The safety of postmenopausal testosterone therapy

Kate Maclaran[1,2] & Nick Panay*[1]

Testosterone is increasingly used as part of postmenopausal HRT regimens. Unfortunately, few androgenic preparations designed specifically for use in women have been approved by regulatory authorities. Ongoing concerns exist surrounding the potential long-term effects of testosterone therapy. Here, we review the most recent data on postmenopausal testosterone therapy, focusing particularly on the effects of testosterone on breast, endometrium and cardiovascular health.

Women's HEALTH

In addition to estrogen and progesterone, testosterone is increasingly used as part of HRT regimens, particularly in women suffering from symptoms of androgen deficiency such as low sexual desire and well-being. Despite increasing evidence in support of postmenopausal androgen therapy, its use remains controversial [1,2] and options for androgen replacement in women are currently very limited.

There are, at present, no androgen products licensed by the US FDA for use in women. The transdermal testosterone patch (TTP; Intrinsa®, Warner Chilcott, NJ, USA) has been approved by the EMA for use in surgically menopausal women with hypoactive sexual desire disorder (HSDD), and subcutaneous testosterone implants are licensed in the UK and Australia.

Regulatory authorities have raised concerns regarding the lack of long-term safety data [3], particularly in terms of breast, cardiovascular and endometrial safety. Here, we review the most recent and relevant data investigating the effects of postmenopausal testosterone replacement.

Androgen physiology

In women, androgens circulate at levels approximately 10–20-times lower than in men [4,5]. Testosterone is produced in women both by direct secretion from the adrenal gland and the ovaries, and through the peripheral conversion of androgen precursors, such as androstenedione, dihydroepiandrosterone and its sulfate dihydroepiandrosterone. In young women, the ovaries produce approximately three- to four-times more testosterone than estrogen per day [6].

Within the circulation, most testosterone is bound to sex hormone-binding globulin (SHBG; 66%) or albumin (33%) [7]. Only a very small amount (~2%) circulates freely. Testosterone only binds to albumin with relatively weak affinity and therefore 'bioavailable' testosterone (i.e., that with the ability to diffuse across cell membranes) is often considered as the free testosterone plus the albumin-bound fraction.

The level of circulating androgens declines gradually with age, owing to a reduction in adrenal production, so the levels at the age of 40 years are approximately half that of a 20 year old [8]. In contrast to estrogen, androgen levels do not appear to fall rapidly during natural menopause, although studies have demonstrated a 40–50% drop in testosterone following surgically induced menopause [9,10]. In postmenopausal women, bioavailable testosterone is primarily determined by SHBG levels and is therefore highly susceptible to the many factors that affect SHBG levels, including obesity or exogenous estrogens.

Testosterone exerts wide-ranging effects via androgen receptors, which are found throughout the body, including in brain tissue, skin, adipose tissue, the vascular tree and bone. Testosterone can also be converted into estradiol by the aromatase enzyme and so it is not entirely clear whether testosterone exerts its actions directly or through the actions of estradiol. Although the exact role of androgens in females remains poorly understood [11], they clearly have an important role in sexual desire and arousal [12]. In addition, exogenous testosterone can affect bone density [13], body composition [14], energy levels and psychological well-being [15].

Testosterone replacement

Testosterone has been used therapeutically in women for over 70 years. It was initially reported to be beneficial in the management of a wide range of gynecological disorders including menorrhagia, dysmenorrhea, mastalgia and even pelvic inflammatory disease [16]. While testosterone is no longer used for these conditions, it was through the use of testosterone in gynecology that the role of androgens in female

[1]*West London Menopause & PMS Centre, Department of Gynaecology, Queen Charlotte's & Chelsea Hospital and Chelsea & Westminster Hospitals, Du Cane Road, London W12 0HS, UK*
[2]*Imperial College London, London, UK*
**Author for correspondence:*
Tel.: +44 208 383 1111
nickpanay@msn.com

Keywords

• androgen • HRT • menopause • sexual desire • testosterone

Future Medicine part of fsg

sexual dysfunction became increasingly clear. Reports of a beneficial effect of testosterone therapy in treatment of menopausal symptoms and female sexual dysfunction were published as early as the 1950s [17]. Many of the early studies reported on the use of injectable, oral or implant therapy, often in supraphysiological doses. Over the last decade, following the development of the TTP, there has been a marked increase in the number of clinical trials investigating the effects of physiological testosterone replacement, predominantly in postmenopausal women for treatment of low libido. These studies have led to a substantial improvement in our understanding of the efficacy of postmenopausal testosterone therapy and potential adverse effects.

Benefits of testosterone therapy

Reduction in testosterone levels have been associated with reduced libido and sexual activity, fatigue and a lesser sense of physical well-being. Terms such as 'relative androgen deficiency' [18] or 'female androgen insufficiency syndrome' [19] have been suggested to characterize this constellation of symptoms (**Box 1**).

Although exogenous testosterone has been recognized for many years to play a role in improving sexual desire, until recently there has been a lack of randomized controlled data to support its use. Many older studies showed that postmenopausal testosterone use in women on estrogen replacement therapy improves desire [20–23], although these studies primarily used oral or intramuscular therapy, often in supraphysiological doses.

More recently, attention has turned towards the use of TTPs. It is thought that this route provides a more physiological and constant serum concentration of the hormone. In addition, the transdermal route avoids first-pass metabolism in the liver, which may help reduce unwanted effects on hepatic proteins. A number of randomized placebo-controlled trials investigating the use of the testosterone patch in treating HSDD have found significant improvements in sexual desire and other sexual function domains both with [24–28] and without [29,30] concurrent HRT use.

Box 1. Symptoms of androgen deficiency.

- Reduced sense of well-being
- Dysphoric mood
- Low energy/reduced motivation
- Low sexual desire
- Bone loss
- Reduced muscle strength
- Poor cognition and memory
- Insomnia

In addition to the TTP, testosterone is now most frequently administered via subcutaneous implant or transdermal gel/cream. The testosterone implants have been used for over 30 years and there are both observational and randomized controlled trial (RCT) data that women receiving estradiol and testosterone implants have a significant benefit in sexual function compared with those receiving estrogen alone [31,32]. Testosterone gel is the least well-studied route in women. There is one randomized, placebo-controlled, crossover study in 53 postmenopausal women with low libido on HRT, which showed that 10 mg daily of testosterone gel was associated with significant improvements in desire, including frequency of sexual activity, fantasies and sexual interest [33]. More extensive reviews on the use of testosterone for the management of low libido have recently been published [34,35].

In addition to the effects of exogenous testosterone on sexual function, several other beneficial effects have been reported, particularly on quality of life, mood and well-being [23,24,26,36]. A recent observational study examined the use of 75–160 mg testosterone implants, without concomitant estrogen, in 300 premenopausal and postmenopausal women reporting symptoms of androgen deficiency such as sexual problems, fatigue, mood disturbance, headaches, insomnia, memory loss and hot flushes. Testosterone implants were found to be effective for the relief of psychological, somatic and urogenital symptoms, in addition to sexual desire [37]. Furthermore, pilot data have indicated that transdermal testosterone may improve cognitive performance, particularly memory and verbal learning in postmenopausal women on transdermal HRT [38].

Androgens can also act to inhibit bone resorption and therefore it has been suggested that testosterone may play a role in the maintenance of bone density. Although no studies have examined fracture rates as a clinical end point, there are data that testosterone implants [13] and combined oral estrogen–methyltestosterone therapy may improve bone density [39,40]. However, conflicting data exist, as a further study found improvements in bone density in both estrogen- and estrogen–androgen-treated groups, with no significant difference observed between groups [41].

Potential adverse effects

Although testosterone replacement can therefore have significant benefit, an ongoing concern frequently cited by regulatory authorities and consensus statements is the apparent uncertainty surrounding the long-term safety of testosterone, especially with regard to the risk of breast or endometrial cancer and cardiovascular disease [3,42]. As will be discussed, many of these concerns are unfounded, particularly when using physiological, transdermal therapy.

Androgenic side effects

Hirsutism and acne are the most common androgenic side effects observed in women receiving physiological doses of testosterone. Androgenic side effects tend to be dose dependent [29] and may take several months to become evident, but are usually mild and resolve when treatment is discontinued.

In the TTP randomized trials, hirsutism was reported by 3.0–19.9% of patients receiving the TTP, although in the majority of studies this was not significantly more frequent than in the placebo group [24–28]. Of note, hirsutism was significantly increased in testosterone users (19.9% cf. 10.5% of controls) in the sole TTP study to continue safety assessment for 52 weeks, in contrast to the other trials, which were 24 weeks in duration [29]. However, participants in this study were not on concomitant HRT, which probably contributed to the findings.

Only one study reported an increase in acne (18% cf. 13% of controls) [28]. In the other TTP trials there was no significant increase in acne, which was experienced by 4.6–7.5% of participants.

Virilization – voice deepening, clitoromegaly and frontal hair loss – is extremely rare and tends to occur only when serum testosterone levels rise significantly above the normal range for reproductive females.

Concern has been expressed that many of these clinical trials were only of relatively short duration and therefore may not reflect clinical practice. Nachtigall *et al.* have recently published a 4-year extended open-label follow-up data from 1094 surgically menopausal women on concomitant estrogen, using testosterone for HSDD [43]. Participants had a mean exposure of 1.1 years of testosterone therapy. They found no increase in the rates of androgenic adverse events (unwanted hair growth, acne, alopecia or voice deepening) over 4 years of treatment, and reported events to be generally mild and not associated with study discontinuation. The observed rates of all androgenic events were 28.4% (0–12 months), 14.3% (12–24 months), 10.5% (24–36 months) and 3.5% (36–48 months).

In an observational case–control study, 8412 women were followed-up for a mean of 4.4 years. The study population included 2103 testosterone users, administered through implants (72.2%), tablets (18.4%) or injections (7.9%). The rate of androgenic events was increased in the testosterone cohort (relative risk [RR]: 1.55, 95% CI: 1.21–1.97), although the overall incidence of androgenic events was only 1.2% in testosterone users compared with 0.7% of controls [44].

Cardiovascular disease

Owing to the marked gender differences in the prevalence of cardiovascular disease, it has previously been thought that androgens increase cardiovascular risk. However, recent studies in men have demonstrated that low circulating testosterone levels are associated with increased cardiovascular risk, even after adjustment for classical cardiovascular risk factors [45]. This has led to increasing speculation regarding the potential cardioprotective role of testosterone.

Unfortunately, in women, the role of androgens on the cardiovascular system is less clear. Potential mechanisms by which androgens may affect cardiovascular risk include effects on lipid profile, insulin resistance, vascular tone and fat distribution.

There have been several studies examining the effects of both endogenous and exogenous testosterone levels on cardiovascular disease, atherosclerosis and cardiovascular risk factors; however, results are frequently conflicting. Methodological limitations of testosterone assays, failure to adjust for estrogen levels, and the varying cardiovascular effects depending on route of sex-hormone administration have contributed to the inconsistent results.

Endogenous testosterone

Many studies have examined the effects of circulating testosterone levels on risk factors for cardiovascular disease. It is well established that in premenopausal women, hyperandrogenism as seen in women with polycystic ovarian syndrome is associated with an adverse cardiovascular risk profile, with increased triglycerides and low-density lipoprotein cholesterol, and reduced high-density lipoprotein (HDL) cholesterol [46,47].

In postmenopausal women, the effects of endogenous androgen levels are less well established. Raised endogenous testosterone has been

linked to multiple cardiovascular risk factors including increased BMI, increased abdominal obesity and elevated blood pressure [48]. Although some studies have demonstrated that increased androgenicity is associated with adverse changes to the lipid profile including increased total cholesterol, increased low-density lipoprotein cholesterol and reduced HDL cholesterol [49,50], the majority of observational data do not support a role for endogenous testosterone and lipid metabolism [48].

Studies investigating the effects of endogenous testosterone on surrogate markers for atherosclerosis have shown conflicting results. Bernini *et al.* demonstrated that endogenous free testosterone levels in 44 postmenopausal women were negatively correlated with carotid intima-media thickness, suggesting a positive effect on the development of atherosclerosis [51]. Subsequent studies [52,53] have also shown an inverse relationship between endogenous testosterone levels and atherosclerosis. A case–control study found that postmenopausal women with proven carotid artery disease were found to have significantly lower levels of free testosterone compared with women with normal carotid arteries, independent of other cardiovascular risk factors [54]. By contrast, other studies have found high, free or bioavailable testosterone to be associated with subclinical atherosclerosis, even after adjustment for estradiol and other cardiovascular risk markers [55,56].

Few studies have examined the impact of endogenous testosterone levels on cardiovascular events and mortality. Patel and colleagues performed a cross-sectional study in 344 older postmenopausal women and found that women in the top quartile for total testosterone had a threefold increased risk of coronary heart disease (odds ratio: 2.95, 95% CI: 1.2–73); however, this association was not true for free testosterone [57]. In another study, the association between the Free Androgen Index and increased cardiovascular risk did not persist after adjustment for BMI and other cardiovascular risk factors [58].

A recent prospective population-based study followed 639 women, who had testosterone levels measured at baseline, for a mean of 12.3 years [59]. There were 134 cardiovascular events during the follow-up. In this study, a U-shaped cardiovascular risk curve was observed, with those in the highest and lowest quintile for bioavailable testosterone having increased cardiovascular risk. This led the authors to suggest that to optimize cardiovascular health, testosterone levels shown be within normal limits [59].

Exogenous testosterone

Therefore, although increased androgenicity is associated with an adverse cardiovascular risk factor profile in postmenopausal women, evidence for an association with cardiovascular events is lacking. Furthermore, it is uncertain whether these associations with endogenous testosterone levels have clinical implications regarding the use of postmenopausal testosterone therapy. Few studies have investigated the cardiovascular risks associated with testosterone administration and no studies have reported on cardiovascular events as a primary outcome.

In animal models, testosterone has been associated with activation of the renin and angiotensin system [60,61] and other cardiovascular risk factors, such as elevated BMI and visceral fat [62]. When looking at surrogate markers of cardiovascular risk such as endothelial function, conflicting results occur. Testosterone administration has been shown to impair endothelium-dependent vasodilatation in hypercholesterolemic rabbits [63], but a further study demonstrated that testosterone caused relaxation of rabbit coronary arteries and aortic rings [64]. High-dose exogenous testosterone in primate models was associated with a doubling of atherosclerosis [62] and similar results of increases in atherosclerotic plaques were observed in rabbit models [65]. Of note, physiological administration of testosterone to androgen-deficient rats led to an improvement in the vasodilator response of the endothelium [66].

Several studies have investigated the effect of exogenous testosterone on traditional cardiovascular risk factors and other surrogate markers of cardiovascular disease.

BMI/weight distribution

High-dose intramuscular testosterone in female-to-male transsexuals has been associated with reductions in subcutaneous fat, but increases in BMI and visceral fat [67–69]. Oral therapy has been associated with increases in lean body mass [23,70] but also with increases in visceral fat mass [71]. Gruber *et al.* examined the effect of transdermal testosterone gel or placebo on body composition in 39 postmenopausal women [72]. Testosterone gel was associated with a significant reduction in total body weight, abdominal fat and BMI. No differences in BMI were observed in a 24-week placebo-controlled study of the TTP [26].

Lipid profile

Studies examining the effects of oral testosterone therapy have consistently demonstrated a positive

effect on triglyceride levels, but at the expense of reductions in HDL [22,23,39,40,71,73–76]. The mechanisms through which testosterone may influence lipid metabolism have not been fully elucidated. In contrast to oral testosterone administration, transdermal therapy does not appear to affect the lipid profile [72]. No disturbances of lipid profiles were found in 4-year follow-up data from 967 surgically menopausal patients who were also on estrogen replacement, each receiving at least one application of the TTP [43]. Thus, the effects of testosterone on lipid profile will depend on the route of administration, dosage and the presence of concomitant estrogen therapy.

Blood pressure

There are no data to suggest a detrimental effect on blood pressure from oral [71], subcutaneous [77] or transdermal therapy [26].

Surrogate cardiovascular markers

The effect of exogenous testosterone on surrogate markers for cardiovascular disease has been studied in three trials. In a trial by Hak *et al.*, intramuscular testosterone and estrogen administration resulted in increased rates of severe aortic atherosclerosis in postmenopausal women, as demonstrated by radiographic detection of calcified aortic deposits [78]. However, this study involved the use of high-dose intramuscular testosterone in a small population and only demonstrated a small effect after 1 year of treatment. Penotti *et al.* examined the effects of 8 months of relatively high-dose oral testosterone undecanoate in addition to transdermal estrogen and medroxyprogesterone acetate on vascular reactivity [79]. They found a small but significant increase in the pulsatility index of the middle cerebral artery in women receiving testosterone. By contrast, Worboys *et al.* showed an improvement in flow-mediated and glyceryl trinitrate-induced vasodilatation following 6 weeks of 50-mg testosterone implant therapy in addition to ongoing estrogen treatment, suggesting a beneficial role for exogenous testosterone in vascular reactivity [77].

No long-term prospective studies have been sufficiently powered to examine cardiovascular risk associated with exogenous testosterone, and there are few data investigating the effect of physiological replacement using transdermal preparations on surrogate markers of cardiovascular disease. A retrospective study in female-to-male transsexuals receiving high doses of testosterone showed no increase in frequency of myocardial infarction, hypertension and no excess of cardiovascular deaths [80]. These studies have involved high dose, oral or intramuscular therapy and, therefore, whether this data can be applied to postmenopausal testosterone use remains controversial.

The recent RCTs using testosterone patches for reduced libido noted no short-term (up to 1 year) increased cardiovascular disease but did not include specific cardiovascular outcome measurements. Some of these studies took further measurements of vital signs, lipid profiles, coagulation, renal and liver function, carbohydrate metabolism and chemistry and hematology; however, no clinically relevant changes were noted in a 24-week [24–26,30] or 52-week follow-up [29].

Therefore, although high-dose parenteral or oral testosterone has been associated with some adverse cardiovascular markers, physiological transdermal replacement does not appear to be detrimental and may even have cardiovascular benefit. A large randomized, placebo-controlled trial designed to investigate cardiovascular outcomes in users of transdermal testosterone gel [81] is currently underway, and should provide useful data regarding the long-term cardiovascular effects of physiological transdermal testosterone replacement.

Glucose metabolism/insulin resistance

Insulin resistance is central in the development of the metabolic syndrome. This syndrome, consisting of insulin resistance, central obesity, hypertension and dyslipidemia [201], is a well-established risk factor for cardiovascular disease. The relationship between insulin resistance and androgens has been recognized for many years, primarily due to observations in patients with polycystic ovarian syndrome who suffer from hyperandrogensim and insulin resistance.

The mechanisms behind the complex relationship between androgens and insulin sensitivity remain a subject of debate. Insulin can promote androgen production by acting as a co-gonadotrophin with lutenizing hormone, to stimulate androgen production from ovarian theca cells. Insulin also causes a reduction in hepatic production of SHBG, resulting in higher free-androgen levels. Conversely, however, it has also been suggested that hyperandrogenism may affect glucose metabolism and insulin sensitivity. It has been shown *in vitro* that adipocytes exposed to testosterone have reduced insulin-mediated glucose uptake [82]. Androgens may indirectly affect insulin sensitivity via effects on lipid balance and body fat distribution. Furthermore, estradiol can affect insulin sensitivity and therefore, due to the peripheral conversion of androgens, their exact effects may be difficult to elicit.

Several clinical studies have demonstrated the relationship between supraphysiological androgen levels and hyperinsulinemia. Polycystic ovary syndrome is strongly associated with hyperinsulinemia and Type II diabetes mellitus [83]. Similar trends have been observed in postmenopausal women, as an elevated Free Androgen Index ratio has been associated with metabolic syndrome [84,85]. A further analysis from the Rancho Bernardo study found that in 233 postmenopausal women not taking estrogen, higher baseline bioavailable testosterone was associated with significantly increased insulin resistance and the risk of Type II diabetes mellitus, with a threefold increased risk of diabetes in women in the highest quartile of bioavailable testosterone (odds ratio: 2.9, 95% CI: 1.1–8.4) [86].

The implications of these findings for exogenous testosterone administration have not been fully explored. Zang *et al.* examined the effects of oral testosterone on insulin sensitivity in 63 postmenopausal women [70]. After 3 months, a small but significant reduction in insulin-mediated glucose disposal was observed in the testosterone groups. However, in obese postmenopausal women, treatment with an anabolic steroid with weak androgenic properties had no effect on fasting glucose or insulin sensitivity [87]. Other studies using oral testosterone therapy also failed to demonstrate any effect on glucose metabolism [71], and physiological replacement using the TTP has been shown to have no detrimental effects on glucose metabolism [25,26,29,36]. In a recent pilot study of elderly women with congestive cardiac failure, 6-month TTP therapy was associated with a significant improvement in insulin resistance [88], a finding which has also been observed in men.

Similarly to the influence on cardiovascular risk, it is possible that at physiological levels, testosterone plays an important role in glucose homeostasis. However, at supraphysiological levels it may result in adverse effects on insulin sensitivity. At present, there is insufficient evidence regarding the use of physiological transdermal testosterone in postmenopausal women to make definitive conclusions upon its effects on insulin sensitivity. Although current data are reassuring, studies are often limited by short treatment periods or small study numbers.

Endometrium

In vitro data suggest that androgens do not act directly to stimulate the endometrium [89]. However, aromatase activity has been observed in endometrial cancer cells [90,91], and so, concerns have been raised that androgens may act indirectly to simulate endometrial proliferation via estrogenic effects.

Very few clinical studies have examined the effects of exogenous testosterone on the endometrium. Only one randomized study could be found that examined the endometrial effects of postmenopausal testosterone replacement as a primary outcome. In this unblinded study, Zang and colleagues randomized 63 naturally postmenopausal women to 2 mg estradiol (n = 22), 40 mg testosterone undecanoate on alternate days (n = 21), or both estradiol and testosterone (n = 20) [92]. Endometrial thickness and proliferation was assessed after 3 months of treatment by ultrasonography and histopathology. They found that endometrial thickness was significantly increased by estrogen therapy, alone or in combination with testosterone. In those receiving testosterone-only therapy, there was no increase in endometrial thickness or proliferation.

In the APHRODITE study, Davis *et al.* investigated the effects of two doses of transdermal testosterone in 814 postmenopausal women with HSDD who were not using concomitant estrogen [29]. Safety outcomes were assessed over a 52-week period. Vaginal bleeding occurred more frequently in the group using 300 µg patches (10.6%) compared with the 150 µg (2.7%) or placebo groups (2.6%), but no cases of endometrial hyperplasia or carcinoma were diagnosed. Other studies using the TTP in conjunction with HRT have not demonstrated increases in vaginal bleeding [26].

In a 6-month double-blinded randomized trial of esterified estrogen, given with or without 1.25 mg methlytestosterone (but no progestogen), endometrial proliferation was observed in both groups but there was no difference in biopsy scores between groups [73]. A double-blinded randomized cross-over study in 53 naturally menopausal women found that 10 mg of testosterone gel daily was not associated with any change in endometrial thickness compared with placebo [33]. In an observational case–control study, 8412 women were followed-up for a mean of 4.4 years. Of the 2103 testosterone users, there were no cases of endometrial cancer compared with five cases in 6309 controls [44]. Finally, there is evidence that even relatively high-dose long-term testosterone therapy, as given to female-to-male transsexuals, does not result in endometrial proliferation and may even have atrophic effects [93].

Breast

The effects of testosterone on the breast and in the etiology of breast cancer remain poorly understood. Androgens may act directly on breast tissue via the androgen receptor but breast tissue also exhibits aromatase activity and therefore, again, the androgenic effects may occur due to conversion to estrogen.

Experimental data have shown that androgens can have both proliferative [94] and antiproliferative [95–97] effects on breast cancer cell lines. In addition, there is evidence that dihydrotestosterone is proapoptotic [98,99] and that androgens may inhibit estrogenic effects on mammary growth [100]. Animal studies have demonstrated that in nonhuman primates, testosterone treatment was associated with a reduction in estrogen-induced breast epithelial cell proliferation [96,101]. Based on these experimental data, it has therefore been suggested that testosterone may act to reduce the adverse effects of estrogen on breast tissue [102].

Many observational studies have examined the relationship between endogenous androgen levels and the risk of postmenopausal breast cancer. A combined analysis of nine prospective studies with 663 incidences of breast cancer cases demonstrated that higher endogenous testosterone was associated with increased risk of postmenopausal breast cancer (RR: 2.22, 95% CI: 1.59–3.10, for those in the highest testosterone quintile compared with the lowest) [103]. A more recent case–control study from the Nurses Health Study with 265 cases and 541 controls reported similar findings with a RR of 1.8 (95% CI: 1.1–2.9) for those in the highest androgen quintile compared with those in the lowest group [104]. Other observational studies have produced conflicting results with no association between endogenous androgens and breast cancer risk observed [105,106]. Methodological limitations with testosterone assays in women, differences in the testosterone fraction measured and failure to adjust for factors such as BMI, estradiol and SHBG levels may, in part, explain the conflicting results. Of note, current evidence in women with hyperandrogenism due to polycystic ovary syndrome is not suggestive of an increased risk of breast cancer [107].

There are relatively few clinical trial data examining the effect of exogenous testosterone on breast cancer risk. Trials to date are summarized in Table 1. Many studies have predominantly investigated the effects of oral methyltestosterone and there is very limited data regarding the breast cancer risk associated with transdermal therapies. Furthermore, many studies have limitations including small case numbers, failure to adjust for confounding factors and the potential for recall bias. Available studies investigating nonoral routes of testosterone replacement have produced more reassuring results than those using oral or intramural, often high-dose, therapy.

An early case–control study suggested that the risk of breast cancer was increased in women using intramuscular testosterone in addition to estrogen or estrogen plus progestogen therapy [108]. A 24 year follow-up of the Nurses Health Study cohort, contributing 1,359,323 person years of data and 4610 incident cases, found an increased breast cancer risk in combined estrogen and testosterone users compared with never users (RR: 2.48, 95% CI: 1.53–4.04) [109]. Data from the Women's Health Initiative observational arm reported nonsignificant increases with combined estrogen and testosterone use, but significant increases in risk when Estratest® (Solvay Pharmaceuticals, GA, USA) was compared separately with other products (hazard ratio: 1.78, 95% CI: 1.05–3.01) [110]. More reassuringly, the other six studies did not find any increased risk of breast cancer associated with postmenopausal testosterone use [44,111–115].

There are no randomized data investigating breast cancer risks with testosterone replacement as a primary outcome. Randomized, placebo-controlled studies on the effects of the TTP on breast cell proliferation [116] and mammographic density [117,118] have shown no adverse effects from transdermal testosterone.

There has been a suggestion from one study that the risk of breast cancer may increase with duration of testosterone treatment [113]. Of the studies that specifically looked at treatment duration, none found an association between duration of treatment and breast cancer risk [109–112,114,115].

Therefore, currently available clinical data are reassuring, particularly regarding physiological replacement via transdermal or subcutaneous routes, although further randomized controlled trials are needed. The large randomized BLISS trial [81] will be examining incidence of invasive breast cancer as a co-primary outcome and should therefore provide much needed safety data.

Conclusion

Testosterone is increasingly used as part of postmenopausal hormone replacement regimens to improve sexual desire and other symptoms of androgen deficiency. As with estrogen therapy, clinical practice is moving more towards physiological replacement with lower dose transdermal

Table 1. Epidemiological studies investigating breast cancer risk with exogenous testosterone therapy.

Author (year)	Study type	n	Primary route of T treatment	Findings	Ref.
Ewertz (1988)	Population-based case–control	1486 cases of invasive breast cancer diagnosed over 1 year, 1336 controls	im. T (50–100 mg) at 3–7-week intervals + HRT	E + T associated with increased breast cancer risk E + P: RR: 1.36 (95% CI: 0.98–1.87) E + T: RR: 2.31 (95% CI: 1.37–3.88; users 56 cases, control 21) E + P + T: RR: 1.26 (95% CI: 0.96–2.24; users 16 cases, control 11)	[108]
Brinton *et al.* (1986)	Population-based case–control	1960 cases, 2258 controls	Oral E/MT combination	No increased risk with E/MT RR: 1.18 (95% CI: 0.70–2.0) 26 cases and 27 controls used E + T	[111]
Dimitrakakis *et al.* (2004)	Retrospective observational study	508 PM women, mean follow-up 5.8 years	Implants 5–150 mg 5-monthly	Incident rates: 7 incident cases in users E + T 115/100,000 E/P + T 293/100,000 cf. to HRT users in WHI (380/100,000) and MWS (520/100,000)	[112]
Tamimi *et al.* (2006)	Prospective cohort study	Nurses Health Study 24-year follow-up 70,444 PM women, 1,359,323 person-years, 4610 incident cases	Oral E/MT combination	T associated with increased breast cancer risk cf. HRT never-users E alone: RR: 1.15 (95% CI: 1.05–1.27) E + T: RR: 1.77 (95% CI: 1.22–2.56) T alone: RR: 2.52 (95% CI: 0.80–7.94) E + P: RR: 1.58 (95% CI: 1.44–1.73)	[109]
Ness *et al.* (2009)	Observational cohort study	WHI observational study 31,842 PM women, mean 4.6-year follow-up	Oral E/MT combination	593 incident cases, 35 in T users, 558 in nonusers E + T: RR: 1.42 (95% CI: 0.95–2.11) Estratest: RR: 1.78 (95% CI: 1.05–3.01)	[110]
Colditz *et al.* (1995)	Prospective cohort study	Nurses Health Study 1992 follow-up 69,566 PM women, 725,550 person-years, 1935 incident cases	Oral E/MT combination	No significant increase in T group E alone: RR: 1.32 (95% CI: 1.14–1.54) E + P: RR: 1.41 (95% CI: 1.15–1.74) E + T: RR: 1.64 (95% CI: 0.53–5.09)	[113]
Jick *et al.* (2009)	Observational case–control	4515 cases, 18,058 controls	Oral E/MT combination	No significant increase in T group compared with nonusers E alone: RR: 0.96 (95% CI: 0.88–1.06) E + P: RR: 1.44 (95% CI: 1.31–1.58) E + T: RR: 1.08 (95% CI: 0.86–1.36; 998 cases, 380 controls) E + P + T: RR: 1.69 (95% CI: 1.03–2.79; 22 cases, 55 controls)	[114]
Davis *et al.* (2009)	Retrospective cohort study	631 PM women, 4015 person-years, 6.7-year follow-up	Implant or transdermal	12 incident cases Age-adjusted incidence rate ratio: 1.35 (95% CI: 0.76–2.38) 299 cases per 100,000	[115]
Van Staa and Sprafka (2009)	Observational case–control	8412 women, 2103 T users, 6309 controls	Implant (72.2%) Oral (18.4%) Injection (7.9%)	16 cases in T users, 52 in controls RR: 0.78 (95% CI: 0.44–1.37)	[44]

E: Estrogen; im.: Intramuscular; MT: Methyltestosterone; MWS: Million Women Study; P: Progestogen; PM: Postmenopausal; RR: Relative risk; T: Testosterone; WHI: Women's Health Initiative.

preparations in an attempt to minimize adverse effects. Current data do not indicate that transdermal preparations are associated with any adverse cardiovascular, breast or endometrial outcomes but further large-scale randomized data are needed. Few testosterone preparations are currently licensed for use in women, and approved products must be used with concomitant estrogen therapy. More data is needed to support the use of testosterone with this population.

Future perspective

In the next 5–10 years, we will hopefully see more data in support of testosterone replacement

and convince regulatory authorities that transdermal testosterone should be approved for postmenopausal women.

Despite more than 50 years of observational data and short-term RCT data, which is generally reassuring, further evidence is needed, particularly regarding the risk of breast cancer. Unfortunately, studies of sufficient length (10–20 years) and power to fully assess breast cancer risk are unlikely to happen, therefore, we must rely on shorter RCTs or epidemiological studies. Importantly, in the next few years, large randomized trials (BLISS) [81] will report on the cardiovascular and breast cancer outcomes associated with transdermal testosterone gel. In licensed products we must continue research through RCTs and postmarketing surveillance. Furthermore, the regulatory authorities must be made aware that testosterone therapy is not merely a 'lifestyle drug' but a drug that is essential in some postmenopausal women

Executive summary

Background

- Testosterone is increasingly used as part of postmenopausal HRT regimens but concerns have been raised regarding the lack of long-term safety data.

Androgen physiology

- Androgens predominantly circulate bound to sex hormone-binding globulin and albumin and therefore biologically active testosterone is highly susceptible to factors that alter sex hormone-binding globulin levels, including oral estrogens and obesity.

Testosterone replacement

- Older methods of testosterone replacement such as parenteral or oral therapy are being increasingly replaced by transdermal or subcutaneous therapies.

Benefits of testosterone therapy

- Testosterone has been shown to improve sexual function, quality of life, mood, cognition and bone density.

Potential adverse effects

- Androgenic side effects
  - Hirsutism & acne are the most common androgenic side effects.
  - Androgenic effects are dose dependent but usually mild and resolve on discontinuation of therapy.
  - Virilization is extremely rare with physiological replacement.
- Cardiovascular disease
  - Endogenous testosterone
    - Both low and high levels of endogenous testosterone have been associated with cardiovascular events.
    - It has been suggested to optimize cardiovascular health that testosterone should be kept within normal limits.
  - Exogenous testosterone
    - High dose or oral therapy can increase lean body mass but may be associated with visceral fat deposition. Transdermal therapy has either a neutral or beneficial effect on body composition.
    - Oral testosterone therapy has a positive effect on triglyceride levels but causes reductions in high-density lipoprotein cholesterol. Transdermal therapy does not appear to affect lipid metabolism.
    - There is currently no evidence that testosterone replacement affects blood pressure.
    - There are no long-term prospective studies sufficiently powered to examine cardiovascular risk associated with exogenous testosterone.
    - Data investigating the effect of testosterone on surrogate markers of cardiovascular disease show that high-dose therapy may be detrimental whereas low-dose subcutaneous therapy may have cardiovascular benefit.
- Glucose metabolism/insulin resistance
  - There is currently insufficient evidence to make definitive conclusions upon the effects of testosterone on insulin sensitivity but there are no data to suggest a deleterious effect from current therapies.

Endometrium

- Available evidence does not support an increased risk of endometrial cancer with testosterone therapy.

Breast

- Epidemiological studies linking elevated endogenous androgens with breast cancer have produced conflicting results.
- Although current clinical data regarding the breast cancer risk with exogenous therapy are reassuring, particularly regarding physiological replacement via transdermal or subcutaneous routes, a lack of randomized trials precludes definitive conclusions.

Conclusion

- As with estrogen therapy, clinical practice is moving more towards physiological replacement with lower dose transdermal testosterone preparations to minimize adverse effects.
- Current data do not indicate that these preparations are associated with any adverse cardiovascular, breast or endometrial outcomes but further large scale randomized data are needed.

to restore or maintain their quality of life. As with estrogen and progestogen therapy, small risks may be accepted if significant benefit is derived.

Should further reassuring long-term safety data become available we hope this would allow more products to be approved and stimulate development of new preparations to improve patient choice. The next few years are also likely to see development of novel selective androgen receptor modulators, which aim to harness the beneficial effects of androgen therapy while minimizing adverse effects.

Financial & competing interests disclosure

The authors have been in receipt of a study grant from Warner Chilcott (NJ, USA) to purchase reagents for a testosterone patch study. N Panay has performed consultancy work for Proctor and Gamble and been involved in previous testosterone patch studies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Basaria S, Dobs AS. Clinical review: controversies regarding transdermal androgen therapy in postmenopausal women. *J. Clin. Endocrinol. Metab.* 91, 4743–4752 (2006).
2. Braunstein GD. The endocrine society clinical practice guideline and the North American menopause society position statement on androgen therapy in women: another one of Yogi's forks. *J. Clin. Endocrinol. Metab.* 92(11), 4091–4093 (2007).
3. Wan Y. Testosterone patches for female sexual dysfunction. *Drug Ther. Bull.* 47(3), 30–34 (2009).
4. Davison SL, Davis SR. Androgenic hormones and aging – the link with female sexual function. *Horm. Behav.* 59(5), 745–753 (2011).
5. Palacios S. Androgens and female sexual function. *Maturitas* 57(1), 61–65 (2007).
6. Panay N, Fenton A. The role of testosterone in women. *Climacteric* 12(3), 185–187 (2009).
7. Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. Clin. Endocrinol. Metab.* 53(1), 58–68 (1981).
8. Zumoff B, Strain GW, Miller LK, Rosner W. Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women. *J. Clin. Endocrinol. Metab.* 80(4), 1429–1430 (1995).
9. Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, von Mühlen D. Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study. *J. Clin. Endocrinol. Metab.* 85(2), 645–651 (2000).
10. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J. Clin. Endocrinol. Metab.* 90(7), 3847–3853 (2005).
11. Basson R. Women's sexual function and dysfunction: current uncertainties, future directions. *Int. J. Impot. Res.* 20(5), 466–478 (2008).
12. Shifren JL. The role of androgens in female sexual dysfunction. *Mayo Clin. Proc.* 79(Suppl. 4), S19–S24 (2004).
13. Davis SR, McCloud P, Strauss BJ, Burger H. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. *Maturitas* 21(3), 227–236 (1995).
14. Davis SR, Walker KZ, Strauss BJ. Effects of estradiol with and without testosterone on body composition and relationships with lipids in postmenopausal women. *Menopause* 7(6), 395–401 (2000).
15. Sherwin BB. Affective changes with estrogen and androgen replacement therapy in surgically menopausal women. *J. Affect Disord.* 14(2), 177–187 (1988).
16. Black EF. The use of testosterone propionate in gynaecology. *Can. Med. Assoc. J.* 47(2), 124–128 (1942).
17. Greenblatt RB, Barfield WE, Garner JF, Calk GL, Harrod JP. Evaluation of an estrogen, androgen, estrogen–androgen combination, and a placebo in the treatment of the menopause. *J. Clin. Endocrinol. Metab.* 10(12), 1547–1558 (1950).
18. Lobo RA. Androgens in postmenopausal women: production, possible role, and replacement options. *Obstet. Gynecol. Surv.* 56(6), 361–376 (2001).
19. Bachmann G, Bancroft J, Braunstein G *et al.* Female androgen insufficiency: the Princeton consensus statement on definition, classification, and assessment. *Fertil. Steril.* 77(4), 660–665 (2002).

• **Clinically useful paper describing guidelines for clinical assessment and diagnosis of female androgen insufficiency.**

20. Sherwin BB, Gelfand MM, Brender W. Androgen enhances sexual motivation in females: a prospective, crossover study of sex steroid administration in the surgical menopause. *Psychosom. Med.* 47(4), 339–351 (1985).
21. Flöter A, Nathorst-Böös J, Carlström K, von Schoultz B. Addition of testosterone to estrogen replacement therapy in oophorectomized women: effects on sexuality and well-being. *Climacteric* 5(4), 357–365 (2002).
22. Lobo RA, Rosen RC, Yang HM, Block B, Van Der Hoop RG. Comparative effects of oral esterified estrogens with and without methyltestosterone on endocrine profiles and dimensions of sexual function in postmenopausal women with hypoactive sexual desire. *Fertil. Steril.* 79(6), 1341–1352 (2003).
23. Dobs AS, Nguyen T, Pace C, Roberts CP. Differential effects of oral estrogen versus oral estrogen–androgen replacement therapy on body composition in postmenopausal women. *J. Clin. Endocrinol. Metab.* 87(4), 1509–1516 (2002).
24. Davis SR, van der Mooren MJ, van Lunsen RH *et al.* Efficacy and safety of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Menopause* 13(3), 387–396 (2006).
25. Simon J, Braunstein G, Nachtigall L *et al.* Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. *J. Clin. Endocrinol. Metab.* 90(9), 5226–5233 (2005).
26. Shifren JL, Davis SR, Moreau M *et al.* Testosterone patch for the treatment of hypoactive sexual desire disorder in naturally menopausal women: results from the INTIMATE NM1 study. *Menopause* 13(5), 770–779 (2006).

27. Buster JE, Kingsberg SA, Aguirre O *et al.* Testosterone patch for low sexual desire in surgically menopausal women: a randomized trial. *Obstet. Gynecol.* 105, 944–952 (2005).
28. Braunstein GD, Sundwall DA, Katz M *et al.* Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. *Arch. Intern. Med.* 165(14), 1582–1589 (2005).
29. Davis SR, Moreau M, Kroll R *et al.* APHRODITE Study Team: testosterone for low libido in postmenopausal women not taking estrogen. *N. Engl. J. Med.* 359, 2005–2017 (2008).
30. Panay N, Al-Azzawi F, Bouchard C *et al.* Testosterone treatment of HSDD in naturally menopausal women: the ADORE study. *Climacteric* 13(2), 121–131 (2010).
31. Chakravarti S, Collins WP, Newton JR, Oram DH, Studd JW. Endocrine changes and symptomatology after oophorectomy in premenopausal women. *Br. J. Obstet. Gynaecol.* 84(10), 769–775 (1977).
32. Burger H, Hailes J, Nelson J, Menelaus M. Effect of combined implants of oestradiol and testosterone on libido in postmenopausal women. *Br. Med. J. (Clin. Res. Ed.).* 294(6577), 936–937 (1987).
33. Nathorst-Böös J, Flöter A, Jarkander-Rolff M, Carlström K, Schoultz B. Treatment with percutanous testosterone gel in postmenopausal women with decreased libido – effects on sexuality and psychological general well-being. *Maturitas* 53(1), 11–18 (2006).
34. Maclaran K, Panay N. Managing low sexual desire in women. *Womens Health* 7(5), 571–581 (2011).
35. Al-Azzawi F, Bitzer J, Brandenburg U *et al.* Therapeutic options for postmenopausal female sexual dysfunction. *Climacteric* 13(2), 103–120 (2010).

•• **Useful review on the psychological and pharmacological management of postmenopausal sexual dysfunction.**

36. Shifren JL, Braunstein GD, Simon JA *et al.* Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. *N. Engl. J. Med.* 343(10), 682–688 (2000).
37. Glaser R, York AE, Dimitrakakis C. Beneficial effects of testosterone therapy in women measured by the validated menopause rating scale (MRS). *Maturitas* 68(4), 355–361 (2011).
38. Davison SL, Bell RJ, Gavrilescu M *et al.* Testosterone improves verbal learning and memory in postmenopausal women: results from a pilot study. *Maturitas* 70(3), 307–311 (2011).
39. Watts NB, Notelovitz M, Timmons MC, Addison WA, Wiita B, Downey LJ. Comparison of oral estrogens and estrogens plus androgen on bone mineral density, menopausal symptoms, and lipid–lipoprotein profiles in surgical menopause. *Obstet. Gynecol.* 85(4), 529–537 (1995).
40. Barrett-Connor E, Young R, Notelovitz M *et al.* A two-year, double-blind comparison of estrogen–androgen and conjugated estrogens in surgically menopausal women. Effects on bone mineral density, symptoms and lipid profiles. *J. Reprod. Med.* 44(12), 1012–1020 (1999).
41. Garnett T, Studd J, Watson N, Savvas M, Leather A. The effects of plasma estradiol levels on increases in vertebral and femoral bone density following therapy with estradiol and estradiol with testosterone implants. *Obstet. Gynecol.* 79(6), 968–972 (1992).
42. Wierman ME, Basson R, Davis SR *et al.* Androgen therapy in women: an Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.* 91(10), 3697–3710 (2006).
43. Nachtigall L, Casson P, Lucas J, Schofield V, Melson C, Simon JA. Safety and tolerability of testosterone patch therapy for up to 4 years in surgically menopausal women receiving oral or transdermal oestrogen. *Gynecol. Endocrinol.* 27(1), 39–48 (2011).

•• **Presents 4-year follow-up safety data from two large randomized controlled trials investigating the use of the transdermal testosterone patch in postmenopausal women with hypoactive sexual desire disorder.**

44. van Staa TP, Sprafka JM. Study of adverse outcomes in women using testosterone therapy. *Maturitas* 62(1), 76–80 (2009).

•• **Observational study on users of testosterone-replacement therapy reporting the risk of a range of adverse outcomes including androgenic events, cardiovascular disease and malignancy.**

45. Malkin CJ, Pugh PJ, Morris PD, Asif S, Jones TH, Channer KS. Low serum testosterone and increased mortality in men with coronary heart disease. *Heart* 96(22), 1821–1825 (2010).
46. Rajkhowa M, Neary RH, Kumpatla P *et al.* Altered composition of high density lipoproteins in women with the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 82(10), 3389–3394 (1997).
47. Pirwany IR, Fleming R, Greer IA, Packard CJ, Sattar N. Lipids and lipoprotein subfractions in women with PCOS: relationship to metabolic and endocrine parameters. *Clin. Endocrinol. (Oxf.)* 54(4), 447–453 (2001).
48. Brand JS, van der Schouw YT. Testosterone, SHBG and cardiovascular health in postmenopausal women. *Int. J. Impot. Res.* 22(2), 91–104 (2010).
49. Lambrinoudaki I, Christodoulakos G, Rizos D *et al.* Endogenous sex hormones and risk factors for atherosclerosis in healthy Greek postmenopausal women. *Eur. J. Endocrinol.* 154(6), 907–916 (2006).
50. Stork S, Bots ML, Grobbee DE, van der Schouw YT. Endogenous sex hormones and C-reactive protein in healthy postmenopausal women. *J. Intern. Med.* 264(3), 245–253 (2008).
51. Bernini GP, Moretti A, Sgró M *et al.* Influence of endogenous androgens on carotid wall in postmenopausal women. *Menopause* 8(1), 43–50 (2001).
52. Karim R, Hodis HN, Stanczyk FZ, Lobo RA, Mack WJ. Relationship between serum levels of sex hormones and progression of subclinical atherosclerosis in postmenopausal women. *J. Clin. Endocrinol. Metab.* 93(1), 131–138 (2008).
53. Golden SH, Maguire A, Ding J *et al.* Endogenous postmenopausal hormones and carotid atherosclerosis: a case–control study of the atherosclerosis risk in communities cohort. *Am. J. Epidemiol.* 155(5), 437–445 (2002).
54. Debing E, Peeters E, Duquet W, Poppe K, Velkeniers B, Van den Brande P. Endogenous sex hormone levels in postmenopausal women undergoing carotid artery endarterectomy. *Eur. J. Endocrinol.* 156(6), 687–693 (2007).
55. Creatsa M, Armeni E, Stamatelopoulos K *et al.* Circulating androgen levels are associated with subclinical atherosclerosis and arterial stiffness in healthy recently menopausal women. *Metabolism* 61(2), 193–201 (2012).
56. Ouyang P, Vaidya D, Dobs A *et al.* Sex hormone levels and subclinical atherosclerosis in postmenopausal women: the multi-ethnic study of atherosclerosis. *Atherosclerosis* 204(1), 255–261 (2009).
57. Patel SM, Ratcliffe SJ, Reilly MP *et al.* Higher serum testosterone concentration in older women is associated with insulin resistance, metabolic syndrome, and cardiovascular disease. *J. Clin. Endocrinol. Metab.* 94(12), 4776–4784 (2009).
58. Rexrode KM, Manson JE, Lee IM *et al.* Sex hormone levels and risk of cardiovascular events in postmenopausal women. *Circulation* 108(14), 1688–1693 (2003).
59. Laughlin GA, Goodell V, Barrett-Connor E. Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. *J. Clin. Endocrinol. Metab.* 95(2), 740–747 (2010).

- **Interesting population-based study suggesting that testosterone should be kept within normal levels to minimize the risk of cardiovascular disease.**

60. Ellison KE, Ingelfinger JR, Pivor M, Dzau VJ. Androgen regulation of rat renal angiotensinogen messenger RNA expression. *J. Clin. Invest*. 83(6), 1941–1945 (1989).
61. Chen YF, Naftilan AJ, Oparil S. Androgen-dependent angiotensinogen and renin messenger RNA expression in hypertensive rats. *Hypertension* 19(5), 456–463 (1992).
62. Adams MR, Williams JK, Kaplan JR. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. *Arterioscler. Thromb. Vasc. Biol*. 15(5), 562–570 (1995).
63. Hutchison SJ, Sudhir K, Chou TM *et al.* Testosterone worsens endothelial dysfunction associated with hypercholesterolemia and environmental tobacco smoke exposure in male rabbit aorta. *J. Am. Coll. Cardiol*. 29(4), 800–807 (1997).
64. Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91(4), 1154–1160 (1995).
65. Bruck B, Brehme U, Gugel N *et al.* Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. *Arterioscler. Thromb. Vasc. Biol*. 17(10), 2192–2199 (1997).
66. Cooper BC, Gokina NI, Osol G. Testosterone replacement increases vasodilatory reserve in androgen-deficient female rats. *Fertil. Steril*. 87(2), 422–425 (2007).
67. Elbers JM, Giltay EJ, Teerlink T *et al.* Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin. Endocrinol. (Oxf.)* 58(5), 562–571 (2003).
68. Elbers JM, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am. J. Physiol*. 276, E317–E325 (1999).
69. Elbers JM, Asscheman H, Seidell JC, Megens JA, Gooren LJ. Long-term testosterone administration increases visceral fat in female to male transsexuals. *J. Clin. Endocrinol. Metab*. 82(7), 2044–2047 (1997).
70. Zang H, Carlström K, Arner P, Hirschberg AL. Effects of treatment with testosterone alone or in combination with estrogen on insulin sensitivity in postmenopausal women. *Fertil. Steril*. 86, 136–144 (2006).
71. Leão LM, Duarte MP, Silva DM, Bahia PR, Coeli CM, de Farias ML. Influence of methyltestosterone postmenopausal therapy on plasma lipids, inflammatory factors, glucose metabolism and visceral fat: a randomized study. *Eur. J. Endocrinol*. 154(1), 131–139 (2006).
72. Gruber DM, Sator MO, Kirchengast S, Joura EA, Huber JC. Effect of percutaneous androgen replacement therapy on body composition and body weight in postmenopausal women. *Maturitas* 29(3), 253–259 (1998).
73. Hickok LR, Toomey C, Speroff L. A comparison of esterified estrogens with and without methyltestosterone: effects on endometrial histology and serum lipoproteins in postmenopausal women. *Obstet. Gynecol*. 82(6), 919–924 (1993).
74. Basaria S, Nguyen T, Rosenson RS, Dobs AS. Effect of methyl testosterone administration on plasma viscosity in postmenopausal women. *Clin. Endocrinol. (Oxf.)* 57(2), 209–214 (2002).
75. Warnock JK, Swanson SG, Borel RW *et al.* ESTRATEST clinical study group: combined esterified estrogens and methyltestosterone versus esterified estrogens alone in the treatment of loss of sexual interest in surgically menopausal women. *Menopause* 12(4), 374–384 (2005).
76. Raisz LG, Wiita B, Artis A *et al.* Comparison of the effects of estrogen alone and estrogen plus androgen on biochemical markers of bone formation and resorption in postmenopausal women. *J. Clin. Endocrinol. Metab*. 81(1), 37–43 (1996).
77. Worboys S, Kotsopoulos D, Teede H, McGrath B, Davis SR. Evidence that parenteral testosterone therapy may improve endothelium-dependent and -independent vasodilation in postmenopausal women already receiving estrogen. *J. Clin. Endocrinol. Metab*. 86(1), 158–161 (2001).
78. Hak AE, Westendorp IC, Pols HA, Hofman A, Witteman JC. High-dose testosterone is associated with atherosclerosis in postmenopausal women. *Maturitas* 56(2), 153–160 (2007).
79. Penotti M, Sironi L, Cannata L *et al.* Effects of androgen supplementation of hormone replacement therapy on the vascular reactivity of cerebral arteries. *Fertil. Steril*. 76(2), 235–240 (2001).
80. van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin. Endocrinol. (Oxf.)* 47(3), 337–342 (1997).
81. White WB, Grady D, Giudice LC, Berry SM, Zborowski J, Snabes MC. A cardiovascular safety study of LibiGel (testosterone gel) in postmenopausal women with elevated cardiovascular risk and hypoactive sexual desire disorder. *Am. Heart J.* 163(1), 27–32 (2012).

- **Description of a randomized controlled trial currently in progress investigating the effects of transdermal testosterone gel on cardiovascular disease and breast cancer risk.**

82. Corbould A. Chronic testosterone treatment induces selective insulin resistance in subcutaneous adipocytes of women. *J. Endocrinol*. 192(3), 585–594 (2007).
83. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for Type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J. Clin. Endocrinol. Metab*. 84(1), 165–169 (1999).
84. Weinberg ME, Manson JE, Buring JE *et al.* Low sex hormone-binding globulin is associated with the metabolic syndrome in postmenopausal women. *Metabolism* 55(11), 1473–1480 (2006).
85. Golden SH, Ding J, Szklo M, Schmidt MI, Duncan BB, Dobs A. Glucose and insulin components of the metabolic syndrome are associated with hyperandrogenism in postmenopausal women: the atherosclerosis risk in communities study. *Am. J. Epidemiol*. 160(6), 540–548 (2004).
86. Oh JY, Barrett-Connor E, Wedick NM *et al.* Rancho Bernardo study: endogenous sex hormones and the development of Type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* 25(1), 55–60 (2002).
87. Lovejoy JC, Bray GA, Greeson CS *et al.* Oral anabolic steroid treatment, but not parenteral androgen treatment, decreases abdominal fat in obese, older men. *Int. J. Obes. Relat. Metab. Disord*. 19, 614–624 (1995).
88. Iellamo F, Volterrani M, Caminiti G *et al.* Testosterone therapy in women with chronic heart failure: a pilot double-blind, randomized, placebo-controlled study. *J. Am. Coll. Cardiol*. 56(16), 1310–1316 (2010).
89. Tuckerman EM, Okon MA, Li T, Laird SM. Do androgens have a direct effect on endometrial function? An *in vitro* study. *Fertil. Steril*. 74(4), 771–779 (2000).
90. Yamaki J, Yamamoto T, Okada H. Aromatization of androstenedione by normal and neoplastic endometrium of the uterus. *J. Steroid Biochem*. 22(1), 63–66 (1985).
91. Legro RS, Kunselman AR, Miller SA, Satyaswaroop PG. Role of androgens in the growth of endometrial carcinoma: an *in vivo* animal model. *Am. J. Obstet. Gynecol*. 184(3), 303–308 (2001).

92. Zang H, Sahlin L, Masironi B, Eriksson E, Lindén Hirschberg A. Effects of testosterone treatment on endometrial proliferation in postmenopausal women. *J. Clin. Endocrinol. Metab.* 92(6), 2169–2175 (2007).
93. Perrone AM, Cerpolini S, Maria Salfi NC *et al.* Effect of long-term testosterone administration on the endometrium of female-to-male (FtM) transsexuals. *J. Sex Med.* 6(11), 3193–3200 (2009).
94. Hackenberg R, Hofmann J, Hölzel F, Schulz KD. Stimulatory effects of androgen and antiandrogen on the *in vitro* proliferation of human mammary carcinoma cells. *J. Cancer Res. Clin. Oncol.* 114(6), 593–601 (1988).
95. Dimitrakakis C, Zhou J, Wang J *et al.* A physiologic role for testosterone in limiting estrogenic stimulation of the breast. *Menopause* 10(4), 292–298 (2003).
96. Poulin R, Baker D, Poirier D, Labrie F. Androgen and glucocorticoid receptor-mediated inhibition of cell proliferation by medroxyprogesterone acetate in ZR-75-1 human breast cancer cells. *Breast Cancer Res. Treat.* 13(2), 161–172 (1989).
97. Ortmann J, Prifti S, Bohlmann MK, Rehberger-Schneider S, Strowitzki T, Rabe T. Testosterone and 5α-dihydrotestosterone inhibit *in vitro* growth of human breast cancer cell lines. *Gynecol. Endocrinol.* 16(2), 113–120 (2002).
98. Lapointe J, Fournier A, Richard V, Labrie C. Androgens down-regulate bcl-2 protooncogene expression in ZR-75-1 human breast cancer cells. *Endocrinology* 140(1), 416–421 (1999).
99. Kandouz M, Lombet A, Perrot JY *et al.* Proapoptotic effects of antiestrogens, progestins and androgen in breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 69(1–6), 463–471 (1999).
100. Dimitrakakis C, Zhou J, Bondy CA. Androgens and mammary growth and neoplasia. *Fertil. Steril.* 77(Suppl. 4), S26–S33 (2002).
101. Zhou J, Ng S, Adesanya-Famuiya O, Anderson K, Bondy CA. Testosterone inhibits estrogen-induced mammary epithelial proliferation and suppresses estrogen receptor expression. *FASEB J.* 14(12), 1725–1730 (2000).
102. Somboonporn W, Davis SR. National Health and Medical Research Council. Testosterone effects on the breast: implications for testosterone therapy for women. *Endocr. Rev.* 25(3), 374–388 (2004).

•• **Detailed discussion regarding the effects of androgens on the breast, summarizing preclinical, animal and human data.**

103. Key T, Appleby P, Barnes I *et al.* Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J. Natl Cancer Inst.* 94(8), 606–616 (2002).
104. Tworoger SS, Rosner BA, Willett WC, Hankinson SE. The combined influence of multiple sex and growth hormones on risk of postmenopausal breast cancer: a nested case–control study. *Breast Cancer Res.* 13(5), R99 (2011).
105. Beattie MS, Costantino JP, Cummings SR *et al.* Endogenous sex hormones, breast cancer risk, and tamoxifen response: an ancillary study in the NSABP breast cancer prevention trial (P-1). *J. Natl Cancer Inst.* 98(2), 110–115 (2006).
106. Danforth KN, Eliassen AH, Tworoger SS *et al.* The association of plasma androgen levels with breast, ovarian and endometrial cancer risk factors among postmenopausal women. *Int. J. Cancer.* 126(1), 199–207 (2010).
107. de França Neto AH, Rogatto S, Do Amorim MM, Tamanaha S, Aoki T, Aldrighi JM. Oncological repercussions of polycystic ovary syndrome. *Gynecol. Endocrinol.* 26(10), 708–711 (2010).
108. Ewertz M. Influence of non-contraceptive exogenous and endogenous sex hormones on breast cancer risk in Denmark. *Int. J. Cancer* 42(6), 832–838 (1988).
109. Tamimi RM, Hankinson SE, Chen WY, Rosner B, Colditz GA. Combined estrogen and testosterone use and risk of breast cancer in postmenopausal women. *Arch. Intern. Med.* 166(14), 1483–1489 (2006).
110. Ness RB, Albano JD, McTiernan A, Cauley JA. Influence of estrogen plus testosterone supplementation on breast cancer. *Arch. Intern. Med.* 169(1), 41–46 (2009).
111. Brinton LA, Hoover R, Fraumeni JF. Menopausal oestrogens and breast cancer risk: an expanded case–control study. *Br. J. Cancer* 54(5), 825–832 (1986).
112. Dimitrakakis C, Jones RA, Liu A, Bondy CA. Breast cancer incidence in postmenopausal women using testosterone in addition to usual hormone therapy. *Menopause* 11(5), 531–535 (2004).
113. Colditz GA, Hankinson SE, Hunter DJ *et al.* The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N. Engl. J. Med.* 332(24), 1589–1593 (1995).
114. Jick SS, Hagberg KW, Kaye JA, Jick H. Postmenopausal estrogen-containing hormone therapy and the risk of breast cancer. *Obstet. Gynecol.* 113(1), 74–80 (2009).
115. Davis SR, Wolfe R, Farrugia H, Ferdinand A, Bell RJ. The incidence of invasive breast cancer among women prescribed testosterone for low libido. *J. Sex Med.* 6(7), 1850–1856 (2009).
116. Hofling M, Hirschberg AL, Skoog L, Tani E, Hägerström T, von Schoultz B. Testosterone inhibits estrogen/progestogen-induced breast cell proliferation in postmenopausal women. *Menopause* 14(2), 183–190 (2007).
117. Hofling M, Lundström E, Azavedo E, Svane G, Hirschberg AL, von Schoultz B. Testosterone addition during menopausal hormone therapy: effects on mammographic breast density. *Climacteric* 10(2), 155–163 (2007).
118. Davis SR, Hirschberg AL, Wagner LK, Lodhi I, von Schoultz B. The effect of transdermal testosterone on mammographic density in postmenopausal women not receiving systemic estrogen therapy. *J. Clin. Endocrinol. Metab.* 94(12), 4907–4913 (2009).

Website

201. WHO. Definition, diagnosis and classification of diabetes mellitus and its complications (1999). http://whqlibdoc.who.int/hq/1999/who_ncd_ncs_99.2.pdf