no PCOS) and VTE risk. The ORs were adjusted for age, body mass index (BMI) (kg/m²) and current smoking (yes vs no).

3 | RESULTS

3.1 | Study population

Figure 1 is a flowchart of the selection of the study population. A total of 6062 women participated in the MEGA study. Of these, 2679 (44%) were cases (i.e., patients with a VTE) and 3356 (56%) were controls, recruited either as partners or by random digit dialing. Of these, women aged more than 45 years (1394 cases and 1900 controls) and women who were pregnant (201 cases and 94 controls) in the year before the index date were excluded. We further excluded participants without a blood sample (562 cases and 838 controls) and participants who used hormones (i.e., oral contraceptives or hormone replacement therapy; 119 cases and 190 controls) or who were pregnant during blood sampling (34 cases and 33 controls). Hence, a total of 369 cases and 296 controls were eligible for the analyses.

The clinical characteristics of this study population are shown in Table 1. On average, the cases, with a mean age of 34.6 years (standard deviation [SD] 7.0 years), were slightly younger than the controls, with a mean age of 36.5 years (SD 6.2). The cases had, on average, a higher BMI (26.7 [SD 5.4]) than the controls (24.8 [SD 4.5]). In addition, current smoking was more common among the cases (43.3%) than among the controls (32.2%).

3.2 | Overall hormone levels

The ORs with 95% CIs for quartiles of hormone levels and VTE risk are shown in Table 2. There was no apparent association between levels of total testosterone and FSH and risk of first VTE. At the fourth quartile of estradiol levels, there was a somewhat increased VTE risk (OR 1.6; 95% CI 1.0-2.5) as compared with levels in the first quartile. For SHBG levels, there was a dose-response association, whereby, with increasing quartiles of hormone levels, the OR for VTE increased up to 2.0 (95% CI 1.2-3.3) at the fourth quartile as compared with the first.

3.3 | Proxy for POI and PCOS

FSH levels of ≥ 40 U/L, as a proxy for POI, were not associated with VTE risk (Table 2). As compared with the first quartile of FSH levels

TABLE 1 Clinical characteristics of the study population

	Cases with VTE	Controls
Total (n)	369	296
Age (years), mean \pm SD	34.6 \pm 7.0	36.5 \pm 6.2
BMI (kg/m ²), mean \pm SD	26.7 \pm 5.4	24.8 \pm 4.5
Current smoking, n (%)	159 (43.3)	95 (32.2)
Deep vein thrombosis, n (%)	218 (59.0)	NA
Pulmonary embolism, n (%)	151 (41.0)	

Abbreviations: BMI, body mass index; NA, not applicable; SD, standard deviation; VTE, venous thromboembolism.

(<4.5 U/L), this yielded an OR of 0.7 (95% CI 0.2-2.1). As compared with levels of <40 U/L (i.e., no POI), this OR was 0.8 (95% CI 0.3-2.1).

Values for the FAI and VTE risk are shown in Table 2. There was an inverse dose-response relationship between quartiles of the FAI and VTE risk, with the lowest risk at the fourth quartile (OR 0.5; 95% CI 0.3-0.8). In contrast, at higher values, i.e., an FAI of >4.5 as a proxy for PCOS, there was an association with increased VTE risk. The ORs for this were 2.3 (95% CI 0.6-9.1) as compared with values at the first quartile, and 3.3 (95% CI 0.9-11.8) as compared with values of ≤ 4.5 (i.e. no PCOS). To further explore the shape of the association, in post hoc analyses we used more arbitrary categories for the FAI; <1.5, 1.5-3, 3-4.5 (reference), and >4.5. On the basis of these analyses, there seemed to be a U-shaped relationship, with increased risk for both the lowest and highest FAI categories. In the fully adjusted model, the ORs for this were 2.3 (95% CI 1.2-4.3), 1.6 (95% CI 2.9-0.8) and 5.4 (95% CI 1.3-22.0) for FAIs of <1.5, 1.5-3.0 and >4.5, respectively, as compared with an FAI of 3-4.5 (Figure 2).

3.4 | Estradiol to testosterone ratio

We observed no association between quartiles of E:T and VTE risk (Table 3).

4 | DISCUSSION

We investigated the association between endogenous sex hormone levels and risk of a first VTE in women aged up to 45 years. Levels of total testosterone and FSH were found not to be associated with VTE risk. For estradiol levels, there was an increased risk at levels in the fourth quartile as compared with those in the first quartile. We observed a dose-response relationship for SHBG levels and VTE risk. For the FAI, we observed a U-shaped relationship, whereby VTE risk was increased at low and high values. Especially at an FAI of >4.5, indicative of PCOS, VTE risk was increased.

Our study has several strengths. First, a large number of young women with a first VTE, for whom detailed information on risk factors and a blood sample was available, could be included in the study. Second, the time frame between the event and the time of blood donation was short, strengthening the assumption that the measured

TABLE 2 Quartiles (based on levels/values in the controls) of endogenous sex hormone levels and the free androgen index and venous thromboembolism risk

	Cases (n)	Controls (n)	OR (95% CI)	OR ^a (95% CI)	OR ^b (95% CI)
Estradiol (pmol/L)					
1st quartile, <186.2	82	73	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 186.2-366.3	103	73	1.3 (0.8-1.9)	1.3 (0.8-2.1)	1.4 (0.9-2.2)
3rd quartile, 366.3-574.0	80	73	1.0 (0.6-1.5)	1.0 (0.7-1.7)	1.1 (0.7-1.7)
4th quartile, >574.0	103	73	1.3 (0.8-1.9)	1.5 (0.9-2.3)	1.6 (1.0-2.5)
Total testosterone (nmol/L)					
1st quartile, <0.69	79	76	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 0.69-0.90	95	72	1.3 (0.8-2.0)	1.2 (0.8-1.9)	1.2 (0.8-1.9)
3rd quartile, 0.90-1.17	82	72	1.1 (0.7-1.7)	0.9 (0.5-1.4)	0.8 (0.5-1.4)
4th quartile, >1.17	106	71	1.4 (0.9-2.2)	1.1 (0.7-1.8)	1.0 (0.6-1.7)
Follicle-stimulating hormone (U/L)					
1st quartile, <4.5	99	75	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 4.5-6.3	113	77	1.1 (0.7-1.7)	1.0 (0.7-1.6)	1.1 (0.7-1.6)
3rd quartile, 6.3-8.7	71	70	0.8 (0.5-1.2)	0.8 (0.5-1.3)	0.8 (0.5-1.2)
4th quartile, >8.7	85	71	0.9 (0.6-1.4)	1.1 (0.7-1.7)	1.1 (0.7-1.7)
Proxy for POI, ≥40	7	10	0.5 (0.2-1.5)	0.8 (0.3-2.3)	0.7 (0.2-2.1)
Sex hormone-binding globulin (nmol/L)					
1st quartile, <42.7	88	74	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 42.7-57.2	87	74	1.0 (0.6-1.5)	1.3 (0.8-2.0)	1.2 (0.8-1.9)
3rd quartile, 57.2-78.4	101	74	1.2 (0.7-1.8)	1.9 (1.2-3.0)	1.8 (1.1-2.9)
4th quartile, >78.4	93	73	1.1 (0.7-1.7)	2.0 (1.2-3.2)	2.0 (1.2-3.2)
Free androgen index					
1st quartile, <1.07	100	73	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 1.07-1.55	86	74	0.8 (0.6-1.3)	0.7 (0.5-1.1)	0.7 (0.4-1.1)
3rd quartile, 1.55-2.27	88	73	0.9 (0.6-1.4)	0.6 (0.4-1.0)	0.6 (0.4-1.0)
4th quartile, >2.77	92	73	0.9 (0.6-1.4)	0.5 (0.3-0.8)	0.5 (0.3-0.8)
Proxy for PCOS, >4.5	19	3	4.6 (1.3-16.2)	2.2 (0.6-8.8)	2.3 (0.6-9.1)

Abbreviations: CI, confidence interval; OR, odds ratio, PCOS, polycystic ovary syndrome; POI, primary ovarian insufficiency.

^aAdjusted for age and body mass index.

^bAdjusted for age, body mass index, and smoking.

hormone levels resemble the situation before the time of the event. Finally, we measured testosterone levels, which are generally low in young women, by means of LC-MS, which is more accurate than conventional immunoassays.²⁰

An important limitation of our study is that we used hormone levels to assign proxies for POI and PCOS, and could not employ the full clinical criteria.^{10,21} Moreover, owing to the study's case-control design, although the blood draw took place only several months from the event, it took place afterwards. Therefore, the results could be susceptible to reverse causation, a situation in which the result of a disease is mistaken for the cause of the disease. For this reason, we included only women who did not use hormones (and were not pregnant) at the time of blood draw, ensuring that the results were not affected by exogenous hormone use or pregnancy. In addition, it is unlikely that the differences in hormonal levels between the cases and controls resulted from the VTE itself.

Our observations on estradiol and total testosterone are mostly in line with a Danish population-based cohort study, in which, after a follow-up of several years, no association was found between estradiol or total testosterone levels and VTE risk.¹⁴ This study was limited, however, by a small number of events in premenopausal women ($n = 58$) and a long period of several years between the measurement of the hormone levels and the events.¹⁴ In our study, we were able to confirm these findings in a large group of young women with VTE and a limited time between blood draw and the actual event (i.e., only months instead of multiple years), strengthening the assumption that, in our study, the studied hormone levels resemble the situation just before and at the time of the event. In addition, we observed an, albeit small, 1.6-fold increased VTE risk at the fourth quartile of estradiol levels, whereby the direction of the effect was similar in all models, but only in the fully adjusted model did the 95% CI not cross unity. An increased VTE risk with higher estradiol levels could be

biologically plausible. Other situations in which estrogen levels are increased, e.g., during oral contraceptive use and during pregnancy, are also associated with an increased VTE risk, presumably because of procoagulant changes.^{4,22,25-28} For pregnancy, these procoagulant changes are evolutionarily beneficial, as they could reduce blood loss during pregnancy and especially after delivery.⁸ It could thus be the case that increases in endogenous levels outside of pregnancy elicit similar (albeit to a smaller extent) procoagulant effects, which may result in the observed increased VTE risk.

We observed a dose-response association between SHBG levels and VTE risk, up to a 2-fold risk in the highest quartile of levels. It should be noted that only in the adjusted models did the 95% CI not cross unity, suggesting that age, BMI and smoking are apparently important confounders. In a previous study in women also not using hormonal contraceptives, SHBG levels were also associated with VTE risk.²⁹ However, in this study, six single-nucleotide polymorphisms in the *SHBG* gene were not associated with VTE risk, suggesting that SHBG levels constitute a marker of the risk but not a causal factor.²⁹ The findings from our study confirm the association between SHBG levels and risk of first VTE, outside of oral contraceptive use. The biological mechanism that explains this association is largely unknown. SHBG is a carrier protein of mostly estradiol and testosterone, and

therefore contributes to the regulation of bioavailability of these sex hormones.^{30,31} In oral contraceptive users, SHBG has previously been identified as a marker of thrombotic risk in several studies.^{5,24,32,33} Here, it was postulated that both the dose of estrogen and the type of progestogen determine changes in SHBG levels.²⁴ In this way, SHBG levels were proposed to reflect the total "estrogenic" effect of estrogen and the total "antiestrogenic" (or androgenic) effect of progestogen.²⁴ This hypothesis may also apply to endogenous sex hormone levels, whereby the SHBG level could reflect the balance between the endogenous estrogens and androgens. In order to test this hypothesis, we calculated E:T and estimated VTE risk for quartiles of its values. Here, we observed no association between this ratio and VTE risk, although the 95% CIs were wide. In oral contraceptive users, the type of progestogen is a strong determinant of VTE risk.^{24,27} As we had no information on endogenous progestogen levels, we could not assess this association, which could also be an important determinant of the association between SHBG and VTE risk. Regardless of the underlying mechanism, SHBG is a VTE risk marker of interest, also outside of oral contraceptive use.

Data on the FAI and VTE risk are limited. In a population-based cohort from the United States, VTE risk was 2-fold higher (hazard ratio 2.12, 95% CI 1.41-3.24) in women with PCOS using combined oral contraceptives than in contraceptive users without PCOS. The hazard ratio in women with PCOS not using oral contraceptives was 1.63 (95% CI 1.13-2.34) as compared with matched controls.³⁴ This is in line with the results from a Danish registry, in which women with PCOS were reported to have a 2-fold increased VTE risk.¹¹ In addition, a high FAI itself has been associated with cardiovascular risk factors in both premenopausal and postmenopausal women.³⁵ Our study allowed a robust confirmation of the results of these studies, as we found that an FAI of >4.5, indicating PCOS, was associated with an increased VTE risk as studied in a large group of young women with VTE. Nevertheless, as the lower limit of the 95% CI just crossed unity, a type I error cannot be excluded. As we suspected a U-shaped relationship, we performed a post hoc analysis with post hoc-defined categories with a middle category as reference (3.0-4.5), to capture this relationship, which we did indeed observe. An explanation for this observation requires consideration of the determinants of the FAI, i.e., SHBG and total testosterone. As high SHBG levels yield a

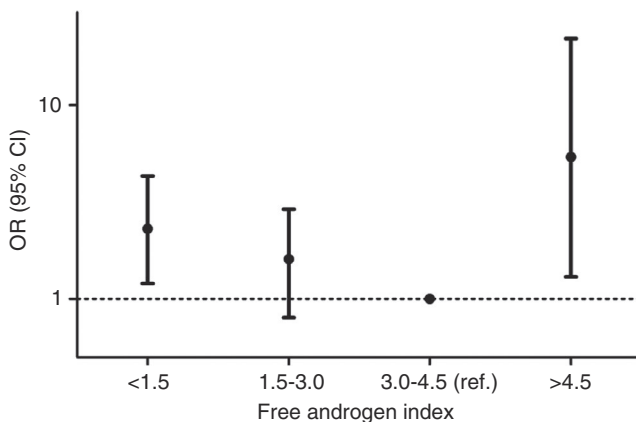


FIGURE 2 Free androgen index and risk of venous thromboembolism in women up to the age of 45 years. Estimates are adjusted for age, body mass index and smoking

	Cases (n)	Controls (n)	OR (95% CI)	OR ^a (95% CI)	OR ^b (95% CI)
Estradiol (pmol/L) to testosterone (nmol/L) ratio					
1st quartile, <231.2	104	72	1 (reference)	1 (reference)	1 (reference)
2nd quartile, 231.2-380.9	66	73	0.6 (0.4-1.0)	0.8 (0.5-1.2)	0.8 (0.5-1.3)
3rd quartile, 380.9-690.0	117	73	1.1 (0.7-1.7)	1.3 (0.9-2.1)	1.4 (0.9-2.1)
4th quartile, >690.0	82	72	0.8 (0.5-1.2)	1.1 (0.7-1.7)	1.2 (0.8-1.9)

TABLE 3 Quartiles (based on values in the controls) of the estradiol to testosterone ratio and venous thromboembolism risk

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and body mass index.

^bAdjusted for age, body mass index, and smoking.

low FAI, this association could be explained by high SHBG levels, as discussed above. Regarding testosterone, the FAI is a marker of androgen status, whereby high values indicate hyperandrogenism.²³ Androgen excess in women, which is the case in PCOS, has been associated with metabolic disturbances that give rise to increased levels of both inflammatory and procoagulant markers.^{36–38} These changes may explain the increased VTE risk that we observed, beyond high BMI, smoking, and hormone use. Moreover, women with PCOS have also been found to have a higher prevalence of non-alcoholic fatty liver disease than women without the syndrome.^{36,39} Non-alcoholic fatty liver disease is associated with increased procoagulant levels, which may also contribute to an increased VTE risk.^{40,41}

Although they are not directly applicable to clinical practice at present, the results of our study are relevant and may contribute to future risk assessment strategies for VTE. Estradiol and SHBG levels and a diagnosis of PCOS or the FAI may prove to be important markers of increased risk. For example, in women with PCOS, in whom important VTE risk factors such as hormonal contraceptive use and obesity are often clustered, risk assessment may be guided by future models including these markers.

In conclusion, we were able to study the levels of endogenous sex hormones measured close to the time of a first VTE in a large group of young women. Total testosterone and FSH levels were not associated with VTE risk in women aged 45 years or less. Estradiol levels, SHBG levels and the FAI indicating hyperandrogenism and as a marker for PCOS were associated with an increased VTE risk. These findings support a role for endogenous sex hormones in the underlying mechanism of VTE. In addition, estradiol levels, SHBG levels and hyperandrogenism (indicated by an FAI of >4.5) are promising potential indicators of VTE risk.

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CONFLICT OF INTERESTS

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AUTHOR CONTRIBUTIONS

L. J. J. Scheres, S. Middeldorp and S. C. Cannegieter designed the research and interpreted the data. L. J. J. Scheres performed the analyses and wrote the manuscript. F. R. Rosendaal designed and performed the MEGA study. B. E. P. B. Ballieux coordinated the laboratory analyses. A. van Hylckama-Vlieg, B. E. P. B. Ballieux, B. C. J. M. Fauser, F. R. Rosendaal, S. Middeldorp and S. C. Cannegieter critically revised the manuscript. All authors take responsibility for the interpretation of the data and critical revision of the manuscript for important intellectual content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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