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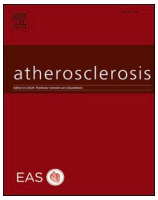

Review article

Cardiovascular disease in transgender individuals

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ABSTRACT

The population of people identifying as transgender has grown rapidly in recent years, resulting in a substantive increase in individuals obtaining gender-affirming medical care to align their secondary sex characteristics with their gender identity. This has established benefits for patients including improvements in gender dysphoria and psychosocial functioning, while reducing adverse mental health outcomes. Despite these potential advantages, recent evidence has suggested that gender-affirming hormone therapy (GAHT) may increase the risk of cardiovascular disease. However, owing to a paucity of research, the mechanisms underpinning these increased risks are poorly understood. Moreover, previous research has been limited by heterogenous methodologies, being underpowered, and lacking appropriate control populations. Consequently, the need for evidence regarding cardiovascular health in LGBTQ + individuals has been recognised as a critical area for future research to facilitate better healthcare and guidance. Recent research investigating the effect of transmasculine (testosterone) GAHT on cardiovascular disease risk points to testosterone effecting the nitric oxide pathway, triggering inflammation, and promoting endothelial dysfunction. Equivalent studies focussing on transfeminine (oestrogen) GAHT are required, representing a crucial area of future research. Furthermore, when examining the effects of GAHT on the vasculature, it cannot be ignored that there are multiple factors that may increase the burden of cardiovascular disease in the transgender population. Such stressors include major psychological stress; increased adverse health behaviours, such as smoking; discrimination; and lowered socioeconomic status; all of which undoubtedly impact upon cardiovascular disease risk and offers the opportunity for intervention.

1. Introduction

Transgender people experience gender dysphoria because the sex they were assigned at birth does not reflect their gender identity (Table 1). The prevalence of transgender individuals in the USA is estimated to be ~0.5% [1,2]. The findings from European studies are similar, with population estimates ranging from 0.6 to 1.1% [3,4]. This population has grown rapidly in recent years relating to an increase in visibility, acceptance and understanding within the general population [5]. Accordingly, there has been a substantial increase in individuals obtaining gender-affirming medical care to align their secondary sex characteristics with their gender identity [1,6].

The term 'transgender' is an umbrella term that includes individuals whose gender identities and expression may not be considered typical for the sex they were assigned at birth [7]. Trans men are people who have gender identities as men and were assigned female at birth, while trans women have gender identities as women and were assigned male at birth. Transgender individuals may also include people who identify as nonbinary, genderqueer, gender neutral, agender, gender fluid, or as a 'third' gender [8].

Not all transgender individuals require or seek out gender-affirming hormone therapy (GAHT). However, transgender individuals who choose to initiate GAHT may receive transmasculine (i.e. testosterone) or transfeminine (i.e. oestrogen ± androgen suppression via gonadotropin-releasing hormone analogues (GnRHa) or cyproterone acetate (CPA)) therapies to facilitate gender congruence with secondary sex characteristics [9]. This may improve gender dysphoria and psychosocial functioning [10], while reducing adverse mental health outcomes [11].

Despite these potential benefits, recent evidence has suggested that GAHT may increase the risk of cardiovascular disease [12]. Owing to a paucity of research, the mechanisms underpinning these increased risks are poorly understood. As is the case for most studies relating to transgender healthcare; the study methodologies are heterogenous, often underpowered, have small patient populations and are frequently not controlled for other co-morbidities. Furthermore, it would be unethical

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Table 1
Glossary of sex and gender definitions [7,8].

Term	Definition
Sex	A person's status as male, female, or intersex based on physical characteristics typically assigned at birth
Gender	Someone's gender expression, gender identity and/or sociocultural gender role
Gender Identity	A person's innate sense of their gender. This may include cisgender, transgender, nonbinary, genderqueer, gender neutral, agender, gender fluid, and 'third' gender identities
Gender Dysphoria	The distress or discomfort derives from a person's gender identity not aligning with the sex they were assigned at birth
Cisgender	A person whose gender identity aligns with the sex they were assigned at birth
Transgender	A person whose gender identity does not align with sex they were assigned at birth
Transgender Woman	A person whose gender identity is a woman who was assigned male sex at birth
Transgender Man	A person whose gender identity is a man who was assigned female sex at birth
Gender-Affirming Care	Medical therapies or surgery used to align a transgender person's primary and/or secondary sex characteristics with their gender

to conduct studies comparing GAHT to placebo, thereby limiting the opportunity to undertake randomised control trials [13].

With substantial increases in the number of transgender individuals seeking GAHT, there is an imperative need to fill the knowledge gap generated by this dearth of data, to facilitate better healthcare and guidance. The lack of evidence regarding cardiovascular health in LGBTQ + individuals has been recognised as a critical area for future research [8]. Dissecting the mechanisms by which GAHT modulates the cardiovascular system is crucial to the development of clinical strategies to mitigate cardiovascular risk and improve the health of transgender individuals. Encouragingly, recent data and engagement with the transgender community has better informed our understanding of cardiovascular effects of GAHT and the potential mechanisms by which they may act.

2. Gender-affirming hormone therapy

Many transgender individuals will initiate GAHT to reduce the presence of their secondary sex characteristics associated with their biological sex, and to develop new characteristics which align with their gender identity. GAHT is provided lifelong to maintain these traits. However, it is unclear whether such therapies should be age-adjusted in later life [9].

In cisgender women, the primary endogenous oestrogen is 17β-oestradiol (E2). Prior to the menopause, E2 in females is produced via ovarian granulosa cells, in response to lutenising hormone, under the regulation of the hypothalamic-pituitary-ovarian axis. Oestrogen acts via genomic and non-genomic mechanisms, through binding oestrogen receptor (ER) alpha, ERβ and the G protein-coupled oestrogen receptor (GPER) [14].

Transgender women with feminine embodiment goals are typically prescribed oestrogen and anti-androgens [15,16]. These therapies promote breast development and decrease facial and body hair growth, testicular volume and alter adipose and muscle mass distribution [17]. Historically ethinyl oestradiol, synthetic oestrogens, or conjugated equine oestrogens have been utilised. However, these are no longer recommended due to immunoassay interference in oestrogen measurement and their associated risk of thrombosis and potentially cardiovascular disease [16,18,19]. Modern GAHT utilises oestrogen hemihydrate or oestrogen ester prodrugs (i.e. oestrogen valerate) that provide oestrogen bioequivalence [20]. In transgender women over the age of 45 years or with a history of thrombosis, transdermal preparations are recommended as these are thought to be considerably less thrombogenic [21–23].

Oestrogen may also be combined with adjuvant androgen lowering therapies that aim to supress testosterone to natal female concentrations and lower oestrogen doses required for physical congruence. This can be achieved via GnRHa, such as leuprorelin; steroidal anti-androgens such as CPA; or partial androgen receptor (AR) antagonists such as spironolactone [24].

In cisgender men, the primary sex steroids are androgens. The primary endogenous male androgen is testosterone, which is synthesised primarily in the Leydig cells of the testes [25] and, in much smaller concentrations, in the adrenal glands. Testosterone, and its more potent metabolite dihydrotestosterone (DHT), bind to the AR [26].

Transgender men with masculine embodiment goals are prescribed testosterone, typically as intramuscular preparations (i.e., testosterone undecanoate or esters). Alternatively, dermal testosterone may be applied as a hydroalcoholic gel [9]. These therapies promote facial and body hair growth, a lowering of voice pitch, cessation of menses and increased lean body mass [15]. Testosterone monotherapy is typically sufficient to supress oestrogen concentrations without the requirement for specific oestrogen lowering therapies [27].

3. Cardiovascular disease in transgender people

Despite GAHT being utilised for decades, its influence on the cardiovascular system of transgender individuals has remained uncertain [12,28]. However, recent longitudinal data and engagement with transgender healthcare needs has provided considerable insight into the role of cross-sex hormones in cardiovascular disease risk.

In 2018 Getahun et al. conducted a large retrospective analysis of transgender cardiovascular health outcomes in 2842 trans women and 2118 trans men [29]. This data was derived from the Kaiser Health system across Georgia and California in the USA. In this analysis, the rates of ischaemic stroke, and to a lesser extent myocardial infarction, were increased in trans women using oestrogen. In a sub analysis of individuals commencing GAHT during the study period, the risk of ischaemic stroke increased following the first 6 years of follow-up, suggesting this association to be driven by long-term oestrogen exposure. Interestingly, trans men were not at increased risk, which may be a consequence of a relatively young, insured, and physician coded transgender population, which may not be fully representative.

In a recent register-based cohort study of 2671 Danish transgender individuals, with a mean follow-up time of 4.5 years, the risk of cardiovascular disease and relative contribute of GAHT was examined [30]. Overall, the transgender population demonstrated a substantive increase in adverse cardiovascular and metabolic outcomes compared to cisgender controls following an age-matched analysis. Cardiovascular disease risk was particularly elevated in transgender individuals assigned female sex at birth (i.e., trans men), who were twice as likely to experience cardiovascular disease compared to cisgender men (aHR 2.20, 95% CI 1.6, 2.9). The risk of any cardiovascular disease was also elevated in trans women compared to either cisgender men (aHR 1.93 95%CI 1.6, 2.3) or women (aHR 1.73, 95% CI 1.5, 2.21). This conflicts with Getahun's analysis, however, is supported by data collected by the Behavioural Risk Factor Surveillance System (BRFSS), which observed a fourfold increase in the risk of myocardial infarction in transgender men, even after accounting for established cardiovascular risk factors [31].

Interestingly, in the Danish registry study only 33.2% of the increased risk of cardiovascular disease in the transmasculine group compared to cisgender men could be attributed to hormonal therapy. Conversely, GAHT was not found to be significant contributor in the transfeminine cohort. However, the cumulative use of transfeminine and transmasculine GAHT was only observed to be 21.3 and 19.2%, respectively. Given the relatively low prevalence of GAHT utilisation within this cohort, it must be considered that GAHT prescriptions were either not captured in the analysis or obtained by external means (i.e., self-obtained). So, the relative contribution of GAHT in this population

must be interpreted with caution [30].

The potential influence of cardiovascular disease on the mortality of people who are transgender has also recently been assessed. In a retrospective cohort study of 4568 transgender individuals attending the Amsterdam University Medical Centre gender identity clinic, a two-fold increased mortality was observed [32,33]. This amounted to 628 deaths per 100,000 person-years. This, importantly, did not differ across the five decades studied, implying that modern, and generally considered safer, GAHT has not improved overall mortality rates in this population. In transgender women, cardiovascular disease, and in particular death due to myocardial infarction, contributed to this increased rate of death when compared to cisgender women, but not men. Cause-specific analysis could not be performed in the transmasculine population owing to limited sample size and event rate.

Hypertension continues to be the principal modifiable risk factor for the development of cardiovascular diseases, worldwide [34]. Importantly, blood pressure is sex-dependent, whereby sex steroids play an integral role in the regulation of blood pressure and prevalence of hypertension in males and females [35]. Hypertension therefore provides a potential mechanism and area of intervention to modulate risk in the transgender population. In a recent systematic review of 14 studies comprising of 1309 transgender individuals there was insufficient data to inform the impact of GAHT on blood pressure owing to the heterogeneity of interventions, lack of outcome uniformity, and the pre-post quasi experimental study design [36]. Subsequently, a longitudinal study of 470 transgender individuals with multiple blood pressure readings demonstrated persistent increases in systolic blood pressures (SBP) in trans men, and a decrease in transgender women. Importantly, significant interindividual variation was observed, thereby highlighting the importance of blood pressure monitoring in transgender individuals commencing GAHT, and a means by which cardiovascular risk may be lowered. Ultimately, further comprehensive prospective data is required across the spectrum of cardiovascular disease and risk factors in order to inform evidence based guidance and practice [28].

3.1. Sex hormone receptors and vascular function

Sex steroids act via their analogous receptors, which are expressed throughout the cardiovascular system, including in the vascular smooth muscle and endothelium [37]. These topics are also discussed in two reviews of this special issue [38,39].

ERα, ERβ and AR are nuclear hormone receptors (NHR), which mediate their actions as ligand-dependent nuclear transcription factors [40]. Inactive NHRs are located within the cytoplasm, bound to heat shock proteins [41], which dissociate upon receptor activation. Activated NHRs then dimerise and undergo nuclear translocation [42].

NHRs have a 5 domain structure, including 3 functional domains: the N-terminal domain, the DNA binding domain; and C-terminal ligand binding domain. The DNA binding domains are highly conserved across all NHRs. They consist of two zinc fingers that mediate direct NHR binding to promotors or enhancers of sex hormone regulated genes, which express an androgen or oestrogen response element [43]. Subsequent downstream NHR-mediated gene regulation remains distinct as a consequence of the heterologous nature of the N-terminal domain, and associated co-regulatory proteins recruited [44]. In addition, NHRs can also indirectly regulate gene expression via interaction with other transcription factors, such as activator protein 1 and specificity protein-1, on the promotor of sex hormone responsive genes [45].

NHRs can also mediate non-genomic responses via activation of membrane bound NHRs, which stimulate secondary messenger systems [46]. GPER can couple G_s proteins, thereby stimulating cAMP production and triggering downstream activation of endothelial nitric oxide synthase (eNOS) and multiple kinases, including ERK1/2 and P13K [47,48].

The effects of NHRs upon the vasculature are therefore numerous, and perturbations of sex steroid function may elicit deleterious effects. Endothelial ERα promotes endothelium-dependent vasodilatation through eNOS activation, endothelial proliferation, and migration, and promotes carotid artery re-endothelialisation [49,50]. This receptor may also modulate the renin-angiotensin-aldosterone system (RAAS) via the activation of oestrogen response elements of renin expressing juxtaglomerular cells [51]. In premenopausal females, ERβ gene variant rs10144225 minor alleles has been demonstrated to be associated with the development of salt-sensitive of blood pressure [52]. Lastly, loss of function of GPER promotes endothelium-dependent vasoconstriction, atherosclerosis and vascular inflammation [53]. Moreover, AR activation may facilitate eNOS activation, endothelium independent vasodilation and promote vascular remodelling via the growth arrest-specific protein 6/Axl pathway and modulate vascular inflammation [54–56] (Fig. 1). However, testosterone may also elicit a RAAS mediated pressor response, contributing to vascular oxidative stress [57–59]. Therefore, dissecting the mechanisms responsible for vascular injury in transgender populations is complex and likely involves multiple effectors (Fig. 2, Table 2).

4. GAHT and mechanisms of cardiovascular dysfunction

4.1. Transmasculine (testosterone) hormone therapy

A study by Gulanski et al. demonstrated that prescribing trans men testosterone was associated with impaired endothelial function, when compared to age-matched cisgender women. Following ≥3 months of testosterone treatment, flow mediated vasodilation in response to simulation of sheer stress in trans men was significantly decreased in comparison with cis women undergoing the follicular phase of their menstrual cycle (4.5 ± 2.7% *vs* 8.1 ± 2.9%; TM *vs* CW; $p = 0.002$). Both groups had comparable cardiovascular disease risk, with no significant difference between BMI, lipid levels or blood pressure [60]. Gulanski et al.'s findings are supported by similar findings in females with androgen-excessive polycystic ovary syndrome (AE-PCOS). Females with AE-PCOS experience significant relative hyperandrogenism, and such a model may provide insight into the effects of testosterone administration in trans men. The supraphysiological levels of testosterone in AE-PCOS have been demonstrated to be responsible for impaired endothelin-1 mediated vasodilation in response to agonists, due to suppression of endothelin B receptor stimulated NO synthesis consequential of androgen binding [61]. However, in AE-PCOS testosterone levels are well below male reference ranges, and oestrogen is not supressed. Additionally, trans men lack enhanced GnRH pulsatility and hyperinsulinemia [62]. Therefore, although AE-PCOS and those utilising transmasculine therapies may share common pathophysiological processes, direct comparisons should be cautiously interpreted.

Gulanski et al.'s study methodology comes with limitations. The study is underpowered (11 trans men, 20 cis women) and heterologous methods were used to study the two groups. Medical records were used to confirm the participants as healthy. However, early endothelial dysfunction is not routinely investigated. Furthermore, there is no baseline data presented for the trans men prior to initiating GAHT, while the GAHT prescribed was heterogenous. In addition, the blood samples obtained from trans men were taken by their physicians, not the study authors, and the analysis methods used on the trans men and cis women's blood samples differ. It is not possible to conclude with certainty that testosterone is solely responsible for the impaired vasodilation witnessed in the trans men in Gulanski et al.'s study.

These findings are supported by Santos et al., who investigated whether testosterone negatively impacted cardiovascular function by affecting Th17 cells in a mouse model of trans masculine GAHT [63]. Th17 cells are implicated in both hypertension and atherosclerosis, due to their role in producing the cytokine IL-17. In mouse models, Il-17 has been shown to phosphorylate the threonine at position 495 in eNOS via Rho kinase-dependent mechanisms, thereby preventing the binding of calmodulin to eNOS, inhibiting the production of NO [64]. Following 8

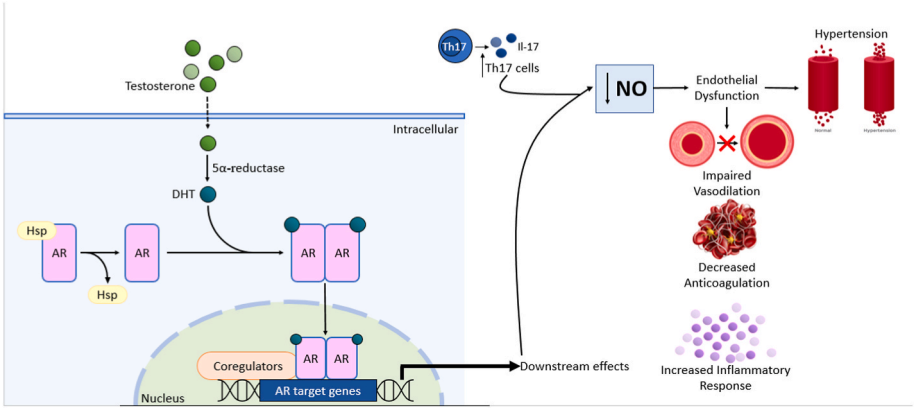

Fig. 1. Androgen mediated signalling and vascular dysfunction in transgender individuals.
Genomic testosterone signalling mediates a series of downstream effects resulting in endothelial dysfunction, coagulation defects and vascular inflammation, leading to the development of hypertension.

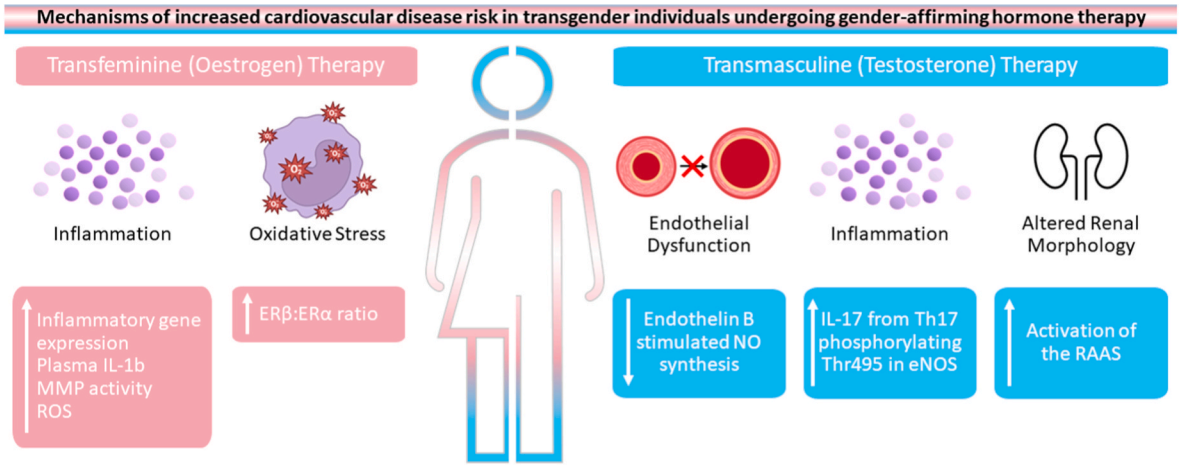

Fig. 2. Mechanisms of increased cardiovascular disease risk in transgender individuals undergoing gender-affirming hormone therapy.
Identification of the causes and potential mechanisms of increased cardiovascular disease risk associated with transfeminine (oestrogen) and transmasculine (testosterone) hormone therapy. eNOS, endothelial nitric oxide synthase; ER, oestrogen receptor; IL-17, interleukin-17; MMP, matrix metalloproteinase; NO, nitric oxide; RAAS, renin angiotensin aldosterone system; ROS, reactive oxygen species; Th17, T-helper (cell) 17.

weeks of testosterone administration in female mice, endothelial dysfunction resulting from decreased vasodilatory responses and an increase in peripheral Th17 cells was observed. In addition, at 24 weeks the mice had developed hypertension, compared to vehicle treated control mice. Using a series of knockout mice, it was concluded that Th17 cells held primary responsibility for the endothelial dysfunction that developed following testosterone treatment, and the associated development of hypertension [63]. This study's methodology is excellent and their conclusion is supported by studies in non-cross sex mouse models [65]. The role of biological sex in CVD models is discussed in the review by Nour et al. in this issue [66].

Many aspects of physiology demonstrate sex-dependent differences, the cardiovascular system being a prime example. Auer et al. sought to gain insight into this relationship and the relative contribution of sex hormones and chromosomes via the measurement of sex-dependent metabolites in transgender persons before and after initiating GAHT. The study concluded that the concentrations of most metabolites remained consistent. However, a decrease in the concentration of ADMA (asymmetric dimethylarginine) was observed in trans women, alongside differences in the citrulline/arginine (C/A) ratio in both trans and women. In trans women the C/A ratio was strongly decreased, while in trans men, the ratio was increased [67]. L-citrulline is the rate determining factor in the conversion of L-arginine to NO. Thus, the C/A ratio has been suggested as a surrogate marker for the rate of NO synthesis *in vivo* by NOS [68]. The increased C/A ratio observed in trans men points to increased NO production and improved endothelial function resulting from testosterone initiation. Conversely, the decreased ratio in trans women indicates decreased NO production and potentially impaired endothelial function following the initiation of oestrogen [67].

However, these findings appear to conflict with Gulanski et al.'s conclusions. Arginine is converted to citrulline in many metabolic cycles beside NO synthesis, including in the urea cycle, which converts arginine to citrulline through the intermediate ornithine [69]. The relevance of the C/A ratio as an indicator of NO production is debated due to comparably lower concentrations of arginine used to produce NO than used in the urea cycle. The decreased C/A ratio observed by Auer et al. in trans women was accompanied by a decrease in the ornithine/arginine ratio, suggesting the altered C/A ratio was the result of decreased activity of the urea cycle, not decreased NO production. This is further supported by a significant decrease in the concentration of the competitive NOS inhibitor, ADMA, in trans women. No changes were observed in ADMA concentration in trans men. Auer et al.'s conclusion was revised such that altered C/A ratios in both trans men and women

Table 2
Studies investigating the causes and mechanisms by which GAHT may affect cardiovascular disease risk.

Transmasculine (testosterone) hormone therapy			
Cause	Evidence	Potential mechanism	Study
Endothelial dysfunction	Impaired vasodilation in response to sheer stress	Suppression of endothelin B receptor stimulated NO synthesis	Gulanski et al. [60]
	Increased l-citrulline/l-arginine ratio	Increased NO synthesis	Auer et al. [67]
	Increased atherosclerotic plaque size in the presence of no oestrogen compared to low dose oestrogen	Oestrogen promoting eNOS activity to synthesise NO	Goetz et al. [73]
Inflammation	Increased Th17 cells leading to the development of hypertension	IL-17 produced by Th17 cells phosphorylating Thr495 in eNOS, preventing the binding of calmodulin to eNOS to inhibit NO synthesis	Santos et al. [63]
Renal morphology	Altered renal morphology coupled with increased systolic blood pressure	Testosterone activation of RAAS	Lichtenecker et al. [71]

Transfeminine (oestrogen) hormone therapy			
Cause	Evidence	Potential mechanism	Author(s)
Oxidative stress	Impaired vasodilation in male resistance arterioles exposed to 100 nM oestrogen and showing predominant ERβ and GPER expression, with undetectable ERα expression	High ERβ:ERα ratio linked to pro-oxidative response of the vasculature to oestrogen	SenthilKumar et al. [79]
Inflammation	Increased AAA incidence	Increased expression of inflammatory genes, plasma IL-1b concentrations, altered matrix metalloproteinase activity and increased oxidative stress	Alsiraj et al. [82] Schutte et al. [77] Wilson et al. [78]

were likely due to differences in the urea cycle, not NOS activity. Thus, NO mediated vascular function was not significantly affected by the initiation of GAHT. As such Auer et al.'s findings do not, in fact, conflict with Gulanski et al.

Auer et al.'s study was part of the European Network for the Investigation of Gender Incongruence study, which aimed to evaluate the clinical and side effects of GAHT; recognising that previous studies investigating safety and clinical effectiveness had been poorly designed [21]. The participants in Auer et al.'s study were studied at baseline and 12 months following the initiation of GAHT, which was intended to be standardised across all participants. However, some trans men were already taking lynestrenol, prescribed to stop their menses, prior to initiating GAHT and continued to do so during the 12 months study period (pre-treatment group). Auer et al. noted significantly lower total testosterone levels at 12 month follow up in the pre-treatment group, compared with the treatment naïve trans men. However, no consideration is given to analysing the pre-treatment and treatment naïve groups separately. The possibility of testosterone interacting with lynestrenol, causing C/A ratios to increase cannot be ruled out, especially given lynestrenol is known to decrease the metabolism of the short-acting testosterone formulations [70].

Lichtenecker et al. conducted a study to investigate the effects of testosterone on renal morphology in a rat model of trans masculine GAHT. The study concluded that testosterone administration increases SBP and induces changes in renal morphology, when compared to male and female vehicle-treated controls [71]. Lichtenecker et al. discussed that the mechanism underlying SBP increases may relate to testosterone activation of RAAS. In a study investigating the effects of androgens on proximal tubule transport in male rats, increased expression of the NHE3 sodium transporter in the proximal tubule in response to androgen exposure was observed [72]. Increased expression of NHE3 would lead to decreased concentrations of sodium in the plasma and the urine. In contrast, Lichtenecker et al. observed no changes to sodium concentrations in either the plasma or urine of the testosterone treated rats, when compared to female controls. Thus, these findings do not support the theory that testosterone upregulates the expression of NHE3 via the RAAS mechanism. Limitations of this study include the absence of an administration and dosing schedule of testosterone administration in female rats, instead opting to cite methods from other sources with inconsistent citations.

A study by Goetz et al. investigating if adding low dose oestradiol to a model of trans masculine GAHT in ovariectomised (OVX) mice would reduce the progression of atherosclerosis found positive results [73]. OVX female mice treated with a combination of testosterone and oestrogen had significant reductions in atherosclerotic plaque size of ~75% and 80% compared to control and testosterone only treated mice, respectively. There was no significant difference in plaque size between testosterone and control treated mice. Interestingly, the combined testosterone + oestrogen treated mice showed plaque size reductions comparable to mice treated with oestrogen alone, when compared to controls. Goetz et al. concluded that the addition of low dose oestrogen to GAHT following gender reassignment surgery in trans men could be beneficial in reducing cardiovascular disease risk. This may seem counterintuitive given the purpose of GAHT. Goetz explains that in males, oestrogen is produced by the aromatization of plasma testosterone into E2, and that this process is only half as effective as in females (0.2% *vs* 0.4% aromatization; female *vs* male) [74]. Post ovariectomy, trans men can only produce oestrogen via aromatization. Goetz et al.'s findings indicate that low oestrogen levels due to impaired aromatization capability in trans men is detrimental to cardiovascular health, as supported by the sharp increase in cardiovascular disease risk observed in females following menopause [75]. Goetz et al.'s findings come with a key caveat: any introduction of oestrogen into the GAHT regimen of trans men post-ovariectomy must not reverse phenotypical changes. Also, due to concern of reversal of phenotypic changes, the compliance of trans men to take oestrogen may be low, with their priority focussed on maintaining the characteristics that align with their gender identity rather than reducing cardiovascular disease risk. Stringent testing would be needed, first in models, then humans, to establish a dose of oestrogen sufficient to reduce cardiovascular disease risk without impacting desired physical attributes. Studies selectively targeting the membrane GPER in this context would also be of merit.

The positive effects observed following oestrogen addition to GAHT in trans men allows for hypothesis generation that the incomplete suppression of endogenous hormones in trans men who have not undergone surgery may positively modify their cardiovascular disease risk. Studies assessing the mechanisms underlying the cardioprotective actions of oestrogen in cisgender women have consistently shown that oestrogen increases NO release by promoting eNOS activity. The effects of oestrogen on eNOS are non-genomic and occur rapidly, as evidenced by *in vitro* studies [76], not because of transcriptional alteration, despite partial estrogen response elements existing in the eNOS gene promotor. It is plausible that this mechanism could explain Goetz et al.'s results and the results of Auer et al.'s study would further support this conclusion. Additionally, it would indicate that despite the juxtaposing effects of oestrogen and testosterone on the NO pathway, oestrogen is the more potent determinant of cardiovascular phenotype.

4.2. Transfeminine (oestrogen) hormone therapy

The number of studies investigating the mechanisms which underpin the effects of feminizing GAHT on the cardiovascular system are more limited than for transmasculine GAHT. Schutte et al. demonstrated a decrease in endothelial and systemic inflammatory markers in trans women 12 months post GAHT initiation (dermal E2 patches and CPA) [77]. This contrasts with Wilson et al. who observed no alterations to inflammatory markers in trans women prescribed dermal E2 patches, but an increase in trans women prescribed oral oestrogen. However, these increases were only observed during months 2–4 following GAHT initiation, and were no longer significant at 6 months [78]. Additionally, Wilson et al.'s study was poorly powered (transdermal E2, n = 6, oral oestrogen, n = 23) whereas Schutte et al. demonstrated results across a population of 48 trans women. Neither study postulated the mechanisms linking oestrogen to changes in inflammatory markers, despite citing these markers as of major influence in modulating cardiovascular disease risk.

SenthilKumar et al. sought to investigate the effects of E2 on the microvasculature using resistance arterioles isolated from tissue discarded during surgery, in both males and females. When exposed to 100 nM E2 in an organ bath, resistance arterioles from males exhibited both endothelial and smooth muscle impaired vasodilation, regardless of diseased or healthy vessel state, contrasting the findings from female arterioles [79]. Additionally, SenthilKumar et al. demonstrated predominant expression of ERβ and GPER in the endothelium of the male arterioles, while ERα was undetectable. In arteries, ERα expression is associated with increased NO production [80], while ERβ expression is positively correlated with atherosclerotic plaque size [81]. A high ERβ: ERα ratio has been connected to a pro-oxidative response of the vasculature to oestrogen, causing increased levels of oxidative stress and associated pro-inflammatory changes, leading to vascular dysfunction. SenthilKumar et al. cites this as a potential mechanism underpinning impaired vascular function in trans women undergoing GAHT; but recognises the limitation of their study as male arterioles are used as a surrogate for arterioles from trans women. In the arterioles from female patients, catalase was used to investigate whether NO or H_2O_2 was the primary vasoactive mediator in the arterioles. NO is anti-inflammatory whereas H_2O_2 is proinflammatory. This was not conducted in the arterioles from male participants, so the primary mediator driving the vasomotor changes in the arterioles could not be concluded.

Such findings are further supported by Alsiraj et al.'s murine model [82]. Phenotypically female mice with XY chromosomes had a doubled incidence of abdominal aortic aneurysms (AAA) following angiotensin II infusion for 28 days. AAAs are sex-dependent, and testosterone is known to promote AAA development. However, the specific impact of chromosomes was not known. The increased AAA incidence was coupled with significantly increased expression of inflammatory genes, plasma IL-1b concentrations, altered matrix metalloproteinase activity and increased oxidative stress, compared to XX female mice. This has relevance for the transgender population as it allows for hypothesis generation regarding the effect of initiating oestrogen as part of feminizing GAHT, and further supports the idea that inflammation underpins the increased cardiovascular disease risk witnessed in trans women following GAHT initiation.

5. Cardiovascular risk beyond GAHT

Beyond the vascular effects of GAHT, there are multiple factors that may increase the burden of cardiovascular disease in the transgender population. Transgender individuals experience major psychological stresses throughout their lifetime, which contributes to a disproportionate risk of developing cardiovascular disease compared with the cisgender population [8]. Based on the Gender Minority Stress and Resilience Model, the contribution of distal stresses specific to the trans population, such as discrimination, gender non-affirmation and stigma, and proximal stresses; internalised transphobia or identity concealment, to the individual's overall stress level modulates their cardiovascular disease risk [28]. In an analysis from Ontario, Canada, 35.1% of transgender individuals considered suicide in the previous year, while 11.2% attempted this [83], demonstrating the considerable psychiatric co-morbidity in this population. Such stressors would undoubtedly impact cardiovascular disease risk [84].

Moreover, adverse health behaviours are prevalent within the transgender population that may modulate risk [85]. Behavioural risks related to cardiovascular disease are discussed in Rajendran et al. [86] and Antza et al. [87] in this issue. The prevalence of tobacco use in the transgender population has been demonstrated to be substantially higher than in cisgender populations, thereby increasing their risk of tobacco-associated cardiovascular disease [88,89]. Furthermore, transgender individuals experience significant obstacles, including discrimination, in accessing healthcare, thereby promoting health disparities [90,91]. There are well established connections between poor socioeconomic status and increased cardiovascular disease [92]. In a national register-based cohort study of 2770 Danish transgender individuals, transgender status was associated with lower socioeconomic status. In this analysis, the relative risk ratio of low versus high income was 5.8 in transgender individuals compared to age-matched cisgender controls. Similarly, the risk of being unemployed or receiving welfare in transgender individual was elevated. Such disparities in equity and access could substantially contribute to the burden of cardiovascular disease in this population.

6. Conclusions and perspectives

As the population of transgender individuals has increased, so too has the number of individuals choosing to undergo GAHT. There are clear benefits of GAHT beyond facilitating gender congruence with secondary sex characteristics [9], including reductions in gender dysphoria, improved psychosocial functioning [10] and reduced adverse mental health outcomes [11]. Novel studies investigating the impact of transmasculine therapy on cardiovascular disease risk point to the shared conclusion that testosterone affects the NO pathway and triggers inflammation, promoting endothelial dysfunction. Equivalent studies are yet to be undertaken focussing on transfeminine therapy, highlighting a crucial area for further research. Additionally, the disproportionate cardiovascular disease burden carried by the transgender population, arising from a multitude of psychological stressors, adverse mental health, socioeconomic disparity, and smoking cannot be ignored when formulating clinical strategies to combat increased cardiovascular disease risk.

Further research is required to understand the long-term effects of GAHT on the cardiovascular system, and to elucidate the mechanisms underpinning the suspected increased cardiovascular disease risk in transgender individuals associated with GAHT, to appropriately inform guidance and practise to reduce risk. Prospective long-term trials (e.g. a 10-year longitudinal analyses) would be of great benefit in understanding the prevalence of cardiovascular disease and long-term effects of GAHT in this patient population, as potential mediators such as enhanced vascular aging and calcification are unlikely to manifest in the short-term. Considering recent evidence, we would highlight the importance of the careful recognition and management of cardiovascular risk factors in this underserved population. However, clinical trials are requires to determine the utility of intensive cardiovascular risk management strategies.

Studies investigating ER and AR subtype expression and signalling in the context of GAHT would assist in examining the contribution of sex steroids in modulating cardiovascular disease risk. Additionally, epigenomics may provide insight into the processes by which minority stress and other social determinants of adverse cardiovascular outcomes act in this population. As many novel mechanisms may contribute, the construction of biobank repositories may be an extremely useful

resource in the identification of transgender-specific biomarkers and mediators.

Of importance is ensuring that future studies are determined in conjunction with the transgender population [93], and are sufficiently powered, well controlled, and investigate mechanisms across the transgender spectrum. A recently published perspective details a road map for improving the inclusion of transgender and nonbinary individuals in the planning, completion, and mobilization of cardiovascular research [94]. Adoption of this procedure, alongside suggested research and clinical practise suggestions in the WPATH Standards of Care [16], would allow for more inclusive practises and assist in both determining and implementing evidence-based interventions to lower cardiovascular disease risk in the transgender population.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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