Journal of the Endocrine Society, 2024, **8**, bvae120
https://doi.org/10.1210/jendso/bvae120
Advance access publication 27 June 2024
Clinical Research Article

Transgender Women With Suppressed Testosterone Display Lower Burden of Coronary Disease Than Matched Cisgender Men

Jordan E. Lake,[1,*] Han Feng,[2,*] Ana N. Hyatt,[1] Hongyu Miao,[3] Paula Debroy,[1] Nicholas Funderburg,[4] Kate Ailstock,[4] Adrian Dobs,[5] Sabina Haberlen,[5] Jared W. Magnani,[6] Joseph B. Margolick,[5] Kate McGowan,[5] Frank J. Palella,[7] Mallory D. Witt,[8] Shalender Bhasin,[9] Matthew J. Budoff,[8] Wendy S. Post,[5] and Todd T. Brown[5]

[1]Department of Medicine, UTHealth Houston, Houston, TX 77030, USA
[2]Tulane Research and Innovation for Arrhythmia Discoveries-TRIAD Center, Tulane University School of Medicine, New Orleans, LA 70112, USA
[3]Department of Statistics and College of Nursing, Florida State University, Tallahassee, FL 32306, USA
[4]Division of Medical Laboratory Science, The Ohio State University, Columbus, OH 43210, USA
[5]Department of Medicine, Johns Hopkins University, Baltimore, MD 21218, USA
[6]Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15261, USA
[7]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
[8]Department of Medicine, Lundquist Institute, Torrance, CA 90502, USA
[9]Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA

Correspondence: Jordan E. Lake, MD, MSc, UTHealth Houston, 6431 Fannin St, MSB 2.112, Houston, TX 77030, USA. Email: Jordan.E.Lake@uth.tmc.edu.
*J.E.L. and H.F. are co-first authors of this work.

Abstract

Context: Cardiovascular disease (CVD) in transgender women (TW) may be affected by gender-affirming hormone therapy (GAHT) and HIV, but few data compare TW on contemporary GAHT to well-matched controls.

Objective: We compared CVD burden and biomarker profiles between TW and matched cisgender men (CM).

Methods: Adult TW on GAHT (n = 29) were recruited for a cross-sectional study (2018-2020). CM (n = 48) from the former Multicenter AIDS Cohort Study were matched 2:1 to TW on HIV serostatus, age ±5 years, race/ethnicity, BMI category and antiretroviral therapy (ART) type. Cardiac parameters were measured by CT and coronary atherosclerosis by coronary CT angiography; sex hormone and biomarker concentrations were measured centrally from stored samples.

Results: Overall, median age was 53 years and BMI 29 kg/m^2; 69% were non-white. All participants with HIV (71%) had viral suppression on ART. Only 31% of TW had testosterone suppression (<50 ng/dL, TW-S). Traditional CVD risk factors were similar between groups, except that TW-S had higher BMI than TW with non-suppressed testosterone (TW-T). TW-S had no evidence of non-calcified coronary plaque or advanced coronary stenosis, whereas TW-T and CM had similar burden. TW had lower prevalence of any coronary plaque, calcified plaque and mixed plaque than CM, regardless of testosterone concentrations and HIV serostatus. Estradiol but not testosterone concentrations moderately and negatively correlated with the presence of coronary plaque and stenosis. Small sample size limited statistical power.

Conclusion: Older TW with suppressed total testosterone on GAHT had no CT evidence of non-calcified coronary plaque or advanced coronary stenosis. Longitudinal studies to understand relationships between GAHT and CVD risk in TW are needed.

Key Words: transgender women, coronary disease, gender-affirming hormone therapy, HIV

Abbreviations: ART, antiretroviral therapy; ASCVD, atherosclerotic cardiovascular disease; AT, adipose tissue; BMI, body mass index; CAC, coronary artery calcium; CD, cluster of differentiation; CM, cisgender men; CT, computed tomography; CVD, cardiovascular disease; CW, cisgender women; EN-RAGE, extracellular newly identified receptor for advanced glycation end products; ET-1, endothelin-1; FABP, fatty acid binding protein; GAHT, gender-affirming hormone therapy; HOMA-IR, homeostatic model assessment of insulin resistance; IL, interleukin; MACS, Multicenter AIDS Cohort Study; PAI, plasminogen activator inhibitor; PWH, people with HIV; SAT, subcutaneous adipose tissue; sCD14, soluble CD14; SHBG, sex hormone–binding globulin; sTNFR, soluble tumor necrosis factor receptor type; TW, transgender women; TW-S, transgender women with total testosterone <50 ng/dL; TW-T, transgender women with total testosterone ≥50 ng/dL; VAT, visceral adipose tissue; VCAM, vascular cell adhesion molecule.

Transgender women (TW) have a high prevalence of modifiable cardiovascular disease (CVD) risk factors [1-8] and are disproportionately affected by HIV, with published HIV prevalence rates ranging from 14% to 42% [9, 10]. Gender-affirming hormone therapy (GAHT), HIV, and antiretroviral therapy (ART) have each been associated with

Received: 29 March 2024. **Editorial Decision:** 10 June 2024. **Corrected and Typeset:** 5 July 2024

altered body composition, inflammatory and coagulation pathway abnormalities, and cardiometabolic disturbances [11-13], creating a potentially unique milieu for CVD development among TW.

Feminizing GAHT (estrogen ± antiandrogen therapy) for transgender and gender-diverse individuals assigned male sex at birth can harmonize gender identity and physical expression, and regimens are highly individualized. For example, suppression of circulating total testosterone concentrations into the normal range for cisgender women (CW) (<50 ng/dL) is not the goal or does not occur for all TW, creating a range of testosterone exposure in the population. Similarly, estrogen dosing varies widely by provider and geography. Together, these may create scenarios where GAHT may alter cardiometabolic risk to varying degrees across populations of TW. GAHT causes fat gain and may increase metabolic disease risk [14], although the longer-term CV effects of GAHT are controversial [15-17], with some studies observing heightened CVD risk [18], systemic inflammation, and metabolic disease [19-21], and others favorable changes in lipid profiles and body composition [22, 23].

Similarly, HIV and ART are associated with altered body composition, metabolic disturbances [11], inflammation and immune activation [24, 25], and multiple coagulation pathway abnormalities [26-28]. Furthermore, HIV is associated with increased CVD risk, with CVD being a leading cause of morbidity and mortality among people with HIV (PWH) on suppressive ART [29-34]. The mechanisms underlying heightened CVD risk in PWH are not well understood, but are believed to involve traditional as well as HIV- and ART-specific risk factors, with fat gain/obesity development after ART initiation as an example of the latter [35-37].

There are limited data addressing the confluence of contemporary GAHT-, HIV-, and ART-induced immunometabolic alterations and their effects on body composition and cardiometabolic risk in TW. We conducted an observational, cross-sectional study to compare coronary atherosclerosis burden and circulating biomarker profiles between TW and matched cisgender men (CM). We hypothesized that TW with circulating total testosterone concentrations in the normal range for CW (<50 ng/dL) would have less atherosclerotic burden than matched CM.

Materials and Methods

Study Design and Participants

TW were recruited between 2018 and 2020 from community-based organizations and clinics in Baltimore, Maryland, USA, and Houston, Texas, USA, for a pilot, observational study. Participants were enrolled sequentially without regard to HIV serostatus. Inclusion criteria for TW included self-identification as a TW or transfeminine person, being aged 40 to 70 years, on current GAHT for 3 months or longer, and ability and willingness to undergo computed tomography (CT) scans and to provide written informed consent. For TW with HIV, written documentation of HIV serostatus was required, and participants were required to be taking ART and have HIV-1 RNA with fewer than 50 copies/mL at screening. For TW believed not to have HIV, documented HIV testing within the prior 90 days and/or testing immediately prior to enrollment was required (latter available at the enrollment sites and most individuals were retested the day of enrollment). Exclusion criteria included living with HIV-1 and not receiving ART, history of coronary artery bypass grafting, heart valve surgery, coronary angioplasty or atrial fibrillation, weight 300 pounds (136 kg) or more, estimated glomerular filtration rate of 60 mL/min or less, and history of contrast allergy or nephropathy.

Matched control CM were selected from participants of the former Multicenter AIDS Cohort Study (MACS) Cardiovascular Substudies 2 or 3 (CVD2, CVD3). These cohorts were chosen due to overlapping clinical and demographic characteristics with the communities of TW enrolled. The MACS was initiated in 1984 to study the natural history of HIV among men who have sex with men and to establish a repository of biologic specimens for future study [38]. Participants were enrolled from 4 sites (Pittsburgh, Pennsylvania, USA; Baltimore, Maryland, USA/Washington, DC, USA; Chicago, Illinois, USA; Los Angeles, California, USA) over 4 time periods (1984-1985, 1987-1990, 2001-2003, 2010+) and completed semiannual visits that included a standardized medical history interview, clinical evaluations, laboratory tests, and storage of specimens. The MACS CVD2 (2010-2013) and CVD3 (2015-2017) substudies were designed to assess metabolic, inflammatory, immunologic, and HIV-specific factors associated with coronary atherosclerosis. Complete methodological details of CVD2 and CVD3 have been described previously [39, 40]; in brief, men were aged 40 to 70 years, did not have a history of heart surgery or coronary angioplasty, weighed less than 300 pounds (136 kg), and were willing and able to provide informed consent.

CM were matched 2:1 (where possible, see "Results" for details) to TW on HIV serostatus, age within 5 years, race/ethnicity, body mass index (BMI) category, and ART type to reduce the potential of confounding from those variables and achieve more comparable groups. All CM with HIV had undetectable HIV-1 RNA on ART. MACS participants identifying on the transfeminine spectrum at the time of CVD2/3 participation were excluded from the pool of potential controls and included as cases. Specimens and data from MACS participants were collected at the time of substudy participation, as described earlier.

Study Procedures

General

The study was approved by the institutional review boards of Johns Hopkins University (Baltimore, Maryland, USA) and UTHealth Houston (Houston, Texas, USA). For TW, written informed consent was obtained prior to the initiation of study procedures. Consent for MACS participants was covered under their MACS CVD 2/3 consent.

TW self-reported basic demographic information, medical history including substance use history, and current medication usage. A separate GAHT questionnaire assessed GAHT usage, including current or past usage, GAHT type and frequency, and GAHT source. For MACS CM, existing variables equivalent to those available for TW were selected.

Blood collection and circulating biomarker and sex hormone concentrations

For TW, blood was collected in the fasting state (nothing to eat or drink except water and medications for at least 8 hours) for comprehensive metabolic panel, lipid panel, complete blood count, HIV-1 RNA, and $CD4^+$ T lymphocyte count measurement at a local certified laboratory. If HIV-1 RNA and $CD4^+$ T lymphocyte count were obtained within 30 days prior to date of entry through routine clinical care, those values were

used instead of redrawing samples for additional laboratory testing at screening. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as fasting insulin [μIU/mL] × fasting glucose [mg/dL])/405, glomerular filtration was estimated by the 2021 Chronic Kidney Disease–Epidemiology Collaboration equation (https://www.kidney.org/professionals/kdoqi/gfr_calculator/formula) using sex assigned at birth for all participants, and 10-year risk for atherosclerotic cardiovascular disease (ASCVD) was estimated by the 2021 ASCVD Risk Estimator Plus by the American College of Cardiology at https://tools.acc.org/ascvd-risk-estimator-plus/#!/calculate/estimate/using sex assigned at birth for CM and current gender for TW [41].

Additionally, serum and plasma were collected from TW and stored at −70 °C in the laboratory of Dr Jordan Lake (UTHealth Houston, Houston, Texas, USA) until batched biomarker measurement could occur. Control serum and plasma samples were sent from the MACS repository (National Institutes of Health, Frederick, Maryland, USA) to the processing laboratories. The measured circulating biomarkers were chosen for their known relationships to CVD, inflammatory processes, and/or coagulation abnormalities. Soluble CD14 (sCD14, R and D Systems catalog No. DC140, RRID:AB_3095885), soluble CD163 (sCD163, R and D Systems catalog No. DC1630, RRID:AB_3096052), interleukin-6 (IL-6, R and D Systems catalog No. HS600C, RRID:AB_2893335), fatty acid-binding protein-4 (FABP-4, (ImmunoDiagnostics catalog No. 31030, RRID:AB_2933962), high-molecular-weight adiponectin (R and D Systems catalog No. DRP300, RRID:AB_2783020), insulin (R and D Systems catalog No. DINS00, RRID:AB_3073852), endothelin-1 (ET-1, R and D Systems catalog No. DET100, RRID:AB_3099470), and vascular cell adhesion molecule-1 (VCAM-1, R and D Systems catalog No. DVC00, RRID:AB_2941366) were analyzed by Quantikine ELISA (enzyme-linked immunosorbent assay), and soluble tumor necrosis factor receptor type (sTNFR, R and D Systems catalog No. LUCAM726, RRID:AB_3099473) I/II, interleukin-8 (IL-8, R and D Systems catalog No. DY208, RRID:AB_2892143), and plasminogen activator inhibitor-1 (PAI-1, R and D Systems catalog No. LOBM1786, RRID:AB_3099472) were measured by Luminex assay. Assays were performed per each manufacturer's instructions. All samples were run in duplicate to assess variability, and the percentage of coefficient variance was consistently less than 15%. Biomarkers assays were performed in the laboratory of Dr Jordan Lake (UTHealth Houston, Houston, Texas, USA), except that extracellular newly identified receptor for advanced glycation end products (EN-RAGE) was measured by DuoSet ELISA (R and D Systems, catalog No. DY1052-05, RRID:AB_3099471) in the laboratory of Dr Nicholas Funderburg (The Ohio State University, Columbus, Ohio, USA).

Sex hormone measurements (estradiol and total testosterone by liquid chromatography–mass spectrometry; free testosterone by equilibrium dialysis; sex hormone–binding globulin [SHBG] by Access Chemluminescent Immunoassay [Beckman Coulter catalog No. A48617, RRID:AB_2893035]) were measured at the Brigham Research Assay Core Laboratory (Brigham and Women's Hospital, Boston, MA, USA) in batch at end of study.

Computed tomography scan performance and interpretation

For CM, cardiac parameters and body composition were previously measured in the CVD2 and CVD3 substudies by non-contrast, cardiac CT imaging and single-slice scans of the abdomen at the level of the umbilicus (L4-L5 vertebral level) and mid-thigh (15 cm above the patellar apex), as well as coronary CT angiography for assessment of coronary luminal narrowing/stenosis, plaque presence, and composition. Complete MACS CVD2/3 CT scanning procedures have previously been published [39, 42]. TW underwent the same protocol as MACS CVD2/3 participants, with measurement of coronary artery calcium (CAC) and adipose tissue (AT, subcutaneous [SAT], visceral [VAT]) areas and densities. Deidentified CT images were electronically transferred to the same reading center used for CM (The Lundquist Institute, Torrance, California, USA) for centralized, blinded analysis. Fat was identified using anatomic landmarks and density ranges of −190 to 0 Hounsfield Units (HU), depending on depot. Fat area was reported in cm^2 and density in HU. Liver and spleen images, when available, allowed assessment of the liver-to-spleen attenuation ratio. Moderate-to-severe hepatic steatosis was defined as liver-to-spleen attenuation ratio less than 1.0.

Each coronary segment was analyzed using the modified 15-segment model of the American Heart Association [43]. Using axial images, multiplanar reconstructions, and maximum-intensity projections, the reader assessed the presence, size, and composition of coronary plaque and the degree of luminal narrowing stenosis in all assessable coronary segments. Plaque size was graded as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Segment stenosis was defined as 0 (none), 1 (1%-29% [minimal]), 2 (30%-49% [mild]), 3 (50%-69% [moderate]), or 4 (≥70% [severe]). The total plaque score was calculated by summing the plaque size score for all assessable coronary segments that showed any plaque (calcified, noncalcified or mixed), up to a maximum score of 45. This measure has been shown to be highly reproducible [44]. The segment involvement score was calculated as the sum of coronary artery segments with plaque, regardless of the degree of stenosis. Each coronary segment was classified as normal or containing noncalcified, mixed (50% of plaque area occupied by calcium) or calcified plaque. Calcified atherosclerotic plaque was defined as any structure with attenuation greater than 130 HU visualized separately from the intravascular lumen, identified in at least 2 independent planes. Noncalcified atherosclerotic plaque was defined as any discernible structure that could be clearly assignable to the vessel wall, with a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue and identified in at least 2 independent planes. Finally, the noncalcified, mixed, and calcified plaque scores were calculated by summing the plaque scores in each plaque segment separately.

Statistical Analysis

For this analysis, a planned sample size of TW (N = 40) was estimated to detect differences in biomarker concentrations with 90% power and differences in noncalcified plaque burden with 80% power, compared to controls. This estimate was based around an achievable and clinically significant between-group difference in $\log_{10}$ sCD14 in PWH of 0.07 (SD 0.09) [45, 46]. Thirty-six evaluable participants per arm were needed to detect a 0.07 $\log_{10}$ sCD14 level difference between any 2 groups with 90% power and a 0.05 2-sided type I error rate, under the assumption that the SD of $\log_{10}$ sCD14 is 0.09 across groups.

Demographic and clinical characteristics were summarized as median (interquartile range, IQR) or frequency. Due to a limited number of participants without HIV, results were not stratified

by HIV serostatus, though sensitivity analyses by HIV serostatus were performed. Given that testosterone in the normal range for CW is not the goal for many TW on GAHT and having total testosterone in the normal range for CW reflects a physiologic state where testosterone is not currently contributing to CVD risk, results from TW were stratified by total testosterone less than 50 mg/mL or greater than or equal to 50 ng/dL [47].

Kruskal-Wallis and Pearson χ^2 tests compared continuous and categorical variables, respectively, between groups. Given the presence of nonnormality in a majority of the variables, bivariate correlations were assessed using the Spearman rank correlation method. Statistical significance was defined as a 2-sided P less than .05. For this hypothesis-generating, pilot study, no adjustments were made for multiple comparisons. Analyses were conducted in R version 4.2.0 (http://www.R-project.org, R Foundation).

Results

Study Population

Twenty-nine participants were enrolled between July 2018 and January 2020, with enrollment stopped short of the target of 40 due to the COVID-19 pandemic. All participants self-identified as TW and were categorized as having total testosterone in the normal range for CW (TW-S, n = 9) vs not (TW-T, n = 20). Two TW identified among MACS CVD substudy participants were included as patient cases. TW were matched 2:1 to CM selected from MACS CVD2 or CVD3 participants by HIV serostatus, age within 5 years, race/ethnicity, BMI category, and ART type (latter when possible). One TW-T participant did not have matched controls due to a lack of specimen availability; all analyses including and excluding this participant had similar results (results including this participant shown).

Clinical and demographic characteristics of the participants are presented in Table 1. Briefly, TW-S had a median (IQR) age of 51 (45-60) years, 67% were Black/African American, 67% were living with HIV, and the median BMI was 33 (28-39). TW-T had a median age of 53 (43-57) years, 45% were Black/African American, 30% Hispanic/Latina and 20% White, 80% were living with HIV, and the median BMI was 29 (26-32). Participants living with HIV had undetectable plasma HIV-1 RNA, a median $CD4^+$ T-cell count greater than 500 cells/μL, and most were receiving integrase strand transfer inhibitor-based ART. All TW reported estrogen use and half in both the TW-S and TW-T groups reported spironolactone use. No other antiandrogen use was reported. Median reported duration of current GAHT use was 3.5 (IQR 2.7) years (N = 18). Overall, 65%, 17%, and 14% reported use of oral, transdermal, and intramuscular formulations, respectively. One TW reported use of a progestin (megestrol acetate). No TW had history of gonadectomy.

Overall, TW had similar metabolic profiles and frequencies of traditional CVD risk factors as CM, regardless of HIV serostatus. Ten-year ASCVD risk scores were also statistically similar to CM, though the median among TW (when using current gender for sex variable), 6%, is classified as borderline risk, whereas the median of 8% among CM is classified as intermediate risk.

Sex Hormone and Biomarker Results

TW-S and TW-T had significantly higher serum estradiol concentrations than CM, though many were below the target range of 100 to 200 pg/mL [47]. Notably, TW-T had similar total testosterone concentrations to CM (see Table 1).

TW-S and TW-T had significantly higher median ET-1 concentrations than CM. TW-T had lower sCD14 concentrations than CM; TW-S had higher IL-6 and lower PAI-1 concentrations than CM (all $P < .05$), and similar concentrations of remaining biomarkers (Table 2). When restricting to TW with HIV, attenuated differences were seen in IL-6 and PAI-1 (data not shown). EN-RAGE concentrations among TW were higher than expected based on general population estimates [48]; concentrations were not measured for CM due to lack of control sample availability.

Testosterone concentrations strongly and inversely correlated with TNFRII ($R = -0.8$; $P = .03$) and VCAM ($R = -0.7$, $P = .04$) concentrations. Though coefficients remained the same when restricting to PWH, the P value for TNFRII remained significant, but the P value for VCAM did not. No other statistically significant correlations were observed between sex hormone and biomarker concentrations.

Body Composition

TW-S and TW-T both had significantly lower intrathoracic and thoracic periaortic AT areas than CM. Additionally, both groups of TW had higher thigh muscle AT area ($P < .01$), but similar epicardial AT, SAT, VAT, and thigh subcutaneous AT areas as CM. Neither TW-S nor their controls had moderate-to-severe hepatic steatosis by liver-to-spleen ratio assessment; frequency of hepatic steatosis was low among TW-T and their controls (8% vs 10%; $P > .05$) (Table 3). Results were similar excluding participants without HIV (data not shown).

Among TW-T, testosterone concentrations moderately and negatively correlated with abdominal SAT ($R = -0.5$, $P = .02$) and VAT ($R = -0.5$, $P = .03$) areas. No other robust correlations were found between sex hormone concentrations and fat quantity.

Cardiac Outcomes

TW-S had no evidence of noncalcified coronary plaque or advanced coronary stenosis. Additionally, all TW had lower prevalence of coronary plaque of any type, calcified coronary plaque, and mixed coronary plaque than CM, regardless of testosterone concentrations and HIV serostatus (all $P > .5$), though differences were generally greater between TW-S vs CM than TW-T vs CM. There were no significant differences in prevalent CAC or median CAC Agatston scores among participants with prevalent CAC (Table 4).

Estradiol but not testosterone concentrations were moderately and negatively correlated with the presence of plaque of any type ($R = -0.5$), calcified plaque ($R = -0.5$), mixed plaque ($R = -0.5$), any coronary stenosis ($R = -0.6$), and having a CAC Agatston score greater than 0 ($R = -0.4$), though none of these achieved statistical significance (all $P > .05$). These correlations weakened when restricting analyses to TW living with HIV.

Discussion

In this pilot study, middle-aged TW on GAHT with serum total testosterone concentrations in the normal range for CW and high rates of HIV and traditional CVD risk factors had zero prevalence of noncalcified coronary plaque and zero prevalence of obstructive coronary plaque (stenosis >50%),

Table 1. Clinical and demographic characteristics

	TW-S (N = 9)	CM (N = 15)	*P* TW-S vs CM	TW-T (N = 20)	CM (N = 33)	*P* TW-T vs CM
Age, y	51 (45-60)	55 (48-57)	[a]	53 (43-57)	55 (50-57)	[a]
Race/ethnicity			[a]			[a]
Black/African	67%	60%		45%	42%	
American						
White	33%	40%		20%	30%	
Hispanic/Latinx	0	0		30%	27%	
Asian/Pacific Islander	0	0		5%	0	
% living with HIV	67%	60%	[a]	80%	76%	[a]
CD4+ T-cell count, cells/μL	599 (402-605)	697 (624-925)	.2	820 (750-901)	609 (497-726)	.01
ART (if living with HIV)						
PI	11%	13%	.8	10%	18%	.4
NNRTI	11%	13%	.8	10%	27%	.1
INSTI	44%	33%	.7	55%	45%	.6
TAF/TDF	44%	53%	.4	55%	64%	.3
ABC	22%	7%	.4	15%	9%	.6
Hepatitis B, history of	0	7%	.4	5%	0	.2
Hepatitis C, history of	22%	7%	.2	20%	9%	.3
10-y ASCVD score[b]	6% (4-7)	8% (6-13)	.1	6% (5-10)	8% (4-10)	.7
BMI	33 (28-39)	32 (27-34)	[a]	29 (26-32)	28 (25-32)	[a]
Current smoker	11%	33%	.2	40%	21%	.1
Hypertension	25%	53%	.2	30%	42%	.4
Diabetes	11%	21%	.5	10%	15%	.6
Fasting glucose, mg/dL	94 (83-103)	95 (90-114)	.8	92 (87-97)	98 (92-105)	.06
HOMA-IR	2.1 (0.9-2.9)	1.9 (1.6-3.1)	.5	1.8 (1.3-7)	1.8 (1.4-3.6)	.7
Hyperlipidemia[c]	33%	71%	.07	55%	82%	.04
Total cholesterol, mg/dL	177 (172-185)	177 (154-198)	.7	162 (156-215)	184 (156-215)	.6
HDL cholesterol, mg/dL	54 (47-61)	46 (39-50)	.01	46 (38-57)	45 (37-56)	.9
LDL cholesterol, mg/dL	104 (91-115)	104 (93-132)	.4	96 (84-142)	115 (91-131)	.8
Triglycerides, mg/dL	89 (75-112)	120 (75-175)		123 (74-190)	131 (93-167)	0.9
Lipid-lowering agent use						
Statins	20%	7%	.3	40%	15%	.05
Fibrates	10%	0	.4	10%	3%	.4
Ezetimibe	0	7%	.6	0	3%	.6
Other	0	0		0	6%[d]	.4
On hormone therapy						
Estrogens (any form)	100%	0		100%	0	
Androgen antagonists	44%	0		50%	0	
Testosterone	0	7%		0	6%	
Hormone concentrations						
Estradiol, pg/mL	78 (59-129)	24 (18-31)	.002	66 (30-116)	22 (17-27)	<.001
Total testosterone, ng/dL	13 (12-17)	400 (346-549)	<.001	453 (294-765)	431 (338-510)	.6
Free testosterone, ng/dL	0.5 (0.4-0.6)	9 (7-15)	<.001	13 (7-18)	13 (11-15)	.9
SHBG, nmol/L	73 (63-166)	32 (26-39)	.003	68 (37-86)	33 (29-43)	.004

Frequency or median (interquartile range) presented.
Abbreviations: ABC, abacavir; ART, antiretroviral therapy; ASCVD, atherosclerotic cardiovascular disease; BMI, Body mass index; CD, cluster of differentiation; CM, cisgender men; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of insulin resistance; INSTI, Integrase strand transfer inhibitor; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SHBG, sex hormone–binding globulin; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TW-S, transgender women with total testosterone <50 ng/dL; TW-T, transgender women with total testosterone ≥50 ng/dL.
[a]Matching factor.
[b]ASCVD risk score was calculated using sex assigned at birth for CM and current gender for TW.
[c]Hyperlipidemia was clinically diagnosed and/or participant was on lipid-lowering agents at screening.
[d]Niacin (N = 1), omega-3 fatty acid (N = 1).

Table 2. Biomarker concentrations

	TW-S (N = 9)	CM (N = 15)	*P* TW-S vs CM	TW-T (N = 20)	CM (N = 33)	*P* TW-T vs CM
IL-6, pg/mL	2.9 (1.9-4.2)	1.5 (0.9-1.9)	.04	1.6 (1.1-2.8)	1.7 (0.8-2.6)	.6
EN-RAGE, pg/mL	30 848 (15 815-49 464)			72 434 (27 194-168 250)		
FABP4, pg/mL	31 423 (25 096-41 057)	27 310 (21 706-31 626)	.6	22 951 (17 217-33 914)	22 073 (16 037-31 099)	.4
sTNFRII, pg/mL	2592 (2291-3226)	3187 (1675-4069)	.8	3378 (2534-4346)	2930 (2250-3788)	.3
Endothelin, pg/mL	3.3 (2.4-5.4)	1.7 (1.3-1.9)	.01	2.9 (1.8-4.8)	1.4 (1.2-1.9)	<.001
CD163, ng/mL	462 (331-566)	476 (410-567)	.6	598 (469-733)	588 (499-696)	.9
CD14, µg/mL	1.65 (1.49-1.84)	1.60 (1.42-1.74)	.3	1.47 (1.33-1.59)	1.71 (1.46-2.02)	.03
Total adiponectin, µg/mL	4.7 (3.4-5.8)	3.3 (2.8-6.1)	.5	6 (3.5-9.1)	4.2 (2.5-5.4)	.07
VCAM-1, ng/mL	505 (397-528)	531 (468-598)	.5	577 (501-747)	662 (542-876)	.1
IL-8, pg/mL	2.8 (2.0-2.8)	0.1 (0.1-1.0)	.06	1.5 (0.2-3.8)	0.4 (0.1-2.5)	.3
PAI-1, pg/mL	15 490 (9454-20 352)	24 828 (14 645-31 134)	.03	20 250 (14 989-40 951)	22 807 (12 838-42 883)	.9

Median (interquartile range) presented.
Abbreviations: CD, cluster of differentiation; CM, cisgender men; EN-RAGE, extracellular newly identified receptor for advanced glycation end products; FABP, fatty acid binding protein; IL, interleukin; PAI, plasminogen activator inhibitor; sTNFR, soluble tumor necrosis factor receptor type; TW-S, transgender women with total testosterone <50 ng/dL; TW-T, transgender women with total testosterone ≥50 ng/dL; VCAM, vascular cell adhesion molecule.

Table 3. Body composition

	TW-S (N = 9)	CM (N = 15)	*P* TW vs CM	TW-T (N = 20)	CM (N = 33)	*P* TW-T vs CM
Abdominal subcutaneous fat, cm^2	450 (316-578)	348 (250-440)	.2	281 (252-420)	277 (169-420)	.5
Abdominal visceral fat, cm^2	161 (114-190)	145 (105-224)	.9	131 (87-199)	160 (123-200)	.3
Thigh muscle fat, cm^2	27 (15-34)	9 (5-11)	.004	15.2 (12-18.0)	7.3 (3.5-10.3)	<.001
Thigh subcutaneous fat, cm^2	78 (44-108)	52 (37-78)	.2	50 (35-59)	55 (25-84)	.9
Epicardial fat, cm^2	59 (52-73)	81 (56-92)	.2	59 (44-69)	73 (47-102)	.1
Intrathoracic fat, cm^2	90 (55-112)	150 (118-220)	.02	77 (64-87)	140 (79-198)	.002
Thoracic periaortic fat, cm^2	8 (6-19)	23 (16-35)	.03	7 (6-10)	20 (10-35)	<.001
Liver-spleen attenuation ratio	1.29 (1.15-1.61)	1.29 (1.13-1.43)	.9	1.3 (1.26-1.32)	1.22 (1.11-1.31)	.2
Moderate-to-severe hepatic steatosis[a]	0	0		8%	10%	.9

Median (interquartile range) or frequency presented.
Abbreviations: CM, cisgender men; TW-S, transgender women with total testosterone <50 ng/dL; TW-T, transgender women with total testosterone ≥50 ng/dL.
[a]Liver-spleen ratio less than 1.0.

compared to 47% and 20% prevalence in CM, respectively. Since noncalcified plaque is more vulnerable, susceptible to rupture, and extensively linked to acute coronary syndromes [49-52], and the presence of obstructive plaque would indicate advanced ASCVD, these 2 findings collectively support our hypothesis of a cardioprotective effect of testosterone suppression in TW on GAHT.

We also observed several findings that support the aforementioned hypothesis but did not reach statistical significance, possibly because of small sample size. Specifically, the prevalences of calcified plaque, CAC (Agatston score >0), and mixed plaque were lower among TW-S than among highly matched CM controls. Additionally, estradiol concentrations in TW moderately and negatively correlated with the presence of any plaque and coronary stenosis.

TW in our study experienced male puberty prior to GAHT initiation. Our provocative preliminary findings may therefore suggest that vascular disease developing early in life, that is, in the face of testosterone exposure, proceeded as expected for CM and TW, but that subsequent testosterone suppression/GAHT minimized the formation of new plaque in TW. Interestingly, TW-S in our cohort had a median serum estradiol concentration (78 pg/mL) lower than might be expected for "optimized" GAHT (100-200 pg/mL) [53], but certainly higher than expected for a CM [54]. Notably, all TW had at least partial testosterone suppression that was likely driven, at least in part, by estrogen therapy. Estrogen is known to lower testosterone by exerting negative feedback at the hypothalamic-pituitary level [55] and increasing hepatic production of SHBG and cortisol-binding globulin [56], the latter of which promotes further reduction of free and bioavailable testosterone [57]. Since the main difference between TW-S and TW-T in our cohort was testosterone exposure and not estradiol exposure, and since TW-T and CM controls had similar testosterone exposure, we hypothesize that testosterone suppression is critical to GAHT-associated cardioprotection in TW.

In seeming consonance with our findings and similar to the general population, the Mechanistic Substudy of the REPRIEVE Trial, which enrolled 800 PWH aged 40 to 75 years who had low-to-moderate ASCVD risk scores from the United States, found that women had a lower prevalence

Table 4. Characteristics of coronary artery disease in transgender women compared to cisgender men

	TW-S (N = 9)	CM (N = 15)	*P* TW-S vs CM	TW-T (N = 20)	CM (N = 33)	*P* TW-T vs CM
Prevalence of CAC (Agatston score >0)	44%	67%	.3	42%	39%	.8
Median CAC Agatston score among participants with calcium (IQR)	166 (65-350)	139 (16-335)	.7	172 (14-417)	246 (72-272)	.9
Prevalence of any coronary plaque	44%	73%	.2	58%	67%	.5
Prevalence of noncalcified plaque	0%	47%	.01	42%	39%	.8
Prevalence of mixed plaque	22%	40%	.4	11%	30%	.1
Prevalence of calcified plaque	44%	60%	.5	42%	46%	.8
Prevalence of coronary stenosis >50%	0%	20%	.2	16%	21%	.6
Prevalence of coronary stenosis >70%	0%	0%		10%	6%	.6
Segment involvement score[a]	0 (0-1)	2 (0-5)	.2	1 (0-2)	2 (0-5)	.2
Total plaque score[a]	0 (0-1)	2 (0-6)	.3	1 (0-3)	2 (0-6)	.3
Noncalcified plaque score[a]	0 (0-0)	0 (0-1)	.02	0 (0-2)	0 (0-2)	.9
Calcified plaque score[a]	0 (0-1)	1 (0-2)	.4	0 (0-1)	0 (0-2)	.5
Mixed plaque score[a]	0 (0-0)	0 (0-2)	.6	0 (0-0)	0 (0-1)	.1

Abbreviations: CAC, coronary artery calcium; CM, cisgender men; IQR, interquartile range; TW-S, transgender women with total testosterone <50 ng/dL; TW-T, transgender women with total testosterone ≥50 ng/dL.
[a]Median (IQR).

than men of any coronary artery plaque and plaque with visible noncalcified portions and/or vulnerable plaque features, after adjusting for ASCVD risk score and BMI [58]. However, in the Women's Health Initiative trial of postmenopausal women on oral conjugated equine estrogens alone, the hazard ratio for all CV events was increased at 1.11 (95% CI, 1.01-1.22) compared to placebo, while the hazard ratios for total myocardial infarction, coronary artery bypass grafting/percutaneous coronary intervention, and CV death were not increased [59]. Additionally, the potential association between profound androgen-deprivation therapy (as experienced by some TW) and CVD has been extensively examined in men with prostate cancer and remains controversial, with several studies showing positive associations and others reporting contrary results [60, 61]. A possible explanation for these conflicting findings may lie in the inclusion of populations with dissimilar baseline rates of CVD risk factors and comorbidities.

Cardiac fat depots mirror VAT, and their quantity and quality contribute to the higher observed CV risk in PWH [62]. Epicardial fat, which has been associated with coronary artery plaque burden in PWH [63] and in the general population [64], was similar across groups in our study. Prevalence of high thoracic periaortic AT area, which has been associated with coronary calcium and CVD risk factors, was higher in women than men with normal VAT in the Framingham Heart Study [65, 66]. Unlike that observed sex difference, TW with and without testosterone in the normal range for CW in our study had significantly lower thoracic periaortic AT than CM.

We found remarkable differences in select circulating biomarker concentrations between TW and age-, race-, BMI-, and HIV serostatus matched CM, with TW-S having higher IL-6 concentrations than CM. Plasma IL-6 concentrations have been independently associated with the risk of major adverse CV events, CV mortality, and all-cause mortality in individuals with and without history of CVD [67-70]. In a recent study, younger TW living with HIV had a significantly higher median IL-6 concentration than CM and CW with a similar frequency of traditional CVD risk factors [71]. Interestingly, TW-S in our cohort had a significantly higher median IL-6 concentration than CM, while TW-T did not. When restricting analysis to PWH, these differences were present but attenuated (data not shown).

ET-1, a vasoconstrictor secreted primarily by endothelial cells, has been independently associated with prevalent and incident CVD, coronary plaque burden and composition, and with poorer outcomes after myocardial infarction [72]. Sexual dimorphism has been documented, with levels being lower in CW of reproductive age than CM [73]. Contrary to these data, TW in our cohort had a significantly higher median ET-1 concentration than CM, regardless of testosterone concentration and HIV serostatus. These results conflict with a recent study that also compared TW with CM from the MACS cohort: While ET-1 levels in that group of TW were similar to those found in the present study, levels in CM were higher than in TW in that cohort [19] and higher than expected in the general population [74]. The cause of high ET-1 concentrations among CM in that study is not known, and additional data are needed to understand how GAHT and/or testosterone suppression in TW may affect ET-1 concentrations.

Circulating EN-RAGE levels have been strongly associated with the risk of coronary artery disease beyond traditional CVD risk factors, especially acute events with plaque instability [75]. Several recent studies of varied design and sample size have reported elevated EN-RAGE concentrations among TW [19, 20, 76]. In our cohort, TW-T had a median EN-RAGE concentration more than twice that of TW-S with similar rates of traditional CVD risk factors. Control samples were not available, but EN-RAGE concentrations in the general population (median [IQR] 10 800 [7600-14 700] pg/mL) are substantially lower than observed in our cohort [48].

PAI-1 overexpression has been associated with metabolic syndrome and increased risk of myocardial infarction in the general population as well as in PWH [77-79]. PAI-1 levels are consistently higher in CM than CW across cohorts [80]. TW-S had a significantly lower median PAI-1 concentration than CM, while concentrations among TW-T and CM were

similar. When restricting to PWH, the difference attenuated (data not shown). Our results are in conflict with those previously published in a large study of TW [19], although that study lacked sex hormone measurements, which may partially account for these differences.

Our study has important strengths, including the use of coronary CT angiography, comprehensive capture of traditional CVD risk factors, a very well-matched CM control group, and GAHT use coupled with sex hormone concentration measurements. There are also important limitations, including the cross-sectional study design, lack of complete GAHT use history for TW (current use required for study participation), CT scan performance across a number of years, small sample size, absence of a CW control group, and the fact that, while matched closely for demographics and HIV serostatus, control CM for the TW-S participants had higher rates of traditional CVD risk factors and use of earlier generations of ART within the same class if living with HIV. To this last fact, it is still surprising given age, HIV prevalence, and CVD risk factor burden (whether similar to controls or not) that none of the TW with suppressed testosterone had evidence of noncalcified plaque or obstructive coronary plaque. ASCVD risk score for TW was lower than for CM, but this could be related to the use of current gender for the score's sex variable [41].

Additionally, while diagnoses associated with CVD risk (hypertension, diabetes, and current smoking) were more prevalent among CM than TW-S, the frequency of antihypertensive and glucose-lowering agent use was similar. This discrepancy may be accounted for by either a diagnosis or medication being used in the frequency reporting for metabolic comorbidities in Table 1. On a related note, we acknowledge that some contribution of spironolactone as an antihypertensive or procardiac remodeling agent on participants' CVD risk cannot be ruled out, but our finding was not limited to participants on spironolactone. Additionally, half of participants in both the TW-S and TW-T groups reported use of spironolactone, so any cardioprotective effects of spironolactone do not account for our results. Statin use was also more frequent among TW with and without total testosterone in the normal range for CW than among CM, but overall statin use was low and any observed benefit on plaque stabilization for TW-S from statin use would likely also be apparent for TW-T. Lastly, time in storage was a few years longer for CM blood samples; however, samples had not previously been thawed and the biomarkers we chose are not anticipated to have had substantial degradation during the additional storage period.

In conclusion, we observed marked differences in CVD burden between TW achieving testosterone suppression on GAHT and well-matched CM. However, many questions remain. For example, would differences between groups be greater if median estradiol was greater than 100 pg/mL for a prolonged period of time? Is total testosterone in the normal range for CW required for cardioprotection with estradiol-based GAHT in TW and, if so, what duration of testosterone suppression to this degree is required to achieve cardioprotection? Additionally, while a growing body of evidence suggests that CVD risk among TW is not accurately estimated by available equations, these equations have not been validated in this population with increased mortality risk [6, 41, 81, 82], results vary depending on the sex variable used [41], and the best way to assess CVD risk among TW on GAHT is unknown. Use of exogenous sex hormones, traditional and nontraditional CVD risk factors, gender minority stressors, high HIV prevalence, and inadequate access to health care all likely affect CVD outcomes in TW, though data are conflicting and mostly stem from observational studies [5, 16, 83-86]. The substantial knowledge gap regarding how best to optimize CVD risk for TW highlights the necessity of prospective studies to longitudinally assess CV health and to determine progression of coronary artery disease among TW receiving standard-of-care GAHT regimens [47]. Future studies should also include both CM and CW as robust comparator/control groups.

Acknowledgments

The authors gratefully acknowledge the contributions of the study participants and dedication of the staff at the MWCCS and Houston sites.

Funding

This work was supported by the National Institutes of Health (NIH grant No. R03 AI141014 to J.E.L., R01 HL095129 to W.S.P., and R01 HL125053 to W.S.P.), with additional support from the National Center for Advancing Translational Sciences (a component of the NIH and National Institutes of Health Roadmap for Medical Research, grant No. UL1 RR 025005) and the Texas Developmental Center for AIDS Research (No. P30 AI161943). The Multicenter AIDS Cohort Study (MACS) was funded by the National Institute of Allergy and Infectious Diseases, with additional supplemental funding from the National Cancer Institute (grant Nos. U01-AI-35042, UL1-RR025005, UM1-AI-35043, U01-AI-35039, U01-AI-35040, and U01-AI-35041). Data in this manuscript were collected by the MACS, now the MACS/WIHS Combined Cohort Study (MWCCS). The contents of this publication are solely the responsibility of the authors and do not represent the official views of the NIH.

MWCCS (principal investigators): Atlanta CRS (Ighovwerha Ofotokun, Anandi Sheth, and Gina Wingood), U01-HL146241; Baltimore CRS (Todd Brown and Joseph Margolick), U01-HL146201; Bronx CRS (Kathryn Anastos, David Hanna, and Anjali Sharma), U01-HL146204; Brooklyn CRS (Deborah Gustafson and Tracey Wilson), U01-HL146202; Data Analysis and Coordination Center (Gypsyamber D'Souza, Stephen Gange and Elizabeth Topper), U01-HL146193; Chicago–Cook County CRS (Mardge Cohen and Audrey French), U01-HL146245; Chicago–Northwestern CRS (Steven Wolinsky, Frank Palella, and Valentina Stosor), U01-HL146240; Northern California CRS (Bradley Aouizerat, Jennifer Price, and Phyllis Tien), U01-HL146242; Los Angeles CRS (Roger Detels and Matthew Mimiaga), U01-HL146333; Metropolitan Washington CRS (Seble Kassaye and Daniel Merenstein), U01-HL146205; Miami CRS (Maria Alcaide, Margaret Fischl, and Deborah Jones), U01-HL146203; Pittsburgh CRS (Jeremy Martinson and Charles Rinaldo), U01-HL146208; UAB-MS CRS (Mirjam-Colette Kempf, Jodie Dionne-Odom, James B. Brock, and Deborah Konkle-Parker), U01-HL146192; UNC CRS (M. Bradley Drummond and Michelle Floris-Moore), U01-HL146194. The MWCCS is funded primarily by the National Heart, Lung, and Blood Institute (NHLBI), with additional co-

funding from the Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institute on Aging (NIA), National Institute of Dental & Craniofacial Research (NIDCR), National Institute of Allergy And Infectious Diseases (NIAID), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Mental Health (NIMH), National Institute on Drug Abuse (NIDA), National Institute of Nursing Research (NINR), National Cancer Institute (NCI), National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institute on Deafness and Other Communication Disorders (NIDCD), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute on Minority Health and Health Disparities (NIMHD), and in coordination and alignment with the research priorities of the NIH, Office of AIDS Research (OAR). MWCCS data collection is also supported by UL1-TR000004 (UCSF CTSA), UL1-TR003098 (JHU ICTR), UL1-TR001881 (UCLA CTSI), P30-AI-050409 (Atlanta CFAR), P30-AI-073961 (Miami CFAR), P30-AI-050410 (UNC CFAR), P30-AI-027767 (UAB CFAR), P30-MH-116867 (Miami CHARM), UL1-TR001409 (DC CTSA), KL2-TR001432 (DC CTSA), and TL1-TR001431 (DC CTSA).

Disclosures

H.F., A.N.H., H.M., P.D., N.F., K.A., A.D., S.H., J.W.M., J.B.M., K.M., F.J.P., M.D.W., S.B., M.J.B., and W.S.P. have nothing to declare. J.E.L. has served as a consultant for Theratechnologies and CytoDyn, Inc, and receives research support from Gilead Sciences. T.T.B. has served as a consultant to Gilead Sciences, ViiV Healthcare, Jannsen, and Merck.

Data Availability

Access to individual-level data from the MWCCS may be obtained on review and approval of a MWCCS concept sheet. Links and instructions for online concept sheet submission are on the study website (http://mwccs.org/). Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Prior Presentation

These data were presented in part at the Conference on Retroviruses and Opportunistic Infections (CROI) 2023; February 2023; Seattle, WA.

References

1. Berger I, Mooney-Somers J. Smoking cessation programs for lesbian, gay, bisexual, transgender, and intersex people: a content-based systematic review. *Nicotine Tob Res*. 2017;19(12):1408-1417.
2. Buchting FO, Emory KT, Scout , *et al*. Transgender use of cigarettes, cigars, and E-cigarettes in a national study. *Am J Prev Med*. 2017;53(1):e1-e7.
3. CDC. Office on smoking and health, national center for chronic disease prevention and health promotion. LGBTQ+ People. Accessed July 14, 2022. https://www.cdc.gov/tobacco/campaign/tips/groups/lgbt.html
4. 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*. 2022;21(1):42-46.
5. Alzahrani T, Nguyen T, Ryan A, *et al*. Cardiovascular disease risk factors and myocardial infarction in the transgender population. *Circ Cardiovasc Qual Outcomes*. 2019;12(4):e005597.
6. Denby KJ, Cho L, Toljan K, Patil M, Ferrando CA. Assessment of cardiovascular risk in transgender patients presenting for gender-affirming care. *Am J Med*. 2021;134(8):1002-1008.
7. Caceres BA, Jackman KB, Edmondson D, Bockting WO. Assessing gender identity differences in cardiovascular disease in US adults: an analysis of data from the 2014-2017 BRFSS. *J Behav Med*. 2020;43(2):329-338.
8. Balcerek MI, Nolan BJ, Brownhill A, *et al*. Feminizing hormone therapy prescription patterns and cardiovascular risk factors in aging transgender individuals in Australia. *Front Endocrinol (Lausanne)*. 2021;12:667403.
9. Becasen JS, Denard CL, Mullins MM, Higa DH, Sipe TA. Estimating the prevalence of HIV and sexual behaviors among the US transgender population: a systematic review and meta-analysis, 2006-2017. *Am J Public Health*. 2019;109(1):e1-e8.
10. CDC. HIV and transgender people: HIV prevalence 2019-2020. Accessed June 18, 2024. https://www.cdc.gov/hiv/data-research/facts-stats/transgender-people.html?CDC_AAref_Val=.
11. Lake JE, Currier JS. Metabolic disease in HIV infection. *Lancet Infect Dis*. 2013;13(11):964-975.
12. Klaver M, de Blok CJM, Wiepjes CM, *et al*. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *Eur J Endocrinol*. 2018;178(2):163-171.
13. Schutte MH, Kleemann R, Nota NM, *et al*. The effect of transdermal gender-affirming hormone therapy on markers of inflammation and hemostasis. *PLoS One*. 2022;17(3):e0261312.
14. Spanos C, Bretherton I, Zajac JD, Cheung AS. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: a systematic review. *World J Diabetes*. 2020;11(3):66-77.
15. Martinez C, Rikhi R, Haque T, *et al*. Gender identity, hormone therapy, and cardiovascular disease risk. *Curr Probl Cardiol*. 2020;45(5):100396.
16. Streed CG Jr, Beach LB, Caceres BA, *et al*. Assessing and addressing cardiovascular health in people who are transgender and gender diverse: a scientific statement from the American Heart Association. *Circulation*. 2021;144(6):e136-e148.
17. SenthilKumar G, Katunaric B, Bordas-Murphy H, Sarvaideo J, Freed JK. Estrogen and the vascular endothelium: the unanswered questions. *Endocrinology*. 2023;164(6):bqad079.
18. Wierckx K, Elaut E, Declercq E, *et al*. 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*. 2013;169(4):471-478.
19. Lake JE, Wang R, Barrett BW, *et al*. Trans women have worse cardiovascular biomarker profiles than cisgender men independent of hormone use and HIV serostatus. *AIDS*. 2022;36(13):1801-1809.
20. Lake JE, Miao H, Bowman ER, *et al*. Gender-affirming hormone therapy decreases d-dimer but worsens insulin sensitivity in transgender women. *HIV Med*. 2023;24(11):1144-1149.
21. Moreira Allgayer RMC, Borba GDS, Moraes RS, Ramos RB, Spritzer PM. The effect of gender-affirming hormone therapy on the risk of subclinical atherosclerosis in the transgender population: a systematic review. *Endocr Pract*. 2023;29(6):498-507.
22. Cocchetti C, Castellini G, Iacuaniello D, *et al*. Does gender-affirming hormonal treatment affect 30-year cardiovascular risk in transgender persons? A two-year prospective European study (ENIGI). *J Sex Med*. 2021;18(4):821-829.
23. Klaver M, van Velzen D, de Blok C, *et al*. Change in visceral fat and total body fat and the effect on cardiometabolic risk factors during transgender hormone therapy. *J Clin Endocrinol Metab*. 2022;107(1):e153-e164.

24. Funderburg NT, Zidar DA, Shive C, *et al.* Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. *Blood*. 2012;120(23):4599-4608.
25. Teer E, Joseph DE, Glashoff RH, Faadiel Essop M. Monocyte/macrophage-mediated innate immunity in HIV-1 infection: from early response to late dysregulation and links to cardiovascular diseases onset. *Virol Sin*. 2021;36(4):565-576.
26. Funderburg NT, Mayne E, Sieg SF, *et al.* Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood*. 2010;115(2):161-167.
27. Baker JV, Brummel-Ziedins K, Neuhaus J, *et al.* HIV replication alters the composition of extrinsic pathway coagulation factors and increases thrombin generation. *J Am Heart Assoc*. 2013;2(4):e000264.
28. Funderburg NT, Lederman MM. Coagulation and morbidity in treated HIV infection. *Thromb Res*. 2014;133(Suppl 1):S21-S24.
29. Masia M, Padilla S, Alvarez D, *et al.* Risk, predictors, and mortality associated with non-AIDS events in newly diagnosed HIV-infected patients: role of antiretroviral therapy. *AIDS*. 2013;27(2):181-189.
30. Weber R, Ruppik M, Rickenbach M, *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV cohort study. *HIV Med*. 2013;14(4):195-207.
31. Deeken JF, Tjen ALA, Rudek MA, *et al.* The rising challenge of non-AIDS-defining cancers in HIV-infected patients. *Clin Infect Dis*. 2012;55(9):1228-1235.
32. Currier JS, Taylor A, Boyd F, *et al.* Coronary heart disease in HIV-infected individuals. *J Acquir Immune Defic Syndr*. 2003;33(4):506-512.
33. Dolan SE, Hadigan C, Killilea KM, *et al.* Increased cardiovascular disease risk indices in HIV-infected women. *J Acquir Immune Defic Syndr*. 2005;39(1):44-54.
34. Oliviero U, Bonadies G, Apuzzi V, *et al.* Human immunodeficiency virus per se exerts atherogenic effects. *Atherosclerosis*. 2009;204(2):586-589.
35. Nou E, Lo J, Hadigan C, Grinspoon SK. Pathophysiology and management of cardiovascular disease in patients with HIV. *Lancet Diabetes Endocrinol*. 2016;4(7):598-610.
36. Hsue PY, Waters DD. HIV infection and coronary heart disease: mechanisms and management. *Nat Rev Cardiol*. 2019;16(12):745-759.
37. Feinstein MJ, Hsue PY, Benjamin LA, *et al.* Characteristics, prevention, and management of cardiovascular disease in people living with HIV: a scientific statement from the American Heart Association. *Circulation*. 2019;140(2):e98-e124.
38. Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR Jr. The multicenter AIDS cohort study: rationale, organization, and selected characteristics of the participants. *Am J Epidemiol*. 1987;126(2):310-318.
39. Post WS, Haberlen SA, Witt MD, *et al.* Suboptimal HIV suppression is associated with progression of coronary artery stenosis: the multicenter AIDS cohort study (MACS) longitudinal coronary CT angiography study. *Atherosclerosis*. 2022;353:33-40.
40. Post WS, Budoff M, Kingsley L, *et al.* Associations between HIV infection and subclinical coronary atherosclerosis. *Ann Intern Med*. 2014;160(7):458-467.
41. Gosiker BJ, Lesko CR, Rich AJ, *et al.* Cardiovascular disease risk among transgender women living with HIV in the United States. *PLoS One*. 2020;15(7):e0236177.
42. Hacioglu Y, Gupta M, Choi TY, *et al.* Use of cardiac CT angiography imaging in an epidemiology study—the methodology of the multicenter AIDS cohort study cardiovascular disease substudy. *Anadolu Kardiyol Derg*. 2013;13(3):207-214.
43. Austen WG, Edwards JE, Frye RL, *et al.* A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation*. 1975;51(4):5-40.
44. Pagali SR, Madaj P, Gupta M, *et al.* Interobserver variations of plaque severity score and segment stenosis score in coronary arteries using 64 slice multidetector computed tomography: a substudy of the ACCURACY trial. *J Cardiovasc Comput Tomogr*. 2010;4(5):312-318.
45. Hunt PW, Sinclair E, Rodriguez B, *et al.* Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *J Infect Dis*. 2014;210(8):1228-1238.
46. Massanella M, Negredo E, Puig J, *et al.* Raltegravir intensification shows differing effects on CD8 and CD4 T cells in HIV-infected HAART-suppressed individuals with poor CD4 T-cell recovery. *AIDS*. 2012;26(18):2285-2293.
47. Hembree WC, Cohen-Kettenis PT, Gooren L, *et al.* Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;102(11):3869-3903.
48. Ligthart S, Sedaghat S, Ikram MA, Hofman A, Franco OH, Dehghan A. EN-RAGE: a novel inflammatory marker for incident coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2014;34(12):2695-2699.
49. Hoffmann U, Moselewski F, Nieman K, *et al.* Noninvasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and stable lesions in stable angina by multidetector computed tomography. *J Am Coll Cardiol*. 2006;47(8):1655-1662.
50. Schuijf JD, Beck T, Burgstahler C, *et al.* Differences in plaque composition and distribution in stable coronary artery disease versus acute coronary syndromes; non-invasive evaluation with multi-slice computed tomography. *Acute Card Care*. 2007;9(1):48-53.
51. Cury RC, Abbara S, Achenbach S, *et al.* CAD-RADS(TM) coronary artery disease—reporting and data system. An expert consensus document of the Society of Cardiovascular Computed Tomography (SCCT), the American College of Radiology (ACR) and the North American Society for Cardiovascular Imaging (NASCI). Endorsed by the American College of Cardiology. *J Cardiovasc Comput Tomogr*. 2016;10(4):269-281.
52. Hell MM, Motwani M, Otaki Y, *et al.* Quantitative global plaque characteristics from coronary computed tomography angiography for the prediction of future cardiac mortality during long-term follow-up. *Eur Heart J Cardiovasc Imaging*. 2017;18(12):1331-1339.
53. Coleman E, Radix AE, Bouman WP, *et al.* Standards of care for the health of transgender and gender diverse people, version 8. *Int J Transgend Health*. 2022;23(Suppl 1):S1-S259.
54. Chadid S, Barber JR, Rohrmann S, *et al.* Age-specific serum total and free estradiol concentrations in healthy men in US nationally representative samples. *J Endocr Soc*. 2019;3(10):1825-1836.
55. Maheshwari A, Nippoldt T, Davidge-Pitts C. An approach to non-suppressed testosterone in transgender women receiving gender-affirming feminizing hormonal therapy. *J Endocr Soc*. 2021;5(9):bvab068.
56. Judd HL. Hormonal dynamics associated with the menopause. *Clin Obstet Gynecol*. 1976;19(4):775-788.
57. Simon JA. Estrogen replacement therapy: effects on the endogenous androgen milieu. *Fertil Steril*. 2002;77(Suppl 4):S77-S82.
58. Zanni MV, Foldyna B, McCallum S, *et al.* Sex differences in subclinical atherosclerosis and systemic immune activation/inflammation among people with human immunodeficiency virus in the United States. *Clin Infect Dis*. 2023;76(2):323-334.
59. Manson JE, Chlebowski RT, Stefanick ML, *et al.* Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's health initiative randomized trials. *JAMA*. 2013;310(13):1353-1368.
60. Levine GN, D'Amico AV, Berger P, *et al.* Androgen-deprivation therapy in prostate cancer and cardiovascular risk: a science advisory from the American Heart Association, American Cancer Society, and American Urological Association: endorsed by the American Society for Radiation Oncology. *Circulation*. 2010;121(6):833-840.

61. Hu J-R, Duncan MS, Morgans AK, *et al.* Cardiovascular effects of androgen deprivation therapy in prostate cancer: contemporary meta-analyses. *Arterioscler Thromb Vasc Biol*. 2020;40(3):e55-e64.
62. Hyatt AN, Lake JE. Ectopic fat and cardiac health in people with HIV: serious as a heart attack. *Curr HIV/AIDS Rep*. 2022;19(5):415-424.
63. Brener M, Ketlogetswe K, Budoff M, *et al.* Epicardial fat is associated with duration of antiretroviral therapy and coronary atherosclerosis. *AIDS*. 2014;28(11):1635-1644.
64. Mancio J, Azevedo D, Saraiva F, *et al.* Epicardial adipose tissue volume assessed by computed tomography and coronary artery disease: a systematic review and meta-analysis. *Eur Heart J Cardiovasc Imaging*. 2018;19(5):490-497.
65. Lehman SJ, Massaro JM, Schlett CL, O'Donnell CJ, Hoffmann U, Fox CS. Peri-aortic fat, cardiovascular disease risk factors, and aortic calcification: the Framingham heart study. *Atherosclerosis*. 2010;210(2):656-661.
66. Britton KA, Pedley A, Massaro JM, *et al.* Prevalence, distribution, and risk factor correlates of high thoracic periaortic fat in the Framingham heart study. *J Am Heart Assoc*. 2012;1(6):e004200.
67. Mossmann M, Wainstein MV, Mariani S, *et al.* Increased serum IL-6 is predictive of long-term cardiovascular events in high-risk patients submitted to coronary angiography: an observational study. *Diabetol Metab Syndr*. 2022;14(1):125.
68. Wainstein MV, Mossmann M, Araujo GN, *et al.* Elevated serum interleukin-6 is predictive of coronary artery disease in intermediate risk overweight patients referred for coronary angiography. *Diabetol Metab Syndr*. 2017;9(1):67.
69. Held C, White HD, Stewart RAH, *et al.* Inflammatory biomarkers interleukin-6 and C-reactive protein and outcomes in stable coronary heart disease: experiences from the STABILITY (stabilization of atherosclerotic plaque by initiation of darapladib therapy) trial. *J Am Heart Assoc*. 2017;6(10):e005077.
70. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101(15):1767-1772.
71. Lake JE, Hyatt AN, Feng H, *et al.* Transgender women with HIV demonstrate unique non-alcoholic fatty liver disease profiles. *Transgender Health*. 2023. Doi:10.1089/trgh.2022.0182
72. Jankowich M, Choudhary G. Endothelin-1 levels and cardiovascular events. *Trends Cardiovasc Med*. 2020;30(1):1-8.
73. Polderman KH, Stehouwer CD, van Kamp GJ, Dekker GA, Verheugt FW, Gooren LJ. Influence of sex hormones on plasma endothelin levels. *Ann Intern Med*. 1993;118(6):429-432.
74. Suzuki N, Matsumoto H, Kitada C, Masaki T, Fujino M. A sensitive sandwich-enzyme immunoassay for human endothelin. *J Immunol Methods*. 1989;118(2):245-250.
75. Ligthart S, Sedaghat S, Ikram MA, Hofman A, Franco OH, Dehghan A. EN-RAGE. *Arterioscler Thromb Vasc Biol*. 2014;34(12):2695-2699.
76. Lake JE, Hyatt AN, Feng H, *et al.* A randomized clinical trial of transgender women switching to B/F/TAF: the (mo)BETTA trial. *Open Forum Infect Dis*. 2023;10(4):ofad178.
77. Alessi MC, Juhan-Vague I. PAI-1 and the metabolic syndrome: links, causes, and consequences. *Arterioscler Thromb Vasc Biol*. 2006;26(10):2200-2207.
78. Knudsen A, Katzenstein TL, Benfield T, *et al.* Plasma plasminogen activator inhibitor-1 predicts myocardial infarction in HIV-1-infected individuals. *AIDS*. 2014;28(8):1171-1179.
79. Wirunsawanya K, Belyea L, Shikuma C, *et al.* Plasminogen activator inhibitor-1 predicts negative alterations in whole-body insulin sensitivity in chronic HIV infection. *AIDS Res Hum Retroviruses*. 2017;33(7):723-727.
80. Suthahar N, Lau ES, Blaha MJ, *et al.* Sex-specific associations of cardiovascular risk factors and biomarkers with incident heart failure. *J Am Coll Cardiol*. 2020;76(12):1455-1465.
81. de Blok CJ, Wiepjes CM, van Velzen DM, *et al.* Mortality trends over five decades in adult transgender people receiving hormone treatment: a report from the Amsterdam cohort of gender dysphoria. *Lancet Diabetes Endocrinol*. 2021;9(10):663-670.
82. Mahowald MK, Maheshwari AK, Lara-Breitinger KM, *et al.* Characteristics of transgender women referred to women's heart clinic. *Am J Prev Cardiol*. 2021;7:100223.
83. Meyer IH, Brown TN, Herman JL, Reisner SL, Bockting WO. Demographic characteristics and health status of transgender adults in select US regions: behavioral risk factor surveillance system, 2014. *Am J Public Health*. 2017;107(4):582-589.
84. Irwig MS. Cardiovascular health in transgender people. *Rev Endocr Metab Disord*. 2018;19(3):243-251.
85. Cetlin M, Fulda ES, Chu SM, *et al.* Cardiovascular disease risk among transgender people with HIV. *Curr HIV/AIDS Rep*. 2021;18(5):407-423.
86. Poteat TC, Divsalar S, Streed CG Jr, Feldman JL, Bockting WO, Meyer IH. Cardiovascular disease in a population-based sample of transgender and cisgender adults. *Am J Prev Med*. 2021;61(6):804-811.