Endogenous oestradiol as a positive correlate of plasma fibrinogen among older postmenopausal women: a population-based study (the Three-City cohort study).

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Serum estradiol as a positive correlate of plasma fibrinogen among **older** postmenopausal women. A population-based study (The Three-City cohort study)

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Abstract

Background. Plasma fibrinogen is a strong predictor of ischemic arterial disease in women. **Sex steroid hormones** including hormone therapy may play an important role in the development of cardiovascular disease. However, whether or not endogenous **sex steroid hormones** influence the plasma fibrinogen concentrations among postmenopausal women remains unclear.

Objectives. To investigate the association of plasma fibrinogen levels with endogenous **sex steroid hormones** and SHBG among postmenopausal women.

Methods. We used data from the French prospective Three-City cohort study which included **9,294** non-institutionalized men and women over 65 years. Total 17β-estradiol (E2, pg/ml), total Testosterone (T, ng/ml), SHBG (nmol/l) and fibrinogen (g/l) were measured in stored **plasmas in a subcohort of 602 randomly selected postmenopausal women who used neither hormone medication nor anticoagulation therapy**. Multivariate linear regression models were used to estimate the regression coefficients assessed in fibrinogen unit by 1 SD increase in log-distribution of **sex steroid hormones** and SHBG. .

Results. E2 but neither T nor SHBG was positively associated with plasma fibrinogen levels (β=0.148, p<0.001). Adjustment for cardiovascular risk factors including diabetes made no substantial change to the results **(β=0.145** p<0.001). The association of fibrinogen with E2 was stronger among women with BMI over 25 kg/m² compared to those with normal weight **(β=0.156**, p<0.001 and **β=0.092**, p=0.02 respectively, p for interaction=0.04).

Conclusion. E2 emerges as a positive and independent correlate of plasma fibrinogen among postmenopausal women, especially in subjects who are overweight. These findings suggest a deleterious effect of endogenous estrogens on cardiovascular risk profile among postmenopausal women.

Key words: Fibrinogen, estradiol, testosterone, sex hormone binding globulin, postmenopausal women

Introduction

Fibrinogen, the fibrin clot precursor, plays a central role in the haemostatic process [1]. Elevated plasma fibrinogen levels have been shown to be associated with established cardiovascular risk factors [2]. In particular, plasma fibrinogen has been positively related to age and body-mass index (BMI) [2]. Plasma fibrinogen has emerged as a strong predictor of arterial disease among both men and women [3] and this association was recently found in a population-based study among elderly subjects [4].

Sex steroid hormones including both **hormone therapy** and endogenous **sex steroid hormones** may play an important role in the development of arterial disease. Despite compelling evidence for a cardioprotective effect of estrogens, randomized clinical trials have reported an increased risk of coronary heart disease among old women using **hormone therapy** as compared to non users [5]. In addition, it is now well demonstrated that **hormone therapy** increases the risk of stroke among postmenopausal women [6, 7]. By contrast, little is known on the association of endogenous sex steroid hormones with cardiovascular disease and results remain non significant [8-10].

The relationship between **sex steroid hormones** and plasma fibrinogen has been studied in some different contexts and results are conflicting. It has been shown that postmenopausal **hormone therapy** slightly affects fibrinogen levels [11] while the association of fibrinogen with endogenous **sex steroid hormones** among peri/postmenopausal women led to inconclusive results [12-15]. Nevertheless, an increase in plasma fibrinogen concentrations has been consistently observed during pregnancy, suggesting a positive and strong correlation between levels of circulating endogenous estrogens and plasma fibrinogen among young women [16-22]. In this context, we hypothesized that plasma levels of endogenous estradiol could be positively related to plasma fibrinogen levels. Therefore, we investigated the association of fibrinogen with total estradiol (E2), total testosterone (T) and Sex Hormone Binding Globulin (SHBG) among postmenopausal women included in the Three-City (3C) study, a large community based prospective cohort study.

Methods

Study population

This study is part of the 3C study, a large ongoing French prospective cohort which aimed to evaluate the risk of dementia attributable to vascular disorders. The study protocol was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre and each participant signed a written informed consent. A detailed methodology of the study has been previously described [23]. Briefly, 3,649 men and 5,645 women over 65 years registered on electoral rolls and not institutionalized were recruited in three French cities (Bordeaux, Dijon and Montpellier) between 1999 and 2001. Baseline data were collected by trained psychologists or nurses during a face to face interview using standardized questionnaires at home or at the study center. These data included information on socio-demographic characteristics, education, medical history, medication use, food consumption and alcohol and tobacco use. Systolic and diastolic blood pressure, weight and height were assessed during a physical examination.

Recently, a case-cohort study has been set-up from the 3C study to investigate the association of biological parameters with cardiovascular and dementia risk. **In practice, the population study consists of a random subsample of the original cohort (the subcohort) and all the incident cases outside the subcohort. The methodology has been described in detail [24]. The present analysis focused on women included in the subcohort (n=759). After exclusion of women who used hormone medication (n=120) or anticoagulation therapy (n=18) and subjects with a missing value regarding the fibrinogen measurement (n=19), this left 602 postmenopausal women.**

Baseline covariates

Smoking status was studied in three categories (never, past, current). Body mass index (BMI) was calculated by dividing the weight by height in meters squared and overweight was defined as BMI superior or equal to 25 kg/m². Glycemia status was considered as "diabetes" if the fasting glycemia value at inclusion was superior or equal

to 1.26 g/L (7.00 mmol/L) and/or a treatment for diabetes, "high glycemia" if the value was between 1.10 and 1.26 g/L (6.10 and 7.00 mmol/L) and "normal glycemia" if glycemia was inferior to 1.10 g/L (6.10 mmol/L). Hypertension status was defined as high blood pressure measurement (systolic blood pressure superior or equal to 140 mm Hg and/or diastolic blood pressure superior or equal to 90 mm Hg) and/or an antihypertensive therapy at baseline. Hypercholesterolemia was considered present if the level of cholesterol was superior to 2.40 g/L at baseline and/or the subject was treated for hypercholesterolemia.

Blood collection and biological measurements

At baseline, fasting blood sample were collected for 90% of the entire cohort, including all the subjects selected for the case/cohort study. Citrated and EDTA plasmas were obtained after one centrifugation at 3.000g and immediately stored at -80°C in 500 microliters and 1 milliliter plastic tubes respectively.

Fibrinogen was centrally measured in citrated plasmas using the kinetic method of Clauss (Dade Behring) with a minimum detectable concentration (MDC) of 0.2 g/l. The intraassay and interassay coefficient of variation (CV) were 0.97% and 2.6% respectively for a fibrinogen concentration of 2.69 g/l. Data on fibrinogen measurement were missing for 19 women recruited in Bordeaux. These subjects did not differ from the other ones in term of age and traditional cardiovascular risk factors.

Total E2, total T and SHBG were centrally measured in EDTA plasmas. Plasma total E2 was measured with a sensitive direct radioimmunoassay (RIA) on an Orion Diagnostica device (Spectria, Espoo, Finland) with a MDC of 2 pg/ml (7.3 pmol/l) that was arbitrarily assigned to the 38 subjects with no detectable estradiol concentration. The intraassay and interassay coefficients of variation (CV) were 17.6% and 18.1% for a total estradiol concentration of 3.2 pg/mL (12 pmol/L), respectively and 2.8% and 5.8% for a total estradiol concentration of 24 pg/mL (88 pmol/L), respectively.

Plasma total T was measured by the same direct RIA as did total E2. The MDC was 0.02 ng/ml (0.06 nmol/l) and the intraassay and interassay CV were 7.5% and 7.0% respectively for a total T concentration of 0.46 ng/ml and 0.35 ng/ml respectively (1.6 nmol/l and 1.2 nmol/l, respectively).

Plasma SHBG was measured with a solid-phase chemiluminescent immunometric assay (Immulite®, Siemens Health Diagnostic Products, **Caernarfon**, Llanberis, UK) with a MDC of 0.02 nmol/l. The intraassay and interassay CV were respectively 3.2% and 4.6% for a SHBG concentration of 56 nmol/l [25].

Statistical analysis and covariates

Baseline characteristics of subjects are presented as absolute numbers and percentages for categorical variables and arithmetic means and standard deviations (SD) for normal continuous variables. Variables which presented a positively skewed distribution were log-transformed and values were expressed as geometric means (GM) and interquartile range (IQR**) as recommended [26].**

Linear regression analyses were used to test the association of fibrinogen with E2, T and SHBG. Regression coefficients were assessed in fibrinogen unit by 1 SD increase in the log-distribution of sex steroid hormones and SHBG. First adjustment included center, age, , hypercholesterolemia, glycemia status, hypertension and smoking status. Models were further adjusted for other sex steroid hormones and SHBG (adjustment 2). **Using a multiplicative linear model, an interaction of BMI with E2 on plasma fibrinogen was detected. Therefore, linear regression of fibrinogen on E2 was separately performed among women with BMI less than 25 kg/m² and among women with an overweight. Then, to assess the independence of the correlation between fibrinogen and E2, final adjustment within each stratum further included BMI as a continuous variable**. In addition, means of plasma fibrinogen were assessed by tertile of the E2 distribution in each stratum. Tests for linear trend across the three categories of sex steroid hormone were used to assess the significance of the variables in the models after having verified the linearity of the associations. To assess the linearity of the relation between E2and plasma fibrinogen levels, we used tests based on the difference in the log-likelihood between 2 models of prediction (one with 2 dummy variables corresponding to the tertile of the

parameter distribution and the other including the qualitative ordinal variable in 3 categories). All tests were not significant, and thus we did not reject the hypothesis of linearity.

Statistical analyses were performed with the Statistical Analysis System software, version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Baseline characteristics of the subjects

Baseline characteristics of the study population are presented in table 1. The final sample included 602 women, aged 74.8 years (SD=5.5) on average. Regarding cardiovascular risk factors, 7.7% of women suffered from diabetes, 77.6% from hypertension, 63.5% from hypercholesterolemia and 48.2% were overweight. In addition, 84 (13.9%) subjects had a prevalent disorder. The mean fibrinogen level was 3.42 g/l and GM of total E2, total T and SHBG were 5.29 pg/ml, 0.28 ng/ml and 26.3 nmol/l, respectively.

*Associations of plasma fibrinogen levels with **sex steroid hormones** and SHBG*

Association of fibrinogen with log-transformed total E2, total T and SHBG are given in the table 2. In univariate analysis, plasma fibrinogen levels were positively and significantly correlated to total E2 (β of fibrinogen unit in g/l by 1 SD increase of total E2 in pg/ml =0.148, p<0.001). Adjustment for center and traditional cardiovascular risk factors including diabetes did not substantially change the result (β of fibrinogen unit in g/l by 1 SD increase of total E2 in pg/ml=0.145, p<0.001). In addition, further adjustment for Total T and SHBG neither modify this association (β of fibrinogen unit in g/l by 1 SD increase of total E2 in pg/ml =0.147, p<0.001).

With respect to T and SHBG, neither crude nor adjusted analysis showed a significant association with plasma fibrinogen. Furthermore, adding BMI in the full models did not change the results (β of fibrinogen unit in g/l by 1 SD increase of total T in ng/ml =0.015, p=0.59 and β of fibrinogen unit in g/l by 1 SD increase of total SHBG in nmol/l =-0.011, p=0.73).

There was a significant interaction of total E2 with being overweight on plasma fibrinogen levels (figure 1). **In a fully adjusted model including BMI**, the association between total E2 and fibrinogen was of borderline significant for **lean** women while it was stronger for subjects who presented an overweight β of fibrinogen unit in g/l by 1 SD increase of total E2 in pg/ml=**0.092, p=0.02** and β of fibrinogen unit in g/l by 1 SD increase of total E2 in pg/ml=**0.156**, p<0.001, respectively, p for

interaction=0.04). Overweight women belonging to the third tertile of total E2 had an approximate 15% increase in plasma fibrinogen concentrations compared to lean women with low total E2 levels.

Stratified analyses showed no striking difference in the association of fibrinogen with **sex steroid hormones** and SHBG according to cardiovascular risk factors, including diabetes, hypercholesterolemia, hypertension and smoking.

Discussion

To our knowledge, this is the first population-based study to show a positive and independent association of endogenous E2 with plasma fibrinogen among postmenopausal women who used neither hormone medication nor anticoagulation therapy. This association was significantly more pronounced among women who were overweight compared to lean ones. By contrast, neither T nor SHBG were associated with plasma fibrinogen levels.

The association of plasma fibrinogen levels with endogenous E2 among peri/postmenopausal women was only investigated in two studies [12, 14]. On one hand, Sowers et al. used the data of the Study of Women's Health Across the Nation (SWAN) to describe the joint changes in haemostatic parameters and hormones concentrations among women transitioning to menopause [14]. Results showed that changes in endogenous E2 during the menopausal transition were not associated with plasma fibrinogen variations. Although this study did not provide direct information on the association of endogenous estradiol with plasma fibrinogen among postmenopausal women, these data did not suggest that variation in endogenous **sex steroid hormones** could induce changes in plasma levels of fibrinogen. **However, women included in the SWAN study were younger than the 3C Study participants and this difference in age between the two studies could in part explain this discrepancy**. On the other hand, Folsom et al. investigated the association between endogenous **sex steroid hormones** and fibrinogen among postmenopausal women recruited in the Atherosclerosis Risk In Communities (ARIC) study [12]. In this study, estrogen was positively associated with plasma fibrinogen and our results are concordant with these previous findings.

With respect to total T and SHBG, our findings showing no relationship with plasma fibrinogen are concordant with the results from three studies which consistently found no association of fibrinogen with total T and SHBG [12, 13, 15].

Several biological mechanisms could explain the positive association of endogenous E2 with plasma fibrinogen. Fibrinogen is strongly implicated in coagulation processing but is also a systemic

inflammation marker. The mechanisms underlying the increase in plasma levels of fibrinogen jointly with an elevated concentration of circulating E2 might include both procoagulant and proinflammatory properties of endogenous E2. As observed in physiological models of hyperestrogeny such as pregnancy or ovarian stimulation, important increases in circulating E2 leads to a dramatically rise in procoagulant factors including fibrinogen and a decrease in fibrinolytic activity [16, 18-21, 27, 28]. In addition, experimental studies in pregnant animals showed an up-regulation of liver fibrinogen biosynthesis by circulating E2 [29]. With respect to inflammatory processing, endogenous E2 has been shown to be positively and significantly associated with plasma levels of C-reactive protein among postmenopausal women [30, 31]. These findings suggest that high levels of endogenous E2 could induce both a procoagulant and a proinflammatory state in postmenopausal women.

Adipose tissue might play a role in modifying the association of fibrinogen with circulating E2. Obesity is characterized by a deleterious haemostatic profile and a low grade inflammation state which can result from both a direct and an indirect processing. On one hand, adipocyte hypertrophy and hyperplasia directly result in a secretion of adipokines which are implicated in both production of proinflammatory proteins and induce a hypofibrinolys and prothrombotic state [32, 33]. On the other hand, an inflammation state in obese subjects can indirectly result from the increase in testosterone aromatization to product E2 which has inflammatory and procoagulant properties [34]. The direct mechanism seems to be predominant in the deleterious haemostatic profile and the low grade inflammation state of obese subjects as showed by our data where the positive correlation between BMI and fibrinogen ($r=0.18$, $p<0.001$) remained significant after adjustment for total E2 ($r=0.14$, $p<0.001$). Since haemostasis and inflammation are closely linked in the context of atherosclerosis, it is conceivable to hypothesize that E2 could interact with inflammatory and procoagulant factors directly secreted by adipose tissue to increase the plasma fibrinogen, the levels of which are enhanced in atherosclerosis [35].

Our study presented strengths and limitations including the study design, the biological measurements and the potential confounders in the statistical analysis.

First, this analysis was carried out on an important number of subjects randomly selected from a population-based cohort study. In addition, baseline data were collected by standardized questionnaires during a face-to-face interview. However, the cross-sectional study design might not be adequate to determine whether changes in plasma fibrinogen levels were a consequence of E2 variations or the reverse.

With respect to the **sex steroid hormones** assays, especially at low levels of estradiol in postmenopausal women, conventional RIAs with preceding purification steps would provide more reliable and accurate measurements of plasma estradiol as compared with direct RIA [36]. However, measurement error related to direct RIA would bias our analysis towards the null hypothesis, resulting in a potential underestimation of the true associations.

Finally, we could not exclude the fact that other confounders could explain our findings of an association between total E2 and plasma fibrinogen. However, adjustment for several cardiovascular risk factors did not change the results.

In conclusion, our results show a positive and independent association of total E2 with plasma fibrinogen levels among postmenopausal women, especially in subjects who were overweight. Plasma fibrinogen is a strong predictor of cardiovascular disease. Therefore, identifying new factors which independently modify plasma fibrinogen levels may be important to improve the cardiovascular risk stratification and disease prevention. **Since the 3C case/cohort study has shown that high level of estradiol represented a new predictor of cardiovascular disease among older postmenopausal women [37], it would be now of interest to investigate whether difference in plasma fibrinogen concentrations could explain part of the association of endogenous estradiol with the risk of ischemic arterial disease among older postmenopausal women.**

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Tables and figure

Table 1. Baseline characteristics of the women selected within the subcohort sample (n=602)

Table 2. Association of fibrinogen with total E2, total T and SHBG assessed in linear regression models

Figure 1. Adjusted means of fibrinogen according to the tertiles of total estradiol by overweight status

Table 1. Baseline characteristics of the women selected within the subcohort sample in the 3C Study (n=602)

Characteristics	Value
Age (years)	74.8 (5.5)
Body-mass index (kg/m²)	25.6 (4.6)
Body-mass index≥25 kg/m²	287 (48.2)
Education	
Less than grade school	229 (38.0)
Grade school or high school	187 (31.1)
High school validated or university	186 (30.9)
Natural menopause	474 (91.2)
Smoking status	
Never	489 (81.2)
Past	87 (14.5)
Current	26 (4.3)
Hypertension	467 (77.6)
Glycemia	
Normal glycemia	534 (88.8)
High glycemia	21 (3.5)
Diabetes	46 (7.7)
Hypercholesterolemia	382 (63.5)
Prevalent disorder*	
Coronary heart disease	53 (8.8)
Stroke	25 (4.2)
Dementia	13 (2.2)
Biological parameters	
Total E2 (pg/ml)	5.29 (3.54-8.32)
Total T (ng/ml)	0.28 (0.20-0.45)
SHBG (nmol/l)	26.3 (18.2-38.1)
Fibrinogen (g/l)	3.42 (0.65)

Data are given as n(%) or arithmetic means (SD) except for steroid sex hormones expressed as geometric means (interquartile range)

* 7 subjects presentend more than one prevalent disorder

E2: estradiol

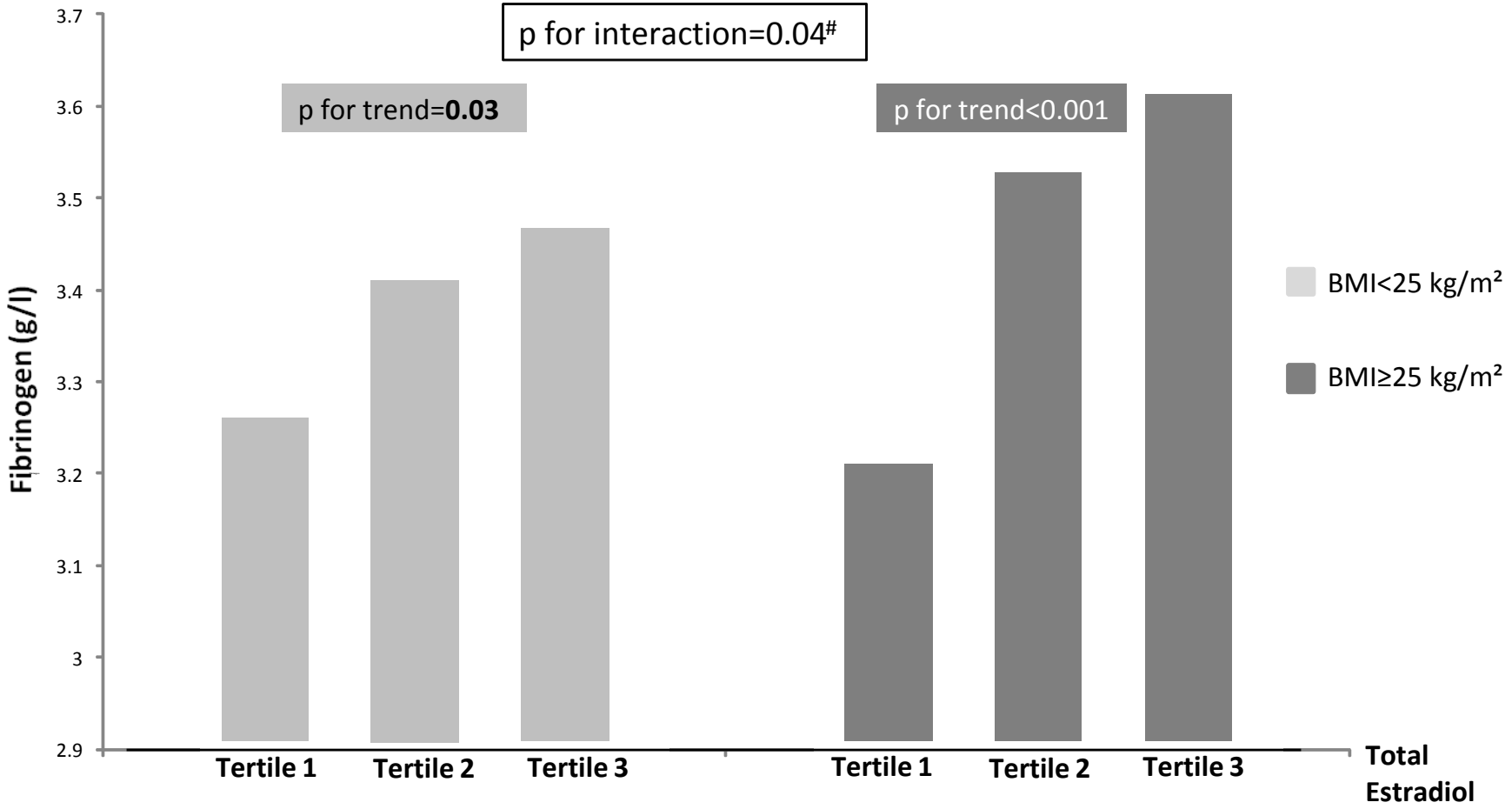
T: testosterone

Table 2. Association of fibrinogen with total E2, total T and SHBG assessed in linear regression models in the 3C study

	Fibrinogen, g/l								
	Crude			Adjustment 1			Adjustment 2		
	β*	SE	p value	β*	SE	p value	β*	SE	p value
Total E2, pg/ml (log)	0.148	0.027	**<0.001**	0.145	0.026	**<0.001**	0.147	0.029	**<0.001**
Total T, ng/ml (log)	0.019	0.027	0.49	0.025	0.027	0.36	0.003	0.027	0.92
SHBG, nmol/l (log)	-0.041	0.027	0.13	-0.050	0.028	0.10	0.008	0.030	0.78

E2: estradiol
T: Testosterone
Adjustment 1: adjustment for center, age, hypertension, diabetes, hypercholesterolemia and smoking status
Adjustement 2: adjustment for center, age, hypertension, diabetes, hypercholesterolemia, smoking status and other steroid sex hormones
* beta-coefficient of fibrinogen unit for 1 SD increment in the log-distribution of steroid sex hormones and SHBG

Figure 1. Adjusted means* of fibrinogen according to the tertiles of total estradiol by overweight status



* Adjusted for center, age, **BMI**, hypertension, diabetes, hypercholesterolemia and smoking status

Total estradiol and BMI were used as continuous variables