# Thrombotic events after starting exogenous testosterone in men with previously undiagnosed familial thrombophilia

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**Our specific aim was to describe thrombosis (osteonecrosis of the hips, pulmonary embolism, and amaurosis fugax) after exogenous testosterone was given to men with no antecedent thrombosis and previously undiagnosed familial thrombophilia. After starting testosterone patch or gel, 50 mg/day or intramuscular testosterone 400 mg IM/month, 2 men developed bilateral hip osteonecrosis 5 and 6 months later, and 3 developed pulmonary embolism 3, 7, and 17 months later. One man developed amaurosis fugax 18 months after starting testosterone gel 50 mg/day. Of these 6 men, 5 were found to have previously undiagnosed factor V Leiden heterozygosity, 1 of whom had ancillary MTHFR C677T homozygosity, and 2 with ancillary MTHFR C677T-A1298C compound heterozygosity. One man had high factor VIII (195%), factor XI (179%), and homocysteine (29.3 umol/L). Thrombotic events after starting testosterone therapy are associated with familial thrombophilia. We speculate that when exogenous testosterone is aromatized to E2, and E2-induced thrombophilia is superimposed on familial thrombophilia, thrombosis occurs. Men sustaining thrombotic events on testosterone therapy should be screened for the factor V Leiden mutation and other familial and acquired thrombophilias. (Translational Research 2011;158:225–234)**

**Abbreviations:** BMI = body mass index; DVT = deep venous thrombosis; MRI = magnetic resonance image; PCR = polymerase chain reaction; PE = pulmonary embolus; TE = thrombotic event; VTE = venous thromboembolism

Familial thrombophilia (factor V Leiden, prothrombin gene mutations, and high factor VIII) seems to be etiologic for "idiopathic" osteonecrosis of the hip and knee in adults,[1-6] and for idiopathic pediatric osteonecrosis of the hip, Legg-Calve-Perthes disease.[7] MTHFR C677T homozygosity is associated with osteonecrosis of the hip in Koreans.[8] Factor V Leiden heterozygosity has also been shown to be etiologic for osteonecrosis of the jaw.[9] It has been speculated that thrombophilia-induced thrombus of the major efferent vein(s) of the head of the femur leads to an increase in intracortical pressure, reducing arterial inflow, with subsequent bone hypoxia and bone death.[1,2,4,10-12]

Amaurosis fugax (retinal artery thrombosis) in the absence of carotid atherosclerosis has been reported to be thrombotic, which is associated with familial thrombophilia.[13]

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## AT A GLANCE COMMENTARY

**Glueck C, et al.**

### Background

Estrogens facilitate development of thromboembolism in patients with familial and acquired thrombophilia. Exogenous and endogenous testosterones are aromatized to estradiol (E2). Of 6 men in the current study who developed thrombotic events 3–18 months after starting testosterone, 5 had previously undiagnosed factor V Leiden heterozygosity, and 1 had high factors VIII and XI and homocysteine.

### Translational Significance

Thromboembolism after starting testosterone is associated with familial thrombophilia. We speculate that when exogenous testosterone is aromatized to E2, with superimposition on familial thrombophilia, thromboembolism may occur. Men sustaining thromboembolism during testosterone therapy should be screened for familial and acquired thrombophilias.

Familial and acquired thrombophilias are more commonly associated with venous thrombotic events, but also they contribute to arterial thrombotic events.[1,14-16]

The major source of estradiol (E2) in men comes from the aromatization of testosterone (endogenous and/or exogenous) to E2.[17-20] In hypogonadal men, before testosterone gel (AndroGel, Abbott, Abbott Park, Ill) treatment, mean E2 levels were in the low-to-normal range, and within 30 days of starting AndroGel (delivering 5 or 10 mg of testosterone/day), mean serum E2 levels increased by an average of 30.9% and 45.5%, $P = 0.0001$.[21] Serum E2 increased progressively from 6 to 24 months on AndroGel (50–100 mg/day testosterone), with levels rising to the upper limit of the male reference range, $P < 0.009$.[22]

High E2 in men is associated with stroke.[23] Although oral estrogen was an effective treatment for prostate cancer, it was abandoned because of increased venous thromboembolism (VTE).[24-26] Ethinyl E2 treatment for transsexual men is associated with increased VTE.[27]

Exogenous estrogens or SERMs (Evista, Tamoxifen) increase the likelihood of thrombosis 3- to 10-fold in subjects with familial thrombophilia.[28-32] Among women taking adjuvant Tamoxifen for early-stage breast cancer, those who had a thrombotic event (TE) were nearly 5 times more likely to carry a factor V Leiden mutation than those who did not have a TE.[33] Postmenopausal women should be evaluated for the factor V Leiden mutation before prescription of adjuvant Tamoxifen if a positive test would alter therapeutic decision-making.[33] Studies comparing aromatase inhibitors to tamoxifen in the adjuvant setting have reported a lower rate of venous thromboembolism with the aromatase inhibitors, yet the incidence of venous thromboembolism with aromatase inhibitors is higher than that expected in the general population.[34]

Thrombophilia associated with E2 treatment is synergistic with the factor V Leiden mutation, substantially increasing the risk of thrombosis.[28] Osteonecrosis of the hip has been reported during pregnancy,[11,35,36] and following exogenous estrogen administration, where the physiologic hyperestrogenemia of pregnancy or exogenous estrogens interact with familial thrombophilia to produce osteonecrosis. When exogenous estrogen is given to women heterozygous for the factor V Leiden mutation, development of osteonecrosis of the jaw is facilitated by the interaction of estrogen-induced thrombophilia superimposed on familial thrombophilia.[9]

In the current report, our specific aim was to describe thrombosis (osteonecrosis of the hips, pulmonary embolism, and amaurosis fugax) after men with no antecedent thrombosis and previously undiagnosed familial thrombophilia were given exogenous testosterone.

## MATERIALS AND METHODS

**Study design.** Informed consent was obtained after the nature of the procedures (blood drawing, measures of coagulation, and medical history) had been fully explained. The procedures followed were in accordance with the ethical standards of the institutional review board of the Jewish Hospital, Cincinnati, Ohio, which approved the research protocol.

In the 6 men who sustained thrombotic events after starting testosterone therapy, at the time of referral, after an overnight fast, blood was drawn in the seated position for assessment of thrombophilia and hypofibrinolysis.[7,37]

History and physical examination was performed, including review of referring physicians' records, X rays, and magnetic resonance images (MRIs).

To assess E2 levels in men given exogenous testosterone, we separately examined the relationships of E2 and total and free testosterone (free T) in 38 men attending our center who were receiving exogenous testosterone prescribed by either their referring family physicians or urologists. In 20 of these 38 men, we were able to measure total testosterone, free T, and E2 before and after testosterone therapy.

**Laboratory assessment of thrombophilia and hypofibrinolysis.** ***Polymerase chain reaction (PCR) assays.*** PCR measures of thrombophilia-hypofibrinolysis (G1691A factor V Leiden, G20210A prothrombin, MTHFR C677T-A1298C, and 4G5G plasminogen

activator inhibitor activity gene mutations) were performed in all cases using previously published methods by laboratory staff blinded to the subjects' status (diagnosis and severity of disease).[7,37-44] Abnormal results of the PCR tests are displayed in Table I.

***Serologic measures of thrombophilia.*** Serologic measures of thrombophilia included anticardiolipin antibodies (IgG and IgM), antigenic protein C, total and free protein S, antithrombin III, resistance to activated protein C (RAPC), activated partial thromboplastin time (APTT), dilute Russel's Viper Venom time (DRVVT), factors VIII and XI, and homocysteine. Established, previously published methods were used.[11,37,44,45]

***Serologic measures of hypofibrinolysis.*** Serologic measures of hypofibrinolysis, lipoprotein, and plasminogen activator inhibitor activity (PAI-Fx) were performed using established, previously published methods.[11,42,45]

***Measurement of total and free testosterone and E2.*** Blood samples were drawn in the morning after an overnight fast, ~2 hours after application of the testosterone gel.

Total and free testosterone and E2 were measured by LabCorp (Burlington, NC) in a single laboratory. Total testosterone and E2 were measured by electrochemiluminescence immunoassay (ECLIA),[46] and free testosterone by equilibrium ultrafiltration.[47,48] Sensitivity of the E2 method was 5.0 pg/mL, and intra-assay coefficients of variation <7% for 50–1000 pg/mL, 1000–3000 pg/mL, <4% for 50–1000 pg, <5% for 1000–3000, and <7% for >3000 pg/mL.

**Statistical methods.** Paired Wilcoxon tests of difference were used to compare E2 before and on testosterone gel therapy (50 mg/day), and Spearman correlations were used to assess correlation between changes in free T and E2 on testosterone therapy as well as correlation between free T and E2 on testosterone therapy. For calculation of T/E2 ratios, with T units ng/dL, we converted E2 units from pg/mL to ng/dL.

## RESULTS

**Interaction of testosterone with familial thrombophilia leading to thrombotic events: osteonecrosis, pulmonary embolus, and amaurosis fugax.** Before our evaluation, triggered by thrombotic events, none of the 6 patients had any history of thrombotic events, although cases 3 and 4 had coronary artery disease. None of the 6 patients had any previous testing of thrombophilia or hypofibrinolysis. The 6 men in the current study had no history of trauma, surgery, overt cancer, or immobilization that would predispose to thrombi, and had no previously diagnosed coagulation disorders, although there was a family history of thrombotic events in 3 of the 6 families (Table I).

**Table I.** Thrombotic events: Pulmonary embolus, osteonecrosis, and amaurosis fugax after starting testosterone therapy in men subsequently found to have familial thrombophilia

| Case # | Age (yr) | Race | Testosterone | Interval in months between testosterone and development of | | | Factor V Leiden | MTHFR C677T-A1298C | Factor VIII levels | Factor XI levels | PAI-1 4G/4G | Homocysteine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ON | PE | AF | | | Normal <150% | Normal <150% | | Normal <16 umol/L |
| 1 | 54 | C | 50 mg/day, patch | 6 | | | Heterozygous | C677T A1298C | | | | |
| 2 | 70 | C | 400 mg/month intramuscular | 5 | | | Wild-type Normal | | 195% | 179% | | 29.3 umol/L |
| 3* | 59 | C | 50 mg/day, gel | | 7 | | Heterozygous | C677T C677T | | | 4G4G | |
| 4† | 48 | C | 50 mg/day, gel | | | 18 | Heterozygous | C677T A1298C | | | | |
| 5 | 38 | C | 50 mg/day, gel | | 17 | | Heterozygous | | | | | |
| 6‡ | 63 | C | 50 mg/day, gel | | 3 | | Heterozygous | | 173% | 174% | | |

*Note:* Only abnormal tests for thrombophilia or hypofibrinolysis are displayed.
*AF*, amaurosis fugax; *ON*, osteonecrosis of hips; *PE*, pulmonary embolus.
*Family history of ischemic stroke.
†Paternal DVT.
‡Family history of ischemic stroke, deep venous thrombosis, and pulmonary embolus.

Intervals between starting testosterone and clinical thrombotic events ranged from 3 to 18 months (Table I). The temporal associations of exogenous testosterone therapy with thrombotic events (Table I) led us to evaluate for underlying familial and acquired thrombophlia-hypofibrinolysis. The major, new, novel finding was that 5 of the 6 patients had previously undiagnosed factor V Leiden heterozygosity. In these 5 patients, ancillary thrombophilic mutations included 1 patient with MTHFR C677T homozygosity, 2 with MTHFR C677T-A1298C compound heterozygosity, and 1 with PAI-1 4G4G homozygosity (Table I). The 1 patient without factor V Leiden heterozygosity (patient 2, Table I) had had high levels of factor VIII (195%), factor XI (179%), and high homocysteine (29.3 umol/L) with normal renal function. Factors VIII and XI were high on repeated sampling.

Family history was positive for thrombotic events (ischemic stroke, deep venous thrombosis [DVT], and pulmonary embolus [PE]) in 3 of the 6 men (patients 3, 4, and 6, Table I).

**Cases 1 and 2: osteonecrosis of the hips.** Case 1, a 54-year-old, nonsmoking, nondrinking Caucasian man, after reporting increasing fatigue and loss of libido (1/08), and after documentation of low serum-free T levels (7 pg/mL, lower normal limit 7.2 pg/mL, Table II), was started on a testosterone patch ([2/08] 50 mg/day). On repeat sampling (8/08), free T had risen to 18 pg/mL, and E2, normal before testosterone therapy (32 pg/mL), rose to a high level (47 pg/mL, Table II). Hematocrit (44.1%) and hemoglobin (15.3 g/dL) were normal on testosterone therapy. After developing severe, bilateral hip pain, X ray and MRI revealed Ficat[49] stage I osteonecrosis of both hips (8/08). The osteonecrosis was thought to be "idiopathic."[12,50] He had not previously taken long-term, high-dose corticosteroids.[3] Coagulation tests (12/08) revealed factor V Leiden heterozygosity and ancillary compound MTHFR C-677T—A1298C heterozygosity (Table I). Testosterone therapy was stopped, and he was treated with Lovenox,[2] 120 mg/day for 3 months, with resolution of pain, and stable, unchanged X rays and MRIs at the Ficat stage I level, 2.5 years later.

Case 2 was a 70-year-old Caucasian man, nonsmoker, nondrinker, with well-controlled type 2 diabetes mellitus without history of long-term, high-dose corticosteroids.[3] In March of 2008, after low free T was found (4 pg/mL, Table II), he was started by his family physician on high-dose intramuscular testosterone enanthate injections (400 mg, once per month), developed severe bilateral hip pain by June 2008, and by August was found to have bilateral osteonecrosis by X ray and MRI, Ficat[49] stage IV with collapse of the head on the femur on the left, and Ficat stage II (without collapse) on the right. Hematocrit (41.8%) and hemoglobin (15.5 g/dL) were

**Table II.** Total and free testosterone and estradiol before and during supplemental testosterone therapy in 6 men with thrombotic events during testosterone therapy

| Case # | Age (yr) | Race | BMI (kg/m$^2$) | Testosterone | Serum testosterone Normal range 280–800 ng/dL | | Serum free testosterone Normal range 7.2–24 pg/mL | | Serum Estradiol Normal range ≤42.6 pg/mL | | Testosterone / Estradiol ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pre Rx | On Rx | Pre Rx | On Rx | Pre Rx | On Rx | Pre Rx | On Rx |
| 1 | 54 | C | 29.3 | 50 mg/day, patch | 291 | 837 | 7 | 18 | 32 | 47 | 9.1 | 17.8 |
| 2 | 70 | C | 40.4 | 400 mg/month intramuscular | 194 | — | 4 | — | — | — | — | — |
| 3 | 59 | C | 23.8 | 50 mg/day, gel | 297 | 624 | 2.7 | 11 | 27 | 33.7 | 11 | 18.5 |
| 4 | 48 | C | 25.0 | 50 mg/day, gel | — | 228 | — | 46 | — | 60 | — | 3.8 |
| 5 | 38 | C | 30.5 | 50 mg/day, gel | 318 | 694 | — | — | — | — | — | — |
| 6 | 63 | C | 32.5 | 50 mg/day, gel | 264 | 462 | — | — | — | — | — | — |

normal on testosterone therapy. Coagulation tests revealed high factor VIII (195%) and high factor XI (179%), both high on repeated tests, and high homocysteine in the presence of normal renal function (29.3 umol/L, Table I). Testosterone therapy was stopped, and high homocysteine was treated with folic acid 5 mg, vitamin B 6 100 mg, and vitamin B12 2000 mcg/day. Total left hip replacement was performed (October 2008) with postoperative maintenance on Arixtra (2.5 mg/day), followed by Coumadin, titrated to an international normalized ratio (INR) of 2.5–3.5. There has been no progression of the right hip Ficat stage II osteonecrosis in 22 months of follow-up on Coumadin.

**Case 3: pulmonary embolus.** A 59-year-old, nonsmoking, nondrinking white man had a myocardial infarction at age 51. Eight years later (May 2007), factor V Leiden heterozygosity and MTHFR C677T homozygosity were diagnosed after angiographic evidence of left anterior descending coronary artery thrombosis (Table I). In May 2007, he was started on daily 50-mg testosterone gel. On testosterone treatment, free T was 11 pg/mL, within the normal range (7.2–24 pg/mL), and E2 rose from 27 to 33.7 pg/mL (Table II). In December 2007, 8 hours after a 23-hour flight to South Africa, he suffered sudden onset of disabling severe dyspnea, chest pain, and arterial hypoxia. Despite a negative computed tomography (CT) angiogram, with a high level of serum D-dimer, he was found to have had a pulmonary embolus (Table I). There was a family history of ischemic stroke.

**Case 4: amaurosis fugax (retinal artery thrombosis).** A 48-year-old, nonsmoking, nondrinking white man developed recurrent amaurosis fugax in the presence of normal carotid and vertebral ultrasounds 18 months after beginning 50-mg testosterone gel/day (Table I). Factor V Leiden heterozygosity was found, accompanied by ancillary findings of compound MTHFR C677T-A1298C heterozygosity, and 4G4G homozygosity for the PAI-1 gene (Table I). On testosterone gel (50 mg/day), serum E2 was high (60 pg/mL, Table II). The testosterone gel was stopped, and anticoagulation with Coumadin was initiated, with resolution of the clinical episodes of amaurosis fugax. The patient's father had documented DVT in the legs (Table I).

**Case 5: pulmonary embolus.** A 38-year-old, nonsmoking, nondrinking white man developed DVT in both legs with subsequent pulmonary embolus 17 months after starting testosterone gel 50 mg/day, which elevated his serum total testosterone from 318 to 694 ng/dL (Table II). Factor V Leiden heterozygosity was found (Table I).

**Case 6: pulmonary embolus.** A 63-year-old, nonsmoking, nondrinking white man developed pulmonary embolus 3 months after starting testosterone gel 50 mg/day, which elevated his total testosterone from 264 to 462 ng/mL (Table II). Factor V Leiden heterozygosity was found along with ancillary thrombophilias (persistently elevated factors VIII and XI, Table I). There was a family history of ischemic stroke.

**Total and free testosterone and E2 in men with and without exogenous testosterone supplementation.** Of 20 men having total and free testosterone and E2 measured before and during exogenous testosterone therapy, 2 (10%) had high pretreatment E2 (≥42.6 pg/mL), but with all men receiving testosterone gel (50 mg testosterone/day, ≥3 months), 9 (45%) had high E2 (≥42.6 pg/mL, Table III).

Of 38 men receiving testosterone gel (50 mg testosterone/day, ≥3 months), E2 was high (≥42.6 pg/mL) in 15 (39%, Table IV). The group mean E2 (41.3 pg/mL) was just below the laboratory upper limit for E2 (42.6 pg/mL, Table IV).

In the 20 men who had measures of testosterone and E2 before and during testosterone therapy, T was positively correlated with E2 before and during therapy, and change in T was correlated with change in E2 (Table V). In these 20 men, E2 did not correlate with body mass index (BMI), either before or during treatment (Table V). Before treatment, free T was positively correlated with BMI, and during treatment, the T/E2 ratio was inversely correlated with BMI (Table V). A change in T/E2 during treatment correlated inversely with BMI (Table V).

In the 38 men studied on testosterone, total and free T were positively correlated with E2. The T/E2 ratio was inversely correlated with BMI (Table V).

## DISCUSSION

Recently, in community-dwelling men ≥ age 65 years, Basaria et al[51] reported that the application of a testosterone gel was associated with an increased risk of cardiovascular adverse events. In a recent meta-analysis and review, Fernandez-Balsells et al concluded that there were no significant effects of testosterone therapy on all-cause mortality or cardiovascular events, but there were decrements in HDL cholesterol.[52] However, they also noted[52] that "current evidence about the safety of testosterone treatment in men in terms of patient-important outcomes is of low quality and is hampered by the brief study follow-up."

Endogenous E2 is associated with ischemic stroke in men,[23] and exogenous estrogen therapy in men is associated with venous thromboembolism and pulmonary embolism.[24-27] Within this frame of reference, the development of thrombotic events (pulmonary emboli, amaurosis fugax, and osteonecrosis), in 6 men 3 to 18 months after starting testosterone therapy alerted us to the possibility of an interaction between exogenous testosterone and familial thrombophilia. The 6 men in the current study had no history of

**Table III.** Total and free testosterone and estradiol before and during supplemental testosterone therapy

| | | | T (ng/dL) | | Free T (pg/mL) | | E2 (pg/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AGE (Yrs) | BMI (kg/m$^2$) | 280–800 | | 7.2–24 | | ≤42.6 | | T/E2 | |
| | Normal range | | Pre | On | Pre | On | Pre | On | Pre | On |
| 1 | 60 | 32.1 | 121 | 154 | 2.7 | *27.0* | 10.0 | 13.0 | 121 | 118 |
| 2 | 55 | 35.8 | 285 | 626 | 6.9 | 13.4 | 18.8 | 32.5 | 151 | 192 |
| 3 | 66 | 24.6 | 84 | 143 | - | - | 11.0 | 11.0 | 76 | 130 |
| 4 | 25 | 37.8 | 249 | 621 | 8.8 | *24.9* | - | *59.0* | - | 105 |
| 5 | 55 | 29.3 | 291 | *837* | 7.0 | 18.0 | *43.0* | *47.0* | 67 | 178 |
| 6 | 55 | 41.1 | 181 | 349 | 5.6 | 9.7 | 21.0 | *46.6* | 86 | 75 |
| 7 | 52 | 26.1 | 70 | 672 | 1.9 | 21.8 | 6.7 | *43.0* | 104 | 156 |
| 8 | 45 | 26.3 | 180 | 570 | 4.3 | 12.9 | 20.9 | 29.3 | 86 | 194 |
| 9 | 54 | 27.4 | 120 | 560 | 3.6 | 15.3 | 30.4 | 41.1 | 39 | 136 |
| 10 | 46 | 45.1 | 162 | 644 | *43.0* | 13.4 | 23.0 | *47.0* | 70 | 66 |
| 11 | 43 | 28.1 | 204 | 158 | 5.6 | 7.0 | 17.1 | 12.6 | 119 | 125 |
| 12 | 62 | 30.0 | 129 | *790* | 4.6 | *24.3* | 20.6 | *49.0* | 62 | 161 |
| 13 | 47 | 32.0 | 567 | 525 | 9.0 | 10.8 | 38.0 | *61.0* | 149 | 86 |
| 14 | 58 | 32.0 | *1021* | *989* | 20.2 | 18.3 | *85.4* | *66.8* | 119 | 148 |
| 15 | 67 | 32.8 | 277 | 224 | 5.2 | 4.4 | 31.6 | 32.6 | 87 | 69 |
| 16 | 52 | 30.0 | 275 | 419 | 14.7 | 8.4 | 26.1 | *57.6* | 105 | 73 |
| 17 | 79 | 29.2 | 22 | 530 | 0.1 | 7.0 | 26.0 | 37.2 | 8 | 142 |
| 18 | 58 | 27.9 | 133 | 183 | - | 8.5 | 14.7 | 25.4 | 90 | 72 |
| 19 | 68 | 23.8 | 297 | 624 | 2.7 | 11.0 | 27.0 | 33.7 | 110 | 185 |
| 20 | 54 | 37.3 | 251 | 278 | 7.3 | 8.5 | 23.2 | 24.5 | 108 | 113 |
| mean ± SD | 55 ± 11 | 31.4 ± 5.6 | 246 ± 217 | 495 ± 247 | 8.5 ± 9.8 | 13.9 ± 6.7 | 26.0 ± 17.0 | 38.5 ± 16.3 | 93 ± 35 | 126 ± 43 |
| change (paired Wilcoxon) | | | $P = 0.0005$ | | 0.016 | | 0.0016 | | 0.036 | |

*Note:* All treated with testosterone 50 mg/day (gel) for ≥ 3 months.

thrombi, no trauma, surgery, overt or covert cancer, or immobilization, which would predispose them to thrombi formation, and had no diagnoses of coagulation disorders, although there was a family history of thrombotic events in 3 of the 6 families. We speculate that as exogenous testosterone is aromatized to E2,[53] thrombophilia associated with the increased E2 interacts with familial and/or acquired thrombophilia to produce thrombotic events, as in the current study.

The major, new, novel finding of our current study was that 5 of 6 men who had thrombotic events 3 to 18 months after starting testosterone therapy were found to be heterozygous for previously undiagnosed factor V Leiden mutations. Moreover, 3 of these 5 men heterozygous for the factor V Leiden mutation also had ancillary findings of other possibly less consequential familial thrombophilias, MTHFR C677T homozygosity, or compound MTHFR C677T-A1298C heterozygosity. The single patient without factor V Leiden heterozygosity who developed thrombi on testosterone therapy was found to have persistently high factors VIII, XI, and high homocysteine, with normal renal function. High factors VIII[54] and XI[55] are major risk factors for thrombosis, as is high homocysteine.[56]

In the current study, 9 of 20 men (45%) studied before and during testosterone supplementation had high E2 during testosterone therapy, which is comparable with 15 of 38 (39%) men studied during testosterone therapy. It is not surprising that 39% to 45% of men on testosterone in the current study had high E2, given the report by Wang et al[22] that serum E2 "levels were significantly higher during treatment with T, compared with baseline ($P = 0.0001$), and increased progressively from 6 ($P = 0.0001$) until 24 months of treatment, remaining at the upper limit of the male reference range." In the current report, the mean E2 on 38 men during testosterone therapy, 41.3 pg/mL, was, congruent with the report by Wang et al,[22] at the very upper limit of the male reference range.

In the 20 men studied before and during testosterone therapy, 8 had pretreatment total and/or free T levels that were normal, and in the 38 men studied during testosterone therapy, 13 had total and/or free T levels that were below the normal range. Because all men had their testosterone therapy prescribed by either their referring family physicians or urologists, we do not know the rationale for treating those whose baseline total and/or free T was normal, nor why those men whose total and/or free T remained below the normal range on testosterone therapy did not have an upward adjustment to their testosterone dose. In the current report, our primary interest was focused on E2 levels in men receiving

**Table IV.** Total and free testosterone and estradiol during supplemental testosterone therapy

| | AGE (Yrs) | B (kg/m$^2$) | T (ng/dL) | Free T (pg/mL) | E2 (pg/mL) | |
|---|---|---|---|---|---|---|
| ID | Normal range | | 280–800 | 7.2–24 | ≤42.6 | T/E2 |
| 1 | 48 | 25.0 | 228 | *46.0* | *60.0* | 38 |
| 2 | 60 | 32.1 | 154 | *27.0* | 13.0 | 118 |
| 3 | 55 | 27.5 | 606 | 16.6 | 35.7 | 170 |
| 4 | 55 | 35.8 | 626 | 13.4 | 32.5 | 193 |
| 5 | 66 | 24.6 | 143 | - | 11.0 | 130 |
| 6 | 21 | 19.3 | 489 | *189.0* | 40.0 | 122 |
| 7 | 25 | 37.8 | 621 | *24.9* | *59.0* | 105 |
| 8 | 44 | 35.9 | 513 | 9.4 | 25.0 | 205 |
| 9 | 55 | 29.3 | *837* | 18.0 | *47.0* | 178 |
| 10 | 55 | 41.1 | 349 | 9.7 | *46.6* | 75 |
| 11 | 52 | 26.1 | 672 | 21.8 | *43.0* | 156 |
| 12 | 54 | 34.7 | 434 | 11.9 | *43.3* | 100 |
| 13 | 54 | 33.6 | 436 | 5.7 | 25.0 | 174 |
| 14 | 24 | 21.3 | *1500* | *63.0* | *110.0* | 136 |
| 15 | 45 | 26.3 | 570 | 12.9 | 29.3 | 195 |
| 16 | 54 | 27.4 | 560 | 15.3 | 41.1 | 136 |
| 17 | 59 | 39.9 | 705 | 22.6 | *61.9* | 114 |
| 18 | 46 | 45.1 | 644 | 22.8 | 23.0 | 280 |
| 19 | 43 | 28.1 | 158 | 7.0 | 12.6 | 125 |
| 20 | 62 | 30.0 | *790* | *24.3* | *49.0* | 161 |
| 21 | 47 | 32.0 | 525 | 10.8 | *61.0* | 86 |
| 22 | 88 | 21.5 | *1173* | 20.1 | *49.7* | 236 |
| 23 | 58 | 32.0 | *989* | 18.3 | *66.8* | 148 |
| 24 | 54 | 38.0 | 344 | 11.4 | 39.5 | 87 |
| 25 | 67 | 32.8 | 224 | 4.4 | 32.6 | 69 |
| 26 | 52 | 30.0 | 419 | 8.4 | *57.6* | 73 |
| 27 | 79 | 29.2 | 530 | 7.0 | 37.2 | 142 |
| 28 | 58 | 27.9 | 183 | 8.5 | 25.4 | 72 |
| 29 | 58 | 32.5 | *884* | *25.2* | *53.0* | 167 |
| 30 | 68 | 23.8 | 624 | 11.0 | 33.7 | 185 |
| 31 | 75 | 35.0 | 452 | 4.1 | 36.6 | 123 |
| 32 | 55 | 30.3 | 670 | 11.4 | 23.0 | 291 |
| 33 | 54 | 37.3 | 278 | 8.5 | 24.5 | 113 |
| 34 | 34 | 40.4 | 245 | 5.4 | 30.0 | 82 |
| 35 | 52 | 22.4 | 251 | 2.7 | 29.6 | 85 |
| 36 | 69 | 27.1 | 586 | 11.8 | 36.8 | 159 |
| 37 | 59 | 40.1 | 514 | 12.9 | *71.0* | 72 |
| 38 | 65 | 29.5 | 344 | 3.7 | 28.7 | 120 |
| **mean ± SD** | **55 ± 14** | **31.1 ± 6.2** | **525 ± 290** | **19.9 ± 30.9** | **41.3 ± 18.9** | **132 ± 53** |

*Note:* All treated with testosterone 50 mg/day (gel) for ≥3 months.

conventional testosterone therapy, and the potential thrombotic interaction of E2 with previously undiagnosed familial thrombophilia.

As noted by Wang et al,[57] the results for serum total testosterone levels vary greatly by assay technique, and it is particularly important to use a single, standardized methodology in prospective follow-up of serum testosterone levels.

If, as in our study 39% to 45% of men on testosterone therapy have high E2, given an estimated Caucasian population prevalence of heterozygosity for the factor V Leiden mutation of 5%,[58] there should be considerable opportunity for men having high E2 on testosterone to be factor V Leiden heterozygotes, with resultant increased risk to thrombosis.

Why was DVT-PE not a marked[22] adverse event feature of AndroGel phase III trials, given our finding of 6 cases who developed thrombotic events 3, 5–7, 17, and 18 months after starting testosterone, 5 of whom had previously undiagnosed factor V Leiden heterozygosity? The explanation speculatively lies in the size of the denominator (men with the factor V mutation also receiving AndroGel), which is unknown in our case series but could be estimated in the AndroGel phase III trials. AndroGel was evaluated in a Phase III multicenter, randomized, parallel-group, active-controlled,

**Table V.** Correlations among testosterone, free t, ratio testosterone/ estradiol (T/E2), and estradiol and BMI

| In 20 men who had measures before and during treatment | | | |
|---|---|---|---|
| | **Mean ± SD** | **Correlation with Estradiol (r)** | **Correlation with BMI (r)** |
| Before treatment | | | |
| Testosterone (ng/dL) | 246 ± 217 | 0.65† | 0.30 |
| Free T (pg/mL) | 8.5 ± 9.8 | 0.39 | 0.63† |
| Estradiol (pg/mL) | 26.0 ± 17.0 | | 0.20 |
| T/E2 | 93 ± 35 | | 0.23 |
| During treatment[2] | | | |
| Testosterone (ng/dL) | 478 ± 248‡ | 0.64† | −0.09 |
| Free T (pg/mL) | 13.9 ± 6.7* | 0.33 | 0.04 |
| Estradiol (pg/mL) | 38.5 ± 16.3† | | 0.32 |
| T/E2 | 126 ± 43* | | −0.52* |
| **Changes** | **Mean ± SD** | **Correlation with change in Estradiol (r)** | **Correlation with BMI (r)** |
| Change in testosterone (ng/dl) | +232 ± 237 | 0.58† | −0.28 |
| Change in free T (pg/mL) | +5.7 ± 12.1 | 0.17 | −0.40 |
| Change in estradiol (pg/mL) | +11.4 ± 13.9 | | 0.11 |
| Change in T/E2 | +35 ± 57 | | −0.53* |
| **In 38 men who had measures during treatment** | | | |
| | **Mean ± SD** | **Correlation with Estradiol (r)** | **Correlation with BMI (r)** |
| During treatment | | | |
| Testosterone (ng/dL) | 525 ± 290 | 0.54‡ | −0.17 |
| Free T (pg/mL) | 19.9 ± 30.9 | 0.54‡ | −0.22 |
| Estradiol (pg/mL) | 41.3 ± 18.9 | | 0.08 |
| T/E2 | 132 ± 53 | | −0.34* |

*Note:* Spearman correlation. Paired Wilcoxon test changes from before to during testosterone treatment.
*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

180-day trial, with evaluable data in 195 men, with a 3-year flexible dose follow-up in 162 men (FDA AndroGel package insert). Wang et al[22] reported evaluable follow-up data of AndroGel use in 123 of these 162 subjects, up to 42 months. Assuming a Caucasian population prevalence of heterozygosity for the factor V Leiden mutation of 5%,[58] then the Phase III AndroGel trials might have included 10 men with the factor V Leiden mutation with 6-month follow-up and 6 men with up to 42-month follow-up. Assuming that like women, 28% of the factor V Leiden heterozygotes might sustain DVT-PE after exposure to increased E2,[59] then the expected number of DVT-PE incidents in the AndroGel Phase III trials would have been very low, occurring in less than 1 single case (ranging from an estimate of 0.2 to 0.3 men). As reported by Wang et al,[22] of 123 evaluable subjects, 1 (0.8%) of the cohort had "deep venous thrombosis deemed to be possibly related to T replacement." Given the small number of men participating in the AndroGel Phase III trials, it would have been difficult to recognize DVT-PE as a common major adverse event. However, the denominator for DVT-PE in the general population could be the total number of men taking AndroGel, a large cohort, 5% of whom might be factor V Leiden heterozygotes,[58] and thus at risk for a thrombotic interaction between high E2 derived from aromatization of testosterone and familial thrombophilia.

In the current study, of 20 men having total and free testosterone and E2 measured before exogenous testosterone therapy, 9 (45%) had high E2 on therapy, and change in total testosterone and in E2 on testosterone therapy were closely correlated ($r = 0.58$, $P = 0.0097$). Moreover, 15 of 38 men (39%) on testosterone therapy had high E2, and total testosterone was closely correlated with E2 on testosterone therapy ($r = 0.54$, $P = 0.0004$). Our findings on increments of E2 during testosterone therapy are congruent with previous reports.[21,22] The publicly available data from the AndroGel phase III trials does not include the number and percentage of subjects who developed high E2 on therapy.[22] The relatively high frequency of high E2 with its E2-associated attendant thrombophilia in men treated with testosterone,[21,22,53] when coupled with common hereditary thrombophilia (factor V Leiden heterozygosity in ~5% of Caucasians),[58,60-62] provides ample synergism for thrombosis. Additional information pertaining to the incidence of thromboembolism in testosterone-treated men with positive screening tests for familial and acquired thrombophilia

versus those with negative screening tests is needed to advise consideration of screening testosterone-treated men for thrombophilia and hypofibrinolysis.

Osteonecrosis of the hip and knee in adults[1-6] and pediatric osteonecrosis of the hip, Legg-Calve-Perthes disease,[7] are associated with factor V Leiden heterozygosity, as is osteonecrosis of the jaw.[9] MTHFR C677T homozygosity is associated with osteonecrosis of the hip in Koreans.[8]

Pulmonary embolism has been reported in a single case study of a man given exogenous testosterone and anabolic steroids,[63] but no assessment of thrombophilia was reported. In our 3 patients with pulmonary emboli 3, 7, and 17 months after starting testosterone, we speculate that the interaction of the factor V Leiden mutation (found in all 3 patients) with testosterone aromatized to E2, which caused the pulmonary embolus.

High factor VIII has been associated with osteonecrosis,[12] and high factors VIII and XI have been associated with increased risk of deep venous thrombosis and pulmonary embolus.[55,64,65] Like other thrombophilias, risk of thrombosis is increased by E2 in subjects with high factor VIII.[66]

**Speculation.** Thrombotic events after starting testosterone therapy seem to be associated with familial thrombophilia, predominantly heterozygosity for the factor V Leiden mutation. We speculate that when exogenous testosterone is aromatized to E2, and E2-induced thrombophilia is superimposed on familial thrombophilia, thrombosis occurs. Men sustaining thrombotic events after testosterone therapy should be screened for the factor V Leiden mutation and for other familial and acquired thrombophilias.

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