Testosterone in women—the clinical significance

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Testosterone is an essential hormone for women, with physiological actions mediated directly or via aromatisation to oestradiol throughout the body. Despite the crucial role of testosterone and the high circulating concentrations of this hormone relative to oestradiol in women, studies of its action and the effects of testosterone deficiency and replacement in women are scarce. The primary indication for the prescription of testosterone for women is loss of sexual desire, which causes affected women substantial concern. That no formulation has been approved for this purpose has not impeded the widespread use of testosterone by women—either off-label or as compounded therapy. Observational studies indicate that testosterone has favourable cardiovascular effects measured by surrogate outcomes; however, associations between endogenous testosterone and the risk of cardiovascular disease and total mortality, particularly in older women, are yet to be established. Adverse cardiovascular effects have not been seen in studies of transdermal testosterone therapy in women. Clinical trials suggest that exogenous testosterone enhances cognitive performance and improves musculoskeletal health in postmenopausal women. Unmet needs include the availability of approved testosterone formulations for women and studies to elucidate the contribution of testosterone to cardiovascular, cognitive, and musculoskeletal health and the risk of cancer.

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Introduction

Testosterone is a critical but enigmatic female hormone. It acts directly as an androgen in addition to being an obligatory precursor for biosynthesis of oestradiol.[1] Control of testosterone production in women is not well understood because no feedback loop governing its production has been described. Testosterone exerts physiological effects in reproductive and non-reproductive tissues in women. Concentrations of testosterone are positively associated with sexual function in women,[2,3] and many randomised placebo-controlled trials have shown that testosterone therapy can be effective in the treatment of female sexual dysfunction.[4] The role of testosterone for the management of female sexual dysfunction has been reviewed in detail elsewhere,[4] and although this Review briefly addresses female sexual dysfunction, its focus is the role of testosterone in women beyond sexual function. We therefore review clinical evidence for the role of testosterone in cardiovascular, musculoskeletal, and vulvovaginal health and in cognitive function, as well as the associations between testosterone and gynaecological cancer in women. We have drawn on studies that have investigated associations between endogenous testosterone and these health outcomes, and clinical trials of testosterone therapy in premenopausal and postmenopausal women.

Androgen physiology in brief

During the reproductive years, testosterone in women is produced by the ovaries and by peripheral conversion of androstenedione and dehydroepiandrosterone (DHEA), which are pre-androgens synthesised by the ovaries and adrenal glands. The pre-androgens contribute about 50% of circulating testosterone in premenopausal women.[5] Concentrations of testosterone first begin to increase in girls at about the age of 6–8 years, when maturation of the adrenal zona reticularis results in increased production of DHEA and its sulphate, DHEAS,[5] heralding the onset of adrenarche. Cyclical production of testosterone by the ovaries starts with the onset of ovulation; concentrations peak mid-cycle and remain high during the luteal phase.[6] Diurnal variation, characterised by higher concentrations in the morning, has also been documented.[7] Maximum concentrations of testosterone are achieved in the third and fourth decades, followed by a steady decline in testosterone and its precursors with increasing age (figure 1).[8] A physiological decline in testosterone with age is unrelated to natural menopause.[8,9] The reason for this decline is not known but most likely is a result of waning production by the ovaries and adrenal glands. Table 1 lists other common causes of low concentrations of testosterone.

Key features of the physiology of testosterone account for its role in women. The enzyme 5α-reductase metabolises testosterone peripherally in target tissues to dihydrotestosterone (DHT),[5] which is the most potent androgen and also has the highest binding affinity for the androgen receptor. Within cells, DHT is further metabolised, such that concentrations of its metabolites provide an index of tissue exposure to androgens. Aromatisation of testosterone to oestradiol occurs within the ovaries and extragonadal tissues, with the extragonadal tissues being the main source of oestrogen production after menopause (figure 2).[1] Circulating testosterone is highly bound to plasma proteins, with about 66% bound to sex hormone-binding globulin (SHBG) and 33% to albumin.[5] The free fraction of testosterone is determined by the rate of production of testosterone, the metabolic clearance rate, and the level of SHBG.[5] Low concentrations of SHBG result in increased clearance of testosterone from the circulation, whereas high concentrations result in reduced clearance.

Testosterone circulates in nanomolar concentrations in women of all ages; although this level is higher than the picomolar concentrations of oestradiol, measurement of concentrations of testosterone in serum in women has

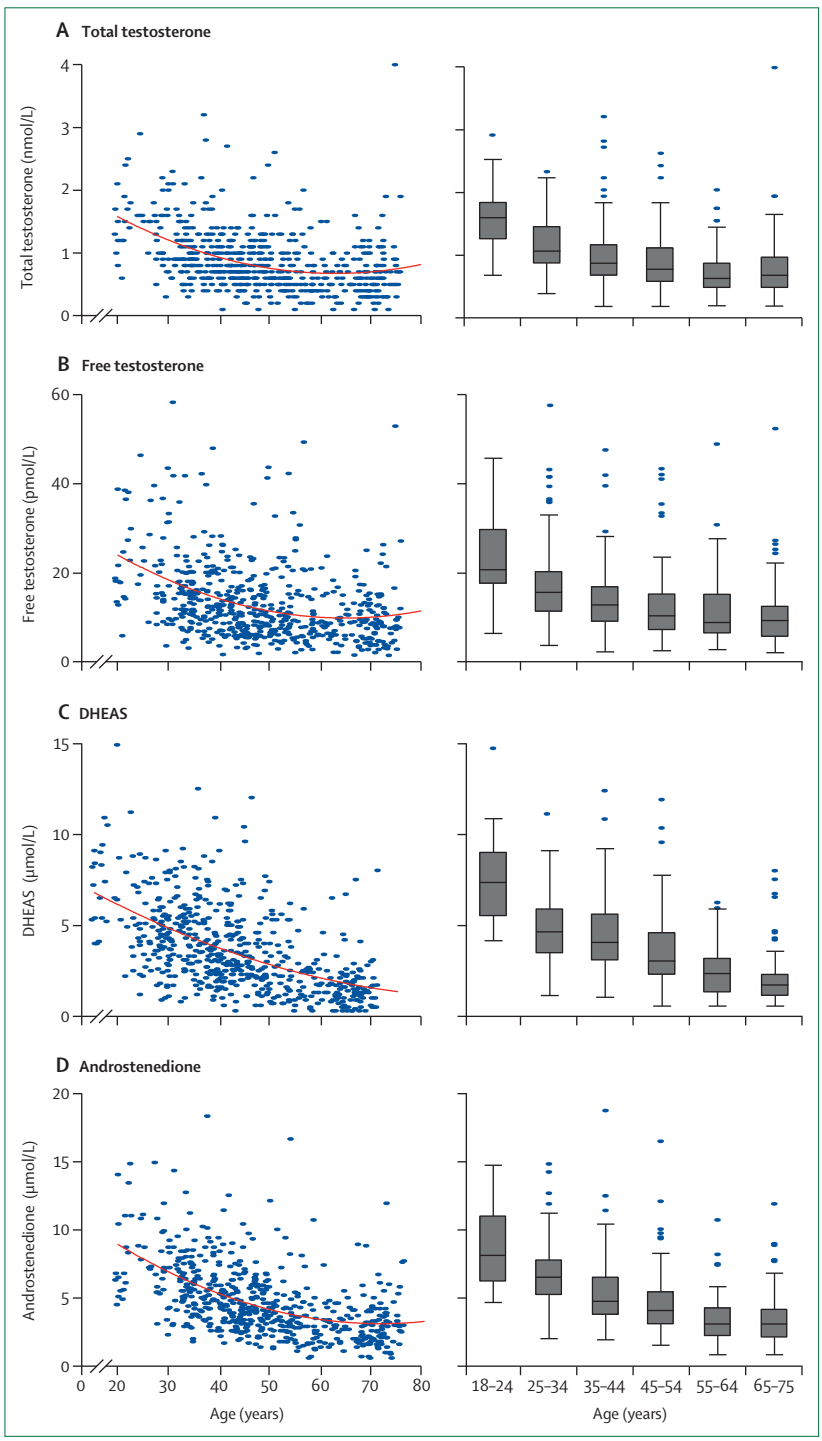

Figure 1: **Relation between age and free and total testosterone and the pre-androgens DHEAS and androstenedione**
Raw data are represented as scattergraphs with fitted regression curves. For the box and whisker plots, the box represents the IQR, and the line in the box is the median. For the whiskers, the upper value is the largest datapoint less than or equal to the 75th percentile + 1·5 × IQR. The lower adjacent value is defined as the smallest datapoint greater than or equal to the 25th percentile −1·5 × IQR. Outliers are any values beyond the whiskers. To convert nmol/L to ng/dL or pmol/L to pg/dL, divide by 0·0347; to convert μmol/L to μg/dL, divide by 0·027. DHEAS=dehydroepiandrosterone sulphate. Reproduced from Davison and colleagues,[8] by permission of The Endocrine Society.

been limited by assay imprecision and wide interassay variability at the low concentrations seen in women.[11] Liquid chromatography-tandem mass spectrometry (LC-MS) is considered to be the most sensitive approach for measuring concentrations of sex steroids[12] and was hoped to translate into greater accuracy and reliability; however, variability between LC-MS assays is substantial in the range relevant to female physiology.[13] Furthermore, much of the action of testosterone is a result of intracellular metabolism, so serum concentrations alone are not a good index of tissue exposure.[4] Sensitivity at the level of the androgen receptor also determines an individual's response to a given level of testosterone. In clinical practice, therefore, concentrations of testosterone in serum are somewhat arbitrary and should always be interpreted in accordance with the clinical presentation and assessment.

The link between testosterone and female sexual function

The multifactorial nature of women's sexual function, the range of approaches used to measure female sexual dysfunction, and the challenges of measuring testosterone, its precursors, and its metabolites have complicated this specialty. Despite these limitations, large cross-sectional and longitudinal studies have shown consistent associations between androgens and sexual function in women. Importantly, sexual function is not related to changes in circulating concentrations of androgens at menopause or early postmenopause, as blood concentrations of androgens do not change during the menopausal transition;[8,9] instead, associations exist between specific androgens and self-reported measures of sexual function in premenopausal and postmenopausal women.

Results of a community-based study[14] of 1021 randomly recruited healthy women showed a direct association between an endogenous level of DHEAS below the tenth percentile and low sexual responsiveness in women aged 45 years or older. In women aged 18–44 years, concentrations of DHEAS below the tenth percentile were directly associated with low sexual desire, arousal, and responsiveness.[14] No associations with androstenedione or total and free testosterone were seen. A small study[15] of women attending a clinic for female sexual dysfunction reported no association between endogenous testosterone and female sexual dysfunction; however, the study was substantially underpowered for this outcome. Subsequently, a prospective longitudinal study[3] of 3266 women aged 42–52 years reported on concurrent concentrations of sex hormones and sexual function. Frequency of masturbation—a sexual function not dependent on partnership status—was a main outcome measure. Endogenous testosterone was associated with masturbation frequency, sexual desire, and arousal, and DHEAS was positively associated with masturbation frequency and desire.[3] In a separate Danish

study[2] of 560 healthy women aged 19–65 years recruited from the community, in which sex steroids were measured by LC-MS and sexual desire by the Female Sexual Function Index,[16] endogenous concentrations of total and free testosterone, androstenedione, and DHEAS were associated with sexual desire after adjustment for age. Observational studies thus have shown the strongest associations between masturbation frequency and sexual desire and endogenous concentrations of testosterone, free testosterone, and testosterone precursors.

Surgical menopause has been used as a model to study the effect of an abrupt reduction in concentrations of testosterone on sexual function. The findings from such studies have been reviewed in detail elsewhere.[4] In essence, the results from observational studies of women who have undergone surgical menopause for benign reasons have been mixed. Preoperative sexual wellbeing predicts postoperative sexual function, and a correlation between postoperative sexual desire and concentrations of androgens has not been shown.[4] Limitations of this model are that concentrations of all sex steroids reduce with surgical menopause and the picture is complicated by the reasons for the surgery, as well as the consequent infertility in young women. The positive associations between endogenous concentrations of androgen and sexual desire and arousal in women suggest potential benefits of treatment with testosterone for those with loss of desire and arousal.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV, hypoactive sexual desire disorder is the absence of sexual fantasies and desire for sexual activity that causes a woman distress. This diagnosis underpins research into the effectiveness of exogenous testosterone as a treatment for female sexual dysfunction, specifically hypoactive sexual desire disorder, over the past 20 years.[17] In DSM-5, hypoactive sexual desire disorder has been merged with female sexual arousal disorders and renamed female sexual interest–arousal disorder, which remains mainly based on sexual desire.[16] Because hypoactive sexual desire disorder can be assessed by validated questionnaires and has been the primary outcome of all clinical trials of testosterone therapy to date, practising clinicians continue to use it as a clinical diagnosis.

Many randomised controlled trials[4,18–20] have shown that administration of testosterone by subcutaneous implant, intramuscular injection, transdermal patch or gel or orally (as for methyltestosterone), with and without concurrent oestrogen, is effective for the treatment of hypoactive sexual desire disorder in surgically and naturally menopausal women. The findings from these studies have been comprehensively summarised in a Cochrane review[20] and a clinical practice guideline from The Endocrine Society.[4] The latter concluded, "Evidence supports the short-term efficacy and safety of high physiological doses of [testosterone] treatment of post-menopausal women with sexual dysfunction due to hypoactive sexual desire disorder".[4] The guideline also recommends considering a 6-month therapeutic trial of transdermal testosterone for women who have been diagnosed with hypoactive sexual desire disorder according to DSM-IV.[17] As described below, many of these studies have contributed to the understanding of the effects of testosterone on other health outcomes.

	Mechanism
Spontaneous causes of androgen insufficiency	
Natural decline with age from mid-to-late reproductive years	Decline in production of androgens by the ovaries and adrenal gland
Hypothalamic amenorrhoea	Anovulation
Primary ovarian insufficiency	Anovulation
Hyperprolactinaemia	Suppression of pituitary gonadotropins; anovulation
Adrenal insufficiency	Loss of adrenal production of pre-androgens
Panhypopituitarism	Loss of adrenal production of pre-androgens and ovarian production of androgens
Other medical conditions (eg, chronic liver disease and HIV infection)	Increased concentrations of SHBG reduce concentrations of free testosterone
Iatrogenic causes of androgen insufficiency	
Surgical menopause at any age	Loss of ovarian production of androgens
Chemotherapy	Ovarian failure
Radiotherapy to the pelvis	Ovarian failure
Systemic glucocorticosteroid therapy	Suppression of adrenal production of pre-androgens
Drug-induced hyperprolactinaemia	Suppression of pituitary gonadotropins; anovulation
Systemic hormonal contraception	Loss of ovarian production of androgens Increased concentrations of SHBG, resulting in reduced concentrations of free testosterone
Oral non-contraceptive therapy (eg, phenobarbital, phenytoin, carbamazepine, and thyroxine)	Increased concentrations of SHBG, resulting in reduced concentrations of free testosterone

SHBG=sex hormone-binding globulin.

Table 1: **Causes of low testosterone in women**

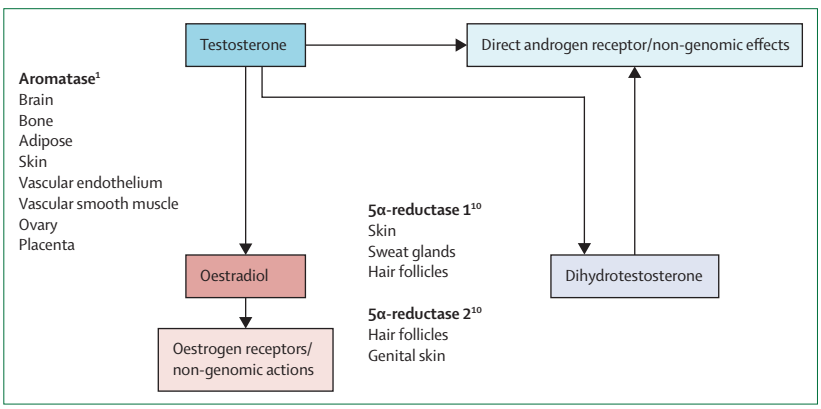

Figure 2: **Modes of androgen action**

Testosterone and vaginal health

Vulvovaginal atrophy is traditionally treated with low-dose oestrogen given vaginally, which is highly effective and safe. As vaginal oestrogen therapy is contraindicated for some women, such as those with breast cancer taking

aromatase inhibitor therapy, intravaginal testosterone has been proposed as an alternative. Androgen receptors have been identified in the vaginal mucosa, submucosa, stroma, smooth muscle, and vascular endothelium.[21,22] The density of androgen receptors in these tissues declines with age, although vaginal expression of the androgen receptor gene increases with administration of testosterone.[21,22] Aromatase, the enzyme that converts testosterone to oestradiol, and 5α-reductase isotypes 1 and 2, which convert testosterone to DHT, are also present in vaginal tissues.[22]

Several studies have investigated the use of intravaginal testosterone in postmenopausal women without breast cancer. Intramuscular testosterone propionate has been shown to induce proliferation of the vaginal epithelium in postmenopausal women.[23] A randomised controlled trial[24] involving 75 postmenopausal women with symptomatic vaginal atrophy and sexual dysfunction compared 1 mg testosterone in combination with 0·625 mg conjugated equine oestrogen given vaginally, 0·625 mg conjugated equine oestrogen alone given vaginally, or placebo. The greatest improvements in vaginal atrophy and sexual function were seen with combined conjugated equine oestrogen–testosterone therapy. In another study,[25] 80 healthy postmenopausal women were randomly assigned to 0·625 mg conjugated equine oestrogen cream, 300 μg of testosterone proprionate, polyacrylic acid, or placebo given intravaginally three times each week for 12 weeks. Compared with placebo, intravaginal testosterone was associated with significant improvements in sexual desire, lubrication, satisfaction, and pain during intercourse, whereas conjugated equine oestrogen only resulted in a significant improvement in desire.[25] The favourable effects of testosterone in these studies could result from local aromatisation of testosterone to oestradiol. A small open-label pilot study reported by Witherby and colleagues[26] showed favourable effects of intravaginal testosterone in postmenopausal women with breast cancer who were taking aromatase inhibitor therapy. Objective and subjective measures of atrophic vaginitis improved and concentrations of oestradiol in serum, measured by a highly sensitive radioimmunoassay, remained undetectable in most women.

These studies are provocative but are limited by their small size. The open-label study by Witherby and colleagues[26] suggests that aromatisation to oestradiol is not required for testosterone to improve vaginal atrophy. However, further studies are needed before vaginal testosterone can be considered for the treatment of vulvovaginal atrophy in women with or without breast cancer in the clinical setting.

Testosterone and cardiometabolic disease

Sex hormones have been suggested to have a critical role in the development and evolution of cardiovascular disease, with the focus mainly on oestrogens in women. The general belief is that testosterone increases the risk of cardiovascular disease, but Spoletini and colleagues[27] concluded in a systematic review that a hypoandrogenic state in women is detrimental to cardiovascular health.

At physiological concentrations, testosterone has favourable effects on vasomotor tone, endothelial function, and peripheral vascular resistance through direct effects on the blood vessel wall.[28] Testosterone improves arterial function in women by enhancing endothelium-dependent (flow-mediated) and endothelium-independent brachial artery vasodilation.[29,30] In in-vitro research, the endothelium-independent effect seems to be independent of aromatase.[31] In support of this, Worboys and colleagues[29] reported that exogenous testosterone enhanced arterial dilation after glyceryl trinitrate (endothelium-independent dilation)—an effect not seen with exogenous estradiol therapy.

Data from observational studies mostly show an inverse relation between endogenous testosterone and the risk of cardiovascular disease (table 2). Whether endogenous testosterone protects against cardiovascular disease (ie, ischaemic heart disease and ischaemic stroke), death from cardiovascular disease, and all-cause mortality remains uncertain. The reported studies listed in table 2 highlight the limitations of research in this field, particularly in older women, which include small sample sizes, recruitment of convenience, clinic-based samples, case-control designs, and long intervals between the time of blood draw and cardiovascular events. Indeed, a longitudinal study reported by Laughlin and colleagues,[40] results of which suggested that low concentrations of endogenous testosterone have an adverse effect on the risk of cardiovascular disease, and findings reported by Benn and colleagues,[41] which suggested an adverse effect of high concentrations of endogenous testosterone (above the 95th percentile) on the risk of cardiovascular disease, each reported on events decades after blood was drawn to measure concentrations of sex steroids. In the study reported by Laughlin and colleagues,[40] low concentrations of both bioavailable and total testosterone were associated with an increased incidence of cardiovascular events. The data reported by Benn and colleagues[41] are hard to reconcile, because the mean concentration of total testosterone in older women was the same as for younger women (1·9 nmol/L), with the ranges for all women being unusually high.[42] They also reported no differences in concentrations of testosterone with age, which is inconsistent with findings in previous studies[8,43] and raises concerns about the concentrations recorded. The women in the upper fifth percentile for testosterone at baseline (then aged 49–65 years) were at greatest risk of subsequent cardiovascular disease.[42] To be in the top fifth percentile, these women would have had extremely high concentrations of testosterone, such that polycystic ovary syndrome or another cause of excess androgens might be suspected.

The association between low concentrations of testosterone and greater incidences of all-cause mortality and cardiovascular events, independent of traditional

	Number of patients	Study sample	Mean (SD) age or age range	Outcome measured	Results for key endpoints
Ankle brachial index					
Maggio et al (2012)[32]	502 postmenopausal women	Population based	75·6 years (7·1)	Ankle brachial index <0·9	Total testosterone (higher *vs* lower log testosterone), OR 2·00 (95% CI 1·1–3·47; p=0·01); no significant associations for SHBG or oestradiol
Carotid artery					
Bernini et al (1999)[33]	101 premenopausal and postmenopausal women	Convenience sample	46·7 years (10·35)	cCIMT	Significant independent inverse relation between free testosterone and maximal total cCIMT (p<0·008), androstenedione and mean cCIMT (p<0·03), and androstenedione and maximal cCIMT (p<0·05); age, BMI, and blood pressure were also inversely related to cCIMT
Golden et al (2002)[34]	182 postmenopausal cases and 182 postmenopausal controls	Case-control	61·6 years	CIMT	In the fully adjusted model (highest *vs* lowest quartile): total testosterone, OR 0·34 (95% CI 0·16–0·70; p=0·004); SHBG, OR 0·48 (0·24–0·97). No associations between CIMT and oestrone, DHEAS, or androstenedione
Ouyang et al (2009)[35]	1947 postmenopausal women	Population based	45–84 years	cCIMT and iCIMT	Total testosterone: cCIMT, β=0·018* (95% CI 0·005 to 0·031; p=0·009); iCIMT, β=0·038 (0·005 to 0·071; p=0·02). Bioavailable testosterone: cCIMT, β=0·018 (0·007 to 0·032; p=0·002); iCIMT, β=0·019 (−0·013 to 0·048; p=0·255). SHBG, oestradiol, and DHEA not significantly associated with CIMT in adjusted model
Calderon-Margalit et al (2010)[36]	1629 premenopausal and postmenopausal women	Population based	37–52 years	CIMT	No association between CIMT and total or free testosterone; SHBG significantly inversely associated with CIMT (p=0·005) across quartiles
Debing et al (2007)[37]	56 postmenopausal women with ICA sclerosis and 56 postmenopausal controls	Case-control convenience sample	70·4 years	ICA sclerosis	Compared with controls, cases had significantly lower mean concentrations of total testosterone (0·23 µg/L [SD 0·12] *vs* 0·31 µg/L [0·20]; p=0·043) and free testosterone (3·42 µg/L [1·94] *vs* 4·59 µg/L [2·97] µg/L; p=0·009); inverse relation between severe ICA sclerosis and free testosterone (β=–0·234; p=0·028) and androstenedione (β=–0·241; p=0·028)
Cardiovascular and coronary artery disease					
Calderon-Margalit et al (2010)[36]	1629 premenopausal and postmenopausal women	Population based	37–52 years	CAC	No association between CAC and total or free testosterone; SHBG significantly inversely associated with CAC (p=0·008) across quartiles
Ouyang et al (2009)[35]	1947 postmenopausal women	Population based	45–84 years	CAC	Bioavailable testosterone negatively associated with extent of CAC (β=–0·182, 95%CI −0·345 to −0·018; p=0·030) and positively associted with SHBG (β=0·298, 0·024 to 0·571; p=0·033); no association between CAC and total testosterone, oestradiol, or DHEA
Naessen et al (2010)[38]	72 postmenopausal women not using hormone therapy	Population based substudy	70 years	Prevalent CVD†	Prevalent CVD associated with indications of lower levels of androgen precursors, increased aromatase activity, and higher levels of oestradiol
Sievers et al (2010)[39]	2914 premenopausal and postmenopausal women	Convenience sample from primary care clinics	58 years (14·4, 18–75)	Cardiovascular events and total mortality at 4·5-year follow-up	When comparing quintile 1 of baseline total testosterone concentrations with quintile 2–5: adjusted HR 0·62 (95% CI 0·42–0·939) for all-cause mortality and 0·68 (0·48–0·97) for cardiovascular events
Laughlin et al (2010)[40]	639 postmenopausal women	Convenience cohort study	73·8 years (50–91) at baseline	CAD events at 30-year follow-up (mean 12·5 years)	When comparing quintile 1 and quintile 5 of baseline bioavailable testosterone (from samples drawn 1984–87) with quintile 2–5: quintile 1 of total testosterone *vs* the higher quintiles, HR 1·72 (95% CI 1·15–2·57; p=0·008); lowest quintile of bioavailable testosterone *vs* quintile 3, HR 1·93 (95% CI 1·09–3·43; p=0·025); and highest quintile of bioavailable testosterone *vs* quintile 3, 1·84 (1·06–3·19; p=0·032)
Scarabin-Carré et al (2012)[41]	628 postmenopausal women, 537 controls, and 106 women with first ischaemic arterial event	Case-cohort study	>65 years at baseline	CAD	No associations with testosterone; total oestradiol, adjusted HR 1·49 (95% CI 1·10–2·02; p=0·01); bioavailable oestradiol, adjusted HR 1·50 (1·11–2·04; p<0·01)
Benn et al (2015)[42]	4716 premenopausal and postmenopausal women	Population-based, nested prospective cohort study	59 years (49–65) at baseline	CAD and total mortality at 30-year follow-up	Multifactorially adjusted risk for baseline testosterone (from blood drawn 1981–83) above 95th percentile *vs* 10th–89th percentile: risk of IHD increased by 68% (95% CI 34–210) and risk of death increased by 36% (18–58). Adjusted risk for oestradiol below 5th percentile *vs* 10th–89th percentiles: risk of IHD increased by 44% (14–81)

OR=odds ratio. SHBG=sex hormone-binding globulin. cCIMT=common carotid artery intima media thickness. CIMT=carotid artery intima media thickness. DHEAS=dehydroepiandrosterone sulphate. iCIMT=internal carotid artery intima media thickness. ICA=internal carotid artery. CAC=coronary artery calcification. DHEA=dehydroepiandrosterone. CVD=cardiovascular disease. HR=hazard ratio. CAD=coronary artery disease. IHD=ischaemic heart disease. *Units of β are log-unit thicker IMT or 1 log-unit greater hormone level. †Testosterone measured by liquid chromatography mass spectrometry.

Table 2: **Studies of the associations between endogenous total and free testosterone and cardiovascular outcomes in women**

risk factors, in women in the study reported by Sievers and colleagues[39] (mean age 58 years) is pertinent for women at midlife. Only one small study[38] used the sensitive method of LC-MS to measure concentrations of testosterone. No study has reported on the association between DHT or any testosterone or DHT metabolites and the risk of cardiovascular disease. The one study[41] to report on ischaemic stroke as an outcome only included 39 patients and showed no significant association with testosterone.

The association between endogenous testosterone and the risk of cardiovascular disease cannot be interpreted in isolation from the effects of SHBG and oestrogen, but few studies have taken concentrations of SHBG or oestradiol into account (panel). Overall, the available observational data suggest that low concentrations of total, free, and bioavailable testosterone (free and albumin-bound testosterone) and SHBG in serum are associated with a greater likelihood of atherosclerotic carotid disease, cardiovascular events, and total mortality. Furthermore, extremely high concentrations of endogenous bioavailable testosterone also seem to increase the future risk of CVD in women.

Randomised controlled trials have consistently shown that transdermal testosterone does not adversely affect known cardiovascular risk factors—namely lipids, C-reactive protein, haematocrit, coagulation proteins, and insulin resistance—in women.[4,20,53] Overall, randomised controlled trials of testosterone therapy have not shown any increase in coronary artery disease, stroke, or thrombosis, although none of the studies were adequately powered to investigate the effects of testosterone on major cardiovascular events. Iellamo and colleagues[54] reported on the effects of testosterone therapy in a randomised controlled trial in which 36 women with pre-existing cardiovascular disease in the form of severe congestive cardiac failure were randomly assigned to treatment with transdermal testosterone or placebo for 6 months. In this study, women given testosterone had significant improvements in the 6-min walk test, oxygen consumption, and insulin resistance compared with those given placebo, and better performance in each of these tests is associated with better prognosis for congestive cardiac failure.[54] This study does not suggest that women with congestive cardiac failure should be given testosterone but rather supports the need for better understanding of the role of testosterone in the pathogenesis of cardiovascular disease in women.

Testosterone and cognition

Evidence from basic and clinical studies suggests that sex steroids affect cognitive decline and progression to dementia in women. Findings from basic studies[55,56] have shown that oestradiol and testosterone are neuroprotective

Panel: Taking sex hormone-binding globulin into account

Testosterone in women is most often considered in the context of excess concentrations and polycystic ovary syndrome. Hyperinsulinaemia, androgen excess, and the metabolic syndrome characterise polycystic ovary syndrome. A hallmark of polycystic ovary syndrome is low concentrations of sex hormone-binding globulin (SHBG), which, in turn, are associated with the higher risk profile for cardiovascular disease seen in this disorder. Strong evidence shows that SHBG is metabolically important and not simply a transport protein for sex steroids. It has emerged as an independent marker of insulin resistance and risk of type 2 diabetes[44] and has been implicated in the pathogenesis of type 2 diabetes and cardiovascular disease in women.[44,45] Strong independent and highly statistically significant inverse associations between insulin resistance and SHBG and between BMI and SHBG have been shown.[46] These associations are independent of endogenous oestrogen and androgen concentrations.[46]

In postmenopausal women, low concentrations of SHBG but not high concentrations of total testosterone are significantly associated with a more adverse lipid profile (high concentrations of triglycerides and low concentrations of HDL cholesterol),[47] visceral fat accumulation,[48] and increased risk of diabetes.[44] Strong inverse associations between SHBG and C-reactive protein and between SHBG and diastolic blood pressure have been reported in postmenopausal women after adjusting for other variables.[46] Single nucleotide polymorphisms (SNPs) in the *SHBG* gene are associated with variations in SHBG concentrations.[44] However, SNP variants explain only about 2% of the variance in SHBG.[44] About 34% of the variance in postmenopausal women can be accounted for by insulin resistance, BMI, and diastolic blood pressure.[46,47]

Accumulation of fat in the liver might modulate hepatic production of SHBG by an insulin-independent mechanism.[49] Furthermore, physiological concentrations of fructose and glucose suppress production of SHBG in animal models, with dietary fructose having a marked effect.[50] The association between insulin resistance and SHBG is abolished after adjusting for fat in the liver, whereas the inverse association between SHBG and fasting glycaemia is not.[44,45] Consistent with this, dietary intervention that reduces liver fat is associated with an increase in SHBG, an effect that seems to be independent of insulin resistance.[44,45] In postmenopausal women, the free androgen index (FAI, calculated as total testosterone ÷ SHBG × 100), but not total testosterone, is associated with the metabolic syndrome and an increased risk of cardiovascular disease.[51,52] Thus, concentrations of SHBG but not testosterone explain the association between FAI and free fraction of testosterone—and other sex steroids—in plasma, but low concentrations of SHBG are an independent risk factor for insulin resistance, type 2 diabetes, and adverse lipid profile in young women and women at midlife.[44,46,47]

and have anti-inflammatory actions within the brain. Concentrations of testosterone in the human female brain during the reproductive years are several times greater than concentrations of oestradiol.[57] As outlined in a previous review,[58] testosterone within the brain is protective against oxidative stress, serum deprivation-induced apoptosis, and soluble amyloid β (Aβ) toxicity. Some of these effects are blocked by inhibition of oestrogen biosynthesis in animal models, which suggests that they are mediated by oestrogen. Protection against Aβ toxicity by testosterone seems to involve an androgen receptor-dependent mechanism that leads to upregulation of the Aβ-catabolising enzyme neprilysin.[58]

Studies of testosterone in women have yielded differing findings according to the age of women studied, dose of testosterone used, and study duration. In a study of 26 healthy premenopausal women, acute replication of male testosterone concentrations (after one dose of testosterone resulting in a ten-times greater concentration of testosterone in serum) enhanced visuospatial ability; effects on verbal learning and memory were not investigated.[59,60]

The effects of low concentrations of testosterone and treatment with testosterone have been explored in observational and clinical trials in postmenopausal women. In a small study[61] of 39 elderly women (aged 65–90 years), higher concentrations of endogenous testosterone, but not oestradiol, were associated with superior verbal fluency but not verbal memory, whereas high concentrations of both hormones in another 38 women (mean age 68 years) were associated with better verbal memory performance.[59,60,62] Two studies investigated the effects of 40 mg/day testosterone undecanoate given orally in postmenopausal women.[63,64] In one study,[63] this dose of testosterone undecanoate resulted in supraphysiological concentrations of testosterone, with a median value in the order of 7 nmol/L—more than three times the upper limit of normal for young women—and more than ten times the concentration of DHT in the first 4 h after dosing. A 4-week placebo-controlled study[64] in postmenopausal women aged 50–65 years reported no effects of testosterone undecanoate treatment on verbal fluency, verbal memory, or spatial ability, although the duration of exposure might have been too short to result in meaningful effects. In another small, randomised, placebo-controlled study[65] in 50 women, addition of the same dose of oral testosterone undecanoate to oral estradiol over 24 weeks was associated with a reduction in immediate memory but no other effects on cognitive performance.

Subsequent studies of cognition in postmenopausal women have investigated restoration of concentrations of testosterone in serum to those seen in healthy premenopausal women. These studies have shown predominantly favourable effects on verbal learning and memory. In an open-label, functional imaging study[66] of the brain in naturally and surgically postmenopausal women aged 47–60 years who were using oestrogen, treatment with transdermal testosterone spray for 6 months was associated with a reduction in neuronal recruitment (assessed by reduced blood oxygen level-dependent MRI signal intensity in the parietal lobe) during mental rotation and verbal fluency tasks but with no change in performance, accuracy, or speed. These effects were not seen in women randomly assigned to oral oestrogen–progestin treatment or placebo in a parallel study in the same setting.[67] A significant improvement in verbal learning and memory, including an improvement in delayed recall, was seen in a pilot study[68] in nine naturally and surgically postmenopausal women using transdermal estradiol who were given transdermal testosterone for 26 weeks, whereas scores were unchanged in the control group of 30 women.

To further investigate the findings from open-label and pilot studies, a double-blind, randomised, placebo-controlled trial[69] investigated the effects of daily testosterone gel in naturally postmenopausal women aged 55–65 years who were not taking concurrent oestrogen. After adjustment for age and baseline score, improvements in verbal learning and memory over 6 months were statistically significant compared with placebo.[69] The participants were not cognitively impaired at baseline and the improvement was within the normal range of cognitive function for age.

Cherrier and colleagues[70] reported on a small study in men that showed that testosterone needs to be converted to oestradiol to exert an effect on verbal memory. In this study, in which hypogonadal men given weekly intramuscular testosterone were randomly allocated to daily aromatase inhibitor therapy or placebo,[70] the placebo group had a significant increase in mean concentration of oestradiol, whereas the aromatase inhibitor group remained oestrogen deplete. In the placebo group, mean verbal memory score was lower at baseline and showed a significant increase from baseline, but the score was not significantly different to that in the aromatase inhibitor group at study end,[70] so the findings were inconclusive. Shah and colleagues[71] studied whether aromatase inhibition impedes testosterone's effects on cognition in postmenopausal women. 76 postmenopausal women, who had been using transdermal estradiol for at least 8 weeks, were started on transdermal testosterone at a dose that resulted in free testosterone concentrations at the upper limit of normal for young women, and randomly allocated to an aromatase inhibitor or identical placebo. Use of an aromatase inhibitor did not modify any measured cognitive outcomes, which included verbal memory and visual reproduction. Whereas the men in Cherrier and colleagues' study[70] were oestrogen deplete at baseline, the women in the study reported by Shah and colleagues[71] were on oestrogen therapy before and during administration of testosterone, such that hormonal effects on verbal memory that require the action of oestradiol were achieved by oestrogen therapy in both groups of women.

In summary, appropriately powered observational and interventional studies have shown an association between verbal learning and memory and physiological concentrations of testosterone given exogenously to postmenopausal women. Simulation of male concentrations of testosterone in premenopausal women enhances visuospatial performance, but the effects of male concentrations of testosterone on verbal learning and memory have not been studied in premenopausal women. The effects of testosterone on verbal learning and memory in postmenopausal women seem not to rely on aromatisation to oestradiol. The statistically significant improvements in verbal memory seen with testosterone therapy in studies of postmenopausal women suggest that further investigation of testosterone to enhance cognitive performance or delay cognitive decline is warranted but currently do not justify the use of testosterone for this purpose.

Musculoskeletal effects of testosterone

The androgen receptor is expressed in osteoblasts and osteocytes.[72] In men, the skeletal effects of androgens seem to be mediated directly through androgen receptors and indirectly by aromatisation to oestrogen, but the latter mechanism seems to dominate in women.[72]

In women in their late reproductive years, lower concentrations of free testosterone but not oestradiol are associated with a statistically significant decline in bone mineral density (BMD) of more than 1% per year.[73] In women aged 67–94 years, endogenous total testosterone has been positively associated with hip and lumbar BMD and free testosterone has been positively associated with hip BMD.[74] In the Women's Health Initiative observational study, higher concentrations of endogenous bioavailable testosterone were associated with lower occurrences of hip fracture independent of concentrations of oestradiol and SHBG.[75] Data for the effects of exogenous testosterone on bone in women are scant.

In a small study[76] in 34 postmenopausal women in whom estradiol plus testosterone implants or estradiol implants alone were inserted every 3 months for 2 years, significantly greater increases in BMD were reported in the combined treatment group (figure 3). In a 2-year randomised controlled trial[77] of oral conjugated equine oestrogen with and without methyltestosterone in 311 postmenopausal women, increases in BMD at the hip and spine were greater for the group given methyltestosterone. In a smaller study[78] of 66 surgically menopausal women, the addition of methyltestosterone to oral estradiol was not associated with increased BMD at the hip, and, although the increase seen at the lumbar spine was greater than at baseline, the increase with methyltestosterone was not significantly different to that with estradiol alone. The women in these studies did not have osteoporosis at baseline.

In a study[79] of 51 women with hypopituitarism, a greater increase in BMD at the hip was seen in those given a 300 µg transdermal testosterone patch plus estradiol than those given estradiol alone; a similar effect was not seen at the lumbar spine. By contrast, treatment with a 150 µg transdermal testosterone patch added to estradiol and progestin therapy in 73 women with primary ovarian insufficiency failed to show benefit for BMD compared with treatment of 72 women with estradiol–progestin alone.[80] No randomised controlled trial has reported on the effect of treatment with testosterone on fracture in women.

With respect to body composition, high concentrations of endogenous free testosterone have been directly associated with greater lean body mass in women aged 67–94 years.[74] Randomised controlled trials[76,81] have shown greater increases in lean body mass and strength and greater reductions in percentage of fat in postmenopausal women given combined oestrogen and testosterone compared with oestrogen alone.

In summary, although findings of epidemiological studies suggest that testosterone has an important anabolic role for muscle and bone, clinical trials have been small and mostly of short duration, so the effects of testosterone therapy on musculoskeletal health and the risk of fracture remain uncertain.

Testosterone and gynaecological cancer

Breast cancer

Breast carcinomas differ in histopathology, expression and bioactivity of hormone receptors, and pathophysiology. High-level evidence links the risk of breast cancer to age, age at menarche, family history, gene mutations, parity, obesity, and smoking.[82] Duration of lifetime exposure to oestrogen has been proposed as a central mechanism in the pathophysiology of breast cancer;[82] however, the relation between androgens and the risk of breast cancer is unclear. Hyperandrogenism in women because of polycystic ovary syndrome or high-dose androgen therapy in female-to-male transsexuals does not increase the risk of breast cancer.[83] High concentrations of bioactivity of the oestrogen receptor α have been proposed to increase the risk of breast cancer, and expression of androgen receptors has been proposed to exert a growth-inhibitory effect in tumours positive for the oestrogen receptor α and is related to better prognosis in tumours negative for the oestrogen receptor α.[84] However, the action of the androgen receptor in different subtypes of breast cancer is incompletely understood.[85]

Preclinical studies have shown testosterone to be antiproliferative and pro-apoptotic in some breast cancer cell lines.[85] Two large epidemiological studies[86,87] have reported on endogenous testosterone and the risk of breast cancer in premenopausal and postmenopausal women. The first was a pooled study[86] of seven prospective observational studies of the risk of breast cancer in premenopausal women, including 767 cases and 1699 matched controls. High endogenous concentrations of total testosterone, androstenedione, DHEAS, total and

free oestradiol, and oestrone were each independently associated with an increased risk of breast cancer but free testosterone was not.[86] In the second study,[87] a collaborative, pooled analysis of 18 prospective observational studies comparing postmenopausal women with breast cancer and postmenopausal controls, positive associations, with statistically significant linear trends, were seen for oestradiol, oestrone, and the risk of breast cancer irrespective of assay methods. An association between total testosterone and the risk of breast cancer was seen when testosterone was measured by extraction and direct assays but not by LC-MS.[87] Although the studies in this pooled analysis[87] that used LC-MS only included 227 cases and 227 controls, the findings do raise doubt about conclusions that can be drawn from studies using less sensitive assays. Additionally, an association identified in epidemiological research does not infer causation.

An important limitation of the studies described above—and of most studies in this field—is the assumption that testosterone, oestradiol, and oestrone can be included in analyses as independent variables without taking concentrations of oestrogen into account. Aromatase is the crucial enzyme that converts androgens to oestrogens within the breast and that drives progression of breast cancer.[88] Along with the risk of breast cancer, aromatase gene expression increases with age.[89] In postmenopausal women, circulating oestrone and oestradiol are derived from non-gonadal biosynthesis, such that concentrations of hormones in plasma provide a crude measure of what is happening at the tissue level.[90] Another limitation of observational studies is that measurement of a single hormone is a poor proxy for lifetime cumulative exposure to that hormone, which will have age-related, cyclical, and circadian fluctuations.

An increased risk of invasive breast cancer was not seen across the clinical trial programme for the testosterone patch, which included studies up to 2 years in duration.[91] Analyses of data from the Nurses Health Study[92] suggested that current but not past users of oral methyltestosterone—a non-aromatisable synthetic androgen—were at increased risk of breast cancer. In total, 32 breast cancers were reported for 5628 years of follow-up, the women's mean age was 61·5 years, and those who reported using methyltestosterone had a worse risk profile for breast cancer, in that they were younger, leaner, more likely to have benign breast disease (55%), and consumed more alcohol than controls. Importantly, the reason women were prescribed methyltestosterone was not known. An increased risk of breast cancer in current users of methyltestosterone has not been supported by other studies[93,94] with this drug that have taken into account concurrent use of oestrogen and progestin. Two observational studies[95,96] of the risk of breast cancer associated with testosterone implant therapy and transdermal testosterone, which included a similar number of users and duration of follow-up as the Nurses Health Study, reported that the risk for current users is not above the background risk in the community. Glaser and Dimitrakakis[97] reported 142 cases of invasive breast cancer per 100 000 person-years for women given testosterone implants compared with a background community rate of 293 per 100 000 person-years; however, the level of testosterone achieved in that study was in the low range for men.

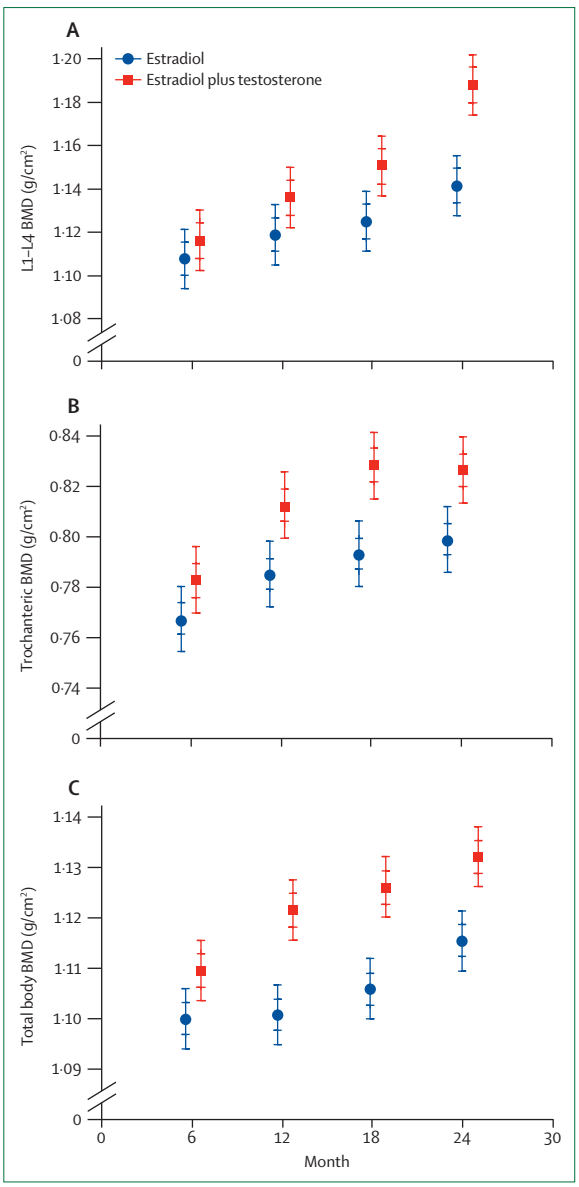

Figure 3: **Effects of estradiol and estradiol plus testosterone implants on bone mineral density at the lumbar spine and femoral trochanter and for total body**
Error bars represent the standard error of the difference (SED). Inner error bars are used to compare means between times for the same treatment and outer error bars for the between-group difference. If error bars do not overlap, the difference is greater than 2 SEDs and the means are significantly different ($p \leq 0{\cdot}05$). Reproduced from Davis and colleagues,[76] by permission of Elsevier.

Search strategy and selection criteria

We did a comprehensive literature search of articles published in English in MEDLINE, Scopus, and the Cochrane Library between February, 2015, and May, 2015. We used the search terms "testosterone women" or "androgens women" in combination with the terms "sexual function", "SHBG", "cardiovascular disease", "bone", "muscle", "vaginal atrophy", "cognition", and "cancer". We largely selected publications from the past 10 years but did not exclude widely referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged to be relevant. Review articles and book chapters are cited to provide readers with more details and more references than could be included in this Review.

In summary, epidemiological studies that have shown positive associations between endogenous concentrations of testosterone and the risk of breast cancer have substantial limitations. Preclinical studies suggest protective effects for some subtypes of cancer. Observational studies have consistently shown no increase in the risk of breast cancer in past users of exogenous testosterone,[92,96] one of three studies[92–94] showed an increased risk in current users of methyltestosterone, and three studies of parenteral testosterone[95–97] have shown no increase in risk for current users. An increased risk of invasive breast cancer was not seen across the reported studies for three doses of the transdermal testosterone patch. No reported clinical trial has been of sufficient duration to provide certainty about the safety of exogenous testosterone in terms of breast cancer, such that the effects of long-term use remain uncertain. A large safety study of transdermal testosterone versus placebo in women at increased risk of cardiovascular disease accrued more than 7000 women-years of data;[98,99] despite the safety monitoring committee recommending continuation of this study after six unblinded reviews, the study was halted because of insufficient funds and the data have not been made public.

Ovarian and endometrial cancer

The role of endogenous androgens in ovarian carcinogenesis is not well understood. A large prospective study[100] that compared 565 cases of invasive epithelial ovarian cancer with 1097 matched controls showed no association between androgens and overall risk of invasive epithelial ovarian cancer. Negative associations between androstenedione and high-grade and type II serous carcinomas and positive associations with low-grade, type I carcinomas were reported, which suggests that androgens could have both protective and procarcinogenic roles.[100] No adjustment for level of oestradiol was made, so it is not possible to conclude whether this is a direct effect of the androgen or occurs via its aromatisation to oestrogens.

Exogenous testosterone has not been independently associated with the risk of endometrial cancer.[101] In female-to-male transsexuals, testosterone is anti-proliferative in the endometrium,[102] with no evidence of endometrial proliferation in a randomised controlled trial of testosterone done over 12 months.[19] Overall, data for this outcome are scarce.

Conclusion

In 2004, an expert panel from the US Food and Drug Administration decided that transdermal testosterone is an effective treatment for hypoactive sexual desire disorder but that documentation of its diverse actions and safety in women was insufficient.[103] This highlights the extent to which research into the role of testosterone and other androgens in women lags behind that in men. Most of the new information is from secondary outcomes of large randomised controlled trials undertaken to investigate testosterone for the treatment of hypoactive sexual desire disorder. Hence, understanding of the physiology and tissue-specific effects of androgens in women remains incomplete. Given that androgen receptors in women have been isolated in most tissues and that testosterone circulates at higher concentrations than oestradiol during the premenopausal and postmenopausal years, better understanding of the action of testosterone and the result of androgen insufficiency is needed.

This Review suggests that testosterone has been overlooked as a hormone with potentially favourable cardiovascular effects in women and that associations between testosterone and the risk of cardiovascular disease and total mortality in older women are yet to be established. Possible beneficial effects of testosterone on cardiovascular function need further investigation. The few studies that suggest favourable effects of testosterone on cognitive performance are provocative, such that further studies are warranted to determine whether testosterone therapy can delay mild cognitive decline and possibly even dementia. High-quality clinical trials assessing the effects of testosterone on musculoskeletal health and the risk of fragility fractures are also needed. Most pressing is the need for research to clarify whether testosterone therapy modifies the risk of breast cancer in premenopausal and postmenopausal women, because this is the issue that concerns most clinicians when considering prescribing testosterone for female sexual dysfunction.

Treatment with testosterone should be considered but should not be routine in the management of women with a premature decline in production of testosterone, specifically women with premature ovarian failure, surgical menopause, and hypopituitarism. The updated clinical practice guideline on androgen therapy from The Endocrine Society[4] includes recommendations for the use of testosterone in women. However, the studies proposed above are needed to inform future guidelines with respect to the role of testosterone in the prevention of diseases of ageing and the safety of such treatment.

Contributors
SRD and SW-J collaboratively searched the scientific literature, interpreted the findings, prepared the report, and reviewed the final report.

Declaration of interests
SRD is a consultant and investigator for Trimel Pharmaceuticals, an investigator for Lawley Pharmaceuticals, and has received honoraria from Abbott Pharmaceuticals. SW-J has received a speaker honorarium from Novo Nordisk Scandinavia.

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