Am J Physiol Heart Circ Physiol 325: H30–H53, 2023.
First published May 5, 2023; doi:10.1152/ajpheart.00015.2023

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
HEART AND CIRCULATORY PHYSIOLOGY

REVIEW

Translational Physiology

The cardiovascular subtleties of testosterone on gender-affirming hormone therapy

Jeimison D. Santos, José T. Oliveira-Neto, and Rita C. Tostes
Department of Pharmacology, Ribeirao Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Abstract

The growing number of people who identify themselves as transgender has gained increased attention in recent years and will certainly impact personalized clinical practices and healthcare worldwide. Transgender and gender-nonconforming individuals frequently undergo gender-affirming hormone therapy (GAHT), i.e., they use sex hormones to align their gender identity with their biological characteristics. Testosterone is the main compound used in GAHT by transmasculine people, leading to the development of male secondary sexual characteristics in these individuals. However, sex hormones, testosterone included, also influence hemodynamic homeostasis, blood pressure, and cardiovascular performance by direct effects in the heart and blood vessels, and by modulating several mechanisms that control cardiovascular function. In pathological conditions and when used in supraphysiological concentrations, testosterone is associated with harmful cardiovascular effects, requiring close attention in its clinical use. The present review summarizes current knowledge on the cardiovascular impact of testosterone in biological females, focusing on aspects of testosterone use by transmasculine people (clinical goals, pharmaceutical formulations, and impact on the cardiovascular system). Potential mechanisms whereby testosterone may increase cardiovascular risk in these individuals are discussed, and the influence of testosterone on the main mechanisms that control blood pressure and that potentially lead to hypertension development and target-organ damage are also reviewed. In addition, current experimental models, which are key to reveal testosterone mechanistic aspects and potential markers of cardiovascular injury, are reviewed. Finally, research limitations and the lack of data on cardiovascular health of transmasculine individuals are considered, and future directions for more appropriate clinical practices are highlighted.

cardiovascular hypertrophy; hypertension; immune system; oxidative stress; PVAT

SEX HORMONES AND THE CARDIOVASCULAR SYSTEM: A VERY BRIEF OVERVIEW

In addition to their fundamental role in the reproductive system and development of primary and secondary sexual characteristics, sex hormones influence practically all body tissues and systems, including the integumentary, skeletal, muscular, lymphatic, respiratory, digestive, nervous, endocrine, urinary, and cardiovascular systems. Moreover, sex hormones impact behavioral, anatomical, physiological, cellular, and molecular processes, very often determining functional and structural differences between males and females, as commonly observed in vertebrate species (1, 2).

In females, the most abundant and active sex hormones are estrogen and progesterone, whereas males predominantly produce androgenic hormones such as testosterone and dihydrotestosterone (DHT). Sex hormones are enzymatically synthesized from cholesterol, which is converted to pregnenolone that, in turn, is converted to progesterone or testosterone. Testosterone is metabolized by 5α-reductase, generating DHT, or converted to estrogens via the enzymatic activity of aromatase. Sex hormones are mainly produced in the gonads, but are also produced in extragonadal peripheral tissues including the adrenal cortex (3), brain (4), skin (5), adipose tissue (6), skeletal muscle (7), kidney (8), and vascular cells (9).

Endogenous secretion of sex hormones is regulated through the action of the hypothalamic gonadotropin-releasing hormone (GnHR), which, in turn, stimulates the release of follicle-stimulating (FSH) and luteinizing (LH) pituitary hormones. In the female reproductive system, FSH stimulates the maturation of ovarian follicles, which produce and secrete estrogen, and LH stimulates ovulation and the release of progesterone by the corpus luteum. In the male reproductive system, LH stimulates the production and release of androgenic hormones, in particular testosterone, by the testicular Leydig cells (10).

Sex hormones exert their biological functions through interaction with their respective receptors. Estrogens activate estrogen receptors (ERs), ERα, ERβ, and also a G protein-coupled estrogen receptor (GPER), whereas testosterone

Correspondence: J. D. Santos (jeimisonds@alumni.usp.br); R. C. Tostes (rtostes@usp.br).
Submitted 10 January 2023 / Revised 13 April 2023 / Accepted 24 April 2023

and DHT activate androgen receptors (ARs). At the cellular level, these receptors are located in the cytosol and plasma membrane, triggering genomic and nongenomic effects, respectively. The nongenomic effects occur faster than genomic-induced mechanisms, do not rely on RNA or protein synthesis, and are usually related to the activation of several intracellular pathways such as increased intracellular calcium (Ca^{2+}), nitric oxide (NO) synthesis, and activation of protein kinase A (PKA), protein kinase C (PKC), and mitogen-activated kinases (MAPK). Receptors for sex hormones are found in both sexes and are present in several cell types and tissues, including cells of the cardiovascular system (11, 12).

In the cardiovascular system, sex-related factors influence hemodynamic homeostasis and blood pressure. Boynton and Todd (13) originally observed that blood pressure is higher in cisgender men than in cisgender women,[1] with many subsequent experimental and clinical studies later showing that sex hormones are major determinants of sex differences in blood pressure in physiological and pathological conditions (14). Sex differences have been reported in not only the hypertension incidence but also the prevalence of hypertension-associated target-organ damage (15).

Female sex hormones, mainly 17β-estradiol, exhibit cardioprotective effects (16). Blood pressure is lower in cisgender women than in cisgender men (17, 18), and arterial blood pressure is increased in postmenopausal cisgender women (19). In experimental models of hypertension, blood pressure is consistently lower in females than in males (20) and ovariectomy (OVX) increases blood pressure values, whereas estrogen treatment reduces blood pressure in females with hypertension (21, 22). In contrast to female sex hormones, androgens are linked to prohypertensive effects. Treatment of rats with DHT increases blood pressure and vascular inflammatory events, leading to endothelial dysfunction (23). In addition, males have higher blood pressure levels compared with females in many experimental models of hypertension. Castration of males with hypertension reduces blood pressure, whereas testosterone treatment increases blood pressure values (20, 24). Androgen-induced increases in blood pressure are also observed in females. Treatment of intact or uninephrectomized female rats with testosterone or DHT raised blood pressure (25, 26), and, in OVX hypertensive rats, testosterone increased blood pressure to levels exhibited by males (27, 28).

Sex hormones influence all mechanisms that control cardiovascular function and blood pressure, including the sympathetic nervous system (SNS) and central cardiovascular reflexes; hormonal systems, such as the renin-angiotensin-aldosterone system (RAAS); immune-related events; and, at the tissue level, the intrinsic components and signaling proteins of cardiac and vascular cells, i.e., cardiomyocytes, fibroblasts, perivascular adipose tissue (PVAT), and endothelial and vascular smooth muscle cells (VSMCs) (29).

Although sex differences and the impact of sex hormones in cardiovascular physiology and pathophysiology have been considered in recent preclinical and clinical studies, data on the cardiovascular effects of exogenous sex hormones in transgender individuals are rare. Therefore, this article will summarize current knowledge on the cardiovascular effects of testosterone in biological females in physiological and pathophysiological conditions. It will focus on aspects of testosterone use by transmasculine people (clinical goals, pharmaceutical formulations, and cardiovascular impact) and discuss potential mechanisms whereby testosterone increases cardiovascular risk in these individuals. The effects of testosterone on the mechanisms that control cardiovascular function and that may contribute to the development of hypertension and hypertension-associated target-organ damage will also be discussed.

TRANSGENDER INDIVIDUALS AND THE GENDER TRANSITION PROCESS

Increasing attention has been given to healthcare and personalized clinical practices aimed at transgender, gender-nonconforming, and intersex individuals. Of importance, the terminology referring to human sexuality and gender aspects (mainly distinguishing them) has changed over the decades because of cultural, social, and political aspects, and advances in human rights. Since the terminology is also critical in scientific writing (30), Table 1 summarizes the umbrella terms that are often used to distinguishing sex and gender aspects, and terms used for transgender people as part of their identities.

Recent data point out that 0.3–4.5% of adults in reproductive age worldwide identify themselves as transgender and gender-diverse people (31). Several reasons, including subjective self-identity and lack of access to primary healthcare, contribute to the paucity of accurate quantification of how many people are identified as transgender. However, because of information diffusion and increasing social acceptance, the number of transgender people seeking healthcare services and specialized centers has grown over the past years (32).

Gender incongruence, which includes physical and social aspects, occurs when the person's self-experience of gender (gender identity) is in discrepancy with the gender assigned to them at birth, usually assigned because of secondary sexual features. Gender incongruence may lead to gender dysphoria, a profound distress or discomfort associated with one's gender incongruence, experienced by many transgender individuals (33). Some transgender individuals seek healthcare services, when available, to relieve gender dysphoria and undergo gender transition. It is important to emphasize that not all transgender individuals experience gender dysphoria or go through gender transition and that gender dysphoria is not an essential condition to undergo gender transition procedures.

From a clinical point of view, different approaches are implemented in the gender transition to promote welfare in transgender individuals, in many cases associated to satisfaction with their bodies. Psychological counseling is strongly recommended for all cases. Pharmacologically, GnRH analogs are used in early adolescents for gonadal suppression and puberty suspension, a procedure known as "block therapy." In older adolescents and adults, desired sexual characteristics are induced by sex hormones, a procedure known as gender-affirming hormone therapy (GAHT), the focus of our review. Many transgender individuals also undergo surgical procedures (34).

[1]Terms distinguishing sexual and gender aspects are detailed in Table 1.

Table 1. *Terminology used in transgender health*

Sex	Biological attributes that characterize an organism as male or female. It includes sex-determining genes, sex chromosomes, the gonads, sex hormones, internal and external genitalia, and secondary sexual characteristics. Male or female "sex-assigned at birth" (before called "natal sex") is usually determined by the external genitalia appearance.
Intersex person	Individual born with variations that fall out of the typical male or female sex attributes (i.e., binary biological features). They may simultaneously exhibit male, female, or exacerbated characteristics of one of them.
Gender	A social construction that encompasses behavior, attitudes, and personality traits as masculine or feminine in a historical period. In a "cis" perspective, masculine attributes are expected from males (people with male-sex characteristics), whereas feminine attributes are expected from females (people with female-sex characteristics).
Gender identity	Individual's feeling of being male, female, both, neither, or other genders. Gender identity is a personal and subjective experience, not visible to others.
Cisgender	Individuals whose gender identity is aligned with the gender assigned at birth (usually attributed based on sex characteristics). Typically, males who identifies with male gender identities (men), and females who identifies with female gender identities (women).
Transgender	An umbrella term that refers to individuals whose gender identity differs from that assigned at birth. Transmasculine, transfeminine, gender nonconforming, nonbinary, gender fluid, agender, and other gender varieties are included. Intersex individuals may identify as transgender, since "transgender" is not a biological trait.
Transmasculine	An umbrella term that refers to individuals who identify themselves with a masculine gender identity and who were assigned as female at birth, before called "female to male" people. Transgender men and other nonbinary masculine gender identities are included.
Transfeminine	An umbrella term that refers to individuals who identify themselves with a feminine gender identity and who were assigned as male at birth, before called "male to female" people. Transgender women and other nonbinary feminine gender identities are included.
Nonbinary	An umbrella term that refers to an individual whose gender identity falls out of the typical binary gender identity. Nonbinary individuals are neither male nor female.
Gender expression	The external manifestations of gender identity. These include name, pronouns, behavior, voice pitch, expressions and appearance of the body, clothing, and other characteristics perceived by others.
Gender incongruence	Self-perception that the gender identity differs from that assigned at birth. Although still controversial, this is an emerging diagnostic term to replace "gender dysphoria" (from DSM-5) in the new edition of ICD-11.
Gender dysphoria	A profound discomfort or distress related to gender incongruence, associated with self (physical and psychological) and social aspects. A diagnostic term in DSM-5. Not all transgender people experience gender dysphoria.
Gender transition	The process that some transgender people undergo, with a set of approaches involving physical, psychological, social, and legal aspects to turn self-perception of gender identity more comfortable. Not all transgender individuals undergo gender transition. Also, gender dysphoria is not a fundamental criterion for offering gender transition procedures. Clinically, psychological counseling, the use of sex hormones and other compounds, and surgical procedures figure out as offered approaches.
Gender-affirming hormone therapy (GAHT)	For older adolescents and adults, the pharmacological use of compounds (mainly sex hormones) to obtain secondary sexual characteristics aligned with the individual's gender identity. Previously called "cross-sex hormone therapy," and then replaced by emerging terms such as "hormonization" (e.g., this term has been perceived as more friendly for the transgender community because of the absence of the term "therapy").
Sexual orientation	An individual's physical and/or emotional attraction to another person. Sexual orientation differs from gender identity. Transgender and cisgender people may have attraction to individuals with multiple gender identities, being heterosexual, homosexual, bisexual, pansexual, and others. They also may be asexual, in case of decreased (based on self-perception) or absent physical and/or emotional attraction for others.

DSM, Diagnostic and Statistical Manual of Mental Disorders; ICD, International Classification of Diseases.

Several criteria influence GAHT prescription, including previous clinical conditions, available healthcare services, and the individual's desire to acquire sexual features of the gender self-identity. The overall GAHT pharmacological aims are *1*) to achieve and maintain sex hormones at levels found in healthy cisgender people aligned with the transgender individual's gender and *2*) to suppress endogenous production and secretion of the predominant biological sex hormones (34). Of importance, GAHT by itself improves global welfare in transgender people, independently of other gender transition procedures (34).

TESTOSTERONE USE BY TRANSMASCULINE PEOPLE

Testosterone is used in several clinical conditions, such as hormone-replacement therapy in cisgender men with hypogonadism, erectile dysfunction and blunted sexual desire, osteoporosis, and anemia. Testosterone is also used by postmenopause cisgender women (35), and in nonpathological contexts such as in physiculturism (body building), to increase athletic performance, and in GAHT by transmasculine people.[2]

Different compounds, such as pregestational agents (contraindicated if ovariectomy is performed) and GnRH analogs, are included in the GAHT portfolio for transmasculine individuals (36). However, testosterone is the main compound used in masculinizing GAHT. Initially, increasing doses of testosterone are administered, with higher maintenance doses then used.

Pharmaceutical Formulations

Overall, testosterone use for transmasculine people aims to achieve testosterone levels similar to those observed in

[2]Beyond the binary gender spectrum, transmasculine individuals, including transgender men, refer to a broad group of people who were assigned a female gender identity at birth, but who identify themselves with a male gender identity.

Table 2. *Testosterone formulations used in gender-affirming hormone therapy*

	Route	Usual Dosing	Advantages	Disadvantages
			Parenteral	
Enanthate or Cypionate	Intramuscular	100–200 mg every 2 wk	- Low cost - successfully achieves target serum concentrations	- Serum concentrations fluctuations (pharmacokinetics features) - Pain in administration - Enhanced erythrocytosis (compared with other formulations)
Undecanoate	Subcutaneously Intramuscular	50% solution, once a week 1,000 mg every 12 wk	- Lower pain in administrations Consistent serum concentrations - Largest intervals between dosing	- Expensive - Large injection volumes - Pain in administration
			Transdermal	
Gel 1.6%	Topical	50–100 mg/day	- Consistent serum concentrations	- Expensive - Potential cross administration in others (skin-to-skin contact)
Patch	Topical	2.5–7.5 mg/day	- Consistent serum concentrations	- Expensive - Achieves subaimed serum concentrations - Skin irritation

The availability of testosterone formulations varies by country.

healthy cisgender men (physiological circulating testosterone range: 300–1,000 ng/dL). Table 2 summarizes testosterone pharmaceutical formulations used in GAHT, according to recent clinical practice guidelines (34). Intramuscular injectable testosterone esters represent the main prescribed formulation, although recent studies have shown that subcutaneous administration (75 mg/wk) is effective and potentially preferred by transmasculine people (37). Because of its long-acting effects, testosterone undecanoate has been used in GAHT (38). Topical androgen gels or transdermal patches are also reported, but oral testosterone formulations and nasal sprays are rarely applied in testosterone GAHT (34). Pelusi and colleagues (39) evaluated three different testosterone formulations for GAHT in transmasculine individuals, and after 12 mo, no differences in short-term safety, body composition changes, or metabolic parameters were found. Of importance, general satisfaction with the gender transition process was reported, independently of the pharmaceutical formulation.

Clinical Goals and Virilization Effects

The major aim of testosterone GAHT is the development of male sexual characteristics (i.e., virilization effects) and changes in body appearance, which attenuate gender dysphoria related to self-recognition or to transmasculine individuals' passing.[3] The development of virilization characteristics vary from individual to individual and occurs from months to years after the beginning of testosterone GAHT (40).

Testosterone effects that directly impact passing are especially desired by transmasculine people. Testosterone changes body composition and shape because of its canonical anabolic role. Testosterone increases lean mass and decreases fat mass, leading to increased body mass index (BMI) in transmasculine individuals (41, 42). Of importance, aspects such as the hormone use adherence, the age of the individual when GAHT is started, and other nonpharmacological aspects (including genetic background) influence the degree/intensity of body changes induced by testosterone in transgender individuals (43, 44). Testosterone also increases facial and body hair growth, often accompanied by acne (45, 46), promotes alopecia (47), and decreases voice pitch (48, 49). Despite its intrinsic anxiogenic and proaggressive behavior effects in females (50), testosterone GAHT decreases anxiety, depression, and other psychological disorders in transmasculine people (51), although studies point out that the GAHT, and not testosterone per se, is related to mental health improvement (52).

Testosterone GAHT also changes breast tissue composition (53), induces amenorrhea (54), decreases endometrium thickness (55), induces clitoral growth (55), and decreases vaginal epithelial thickness with reduced proliferation of vaginal stromal cells with decreased intracellular glycogen content, and ERα and ERβ expression (56). Testosterone also induces polycystic ovary syndrome (PCOS)-like histopathological changes, including cystic follicles, stromal hyperplasia, and tunica albuginea fibrosis in the ovaries of transmasculine people and experimental models (57, 58). Because of these and other reproductive functional changes, counseling focusing on fertility preservation is strongly recommended before starting testosterone GAHT in transmasculine individuals (34). Of note, reversibility of the menses cycle and reproductive system architecture after testosterone GAHT suspension has been reported in clinical and experimental studies, leading to successful birth outcomes (59, 60). Figure 1 summarizes the main effects, desired and unwanted cardiovascular effects, of testosterone GAHT.

CARDIOVASCULAR RISK IN TRANSMASCULINE PEOPLE ON TESTOSTERONE GAHT

Asscheman and colleagues published in 1989, for the first time, a study addressing whether GAHT is related to

[3]The term "passing" refers to whether someone is perceived as man or woman (or another gender) in the society and frequently related to gender dysphoria in transgender individuals.

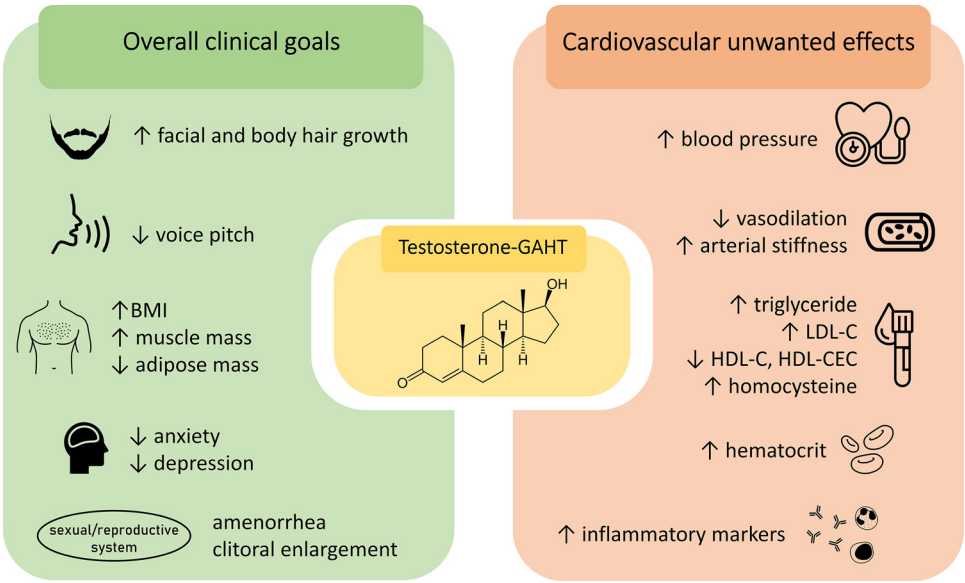

Figure 1. Testosterone effects in gender-affirming hormone therapy (GAHT). Testosterone promotes virilization effects including increased facial and body hair volume and growth rate, decreased voice pitch, and changes in body composition through increased lean mass and decreased adipose mass. Testosterone also induces menses cessation and clitoral enlargement and decreases harmful psychological symptoms. These effects relieve gender dysphoria (if present), improving general welfare in transmasculine people. Side effects include acne, alopecia, and transient infertility. Although considered safe, testosterone GAHT negatively impacts the cardiovascular system, increasing cardiovascular risk for transmasculine individuals. Harmful cardiovascular effects include increased blood pressure, endothelial dysfunction, arterial stiffness, and dyslipidemia and increased cardiovascular damage markers, such as homocysteine, hematocrit, and inflammatory molecules. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HDL-CEC, HDL-cholesterol efflux capacity; LDL-C, low-density lipoprotein cholesterol.

cardiovascular disease (CVD) (61). In a retrospective analysis, the authors reported that myocardial infarction cases or myocardial infarction-related deaths were similar between transfeminine people and cisgender men, while there were no myocardial infarction cases in the transmasculine group. However, over the past decades, numerous studies have further evaluated potential harmful cardiometabolic effects and potential cardiovascular outcomes induced by testosterone GAHT in transmasculine people. Although considered safe, testosterone GAHT has been linked to undesired cardiovascular effects, such as increased blood pressure and dyslipidemia, as discussed in the next section and summarized in Fig. 1.

Testosterone GAHT and Cardiovascular Risk Markers

High blood pressure.

High blood pressure is a leading risk factor for heart disease and receives great attention in testosterone GAHT monitoring. Several studies report that testosterone GAHT increases blood pressure in transmasculine people (47, 62–66). In a prospective study evaluating 53 transmasculine people on intramuscular testosterone administration for 12 mo, testosterone GAHT increased mean systolic blood pressure (SBP) from 111.5 to 115.6 mmHg, but did not modify mean diastolic blood pressure (DBP) (47). Increased mean SBP (from 129.0 to 135.0 mmHg) induced by intramuscular testosterone for 24 mo was also reported in 45 transmasculine people. Again, testosterone did not change mean DBP (63). In 43 transmasculine individuals (mean age 28) receiving intramuscular testosterone injections, SBP increased after 1 yr [(mean SBP ± SD, mmHg) 123.6 ± 6.3] and 2 yr (124.6 ± 6.3) of starting GAHT (basal, 111.1 ± 10.5, $P < 0.001$), with no differences between the evaluated time points (66). A prospective study with 188 transmasculine people (mean age 26 yr) on three different testosterone GAHT regimens: intramuscular undecanoate 1,000 mg every 12 wk, topical gel dosed at 50 mg/day, and intramuscular esters dosed at 250 mg every 2 wk, showed that testosterone GAHT increased mean DBP in 2.5% (95% CI: 0.6–4.4) from baseline levels, although no differences were found in mean SBP (64). When compared with 63 nonhormone users transmasculine people, SBP was higher in 48 transmasculine people on testosterone GAHT for ~45 mo [(mean SBP ± SD, mmHg): 117.4 ± 10.2 vs. 110.4 ± 9.4, $P < 0.01$] (65), corroborating prohypertensive effects of testosterone GAHT. In addition, using a mouse model of GAHT, we recently showed that, after 24 wk of treatment, testosterone administration to female mice increased mean arterial pressure (MAP) [(mean MAP ± SE, mmHg) 135.2 ± 3.3 vs. vehicle-treated female mice: 116.2 ± 2.8, $P = 0.0072$] (67). Similarly, testosterone treatment for 4 mo increased SBP in females, in a rat model of GAHT [(means ± SE, mmHg) 131 ± 1.4 vs. vehicle-treated female rats: 119 ± 1.0, $P < 0.0001$] (68).

Chronic and sustained increased blood pressure leads to hypertension, whose prevalence in transmasculine community ranges from 1.5 to 25.2% (69). However, the few studies evaluating whether testosterone GAHT directly plays a role in this clinical outcome are inconclusive. Using data from the Behavioral Risk Factor Surveillance System (BRFSS) study from 2015, Nokoff and colleagues (70) found that the prevalence of hypertension is similar between transgender (transmasculine included) [27.9% (95% CI: 22.0–33.8)] and cisgender [29.4% (95% confidence interval, CI: 29.0–29.9)] people. On the other hand, using propensity score-matched samples, data from 2017–2019 show that hypertension

prevalence is higher in transgender individuals (31.3%) versus cisgender people (27.6%, $P < 0.001$) (71).

Changes in lipid metabolism.

Changes in lipid metabolism are also induced by testosterone GAHT. A meta-analysis with 29 studies addressing whether GAHT modifies serum lipid levels in transgender people showed that, under different administration regimens, testosterone increased triglycerides levels after 3 to 6 mo of hormone use [(change to baseline, mg/dL) +9, 95% CI: 2.5–15.5] and also up to 24 mo (+21.4, 95% CI: 0.1–42.6). Serum low-density lipoprotein cholesterol (LDL-C) levels increased at 12 [(mg/dL) +11.3, 95% CI: 5.5–17.1] and 24 mo or more (+17.8, 95% CI: 3.5–32.1) of GAHT. Furthermore, serum high-density lipoprotein cholesterol (HDL-C) levels decreased across all follow-up periods [(3–6 mo): −6.5, 95% CI: −11.9 to −1.0; (12 mo): −8.1, 95% CI: −10.6 to −5.7; (≥24 mo): −8.5, 95% CI: −13.0 to −3.9] (72). Testosterone GAHT also increased total serum cholesterol [(% to baseline): 4.1%; 95% CI 1.5–6.6], LDL-C (13%; 95% CI 9.2–16.8), and triglyceride (36.9%; 95% CI 29.8–44.1) levels and decreased 10.8% serum HDL-C levels (95% CI: −14.0 to −7.6) (64). Transmasculine individuals that received intramuscular injections of testosterone showed increased total serum cholesterol [(means ± SD, mg/dL) 189.5 ± 28.4 vs. 178.9 ± 31.0, $P < 0.05$] and triglyceride (69.2 ± 63.9 vs. 103.0 ± 78.1, $P < 0.01$) levels, and decreased circulating HDL-C (69.3 ± 17.7 vs. 60.9 ± 15.8, $P < 0.02$) compared with nontestosterone users transmasculine people (65).

Testosterone administration route appears to be critical in lipid metabolism changes in transmasculine people. Increased serum LDL-C levels after 54 wk of GAHT were lower in transmasculine individuals using transdermal testosterone gels [(means ± SD, mg/dL): 84.9; 95% CI: 66.6–103.1] compared with individuals using intramuscular esters formulations (107.0; 95% CI: 88.7–125.3 for testosterone enanthate, and 98.9; 95% CI: 79.8–118.2, for testosterone undecanoate, $P = 0.025$) (39).

HDL cholesterol efflux capacity (HDL-CEC), a functional parameter that measures the capacity of HDL-C to remove cholesterol from the periphery and transfer it to the liver, is an independent marker for cardiovascular risk and is considered a better CVD predictor than circulating HDL-C levels in coronary heart disease (73). Data on HDL-CEC in 15 transmasculine people under three different formulations of testosterone show that, after 12 mo, testosterone decreased circulating HDL-C levels by 19.6% (95% CI: − 33.5 to − 5.6, $P < 0.05$) and global HDL-CEC [− 6.7% (95% CI: −13.7 to 0.2), $P = 0.06$)] (74).

Hematological and biochemical parameters.

Testosterone GAHT also impacts other cardiovascular risk factors, such as hematological parameters, immune system components, and endothelial function. Testosterone GAHT for 12 mo increased hematocrit [(means ± SD, %) from 40.8 ± 2.9 to 45.8 ± 3.0, $P < 0.001$] (47). Intramuscular administration of testosterone esters for 45 mo increased hematocrit in 48 transmasculine individuals compared with 63 transmasculine nontestosterone users [(means ± SD, %) 44.9 ± 3.5 vs. 38.8 ± 3.2, $P < 0.01$] (65). In a prospective study, intramuscular testosterone esters injections for 12 wk increased hemoglobin [(means ± SD, g/mL) from 13.8 ± 1.22 to 15.1 ± 0.68, $P = 0.02$] and the hematocrit mean [(means ± SD, %) from 41 ± 3.6 to 44.3 ± 1.4, $P = 0.01$] in 12 transmasculine people (mean age, 33 yr) (75). Testosterone GAHT similarly increased hematocrit and hemoglobin in transmasculine people, under transdermal or intramuscular testosterone administration (39). Moreover, 17 transmasculine people (median age 22) on intramuscular testosterone injections for 4 mo exhibited increased plasma total homocysteine by 17% ($P = 0.005$). Interestingly, homocysteine positively correlated with plasma creatinine levels in those subjects (76).

Hyperactivation of the immunological system.

The relationship between hyperactivation of the immunological system and CVD is extensively reviewed (77, 78). A prospective study with 30 transmasculine individuals (median age 23) on intramuscular esters injections or oral testosterone use showed that after 4 mo of GAHT, testosterone decreased serum immunoglobulin A (IgA) levels [(geometric mean, g/L) 1.47; 95% CI: 1.27–1.70 vs. 1.57; 95% CI: 1.35–1.82 at baseline; $P = 0.001$] and increased serum IgM [(geometric mean, g/L) 1.48, 95% CI: 1.22–1.79 vs. 1.38, 95% CI: 1.15–1.66, $P = 0.009$] and peripheral blood mononuclear cell-released tumor necrosis factor-α (TNF-α) levels [(geometric mean, ng/mL) 1.75, 95%: 0.90–3.38 vs. 1.26, 95% CI: 0.71–2.26, $P = 0.03$] (79), indicating that GAHT influences primary inflammatory markers in transmasculine people. More recently, a study with 157 transmasculine people on testosterone use (intramuscular injections) for 12 mo showed that testosterone increased serum interleukin-6 (IL-6) ($P < 0.05$), TNF-α ($P < 0.01$), soluble E-selectin ($P < 0.05$), and soluble-vascular cell adhesion molecule-1 (VCAM-1) ($P < 0.05$) levels in subjects on GAHT (80). Moreover, testosterone GAHT modulates leukocyte-endothelium cell interactions, since polymorphonuclear cells (PMNs) from transmasculine individuals on testosterone exhibited lower rolling velocity ($P < 0.01$) and increased rolling flux rate ($P < 0.001$) and adhesion ($P < 0.001$).

Testosterone GAHT and Cardiovascular Outcomes or CVD-Related Mortality

Although testosterone GAHT promotes harmful cardiometabolic changes, these do not directly lead to cardiovascular outcomes or CVD-related mortality. An observational study evaluated cardiovascular events in 293 transmasculine people (mean age 39 yr) on testosterone GAHT for 2 yr. Standardized incidence ratio of mortality and myocardial infarction was similar in transmasculine individuals and cisgender women (81). In a retrospective study, only one myocardial infarction case was reported within 365 transmasculine people on testosterone GAHT (82). Moreover, the ischemic heart-related mortality standardized ratio was within the expected range (1.19, 95% CI: 0.39–2.74). Myocardial infarction and cerebrovascular events ratio did not change in a cohort study including 214 transmasculine people under different patterns of testosterone GAHT (83). In addition, the frequency of self-reported myocardial infarction, stroke, and angina was not different between transmasculine and cisgender women or cisgender men groups (70). In a retrospective study, clinical data from 2,118

transmasculine people that received testosterone GAHT for 3.6 yr showed that the incidence ratio of venous thromboembolism and ischemic stroke was not different between transmasculine people and cisgender women or cisgender men [3.1 (95% CI: 2.0–4.6), 2.1 (95% CI: 1.3–3.5), and 1.2 (95% CI: 0.6–2.3), respectively] (84). Despite these findings, the relatively young age of the patients and the short time of testosterone use represent major limitations for precise inferences on the impact of testosterone GAHT in cardiovascular events, as will be addressed next.

The reduced number of studies addressing mortality and outcomes related to cardiovascular events in transgender people on GAHT also limits accurate conclusions. The meta-analysis published by Maraka and colleagues, with 29 studies, showed that few studies evaluated mortality, myocardial infarction, and stroke (only 4, 3, and 2 studies, respectively). Mortality was reported in 13 of 651 transmasculine people, myocardial infarction in 1 of 478, and no stroke cases were reported in 340 evaluated individuals (72).

Testosterone GAHT and Cardiovascular Target-Organ Damage

Despite increased cardiovascular risk by testosterone GAHT, very few studies have addressed whether the hormone directly harms cardiovascular target organs in transmasculine people. A study within 12 transmasculine people (mean age, 33 yr), who received different testosterone formulations for at least 12 mo, reported no differences in flow-mediated dilatation (FMD), an indirect measurement of endothelial function, between transmasculine people and age-matched cisgender women (85). However, the increased brachial artery diameter induced by sublingual nitroglycerin administration was lower in transmasculine individuals [(means ± SD, %) 15.9 ± 4.9 vs. 22 ± 5.8, $P = 0.01$], suggesting decreased vascular responsiveness to vasodilator agents. In a group of 17 transmasculine people (mean age, 28 yr) on testosterone injections for at least 3 mo, FMD was lower compared with cisgender women [(means ± SD, %) 4.5 ± 2.7 vs. 8.1 ± 2.9, $P = 0.002$] and related to a mild increase in blood pressure (86). Accordingly, in a testosterone GAHT mouse model, testosterone induced endothelial dysfunction, i.e., it reduced vasodilation to acetylcholine (ACh), after 8 and 24 wk of treatment (67). Although very few studies have addressed vascular structure under testosterone GAHT, brachial-ankle pulse wave velocity was higher in testosterone transmasculine users [(means ± SD, cm/s) 1,202.8 ± 138.2] compared with nonhormonized transmasculine individuals (1,080.0 ± 113.7, $P < 0.01$) (65), suggesting a vascular prohypertrophic role for testosterone.

In addition to testosterone itself, other aspects related to masculinizing gender transition, such as gonadectomy and use of other drugs, may modulate vascular function and structure in transmasculine people. Carotid intima-media thickness (C-IMT) and brachial artery FMD were evaluated in 56 transgender people on GAHT, transmasculine individuals included. The group that underwent gonadectomy showed increased C-IMT and lower FMD ($P < 0.0001$) compared with people who did not have surgery (87), suggesting a critical role of gonadal-derived mediators in cardiovascular function in transgender people. Bunck and colleagues evaluated 30 ovariectomized testosterone users transmasculine people (range aged 21–46 yr) for at least 18 mo. Individuals received anastrozole, an aromatase inhibitor, or placebo for 3 mo. Anastrozole administration did not change FMD. However, serum 17β-estradiol levels positively correlated with carotid compliance ($r = 0.62$, $P = 0.018$) and distensibility coefficients ($r = 0.61$, $P = 0.021$), markers of vascular health (88), suggesting an important estrogen-mediated cardiovascular protective role in those subjects. Similarly, vasodilation induced by ACh was further decreased by OVX in testosterone-treated female mice, indicating that removal of the ovaries exacerbates testosterone-induced vascular dysfunction (67). Therefore, multiple factors modulate vascular function and structure in transmasculine people in the gender transition perspective, warranting further studies.

In a testosterone GAHT experimental model, female rats treated with testosterone for 4 mo exhibited increased kidney glomerular area [(means ± SE, μm^2) 9,078 ± 133.4 vs. 7,884 ± 112.8, $P < 0.0001$] and decreased glomeruli number/mm^3 compared with vehicle-treated female rats. Also, testosterone decreased intralobular arteries thickness [(means ± SE, μm) 15.4 ± 0.5 vs. 12.1 ± 0.4, $P < 0.0001$] and glomerular filtration rate [(means ± SE, mL/min/g kw) 0.67 ± 0.03 vs. 0.78 ± 0.02, $P = 0.0228$] (68). However, studies addressing whether GAHT affects kidney structure and function in humans are missing.

As shown in Table 3, very few current clinical trials address the cardiometabolic impact of testosterone GAHT. Very often, cardiovascular alterations are addressed as secondary outcomes. The Gender Dysphoria Treatment in Sweden (GETS) clinical trial is an example. Aimed to evaluate the potential impact of testosterone GAHT on metabolic profile and body composition, GETS primarily evaluates GAHT-induced physiological changes in the skeletal muscle and adipose tissue. Carotid augmentation index, coronary flow reserve, and C-IMT are being determined as secondary outcomes (89).

MECHANISMS WHEREBY TESTOSTERONE MAY INCREASE CARDIOVASCULAR RISK IN TRANSMASCULINE INDIVIDUALS

There is paucity of studies exploring the mechanisms involved in the effects of testosterone in the cardiovascular system in individuals and experimental animals under GAHT. In this review, we tried to cover all main preclinical and clinical studies on the cardiovascular effects of testosterone from a transgender perspective. Whenever available, studies reporting testosterone cardiovascular effects in transmasculine people or in testosterone GAHT experimental models were preferably evaluated. Studies on androgen administration to biological females leading to supraphysiological levels of testosterone were therefore prioritized. Next, studies evaluating physiological testosterone levels or the impact of testosterone in cardiovascular injury models using female animals, as well as in vitro studies addressing the effects of testosterone in cardiac and vascular samples from biological females were mentioned. Sex differences in cardiovascular parameters in health and CVD-related conditions, or dual testosterone effects in

Table 3. *Clinical trials aiming to evaluate cardiometabolic parameters in transmasculine people on testosterone gender-affirming hormone therapy*

Authors and ID	Study Type, Observational/ Interventional Model, and Time Perspective	Participants	GAHT	Primary End Points	Secondary End Points	Interventions
Wiik et al. (GETS study) NCT02518009	Observational, cohort, and prospective study	Transmasculine individuals (20–40 yr old) with gender dysphoria. Exclusion criteria: previous infectious and cardiovascular diseases, treatment with anticoagulants, serious illness or mental disorder, and DM1. People enrollment: 25	Testosterone (other pharmaceutical aspects not detailed)	Association between epigenetic factors in skeletal muscle, skin and adipose tissue and metabolism markers, insulin sensitivity, muscle strength, and adipokines. Body composition changes and adipose tissue morphology (time frame: 5 yr from baseline).	Arterial stiffness, endothelial and cardiac function and vascular morphology, body composition with lean and adipose body mass, general immune system markers, glucose and lipid metabolism markers, and morphological and functional analysis on areas in the brain (time frame: 5 yr from baseline).	-
Cabrera et al. NCT04977765	Observational, cohort, and prospective study	Transmasculine individuals (12–30 yr old) considering GAHT. Exclusion criteria: previous hysterectomy or GnRH agonist ("bock-therapy") use. Estimated enrollment: 90	Testosterone and other masculinizing therapies (pharmaceutical formulations not detailed)	Endothelial function through FMD measurement, blood pressure, and circulating inflammatory markers (time frame: 1 yr from baseline)	Body composition changes, plasma lipid profile, and glucose metabolism (time frame: 1 yr from baseline)	
Ahmed et al. NCT05442463	Observational, case-control, and prospective study	Transmasculine individuals (18–90 yr old) on GAHT. Exclusion criteria: previous cardiometabolic events or diseases, and current smokers. Estimated enrollment: 200	Oral or nonoral testosterone formulations (other pharmaceutical aspects not detailed)	ANG II-dependent arterial and renal vasoconstriction		Iohexol (5 mL, iv) followed by 10 mL of saline are administered to determinate baseline GFR. FF is calculated as follows: GFR/RPF = FF. Then, ANG II is administered (iv) as follows: 3 ng/kg/min (30 min) followed by 6 ng/kg/min (30 min).
Central Hospital, Nancy, France NCT04971447	Interventional, parallel assignment, and nonmasking study	Transmasculine individuals (18–49 yr old) considering GAHT. Exclusion criteria: previous hormone use, chronic diseases, cancer, pregnancy, obesity, and illicit drugs, alcohol and tabacco abuse. Estimated enrollment: 34	Testosterone enanthate (other pharmaceutical aspects not detailed)	Arterial stiffness through PWV measurement (time frame: 2 yr)		

Continued

Table 3.— *Continued*

Authors and ID	Study Type, Observational/ Interventional Model, and Time Perspective	Participants	GAHT	Primary End Points	Secondary End Points	Interventions
Iwamoto et al. NCT04237467	Observational, cohort, and cross-sectional study	Transmasculine people (two distinct groups: 18–40 and 50–75 yr old) on testosterone GAHT for ≥ 1 yr. Exclusion criteria: previous hysterectomy or oophorectomy, estrogen-dependent neoplasms, acute liver or gallbladder disease, venous thromboembolism, dyslipidemia, diabetes, hypertension, and alcohol and tabacco abuse. Estimated enrollment: 30	Parenteral or transdermal testosterone formulations (other pharmaceutical aspects not detailed)	Endothelial function through FMD (time frame from baseline)	Carotid artery compliance, carotid artery β stiffness index, carotid artery intimal-medial thickness, oxidant burden (oxidized LDL-C, and nitrotyrosine), endothelial cell inflammation (NF-κB, MCP-1, IL-6, and CRP), blood pressure, plasma lipid and d-dimer concentrations, and body composition (time frame from baseline). Other time frames: physical activity monitoring (7 days), and energy intake (3 days)	
Blueher et al. NCT04838249	Observational, case-control, and prospective study	Transmasculine people (≥ 18 yr old) on GAHT. Exclusion criteria: previous cardiovascular, endocrine, pulmonary, and gastrointestinal diseases, HIV infection, or ongoing chronic infection. Estimated enrollment: 20	Testosterone (pharmaceutical formulations not detailed)	Vascular stiffness, and insulin sensitivity (time frame: up to 5 yr from baseline:). Body weight changes (time frame: up to 1 yr from baseline)		

The list was constructed based on information available at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) using the following entries: testosterone, cardiovascular, transgender, transgenderism, transsexualism, and gender identity. For all studies, primary outcomes are ongoing. ANG II, angiotensin II; CRP, C-reactive protein; FF, filtration fraction; FMD, flow-mediated dilation; GFR, glomerular filtration rate; GAHT, gender-affirming hormone therapy; IL-6, interleukin-6; iv, intravenously; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor κ-B; PWV, pulse wave velocity.

cisgender men and male animals (when no sex is mentioned) were also included to discuss potential harmful cardiovascular effects in females. Therefore, the next section was organized into three topics: *1*) direct effects of the hormone in the heart and blood vessels, including signaling mechanisms that control cardiac and vascular cells' function and remodeling/hypertrophy, such as reactive oxygen species (ROS) generation; *2*) indirect effects of the hormone, via activation of central, hormonal, and immune mechanisms, lipid metabolic changes; and *3*) impact of testosterone on the PVAT. A very brief discussion on the mechanisms whereby psychosocial issues may also impact the cardiovascular system is provided. The potential mechanisms whereby testosterone may increase cardiovascular risk are highlighted in Fig. 2.

Direct Effects in the Heart and in the Vasculature

In vitro, testosterone increases β_1-adrenergic receptor, L-type Ca^{2+} channels, and Na^{+}/Ca^{2+} exchanger mRNA levels in cardiomyocytes from male and female rats suggesting positive inotropic effects (95). In female rats, testosterone treatment enhances contractile function and increases lusitropic effects in the left ventricle (96). Testosterone also enhances cardiac contractility by increasing G_s protein levels in the heart, attenuating cardiac failure induced by castration in male rats (97).

In males, increased cardiac contraction strength induced by supraphysiological levels of testosterone leads to maladaptive morphofunctional changes. Athletes using testosterone showed decreased left ventricular dimensions with thicker walls, leading to diastolic dysfunction, when compared with nontestosterone user athletes (98). Testosterone also increased SBP, induced biventricular dysfunction, and increased pulmonary artery systolic pressure, leading to lung congestion in athletes on testosterone use (99). In male rats treated with testosterone, areas of cardiac degeneration and vacuolation were identified and ultrastructural analyses revealed disturbance of the banding pattern of the cardiac muscle fiber with disintegration, loss of striations, dehiscent intercalated disk, and interrupted Z-bands (98). It is important to emphasize that cardiac trophic mechanisms differ between the sexes (100), and testosterone in transmasculine people may lead to distinctive cardiovascular outcomes, guaranteeing further studies.

Testosterone increases protein synthesis and atrial natriuretic peptide secretion in cardiomyocytes from male and female rats, suggesting a direct hypertrophic effect in these cells (101). In vivo, testosterone induces maladaptive ventricular hypertrophy, mainly because of hemodynamic changes. In male rats, supraphysiological testosterone levels increased cardiac mass and cardiomyocyte cross-sectional area and increased cardiac collagen deposition (102). After 12 wk, testosterone decreased phosphorylated forms of extracellular signal-regulated kinase 1/2 [ERK1/2 (Thr^{202} and Tyr^{204})] and mammalian target of rapamycin [mTOR (Ser^{2448})] in the cardiac tissue without changing myocardial stiffness, suggesting that long-term testosterone induces eccentric cardiac hypertrophy.

Testosterone-induced maladaptive cardiac morphological changes are also reported in females. Testosterone-induced cardiac hypertrophy was associated with vascular dysfunction and increased blood pressure in female rats (103). Harmful testosterone-induced cardiac effects in females are especially reported in pathophysiological conditions. Cisgender women with PCOS show increased cardiac concentric hypertrophy prevalence (104). Experimentally, testosterone increased cardiac hypertrophy and intensified cardiac dysfunction in female mice that underwent ischemia-reperfusion (I/R) cardiac injury, leading to increased blood pressure and mortality (105). Estrogen treatment had

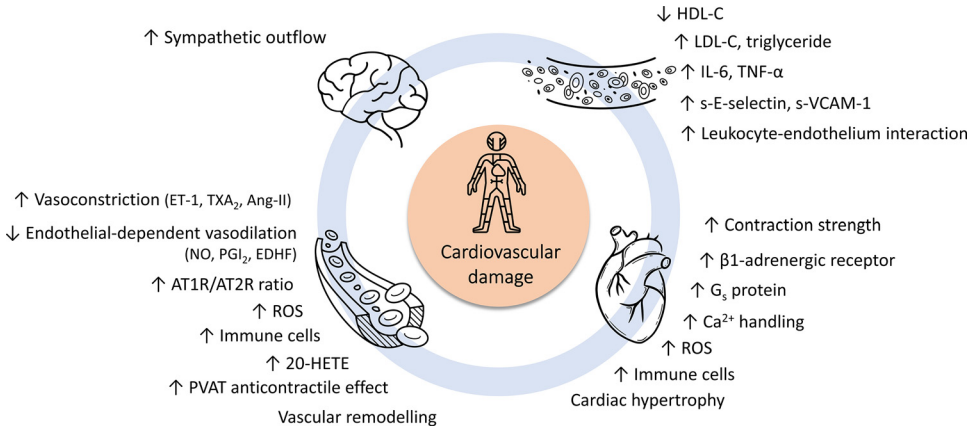

Figure 2. Potential mechanisms whereby testosterone gender-affirming hormone therapy (GAHT) impacts the cardiovascular system. Supraphysiological levels of testosterone in biological females may induce end-target organ damage. In the neural system, testosterone enhances sympathetic outflow, favoring increased blood pressure. Testosterone induces dyslipidemia, increases circulating inflammatory cytokines, and enhances leukocyte-endothelium interactions. In the heart, testosterone intensifies contraction strength by increasing calcium flux and increases ROS generation and infiltration of immune cells, all related to cardiac hypertrophy. In the vasculature, testosterone enhances vasoconstriction and impairs endothelium-dependent vasodilation, through increased ROS generation and activation of inflammatory mechanisms. Testosterone induces PVAT dysfunction through immune cell-linked mechanisms and promotes vascular remodeling. 20-HETE, 20-hydroxyeicosatetraenoic acid; ANG II, angiotensin II; AT_1R and AT_2R, angiotensin receptor type 1 and type 2, respectively; Ca^{2+}, calcium; EDHF, endothelium-dependent hyperpolarizing factor; ET-1, endothelin-1; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin 6; LDL-C, low-density lipoprotein cholesterol; NO, nitric oxide; PGI_2, prostacyclin; PVAT, perivascular adipose tissue; ROS, reactive oxygen species; s-E-selectin, soluble E-selectin; s-VCAM-1, soluble vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor-α; TXA_2, thromboxane A_2.

opposite effects in males, reinforcing potential deleterious cardiovascular effects of male hormones. Moreover, testosterone increased cardiac apoptotic markers in female rats, aggravating I/R-induced cardiac injury (106).

In males (blood vessels and cells from male rodents), testosterone induces vasodilation through mechanisms that include NO release by endothelial cells, as well as activation of potassium (K^+) channels and inhibition of Ca^{2+} channels in VSMCs (107). However, vasodilation induced by high concentrations of testosterone in vitro is lower in pulmonary arteries from cisgender women compared with cisgender men arteries, suggesting limited testosterone vasodilator effects in biological females, especially under supraphysiological levels (108).

Females exposed to higher androgen levels have vascular dysfunction characterized by increased vascular contractile responses to different agents and by impaired vasodilation. Testosterone increases thromboxane-prostanoid (TP) receptor density in VSMCs from female rats (109), and increases vasoconstrictor responses to U46619, a thromboxane A_2 (TXA_2) analog, in arteries from pregnant rats (110). Testosterone also increases vasoconstriction in biological females via endothelium-derived constrictor agents. In transmasculine people on GAHT for 4 mo, testosterone increased plasma endothelin-1 (ET-1) levels [(means ± SD, pg/mL) from 6.2 ± 1.1 to 7.8 ± 1.2, $P < 0.01$] (111). Of note, ET-1 is also increased in cisgender women with PCOS, leading to vascular dysfunction (112). However, studies addressing whether TXA_2 and ET-1 mediate vascular dysfunction in transmasculine people are missing, warranting further studies.

Testosterone also decreased endothelium-dependent vasodilation in females in various conditions (67, 103, 113). In these studies, vascular responses to NO donors were not affected by testosterone treatment, suggesting a crucial role of endothelial cells in testosterone-induced vascular dysfunction in females. Testosterone decreased total and phosphorylated (Ser^{1177}) endothelial nitric oxide synthase (eNOS), leading to decreased NO-mediated dilation in uterine arteries from pregnant rats. Moreover, testosterone decreased vasodilation mediated by prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF) and decreased mRNA expression of small conductance Ca^{2+}-activated K^+ channel-3 (SK3) (110), which plays a critical role in EDHF-mediated vascular tonus maintenance in small arteries and in blood pressure regulation (114).

Increased generation of ROS, or oxidative stress, figures out as a major contributor to endothelial dysfunction induced by testosterone in males and females. Testosterone increases ROS in endothelial cells (115) and in VSMCs (116). Testosterone increases ROS generation via nongenomic mechanisms and independently of its conversion to estradiol in rat VSMCs (116). In vivo, acute testosterone treatment increases leucocyte migration, an effect inhibited by antioxidant agents, suggesting that ROS contribute to immune and vascular changes induced by testosterone (117). In agreement, testosterone-induced vascular oxidative stress is linked to immune-mediated endothelial dysfunction in a GAHT experimental model (67).

In female rats, testosterone increases systemic and cerebral blood vessels ROS generation, aggravating cerebral vascular injury (118). Mechanistically, testosterone-induced oxidative stress in females occurs mainly via NADPH oxidases (Nox), the main ROS source in the cardiovascular system. Testosterone increases Nox subunits $p22^{phox}$, $p47^{phox}$, and $gp9^{phox}$ mRNA expression in the kidney of female rats, leading to increased glomerular filtration rate and albuminuria (119). Moreover, testosterone increases phosphorylated (Ser^{345}) $p47^{phox}$ in vessels from hypertensive female rats, suggesting that increased Nox activity is linked to testosterone-induced vascular dysfunction (113). Regarding cardiac function, testosterone increases ROS in coronary venous effluent in the heart from female rats, which is related to increased contractile responses, cardiac hypertrophy, and elevated blood pressure in vivo (96). An overview of the relationship between testosterone-induced ROS generation and CVD can be found in other comprehensive reviews (120, 121).

Testosterone also affects cardiovascular function through maladaptive changes in tissue morphology, triggering profibrotic and proproliferative signaling. In VSMCs from the male rat aorta, testosterone induces cellular proliferation (122), leading to cellular migration, in a ROS-dependent manner (116). Furthermore, testosterone increases phosphate-induced calcification in VSMCs from male mice aorta (123). In agreement with these in vitro findings, testosterone GAHT increases vascular profibrotic parameters in transmasculine people (65). Testosterone treatment also increases cross-sectional area and aortic arch intimal thickness in female mice with atherosclerosis (124). Compared with females, genes involved in aortic valve mineralization are upregulated in male mice, which is related to aortic stenosis progression (125).

Although data still remain inconclusive, antihypertrophic effects of testosterone have also been reported. Testosterone reduced atherosclerotic lesions in the aortic root in male mice, while the lesions were increased by orchidectomy. Administration of anastrozole, a selective aromatase inhibitor, blunted the testosterone protective effect, suggesting that testosterone conversion to estradiol (and not testosterone per se) is key to its vascular antihypertrophic effects (126). Of note, most studies on the vascular effects of testosterone were performed in males, which might not reflect conditions or events in females. AR deletion increased atherosclerotic lesion area in female mice aorta, suggesting an antiatherogenic role for physiological levels of testosterone in females (127). Studies addressing vascular morphological changes induced by exogenous administration of testosterone in females are lacking, warranting further studies.

Indirect Effects via Activation of Central, Hormonal, and Immune Mechanisms and Lipid Metabolic Changes

Sympathetic nervous system activity.

Female hormones exert cardioprotective effects, preventing, e.g., hypertension development, via central nervous system-related mechanisms (128). On the other hand, cardiovascular effects induced by direct effects of androgens on neuronal structures are limited.

Androgens increase sympathetic nervous system activity. Postexercise heart rate recovery is impaired in cisgender men athletes on androgen use (129), and cisgender women with PCOS have increased sympathetic outflow (130). Experimentally, DHT increased blood pressure in female

rats, which was blunted by renal denervation or simultaneous treatment with antagonists of α_1 (α_1) and β-adrenergic receptors, suggesting that increased sympathetic activity contributes to androgen-induced hypertension (131). Mechanistically, DHT increases melanocortin-4 receptor (MC4R) protein expression in the hypothalamus, and intracerebroventricular administration of SHU-9119, an MC4R antagonist, reduced blood pressure in DHT-induced hypertensive female rats. Also, DHT did not induce hypertension in female rats lacking MC4R, further supporting that MCR has a fundamental role in sympathetic nervous system-mediated increased blood pressure by androgens in females. In accordance, MCR activation in the central nervous system exerts an excitatory effect on sympathetic outflow, increasing blood pressure in male rats (132).

Hyperactivation of the classical RAAS.

Cardiovascular damage in males is also related to hyperactivation of the classical RAAS. Overall, male-related factors trigger classical RAAS components [i.e., angiotensin-converting enzyme (ACE), angiotensin II (ANG II), and angiotensin type 1 receptor (AT_1R)], leading to prohypertensive effects. On the other hand, female-related factors elicit cardioprotective effects via activation of the antihypertensive RAAS axis {ACE2, angiotensin-1–7 [Ang-(1–7)], Mas receptor (MasR), and AT_2R). A comprehensive overview on the role of the RAAS components in sex differences in the cardiovascular system and CVD can be found in excellent reviews (133, 134).

Testosterone enhances the prohypertensive RAAS axis in females. Reckelhoff and colleagues (135) demonstrated that testosterone increased blood pressure in OVX spontaneously hypertensive rats (SHR) to levels found in male SHR, and enalapril, an ACE inhibitor, dropped blood pressure by 65% in testosterone-treated OVX SHR. Interestingly, enalapril reduced blood pressure by 40% in intact female SHR or untreated OVX SHR, suggesting that testosterone modulates RAAS components that are key to hypertension development independently of other sex factors. Accordingly, androgens in males increase angiotensinogen and ACE gene expression in the kidney and in the liver, increase plasma renin activity, and, in hypertensive female rats, increase ANG II-induced vasoconstriction (113, 119, 136).

Imbalances in AT_1R/AT_2R ratio contribute to testosterone-induced cardiovascular damage in females. In pregnant rats, AT_2R activation attenuates increased blood pressure induced by testosterone (137). Moreover, testosterone in vitro and in vivo decreases AT_2R protein expression in conductance arteries from female rats (138), suggesting that testosterone impairs systemic AT_2R function in females. Testosterone increases ANG II-induced vasoconstriction by increasing the AT_1R/AT_2R ratio in uterine arteries from pregnant rats (110).

Immune mechanisms.

Immune mechanisms also play a fundamental role in sex differences observed in several cardiovascular disorders. Experimental studies point out that the predominance of an "inflammatory status" in males contributes to greater cardiovascular damage. Male SHR exhibited increased proteinuria and higher blood pressure levels, which was associated with kidney proinflammatory status. Males exhibited more Th17 cells in the kidney (a proinflammatory $CD4^+$ T-cell subset), whereas females showed more T regulatory (Treg) cells (anti-inflammatory cells) (139). In addition, transplanted female mice that received kidneys from male donors exhibited increased blood pressure and higher proinflammatory cytokines in response to ANG II. However, blood pressure and renal inflammatory markers were attenuated when female transplanted mice received kidneys from female donors (140). Together, these results suggest that a male-related proinflammatory profile is crucial to hypertension development and cardiovascular damage.

Androgens trigger proinflammatory mechanisms that contribute to CVD development in males. In human endothelial cells, DHT increased VCAM-1 gene expression via nuclear transcription factor κ B (NF-κB) activation, a mechanism that also contributes to androgen-induced vascular dysfunction (23, 141). In murine macrophages, testosterone increased TNF-α and monocyte chemoattractant protein-1 (MCP-1) release, which contributes to cerebral vascular injury (118). In vivo, testosterone increased cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) protein expression, and prostaglandin E_2 (PGE_2) production in brain vessels, contributing to cerebrovascular inflammation (142). In addition, acute testosterone administration increased vascular leukocyte migration in a COX-2-dependent manner (117), and long-term testosterone treatment promoted vascular dysfunction via NLRP3 inflammasome activation (143). Pharmacological AR blockade and genetic deletion of AR decreased interferon-γ (IFN-γ), IL-1α, IL-6, and IL-17 vascular expression, and reduced vascular macrophages infiltration, attenuating aortic aneurysm in mice (144), all reported in male animals.

Androgen-induced deleterious cardiovascular effects mediated by inflammatory mechanisms are also reported in females, but studies addressing this topic are still insufficient. Lipid uptake induced by DHT is higher in macrophages from cisgender men than in macrophages from pre- and postmenopause cisgender women, suggesting that male-related immune mechanisms contribute to increased prevalence and severity of atherosclerosis in cisgender men (145). Accordingly, testosterone increased vascular atheroma plaque deposition and vascular lesion area in female rabbits (124).

In transmasculine people on GAHT, testosterone increased circulating inflammatory cytokines and leukocyte-endothelial cell interactions (80). Experimentally, plasma TNF-α levels were increased in females treated with testosterone as well as in a PCOS-induced experimental model (118, 119). In addition, 20-hydroxyeicosatetraenoic acid (20-HETE), an arachidonic acid metabolite, contributed to renal inflammation and DHT-induced hypertension in female rats (146). 20-HETE also increased in the vasculature of testosterone-treated hypertensive female rats, which is related to vascular hypercontractility (147).

Recently, we showed that testosterone-induced vascular dysfunction in a GAHT experimental model relies on activation of adaptive immune system components (67). Testosterone increased vascular *Rorc* expression (or RORγt coding gene, a crucial Th17 cell marker, the main IL-17-secretory $CD4^+$ T-cell subset) in female mice. $CD4^+$ T cells and IL-17 receptor (IL-17R) were key to the decreased endothelium-dependent vasodilation induced by testosterone. γδ T cells, another T-cell subset that releases IL-17, did not

contribute to testosterone-induced vascular dysfunction. From a translational perspective, those data suggest that Th17 cells may contribute to increased cardiovascular risk in transmasculine people. Of note, activated $CD4^+$ T cells from healthy cisgender men have a predilection toward IL-17 production compared with $CD4^+$ T cells from cisgender woman (148). In addition, Th17 cells are increased in cisgender women with PCOS (149).

Few studies have investigated whether immune system components contribute to cardiac dysfunction induced by testosterone in females. Female mice with myocarditis had lower cardiac histopathological damage, reduced global inflammation, and a higher survival rate than males (150). Although estrogen treatment reduced cardiac damage, attenuated inflammation, and improved survival rate in male mice, testosterone increased neutrophil infiltration in the heart from female mice, aggravating cardiac hypertrophy and dysfunction induced by myocardial infarction (151). Despite these preliminary evidences, the immune mechanisms triggered by testosterone and how they affect the cardiovascular system in females are not completely elucidated.

Lipid metabolism changes.

As already mentioned, testosterone GAHT induces dyslipidemia, characterized by increased triglycerides and LDL-C levels, and decreased HDL-C levels, in transmasculine people under different regimens (39, 64, 65, 72). Moreover, HDL-CEC is reduced in transmasculine people on testosterone GAHT, increasing cardiovascular risk in those subjects (74). Despite these clinical findings, testosterone failed to increase vascular atherosclerotic plaque deposition in a GAHT experimental model (152). The low dose of testosterone used, which does not mimic testosterone GAHT, might explain these discrepancies.

Mechanistically, changes in lipid metabolism induced by testosterone have been attributed to increased expression of the scavenger receptor class B type 1 (SR-B1) protein and plasma lipase activity. In human hepatocytes, testosterone increases SR-B1 mRNA expression (153). Testosterone therapy increases plasma hepatic lipase activity, which was positively correlated to DHT serum levels, in cisgender men with primary testicular failure (154). SR-B1 plays a crucial role in the influx of HDL-C into the cells and facilitates the efflux of cholesterol from peripheral tissues back to the liver (155). Also, increased plasma lipase activity may lead to hydrolysis of triglycerides and phospholipase on circulating HDL-C, resulting in ApoA-1 release and degradation. In association, these mechanisms may contribute to reduced HDL-C levels, which may contribute to the testosterone-protective atherogenic role by enhancing reverse cholesterol transport from HDL. Whether those mechanisms also occur in biological females is unknown, guaranteeing further studies.

Testosterone effects in the PVAT.

The adipose tissue surrounding most blood vessels, the PVAT, plays a critical role in vascular homeostasis. The PVAT exerts anticontractile effect in the vasculature through the release of vasoactive agents such as NO, hydrogen peroxide (H_2O_2), hydrogen sulfide (H_2S), Ang-(1–7), and adiponectin. Moreover, the cellular arrangement of the PVAT contributes to vascular structure maintenance. On the other hand, PVAT dysfunction, which may result from an imbalance in the release of vasoactive compounds, changes in PVAT morphology, and inflammatory cells infiltration in the PVAT dysfunction, on the other hand, leads not only to an imbalance in the release of vasoactive compounds but also to variations in PVAT morphology and inflammatory cells infiltration, pathophysiological processes found in cardiometabolic diseases (156).

Sex differences in PVAT function are reported in experimental models of hypertension and obesity. In physiological conditions, female-related hormones play protective effects on PVAT structure and function (157). On the other hand, similar noradrenaline-induced vasoconstriction in PVAT-intact vessels from orchiectomized and intact (sham) male mice, suggests that physiological levels of testosterone do not influence PVAT function (158). However, studies addressing the impact of androgen administration on the PVAT function, especially in females, are missing.

We addressed testosterone effects on PVAT function in a testosterone-GAHT mouse model (67). Female 8-wk-old C57BL/6J wild-type (WT) mice received testosterone cypionate for 8 wk (48 mg/kg/wk) or peanut oil, used as vehicle. To determine whether OVX further impacts vascular actions of testosterone, a group of female WT mice was bilaterally ovariectomized (OVX) or fictitiously operated (sham). Sham and OVX female mice received testosterone or vehicle. Age-matched WT male mice were also treated with vehicle for sex comparisons. All the experimental protocols were performed in accordance with the National Council for Animal Experimentation Control and approved by the Ethics Committee on Animal Use of the University of São Paulo, Ribeirao Preto, Brazil (Protocol No. 079/2019). After the treatment period, the animals were euthanized by cardiac exsanguination under isoflurane anesthesia, the thoracic aorta was carefully harvested, and the PVAT was either removed or left intact. Phenylephrine (PE)-induced contractions were evaluated in endothelium-intact aortic rings by classic vascular reactivity studies. The PVAT influence on PE-induced vasoconstriction was inferred by the difference (Δ) between the responses [area under the concentration-effect curve (AUC)] in aortas without and with the PVAT (ΔAUC). All the in vivo procedures, vascular reactivity studies, and statistical analyses were performed as previously described (67). Testosterone increased the PVAT anticontractile effect (i.e. testosterone down-shifted PE-induced contractile responses in PVAT-intact vessels, increasing the ΔAUC) (Fig. 3). Changes in PVAT function induced by testosterone were not modified by OVX (Fig. 3*A*). Interestingly, arteries from male mice exhibited a PVAT functional phenotype that resembles the one displayed by testosterone-treated female mice (Fig. 3*B*). Since the increased anticontractile effect of PVAT induced by testosterone is not observed in arteries of female mice treated with testosterone for longer periods of time (Supplemental Fig. S1; https://doi.org/10.6084/m9.figshare.22811462.v1), it seems that increased PVAT function at earlier time points of testosterone-GAHT represents an adaptive and transitory compensatory mechanism for the vascular changes induced by testosterone.

In many CVD, the PVAT releases proinflammatory cytokines, adipokines, and ROS (159). Thus, we addressed whether immune mechanisms are involved in the effects of testosterone in the PVAT. Female mice lacking T and B cells ($Rag1^{-/-}$ mice), female $Rag1^{-/-}$ that received $CD4^+$ T cells from WT

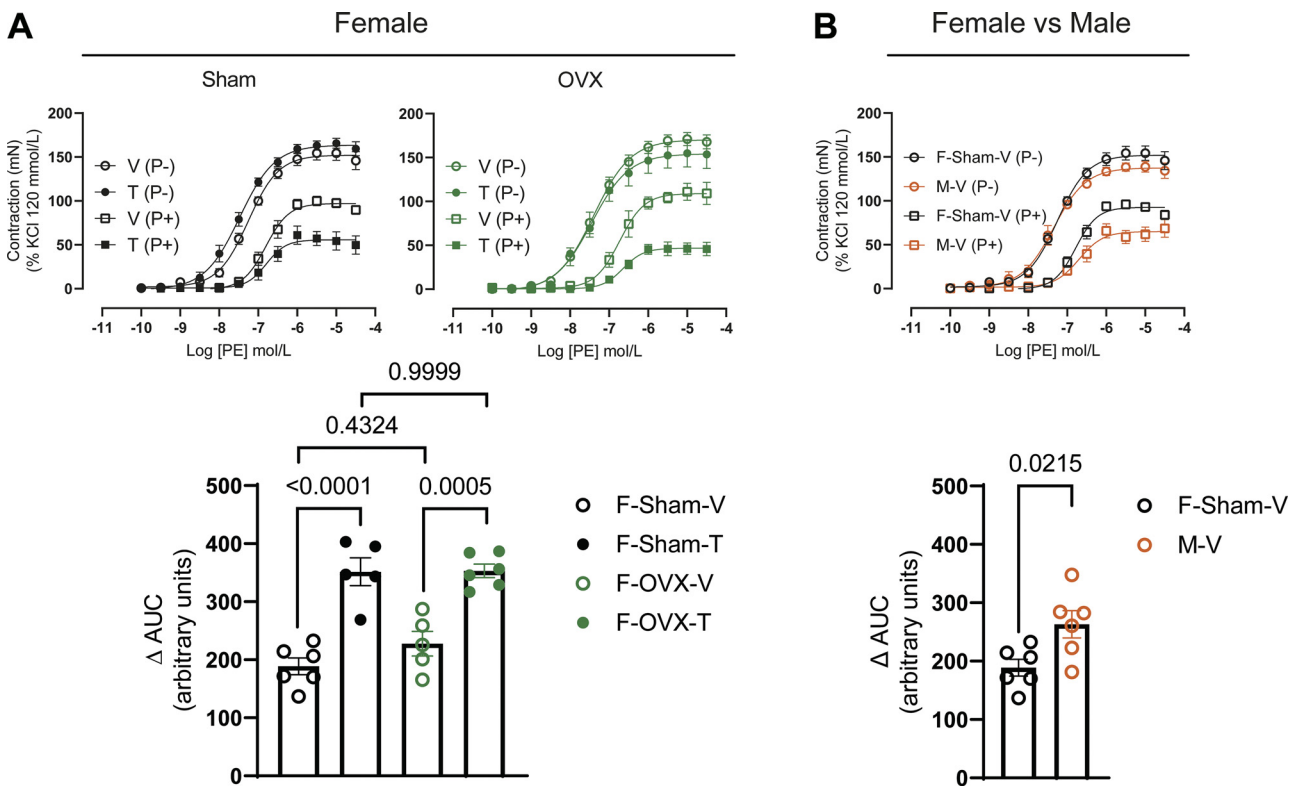

Figure 3. Testosterone for 8 wk increases anticontractile effect of the perivascular adipose tissue (PVAT) in an experimental gender-affirming hormone therapy (GAHT) model. Vasoconstrictor responses were assessed through concentration-effect curves to phenylephrine (PE) in PVAT-intact (P+) or PVAT-denuded (P−) aortic rings (*top*) from intact (sham, n = 5–6) or ovariectomized (OVX, n = 5–6) C57BL/6J female (F) mice (*A*) or male (M, n = 6) mice (*B*), treated with testosterone cypionate (T, 48 mg/kg/wk) or vehicle (V) for 8 wk. PVAT anticontractile effect was inferred by the difference between the area under the curve in the P− and P+ groups (ΔAUC, *bottom*). Data are expressed as means ± SE; dots covering bars indicate biological replicates (n). Statistical differences were determined by two-way ANOVA followed by Tukey's test within female groups, and Student's t test between F-sham-V and M-V. $P < 0.05$, statistically significant values.

female mice ($CD4^+$ T cells adoptive transfer), and female mice lacking the IL-17 receptor ($IL\text{-}17Ra^{-/-}$) were treated with testosterone for 8 wk. All the in vivo protocols were carried out as previously described (67). Testosterone administration did not change PVAT function in vessels from female $Rag\text{-}1^{-/-}$ or $IL\text{-}17Ra^{-/-}$ mice. However, $CD4^+$ T cells transfer into $Rag\text{-}1^{-/-}$ female mice restored testosterone-induced increased PVAT anticontractile effect (Fig. 4*A*). After euthanasia of testosterone-treated female WT mice, the PVAT was removed and cDNA was obtained from 1 μg of total RNA for Rorc expression analysis by qRT-PCR. RNA isolation, conversion to cDNA, and gene expression analysis, including the primer sequences, were performed as previously described (67). Testosterone increased *Rorc* expression in the PVAT from WT female mice (Fig. 4*B*), suggesting the presence of Th17 cells in the PVAT. Together, these data point to Th17 cells as crucial to PVAT dysfunction in a testosterone-GAHT mouse model. However, mechanisms involved in testosterone-induced PVAT functional changes should be further addressed.

Psychosocial and environmental aspects influencing cardiovascular risk in transmasculine individuals.

As discussed in earlier sections, testosterone GAHT relieves gender dysphoria, usually by improving physical aspects (i.e., by aligning gender identity and the primary and secondary sexual features). However, the social component of gender incongruence (i.e., regardless of physical traits, whether other people in the society recognize transgender individuals by their current self-affirming gender identity and not by the gender assigned to them at birth) plays a critical role in gender dysphoria development. In addition, transgender people (transmasculine included) usually suffer social and economic historical marginalization, disease-linked perception by others, social stigma, discrimination, and, many times, violence (33). This adverse environment may impact cardiovascular health, since poor social determinants of health (SDoH), including access to healthcare services, are linked to higher cardiovascular risk factors and worse clinical outcomes such as stroke, myocardial infarction, coronary heart disease, and heart failure (160). Of importance, many SDoH domains or individual social aspects are not interchangeable among different populations. From a transgender healthcare perspective, a refined look at the relationship between the SDoH and cardiovascular risk, considering peculiar intersectional factors and aiming for more accurate clinical inferences, is warranted.

Chronic stress experienced by transmasculine individuals triggers mechanisms that might influence cardiovascular function. Activation of the biological stress response system, which comprises the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (mainly the sympathetic nervous systems, including the sympathoadrenal-medullary axis) (161), releases several modulators of cardiovascular performance,

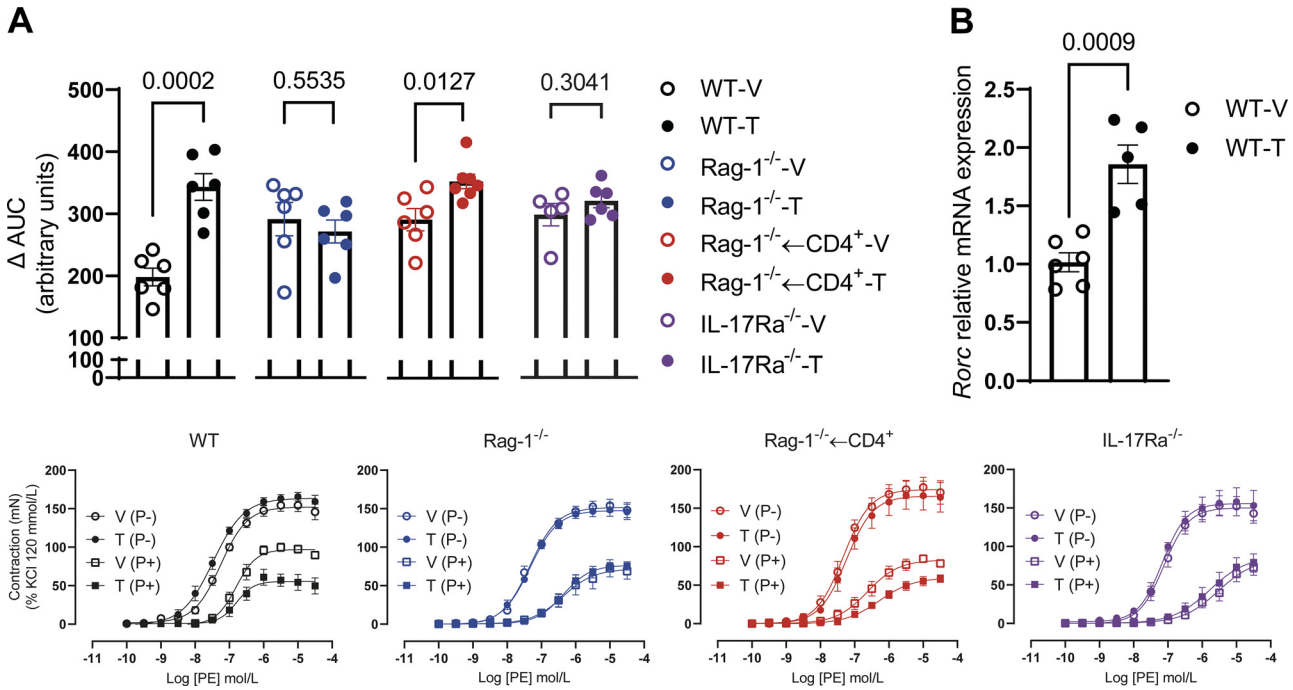

Figure 4. Th17 cell-linked mechanisms are involved in perivascular adipose tissue (PVAT) alterations induced by testosterone in female mice. *A*: PVAT anticontractile effect was inferred by the difference between the area under the concentration-effect curves (ΔAUC, *top*) to phenylephrine (PE)-induced vasoconstriction in PVAT-denuded (P−) and PVAT-intact (P+) aortic rings (*bottom*) from wild-type (WT, $n = 6$), Rag1$^{-/-}$ (after CD4$^+$ T cells adoptive transfer, $n = 6–7$), or IL-17Ra$^{-/-}$ ($n = 5–6$) female mice, treated with testosterone cypionate (T, 48 mg/kg/wk) or vehicle (V) for 8 wk. *B*: analysis of *Rorc* gene expression (gene coding RORγt, by qRT-PCR) in the PVAT isolated from vehicle- and testosterone-treated WT female mice ($n = 5–6$). Data are expressed as means ± SE; dots covering bars indicate biological replicates (n). Statistical differences were determined by Student's t test within each mouse strain. $P < 0.05$, statistically significant values. ←CD4$^+$, Rag1$^{-/-}$ female mice after adoptive transfer of CD4$^+$ T cells (from WT female mice).

such as catecholamines, serotonin, ANG II, mineralocorticoids, cortisol, and dehydroepiandrosterone (DHEA) (162, 163). In addition to their direct cardiac, vascular, and renal effects, stress response-derived mediators also activate proinflammatory mechanisms and induce oxidative stress. A detailed discussion concerning molecular mechanisms whereby chronic stress and adverse environmental factors impair cardiovascular function and increases CVD prevalence can be found in comprehensive reviews (163, 164). Highlighting the importance of this topic, it is not known whether testosterone GAHT has a greater cardiovascular impact in transmasculine individuals who have gender dysphoria, warranting further studies.

EXPERIMENTAL MODELS OF TESTOSTERONE-GAHT

An important limiting factor in cardiovascular research linked to testosterone GAHT is the paucity of appropriate experimental animal models. Although androgens-induced cardiovascular effects in female models have been explored, very few preclinical studies addressed testosterone effects in the GAHT perspective.

Slight differences in experimental models aimed to mimic testosterone GAHT should be noted. A GAHT experimental model is distinct from other approaches where females are exposed to androgen excess. First, testosterone is preferably chosen among other androgens, since it is the main compound used in GAHT. Pharmacological aspects including dose, route, volume, and frequency of administration of the different pharmaceutical formulations should also be considered. Efficacy of the treatment should be addressed according to laboratorial and clinical testosterone GAHT aims, considering peculiarities and intrinsic limitations among experimental species. To mimic overall GAHT clinical aims, testosterone administration to females in adulthood should increase circulating testosterone to levels found in healthy males (e.g., in healthy male mice or rats, when rodents are used) and decrease estrogen's endogenous secretion. To improve the model prediction, testosterone should also change body appearance/body composition (considering that detection and measurements are possible). The "male"-like phenotype should be confirmed through comparisons with male-control groups. "Defeminization" effects by testosterone (i.e., reduction or interruption of physiological female-related processes) should also be addressed. Figure 5 summarizes important aspects regarding experimental approaches and translational perspectives (detailed in later sections) related to testosterone GAHT in preclinical studies.

Goetz and colleagues suggested a testosterone GAHT model for the first time in 2017 (165). C57BL/6, 6-wk-old, female mice underwent bilateral OVX and received weekly subcutaneous testosterone injections (0.0031 mg/wk) for 6 or 10 wk. As determined by dual-energy X-ray absorptiometry measurement, the two treatment periods did not change body composition, despite increasing circulating testosterone levels. No intact-female or male control groups were included in the analysis. In a later study, the authors did not report anthropometric parameters after 16 wk of testosterone treatment in OVX ApoE$^{-/-}$ female mice (152).

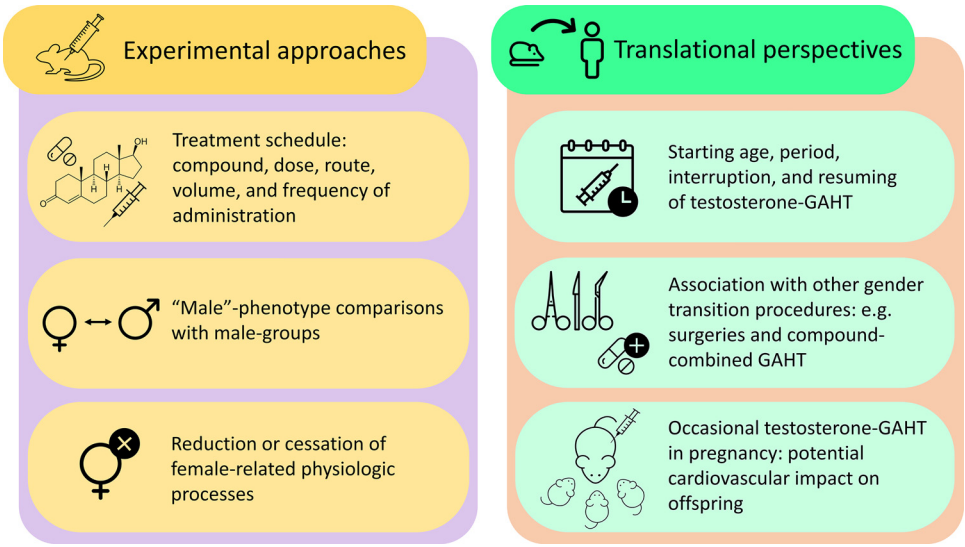

Figure 5. Critical points in experimental models and preclinical studies on testosterone gender-affirming hormone therapy (GAHT). Peculiarities in experimental models of testosterone GAHT that should be considered to increase translational data: *1*) testosterone should be preferably chosen among other androgens, and pharmacological aspects such as dose, route, volume, and frequency of administration in female models should mimic clinical use; *2*) GAHT should induce a "male" phenotype (e.g., increased circulating testosterone levels, and increased body mass index and lean mass, for rodents) verified by comparisons with male-control groups; *3*) "defeminization" effects (i.e., reduction or interruption of physiological female-related processes) are also desired (e.g., dropping of circulating estrogen levels, and estrus cycle cessation); *4*) the diversity of testosterone GAHT adherence by transmasculine population; and *5*) different starting age and duration of the GAHT. Experimental studies have the potential to address whether testosterone effects remain after interruption or resumption of the treatment or are aggravated by association with other gender transition procedures (e.g., surgeries, other compounds offered in masculinizing-GAHT). Experimental studies also allow investigation of potential cardiovascular impact on offspring, if testosterone GAHT is performed during pregnancy or lactating period.

Another testosterone GAHT model was proposed by Kinnear and colleagues in 2019 (58). Female, 8–9-wk-old, C57BL/6 mice received subcutaneous testosterone enanthate injections (0.9 mg/wk, divided into two doses of 0.45 mg/day), increasing testosterone circulating levels. After 6 wk of treatment, testosterone promoted clitoromegaly and cessation of estrus cycles. Testosterone promoted changes in the reproductive system (e.g., PCOS-like ovaries changes), which were reverted after 5 wk of treatment interruption (59). Low doses of testosterone (0.45 mg/wk) did not promote "defeminization" effects (i.e., cessation of estrus cycles), whereas higher testosterone doses (1.8 mg/wk) induced vaginal prolapse and were, therefore, not used in further studies. Anthropometric measurements were not assessed, and a male-control phenotype group was not included for comparisons in these studies.

A more refined testosterone GAHT experimental model was suggested by Bartels and colleagues in 2020 (166). Female, 6-wk-old, CF-1 mice were treated weekly with subcutaneous cypionate testosterone injections (0.4 mg/wk). The treatment increased testosterone circulating concentrations in female mice to levels found in age-matched male CD-1 mice. Similar to Kinnear's model, testosterone induced clitoral enlargement and cessation of estrus cycles after 6 wk of treatment. Interruption of testosterone treatment for 5 wk reverted those alterations. Despite the inclusion of a male-control group, no "male"-phenotype parameters were assessed.

Lichtenecker and colleagues (68) described a testosterone GAHT model using 8-wk-old female Wistar rats, which received intramuscular cypionate testosterone injections [3 mg/kg, every 10 days (weight and time adjusted: ~0.58 mg/wk)]. After 16 wk, the treatment increased circulating testosterone to levels found in male rats and promoted cessation of estrus cycles. Testosterone increased body weight and lean body mass, and decreased adipose mass, determined by dual-energy X-ray absorptiometry. However, comparisons to male-control rats were not reported.

Finally, we recently reported a mouse model that more closely mimics testosterone GAHT in mice (67). After preliminary treatment tests, 8-wk-old, female C57BL/6 mice received weekly subcutaneous cypionate testosterone injections [48 mg/kg/wk, divided into two doses of 24 mg/kg/day (weight adjusted: ~1.0 mg/wk)]. After 8 wk, testosterone administration to female mice increased serum testosterone concentrations and BMI to levels found in male mice and decreased adipose tissue weight. Corroborating the anabolic effects of testosterone, the treatment induced skeletal muscle fiber enlargement (to levels observed in control male mice) and increased muscle tissue weight (dry weight) in female mice, suggesting increased body lean mass. OVX and a longer testosterone treatment period (24 wk) did not alter the "male" phenotype induced by testosterone.

Although testosterone GAHT models are currently available, important clinical questions are unresolved, and further studies will certainly help to fill the gaps, as will be discussed in the next section.

LIMITATIONS IN CARDIOVASCULAR RESEARCH IN TRANSMASCULINE PEOPLE AND FUTURE DIRECTIONS

Age When GAHT Starts and Evaluation of End Points for Short Times of GAHT

Important aspects limit the full comprehension of GAHT-related cardiovascular health and risk and represent major

challenges for future studies. First, the individuals' age when GAHT starts and evaluation of end points only for short times of GAHT are important restraining aspects in the cardiovascular assessment of transgender people. Aging itself leads to cardiovascular dysfunction, increasing CVD prevalence (167), and studies evaluating whether aging impacts cardiovascular outcomes induced by testosterone GAHT are necessary. As an example, a retrospective study on GAHT-induced cognitive effects after long periods (over 10 yr) was performed only in transfeminine individuals, and no data on transmasculine people are available (168). In addition, increased carotid intima-media thickness in transmasculine people on testosterone GAHT was positively related to age ($r = 0.390$, $P = 0.04$) (169). In an observational study with 365 transmasculine on testosterone GAHT, only six individuals were >65 yr old, and myocardial infarction was reported in a 72-yr-old person (82). Cardiovascular events or cardiovascular-related mortality rates were higher in transfeminine than in the transmasculine group. However, the transfeminine group was older than the transmasculine subjects (≥40 yr age range: 21.5 vs. 16%, respectively).

Regarding GAHT duration, increased cardiovascular risk in transfeminine individuals was related to a longer time GAHT follow-up ratio (over 30 yr) compared with the transmasculine group (9.5 vs. 5.2%, respectively) (82). Although GAHT may start at different time points in adulthood, in most cases, the use of the hormones is maintained across the lifespan of transgender individuals, and experimental studies suggest that longer exposure times to male-related factors blunt female-related cardioprotective mechanisms (67). Thus, further studies are needed to evaluate the long-term impact of testosterone GAHT.

Sample Sizes

Small sample sizes limit accurate evaluation of GAHT-related cardiovascular risk. A recent systematic review analyzed 13 pre- and postobservational studies on blood pressure in transmasculine people and showed incongruent data; three studies showed that testosterone increased SBP, whereas nine studies reported no differences in SBP. Furthermore, 10 studies did not show differences in DBP. However, only two studies demonstrated good quality ratings and were powered to show significant changes in blood pressure, limiting precise conclusions (170). Ongoing studies also have limited sample sizes (Table 3). Of importance, people enrollment and permanence in the studies throughout time evaluation greatly impact sample size, and the paucity of friendly and specialized healthcare centers reduces the number of transgender people seeking assistance (33).

Data Analysis

Data analysis design is another point. In a retrospective study, transmasculine people showed a similar myocardial infarction rate compared with cisgender men (7.2 vs. 5.6%, respectively, $P = 0.30$), but higher than cisgender woman (3.1%, $P < 0.01$) (171). Serum HDL-C levels in transmasculine people and cisgender men were similar, but lower than in cisgender women (44). In a testosterone GAHT experimental model, testosterone-treated female rats showed increased SBP [(means ± SE, mmHg) 131 ± 1.4] compared with vehicle-treated female rats (119 ± 1.0, $P < 0.0001$), but similar to vehicle-treated male rats (126 ± 3.3) (68). Moreover, vehicle-treated female and male mice exhibited similar endothelial-dependent vasodilation, whereas testosterone-treated female mice showed endothelial dysfunction compared with vehicle-treated females (67). Endothelial function in transmasculine individuals on testosterone GAHT is also impaired compared with cisgender women (86). Importantly, although testosterone GAHT in transmasculine people aims to achieve male physiological circulating levels of the hormone (i.e., testosterone levels found in healthy cisgender men), the fact that those individuals are influenced by embryological and developmental female-related factors, should be considered. Sex-related factors in host organisms are key to testosterone-induced cardiovascular effects and, therefore, comparisons with cisgender female individuals may reveal substantial differences on the biological factors being evaluated. Furthermore, paired analysis within the same transmasculine group may offer a more precise cardiovascular risk evaluation induced by testosterone GAHT, since the paucity of access to healthcare services by transgender individuals greatly contrasts with the healthcare available to the cisgender population (33). Of note, some nonbinary transmasculine individuals do not wish body changes; they undergo gender transition to defeminize themselves rather than masculinize their body appearance, requiring lower doses of testosterone (172). Thus, the data heterogenicity within the transmasculine groups should be also considered.

Comparisons with Other Conditions in Which Biological Females Receive or Display High Levels of Androgens

Occasionally, evaluation of testosterone-induced cardiovascular effects in transmasculine people is extrapolated from other conditions in which female individuals received or display high levels of androgens. However, peculiarities between pathophysiological processes versus GAHT should be noted. For example, serum testosterone levels in women with PCOS or testosterone-recommended doses in hormone replacement therapy for postmenopause women are lower than those used by transmasculine individuals on GAHT (40), potentially reflecting different clinical outcomes. A study evaluating metabolic parameters of 29 transmasculine people, who received intramuscular testosterone injections for 1 yr, noted that serum HDL-C levels were lower in those subjects [(means, mg/dL) 43, 95% CI: 40–47] versus age-matched cisgender woman with PCOS (65, 50% CI: 58–73, $P < 0.001$) (173). Recently, it was shown that carotid intima-media thickness is higher in transmasculine individuals on testosterone GAHT for at least 1 yr [(means ± SD, mm) 0.48 ± 0.09] than on PCOS (0.41 ± 0.09, $P = 0.005$) or healthy cisgender women (0.38 ± 0.7, $P = 0.001$) (169). Regarding postmenopause therapy comparisons, testosterone decreased endothelial-mediated vasodilation in transmasculine people [(FMD, means ± SD, %) 4.5 ± 2.7 vs. 8.1 ± 2.9 in cisgender woman, $P = 0.002$] (86) and improved endothelial function in healthy cisgender woman on postmenopause testosterone replacement therapy for 6 mo [(FMD, means ± SE, %) 9.1 ± 1.1 vs. 6.4 at baseline, $P = 0.03$] (174).

Previous Cardiometabolic Comorbidities and Lifestyle

Cardiometabolic comorbidities that increase cardiovascular risk are also an important limitation in transgender people-related cardiovascular research. A retrospective study reported greater obesity prevalence in transfeminine (9.9%) and

transmasculine (6.6%) adolescents than in cisgender girls (2.2%) and boys (3.0%) (175). Moreover, 80.8% from 277 transgender people showed overweight and obesity, and transmasculine individuals showed a higher BMI z-score (BMIz) than transfeminine people (176). In a group with 69 transmasculine people, 58% had PCOS before starting testosterone GAHT (177). Concerning lifestyle, habits that increase cardiovascular risk are especially experienced by the transgender community. Alcohol use and smoking ratio was higher in transgender than cisgender individuals (6.7 vs. 6.0%, and 22.4 vs. 20.0%, respectively) (71). Daily smoking rate was also higher in transmasculine people (14.3%) than in cisgender woman (10.2%) (171). In addition, of 138 transgender patients with CVD, 60.5% were current or former smokers, 35.9% used alcohol in the past 30 days of data analysis, and 61.1% did not regularly practice physical exercises (178).

Paucity of Experimental Studies

Finally, the paucity of experimental studies limits knowledge of mechanisms involved in cardiovascular damage induced by testosterone GAHT. Furthermore, essential aspects related to testosterone use by transmasculine people, from a translational perspective, are not covered by available study designs. For example, starting testosterone GAHT at different time points in adulthood would closely mimic the variability seen in transmasculine people who undergo gender transition. Also, longer testosterone-treatment periods certainly will elucidate current gaps, especially those associated with other gender transition approaches, such as surgical procedures. Testosterone treatment in association with progestational agents (compounds also included in the masculinizing-GAHT collection) should also be considered, because of their cardiovascular effects (107).

The pattern of testosterone treatment is a special aspect that should be considered in experimental designs. Studies addressing cardiovascular effects at different time points of testosterone-treatment interruption may provide answers concerning the maintenance, or not, of the testosterone-induced cardiovascular phenotype. Of clinical importance, at least 39% of transmasculine individuals have current or future parental desire (179). Considering the testosterone-induced alterations in the reproductive system, testosterone GAHT suspension is essential before pregnancy attempts (34). However, transmasculine people with biological children may experience increased gender dysphoria during pregnancy or postpartum, e.g., because of the development of lactating mammary tissue, leading them to resume testosterone GAHT (180). The cardiovascular impact of interrupting and resuming testosterone use, i.e., whether deleterious cardiovascular effects are intensified) is still unknown. Furthermore, experimental evidence shows increased adulthood cardiovascular events in offspring prenatally exposed to testosterone (181–186), suggesting that cardiovascular monitoring in biological children from transmasculine individuals who used testosterone during pregnancy could prevent harmful cardiovascular outcomes.

CONCLUSION AND CLINICAL PERSPECTIVES

The worldwide population who identifies as transgender and seek healthcare services represents an important clinical demand. The cardiovascular impact of testosterone GAHT is debatable and still very preliminary. Reported harmful cardiovascular effects induced by testosterone GAHT include increased blood pressure, impaired vasodilation, arterial stiffness, dyslipidemia, maladaptive cardiac hypertrophy, and increased inflammatory markers. These events can potentially increase cardiovascular risk in those individuals and may significantly impact the healthcare system worldwide. In addition, unsupervised or indiscriminate use of testosterone by transmasculine people, especially in low- and middle-income countries, increases the risk of adverse events and can further impact the health system (187).

Current clinical data assessing the cardiovascular effects of testosterone GAHT are mainly derived from cross-sectional studies, and data from longitudinal studies are missing. GAHT duration and starting age of individuals, the existence of cardiometabolic comorbidities, and sample size should be considered for more accurate evaluation. Preclinical studies addressing the cardiovascular effects induced by testosterone GAHT, especially studies aimed to investigate mechanisms that mediate testosterone effects, are essential to prevent or mitigate adverse events associated with testosterone GAHT. Preclinical studies should consider *1*) pharmacological aspects such as the choice of the androgen, dose, route, volume, and frequency of administration to closely mimic clinical use; *2*) the induction of a "male" phenotype; *3*) "defeminization" effects of the GAHT; and *4*) starting age and duration of GAHT. Together, these aspects certainly will contribute to testosterone GAHT optimization, decreasing the cardiovascular risk for transmasculine individuals.

SUPPLEMENTAL DATA

Supplemental material: https://doi.org/10.6084/m9.figshare.22811462.v1.

GRANTS

This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-CRID 2013/08216-2 to R.C.T.), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.D.S. and R.C.T. conceived and designed research; J.D.S. performed experiments; J.D.S. analyzed data; J.D.S., J.T.O-N., and R.C.T. interpreted results of experiments; J.D.S. prepared figures; J.D.S. and J.T.O-N. drafted manuscript; J.D.S. and R.C.T. edited and revised manuscript; J.D.S., J.T.O-N., and R.C.T. approved final version of manuscript.

REFERENCES

1. **Tokatli MR**, **Sisti LG**, **Marziali E**, **Nachira L**, **Rossi MF**, **Amantea C**, **Moscato U**, **Malorni W.** Hormones and sex-specific medicine in human physiopathology. *Biomolecules* 12: 413, 2022. doi:10.3390/biom12030413.

2. **McEwen BS**, **Milner TA.** Understanding the broad influence of sex hormones and sex differences in the brain. *J Neurosci Res* 95: 24–39, 2017. doi:10.1002/jnr.23809.
3. **Turcu A**, **Smith JM**, **Auchus R**, **Rainey WE.** Adrenal androgens and androgen precursors: definition, synthesis, regulation and physiologic actions. *Compr Physiol* 4: 1369–1381, 2014. doi:10.1002/cphy.c140006.
4. **Fester L**, **Rune GM.** Sex neurosteroids: hormones made by the brain for the brain. *Neurosci Lett* 753: 135849, 2021. doi:10.1016/j.neulet.2021.135849.
5. **Chen W**, **Tsai S-J**, **Sheu H-M**, **Tsai J-C**, **Zouboulis CC.** Testosterone synthesized in cultured human SZ95 sebocytes derives mainly from dehydroepiandrosterone. *Exp Dermatol* 19: 470–472, 2010. doi:10.1111/j.1600-0625.2009.00996.x.
6. **Amer SA**, **Alzanati NG**, **Warren A**, **Tarbox R**, **Khan R.** Excess androgen production in subcutaneous adipose tissue of women with polycystic ovarian syndrome is not related to insulin or LH. *J Endocrinol* 241: 99–109, 2019. doi:10.1530/JOE-18-0674.
7. **Schuppe ER**, **Tobiansky D**, **Goller F**, **Fuxjager MJ.** Specialized androgen synthesis in skeletal muscles that actuate elaborate social displays. *J Exp Biol* 225: jeb243730, 2022. doi:10.1242/jeb.243730.
8. **Quinkler M**, **Bumke-Vogt C**, **Meyer B**, **Bähr V**, **Oelkers W**, **Diederich S.** The human kidney is a progesterone-metabolizing and androgen-producing organ. *J Clin Endocrinol Metab* 88: 2803–2809, 2003. doi:10.1210/jc.2002-021970.
9. **Milewich L**, **Kaimal V**, **Johnson AR.** Steroid 5α-reductase activity in endothelial cells from human umbilical cord vessels. *J Steroid Biochem* 26: 561–567, 1987. doi:10.1016/0022-4731(87)90008-2.
10. **Burger HG.** Androgen production in women. *Fertil Steril* 77: S3–S5, 2002. doi:10.1016/S0015-0282(02)02985-0.
11. **Negro-Vilar A.** Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J Clin Endocrinol Metab* 84: 3459–3462, 1999. doi:10.1210/jcem.84.10.6122.
12. **Nilsson S**, **Mäkelä S**, **Treuter E**, **Tujague M**, **Thomsen J**, **Andersson G**, **Enmark E**, **Pettersson K**, **Warner M**, **Gustafsson J-Å.** Mechanisms of estrogen action. *Physiol Rev* 81: 1535–1565, 2001. doi:10.1152/physrev.2001.81.4.1535.
13. **Boynton RE**, **Todd RL.** Blood pressure readings of 75,258 university students. *Arch Intern Med (Chic)* 80: 454–462, 1947. doi:10.1001/archinte.1947.00220160033003.
14. **Joyner MJ**, **Barnes JN**, **Hart EC**, **Wallin BG**, **Charkoudian N.** Neural control of the circulation: how sex and age differences interact in humans. *Compr Physiol* 5: 193–215, 2015. doi:10.1002/cphy.c140005.
15. **Gerdts E**, **Sudano I**, **Brouwers S**, **Borghi C**, **Bruno RM**, **Ceconi C**, **Cornelissen V**, **Diévart F**, **Ferrini M**, **Kahan T**, **Løchen M-L**, **Maas AHEM**, **Mahfoud F**, **Mihailidou AS**, **Moholdt T**, **Parati G**, **de Simone G.** Sex differences in arterial hypertension. *Eur Heart J* 43: 4777–4788, 2022. doi:10.1093/eurheartj/ehac470.
16. **Ashraf MS**, **Vongpatanasin W.** Estrogen and hypertension. *Curr Hypertens Rep* 8: 368–376, 2006. doi:10.1007/s11906-006-0080-1.
17. **Burt VL**, **Whelton P**, **Roccella EJ**, **Brown C**, **Cutler JA**, **Higgins M**, **Horan MJ**, **Labarthe D.** Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988–1991. *Hypertension* 25: 305–313, 1995. doi:10.1161/01.HYP.25.3.305.
18. **Kapil V**, **Milsom AB**, **Okorie M**, **Maleki-Toyserkani S**, **Akram F**, **Rehman F**, **Arghandawi S**, **Pearl V**, **Benjamin N**, **Loukogeorgakis S**, **MacAllister R**, **Hobbs AJ**, **Webb AJ**, **Ahluwalia A.** Inorganic nitrate in supplementation lowers blood pressure in humans: role for nitrate-derived NO. *Hypertension* 56: 274–281, 2010 [Erratum in *Hypertension* 56: e37–e39, 2010]. doi:10.1161/HYPERTENSIONAHA.110.153536.
19. **Virani SS**, **Alonso A**, **Benjamin EJ**, **Bittencourt MS**, **Callaway CW**, **Carson AP**, **et al.** Heart Disease and Stroke Statistics—2020 Update: a report from the American Heart Association. *Circulation* 141: e139–e596, 2020. doi:10.1161/CIR.0000000000000757.
20. **Dubey R**, **Oparil S**, **Imthurn B**, **Jackson EK.** Sex hormones and hypertension. *Cardiovasc Res* 53: 688–708, 2002. doi:10.1016/S0008-6363(01)00527-2.
21. **Crofton JT**, **Share L.** Gonadal hormones modulate deoxycorticosterone-salt hypertension in male and female rats. *Hypertension* 29: 494–499, 1997. doi:10.1161/01.HYP.29.1.494.
22. **Harrison-Bernard LM**, **Schulman IH**, **Raij L.** Postovariectomy hypertension is linked to increased renal AT1 receptor and salt sensitivity. *Hypertension* 42: 1157–1163, 2003. doi:10.1161/01.HYP.0000102180.13341.50.
23. **Wu C-C**, **Cheng J**, **Zhang FF**, **Gotlinger KH**, **Kelkar M**, **Zhang Y**, **Jat JL**, **Falck JR**, **Schwartzman ML.** Androgen-dependent hypertension is mediated by 20-hydroxy-5,8,11,14-eicosatetraenoic acid-induced vascular dysfunction: role of inhibitor of kappaB kinase. *Hypertension* 57: 788–794, 2011. doi:10.1161/HYPERTENSIONAHA.110.161570.
24. **Bubb KJ**, **Khambata RS**, **Ahluwalia A.** Sexual dimorphism in rodent models of hypertension and atherosclerosis. *Br J Pharmacol* 167: 298–312, 2012. doi:10.1111/j.1476-5381.2012.02036.x.
25. **Colby HD**, **Skelton FR**, **Brownie AC.** Testosterone-induced hypertension in the rat. *Endocrinology* 86: 1093–1101, 1970. doi:10.1210/endo-86-5-1093.
26. **Singh H**, **Schwartzman ML.** Renal vascular cytochrome P450-derived eicosanoids in androgen-induced hypertension. *Pharmacol Rep* 60: 29–37, 2008.
27. **Chen YF**, **Meng QC.** Sexual dimorphism of blood pressure in spontaneously hypertensive rats is androgen dependent. *Life Sci* 48: 85–96, 1991. doi:10.1016/0024-3205(91)90428-E.
28. **Reckelhoff JF**, **Zhang H**, **Granger JP.** Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* 31: 435–439, 1998. doi:10.1161/01.HYP.31.1.435.
29. **Willemars MMA**, **Nabben M**, **Verdonschot JAJ**, **Hoes MF.** Evaluation of the Interaction of Sex Hormones and Cardiovascular Function and Health. *Curr Heart Fail Rep* 19: 200212, 2022. doi:10.1007/s11897-022-00555-0.
30. **Robinson AT**, **Wenner MM**, **Bunsawat K**, **Watso JC**, **Giersch GEW**, **Charkoudian N.** When it's time for the sex talk, words matter. *Am J Physiol Heart Circ Physiol* 322: H66–H70, 2022. doi:10.1152/ajpheart.00556.2021.
31. **Coleman E**, **Radix AE**, **Bouman WP**, **Brown GR**, **de Vries ALC**, **Deutsch MB**, **et al.** Standards of care for the health of transgender and gender diverse people, version 8. *Int J Transgend Health* 23: S1–S259, 2022. doi:10.1080/26895269.2022.2100644.
32. **Goodman M**, **Adams N**, **Corneil T**, **Kreukels B**, **Motmans J**, **Coleman E.** Size and distribution of transgender and gender nonconforming populations. *Endocrinol Metab Clin North Am* 48: 303–321, 2019. doi:10.1016/j.ecl.2019.01.001.
33. **Winter S**, **Diamond M**, **Green J**, **Karasic D**, **Reed T**, **Whittle S**, **Wylie K.** Transgender people: health at the margins of society. *Lancet* 388: 390–400, 2016. doi:10.1016/S0140-6736(16)00683-8.
34. **Hembree WC**, **Cohen-Kettenis PT**, **Gooren L**, **Hannema SE**, **Meyer WJ**, **Murad MH**, **Rosenthal SM**, **Safer JD**, **Tangpricha V**, **T'Sjoen GG.** Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 102: 3869–3903, 2017 [Erratum in *J Clin Endocrinol Metab* 103: 699, 2018]. doi:10.1210/jc.2017-01658.
35. **Petering RC**, **Brooks NA.** Testosterone therapy: review of clinical applications. *Am Fam Physician* 96: 441–449, 2017.
36. **T'Sjoen G**, **Arcelus J**, **Gooren L**, **Klink DT**, **Tangpricha V.** Endocrinology of transgender medicine. *Endocr Rev* 40: 97–117, 2019. doi:10.1210/er.2018-00011.
37. **Spratt DI**, **Stewart II**, **Savage C**, **Craig W**, **Spack NP**, **Chandler DW**, **Spratt LV**, **Eimicke T**, **Olshan JS.** Subcutaneous injection of testosterone is an effective and preferred alternative to intramuscular injection: demonstration in female-to-male transgender patients. *J Clin Endocrinol Metab* 102: 2349–2355, 2017. doi:10.1210/jc.2017-00359.
38. **Jacobeit JW**, **Gooren LJ**, **Schulte HM.** Safety aspects of 36 months of administration of long-acting intramuscular testosterone undecanoate for treatment of female-to-male transgender individuals. *Eur J Endocrinol* 161: 795–798, 2009. doi:10.1530/EJE-09-0412.
39. **Pelusi C**, **Costantino A**, **Martelli V**, **Lambertini M**, **Bazzocchi A**, **Ponti F**, **Battista G**, **Venturoli S**, **Meriggiola MC.** Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med* 11: 3002–3011, 2014. doi:10.1111/jsm.12698.
40. **Irwig MS.** Testosterone therapy for transgender men. *Lancet Diabetes Endocrinol* 5: 301–311, 2017 [Erratum in *Lancet Diabetes Endocrinol* 5: e2, 2017]. doi:10.1016/S2213-8587(16)00036-X.
41. **Klaver M**, **Dekker MJHJ**, **de Mutsert R**, **Twisk JWR**, **den Heijer M.** Cross-sex hormone therapy in transgender persons affects total body weight, body fat and lean body mass: a meta-analysis. *Andrologia* 49: e12660, 2017. doi:10.1111/and.12660.

42. **Velho I**, **Fighera TM**, **Ziegelmann PK**, **Spritzer PM.** Effects of testosterone therapy on BMI, blood pressure, and laboratory profile of transgender men: a systematic review. *Andrology* 5: 881–888, 2017. doi:10.1111/andr.12382.
43. **Gooren LJ.** Management of female-to-male transgender persons. *Curr Opin Endocrinol Diabetes Obes* 21: 233–238, 2014. doi:10.1097/MED.0000000000000064.
44. **Nokoff NJ**, **Scarbro SL**, **Moreau KL**, **Zeitler P**, **Nadeau KJ**, **Juarez-Colunga E**, **Kelsey MM.** Body composition and markers of cardiometabolic health in transgender youth compared with cisgender youth. *J Clin Endocrinol Metab* 105: e704–e714, 2020. doi:10.1210/clinem/dgz029.
45. **Giltay EJ**, **Gooren LJG.** Effects of sex steroid deprivation/administration on hair growth and skin sebum production in transsexual males and females. *J Clin Endocrinol Metab* 85: 2913–2921, 2000. doi:10.1210/jcem.85.8.6710.
46. **Wierckx K**, **Van de Peer F**, **Verhaeghe E**, **Dedecker D**, **Van Caenegem E**, **Toye K**, **Kaufman JM**, **T'Sjoen G.** Short- and long-term clinical skin sffects of testosterone treatment in trans men. *J Sex Med* 11: 222–229, 2014. doi:10.1111/jsm.12366.
47. **Wierckx K**, **Van Caenegem E**, **Schreiner T**, **Haraldsen I**, **Fisher A**, **Toye K**, **Kaufman JM**, **T'Sjoen G.** Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: Results from the European network for the investigation of gender incongruence. *J Sex Med* 11: 1999–2011, 2014 [Erratum in *J Sex Med* 13: 732, 2016]. doi:10.1111/jsm.12571.
48. **Nygren U**, **Nordenskjöld A**, **Arver S**, **Södersten M.** Effects on voice fundamental frequency and satisfaction with voice in trans men during testosterone treatment—a longitudinal study. *J Voice* 30: 766.e23–766–e34, 2016. doi:10.1016/j.jvoice.2015.10.016.
49. **Cosyns M**, **Van Borsel J**, **Wierckx K**, **Dedecker D**, **Van de Peer F**, **Daelman T**, **Laenen S**, **T'Sjoen G.** Voice in female-to-male transsexual persons after long-term androgen therapy. *Laryngoscope* 124: 1409–1414, 2014. doi:10.1002/lary.24480.
50. **Costine BA**, **Oberlander JG**, **Davis MC**, **Penatti CAA**, **Porter DM**, **Leaton RN**, **Henderson LP.** Chronic anabolic androgenic steroid exposure alters corticotropin releasing factor expression and anxiety-like behaviors in the female mouse. *Psychoneuroendocrinology* 35: 1473–1485, 2010. doi:10.1016/j.psyneuen.2010.04.015.
51. **Keo-Meier CL**, **Herman LI**, **Reisner SL**, **Pardo ST**, **Sharp C**, **Babcock JC.** Testosterone treatment and MMPI–2 improvement in transgender men: a prospective controlled study. *J Consult Clin Psychol* 83: 143–156, 2015. doi:10.1037/a0037599.
52. **Colizzi M**, **Costa R**, **Todarello O.** Transsexual patients' psychiatric comorbidity and positive effect of cross-sex hormonal treatment on mental health: results from a longitudinal study. *Psychoneuroendocrinology* 39: 65–73, 2014. doi:10.1016/j.psyneuen.2013.09.029.
53. **Grynberg M**, **Fanchin R**, **Dubost G**, **Colau JC**, **Brémont-Weil C**, **Frydman R**, **Ayoubi J-M.** Histology of genital tract and breast tissue after long-term testosterone administration in a female-to-male transsexual population. *Reprod Biomed Online* 20: 553–558, 2010. doi:10.1016/j.rbmo.2009.12.021.
54. **Nakamura A**, **Watanabe M**, **Sugimoto M**, **Sako T**, **Mahmood S**, **Kaku H**, **Nasu Y**, **Ishii K**, **Nagai A**, **Kumon H.** Dose-response analysis of testosterone replacement therapy in patients with female to male gender identity disorder. *Endocr J* 60: 275–281, 2013. doi:10.1507/endocrj.EJ12-0319.
55. **Mueller A**, **Kiesewetter F**, **Binder H**, **Beckmann MW**, **Dittrich R.** Long-term administration of testosterone undecanoate every 3 months for testosterone in supplementation in female-to-male transsexuals. *J Clin Endocrinol Metab* 92: 3470–3475, 2007. doi:10.1210/jc.2007-0746.
56. **Baldassarre M**, **Giannone FA**, **Foschini MP**, **Battaglia C**, **Busacchi P**, **Venturoli S**, **Meriggiola MC.** Effects of long-term high dose testosterone administration on vaginal epithelium structure and estrogen receptor-α and -β expression of young women. *Int J Impot Res* 25: 172–177, 2013. doi:10.1038/ijir.2013.9.
57. **Spinder T**, **Spijkstra JJ**, **Van Den Tweel JG**, **Burger CW**, **VAN Kessel H**, **Hompes PGA**, **Gooren LJG.** The effects of long term testosterone administration on pulsatile luteinizing hormone secretion and on ovarian histology in eugonadal female to male transsexual subjects. *J Clin Endocrinol Metab* 69: 151–157, 1989. doi:10.1210/jcem-69-1-151.
58. **Kinnear HM**, **Constance ES**, **David A**, **Marsh EE**, **Padmanabhan V**, **Shikanov A**, **Moravek MB.** A mouse model to investigate the impact of testosterone therapy on reproduction in transgender men. *Hum Reprod* 34: 2009–2017, 2019. doi:10.1093/humrep/dez177.
59. **Kinnear HM**, **Hashim PH**, **Dela Cruz C**, **Rubenstein G**, **Chang FL**, **Nimmagadda L**, **Brunette MA**, **Padmanabhan V**, **Shikanov A**, **Moravek MB.** Reversibility of testosterone-induced acyclicity after testosterone cessation in a transgender mouse model. *F S Sci* 2: 116–123, 2021. doi:10.1016/j.xfss.2021.01.008.
60. **Light AD**, **Obedin-Maliver J**, **Sevelius JM**, **Kerns JL.** Transgender men who experienced pregnancy after female-to-male gender transitioning. *Obstet Gynecol* 124: 1120–1127, 2014. doi:10.1097/AOG.0000000000000540.
61. **Asscheman H**, **Gooren LJ**, **Eklund PL.** Mortality and morbidity in transsexual patients with cross-gender hormone treatment. *Metabolism* 38: 869–873, 1989. doi:10.1016/0026-0495(89)90233-3.
62. **Banks K**, **Kyinn M**, **Leemaqz SY**, **Sarkodie E**, **Goldstein D**, **Irwig MS.** Blood pressure effects of gender-affirming hormone therapy in transgender and gender-diverse adults. *Hypertension* 77: 2066–2074, 2021. doi:10.1161/HYPERTENSIONAHA.120.16839.
63. **Mueller A**, **Haeberle L**, **Zollver H**, **Claassen T**, **Kronawitter D**, **Oppelt PG**, **Cupisti S**, **Beckmann MW**, **Dittrich R.** Effects of intramuscular testosterone undecanoate on body composition and bone mineral density in female-to-male transsexuals. *J Sex Med* 7: 3190–3198, 2010. doi:10.1111/j.1743-6109.2010.01912.x.
64. **van Velzen DM**, **Paldino A**, **Klaver M**, **Nota NM**, **Defreyne J**, **Hovingh GK**, **Thijs A**, **Simsek S**, **T'Sjoen G**, **den Heijer M.** Cardiometabolic effects of testosterone in transmen and estrogen plus cyproterone acetate in transwomen. *J Clin Endocrinol Metab* 104: 1937–1947, 2019. doi:10.1210/jc.2018-02138.
65. **Emi Y**, **Adachi M**, **Sasaki A**, **Nakamura Y**, **Nakatsuka M.** Increased arterial stiffness in female-to-male transsexuals treated with androgen. *J Obstet Gynaecol Res* 34: 890–897, 2008. doi:10.1111/j.1447-0756.2008.00857.x.
66. **Colizzi M**, **Costa R**, **Scaramuzzi F**, **Palumbo C**, **Tyropani M**, **Pace V**, **Quagliarella L**, **Brescia F**, **Natilla LC**, **Loverro G**, **Todarello O.** Concomitant psychiatric problems and hormonal treatment induced metabolic syndrome in gender dysphoria individuals: a 2 year follow-up study. *J Psychosom Res* 78: 399–406, 2015. doi:10.1016/j.jpsychores.2015.02.001.
67. **Santos JD**, **Oliveira-Neto JT**, **Barros PR**, **Damasceno LEA**, **Lautherbach N**, **Assis AP**, **Silva CAA**, **Sorgi CA**, **Faccioli LH**, **Kettelhut IC**, **Salgado HC**, **Carneiro FS**, **Alves-Filho JC**, **Tostes RC.** Th17 cell-linked mechanisms mediate vascular dysfunction induced by testosterone in a mouse model of gender-affirming hormone therapy. *Am J Physiol Heart Circ Physiol* 323: H322–H335, 2022. doi:10.1152/ajpheart.00182.2022.
68. **Lichtenecker DCK**, **Argeri R**, **Castro Ch de M**, **Dias-da-Silva MR**, **Gomes GN.** Cross-sex testosterone therapy modifies the renal morphology and function in female rats and might underlie increased systolic pressure. *Clin Exp Pharmacol Physiol* 48: 978–986, 2021. doi:10.1111/1440-1681.13495.
69. **Irwig MS.** Hypertension in transgender individuals. *J Hum Hypertens*. In Press. 2022. doi:10.1038/s41371-022-00721-w.
70. **Nokoff NJ**, **Scarbro S**, **Juarez-Colunga E**, **Moreau KL**, **Kempe A.** Health and cardiometabolic disease in transgender adults in the United States: behavioral risk factor surveillance system 2015. *J Endocr Soc* 2: 349–360, 2018. doi:10.1210/js.2017-00465.
71. **Pharr JR**, **Batra K.** Propensity score analysis assessing the burden of non-communicable diseases among the transgender population in the United States using the behavioral risk factor surveillance system (2017–2019). *Healthcare (Basel)* 9: 696, 2021. doi:10.3390/healthcare9060696.
72. **Maraka S**, **Ospina NS**, **Rodriguez-Gutierrez R**, **Davidge-Pitts CJ**, **Nippoldt TB**, **Prokop LJ**, **Hassan MM.** Sex steroids and cardiovascular outcomes in transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 102: 3914–3923, 2017. doi:10.1210/jc.2017-01643.
73. **Saleheen D**, **Scott R**, **Javad S**, **Zhao W**, **Rodrigues A**, **Picataggi A**, **Lukmanova D**, **Mucksavage ML**, **Luben R**, **Billheimer J**, **Kastelein JJP**, **Boekholdt SM**, **Khaw K-T**, **Wareham N**, **Rader DJ.** Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. *Lancet Diabetes Endocrinol* 3: 507–513, 2015. doi:10.1016/S2213-8587(15)00126-6.
74. **van Velzen DM**, **Adorni MP**, **Zimetti F**, **Strazzella A**, **Simsek S**, **Sirtori CR**, **Heijer M. D**, **Ruscica M.** The effect of transgender

hormonal treatment on high density lipoprotein cholesterol efflux capacity. *Atherosclerosis* 323: 44–53, 2021. doi:10.1016/j.atherosclerosis.2021.03.008.

75. **Jacobeit JW**, **Gooren LJ**, **Schulte HM.** Long-acting intramuscular testosterone undecanoate for treatment of female-to-male transgender individuals. *J Sex Med* 4: 1479–1484, 2007. doi:10.1111/j.1743-6109.2007.00556.x.
76. **Giltay EJ**, **Hoogeveen EK**, **Elbers JM**, **Gooren LJ**, **Asscheman H**, **Stehouwer CD.** Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females. *J Clin Endocrinol Metab* 83: 550–553, 1998. doi:10.1210/jcem.83.2.4574.
77. **Rodriguez-Iturbe B**, **Pons H**, **Johnson RJ.** Role of the immune system in hypertension. *Physiol Rev* 97: 1127–1164, 2017. doi:10.1152/physrev.00031.2016.
78. **Bomfim GF**, **Cau SBA**, **Bruno AS**, **Fedoce AG**, **Carneiro FS.** Hypertension: a new treatment for an old disease? Targeting the immune system. *Br J Pharmacol* 176: 2028–2048, 2019. doi:10.1111/bph.14436.
79. **Giltay EJ**, **Fonk JC**, **von Blomberg BM**, **Drexhage HA**, **Schalkwijk C**, **Gooren LJ.** In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. *J Clin Endocrinol Metab* 85: 1648–1657, 2000. doi:10.1210/jcem.85.4.6562.
80. **Iannantuoni F**, **Salazar JD**, **Martinez de Marañon A**, **Bañuls C**, **López-Domènech S**, **Rocha M**, **Hurtado-Murillo F**, **Morillas C**, **Gómez-Balaguer M**, **Víctor VM.** Testosterone administration increases leukocyte-endothelium interactions and inflammation in transgender men. *Fertil Steril* 115: 483–489, 2021. doi:10.1016/j.fertnstert.2020.08.002.
81. **Van Kesteren PJ**, **Asscheman H**, **Megens JA**, **Gooren LJ.** Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin Endocrinol (Oxf)* 47: 337–342, 1997. doi:10.1046/j.1365-2265.1997.2601068.x.
82. **Asscheman H**, **Giltay EJ**, **Megens JAJ**, **De Ronde W**, **Van Trotsenburg MAA**, **Gooren LJG.** A long-term follow-up study of mortality in transsexuals receiving treatment with cross-sex hormones. *Eur J Endocrinol* 164: 635–642, 2011. doi:10.1530/EJE-10-1038.
83. **Wierckx K**, **Elaut E**, **Declercq E**, **Heylens G**, **De Cuypere G**, **Taes Y**, **Kaufman JM**, **T'Sjoen G.** Prevalence of cardiovascular disease and cancer during cross-sex hormone therapy in a large cohort of trans persons: a case-control study. *Eur J Endocrinol* 169: 471–478, 2013. doi:10.1530/EJE-13-0493.
84. **Getahun D**, **Nash R**, **Flanders WD**, **Baird TC**, **Becerra-Culqui TA**, **Cromwell L**, **Hunkeler E**, **Lash TL**, **Millman A**, **Quinn VP**, **Robinson B**, **Roblin D**, **Silverberg MJ**, **Safer J**, **Slovis J**, **Tangpricha V**, **Goodman M.** Cross-sex hormones and acute cardiovascular events in transgender persons. *Ann Intern Med* 169: 205–213, 2018. doi:10.7326/M17-2785.
85. **McCredie RJ**, **McCrohon JA**, **Turner L**, **Griffiths KA**, **Handelsman DJ**, **Celermajer DS.** Vascular reactivity is impaired in genetic females taking high-dose androgens. *J Am Coll Cardiol* 32: 1331–1335, 1998. doi:10.1016/S0735-1097(98)00416-1.
86. **Gulanski BI**, **Flannery CA**, **Peter PR**, **Leone CA**, **Stachenfeld NS.** Compromised endothelial function in transgender men taking testosterone. *Clin Endocrinol (Oxf)* 92: 138–144, 2020. doi:10.1111/cen.14132.
87. **Ciccone MM**, **Loverro G**, **Scicchitano P**, **Loverro M**, **Ricci G**, **Scaramuzzi F**, **Gesualdo M**, **Zito A**, **Campagna M**, **Moncelli M**, **Nicolardi V**, **Manca F**, **Boninfante B**, **Carbonara S**, **Cortese F**, **Todarello O**, **Bettocchi C.** Surgical and pharmacological reassignment: influence on transsexual cardiovascular risk profile. *Intern Med J* 47: 1255–1262, 2017. doi:10.1111/imj.13597.
88. **Bunck MCM**, **Toorians AWFT**, **Lips P**, **Gooren LJG.** The effects of the aromatase inhibitor anastrozole on bone metabolism and cardiovascular risk indices in ovariectomized, androgen-treated female-to-male transsexuals. *Eur J Endocrinol* 154: 569–575, 2006. doi:10.1530/eje.1.02126.
89. **Wiik A**, **Andersson DP**, **Brismar TB**, **Chanpen S**, **Dhejne C**, **Ekström TJ**, **Flanagan JN**, **Holmberg M**, **Kere J**, **Lilja M**, **Lindholm ME**, **Lundberg TR**, **Maret E**, **Melin M**, **Olsson SM**, **Rullman E**, **Wåhlén K**, **Arver S**, **Gustafsson T.** Metabolic and functional changes in transgender individuals following cross-sex hormone treatment: design and methods of the GEnder dysphoria treatment in Sweden (GETS) study. *Contemp Clin Trials Commun* 10: 148–153, 2018. doi:10.1016/j.conctc.2018.04.005.
95. **Golden KL**, **Marsh JD**, **Jiang Y.** Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. *Horm Metab Res* 36: 197–202, 2004. doi:10.1055/s-2004-814445.
96. **Joksimovic Jovic J**, **Sretenovic J**, **Jovic N**, **Rudic J**, **Zivkovic V**, **Srejovic I**, **Mihajlovic K**, **Draginic N**, **Andjic M**, **Milinkovic M**, **Milosavljevic Z**, **Jakovljevic V.** Cardiovascular properties of the androgen-induced PCOS model in rats: the role of oxidative stress. *Oxid Med Cell Longev* 2021: 8862878, 2021. doi:10.1155/2021/8862878.
97. **Sun J**, **Fu L**, **Tang X**, **Han Y**, **Ma D**, **Cao J**, **Kang N**, **Ji H.** Testosterone modulation of cardiac β-adrenergic signals in a rat model of heart failure. *Gen Comp Endocrinol* 172: 518–525, 2011. doi:10.1016/j.ygcen.2011.04.019.
98. **Hassan NA**, **Salem MF**, **Sayed MAEL.** Doping and effects of anabolic androgenic steroids on the heart: Histological, ultrastructural, and echocardiographic assessment in strength athletes. *Hum Exp Toxicol* 28: 273–283, 2009. doi:10.1177/0960327109104821.
99. **D'Andrea A**, **Radmilovic J**, **Russo V**, **Sperlongano S**, **Carbone A**, **Di Maio M**, **Ilardi F**, **Riegler L**, **D'Alto M**, **Giallauria F**, **Bossone E**, **Picano E.** Biventricular dysfunction and lung congestion in athletes on anabolic androgenic steroids: a speckle tracking and stress lung echocardiography analysis. *Eur J Prev Cardiol* 28: 1928–1938, 2022. doi:10.1093/eurjpc/zwab086.
100. **Wu J**, **Dai F**, **Li C**, **Zou Y.** Gender differences in cardiac hypertrophy. *J Cardiovasc Transl Res* 13: 73–84, 2020. doi:10.1007/s12265-019-09907-z.
101. **Marsh JD**, **Lehmann MH**, **Ritchie RH**, **Gwathmey JK**, **Green GE**, **Schiebinger RJ.** Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation* 98: 256–261, 1998. doi:10.1161/01.CIR.98.3.256.
102. **Pirompol P**, **Teekabut V**, **Weerachatyanukul W**, **Bupha-Intr T**, **Wattanapermpool J.** Supra-physiological dose of testosterone induces pathological cardiac hypertrophy. *J Endocrinol* 229: 13–23, 2016. doi:10.1530/JOE-15-0506.
103. **Frantz S**, **Hu K**, **Widder J**, **Weckler B**, **Scheuermann H**, **Bauersachs J**, **Ertl G**, **Callies F**, **Allolio B.** Detrimental effects of testosterone on post-myocardial infarction remodeling in female rats. *J Physiol Pharmacol* 58: 717–727, 2007.
104. **De Jong KA**, **Berisha F**, **Naderpoor N**, **Appelbe A**, **Kotowicz MA**, **Cukier K**, **McGee SL**, **Nikolaev VO.** Polycystic ovarian syndrome increases prevalence of concentric hypertrophy in normotensive obese women. *PLoS One* 17: e0263312, 2022. doi:10.1371/journal.pone.0263312.
105. **Cavasin MA**, **Sankey SS**, **Yu A-L**, **Menon S**, **Yang X-P.** Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol* 284: H1560–H1569, 2003. doi:10.1152/ajpheart.01087.2002.
106. **Le TYL**, **Ashton AW**, **Mardini M**, **Stanton PG**, **Funder JW**, **Handelsman DJ**, **Mihailidou AS.** Role of androgens in sex differences in cardiac damage during myocardial infarction. *Endocrinology* 155: 568–575, 2014. doi:10.1210/en.2013-1755.
107. **dos Santos RL**, **da Silva FB**, **Ribeiro RF Jr**, **Stefanon I.** Sex hormones in the cardiovascular system. *Horm Mol Biol Clin Investig* 18: 89–103, 2014. doi:10.1515/hmbci-2013-0048.
108. **Rowell KO**, **Hall J**, **Pugh PJ**, **Jones TH**, **Channer KS**, **Jones RD.** Testosterone acts as an efficacious vasodilator in isolated human pulmonary arteries and veins: Evidence for a biphasic effect at physiological and supra-physiological concentrations. *J Endocrinol Invest* 32: 718–723, 2009. doi:10.1007/BF03346526.
109. **Matsuda K**, **Mathur RS**, **Ullian ME**, **Halushka PV.** Sex steroid regulation of thromboxane A2 receptors in cultured rat aortic smooth muscle cells. *Prostaglandins* 49: 183–196, 1995. doi:10.1016/0090-6980(95)00020-B.
110. **Chinnathambi V**, **Blesson CS**, **Vincent KL**, **Saade GR**, **Hankins GD**, **Yallampalli C**, **Sathishkumar K.** Elevated testosterone levels during rat pregnancy cause hypersensitivity to angiotensin II and attenuation of endothelium-dependent vasodilation in uterine arteries. *Hypertension* 64: 405–414, 2014. doi:10.1161/HYPERTENSIONAHA.114.03283.
111. **Polderman KH**, **Stehouwer CDA**, **Van Kamp GJ**, **Dekker GA**, **Verheugt FWA**, **Gooren LJG.** Influence of sex hormones on plasma endothelin levels. *Ann Intern Med* 118: 429–432, 1993. doi:10.7326/0003-4819-118-6-199303150-00006.
112. **Diamanti-Kandarakis E**, **Spina G**, **Kouli C**, **Migdalis I.** Increased endothelin-1 levels in women with polycystic ovary syndrome and

the beneficial effect of metformin therapy. *J Clin Endocrinol Metab* 86: 4666–4673, 2001. doi:10.1210/jcem.86.10.7904.

113. **Costa TJ**, **Ceravolo GS**, **dos Santos RA**, **de Oliveira MA**, **Araújo PX**, **Giaquinto LR**, **Tostes RC**, **Akamine EH**, **Fortes ZB**, **Dantas AP**, **Carvalho MHC.** Association of testosterone with estrogen abolishes the beneficial effects of estrogen treatment by increasing ROS generation in aorta endothelial cells. *Am J Physiol Heart Circ Physiol* 308: H723–H732, 2015. doi:10.1152/ajpheart.00681.2014.
114. **Taylor MS**, **Bonev AD**, **Gross TP**, **Eckman DM**, **Brayden JE**, **Bond CT**, **Adelman JP**, **Nelson MT.** Altered expression of small-conductance Ca2 + -activated K + (SK3) channels modulates arterial tone and blood pressure. *Circ Res* 93: 124–131, 2003. doi:10.1161/01.RES.0000081980.63146.69.
115. **Koukoulis GN**, **Filiponi M**, **Gougoura S**, **Befani C**, **Liakos P**, **Bargiota A.** Testosterone and dihydrotestosterone modulate the redox homeostasis of endothelium. *Cell Biol Int* 46: 660–670, 2022. doi:10.1002/cbin.11768.
116. **Chignalia AZ**, **Schuldt EZ**, **Camargo LL**, **Montezano AC**, **Callera GE**, **Laurindo FR**, **Lopes LR**, **Avellar MCW**, **Carvalho MHC**, **Fortes ZB**, **Touyz RM**, **Tostes RC.** Testosterone induces vascular smooth muscle cell migration by NADPH oxidase and c-Src-dependent pathways. *Hypertension* 59: 1263–1271, 2012. doi:10.1161/HYPERTENSIONAHA.111.180620.
117. **Chignalia AZ**, **Oliveira MA**, **Debbas V**, **Dull RO**, **Laurindo FRM**, **Touyz RM**, **Carvalho MHC**, **Fortes ZB**, **Tostes RC.** Testosterone induces leucocyte migration by NADPH oxidase-driven ROS- and COX2-dependent mechanisms. *Clin Sci (Lond)* 129: 39–48, 2015. doi:10.1042/CS20140548.
118. **Jin T**, **Wang L**, **Li D**, **Yang T**, **Zhou Y.** Testosterone aggravates cerebral vascular injury by reducing plasma HDL levels. *Open Life Sci* 15: 1042–1048, 2020. doi:10.1515/biol-2020-0107.
119. **Yanes LL**, **Romero DG**, **Moulana M**, **Lima R**, **Davis DD**, **Zhang H**, **Lockhart R**, **Racusen LC**, **Reckelhoff JF.** Cardiovascular-renal and metabolic characterization of a rat model of polycystic ovary syndrome. *Gend Med* 8: 103–115, 2011. doi:10.1016/j.genm.2010.11.013.
120. **Tostes RC**, **Carneiro FS**, **Carvalho MHC**, **Reckelhoff JF.** Reactive oxygen species: players in the cardiovascular effects of testosterone. *Am J Physiol Regul Integr Comp Physiol* 310: R1–R14, 2016. doi:10.1152/ajpregu.00392.2014.
121. **Lucas-Herald AK**, **Alves-Lopes R**, **Montezano AC**, **Ahmed SF**, **Touyz RM.** Genomic and non-genomic effects of androgens in the cardiovascular system: clinical implications. *Clin Sci (Lond)* 131: 1405–1418, 2017. doi:10.1042/CS20170090.
122. **Fujimoto R**, **Morimoto I**, **Morita E**, **Sugimoto H**, **Ito Y**, **Eto S.** Androgen receptors, 5 alpha-reductase activity and androgen-dependent proliferation of vascular smooth muscle cells. *J Steroid Biochem Mol Biol* 50: 169–174, 1994. doi:10.1016/0960-0760(94)90025-6.
123. **Zhu D**, **Hadoke PWF**, **Wu J**, **Vesey AT**, **Lerman DA**, **Dweck MR**, **Newby DE**, **Smith LB**, **MacRae VE.** Ablation of the androgen receptor from vascular smooth muscle cells demonstrates a role for testosterone in vascular calcification. *Sci Rep* 6: 24807, 2016. doi:10.1038/srep24807.
124. **Bruck B**, **Brehme U**, **Gugel N**, **Hanke S**, **Finking G**, **Lutz C**, **Benda N**, **Schmahl FW**, **Haasis R**, **Hanke H.** Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol* 17: 2192–2199, 1997. doi:10.1161/01.ATV.17.10.2192.
125. **Fleury M-A**, **Annabi M-S**, **Voisine M**, **Hervault M**, **Boilard A-J**, **Shen M**, **Marette A**, **Côté N**, **Clavel M-A.** Impact of sex and sex hormones on pathophysiology and progression of aortic stenosis in a murine model. *Physiol Rep* 10: e15433, 2022. doi:10.14814/phy2.15433.
126. **Nathan L**, **Shi W**, **Dinh H**, **Mukherjee TK**, **Wang X**, **Lusis AJ**, **Chaudhuri G.** Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci USA* 98: 3589–3593, 2001. doi:10.1073/pnas.051003698.
127. **Fagman JB**, **Wilhelmson AS**, **Motta BM**, **Pirazzi C**, **Alexanderson C**, **De Gendt K**, **Verhoeven G**, **Holmäng A**, **Anesten F**, **Jansson J-O**, **Levin M**, **Borén J**, **Ohlsson C**, **Krettek A**, **Romeo S**, **Tivesten Å.** The androgen receptor confers protection against diet-induced atherosclerosis, obesity, and dyslipidemia in female mice. *FASEB J* 29: 1540–1550, 2015. doi:10.1096/fj.14-259234.
128. **Xue B**, **Badaue-Passos D Jr**, **Guo F**, **Gomez-Sanchez CE**, **Hay M**, **Johnson AK.** Sex differences and central protective effect of 17β-estradiol in the development of aldosterone/NaCl-induced hypertension. *Am J Physiol Heart Circ Physiol* 296: H1577–H1585, 2009. doi:10.1152/ajpheart.01255.2008.
129. **dos Santos MR**, **Dias RG**, **Laterza MC**, **Rondon MUPB**, **Braga AMFW**, **de Moraes Moreau RL**, **Negrão CE**, **Alves M-JNN.** Impaired post exercise heart rate recovery in anabolic steroid users. *Int J Sports Med* 34: 931–935, 2013. doi:10.1055/s-0032-1331741.
130. **Lansdown A**, **Rees DA.** The sympathetic nervous system in polycystic ovary syndrome: a novel therapeutic target? *Clin Endocrinol (Oxf)* 77: 791–801, 2012. doi:10.1111/cen.12003.
131. **Maranon R**, **Lima R**, **Spradley FT**, **do Carmo JM**, **Zhang H**, **Smith AD**, **Bui E**, **Thomas RL**, **Moulana M**, **Hall JE**, **Granger JP**, **Reckelhoff JF.** Roles for the sympathetic nervous system, renal nerves, and CNS melanocortin-4 receptor in the elevated blood pressure in hyperandrogenemic female rats. *Am J Physiol Regul Integr Comp Physiol* 308: R708–R713, 2015. doi:10.1152/ajpregu.00411.2014.
132. **da Silva AA**, **do Carmo JM**, **Kanyicska B**, **Dubinion J**, **Brandon E**, **Hall JE.** Endogenous melanocortin system activity contributes to the elevated arterial pressure in spontaneously hypertensive rats. *Hypertension* 51: 884–890, 2008. doi:10.1161/HYPERTENSIONAHA.107.100636.
133. **Komukai K**, **Mochizuki S**, **Yoshimura M.** Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol* 24: 687–698, 2010. doi:10.1111/j.1472-8206.2010.00854.x.
134. **Hilliard LM**, **Sampson AK**, **Brown RD**, **Denton KM.** The "his and hers" of the renin-angiotensin system. *Curr Hypertens Rep* 15: 71–79, 2013. doi:10.1007/s11906-012-0319-y.
135. **Reckelhoff JF**, **Zhang H**, **Srivastava K.** Gender differences in development of hypertension in spontaneously hypertensive rats: role of the renin-angiotensin system. *Hypertension* 35: 480–483, 2000. doi:10.1161/01.hyp.35.1.480.
136. **Chen YF**, **Naftilan AJ**, **Oparil S.** Androgen-dependent angiotensinogen and renin messenger RNA expression in hypertensive rats. *Hypertension* 19: 456–463, 1992. doi:10.1161/01.HYP.19.5.456.
137. **Mishra JS**, **Kumar S.** Activation of angiotensin type 2 receptor attenuates testosterone-induced hypertension and uterine vascular resistance in pregnant rats. *Biol Reprod* 105: 192–203, 2021. doi:10.1093/biolre/ioab051.
138. **Mishra JS**, **Hankins GD**, **Kumar S.** Testosterone downregulates angiotensin II type-2 receptor via androgen receptor-mediated ERK1/2 MAP kinase pathway in rat aorta. *J Renin Angiotensin Aldosterone Syst* 17: 1470320316674875, 2016. doi:10.1177/1470320316674875.
139. **Tipton AJ**, **Baban B**, **Sullivan JC.** Female spontaneously hypertensive rats have greater renal anti-inflammatory T lymphocyte infiltration than males. *Am J Physiol Regul Integr Comp Physiol* 303: R359–R367, 2012. doi:10.1152/ajpregu.00246.2012.
140. **Wang L**, **Wang X**, **Qu HY**, **Jiang S**, **Zhang J**, **Fu L**, **Buggs J**, **Pang B**, **Wei J**, **Liu R.** Role of kidneys in sex differences in angiotensin II-induced hypertension. *Hypertension* 70: 1219–1227, 2017. doi:10.1161/HYPERTENSIONAHA.117.10052.
141. **Death AK**, **McGrath KCY**, **Sader MA**, **Nakhla S**, **Jessup W**, **Handelsman DJ**, **Celermajer DS.** Dihydrotestosterone promotes vascular cell adhesion molecule-1 expression in male human endothelial cells via a nuclear factor-κB-dependent pathway. *Endocrinology* 145: 1889–1897, 2004. doi:10.1210/en.2003-0789.
142. **Razmara A**, **Krause DN**, **Duckles SP.** Testosterone augments endotoxin-mediated cerebrovascular inflammation in male rats. *Am J Physiol Heart Circ Physiol* 289: H1843–H1850, 2005. doi:10.1152/ajpheart.00465.2005.
143. **Alves JV**, **da Costa RM**, **Pereira CA**, **Fedoce AG**, **Silva CAA**, **Carneiro FS**, **Lobato NS**, **Tostes RC.** Supraphysiological levels of testosterone induce vascular dysfunction via activation of the NLRP3 inflammasome. *Front Immunol* 11: 1647, 2020. doi:10.3389/fimmu.2020.01647.
144. **Davis JP**, **Salmon M**, **Pope NH**, **Lu G**, **Su G**, **Meher A**, **Ailawadi G**, **Upchurch GR Jr.** Pharmacologic blockade and genetic deletion of androgen receptor attenuates aortic aneurysm formation. *J Vasc Surg* 63: 1602–1612.e2, 2016. doi:10.1016/j.jvs.2015.11.038.
145. **McCrohon JA**, **Death AK**, **Nakhla S**, **Jessup W**, **Handelsman DJ**, **Stanley KK**, **Celermajer DS.** Androgen receptor expression is greater in macrophages from male than from female donors: a sex difference with implications for atherogenesis. *Circulation* 101: 224–226, 2000. doi:10.1161/01.CIR.101.3.224.
146. **Dalmasso C**, **Maranon R**, **Patil C**, **Moulana M**, **Romero DG**, **Reckelhoff JF.** 20-HETE and CYP4A2 ω-hydroxylase contribute to the elevated

blood pressure in hyperandrogenemic female rats. *Am J Physiol Renal Physiol* 311: F71–F77, 2016. doi:10.1152/ajprenal.00458.2015.

147. **Costa TJ**, **Ceravolo GS**, **Echem C**, **Hashimoto CM**, **Costa BP**, **Santos-Eichler RA**, **Oliveira MA**, **Jiménez-Altayó F**, **Akamine EH**, **Dantas AP**, **Carvalho MHC.** Detrimental effects of testosterone addition to estrogen therapy involve cytochrome P-450-induced 20-HETE synthesis in aorta of ovariectomized spontaneously hypertensive rat (SHR), a model of postmenopausal hypertension. *Front Physiol* 9: 490, 2018. doi:10.3389/fphys.2018.00490.
148. **Zhang MA**, **Rego D**, **Moshkova M**, **Kebir H**, **Chruscinski A**, **Nguyen H**, **Akkermann R**, **Stanczyk FZ**, **Prat A**, **Steinman L**, **Dunn SE.** Peroxisome proliferator-activated receptor (PPAR)α and -γ regulate IFN and IL-17A production by human T cells in a sex-specific way. *Proc Natl Acad Sci USA* 109: 9505–9510, 2012. doi:10.1073/pnas.1118458109.
149. **Ma M**, **Wang M**, **Xu F**, **Hao S.** The imbalance in Th17 and Treg cells in polycystic ovarian syndrome patients with autoimmune thyroiditis. *Immunol Invest* 51: 1170–1181, 2022. doi:10.1080/08820139.2021.1915329.
150. **Li Z**, **Yue Y**, **Xiong S.** Distinct Th17 inductions contribute to the gender bias in CVB3-induced myocarditis. *Cardiovasc Pathol* 22: 373–382, 2013. doi:10.1016/j.carpath.2013.02.004.
151. **Cavasin MA**, **Tao Z-Y**, **Yu A-L**, **Yang X-P.** Testosterone enhances early cardiac remodeling after myocardial infarction, causing rupture and degrading cardiac function. *Am J Physiol Heart Circ Physiol* 290: 2043–2050, 2006. doi:10.1152/ajpheart.01121.2005.
152. **Goetz LG**, **Mamillapalli R**, **Sahin C**, **Majidi-Zolbin M**, **Ge G**, **Mani A**, **Taylor HS.** Addition of estradiol to cross-sex testosterone therapy reduces atherosclerosis plaque formation in female ApoE−/− mice. *Endocrinology* 159: 754–762, 2018. doi:10.1210/en.2017-00884.
153. **Langer C**, **Gansz B**, **Goepfert C**, **Engel T**, **Uehara Y**, **Von Dehn G**, **Jansen H**, **Assmann G**, **Von Eckardstein A.** Testosterone up-regulates scavenger receptor bi and stimulates cholesterol efflux from macrophages. *Biochem Biophys Res Commun* 296: 1051–1057, 2002. doi:10.1016/S0006-291X(02)02038-7.
154. **Tan KC**, **Shiu SW**, **Kung AW.** Alterations in hepatic lipase and lipoprotein subfractions with transdermal testosterone replacement therapy. *Clin Endocrinol (Oxf)* 51: 765–769, 1999. doi:10.1046/j.1365-2265.1999.00882.x.
155. **Shen W-J**, **Azhar S**, **Kraemer FB.** SR-B1: a unique multifunctional receptor for cholesterol influx and efflux. *Annu Rev Physiol* 80: 95–116, 2018. doi:10.1146/annurev-physiol-021317-121550.
156. **Scheja L**, **Heeren J.** The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat Rev Endocrinol* 15: 507–524, 2019. doi:10.1038/s41574-019-0230-6.
157. **Victorio JA**, **da Costa RM**, **Tostes RC**, **Davel AP.** Modulation of vascular function by perivascular adipose tissue: sex differences. *Curr Pharm Des* 26: 3768–3777, 2020. doi:10.2174/1381612826666200701211912.
158. **Boydens C**, **Pauwels B**, **Van de Voorde J.** Effect of resveratrol and orchidectomy on the vasorelaxing influence of perivascular adipose tissue. *Heart Vessels* 31: 608–615, 2016. doi:10.1007/s00380-015-0664-2.
159. **Nosalski R**, **Guzik TJ.** Perivascular adipose tissue inflammation in vascular disease. *Br J Pharmacol* 174: 3496–3513, 2017. doi:10.1111/bph.13705.
160. **Jilani MH**, **Javed Z**, **Yahya T**, **Valero-Elizondo J**, **Khan SU**, **Kash B**, **Blankstein R**, **Virani SS**, **Blaha MJ**, **Dubey P**, **Hyder AA**, **Vahidy FS**, **Cainzos-Achirica M**, **Nasir K.** Social determinants of health and cardiovascular disease: current state and future directions towards healthcare equity. *Curr Atheroscler Rep* 23: 55, 2021. doi:10.1007/s11883-021-00949-w.
161. **Murison R.** The neurobiology of stress. In: *Neuroscience of Pain, Stress, and Emotion*. Elsevier, 2016, p. 29–49.
162. **Kubzansky LD**, **Adler GK.** Aldosterone: a forgotten mediator of the relationship between psychological stress and heart disease. *Neurosci Biobehav Rev* 34: 80–86, 2010. doi:10.1016/j.neubiorev.2009.07.005.
163. **Baumer Y**, **Pita MA**, **Baez AS**, **Ortiz-Whittingham LR**, **Cintron MA**, **Rose RR**, **Gray VC**, **Osei Baah F**, **Powell-Wiley TM.** By what molecular mechanisms do social determinants impact cardiometabolic risk? *Clin Sci (Lond)* 137: 469–494, 2023. doi:10.1042/CS20220304.
164. **Emeny RT**, **Carpenter DO**, **Lawrence DA.** Health disparities: intracellular consequences of social determinants of health. *Toxicol Appl Pharmacol* 416: 115444, 2021. doi:10.1016/j.taap.2021.115444.
165. **Goetz TG**, **Mamillapalli R**, **Devlin MJ**, **Robbins AE**, **Majidi-Zolbin M**, **Taylor HS.** Cross-sex testosterone therapy in ovariectomized mice: addition of low-dose estrogen preserves bone architecture. *Am J Physiol Endocrinol Metab* 313: E540–E551, 2017 [Erratum in *Am J Physiol Endocrinol Metab* 319: E658, 2020]. doi:10.1152/ajpendo.00161.2017.
166. **Bartels CB**, **Uliasz TF**, **Lestz L**, **Mehlmann LM.** Short-term testosterone use in female mice does not impair fertilizability of eggs: implications for the fertility care of transgender males. *Hum Reprod* 36: 189–198, 2021. doi:10.1093/humrep/deaa282.
167. **Barros PR**, **Costa TJ**, **Akamine EH**, **Tostes RC.** Vascular aging in rodent models: contrasting mechanisms driving the female and male vascular senescence. *Front Aging* 2: 727604, 2021. doi:10.3389/fragi.2021.727604.
168. **van Heesewijk JO**, **Dreijerink KMA**, **Wiepjes CM**, **Kok AAL**, **van Schoor NM**, **Huisman M**, **den Heijer M**, **Kreukels BPC.** Long-term gender-affirming hormone therapy and cognitive functioning in older transgender women compared with cisgender women and men. *J Sex Med* 18: 1434–1443, 2021. doi:10.1016/j.jsxm.2021.05.013.
169. **Balık AÖ**, **Vural F**, **Alpogan O**, **Özoğul M**, **Dönmez EE.** The effects of testosterone on transgender males on carotid intima-media thickness and serum inflammatory markers compared within patients with polycystic ovary syndrome. *Gynecol Endocrinol* 38: 771–775, 2022. doi:10.1080/09513590.2022.2112942.
170. **Connelly PJ**, **Clark A**, **Touyz RM**, **Delles C.** Transgender adults, gender-affirming hormone therapy and blood pressure: a systematic review. *J Hypertens* 39: 223–230, 2021. doi:10.1097/HJH.0000000000002632.
171. **Alzahrani T**, **Nguyen T**, **Ryan A**, **Dwairy A**, **McCaffrey J**, **Yunus R**, **Forgione J**, **Krepp J**, **Nagy C**, **Mazhari R**, **Reiner J.** Cardiovascular disease risk factors and myocardial infarction in the transgender population. *Circ Cardiovasc Qual Outcomes* 12: e005597, 2019. doi:10.1161/CIRCOUTCOMES.119.005597.
172. **Schaeff C.** Assigned-Female-at-Birth Trans Masculine and Non-Binary Individuals Differ in Their Body Satisfaction and Desire for Gender Affirming Treatments. *Arch Sex Behav* 51: 20372047, 2022. doi:10.1007/s10508-021-02178-3.
173. **Cupisti S**, **Giltay EJ**, **Gooren LJ**, **Kronawitter D**, **Oppelt PG**, **Beckmann MW**, **Dittrich R**, **Mueller A.** The impact of testosterone administration to female-to-male transsexuals on insulin resistance and lipid parameters compared with women with polycystic ovary syndrome. *Fertil Steril* 94: 2647–2653, 2010. doi:10.1016/j.fertnstert.2010.03.048.
174. **Worboys S**, **Kotsopoulos D**, **Teede H**, **McGrath B**, **Davis SR.** Evidence that parenteral testosterone therapy may improve endothelium-dependent and-independent vasodilation in postmenopausal women already receiving estrogen. *J Clin Endocrinol Metab* 86: 158–161, 2001. doi:10.1210/jcem.86.1.7103.
175. **Klaver M**, **de Mutsert R**, **van der Loos MATC**, **Wiepjes CM**, **Twisk JWR**, **den Heijer M**, **Rotteveel J**, **Klink DT.** Hormonal treatment and cardiovascular risk profile in transgender adolescents. *Pediatrics* 145: e20190741, 2020. doi:10.1542/peds.2019-0741.
176. **Fornander MJ**, **Roberts T**, **Egan AM**, **Moser CN.** Weight status, medication use, and recreational activities of treatment-naïve transgender youth. *Child Obes* 18: 228–236, 2022. doi:10.1089/chi.2021.0155.
177. **Baba T**, **Endo T**, **Honnma H**, **Kitajima Y**, **Hayashi T**, **Ikeda H**, **Masumori N**, **Kamiya H**, **Moriwaka O**, **Saito T.** Association between polycystic ovary syndrome and female-to-male transsexuality. *Hum Reprod* 22: 1011–1016, 2007. doi:10.1093/humrep/del474.
178. **Malhotra A**, **Kort S**, **Lauther T**, **Mann N**, **Skopicki HA**, **Parikh PB.** Prevalence and predictors of cardiovascular disease and risk factors in transgender persons in the United States. *Crit Pathw Cardiol* 21: 42–46, 2022. doi:10.1097/HPC.0000000000000271.
179. **Defreyne J**, **Van Schuylenbergh J**, **Motmans J**, **Tilleman KL**, **Rik T'Sjoen GG.** Parental desire and fertility preservation in assigned female at birth transgender people living in Belgium. *Fertil Steril* 113: 149–157.e2, 2020. doi:10.1016/j.fertnstert.2019.09.002.
180. **Oberhelman-Eaton S**, **Chang A**, **Gonzalez C**, **Braith A**, **Singh RJ**, **Lteif A.** Initiation of gender-affirming testosterone therapy in a lactating transgender man. *J Hum Lact* 38: 339–343, 2022. doi:10.1177/08903344211037646.
181. **Zhang X**, **Thatcher SE**, **Rateri DL**, **Bruemmer D**, **Charnigo R**, **Daugherty A**, **Cassis LA.** Transient exposure of neonatal female mice to testosterone abrogates the sexual dimorphism of abdominal aortic

aneurysms. *Circ Res* 110: e73–e85 2012. doi:10.1161/CIRCRESAHA.111.253880.

182. **More AS**, **Mishra JS**, **Hankins GD**, **Kumar S.** Prenatal testosterone exposure decreases aldosterone production but maintains normal plasma volume and increases blood pressure in adult female rats. *Biol Reprod* 95: 42, 2016. doi:10.1095/biolreprod.116.141705.
183. **Sathishkumar K**, **Elkins R**, **Yallampalli U**, **Balakrishnan M**, **Yallampalli C.** Fetal programming of adult hypertension in female rat offspring exposed to androgens in utero. *Early Hum Dev* 87: 407–414, 2011. doi:10.1016/j.earlhumdev.2011.03.001.
184. **Blesson CS**, **Chinnathambi V**, **Hankins GD**, **Yallampalli C**, **Sathishkumar K.** Prenatal testosterone exposure induces hypertension in adult females via androgen receptor-dependent protein kinase Cδ-mediated mechanism. *Hypertension* 65: 683–690, 2015. doi:10.1161/HYPERTENSIONAHA.114.04521.
185. **Hou M**, **Gu H-C**, **Wang H-H**, **Liu X-M**, **Zhou C-L**, **Yang Q**, **Jiang Z-R**, **Lin J**, **Wu Y-M**, **Wu Y-T**, **Sheng J-Z**, **Huang H-F.** Prenatal exposure to testosterone induces cardiac hypertrophy in adult female rats through enhanced Pkcδ expression in cardiac myocytes. *J Mol Cell Cardiol* 128: 1–10, 2019. doi:10.1016/j.yjmcc.2019.01.008.
186. **Manti M**, **Fornes R**, **Pironti G**, **McCann Haworth S**, **Zhengbing Z**, **Benrick A**, **Carlström M**, **Andersson D**, **Stener-Victorin E.** Maternal androgen excess induces cardiac hypertrophy and left ventricular dysfunction in female mice offspring. *Cardiovasc Res* 116: 619–632, 2020. doi:10.1093/cvr/cvz180.
187. **Scheim A**, **Kacholia V**, **Logie C**, **Chakrapani V**, **Ranade K**, **Gupta S.** Health of transgender men in low-income and middle-income countries: a scoping review. *BMJ Glob Heal* 5: e003471, 2020. doi:10.1136/bmjgh-2020-003471.