CLINICAL STUDY

Endogenous sex hormone levels in men are not associated with risk of venous thromboembolism: the Tromsø study

Johan Svartberg^{1,2}, Sigrid K Brækkan³, Gail A Laughlin⁴ and John-Bjarne Hansen³

¹Section of Endocrinology, Department of Medicine, University Hospital of North Norway, 9038 Tromsø, Norway, ²Department of Medicine, Institute of Clinical Medicine, University of Tromsø, 9037 Tromsø, Norway, ³Department of Medicine, Institute of Clinical Medicine, Center for Atherothrombotic Research in Tromsø (CART), University of Tromsø, 9037 Tromsø, Norway and ⁴Department of Family and Preventive Medicine, School of Medicine, University of California, San Diego, La Jolla, California 92093, USA

(Correspondence should be addressed to J Svartberg; Email: johan.svartberg@unn.no)

Abstract

Objectives: Low testosterone levels in men have been associated with cardiovascular risk factors and atherosclerosis and lately also an increased risk of both cardiovascular disease (CVD) and all-cause mortality. As arterial CVDs and venous thromboembolism (VTE) have been shown to share common risk factors, the purpose of the present study was to determine the impact of endogenous sex hormone levels on the incidence of VTE in a cohort of men.

Design: A prospective, population-based study.

Methods: Sex hormone measurements were available in 1350 men, aged 50–84, participating in the Tromsø study in 1994–1995. First, lifetime VTE-events during the follow-up were registered up to September 1 2007.

Results: There were 63 incident VTE-events (4.5 per 1000 person-years) during a mean of 10.4 years of follow-up. Age was significantly associated with increased risk of VTE; men 70 years or older had a 2.5-fold higher risk of VTE (HR 2.47, 95% CI 1.19–5.12), compared with those between 50 and 60 years of age. In age-adjusted analyses, endogenous sex hormones levels were not associated with risk of VTE; for each s.d. increase, hazards ratios (95% CI) were 1.06 (0.83–1.35) for total testosterone, 1.02 (0.79–1.33) for free testosterone, and 1.27 (0.94–1.71) for ln-estradiol. In dichotomized analyses comparing men in the lowest total and free testosterone quartile with men in the higher quartiles, hypoandrogenemia was not associated with risk of VTE.

Conclusions: In this population-based study of middle-aged and older men, endogenous sex hormone levels were not associated with 10-year risk of VTE.

European Journal of Endocrinology 160 833–838

Introduction

Epidemiological studies have shown that low testosterone levels are associated with several cardiovascular disease (CVD) risk factors such as abdominal obesity (1), blood pressure (2), lipid levels (3), and left ventricular mass (2). Similarly, lower testosterone levels have also been found in men with hypertension (2, 4), stroke (5), diabetes mellitus (6), and atherosclerosis (7, 8). Recently, low testosterone levels were also shown to be associated with an increased risk of both CVD and all-cause mortality in men (9, 10).

Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease with serious short and long term complications, and a potential fatal outcome (11). Although surgery, trauma, hospitalization, malignancy, immobilization, pregnancy, use of estrogen, and inherited thrombophilia are each associated with VTE (11, 12), 30–50% of VTE events occur in the absence of

apparent predisposing factors (13). The concept that VTE and arterial CVD are distinct entities has been challenged during the last years. Patients with unprovoked VTE are reported to have a higher frequency of carotid plaques (14) and increased incidence of CVD (15). A recent meta-analysis, based on selected prospective studies and case-control studies with verified endpoints, concluded that several CVD risk factors such as hypertension, dyslipidemia, and diabetes mellitus were associated with VTE (16). Furthermore, Brækkan *et al.* (17) recently reported that family history of myocardial infarction was an independent risk factor for VTE.

As low testosterone levels have been associated with CVD risk factors and increased risk of CVD events in men, and VTE and arterial thromboembolism share some of these risk factors, it seems relevant to investigate the impact of testosterone levels on incident VTE. Furthermore, as testosterone is converted by aromatase to estradiol it would also be important to assess the

association of endogenous estradiol and VTE. Accordingly, we performed a prospective, population-based study in men from the Tromsø study to assess the impact of endogenous sex hormones on the incidence of VTE.

Methods

Study population

The participants of the study were men recruited from a population-based health study, the fourth survey of the Tromsø study (1994–1995). The study population has been described in detail previously (5). In a random sample of 1605 non-fasting men, aged 25–84 years, blood samples were drawn between 0800 and 1600 h. Serum samples were stored at -70°C , until they were first thawed for analyses of sex hormones in 2001. Determination of total testosterone, estradiol, and sex hormone-binding globulin (SHBG) was performed on Immulite 2000 (Diagnostic Product Corp., Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation (CV) for the analyses were between 5 and 10%. Free testosterone values were calculated from total testosterone and SHBG using a fixed albumin concentration according to Vermeulen *et al.* (18). Height and weight were measured in standing subjects wearing light clothing without shoes, and body mass index (BMI ; kg/m^2) was calculated. Self-administered questionnaires that included information about smoking habits and medical history were completed and checked by trained nurses. Men of 50 years and older with a valid testosterone measure who had not previously been diagnosed with VTE were selected for these analyses ($n=1350$).

The Tromsø study has been approved by the National Data Security Board as a registry custodian and the study was approved by the Regional Committee for Medical and Health Research Ethics, North Norway. All subjects were asked for an informed consent for further scientific use of data and linkage to existing health registries. Subjects not consenting have been excluded from follow-up.

Outcome assessment

First, lifetime events of VTE during follow-up were identified by searching the computerized index of medical diagnoses, the autopsy register, and the radiology procedure register at the University Hospital of North Norway. The University Hospital of North Norway is the only hospital in the region, and all hospital care and relevant diagnostic radiology in the Tromsø community is provided exclusively by this hospital. The relevant discharge codes were ICD-9 codes 325, 415.1, 451, 452, 453, 671.3, 671.4, 671.9, for the period 1994–1998, and ICD-10 codes

I26, I80, I81, I82, I67.6, O22.3, O22.5, O87.1, O87.3 for the period 1999–2007. The index of medical diagnoses included diagnoses from outpatient clinic visits and hospitalizations. An additional search through the computerized index of autopsy diagnoses was conducted, and cases diagnosed with VTE, either as a cause of death (part one of the death certificate), or as a significant condition (part two of the death certificate), were identified. The radiology database was also searched to identify potential cases of objectively confirmed VTE that may have been missed because of coding errors in the index of medical diagnoses. All relevant diagnostic procedures performed at the Department of Radiology to diagnose VTE during the 13-year period were systematically reviewed by trained personnel, and cases with objectively confirmed VTE were identified.

The medical records for each potential VTE-case, derived from the medical diagnostic index, the autopsy register or the radiology procedure register were reviewed by trained personnel. The personnel were blinded to the baseline variables. For subjects derived from the medical diagnostic index and the radiology procedure register, an episode of VTE was verified and recorded as a validated outcome when all the following four criteria were fulfilled: i) objectively confirmed by diagnostic procedures (compression ultrasonography, venography, spiral-CT, perfusion-ventilation scan, pulmonary angiography or autopsy), ii) the medical record indicated that a physician had made a diagnosis of DVT or PE, iii) signs and symptoms consistent with DVT or PE were present, and iv) the patient underwent therapy with anticoagulants (heparin, warfarin or a similar agent), thrombolytics or vascular surgery. For subjects derived from the autopsy registry, a VTE-event was recorded as an outcome when the autopsy record indicated VTE as cause of death or as a significant condition.

A VTE-event was further classified as unprovoked or provoked, based on the presence of provoking factors at the time of diagnosis. The following were regarded as provoking factors: surgery or trauma within 8 weeks before the event, acute medical condition (MI, ischemic stroke, major infectious disease), cancer, marked immobilization (bed rest >3 days, wheelchair patient, long distance travel ≥ 4 h within the last 14 days), or other potential provoking factors described by a physician in the medical record (e.g., intravascular catheter).

Statistical analyses

For each participant the follow-up time was accrued from the date of enrolment in the Tromsø study (1994–1995) to the date a VTE-event was first diagnosed, to the date the participant died or moved from the municipality of Tromsø, or to the end of the study-period (September 1 2007).

Power was estimated using PASS (Number Cruncher Statistical Systems, Kaysville, UT, USA). Statistical analysis was carried out using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The significance level was 0.05.

Univariate associations between sex hormones and baseline characteristics were assessed by Pearson or Spearman correlations, as appropriate. The association between baseline sex hormones and VTE was determined using Cox proportional hazards regressions. Age and BMI were categorized into three intervals (<60, 60–69 and ≥ 70 years and <25, 25–29.9 and ≥ 30 kg/m² respectively). Hormone levels were analyzed as continuous variables, as quartiles based on the whole population, and as dichotomous variables comparing the lowest quartile to the three higher. Estradiol levels were not normally distributed and when used as a continuous variable they were transformed to natural logarithms, and assumed normal distribution. Two separate regression models were evaluated in VTE analyses: the first adjusted for age and the second also adjusted for BMI and smoking status. The sample size and event rate in this study provided greater than 90% power to detect a hazards ratio as low as 1.1 with 20% of variance explained by covariates and $\alpha < 0.05$.

Results

Baseline characteristics of the study population are presented in Table 1. Mean age was 63 ± 7 years and BMI 26.1 ± 3.5 kg/m². Approximately, one third of the population smoked and 19 percent reported having CVD. During the study period, 97 subjects moved from the municipality of Tromsø, and 390 subjects died. There were 63 validated, first VTE-events during a mean follow-up of 10.4 ± 3.6 years (Fig. 1). The crude incidence rate of VTE was 4.5 per 1000 persons-years. As shown in Fig. 2, the incidence rate increased markedly with age.

Characteristics of the VTE-events are shown in Table 2. Among the subjects with incident VTE, 61.9% had DVT and 38.1% had PE, with or without

Table 1 Baseline characteristics of 1350 men 50–84 years of age participating in the fourth Tromsø study (1994–95).

Age (years)	63 $\pm$ 7
Body mass index (kg/m ²)	26.1 $\pm$ 3.5
Total testosterone (nmol/l)	13.0 $\pm$ 5.2
Free testosterone (pmol/l)	194 $\pm$ 69
Estradiol (nmol/l)	0.06 $\pm$ 0.03
SHBG (nmol/l)	54.3 $\pm$ 24.3
Smoking (%)	32.1 (434)
Self-reported diabetes (%)	3.8 (51)
Self-reported cardiovascular disease (%) ^a	19.0 (257)

Values are mean $\pm$ s.d. or percentage with number in brackets.

^aSelf-reported cardiovascular disease includes myocardial infarction and/or angina and/or stroke.

Figure 1 Number and time-point of venous thromboembolic events during a mean follow-up time of 10.4 years in 1350 men participating in the Tromsø study 1994–2007.

concurrent DVT. Twenty-three events (36.5%) were unprovoked. Cancer was the most common provoking factor.

Crude and age adjusted hazard ratios (HR) for VTE were calculated and age was significantly associated with increased risk of VTE. Subjects who were of 70 years or older had a 2.5-fold higher risk of VTE (HR 2.47, 95% CI 1.19–5.12) compared with those between 50 and 60 years of age. The HR for VTE by endogenous sex hormone levels are presented in Table 3. As age and BMI are strong predictors of VTE, and together with smoking correlated to total ($r = -0.038$, $P = 0.165$, $r = -0.310$, $P < 0.001$ and $r = 0.226$, $P < 0.001$ respectively) and free testosterone ($r = -0.267$, $P < 0.001$, $r = -0.020$, $P = 0.464$, and $r = 0.137$, $P < 0.001$ respectively), they were included in the regression models. Endogenous sex hormone levels were not associated with risk of VTE either as continuous variables or as quartiles. Dichotomized analyses comparing men in the lowest quartiles of total and free testosterone with men in the higher

Figure 2 The incidence of venous thromboembolism with increasing age in 1350 men. The Tromsø study 1994–2007.

Table 2 Characteristics of venous thromboembolic events (VTE; $n=63$) among 1350 men, the Tromsø study 1994–2007.

	Percentage (n)
Deep vein thrombosis	61.9 (39)
Pulmonary embolism	38.1 (24)
Unprovoked	36.5 (23)
Clinical risk factors	
Heredity ^a	1.6 (1)
Other medical conditions ^b	22.2 (14)
Provoking factors	
Surgery	19.0 (12)
Trauma	3.2 (2)
Acute medical conditions	20.6 (13)
Cancer	33.3 (21)
Immobilization (bed rest > 3 days, wheelchair, long distance travel)	22.2 (14)
Other ^c	4.8 (3)

^aHeredity: family history of VTE (in first degree relative before 60 years of age).

^bOther diseases within the previous year (myocardial infarction, ischemic stroke, heart failure, inflammatory bowel disease, chronic infections, chronic obstructive pulmonary disease, or myeloproliferative disorder).

^cOther provoking factors described by a physician in the medical record (e.g. intravascular catheter).

quartiles also failed to identify any significant association of sex hormones with VTE risk (data not shown). In stratified analyses, endogenous sex hormone levels were not associated with the risk of unprovoked VTE or provoked VTE (data not shown). Finally, all analyses were adjusted for sampling time of the sex hormones, but the lack of associations persisted.

Discussion

Previous studies have shown that low testosterone levels in men are associated with CVD risk factors, CVD events and all-cause mortality (9, 10). As CVD and VTE share several risk factors, we investigated whether endogenous sex hormones in middle-aged and elderly men were risk factors for VTE. In this prospective, population-based study, neither testosterone levels nor estradiol levels were associated with the risk of VTE. Even men with the lowest testosterone levels, who were presumably androgen deficient, were not at increased risk of VTE. As the study was adequately powered, we feel confident that our results were not caused by a type 2 error.

Our results are unchallenged as this is the first population-based study to investigate these associations. Although, CVD and VTE share risk factors (16) our study suggests that low endogenous testosterone levels are not one of them. Low levels of endogenous testosterone have previously been shown to be associated with increased risk of CVD and all-cause mortality (9, 10), but not associated with risk of thromboembolic stroke (19). Likewise, higher levels of testosterone were not related to VTE risk. Anabolic steroid abuse has been linked to acute vascular events in athletes. Acute thrombosis risk in such athletes could be mediated through androgen-induced abnormalities of coagulation (20) or supraphysiologic testosterone levels could be prothrombotic. There exists no direct evidence that physiological testosterone levels or treatment doses are thrombogenic. For instance, in female to male transsexuals with a mean age of 34 (range 17–70 years),

Table 3 Hazard ratios of endogenous sex-hormones for incident venous thromboembolism.

Hormones	Number of events	Mean hormone level	Model 1 HR (95% CI) ^b	Model 2 HR (95% CI) ^c
Testosterone (nmol/l)				
Whole cohort per 1 s.d.	63	13.0	1.06 (0.83, 1.35)	1.12 (0.86, 1.44)
Quartiles				
Q1 (<9.6)	16	7.1	1.0 (ref)	1.0 (ref)
Q2 (9.6–12.4)	17	11.1	1.05 (0.53, 2.08)	1.06 (0.53, 2.11)
Q3 (12.5–15.8)	12	14.0	0.72 (0.34, 1.52)	0.80 (0.36, 1.62)
Q4 (>15.8)	18	20.0	1.09 (0.56, 2.14)	1.21 (0.60, 2.44)
Free testosterone (pmol/l)				
Whole cohort per 1 s.d.	63	194	1.02 (0.79, 1.33)	1.03 (0.80, 1.35)
Quartiles				
Q1 (<152)	18	115	1.0 (ref)	1.0 (ref)
Q2 (152–188)	20	171	1.10 (0.58, 1.98)	1.10 (0.57, 2.08)
Q3 (189–231)	8	209	0.44 (0.19, 1.02)	0.45 (0.19, 1.04)
Q4 (231)	17	281	1.04 (0.53, 2.06)	1.06 (0.53, 2.10)
Estradiol ^a (nmol/l)				
Whole cohort per 1 s.d.	63	0.06	1.27 (0.94, 1.71)	1.27 (0.94, 1.72)
Quartiles				
Q1 (<0.04)	9	0.021	1.0 (ref)	1.0 (ref)
Q2 (0.04–0.05)	17	0.046	1.45 (0.64, 3.26)	1.45 (0.64, 3.25)
Q3 (0.06–0.07)	11	0.065	0.94 (0.40, 2.27)	0.94 (0.39, 2.28)
Q4 (>0.07)	26	0.098	1.90 (0.87, 4.04)	1.89 (0.88, 4.07)

^aWhen analyzing the whole cohort estradiol levels were transformed to natural logarithms, hazards ratios are for 1 s.d. increase of the log.

^bAdjusted for age.

^cAdjusted for age, BMI and smoking.

treatment with testosterone has not been associated with an increased rate of VTE (21). Moreover, *in vitro* studies indicate that testosterone in physiological doses may actually have beneficial effects on the haemostatic system through enhancement of anticoagulant activity in endothelial cells (22) and via modulation of platelet activation (23). The latter experimental studies support the notion that physiological levels of testosterone would be superior to low testosterone levels in protecting against thrombosis, however, our results in men from a general population do not support this thesis.

It is well known that hormone replacement therapy in menopausal or postmenopausal women is associated with an increased risk of VTE (24), and supraphysiological estrogen administration to men has been reported to provoke VTE (25), however, endogenous estrogens were not related to VTE risk in the older men in the present study. By contrast, both total and bioavailable estradiol has previously been associated with an elevated risk of stroke in elderly men (19).

The search for eternal youth has created a market for drugs/hormones that possibly could affect the process of aging; testosterone is one of the hormones that have been targeted. Testosterone treatment in aging men has increased rapidly during the last few years (26). We failed to identify either a harmful or protective effect of higher levels of endogenous testosterone with VTE risk, suggesting this outcome may not be affected by testosterone therapy. However, this is the first prospective cohort study of this question and confirmation in other epidemiologic studies is required. In addition, further clinical studies are warranted to establish hemostatic changes in men with prolonged androgen deficiency, and subsequently the impact of testosterone treatment on the hemostatic system and risk of venous thrombosis.

The strengths of our study are its prospective design, long term follow-up, large number of participants, and validated VTE-events. All hospital-care in the region is exclusively provided by a single hospital, which increases the possibility of a complete VTE-register. However, the study also has some limitations. Hormone levels were based on a single sample drawn between 0800 and 1600 h. Preferably, samples should have been drawn in the morning, due to the diurnal variation of total and free testosterone. However, the diurnal variation is reduced markedly with age (27) and adjusting for time of venipuncture did not change the lack of associations. Our samples were kept frozen for ~6.5 years at -70 °C, and hormone levels were measured when samples were thawed for the first time. Levels of steroid hormones have been shown to be relatively stable in frozen serum for up to 10 years (28). We did not measure free testosterone, but used calculated values. This calculation has been found to be a reliable index of free testosterone (18).

In summary, although physiological testosterone levels seem to be beneficial for the haemostatic system *in vitro* studies, in our population-based study, endogenous levels of total and free testosterone and estradiol were not associated with the risk of VTE in middle-aged and older men. As testosterone treatment in aging men is increasing, more studies are warranted to establish the impact of testosterone treatment on the hemostatic system and the risk of both arterial and venous thrombosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a traveling grant from the Norwegian Society of Endocrinology to the first author.

References

- 1 Svartberg J, von Muhlen D, Sundsfjord J & Jorde J. Waist circumference and testosterone levels in community dwelling men: the Tromsø study. *European Journal of Epidemiology* 2004 **19** 657–663.
- 2 Svartberg J, von Muhlen D, Schirmer H, Barrett-Connor E, Sundsfjord J & Jorde R. Association of endogenous testosterone with blood pressure and left ventricular mass in men: the Tromsø study. *European Journal of Endocrinology* 2004 **150** 65–71.
- 3 Agledahl I, Skjaerpe P-A, Hansen JB & Svartberg J. Low serum testosterone in men is inversely associated with non-fasting serum triglycerides: the Tromsø study. *Nutrition, Metabolism, and Cardiovascular Diseases* 2008 **18** 256–262.
- 4 Khaw KT & Barrett-Connor E. Blood pressure and endogenous testosterone in men: an inverse relationship. *Journal of Hypertension* 1988 **6** 329–332.
- 5 Svartberg J, Midtby M, Bonna KH, Sundsfjord J, Joakimsen RM & Jorde R. The associations of age, lifestyle factors and chronic disease with testosterone in men: the Tromsø study. *European Journal of Endocrinology* 2003 **149** 145–152.
- 6 Ding EL, Song Y, Malik VS & Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *Journal of the American Medical Association* 2006 **295** 1288–1299.
- 7 Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A & Pols HA. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3632–3639.
- 8 Svartberg J, Von Muhlen D, Mathiesen E, Joakimsen O, Bonna KH & Stensland-Bugge E. Low testosterone levels are associated with carotid atherosclerosis in men. *Journal of Internal Medicine* 2006 **259** 576–582.
- 9 Khaw KT, Dowsett M, Fokerd E, Bingham S, Wareham N, Luben R, Welch A & Day N. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men. *Circulation* 2007 **116** 2694–2701.
- 10 Laughlin GA, Barrett-Connor E & Bergstrom J. Low serum testosterone and mortality in older men. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 68–75.
- 11 Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. *Journal of Thrombosis and Haemostasis* 2005 **3** 1611–1617.

- 12 Rosendaal FR. Venous thrombosis: a multicausal disease. *Lancet* 1999 **353** 1167–1173.
- 13 White RH. The epidemiology of venous thromboembolism. *Circulation* 2003 **107** 14–18.
- 14 Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, Prins MH & Girolame A. An association between atherosclerosis and venous thrombosis. *New England Journal of Medicine* 2003 **348** 1435–1441.
- 15 Sorensen HT, Horvath-Puho E, Pedersen L, Baron JA & Prandoni P. Venous thromboembolism and subsequent hospitalization due to acute arterial cardiovascular event: a 20-year cohort study. *Lancet* 2007 **370** 1773–1779.
- 16 Ageno W, Becattini C, Brighton T, Selby R & Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. *Circulation* 2008 **117** 93–102.
- 17 Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J & Hansen JB. Family history of myocardial infarction is an independent risk factor for venous thromboembolism: the Tromsø study. *Journal of Thrombosis and Haemostasis* 2008 **6** 1851–1857.
- 18 Vermeulen A, Verdonck L & Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3666–3672.
- 19 Abbott RD, Launer LJ, Rodriguez BL, Ross GW, Wilson PW, Masaki KH, Strozyk D, Curb JD, Yano K, Popper JS & Petrovitch H. Serum estradiol and risk of stroke in elderly men. *Neurology* 2007 **68** 563–568.
- 20 Ferenchick GS, Hirokawa S, Mammen EF & Schwartz KAA. Anabolic-androgenic steroid abuse in weight lifters: evidence for activation of the hemostatic system. *American Journal of Hematology* 1995 **49** 282–288.
- 21 Van Kesteren PJM, Asscheman H, Megens JAJ & Gooren LJG. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clinical Endocrinology* 1997 **47** 337–342.
- 22 Jin H, Lin J, Fu L, Mei YF, Peng G, Tan X, Wang DM, Wang W & Li YG. Physiological testosterone stimulates tissue plasminogen activator and tissue factor pathway inhibitor and inhibits plasminogen activator inhibitor type 1 release in endothelial cells. *Biochemistry and Cell Biology* 2007 **85** 246–251.
- 23 Li SJ, Li XY, Li J, Deng XL, Li Y & Cong YL. Experimental arterial thrombosis regulated by androgen and its receptor via modulation of platelet activations. *Thrombosis Research* 2007 **121** 127–134.
- 24 Rosendaal FR, Helmerhorst FM & Vandenbroucke JP. Female hormones and thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2002 **22** 201–210.
- 25 The Coronary Drug Project: initial findings leading to modifications of its research protocol. *Journal of the American Medical Association* 1970 **214** 1303–1313.
- 26 Barrett-Connor E & Bhasin S. Time for (more research on) testosterone. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 501–502.
- 27 Bremner WJ, Vitiello MV & Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *Journal of Clinical Endocrinology and Metabolism* 1983 **56** 1278–1281.
- 28 Kley HK, Schlaghecke R & Kruskemper HL. Stability of steroids in plasma over a 10-year period. *Journal of Clinical Chemistry and Clinical Biochemistry* 1985 **23** 875–878.

Received 3 February 2009

Accepted 7 February 2009