Menopause, androgens, and cardiovascular ageing: a narrative review

Eleni Armeni and Irene Lambrinoudaki

Abstract: Cardiovascular disease is the leading cause of death worldwide; however, women tend to be less affected than men during their reproductive years. The female cardiovascular risk increases significantly around the time of the menopausal transition. The loss of the protective action of ovarian oestrogens and the circulating androgens has been implicated in possibly inducing subclinical and overt changes in the cardiovascular system after the menopausal transition. *In vitro* studies performed in human or animal cell lines demonstrate an adverse effect of testosterone on endothelial cell function and nitric oxide bioavailability. Cohort studies evaluating associations between testosterone and/or dehydroepiandrosterone and subclinical vascular disease and clinical cardiovascular events show an increased risk for women with more pronounced androgenicity. However, a mediating effect of insulin resistance is possible. Data on cardiovascular implications following low-dose testosterone treatment in middle-aged women or high-dose testosterone supplementation for gender affirmatory purposes remain primarily inconsistent. It is prudent to consider the possible adverse association between testosterone and endothelial function during the decision-making process of the most appropriate treatment for a postmenopausal woman.

Keywords: androgens, cardiovascular disease, endothelial function, menopause, oxidative stress

Received: 31 January 2022; revised manuscript accepted: 9 September 2022.
mortality, estimated as 1.5-times and 1.19-times higher, respectively, compared with women experiencing spontaneous menopause at an age more than 45 years.7 Similarly, women experiencing menopause at the age of 50–54 years had a 13% lower risk of fatal coronary heart disease than women experiencing menopause at 50 years.7

According to the latest statement of the American Heart Association, the menopausal transition is an essential window of accelerated cardiovascular risk suggesting the acquisition of primary prevention strategies.5 Recent evidence highlighted the importance of both the traditional risk factors and additional sex-specific factors, including the possible effect of sex hormones in determining the overall cardiovascular risk.8 A large amount of data support a possible association between hormonal changes associated with menopause and age-related alterations of the cardiovascular system, further contributing to the sharp increase of CVD observed at midlife.5,8 However, the available literature seems to be characterized by heterogeneity, determined by the study design.

The aim of this review is to summarize the evidence on the association between androgens and subclinical as well as clinical CVD in ageing women.

Cardiovascular ageing: the effect of menopause

The process of cardiovascular ageing is comprised of a series of changes affecting both the left ventricle as well as the overall arterial tree. The effect of ageing on the left ventricle seems to range from concentric hypertrophy with or without diastolic dysfunction, aortic valvulopathy, reduced variability of the heart rate, and annular calcification of the mitral valve as well as conduction abnormalities.9 The arterial tree itself is characterized by progressively enlarging vascular lumen as well as thickening of the vessel wall.9,10 Simultaneously, early stages of vasodilatation become apparent in the aorta, the large branches proximal to the myocardium.9,10 The raised aortic stiffness results in a further rise in reflected waves and aortic pulse wave velocity, which became apparent as elevated systolic blood pressure and increased left ventricular afterload.10 Diastolic blood pressure, on the contrary, tends to decline with age.10 The raised systolic blood pressure in the context of low diastolic blood pressure results in increased oxygen demand by the myocardium, the supply of which is likely to be compromised in the context of advancing atherosclerosis of coronary arteries.10

At a cellular level, ageing endothelial cells and cardiomyocytes produce increasing amounts of reactive oxygen species (ROS)11–13 This process takes place principally in the mitochondria, which constitute up to 45% of the cardiac cell volume.14,15 Ultimately, ageing-mediated increased production of ROS compromises vasorelaxation, increases vascular stiffness and impairs endothelial cell turnover and release of nitric oxide.16,17 At a cellular level, oxidative stress and marked production of ROS result in the following changes, which contribute to the development of heart failure, valvular degeneration, and atrial fibrillation: (1) epigenetic alterations and DNA damage, (2) increased expression of tumour necrosis factor-alpha and matrix metalloproteinase’s, (3) impaired function of the sarcoplasmic/endoplasmic reticulum calcium-ATPase and consequently insufficient reuptake of ionized calcium, (4) impaired bioavailability of nitric oxide and suppressed function of endothelial nitric oxide synthase.16,17

Menopause-associated hormone changes contribute to the age-related alterations evident in the cardiovascular system. Oestrogen deficiency is implicated in endothelial dysfunction and reduced NO bioavailability.18 The postmenopausal ovary continues to produce testosterone, the levels of which decline progressively with ageing.19,20 The adrenal glands contribute to the pool of testosterone through an ongoing secretion of dehydroepiandrosterone sulphate (DHEAS), the levels of which also decline with ageing, as well as androstenedione.19,20 Androgen production during the female reproductive life is evident as follows:20 (1) in the ovarian theca cells, which are secreting up to 20% of circulating DHEAS, (2) in the ovarian stroma, which is producing 50% of the circulating androstenedione and up to 25% of the circulating testosterone, (3) the adrenal zona reticularis which is secreting up to 50% of circulating DHEAS, androstenedione and up to 25% of circulating testosterone. The remaining portion of testosterone is produced with the direct conversion from circulating androstenedione.20
Increasing age, as well as the menopause-related hormonal changes, result in changes that directly or indirectly contribute to increase in cardiovascular risk21 (Figure 1). Following ovarian senescence, the production of oestrogen decreases rapidly.22 Simultaneously, levels of sex hormone-binding globulin also decrease due to the subsequent reduction in oestrogen-liver interaction,23 a change that results in an increase in circulating levels of sex hormones.24 The rate of androgen production declines to a lesser extent, given the ongoing contribution of the adrenal glands to the pool of circulating sex hormones.25 Consequently, the androgen-to-oestrogen ratio increases, resulting in an environment of relative androgenicity.25 These hormonal alterations, as well as ageing per se, are known to induce pathophysiological manifestations, which consist of impaired fibrinolysis,26 insulin resistance,27 and visceral adiposity.28 In this context, impaired fibrinolysis contributes to the origin and progression of atherosclerotic changes.29,30 Hyperandrogenism, visceral adiposity, and insulin resistance contribute to metabolic dysfunction, generation of oxidative stress, and low-grade inflammation, which result into endothelial dysfunction. Hyperandrogenemia has also been demonstrated to contribute to endothelial dysfunction, which eventually result into more advanced atherosclerotic changes.

**The effect of androgens on cardiovascular tissues: preclinical studies**

Androgen receptors are widely distributed in different cell types of the cardiovascular tissue; their presence has been confirmed in endothelial cells, cardiomyocytes and vascular smooth muscle cells (VSMCs).40 The interaction between androgens and endothelial cells is of particular significance, as these represent key players in cardiovascular pathophysiology, with a fundamental role in cardiovascular health and disease.41

Emerging data from *in vitro* studies suggest cardiovascular implications of testosterone to cells of the cardiovascular system (Table 1). Overall,
Table 1. Experimental data on the effect of testosterone on cells of the cardiovascular system.

| Ref.          | Target tissue                                                                 | Messenger or receptor involved                                                                 | Experimental response                                                                 |
|--------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Preclinical studies on the effect of testosterone administration                                                                                   |
| *Endothelial cells*                                                                                                                                   |                                                                                                 |                                                                                        |
| Arapa-Diaz et al.\(^{42}\) | ECs derived from the coronary vascular bed of male hypertensive rats          | Bradykinin/NO-dependent pathway                                                                 | ↑ Endothelium-dependent relaxation                                                   |
| Campelo et al.\(^{43}\)   | ECs derived from aortic strips of female, sexually mature Wistar rats          | MAPK and PKC pathway mediating NO production                                                   | Modulation of EC growth                                                              |
| Goglia et al.\(^{44}\)     | HUVECs from female and male foetuses, ECs from ovariectomized Wistar rats     | Activation of eNOS and regulation of t-PA and PAI-I expression                                  | Modulation of ECs function                                                           |
|                           |                                                                             |                                                                                                 | • Evident in rats administered physiological concentration of T                       |
|                           |                                                                             |                                                                                                 | • Not evident in rats administered supraphysiological doses of T                      |
|                           |                                                                             |                                                                                                 | • No association with DHT of either physiological or supraphysiological dose          |
| Sieveking et al.\(^{45}\)  | HUVEC                                                                       | The effect of AR activation depends on the gender:                                               | ↑ neovascularization after ischemic injury in males only                              |
|                           |                                                                             | Male ECs: antagonism of AR leads to reduced neovascularization, which is restored with T administration |
|                           |                                                                             | Female ECs: ↑ expression of AR resulted in androgen sensitivity and angiogenesis                |                                                                                        |
| Alves et al.\(^{46}\)      | Thoracic aorta derived from male 12 week-old NLRP3 knockout mice             | mROS generation and NLRP3 inflammasome activation                                              | ↑ contractile responses                                                               |
|                           |                                                                             |                                                                                                 | ↓ endothelium-dependent vasodilation                                                   |
| *Vascular smooth muscle cells*                                                                                                                      |                                                                                                 |                                                                                        |
| Montaño et al.\(^{47}\)    | Myocytes derived from thoracic aortas of adult male Wistar rats              | ↑ T concentration induces ↑ intracellular Ca\(^{2+}\) and cAMP production                      | ↑ vasodilation                                                                        |
|                           |                                                                             | ↓ T concentration results in L-VOCCs antagonism                                                 |                                                                                        |
| Chignalia et al.\(^{48}\)  | VSMCs derived from Wistar-Kyoto rat and spontaneously hypertensive rat      | cSrc-dependent pathway and NADPH oxidase-derived ROS by genomic/non-genomic mechanisms         | Induction of ROS generation                                                          |
|                           |                                                                             |                                                                                                 | Stimulation of VSMCs migration                                                        |
| Lopes et al.\(^{49}\)      | VSMCs derived from male Wistar rats                                         | Activation of MAPK and tyrosine kinase pathway                                                | VSMCs apoptosis via ROS generation                                                   |
|                           |                                                                             | Modulation of the NADPH oxidase complex                                                        |                                                                                        |
| Zhu et al.\(^{50}\)        | VSMCs were derived from primary murine AR-ablated compared to WT-mice        | Non-specific alkaline phosphatase mRNA expression                                              | ↓ calcification in VSMCs from AR- vs WT-mice                                         |

(Continued)
Table 1. (Continued)

| Ref.         | Target tissue                                         | Messenger or receptor involved                                                                 | Experimental response |
|--------------|-------------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------|
| Son et al.51 | Human aortic smooth muscle cells                      | AR signalling regulates Growth arrest-specific gene 6 transcription                           | Inhibition of VSMCs calcification |
| Cardiomyocytes |                                                      |                                                |                       |
| Xiao et al.52 | Cardiomyocytes derived from embryonic rat heart       | • AR and NF-κB signalling pathways (↑) Akt activity and (↓) caspase-3 expression in cardiac myocytes | Protection of cardiomyocytes from superoxide injury and apoptosis |
| Vicencio et al.53 | Cardiomyocytes derived from neonatal male Sprague Dawley rats | PTX sensitive G protein – phospholipase C / inositol 1,4,5 triphosphate signalling pathway | (↑) intracellular Ca²⁺ signalling |
| Akdis et al.54 | Cardiomyocyte-based model derived from human stem cells in ARVC/D model | Not specified | (↑) T-induced cardiomyocyte apoptosis and lipid accumulation |
| Zhang et al.55 | Cardiomyocytes derived from testicular feminized and castrated male mice | AR-independent pathway (↑) activities of SOD and GSH-Px enzyme (↓) MDA levels (↓) mtDNA mutations | suppression of oxidative stress |
| Preclinical studies on the effect of combined testosterone and oestrogen administration |                                                      |                                                |                       |
| Costa et al.56 | Ovariectomized spontaneously hypertensive rats received CEE + T | Phosphorylation of the cytosolic NADPH-oxidase subunit p47phox | CEE + T vs CEE-only therapy resulted in (↑) ROS |
| Costa et al.57 | Hypertensive ovariectomized female rats received CEE + T | CYP4 F3/20-HETE pathway | (↑) adrenergic vasoconstriction in isolated aorta (↑) ROS generation in isolated aorta |
| Xu et al.58 | Cardiomyocytes treated with estradiol (50 nM) and testosterone (10 nM) | TGF-β1 pathway | The optimal E2/T ratio is associated with (↓) apoptosis of cardiomyocytes |
| Dai et al.59 | HUVECs derived from female mice were treated with 17β-estradiol (1 mg/day) ± testosterone (7 mg/day) for 120 days | • PI3K/Akt pathway (↑) Expression of cleaved caspase 3, Bax, cleaved PARP (↑) Expression of Bcl-2 | The optimal E2/T ratio is associated with • (↓) Apoptosis of cardiomyocytes • (↓) Lipid lesions, and formation of foam cells, • (↑) Endothelial injury, Modulating the coagulation system function • (↓) Inflammation |

A, androgens; AR, androgen receptor; cAMP, cyclic adenosine monophosphate; CEE, conjugated equine oestrogen; CP4 F3, cytochrome P450 4F3B; DHT, dihydrotestosterone; EC, endothelial cells; GSH-Px, glutathione peroxidase; HUVEC, human umbilical vein endothelial cells; iPSC-CMs, pluripotent stem cell-derived cardiomyocytes; L-VGCCs, L-type voltage operated Ca²⁺ channels; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; mtDNA, mitochondrial deoxyribonucleic acid; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; PAI-I, plasminogen activator inhibitor I; PI3 K/Akt, phosphoinositide-3-kinase/protein kinase B; PTX, pertussis toxin; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; NO, nitric oxide; PKC, protein kinase C; ROS, Reactive oxygen species; SOD, superoxide dismutase; Src, regulator of redox-sensitive migration; T, testosterone; t-PA, tissue plasminogen activator; VSMC, vascular smooth muscle cell; (↑), tendency to increase; (↓), tendency to decrease; 20-HETE, 20-hydroxyeicosatetraenoic acid.
animal studies support that testosterone improves endothelial mediated vasodilation, increases ROS generation and oxidative stress and promotes apoptosis and migration of VSMCs. Data on angiogenesis are conflicting, with evidence supporting a positive effect of testosterone in male animals only, with no effect in females. The results on a possible link between testosterone and VSMC calcification remain conflicting, with studies supporting stimulation of VSMCs migration, induction of VSMCs-apoptosis, or suppression of VSMCs-calcification. Studies addressing the effect of testosterone on cardiomyocytes report protection from apoptosis, but also induction of apoptosis, suppression of oxidative stress and increased intracellular calcium ion signalling. Moreover, preclinical studies describe the effect of the synergistic action of estradiol and testosterone on cells of the cardiovascular system. Accordingly, spontaneously hypertensive ovariectomized rats treated with combined equine oestradiol (CEE) showed suppression of oxidative stress (ROS). This beneficial effect of CEE-treatment was reversed after the addition of testosterone, with induction of ROS. Others reported that the optimal estradiol to testosterone ratio following treatment with a combination of estradiol and testosterone is associated with a decrease in apoptosis, endothelial injury, vascular inflammation, coagulation, and formation of foam cells and vascular lipid lesions.

Table 2. Experimental data on the effect of DHEAS on cardiovascular tissues.

| Ref. | Target tissue | Messenger or receptor involved | Experimental response |
|------|---------------|--------------------------------|-----------------------|
| **Endothelial cells** | | | |
| Gündoğan et al. | Primary HUVECs were treated with DHEA (0–200 μM, 24–72 h) | Not clearly described | • Reduced proliferation rate of endothelial cells at 24 h, 48 h, 72 h • Inhibition of proliferation and induction of necrosis |
| Altman et al. | Human aortic ECs were treated with DHEAS (48 h) and then with TNF-alpha | • TNF-alpha mediated activation of NF-kappaB • PPARα inhibition | • Dose-dependent inhibition of the expression of VCAM-1 |
| Curatola et al. | Human coronary ECs from men and women | • Increased expression of polysialylated NCAM | • Inhibiting adhesion of monocytes |
| Liu et al. | Bovine aortic ECs | • Galphai-PI3K/Akt-Bcl-2 pathway | • Protecting against cell ECs-apoptosis |
| Huerta-García et al. | HUVEC exposed to PM and NPs and subsequently treated with DHEA | Not clearly described | • Anti-oxidative and anti-inflammatory effects |
| Huerta-García et al. | HUVEC treated with a high concentration of DHEA and/or glucose | • NF-κB | • (↓) Oxidative stress • (↓) Activation of endothelial cells |
| Liu et al. | Bovine aorta ECs | • PTX-sensitive G proteins • ERK1/2 | • Enhanced angiogenesis • Increased proliferation of vascular ECs |
| Wang et al. | *In vivo*: Ovariectomized rabbits *In vitro*: Injured HUVECs due to oxidized LDL particles | • Not mediated through oestrogen receptor alpha or beta • (↓) the lipopolysaccharide-induced NF-κB transcription | *In vivo*: (↑) EC-oestrogen receptor and (↑) serum NO *In vitro*: (1) (↑) NO synthesis, (2) (↓) expression of adhesion molecules (VCAM-1, ICAM-1 and E-selectin), (3) (↓) expression of inflammatory molecules (MCP-1 and MDA) |

(Continued)
Table 2. (Continued)

| Ref.                  | Target tissue                                                                 | Messenger or receptor involved                                                                 | Experimental response                                                                 |
|-----------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Williams et al.⁶⁹     | Bovine aortic ECs derived from the aorta of young calves, HUVEC                |  Coronavirus expression of eNOS, MAPK ERK 1 / 2 kinase                                            |  Poliferation of ECs                                                                |
| **Vascular smooth muscle cells** |                                                                                 |                                                                                                |                                                                                      |
| Ochi et al.⁷⁰         | A7r5 aortic smooth muscle cells from the thoracic aorta of rat embryos were treated with DHEA. Cells lines were pretreated with 6-AN for competitive inhibition of G6PD | • Voltage-independent inhibition: mediated by G6PD                                                |  Voltage-dependent and -independent inhibition of ion-channels (L-type Ca²⁺) and window current (I_{WD}) |
| Chen et al.⁷¹         | VSMC were retrieved from the thoracic aorta of SD rats                         | • Inhibition of the ERK 1 / 2 signalling pathway                                                |  Reduction of inflammation and oxidative stress in VSMCs                            |
| Li et al.⁷²           | Baloon injury of rabbit carotid artery in adult males treated with DHEAS or saline | PPARα signalling                                                                                 |  Medial carotid layer cell death and apoptosis                                        |
| Urata et al.⁷³        | Thoracic aorta VSMC derived from embryonic rat models A7r5                     | • Upregulation of PPAR-α                                                                         |  • VSMC migration                                                                    |
|                       |                                                                                | • Inhibition of platelet-derived growth factor-β phosphorylation                                |  • VSMC proliferation                                                                |
|                       |                                                                                | • Up-regulation of glutathione/glutaredoxin-1 redox system                                     |                                                                                        |
|                       |                                                                                | • Control of low molecular weight-protein tyrosine phosphatase                                |                                                                                        |
|                       |                                                                                | • No interaction with androgen or oestrogen receptor                                             |                                                                                        |
|                       |                                                                                | • Suppression of VSMC phenotypic proliferation                                                  |                                                                                        |
| **Cardiomyocytes**    |                                                                                 |                                                                                                |                                                                                      |
| Dumas de La Roque et al.⁷⁴ | Juvenile Wistar rat models of pulmonary hypertension were treated with DHEA or control | [†] The expression and activity of the large-conduction BKCa-inhibited KCL- and serotonin-induced VSMC contraction and proliferation |  • Pulmonary arterial pressure                                                       |
|                       |                                                                                |                                                                                                |  • Preventing pulmonary artery hyper-reactivity and remodelling                        |
|                        |                                                                                |                                                                                                |  • Preventing RV hypertrophy                                                          |
| Alzoubi et al.⁷⁵      | Adult chronically hypoxic, adult male Sprague-Dawley rats received monocrotaline injection followed by 5-week treatment with DHEA | • [†] NADPH levels in RV tissue                                                                 |  Preservation of contractile RV-function against fibrosis and apoptosis                |
| Mannic et al.⁷⁶       | Ventricles retrieved from 1–2 day old Wistar rats; assessment of the effect of DHEAS following exposure to dexamethasone or aldosterone | • Cellular pathways, likely including MAPK                                                     |  • [†] Hypertrophic response of cardiomyocytes                                        |
Table 2. (Continued)

| Ref.                  | Target tissue                                                                 | Messenger or receptor involved                                                                 | Experimental response                                                                 |
|----------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Nakamura et al.77    | In vivo: Human LV-tissue autopsy samples in patients with HF vs control        | Not clearly described                                                                          | In vivo: DHEA levels in LV tissue are higher in control vs HF-patients                  |
|                      | In vitro: Neonatal cardiocyte culture                                          |                                                                                                 | In vitro: (1) (↓) Increase in the size of myocytes; (2) (↓) mRNA levels for BNP production |
|                      |                                                                                |                                                                                                 |                                                                                         |

Data from preclinical studies describing the effect of dehydroepiandrosterone (DHEAS) on cardiovascular tissues are presented in Table 2. Experimental data on the effect of DHEAS administered to endothelial cells (ECs) describe that DHEAS-treatment is reducing the proliferation rate,61 or increasing the proliferation rate of ECs.67,69 DHEAS has, furthermore, been reported to induce endothelial cell necrosis.61,64 Others described reduction of vascular inflammation,62 exertion of antioxidative and anti-inflammatory effects,65,66 increased angiogenesis,67 and improved endothelial function.68 DHEAS treatment administered to VSMCs results in reduced inflammation and oxidative stress in healthy VSMCs.71 In studies describing balloon injury models, DHEAS treatment is linked with reduced VSMC migration and proliferation72,73 and increased apoptosis.72 The effect of DHEAS-treatment on cultures of cardiomyocytes consists of a tendency towards a lower chronotropic and hypertrophic response,76 reduced pulmonary artery pressure, prevention of pulmonary artery hyperreactivity and remodelling and prevention of the remodelling of the right ventricle.74

**Pathophysiological mechanisms determining the interaction between menopause and endothelial cells**

Endothelial cells are known to express both oestrogen (ER) and androgen receptors (AR), which are known to mediate their functional capacity.40 The binding of oestrogen on the ER has been linked with decreased expression of adhesion molecules (e.g. CD40, P-selectin, E-selectin, ICAM-1) as a response to atherogenic promoting factors, ensuing in increased NO release and VSMC relaxation.78 The chronic oestrogen deficiency following ovarian senescence contributes to endothelial dysfunction with a decrease in NO bioavailability, secondary to decreased NO synthesis and increased NO inactivation.79 Moreover, the post-menopausal environment of hypoestrogenemia induces an altered redox balance, contributing to oxidative stress,37 while also inducing the expression of homocysteine and cysteine molecules, with adverse effects on endothelial function.81

Focusing on the interactions mediated by androgens, testosterone has been linked with a dual effect on cardiovascular cells, both pro-oxidant and antioxidant.60 More specifically, the pro-oxidant effect consists of suppressed activation of the endothelial NO synthase (eNOS), resulting in impaired endothelial NO release in women.37,82 The antioxidant effect is maintained through conversion to estradiol, which also contributes to mediating high levels of antioxidant enzymes.60 Earlier data suggested that the degree of oxidative stress is an essential factor regulating testosterone action; thus, an environment of high oxidative stress is related to adverse testosterone effects and vice versa.55 These observations highlight the
need for further research to determine factors driving oxidative stress during and after the menopausal transition.

Data on the cardiovascular alterations observed in women during the menopausal transition is rather sparse. Samargandy et al. evaluated 339 women retrieved from the Study of Women’s Health Across the Nation, aiming to measure the annual percentage change in carotid-femoral PWV in relation to the final menstrual period (FMP). This study highlighted the pattern of statistically significant changes in values of cfPWV around the FMP. Accordingly, cfPWV values increased at a steady rate up to 12 months prior to the FMP (0.9%, 95% CI: −0.6% to 2.3%), continued to increase more rapidly up to 1 year after the FMP (7.5%, 95% CI: 4.1% to 11.1%), and subsequently decreased at a steady rate (−1.0%, 95% CI: −2.8% to 0.8%).

### Endogenous androgens and CVD: data from human cohort studies

#### Subclinical CVD

Data on cohort studies assessing the possible associations between endogenous androgens and markers of subclinical vascular disease are presented in Table 3.

The possible association between endogenous androgens and endothelial function in middle-aged women has been investigated by one cohort study.

| Ref. | Study sample and Design | Primary outcome | FU | Results | Discussion |
|------|------------------------|----------------|----|---------|------------|
| Georgiopoulos et al. | 180 postmenopausal women with no overt CVD or diabetes | New-onset HTN, FMD | Median: 29 m | Baseline FAI was associated with: • New-onset HTN (OR 2.71, 95% CI: 1.14–6.41) • change in FMD (−0.42% per SD of FAI) | (↑) Baseline FAI was associated with: • new-onset HTN • change in FMD |
| Subramanya et al. | Multi-ethnic study of atherosclerosis (2018) 457 postmenopausal women and 548 men aged 45–84 years | AAD, PWV | Median: 10 y | Baseline: Highest tertile vs lowest tertile of FT, • AAD, b-coefficient = −0.10, 95% CI: −0.19 to −0.01 • PWV, b-coefficient = −0.04, 95% CI: −0.10 to 0.02 | CS: (↑) FT was associated with (↓) AAD and (↓) PWV L: no association |
| Lambrinoudaki et al. | 411 consecutively recruited healthy postmenopausal women | PWV, SI index, FMD, CAS | NA | FAI was associated with • PWV (β = 0.149, p value = 0.014), • Slindex (β = 0.154, p value = 0.022), • CAS (β = 0.193, p value = 0.02) | (↑) FAI was associated with • (↑) PWV • (↑) Slindex • (↑) CAS |
| Georgiopoulos et al. | 180 postmenopausal women with no overt CVD or diabetes | PWV | Median: 29 m | Baseline FAI [high vs lower quartile] and transition into abnormal PWV: NRI = 53.4 ± 23.2%, p value = 0.021, across follow-up period | (↑) FAI at baseline was associated with (↑) risk for abnormal PWV |

Table 3. Cohort studies exploring the possible associations between endogenous androgens and subclinical vascular disease.
Table 3. (Continued)

| Ref. | Study sample and Design | Primary outcome | FU | Results | Discussion |
|------|-------------------------|-----------------|----|---------|------------|
| **Atherosclerosis** | | | | | |
| Kische et al.87 | Study of Health in Pomerania (2016), 2,140 individuals (mean age: 60.8 years) Design: CS and L | Carotid IMT | Median: 5 year | - Association between TT and IMT in women (beta per log unit increase: 0.02; 95% CI: 0.007–0.45)  
- Association between SHBG and incident plaques (Q1 vs Q3: beta 1.35, 95% CI: 1.04–1.74)  
- No consistent association between TT and subclinical CVD | CS: [↑] TT was associated with [↑] IMT; [↓] SHBG was associated with the incidence of atherosclerotic plaques  
L: no consistent association between subclinical CVD and TT |
| Lee et al.88 | The CARDIA study: 367 middle-aged women Design: L | Carotid bulb IMT, FU points: 15 and 20 years | Carotid bulb IMT, multivariable adj. model  
Cortisol to free testosterone ratio  
- Tertile T3 vs T1: beta = 0.088, p = 0.006 | [↑] Cortisol-to-free T ratio was associated with higher carotid bulb IMT |
| Calderon-Margalit et al.89 | The CARDIA study 1629 women Design: L | Carotid IMT | 20 years | Coronary – IMT, highest quartile:  
- SHBG levels, Q4, OR = 0.56, 95% CI: 0.37–0.84, p for linear trend across quartiles 0.005 | [↑] Carotid IMT was associated with [↓] SHBG  
No association between carotid IMT and TT or FT |
| **Coronary artery calcium** | | | | | |
| Subramanya et al.90 | Multi-Ethnic Study of Atherosclerosis (2019)  
2759 postmenopausal women (age 65 ± 9 years) free from baseline CVD Design: CS and L | CAC | Median 4.7 y (maximum 10 years) | - [↑] FT and relative CAC progression ratio (2.16, 95% CI: 1.011–1.56)  
- [↑] SHBG and CAC progression ratio (0.80, 95% CI: 0.64–0.99) | CS: No association between CAC and sex hormones  
L: CAC progression ratio was associated with [↑] FT and [↓] SHBG |
| Calderon-Margalit et al.89 | The CARDIA study 1629 women Design: L | CAC | 20 years | Incidence of CAC, multivariable adj. models  
- SHBG above vs below median, adjusted OR = 0.59, 95% CI: 0.60–0.87 | [↑] CAC-incidence was associated with [↓] SHBG but not with TT or FT |
| Lee et al.88 | The CARDIA study 367 middle-aged women Design: L | New-onset CAC, FU points: 15 and 20 years | Incident CAC,  
Cortisol to free testosterone ratio  
- Tertile T3 vs T1: OR = 3.45 (95% CI: 1.18–10.06) | [↑] CAC-incidence was associated with [↑] cortisol-to-FT ratio, but not with cortisol-to-FT ratio or with the cortisol-to-SHBG ratio |

AAD, ascending aortic distensibility; CAC, coronary artery calcification; CARDIA, coronary artery risk development in young adults; CAS, cumulative marker combining pulse wave velocity and stiffness index; CI, confidence interval; CS, cross-sectional; CVD, cardiovascular disease; FAI, free androgen index; FT, free testosterone; FU, follow-up; IMT, intima media thickness; m, months; max, maximum; NA, non applicable; NRI, net reclassification index; NS, non-significant; OR, odds ratio; PWV, pulse wave velocity; SHBG, sex hormone binding globulin; SIindex, stiffness index; TT, total testosterone; y, years.
The possible association between endogenous androgens and markers of arterial stiffness in middle-aged women has been investigated by three cohort studies. Subramanya et al.\(^91\) investigated 1345 postmenopausal women aged 45–84 years, retrieved from the MESA (multi-ethnic study in atherosclerosis), describing evidence of cross-sectional but not longitudinal associations between free testosterone and ascending aortic distensibility (AAD), during a follow-up of 10 years. In fact, at baseline, women with free testosterone at the highest tertile \textit{versus} lowest tertiles had lower values of AAD, adjusting for risk factors (b-coefficient = −0.10, 95% CI: −0.19 to −0.01). This study\(^91\) could not demonstrate cross-sectional or longitudinal associations between free testosterone in tertiles and PWV. In an analysis of 411 consecutive middle-aged women, levels of FAI were directly associated with arterial stiffness in a study of non-causal design.\(^86\) More specifically, FAI was shown to directly predict PWV (\(\beta = 0.149, p \text{ value } = 0.014\)), stiffness index (\(\beta = 0.154, p \text{ value } = 0.022\)), combined measure of local and aortic arterial elastic properties (\(\beta = 0.193, p \text{ value } = 0.02\)). Using structural equation modelling analysis, this study also highlighted that the link between FAI and arterial stiffness is not mediated by FAI. The small cohort study by Georgiopoulos \textit{et al.}\(^84\) reported that higher values of FAI at baseline are linked with a greater risk for transition into the category of abnormal PWV during a follow-up period of 29 months, taking cardiovascular risk factors into account. The results of longitudinal analyses seem to be supportive of a direct link between higher serum levels of testosterone and arterial stiffness in middle-aged women.

The possible link between serum levels of androgens and subclinical atherosclerosis was assessed by two cohort studies. Kische \textit{et al.}\(^92\) provided cross-sectional and longitudinal associations between circulating androgens and carotid IMT as well as incident atherosclerotic plaque burden in a total of 2140 individuals aged on average 60.8 years and retrieved from the Study of Health in Pomerania. They showed a cross-sectional association between higher total testosterone and IMT, as well as a significant negative association between SHBG and incident plaque burden.\(^92\) However, they could not confirm any consistent longitudinal association between total testosterone and subclinical CVD.\(^92\) The earlier sub-analysis of the CARDIA (Coronary Artery Risk Development in Young Adults) study evaluated 1629 women who were followed up for 20 years.\(^89\) Coronary IMT measures within the highest quartile were associated inversely with levels of SHBG (Q4 levels, \(OR = 0.56\), 95% CI: 0.37–0.84, \(p \text{ value } = 0.005\)), while no associations were observed with total and free testosterone. Overall, longitudinal studies suggest that higher SHBG levels protect from the progression of carotid wall thickening, while there is no convincing evidence on the effect of total or free testosterone.

The possible interrelation between serum levels of circulating androgens and coronary artery calcium (CAC) has been investigated by three studies. The MESA study\(^90\) described a lack of cross-sectional association between prevalent CAC and serum androgen. The longitudinal analysis\(^90\) showed that the CAC-progression ratio increased in parallel with higher levels of free testosterone (2.16, 95% CI: 1.01–1.56), and the CAC-progression risk decreased with higher levels of SHBG (0.80, 95% CI: 0.64–0.99). Two sub-analyses of the CARDIA study tried to identify hormonal predictors of CAC.\(^88,89\) In a sample of 1629 women who were followed up for 20 years,\(^89\) the incidence of CAC was inversely associated with values of SHBG above rather than below the median (adjusted \(OR = 0.59\), 95% CI: 0.40–0.86, \(p \text{ value } = 0.008\)), but not with free or total testosterone. More recently, in another subanalysis of the CARDIA study,\(^88\) the incidence of CAC was found to be associated with values of the cortisol to free testosterone ratio within the highest tertile \textit{versus} the lowest tertile (\(OR = 3.45\), 95% CI: 1.18–10.06). Overall, evidence retrieved from longitudinal studies shows a protective effect of higher SHBG levels regarding the CAC progression. Evidence on the effect of testosterone levels with regards to CAC risk and progression is largely inconsistent.

### Clinical CVD

Data on cohort studies assessing the possible associations between endogenous androgens and prevalent or incident rates of CVD is presented in Table 4.
Table 4. Cohort studies exploring associations between endogenous androgens and cardiovascular events as well as all-cause mortality.

| Ref. | Study sample and design | Primary outcome | FU | Results | Discussion |
|------|-------------------------|----------------|-----|---------|------------|
| **Clinical cardiovascular disease** | | | | | |
| Di et al.⁹³ | Multi-ethnic study of atherosclerosis N = 2834 postmenopausal women CVD-free at baseline Design: L | CV risk | Median 12.1 years | Incident CV risk, per 1 SD of log-T/E2 ratio:  
- For CVD, HR 1.19 (95% CI, 1.02–1.40)  
- For CHD, HR 1.45 (95% CI, 1.19–1.78)  
- For HF, HR 1.31 (95% CI, 1.01–1.70) | [↑] Log-T/E2 ratio was associated with [↑] incidence of CVD, CHD and HF |
| Zhao et al.⁹⁴ | Atherosclerosis risk in communities (ARIC) study N = 4839 postmenopausal women, mean age 62.8 ± 5.5 years and 4107 men Design: L | HF-risk | Median 19.2 years | Incident HF events and log-transformed:  
- TT, HR 1.05 (95% CI: 0.99–1.13)  
- DHEAS, HR 1.17 (95% CI: 1.09–1.24)  
- SHBG, HR 0.93 (95% CI: 0.85–1.01) | [↑] HF incidence was associated with [↑] TT, DHEAS and [↓] SHBG |
| Wang et al.⁹⁵ | UK Biobank N = 154,965 men and N = 93,314 postmenopausal women Design: L | All-cause mortality Cancer mortality | Median 8.9 years | Levels of TT and:  
- All-cause mortality (HR for Q5 vs Q1: 1.20; 95% CI: 1.06–1.37)  
- Cancer mortality (p value 0.03) | [↑] TT was associated with [↑] All-cause- and cancer-related mortality |
| Jia et al.⁹⁶ | The atherosclerosis risk in communities study N = 8143 individuals (3,650 men and 4,493 women) with prevalent CVD, mean age of 63 years Design: L | Incident risk for HF-hospitalization or death in women DHEAS levels:  
- Change: 5.5 years | HF-hospitalization in women, DHEAS < 27.4 μg/dL (15th sex specific percentile): HR = 1.42, 95% CI: 1.13–1.79  
Risk of death, DHEAS < 37.1 μg/dL (25th sex-specific percentile): HR = 1.19, 95% CI: 1.03–1.37 | [↓] DHEAS was associated with:  
- [↑] Risk for HF-hospitalization  
- [↑] Risk of death |
| Schederecker et al.⁹⁷ | KORA F4 study N = 1086 men and N = 709 peri- and postmenopausal women from a German population-based study Design: L | All-cause or other disease-related mortality | Median 8.7 years | All-cause mortality in women,  
- SHBG, HR = 1.54, 95% CI: 1.16–2.04  
- DHT, HR = 1.32, 95% CI: 1.00–1.73  
Other disease-related mortality in women  
- SHBG, HR = 1.86, 95% CI: 1.08–3.20 | All-cause mortality was associated with [↑] SHBG and [↑] DHT. Other disease-related mortality was associated with [↑] SHBG |
| Schaffrath et al.⁹⁸ | Study of Health in Pomerania 2129 middle-aged women with a mean age of 49 years (baseline) Design: L | Incident CVD risk | Median 10.9 years | Incident CVD and baseline sex hormones (multivariable adj models)  
- TT, RR = 1.00 (95% CI: 0.96–1.06)  
- SHBG, RR = 1.02 (95% CI: 0.97–1.07)  
- Androstenedione, RR = 1.07 (95% CI: 0.93 to 1.09)  
- FT, RR = 0.99 (95% CI: 0.95–1.05)  
- FAI, RR = 0.99 (95% CI: 0.94–1.05) | Future CVD risk was not associated with baseline TT, SHBG, androstenedione, FT or FAI levels |

(Continued)
### Table 4. (Continued)

| Ref. | Study sample and design | Primary outcome | FU | Results | Discussion |
|------|-------------------------|-----------------|----|---------|------------|
| **Daka et al.** | $N = 1109$ men and women, aged at least 40 years (mean age $62 \pm 12$ years), with T2DM at baseline Design: L | MI-risk | Mean 14.1 $\pm$ 5.3 years | MI-risk per change of FT levels (by 0.01 mmol/L), multivariable adjusted: all subjects, HR 0.72, 95% CI: 0.52–1.00, $p$ value = 0.046 T2DM, HR 0.57, 95% CI: 0.26–1.31 | (↑) FT levels were associated with (↓) MI-risk in females No association between MI-risk and androgens for T2DM females |
| **Holmegard et al.** | Copenhagen City Heart Study $N = 4724$ pre and perimenopausal women, $N = 4615$ men Design: L and meta-analysis | Incident risk for ischemic stroke | Median 29 years | Ischemic stroke according to T levels T $\leq$ 10th percentile vs 11th–90th percentile, • Premenopausal: HR 0.93, 95% CI: 0.28–3.08 • Postmenopausal, HR 0.98, 95% CI: 0.72–1.33 T $> 91$st to 100th percentile vs 11–90th percentile, • Premenopausal women: HR 1.26, 95% CI: 0.48–3.27; • Postmenopausal women: HR 1.22, 95% CI: 0.90–1.66 | Incident risk for ischemic stroke was not associated with extremes of T levels |
| **Laughlin et al.** | 639 postmenopausal women (mean age 73.8 years) Design: L | Incident risk for first time CHD | Median 12.3 years | Incident first time CHD and TT, $\geq 80$ pg/mL vs $> 80$ pg/mL, HR 1.62, 95% CI: 1.10–2.39 Incident CHD and bioavailable T, • 1st vs 3rd quintile: HR = 1.79, 95% CI: 1.01–3.16 • 5th vs 3rd quintile: HR = 1.96, 95% CI: 1.13–3.16 | Incident risk for first time CHD was associated with extreme levels of bioavailable T (U-shaped association) |
| **Wehr et al.** | $N = 875$ postmenopausal diabetic women Design: L | Risk for all-cause mortality and CV-mortality | Median 7.7 years | Mortality per FT Q4 vs Q1, multivariable adjusted: • All-cause mortality, HR = 0.38, 95% CI: 0.08–0.90, $p$ value = 0.025; • CV-mortality, HR = 0.28, 95% CI: 0.08–0.90, $p$ value = 0.032 | (↑) FT was associated with (↑) all-cause and CV-mortality |
| **Benn et al.** | Copenhagen City Heart Study Nested study ($N = 4,716$ women) not on HRT or OC Design: L | Risk for IHD or any death | Max. $\leq 30$ y | T $> 95$th percentile vs 10th–89th percentile (multifactorial adjusted) • IHD, +88%, 95% CI: 34%–210% • Any death, +36%, 95% CI: 18%–58% | (↑) T was associated with (↑) risk for IHD or risk for death of any cause |

CHD, coronary heart disease; CV, cardiovascular; CVD, cardiovascular disease; DHEAS, dehydroepiandrosterone; DHT, dihydrotestosterone; FT, free testosterone; FU, follow-up; HF, heart failure; HR, hazard ratio; HRT, hormone replacement therapy; IHD, ischemic heart disease; MI, myocardial infarction; OC, oral contraceptives; RR, relative risk; SD, standard deviation; T, testosterone; T/E2, testosterone to oestrogen ratio; TT, total testosterone.
Five of the available cohort studies support a direct association between a more androgenic profile and cardiovascular and/or cerebrovascular events. The possible link between serum levels of androgens and clinical CVD has been investigated in 2834 postmenopausal women from the MESA. This study showed that the risk for future events associated with more pronounced androgenicity, reflected by the testosterone to oestrogen ratio as estimated at 12 years of follow-up (incident CVD, HR = 1.19, 95% CI: 1.02–1.40; incident CHD, HR = 1.45, 95% CI: 1.19–1.78; incident HF, HR = 1.31, 95% CI: 1.01–1.70). Similar results were presented by the Atherosclerosis Risk in Communities study (ARIC), which showed that the risk for incident HF was associated with higher levels of DHEAS (HR = 1.40; incident CHD, HR = 1.01–1.70). Similar results were presented by the ARIC study demonstrated that the possible link between DHEAS and risk for hospitalizations due to heart failure or the risk of death was evaluated in a total of 8143 individuals with prevalent CVD. The incident risk for hospitalization due to HF was higher in women with DHEAS < 15th sex-specific percentile (HR = 1.42, 95% CI: 1.13–1.17). Similarly, the risk of death was higher for women with levels of DHEAS < 25th sex-specific percentile (HR = 1.19, 95% CI: 1.03–1.37). Investigating a sample of 1109 men and women aged at least 40 years, one more study reported a borderline significant association between low androgenicity and increasing risk of acute myocardial infarction, at least in women with type 2 diabetes, adjusting for cardiovascular risk factors. Similarly, a study of 875 postmenopausal women followed up for up to 7.7 years reported an inverse association between high free testosterone and low cardiovascular and all-cause mortality in postmenopausal women diagnosed with diabetes mellitus.

Different results were presented in three more cohort studies. Interestingly, an earlier study described a U-shaped association between levels of testosterone and coronary heart disease in a sample of 639 postmenopausal women, followed up for up to 12.3 years. In conclusion, data retrieved from cohort studies seems to be supportive of a direct link between more pronounced androgenicity and the risk for clinically evident CVD. Finally, two cohort studies of 2129 middle-aged women and 4724 pre- and perimenopausal women could not find longitudinal associations between circulating androgens and clinically evident CVD.

**Exogenous androgens and CVD**

 Trials of testosterone replacement in middle-aged women

Data retrieved from trials of testosterone replacement in middle-aged women are presented in Table 5. In an early trial, administration of a testosterone implant of 50 mg for a total of 6 weeks in postmenopausal women already on HRT described that testosterone administration resulted in improvement of endothelium-dependent and independent vasodilation (increase in FMD, 6.4% ± 0.7% to 9.1% ± 1.1, p value = 0.003; GTN induced, 14.9% ± 0.9 to 17.8 ± 1.2,
| Ref.         | Study sample and Design                                                                 | Treatment                                                                 | Primary outcome     | FU    | Results                                                                 | Discussion                                                                 |
|-------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------|-------|-------------------------------------------------------------------------|----------------------------------------------------------------------------|
| **Testosterone treatment**                                                                                                                                           |                                                                          |                     |       |                                                                         |                                                                            |
| Worboys et al. 106 | postmenopausal women, Cases (N = 33) HRT-users (>6 months) who also received T-treatment; Controls (N = 15) not on HRT | E2 ± progestin, Exogenous T implant 50 mg                                  | FMD GTN-induced vasodilation | 6 weeks | Change in FMD from baseline:                                         | T-treatment was associated with ↑ endothelium-dependent and independent vasodilation |
| Hak et al. 107   | Population-based study (N = 513) naturally postmenopausal women aged 54–67y; Cases: hormone therapy users, Controls: never-users of hormone therapy | IM 2–5 mg estradiol esters, IM 50–100 mg testosterone esters               | Aortic atherosclerosis | Min. 1 y | Cases vs controls, adjusted for CV risk factors                        | Hormone therapy use was associated with ↑ risk for aortic atherosclerosis |
| Penotti et al. 108 | Healthy postmenopausal women; Cases: sequential HRT-users (N = 20), sequential HRT-users randomized to T-treatment (N = 20); Controls: non-users of HRT (N = 20) | transdermal E₂ 50 µg/d + MPA 10 mg/d for 12 days every other month Testosterone undecanoate 40 mg/day | PI index of middle cerebral artery | 8 m    | Only HRT treatment, no significant difference between baseline vs 4 months vs 8 months Combined HRT + T treatment, at 8 months vs baseline, 0.822 (95% CI: 0.740–1.065) vs 0.780 (95% CI: 0.695–0.955), p value < 0.001 at 8 months vs HRT-only, 0.822 (95% CI: 0.740 to 1.065) vs 0.777 (95% CI: 0.700–0.965), p value < 0.05 | HRT + T-treatment was associated with a significant increase in the PI of the middle cerebral artery |
| Williams et al. 69 | Healthy postmenopausal women vs placebo; Cases: DHEA-treatment (N = 18); Controls: placebo treatment (N = 18) | DHEA 100 mg/day or placebo                                                 | FMD GTN-induced vasodilation | 3 m    | FMD:                                                                   | DHEAS treatment vs placebo was associated with ↑ FMD but had no effect on GTN induced vasodilation |

CI, confidence interval; CV, cardiovascular; DHEA(S), dehydroepiandrosterone (sulphate); E + T, oestrogen and testosterone; FMD, flow-mediated dilation; FU, follow-up; GTN, glyceryl trinitrate; HRT, hormone replacement therapy; IM, intramuscular; m, months; MPA, medoxyprogesterone acetate; NS, not significant; OR, odds ratio; PI, pulsatility index; T, testosterone.
\( p \text{ value } = 0.03 \). One more trial\(^{107} \) estimated the impact of intramuscular hormone replacement with 2–5 mg of estradiol esters and 50–100 mg testosterone esters in postmenopausal women compared to never users of HRT. The study\(^{107} \) concluded that combined estradiol and testosterone treatment for at least 1 year was associated with a significantly higher risk of severe aortic calcification (OR = 3.1, 95% CI: 1.1–8.5). Penotti \textit{et al.}\(^{108} \) described the effect on arterial resistance of treatment with testosterone undecanoate 40 mg/day in combination with sequential HRT for 8 months as opposed to HRT only in a small sample of postmenopausal women. The combined oestrogen and testosterone administration resulted in an increase in the pulsatility index of the middle cerebral artery as opposed to baseline. The effect of the oestrogen-testosterone combination was also significantly higher than the equivalent pulsatility index estimated in HRT-only users. One more intervention trial\(^{109} \) investigated the possible effect of DHEA administration of 100 mg per day in 36 healthy postmenopausal women compared to placebo and concluded that DHEA treatment was related to significant improvement in FMD values (intervention group, 8.4% ± 0.7% to 14.5% ± 1.1, \( p \text{ value } < 0.05 \); placebo, 10.8% ± 1.1% to 10.9% ± 0.6, \( p \text{ value } = \text{NS} \)), but had no effect on the GTN-induced vasodilation.

\textbf{Data on transgender people}

Insight into the possible cardiovascular implications of testosterone treatment can be gained from studies on female-to-male transgender. The behavioural risk factor surveillance system (BRFSS, 2014–2017)\(^{109} \) evaluated the risk of myocardial infarction in a large sample of transgender men and women as well as cisgender women (transgender men and cisgender women 410,828; transgender women 1788, cisgender men 306,046). This analysis\(^{109} \) showed that female-to-male transgenders had higher rates of myocardial infarction compared with cisgender men (OR 2.53, 95% CI: 1.14–5.63, \( p \text{ value } = 0.02 \)) and also compared with cisgender women (OR 4.9, 95% CI: 2.21–10.90, \( p \text{ value } < 0.01 \)). Additional data retrieved from the BRFSS analysis\(^{110} \) showed that the elevated crude risk for myocardial infarction observed in transgender men as opposed to cisgender women is largely affected by confounders and highlighted differences in health behaviours between female-to-male transgenders and cisgender women. Transgenders are more frequently overweight (female-to-male vs cisgender women, adjusted odds ratio 1.54, 95% CI: 1.07–2.24), smokers (female-to-male vs cisgender women, OR 1.64, 95% CI: 1.17–2.31), with likely lower educational attainment (combined rates, 30.5% high school graduates; 13.3% never graduated from high-school) and likely to face challenges on employment (5.8% unemployed and 5.8% not able to work).\(^{110} \) A recent study evaluated the incidence of cardiovascular events in a large transgender cohort,\(^{111} \) consisting of 2517 male-to-female and 1358 female-to-male (median age of 30 years and 23 years, respectively). Results in male-to-female transgender showed that the standardized incident ratios (SIR) for the development of acute cardiovascular events were higher for stroke, myocardial infarction, and venous thromboembolism (VTE) as opposed to the equivalent risk for cis women (standardized incidence ratio, stroke: 2.42, 95% CI: 1.65–3.42; MI, 2.64, 95% CI: 1.81–3.72; VTE, 5.52, 95% CI: 4.36–6.90).\(^{111} \) A recent observational analysis of 114 transgender and 964 cisgender individuals\(^{112} \) described a higher risk of VTE in male-to-female transgenders compared to cis women (adjusted OR 3.94, 95% CI: 1.24–12.51), but comparable rates of any CVD condition in transgender individuals vs cis women or cis men. A prospective cohort study assessed 2842 male-to-female transgenders (followed up for 4 years) and 2118 female-to-male transgenders (followed up for 3.6 years) and compared the cohort with a matched group of 48,686 cisgender men and 48,775 cisgender women.\(^{113} \) The results showed\(^{113} \) that the rates of VTE, ischemic stroke or myocardial infarction did not differ between the female-to-male transgenders and the cisgender women (adjusted HR 1.1, 95% CI: 0.6–2.1; 1.3, 95% CI: 0.7–2.5; 1.3, 95% CI: 0.5–3.9; for VTE, stroke and MI, respectively). An earlier meta-analysis of 16 studies, including 1471 male-to-female and 652 female-to-male individuals, showed a low incidence of cardiovascular events in the female-to-male transgender group (death, two cases; myocardial infarction, two cases; VTE, two cases; stroke, no case).\(^{114} \)

Data on the cardiovascular implications of female-to-male transgenders remains limited, and a detailed review of studies on the topic is outside the scope of this study, mainly due to the methodological differences between women on gender-affirming hormone treatment and the peri- or postmenopausal population. The majority of
studies on female-to-male transgenders were observational and usually consisted of a small sample size of younger individuals, while the effect of health behaviour possibly modifying the overall cardiovascular risk was not always accounted for. A recent meta-analysis consisting of 29 studies with moderate risk of bias reported that sex hormone treatment in transgender men is usually accompanied by higher rates of dyslipidaemia (at ≥24 months, mean difference, low-density lipoprotein (LDL) cholesterol, +17.8 mg/dL; 95% CI: 3.5–32.1; serum triglycerides, +21.4 mg/dL, 95% CI: 0.14–42.6). These observations were also supported by a systematic review of 13 studies, which described the metabolic effects of parenteral testosterone treatment (undecanoate 1000 mg per 12 weeks) for up to 60 months in transgender men. The same study reported that transgender men on testosterone treatment have consistently higher levels of LDL-cholesterol and also increased body mass index (from 1.3% to 11.4%). In any case, data on the effect of testosterone treatment in transgender individuals should not be considered directly comparable with the effect of lower-dose testosterone replacement in middle-aged women, considering the difference in age, health behavior, and overall metabolic burden.

Conclusion
Data on the possible effect of testosterone on cardiovascular cells and tissues remain contradictory. Preclinical studies highlight a possibly beneficial effect on vascular cell lines, which fails to be translated into an actual clinical benefit. The results of a recent study on cardiomyocytes and human umbilical vein endothelial cells have revealed that the optimal oestrogen-to-testosterone ratio is ideal, as sex hormones at this level have been found to reduce apoptosis of cardiac myocytes and improve the overall metabolism. Even though the idea of an ‘optimal’ oestrogen-to-testosterone ratio sounds promising, this balance is extremely difficult to be achieved in clinical praxis due to the existence of other counterregulatory factors, which are not present at the preclinical setting. In this context, factors like weight gain and adipose tissue, metabolic syndrome or even insulin resistance and diabetes mellitus are pathophysio logically interrelated with the oestrogen-to-testosterone ratio and are, therefore, likely to modify the effect of sex hormones on the cardiovascular system. Evidence on the effect of testosterone treatment remains insufficient to draw firm conclusions. In any case, cardiovascular risk stratification should be incorporated in the management of postmenopausal women, especially when indications for testosterone treatment are present, to minimize the possible future cardiovascular risk.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions

Eleni Armeni: Conceptualization; Data curation; Investigation; Methodology; Visualization; Writing – original draft.

Irene Lambrinoudaki: Conceptualization; Project administration; Validation; Visualization; Writing – review & editing.
Acknowledgements
None.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials
Not applicable.

ORCID iD
Irene Lambrinoudaki https://orcid.org/0000-0003-1488-2668

References
1. Benjamin EJ, Muntner P, Alonso A, et al. Heart disease and stroke statistics-2019 update: a report from the American Heart Association. Circulation 2019; 139: e56–e528.
2. Virani SS, Alonso A, Aparicio HJ, et al. Heart disease and stroke statistics-2021 update: a report from the American Heart Association. Circulation 2021; 143: e254–e743.
3. Walli-Attaei M, Joseph P, Rosengren A, et al. Variations between women and men in risk factors, treatments, cardiovascular disease incidence, and death in 27 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. Lancet 2020; 396: 97–109.
4. Kannel WB, Hjortland MC, McNamara PM, et al. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 1976; 85: 447–452.
5. El Khoudary SR, Aggarwal B, Beckie TM, et al. Menopause transition and cardiovascular disease risk: implications for timing of early prevention: a scientific statement from the American Heart Association. Circulation 2020; 142: e506–e532.
6. Zhu D, Chung H-F, Dobson AJ, et al. Type of menopause, age of menopause and variations in the risk of incident cardiovascular disease: pooled analysis of individual data from 10 international studies. Hum Reprod 2020; 35: 1933–1943.
7. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: a systematic review and meta-analysis. JAMA Cardiol 2016; 1: 767–776.
8. Connelly PJ, Azizi Z, Alipour P, et al. The importance of gender to understand sex differences in cardiovascular disease. Can J Cardiol 2021; 37: 699–710.
9. Karavidas A, Lazaros G, Tsiachris D, et al. Aging and the cardiovascular system. Hellenic J Cardiol 2010; 51: 421–427.
10. McEniery CM, Wilkinson IB and Avolio AP. Age, hypertension and arterial function. Clin Exp Pharmacol Physiol 2007; 34: 665–671.
11. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018; 13: 757–772.
12. Cosentino F, Francia P, Camici GG, et al. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. Arterioscler Thromb Vasc Biol 2008; 28: 622–628.
13. Papaconstantinou J. The role of signaling pathways of inflammation and oxidative stress in development of senescence and aging phenotypes in cardiovascular disease. Cells 2019; 8: 1383.
14. Kaasik A, Kuum M, Joubert F, et al. Mitochondria as a source of mechanical signals in cardiomyocytes. Cardiovasc Res 2010; 87: 83–91.
15. North BJ and Sinclair DA. The intersection between aging and cardiovascular disease. Circ Res 2012; 110: 1097–1108.
16. Izzo C, Carrizzo A, Alfano A, et al. The impact of aging on cardio and cerebrovascular diseases. Int J Mol Sci 2018; 19: 481.
17. Paneni F, Diaz Cañestro C, Libby P, et al. The aging cardiovascular system: understanding it at the cellular and clinical levels. J Am Coll Cardiol 2017; 69: 1952–1967.
18. Pourbagher-Shahri AM, Farkhondeh T, Talebi M, et al. An overview of NO signaling pathways in aging. Molecules 2021; 26: 4533.
19. Davis SR, Lambrinoudaki I, Lumsden M, et al. Menopause. Nat Rev Dis Prim 2015; 1: 15004.
20. Burger HG. Androgen production in women. Fertil Steril 2002; 77(Suppl. 4): S3–S5.
21. Monteleone P, Mascagni G, Giannini A, et al. Symptoms of menopause – global prevalence, physiology and implications. Nat Rev Endocrinol 2018; 14: 199–215.
22. Lambrinoudaki I, Paschou SA, Lumsden MA, et al. Premature ovarian insufficiency: a toolkit for the primary care physician. Maturitas 2021; 147: 53–63.
23. Hammond GL. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. *J Endocrinol* 2016; 230: R13–R25.

24. Laurent MR, Helsen C, Antonio L, et al. Effects of sex hormone-binding globulin (SHBG) on androgen bioactivity in vitro. *Mol Cell Endocrinol* 2016; 437: 280–291.

25. Sutton-Tyrrell K, Zhao X, Santoro N, et al. Reproductive hormones and obesity: 9 years of observation from the Study of Women’s Health Across the Nation. *Am J Epidemiol* 2010; 171: 1203–1213.

26. Bucciarelli P and Mannucci PM. The hemostatic system through aging and menopause. *Climacteric* 2009; 12(Suppl. 1): 47–51.

27. Barbieri RL, Smith S and Ryan KJ. The role of inflammatory and oxidative stress: the Framingham Heart Study. *Circulation* 2007; 116; 1203–1213.

28. Donato AJ, Morgan RG, Walker AE, et al. Cellular and molecular biology of aging endothelial cells. *J Mol Cell Cardiol* 2015; 89(Pt. B): 122–135.

29. Arapa-Diaz JC, Rouver WDN, Giesen JAS, et al. Endothelial dysfunction and atherosclerosis: focus on novel therapeutic approaches. *Recent Pat Cardiovasc Drug Discov* 2012; 7: 21–32.

30. Takov K, Wu J, Denvir MA, et al. The role of androgen receptors in atherosclerosis. *Mol Cell Endocrinol* 2018; 465: 82–91.

31. Kim CJ. Oxidative and cellular stress markers in women at midlife: the Study of Women’s Health Across the Nation. *Atherosclerosis* 2013; 231: 54–58.

32. Wang NC, Matthews KA, Barinas-Mitchell EJ, et al. Inflammatory/hemostatic biomarkers and coronary artery calcification in midlife women of African-American and White race/ethnicity: the Study of Women’s Health Across the Nation (SWAN) heart study. *Menopause* 2016; 23: 653–661.

33. Schmiegelow MD, Hedlin H, Mackey RH, et al. Race and ethnicity, obesity, metabolic health, and risk of cardiovascular disease in postmenopausal women. *J Am Heart Assoc* 2015; 4: e001695.

34. Pou KM, Massaro JM, Hoffmann U, et al. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. *Circulation* 2007; 116: 1234–1241.

35. Incalza MA, D’Oria R, Natalicchio A, et al. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul Pharmacol* 2018; 100: 1–19.

36. Staniewicz AE, Wenner MM and Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol* 2018; 315: H1569–H1588.

37. E Armeni and I Lambrinoudaki.
5beta-dihydrotestosterone restricted to L-type Ca2+ channel blockade. Endocrinology 2008; 149: 2517–2526.

48. Chignalia AZ, Schuldt EZ, Camargo LL, et al. Testosterone induces vascular smooth muscle cell migration by NADPH oxidase and c-Src-dependent pathways. Hypertension 2012; 59: 1263–1271

49. Lopes RAM, Neves KB, Pestana CR, et al. Testosterone induces apoptosis in vascular smooth muscle cells via extrinsic apoptotic pathway with mitochondria-generated reactive oxygen species involvement. Am J Physiol Heart Circ Physiol 2014; 306: H1485–H1494.

50. Zhu D, Hadoke PWF, Wu J, et al. Ablation of the androgen receptor from vascular smooth muscle cells demonstrates a role for testosterone in vascular calcification. Sci Rep 2016; 6: 24807.

51. Son B-K, Akishita M, Iijima K, et al. Androgen receptor-dependent transactivation of growth arrest-specific gene 6 mediates inhibitory effects of testosterone on vascular calcification. J Biol Chem 2010; 285: 7537–7544.

52. Xiao F-Y, Nheu L, Komesaroff P, et al. Testosterone protects cardiac myocytes from superoxide injury via NF-kB signalling pathways. Life Sci 2015; 133: 45–52.

53. Vicencio JM, Ibarra C, Estrada M, et al. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. Endocrinology 2006; 147: 1386–1395.

54. Akdis D, Saguner AM, Shah K, et al. Sex hormones affect outcome in arrhythmogenic right ventricular cardiomyopathy/dysplasia: from a stem cell derived cardiomyocyte-based model to clinical biomarkers of disease outcome. Eur Heart J 2017; 38: 1498–1508.

55. Zhang L, Wu S, Ruan Y, et al. Testosterone suppresses oxidative stress via androgen receptor-independent pathway in murine cardiomyocytes. Mol Med Rep 2011; 4: 1183–1188.

56. Costa TJ, Ceravolo GS, dos Santos RA, et al. Association of testosterone with estrogen abolishes the beneficial effects of estrogen treatment by increasing ROS generation in aorta endothelial cells. Am J Physiol Hear Circ Physiol 2015; 308: H723–H732.

57. Costa TJ, Ceravolo GS, Echem C, et al. Detrimental effects of testosterone addition to estrogen therapy involve cytochrome P-450-induced 20-HETE synthesis in aorta of ovarietomized spontaneously hypertensive rat (SHR), a model of postmenopausal hypertension. Front Physiol 2018; 9: 490.

58. Xu S, Dai W, Li J, et al. Synergistic effect of estradiol and testosterone protects against IL-6-induced cardiomyocyte apoptosis mediated by TGF-β1. Int J Clin Exp Pathol 2018; 11: 10–26.

59. Dai W, Ming W, Li Y, et al. Synergistic effect of a physiological ratio of estradiol and testosterone in the treatment of early-stage atherosclerosis. Arch Med Res 2015; 46: 619–629.

60. Cruz-Topete D, Dominic P and Stokes KY. Uncovering sex-specific mechanisms of action of testosterone and redox balance. Redox Biology 2020; 31: 101490.

61. Gündoğan GI, Keg C, Karacan M, et al. Investigation of physiological effects induced by dehydroepiandrosterone in human endothelial cells and ovarian cancer cell line. Turkish J Pharm Sci 2021; 18: 185–191.

62. Altman R, Motton DD, Kota RS, et al. Inhibition of vascular inflammation by dehydroepiandrosterone sulfate in human aortic endothelial cells: roles of PPARalpha and NF-kappaB. Vascular Pharmacol 2008; 48: 76–84.

63. Curatola AM, Huang K and Naftolin F. Dehydroepiandrosterone (DHEA) inhibition of monocyte binding by vascular endothelium is associated with sialylation of neural cell adhesion molecule. Reprod Sci 2012; 19: 86–91.

64. Liu D, Si H, Reynolds KA, et al. Dehydroepiandrosterone protects vascular endothelial cells against apoptosis through a Gαi2α protein-dependent activation of phosphatidylinositol 3-kinase/Akt and regulation of antiapoptotic Bcl-2 expression. Endocrinology 2007; 148: 3068–3076.

65. Huerta-García E, Montiel-Dávalos A, Alfaro-Moreno E, et al. Dehydroepiandrosterone protects endothelial cells against inflammatory events induced by urban particulate matter and titanium dioxide nanoparticles. Biomed Res Int 2013; 2013: 382058.

66. Huerta-García E, Ventura-Gallegos JL, Victoriano ME, et al. Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. Endocrinology 2018; 149: 889–898.

67. Liu D, Iruthayanathan M, Homan LL, et al. Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. Endocrinology 2018; 149: 889–898.

68. Wang L, Hao Q, Wang YD, et al. Protective effects of dehydroepiandrosterone on atherosclerosis in ovarietomized rabbits via...
alleviating inflammatory injury in endothelial cells. *Atherosclerosis* 2011; 214: 47–57.

69. Williams MR, Dawood T, Ling S, et al. Dehydroepiandrosterone increases endothelial cell proliferation in vitro and improves endothelial function in vivo by mechanisms independent of androgen and estrogen receptors. *J Clin Endocrinol Metab* 2004; 89: 4708–4715.

70. Ochi R, Chettimada S, Kizub I, et al. Dehydroepiandrosterone inhibits I(Ca, L) and its window current in voltage-dependent and -independent mechanisms in arterial smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2018; 315: H1602–H1613.

71. Chen J, Xu L and Huang C. DHEA inhibits vascular remodeling following arterial injury: a possible role in suppression of inflammation and oxidative stress derived from normal smooth muscle cells. *Mol Cell Biochem* 2014; 388: 75–84.

72. Alzoubi A, Toba M, Abe K, et al. Dehydroepiandrosterone restores right ventricular structure and function in rats with severe pulmonary arterial hypertension. *Am J Physiol Heart Circ Physiol* 2013; 304: H1708–H1718.

73. Mannic T, Mouffok M, Python M, et al. DHEA prevents mineralo- and glucocorticoid receptor-induced chronotropic and hypertrophic actions in isolated rat cardiomyocytes. *Endocrinology* 2013; 154: 1271–1281.

74. Nakamura S, Yoshimura M, Nakayama M, et al. Possible association of heart failure status with synthetic balance between aldosterone and dehydroepiandrosterone in human heart. *Circulation* 2004; 110: 1787–1793.

75. Boese AC, Kim SC, Yin K-J, et al. Sex differences in vascular physiology and pathophysiology: estrogen and androgen signaling in health and disease. *Am J Physiol Heart Circ Physiol* 2017; 313: H524–H545.
90. Subramanya V, Zhao D, Ouyang P, et al. Association of endogenous sex hormone levels with coronary artery calcium progression among post-menopausal women in the Multi-Ethnic Study of Atherosclerosis (MESA). *J Cardiovasc Comput Tomogr* 2019; 13: 41–47.

91. Subramanya V, Zhao D, Ouyang P, et al. Sex hormone levels and change in left ventricular structure among men and postmenopausal women: the Multi-Ethnic Study of Atherosclerosis (MESA). *Maturitas* 2018; 108: 37–44.

92. Kischke H, Gross S, Wallaschofski H, et al. Clinical correlates of sex hormones in women: the study of health in Pomerania. *Metabolism* 2016; 65: 1286–1296.

93. Di Z, Eliseo G, Pamela O, et al. Endogenous sex hormones and incident cardiovascular disease in post-menopausal women. *J Am Coll Cardiol* 2018; 71: 2555–2566.

94. Zhao D, Guallar E, Ballantyne CM, et al. Sex hormones and incident heart failure in men and postmenopausal women: the atherosclerosis risk in communities study. *J Clin Endocrinol Metab* 2020; 105: e3798–e3807.

95. Wang J, Fan X, Yang M, et al. Sex-specific associations of circulating testosterone levels with all-cause and cause-specific mortality. *Eur J Endocrinol* 2021; 184: 723–732.

96. Jia X, Sun C, Tang O, et al. Plasma dehydroepiandrosterone sulfate and cardiovascular disease risk in older men and women. *J Clin Endocrinol Metab* 2020; 105: e4304–e4327.

97. Scherederck F, Cecil A, Prehn C, et al. Sex hormone-binding globulin, androgens and mortality: the KORA-F4 cohort study. *Endocr Connect* 2020; 9: 326–336.

98. Schaffrath G, Kischke H, Gross S, et al. Association of sex hormones with incident 10-year cardiovascular disease and mortality in women. *Maturitas* 2015; 82: 424–430.

99. Daka B, Langer RD, Larsson CA, et al. Low concentrations of serum testosterone predict acute myocardial infarction in men with type 2 diabetes mellitus. *BMC Endocr Disord* 2015; 15: 35.

100. Holmegard HN, Nordestgaard BG, Jensen GB, et al. Sex hormones and ischemic stroke: a prospective cohort study and meta-analyses. *J Clin Endocrinol Metab* 2016; 101: 69–78.

101. Laughlin GA, Goodell V and Barrett-Connor E. Extremes of endogenous testosterone are associated with increased risk of incident coronary events in older women. *J Clin Endocrinol Metab* 2010; 95: 740–747.

102. Wehr E, Pilz S, Boehm BO, et al. Low free testosterone levels are associated with all-cause and cardiovascular mortality in postmenopausal diabetic women. *Diabetes Care* 2011; 34: 1771–1777.

103. Benn M, Voss SS, Holmegard HN, et al. Extreme concentrations of endogenous sex hormones, ischemic heart disease, and death in women. *Arterioscler Thromb Vasc Biol* 2015; 35: 471–477.

104. Wallace IR, McKinley MC, Bell PM, et al. Sex hormone binding globulin and insulin resistance. *Clin Endocrinol* 2013; 78: 321–329.

105. Laurent MR, Hammond GL, Blokland M, et al. Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis. *Sci Rep* 2016; 6: 35539.

106. Worboys S, Kotsopoulos D, Teede H, et al. Evidence that parenteral testosterone therapy may improve endothelium-dependent and -independent vasodilation in postmenopausal women already receiving estrogen. *J Clin Endocrinol Metab* 2001; 86: 158–161.

107. Hak AE, Westendorp ICD, Pols HAP, et al. High-dose testosterone is associated with atherosclerosis in postmenopausal women. *Maturitas* 2007; 56: 153–160.

108. 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* 2001; 76: 235–240.

109. Alzahrani T, Nguyen T, Ryan A, et al. Cardiovascular disease risk factors and myocardial infarction in the transgender population. *Circ Cardiovasc Qual Outcomes* 2019; 12: e005597.

110. Caceres BA, Jackman KB, Edmondson D, et al. Assessing gender identity differences in cardiovascular disease in US adults: an analysis of data from the 2014-2017 BRFSS. *J Behav Med* 2020; 43: 329–338.

111. Nota NM, Wiepjes CM, de Blok CJM, et al. Occurrence of acute cardiovascular events in transgender individuals receiving hormone therapy. *Circulation* 2019; 139: 1461–1462.

112. Potat TC, Divsalar S, Streed CGJ, et al. Cardiovascular disease in a population-based sample of transgender and cisgender adults. *Am J Prev Med* 2021; 61: 804–811.
113. Getahun D, Nash R, Flanders WD, et al. Cross-sex hormones and acute cardiovascular events in transgender persons: a cohort study. *Ann Intern Med* 2018; 169: 205–213.

114. Elamin MB, Garcia MZ, Murad MH, et al. Effect of sex steroid use on cardiovascular risk in transsexual individuals: a systematic review and meta-analyses. *Clin Endocrinol* 2010; 72: 1–10.

115. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, et al. Sex steroids and cardiovascular outcomes in transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2017; 102: 3914–3923.

116. Velho I, Fighera TM, Ziegelmann PK, et al. Effects of testosterone therapy on BMI, blood pressure, and laboratory profile of transgender men: a systematic review. *Andrology* 2017; 5: 881–888.

117. Capellino S, Straub RH and Cutolo M. Aromatase and regulation of the estrogen-to-androgen ratio in synovial tissue inflammation: common pathway in both sexes. *Ann N Y Acad Sci* 2014; 1317: 24–31.