

BMJ Open Sex hormone-dependent host-microbiome interactions and cardiovascular risk (XCVD): design of a longitudinal multi-omics cohort study

Kristina Franz ^{1,2,3,4,5}, Lajos Markó,^{1,2,3,5,6} Anja Mähler,^{1,2,5} Rima Chakaroun,^{7,8} Sascha Heinitz,⁷ Haiko Schlögl,^{7,9} Julia Sacher,¹⁰ Nico Steckhan,¹¹ Ralf Dechend,^{1,2,3,5,12} Noah Adams,^{13,14,15} Marianne Andersen,^{16,17} Dorte Glintborg,^{17,18} Martin Viehweger,¹⁹ Lina Samira Bahr,^{1,2,3,5} Sofia Kirke Forslund-Startceva ^{1,2,3,5,15,20}

To cite: Franz K, Markó L, Mähler A, *et al.* Sex hormone-dependent host-microbiome interactions and cardiovascular risk (XCVD): design of a longitudinal multi-omics cohort study. *BMJ Open* 2025;**15**:e087982. doi:10.1136/bmjopen-2024-087982

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<https://doi.org/10.1136/bmjopen-2024-087982>).

LSB and SKF-S contributed equally.

Received 24 April 2024
Accepted 23 December 2024



© Author(s) (or their employer(s)) 2025. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ Group.

For numbered affiliations see end of article.

Correspondence to

Dr Kristina Franz;
kristina.franz@charite.de

ABSTRACT

Introduction Cardiovascular diseases (CVDs) present differently in women and men, influenced by host-microbiome interactions. The roles of sex hormones in CVD outcomes and gut microbiome in modifying these effects are poorly understood. The XCVD study examines gut microbiome mediation of sex hormone effects on CVD risk markers by observing transgender participants undergoing gender-affirming hormone therapy (GAHT), with findings expected to extrapolate to cisgender populations.

Methods and analyses This observational, longitudinal cohort study includes baseline, 1- and 2-year follow-ups with transgender participants beginning GAHT. It involves comprehensive phenotyping and microbiome genotyping, integrating computational analyses of high-dimensional data. Microbial diversity will be assessed using gut, skin, and oral samples via 16S rRNA and shotgun metagenomic sequencing of gut samples. Blood measurements will include sex hormones, CVD risk markers, cardiometabolic parameters, cytokines, and immune cell counts. Hair samples will be analysed for cortisol. Participants will complete online questionnaires on physical activity, mental health, stress, quality of life, fatigue, sleep, pain, and gender dysphoria, tracking medication use and diet to control for confounders. Statistical analyses will integrate phenomic, lifestyle, and multi-omic data to model health effects, testing gut microbiome mediation of CVD risk as the endocrine environment shifts between that typical for cisgender men to women and vice versa.

Ethics and dissemination The study adheres to Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the Charité Ethical Committee (EA1/339/21). Signed informed consent will be obtained. Results will be published in peer-reviewed journals and conferences and shared as accessible summaries for participants, community groups, and the public, with participants able to view their data securely after public and patient involvement review for accessibility.

Trial registration number The XCVD study was registered on ClinicalTrials.gov (NCT05334888) as 'Sex-differential host-microbiome CVD risk — a longitudinal cohort approach (XCVD)' on 4 April 2022. Data set link

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Where most clinical studies cannot separate social, genetic, and endocrine drivers of sex and gender, and where animal studies only capture human biology partially, our study design allows to investigate one such driver while keeping others constant.
- ⇒ By ensuring the involvement of members of the studied demographic at all stages of study design, execution, analysis, and interpretation, we showcase best practices for public and patient involvement and overcome obstacles otherwise faced in enrolling participants from marginalised groups.
- ⇒ By recruiting participants through the multiple private endocrinology clinics providing gender-affirming hormone therapy in Berlin while also leveraging two of Charité's highly specialised Clinical Research Units (Neuroscience Clinical Research Center and Experimental and Clinical Research Center), we successfully recruit from marginalised care recipient populations while carrying out comprehensive deep phenotyping and multi-omics analysis.
- ⇒ The study is fully designed to take advantage of the tools for longitudinal confounder adjustment and mediation testing that we have developed and benchmarked, enabling longitudinal effect disentangling in an observational context for treatment with wide-ranging effects.
- ⇒ The geographical uptake range (Germany, based on Berlin) limits population diversity (though somewhat mitigated through the many anticipated participants with migrant backgrounds), and the 2-year timeframe similarly limits the scope of the observed effect, motivating subsequent further follow-up.

can be found at <https://classic.clinicaltrials.gov/ct2/show/NCT05334888>.

INTRODUCTION

Background and rationale

Cardiovascular diseases (CVDs) account for approximately 31% of global mortality,

according to the WHO,¹ making them one of the leading causes of death worldwide.^{2–3} Despite improvements in preventive and therapeutic care for CVDs, including substantial technological and pharmaceutical developments, CVDs continue to burden healthcare systems substantially and incur exorbitant care costs.^{4,5}

Pronounced differences between women and men exist in the epidemiology, pathophysiology, manifestation, progression and outcomes of CVDs.^{6–8} Consistently, there is a documented trend of lower rates of CVD in premenopausal women compared with men of the same age.^{9,10} However, following menopause, the incidence of CVD development and mortality in women is higher than that observed in men.⁹ Cardiometabolic diseases (CMDs) such as hypertension, insulin resistance, diabetes mellitus, dyslipidaemia, obesity and atherosclerosis,^{11–12} health-impairing behaviours like smoking, alcohol consumption, physical inactivity, poor sleep patterns and unhealthy dietary habits,¹³ as well as mental disorders,¹⁴ compound these risk factors of CVDs.¹⁵ These factors are also distributed differentially between women and men.¹⁶ Research into disease mechanisms, including our own and others, shows circulating serum metabolites,¹⁷ immune markers¹⁸ and the gut microbiome¹⁹ to mediate some of both genetic and environmental risk factors.

Notably, *gendered* lifestyle factors and *sexed* properties of bodies may influence symptom presentation, disease risk and speed of progression, as well as response to pharmacological therapies in CVDs,^{20–21} thus altogether driving higher mortality in men or women in different cases.^{22–23} Here, we use sex to mean a context-dependent summary of bodily sex characteristics,²⁴ usually assigned at birth as male or female, and gender to similarly summarise gender expression, identity and/or sociocultural characteristics.²⁵ The proposed factors underlying sex-differential CVD risks and presentations include different genetic and epigenetic mechanisms^{26–27} that act through inflammatory, metabolic and immunological processes.^{28–30} These processes are also shaped by the organising and activating influences of sex hormones, including variable expression and sensitivity of hormone receptors.^{31–32} While evidence remains inconclusive, the literature suggests that both higher and lower testosterone levels are linked to increased cardiovascular risk. Elevated testosterone presumably manifested by insulin resistance, higher visceral body fat and increased total homocysteine concentrations.^{33–34} Conversely, lower testosterone concentrations are also associated with heightened cardiovascular risk.^{35–37} Oestrogen, in both bioidentical forms and drug formulations (eg, estradiol valerate, conjugated equine oestrogen or esterified oestrogen), plays a critical role. It increases the risk of thromboembolic events through procoagulant shifts in the haemostatic system and is linked to the development of resistance to activated protein C,³⁸ alongside immunomodulatory activity.

The gut microbiome as a bacterial landscape and integrator of environmental risk factors over the life span

has been increasingly recognised as a long-term indicator of health, a source of variation of risk and health outcomes.^{39–41} Using integrated high-dimensional multi-omics analyses of biosamples from 2173 European residents in the MetaCardis cohort, we mined this dense data set for interlocking and dynamic impacts of CMD phenotype, risk factors, medication, gut microbiome and circulating metabolite levels,⁴² also highlighting potential gut microbiome-mediated disease improvement through medication.⁴³ Bioactive metabolites are produced in large quantities by the gut microbiome, further metabolised by host enzymes, and enter the systemic circulation alongside similarly metabolised xenobiotics and drugs.⁴² Furthermore, the gut microbiome can impact metabolism and regulation of sex hormones through various mechanisms.^{44–46} These metabolic processes can affect the cardiovascular system and the development of CVDs.^{47–48} Of note, changes in microbiome and metabolome characteristics are present before clinical onset/manifestation of ischaemic heart disease and may have predictive value.⁴⁹ There are also considerable sex differences in the host microbiome as a contributing factor to CVDs.⁴⁸ While the microbiome of the gut has been most frequently linked to health,⁵⁰ other body sites also host microbiome with such impacts as the skin and the mouth.^{50–51} Dysregulated skin microbiome composition has been correlated with increased risk for CVD by potentially affecting immune cell activation and cytokine production,⁵² and may be strongly responsive to sex hormone changes. An imbalance in the oral microbiome can result in periodontal disease, which has been linked to an increased risk of CVD,^{50–53} for example, through its immune privileges and as a potential site for microbial translocation into the bloodstream. These ecosystems may interact with further potential implications for the host, including through increased risk of CVDs and other illnesses, and would also be expected to undergo parallel changes from changes in circulating sex hormone levels.

While endogenous hormone action likely underlies much of the observed sex phenotypes in cisgender persons, changes in these phenotypes in transgender persons undergoing gender-affirming hormone replacement therapy (GAHT) represent a largely unexplored approach to understanding the mechanisms and correlates of sex. Here, we present the protocol of the ongoing XCVD study, designed according to the Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines.⁵⁴ The study focuses on individuals administered exogenous sex hormones associated with the sex they were not assigned at birth to revert effects of endogenous puberty and instead induce pubertal development and subsequent maintenance of phenotype as typical of their target sex. We emphasise that while in this article we refer to such persons as transgender individuals undergoing GAHT, not all transgender persons seek this treatment. Transgender modality is defined simply as not identifying with the sex and gender assigned at birth.^{55–56} GAHT most commonly

consists of the administration of testosterone in transgender men (or non-binary persons assigned female at birth) and oestrogens and antiandrogens in transgender women (or non-binary persons assigned male at birth).⁵⁷ Such GAHT is often standardised according to approved recommendations, routinely monitored and prescribed only to individuals without evidence of serious CMD.^{57 58}

To date, no studies using high-throughput methods have examined the immune system, metabolism or gut microbiome in transgender persons undergoing GAHT. Although the role of circulating sex hormone levels in CVDs is increasingly understood, the extent to which the gut microbiome mediates or modulates this relationship remains unclear.

Aim

We will explore the gut microbiome's potential role as a mediator of sex hormone effects on CVD risk markers. This study will focus on transgender participants undergoing GAHT, with the expectation that findings also largely may apply to cisgender populations.

We hypothesise that the gut microbiome influences sex hormone-driven changes in CVD risk markers, specifically circulating NT-pro brain natriuretic peptide (BNP) and Hs-Troponin T. Secondary endpoints include concomitant variables including measurements of skin, and oral microbiome composition, immune system state, assessment of medication intake and questionnaire data of dietary habits and physical activity. Patient-centred outcomes will also be assessed, including mental health, perceived stress, quality of life, fatigue, sleep disturbance, pain, extent of gender dysphoria and risk of depression.

METHODS AND ANALYSES

Study design and setting

This is a longitudinal observational, non-interventional cohort study with baseline, 2-year follow-up study visits and an optional 1-year follow-up visit in between. The study takes place at the Neuroscience Clinical Research Center at Charité — Universitätsmedizin Berlin, Germany, and the Experimental Clinical Research Center (a cooperation between Charité — Universitätsmedizin Berlin and Max Delbrück Center for Molecular Medicine, Berlin, Germany). The study design is shown in [figure 1](#).

Measurements at baseline (1–4 weeks before starting GAHT), 1-year and 2-year visits after the start of GAHT are performed using the same standardised protocol for all participants ([table 1](#)). We plan to offer, where possible, an optional 1-year follow-up visit as part of the longitudinal evaluation of the microbiome, including faeces, saliva and skin samples, as well as selected blood tests. Remote follow-up visits occur 6, 12 and 18 months after GAHT initiation.

Patient and public involvement statement

The associations in Berlin, such as Berufsverband Trans* and the AHA Berlin e.V., as well as the ViRo outpatient practice, were involved in the design of some study instruments (e.g. questionnaires, interviews), investigations (e.g. measurements of sex hormones) and communication with transgender persons (e.g. terminology). They have read and commented on the study materials and will be contacted again for further changes. Every aspect of study design, execution and planned analysis also has been either initially proposed or reviewed by at least one member of the research team who has own lived trans experience (SKF, NA).

Participants

A total of 200 healthy transgender persons (50% undergoing feminising GAHT, 50% undergoing masculinising GAHT) who are planning to initiate but have not yet commenced such therapy will be recruited for this study. Accordingly, we recruit persons seeking GAHT and group them based on the direction of this treatment relative to baseline as transfeminine (binary trans women or non-binary persons assigned male at birth) or transmasculine (binary trans men or non-binary persons assigned female at birth).

Eligibility criteria

Individuals are eligible for inclusion if they plan to start GAHT treatment under the supervision and monitoring of their healthcare providers. The study is purely observational, and collaborating endocrinology practices solely mediate contact for enrollment. The study inclusion criteria are age from 18 to 50 years, imminent GAHT commencement (with no recent or long-term history of prior self-medicating) and without serious acute or

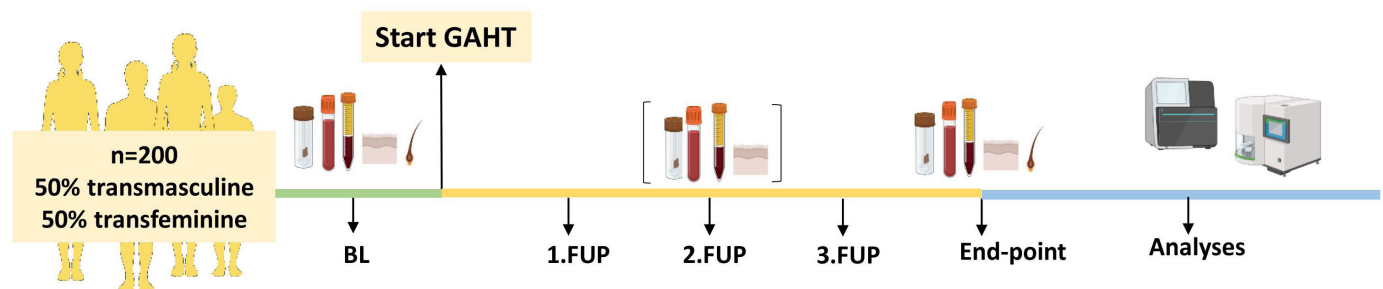


Figure 1 XCVd study design with on-site study visits at BL, 1 and 2 years and online interview FUP at 6 months (1. FUP), 12 months (2. FUP), and 18 months (3. FUP) after starting the GAHT as well as the final endpoint 24 months after starting the GAHT. BL, baseline; FUP, follow-up; GAHT, gender-affirming hormone therapy.

Table 1 Overview of XCVD study assessments and study visits

Study visit	Screening	BL	Start GAHT	1. FUP	2. FUP	3. FUP	Endpoint
Time	-8 weeks to -4 weeks	-4 weeks to -1 weeks	0 week	+6 months	+12 months	+18 months	+24 months
Inclusion/exclusion criteria	x						
Informed consent		x					
Faeces, saliva and skin biosamples		x			Optional		x
Blood samples		x			Optional		x
Hair samples		x					x
Medical history		x		x	x	x	x
Clinical examination		x			Optional		x
Standardised questionnaires		x		x	x	x	x
Sociodemographics		x					x
Lifestyle parameters		x					x
Dietary habits		x		x	x	x	x
GAHT and transition-relevant surgery			x	x	x	x	x

BL, baseline; FUP, follow-up; GAHT, gender-affirming hormone replacement therapy.;

chronic diseases requiring continuous medical care and/or a risk of life-threatening exacerbation of the illness. The exclusion criteria are age ≥ 51 years (as CVD risk increases independently with age, a narrower range reduces variance resulting from this factor to more clearly reveal causal effects of GAHT), already having started GAHT or already having self-medicated equivalent to GAHT for an extended duration prior to the study, serious acute or chronic diseases requiring continuous medical care and/or a risk of life-threatening exacerbation of the illness as well as cardiovascular events (CVEs) in the last 1 year (such as stroke or ST-elevation myocardial infarction).

Recruitment

The recruitment strategy is established in medical practices and clinics in Berlin, as well as associations such as AHA, Bundesverband Trans* and Schwulenberatung Berlin, either by physicians mentioning the study to eligible participants and/or by flyers provided in waiting rooms. Participants also are welcome to refer other interested eligible persons to apply, for example, through peer group networks, and the study flyer is visible online at the sponsoring institute's web page. The volunteers are requested to contact the study team by phone or email. Potential participants receive study information before participation.

Outcome parameters and participant timeline

Endpoints

Primary endpoint

Change in the relative abundance of intestinal bacterial taxa from faeces samples compared with baseline and 2 years after the initiation of GAHT (and at the 1-year follow-up time point, if applicable).

Secondary endpoints

Changes in microbiome parameters compared with baseline, 1 year (optional) and 2 years after the initiation of GAHT:

- ▶ Relative species abundances from skin and saliva samples.

Changes in the blood-based parameters compared with baseline, 1 year (optional) and 2 years after the initiation of GAHT:

- ▶ Concentrations of markers for CVD risk such as NT-pro BNP and Hs-Troponin T (from sera).
- ▶ Sex hormones such as free androgen index, beta-cross laps, carboxy-terminal propeptide of procollagen I, luteinising hormone, estradiol, progesterone, testosterone, free testosterone, dihydrotestosterone, estrone and free estriol (from sera), as well as sex hormone-binding globulin (from sera).
- ▶ Fasting blood glucose (from sodium fluoride blood).
- ▶ Markers of liver health such as gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (from sera).
- ▶ Kidney function markers such as creatinine, urea and uric acid (from sera).
- ▶ Immunological markers such as immune cell populations from peripheral blood mononuclear cells and cytokines (from whole blood, sera, and plasma samples).
- ▶ Cardiometabolic such as adipokines, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein-cholesterol, lipoprotein (a) and triglycerides, as well as inflammation marker such as C reactive protein (from sera).

Changes in the blood-based parameters compared with baseline and 2 years after the initiation of GAHT:

- ▶ Steroid analysis from hair samples to assess the concentration of cortisol, progesterone, testosterone and dehydroepiandrosterone.

Changes in clinical data and health-related symptoms compared with baseline, 1 year (optional) and 2 years after the initiation of GAHT:

- ▶ Vital signs and anthropometric data such as body weight, height, waist and hip circumference.
- ▶ Framingham risk scores (FRS) and Systematic Coronary Risk Evaluation (SCORE).
- ▶ Gender identity and gender dysphoria questionnaires.
- ▶ Standardised questionnaires on quality of life, physical activity, mental disorder symptoms, depressive symptoms and perceived stress.
- ▶ Dietary habits, special diet use and intake of dietary supplements, prebiotics and probiotics.

Further secondary parameters are evaluated at baseline and 2 years after the initiation of GAHT:

- ▶ Sociodemographics: family status, education level, current working status and living situation.
- ▶ Lifestyle parameters: smoking status, alcohol consumption and use of recreational drugs.

Medication documentation in a descriptive manner includes GAHT product, dosing, regimens, progress and any transition-relevant surgery or other physical interventions.

Assessments

The assessments and study visits are outlined in [table 1](#).

Biosample collection

Each participant receives verbal and written instructions for the self-collection of faeces and hair samples at home. The faeces samples are collected in OMNIgene GUT (OM-200, DNA Genotek, Ottawa, Ontario, Canada) and OMNImet GUT kits (ME-200, DNA Genotek, Ottawa, Ontario, Canada) under standardised conditions, such as immediate collection after defaecation, requiring no more than 1 min of exposure to air and room temperature, followed by rapid homogenisation and stabilisation at point of collection at baseline, 1 and 2 years after starting the GAHT. We have previously shown that such stabiliser kits are effective at controlling sample quality and reproducibility under these conditions, as well as under prolonged room temperature exposure kit application.⁵⁹ The hair samples are taken from the occipital region at room temperature (3–4 strands) at baseline and 2 years after starting the GAHT. These two self-collected biosamples are subsequently sent at room temperature using light- and temperature-protected envelopes via standard postal services and are supposed to arrive within 4–5 days after sampling at the study centre. The faeces samples are then stored at -80°C for microbiome shotgun sequencing as well as 16S rRNA gene sequencing for validation purposes. Accordingly, our protocol benefits from multiple redundant safeguards against sample

degradation (primarily through stabiliser kits, secondarily through moderate cold chain maintenance) and against sequencing artefacts (complementary shotgun and 16S sequencing). The collected hair samples will be stored at room temperature until hair steroid analyses by liquid chromatography-mass spectrometry.

Blood samples are obtained in the morning after a 12-hour overnight fast at the on-site study visit (baseline, 1 and 2 years after starting the GAHT). Blood is collected in serum ($3\times 5\text{ mL}$) and sodium fluoride tubes ($1\times 2\text{ mL}$) for the measurements of sex hormones, cardiovascular risk markers, cardiometabolic markers, liver and kidney markers, and blood glucose using standard procedures and is untreated sent at room temperature to the internal lab (Labor Berlin — Charité Vivantes GmbH, Sylter Str. 2, 13353 Berlin, Germany) for immediate analysis on the study day. Further blood is collected in serum ($1\times 9\text{ mL}$) and lithium-heparin tubes ($1\times 9\text{ mL}$) using standard procedures. While the serum tube is left to stand vertically at room temperature for 30 min before centrifugation (10 min at $3000\times g$, 4°C), the lithium-heparin tube is centrifuged within 10 min after collection (10 min at $3000\times g$, 4°C). This obtained plasma and sera are immediately aliquoted in smaller tubes (sera: $3\times 1\text{ mL}$; plasma: $3\times 1\text{ mL}$ and $4\times 600\text{ }\mu\text{L}$) and then stored at -80°C until later analysis of metabolomics, proteomics, as well as pro- and anti-inflammatory cytokines and stored in liquid nitrogen (long-term storage). Serum metabolomics will be undertaken using two primary platforms, OLINK analysis in-house and through Metabolon's (North Carolina, USA) global discovery metabolomics panel through subcontracting with strict care taken to ensure the safety and integrity of samples. These platforms were chosen to ensure good compatibility with important reference data sets from studies of CVD risk in cisgender study participants we have access to.⁴³ We also collect whole blood in lithium-heparin ($1\times 9\text{ mL}$) and EDTA tubes ($4\times 10\text{ mL}$) for later immune cell analysis; one part of this blood ($4\times 500\text{ }\mu\text{L}$ from lithium-heparin and $4\times 500\text{ }\mu\text{L}$ from EDTA whole blood) is immediately incubated with Smart tube buffer ($700\text{ }\mu\text{L}$ per each incubation) at room temperature for 12 min before being frozen at -80°C . Another part of the whole blood sample is immediately used to obtain peripheral blood mononuclear cells for later analyses of regulatory T-cells and stored in liquid nitrogen (long-term storage).

There is strong evidence supporting the roles of gut and oral microbiota in CVD risk. Additionally, variations in the skin microbiome have been noted in response to changing circulating hormone levels. Thus, these sites provide accessible, non-invasive and independent readouts of host-microbiome shifts under GAHT. We are also interested in exploring whether alterations in the microbiome at one location may drive changes at other sites, indicative of system-wide impact. The samples for analyses of the oral microbiome at baseline, 1 and 2 years after starting the GAHT are collected using the OMNIgene ORAL kits (OME-505, DNA Genotek, Ottawa, Ontario,

Canada) at least 30 min apart from the last meal, chewing gum or drink. The saliva samples are stabilised by the pre-filled liquid in the kits at room temperature, and these kits are then incubated with a dry incubator (at 50°C, for 2 hours). After incubation, the saliva liquid is aliquoted in a smaller tube (1×1.5 mL) and stored at -80°C. The skin swabs for analysing the skin microbiome are taken from the left subscapular region (5×5 cm square area) with the DNA/RNA Shield Collection tube (with Swab, R1107-E, Zymo Research Europe GmbH, Freiburg, Germany). The swabs with the skin samples are stabilised in a pre-filled kit vial of DNA/RNA Shield that preserves nucleic acids in samples at room temperature and stored at -80°C for later gene sequencing. The same safeguards against sample degradation as for the faeces samples thus apply to the saliva and skin samples. Both of these microbiomes differ from the gut in being a low bacterial cell mass environment with high host contamination. Accordingly, here only 16S rRNA sequencing, which better maintains sensitivity under such conditions, will be employed.

Medical history and clinical examination

Medical history at baseline and during the entire study period is recorded and includes current concomitant diseases, the presence of first-degree relative(s) with any of the tracked CVDs and medication intake. Clinical vital signs such as systolic and diastolic blood pressure and heart rate are measured using a digital blood pressure monitor (OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands).

Body weight (digital Seca 799) and body height (Seca 206) are measured using a standardised procedure. Body mass index is calculated as the weight in kilograms divided by the square of height in metres. Waist circumference is evaluated with a measuring tape placed on the midpoint between the lowest rib and the iliac crest, at the level of the navel, and passed once around the entire waist. Hip circumference is measured at the largest circumference around the buttocks.

CVD risk is measured using two established scores, *FRS*⁶⁰ and *SCORE*,⁶¹ for the estimation of CVD risk calculated using both male and female reference values for each participant, with both resulting scores tracked separately.

Online standardised questionnaires

Participants are administered online self-reported standardised questionnaires via the RedCap database that include assessment of

- ▶ Gender identity (*Transgender Congruence Scale*⁶²).
- ▶ Physical activity (*International Physical Activity Questionnaire*⁶³).
- ▶ Mental disorder symptoms (*Generalized Anxiety Disorder*⁶⁴).
- ▶ Depressive symptoms (*Beck-Depressions-Inventory-II, Patient Health Questionnaire*⁶⁵).
- ▶ Stress (*Perceived Stress Questionnaire*⁶⁷).
- ▶ Quality of life (including items for physical function, anxiety, depression, fatigue, sleep disturbance, ability

to participate in social roles and activities, pain interference, pain intensity and cognitive function in the past) (*PROMIS 29+2 profile V.2.1*, Clinical Trial Unit Charité⁶⁸).

- ▶ Chronotype (*Munich Chronotype Questionnaire*⁶⁹).
- ▶ Self-administered food frequency questionnaire, established in the *German Health Examination Survey* for adults 18–79 years of age from the Robert Koch Institute, to evaluate the dietary habits and the amount of 53 food items consumed during the past 4 weeks.⁷⁰

Online interview

Sociodemographic data include family status, education level, current working status and living situation. Evaluated lifestyle parameters include habitual intake of dietary supplements, prebiotics and probiotics, smoking status (current and previous smoking in pack-years), alcohol consumption (in the last 3 months) as well as the use of recreational drugs. Further, the use of a special form of diet is asked (omnivore, vegetarian, vegan, fasting). Moreover, we ask for experience on gender dysphoria about stress and day-to-day impairment in interpersonal, social, occupational and other important areas of functioning.

Sample size and power calculation

To assess statistical power in examining sex differences in the gut microbiome during GAHT, we consider $n=200$ (100 persons undergoing testosterone-based GAHT vs oestrogen-based GAHT, respectively) at baseline with those at follow-up in terms of the amount of N_{taxa} (gut bacterial taxa). In a t-test, these values are compared in two groups, applying the Bonferroni correction for N_{taxa} hypotheses, resulting in an adjusted alpha threshold of $0.05/N_{\text{taxa}}$. We assume $N_{\text{taxa}}=50$ core bacterial genera. Assuming a maximum of 20% of participants terminate the study early, a statistical power of 82% is achieved, which is usually considered an acceptable target.

Data management, oversight and monitoring

All data collection is conducted using the electronic RedCap database (www.project-redcap.org), running on a secure data platform fulfilling all criteria outlined by a data protection concept. Data are stored with a pseudonym. RedCap automatically checks for value range, format and one or two decimal values. Missing data are marked as 'not asked' or 'answer unknown'. Pseudonyms are also used for labelling biosamples. Only the study team has access to data in RedCap and also to stored biosamples.

Our team, which is responsible for internal data management, routinely verifies data quality in the RedCap database to identify missing data. As a result, the risk of missing data is minimal, and there is no need for an extra data monitoring committee. We will submit modifications and updates on the study protocol to the institutional review board and study participants. The follow-ups are used to motivate the participants and stay in contact. The study ends after completion of the endpoint examination

(2 years after the start of GAHT) of the last participant included. Only the study team has access to consent form and final data set. After completion of the study and the final analyses, all data will be stored with a retention period of 10 years.

Data analysis

The subsequent analysis is done solely on fully pseudonymised RedCap data sets. All analyses will be done in the R statistical programming environment, primarily using previously published software packages (*longDat* and *metadeconfoundR* described in more detail below). Should additional exploratory analyses be called for once data have been curated, then unless already published and citable, any further statistical workflows will be freely provided alongside any publication of data or results from this study to ensure transparency and reproducibility. Descriptive methods will be used to analyse sociodemographic and clinical parameters with measures of 95% CIs and p-values relative to corresponding null hypotheses of no impact. Most measured traits (eg, microbiome, immune or metabolome measures) are not normally distributed and will be analysed using either non-parametric tests or tests employing error models adjusted for the type of data in question (eg, Poisson or zero-inflated models for count data). All these tests require rigorous correction for multiple tests, which we will consistently apply (defaulting to Benjamini-Hochberg correction). Missing data will be handled using best practices, that is, through multiple imputations for those tests that require complete data to be applied.

To statistically operationalise the study observations, GAHT treatment recipients are grouped as either transfeminine (binary trans women or nonbinary persons assigned male at birth) or transmasculine (binary trans men or nonbinary persons assigned female at birth) based on the type of GAHT they primarily receive. This is in all cases expected to be associated with pubertal development of whichever sex the participant was not assigned at birth. These are operational categories to understand the roles of androgens versus oestrogens and are not intended nor expected to exhaustively describe the identity of all participants. In (rare) cases where a participant receives both androgen and oestrogen GAHT (eg, where a transfeminine participant is prescribed testosterone in micro dosage to achieve levels typical for cisgender women in case of complete suppression of its production through anti-androgens or gender-confirming surgery), we operationalise the overall direction as that which opposes the endogenous puberty the participant has undergone, but we also track and test statistically for the separate GAHT treatment regimen components in detail. Taking a human systems biology approach, we seek to link participant demographics, risk factors and treatment effects with clinical outcomes and readouts and quantitative microbial and molecular data. Our study will investigate how different biological factors interact and influence the risk, prevalence and progression of

CVDs. We aim to understand the variations in treatment responses to GAHT between individuals with oestrogen-dominant and testosterone-dominant sex hormone profiles. One of XCVD's primary goals is to phenotype transgender individuals, focusing on cardiac parameters. We will also employ multiomics technologies to gain insights into these parameters, taking into account the complex profiles of our participants. We plan to analyse a variety of sample types. This includes metagenomic analysis of faecal, skin and saliva samples and metabolomic, proteomic, epigenomic assessments of whole, blood, sera and plasma. These samples will be collected both before the initiation of GAHT and 2 years following the start of treatment (optional after 1 year). Finally, we will use computational methods to integrate all these data types. Our goal is to untangle the web of confounding factors and provide clear answers to the questions set forth by our study objectives. To distinguish direct from indirect influences and test for mediation, we have previously developed tools that can conservatively assess this using comparative mixed-effects modelling ('*metadeconfoundR*' R software package for case-control analyses, '*longDat*' R software package for longitudinal analyses⁷¹) that we previously developed for multiomics data.^{72 73} Mediation analyses and assessment of direct and indirect effects are also performed within the framework of these tools, as well as descriptive statistics and data visualisation. Broadly speaking, they each implement multiple parallel tests for investigating association between a per-subject or time-course variable (such as GAHT status in the longitudinal analysis enabled through *longDat* or type of GAHT planned in the cross-sectional status enabled through *metadeconfoundR*) and any of the measured clinical or -omics features, allowing untransformed or transformed variable representations. Each association achieving statistical significance following multiple testing correction is then evaluated through multiple comparative pairwise mixed-effects linear modelling together with each tracked baseline (*metadeconfoundR*) or time course (*longDat*) covariate that also achieved such initial marginal significance. Any initial association to a main variable of interest that fails to statistically improve model fit beyond what a model for each covariate affords is filtered out as likely either confounded or mediated by that other variable, to be determined based on more detailed descriptive analysis and domain knowledge. Both tools can likewise be used to generate covariate-filtered association networks and to test for variable interaction effects and differential slopes by expansion of proxy variables. For further details, please see the original publications of these tools as well as their online documentation in CRAN and BioConductor, respectively. All overall data processing, filtering, conversion and visualisation are likewise done with R packages such as *dplyr* and *ggplot2*.

Observational matched cohorts

Data from the XCVD study will be combined with that from two other studies to harmonise evaluation methods

and results. The results will be combined and validated after the end of the three studies to increase the overall validity. For these observational matched cohorts approach, we will integrate data from

- ▶ The ongoing Hormones and Health Study (HHS, title ‘Investigation of the Effects of testosterone and estrogen on eating behavior, metabolism, energy balance, and cardiovascular system in transsexual patients undergoing cross-sex hormone therapy’, Leipzig University Hospital, Germany, NCT04838249).
- ▶ The ongoing observational cohort study in Denmark at the Body Identity Clinic (BIC cohort study, title ‘Masculinizing testosterone treatment and effects on preclinical cardiovascular disease, muscle strength and power, aggression, physical fitness, and respiratory function in transgender men: protocol for a 10-year, prospective, observational cohort study in Denmark at the Body Identity Clinic’, Odense University Hospital, Denmark, NCT04254354).

HHS cohort study

The study was approved by the ethics committee at the University of Leipzig and focuses on individuals undergoing GAHT with an ICD F64.1 diagnosis. This prospective case-control study aims to assess five interconnected physiological systems: glucose and lipid metabolism, energy homeostasis, eating behaviour and the cardiovascular system. It plans to recruit 80 individuals aged 18 and older, analysing results longitudinally, comparing trans men, trans women and a cisgender control group not receiving GAHT. Participants will undergo a comprehensive evaluation, including medical history, physical exams, fasting blood samples, body composition analysis, resting energy expenditure measurement and various cardiovascular assessments. Food intake situations and taste tests will evaluate dietary preferences. Additional assessments will measure thermic perception, taste perception, smell and sex hormone deposition in hair and collection of gut and oral microbiome samples.

BIC cohort study

There is approval from the Regional Committee on Health Research Ethics for Southern Denmark, as well as the Danish Data Protection Agency (19/27572). The *BIC cohort* study recruits transgender men (n=200, aged 18 years and older) to assess preclinical coronary disease by estimating non-calcified plaque volume and calcium score using coronary CT angiography.⁷⁴ The study aims to investigate the short-term as well as long-term effects of masculinising testosterone-based GAHT on preclinical and clinical coronary disease, muscle strength and power, VO₂ max, cardiac and respiratory function, and quality of life, including aggression in transgender men. Serum levels of testosterone, estradiol, cortisol and circulating markers of cardiovascular risk and inflammation will be collected. Furthermore, standardised questionnaires such as quality of life (SF-36),⁷⁵ Inventory of Interpersonal Problems, Gender Q (in development in progress),

General Anxiety Disorder-7⁶⁴ and Patient Health Questionnaire-8⁷⁶ will be collected.

ETHICS AND DISSEMINATION

Recruitment was started on 1 August 2022 (ClinicalTrials.gov identifier: NCT05334888). The XCVD study is conducted following the ethical principles of the International Conference on Harmonization of Good Clinical Practice, the Declaration of Helsinki, applicable German laws and regulations of the general data protection, approved by the Ethical Committee of the Charité (EA1/339/21). All study participants must give their written consent before participating. The study results will be made available to the participants in summarised form following consultation for accessibility by our PPI affiliates. Results will be published in peer-reviewed journals and presented at scientific conferences in an anonymised form that will rule out the possibility of drawing any conclusions on individuals. The publications will follow the International Committee of Medical Journal Editors recommendations. Here, fully anonymised data can be made available after having undergone a peer review process and after having been published in public repositories to allow for the possibility of reproducing the results by scientists who have not been working within the framework of this project, following the FAIR-Data Principles (Open Data Approach). Specific participant-informed study consent supports this. In addition to analysis for the main purposes of the XCVD study, the data will be provided securely through a research platform and used for future projects involving the gut microbiome, immune system and CVD (additional informed consent was received for this by the participants). We collect additional declarations from the participants to consent to possible further contact to inform them about follow-up studies that may build on or extend the current research.

DISCUSSION

The XCVD study explores sex-differential host-microbiome interplay, seeking to trace the gut microbiome-mediated impact of sex hormones on CVD risk. Observation of transgender individuals undergoing GAHT offers a unique opportunity to test these associations in the human setting while further advancing knowledge of transgender health.

The administration of exogenous sex hormones, although with unverifiable accounts from antiquity, has a long history. In modern medicine, it has been used to enhance the quality of life for transgender individuals⁷⁷ and has largely been shown to be safe and efficacious for these purposes.⁵⁸ Like other marginalised or minority populations, transgender persons have been reported to be at slightly elevated cardiovascular risk compared with the cisgender average, though there is little to no evidence that GAHT plays a part in this risk elevation. Several studies have investigated persons undergoing

GAHT cardiovascular research.^{58 78} While several studies have sought to assess the cardiovascular health risk impact of GAHT regimens in both transgender and cisgender populations using different approaches, results have been inconsistent. Adverse events associated with exogenous sex hormones have been reported, but conclusive evidence regarding whether the administration of GAHT increases subsequent risk of CVEs or death in transgender individuals is lacking.⁷⁸ Adverse events reported include negative impacts on lipid profiles and CVEs such as venous thromboembolism, stroke and myocardial infarction.^{57 78} A recent literature review, including meta-analyses and cohort studies, suggests that oestrogen administration may elevate the risk of CVE in transgender women.⁷⁹ The evidence regarding androgen administration and CVE risk in transgender men is inconclusive.⁷⁹ Not least, this is because of the heterogeneous regimens of GAHT used in these trials, as well as because of the relatively short-term follow-up times in trials so far assessing cardiovascular mortality and morbidity, as any effects on cardiovascular mortality/morbidity may need long-term observation to manifest.^{58 78}

However, to our knowledge, this is the first study performing a comprehensive collection of phenotype, health, lifestyle and multi-omics data, including immune phenotyping and microbiome quantification, and the first defined explicitly for use with the kind of systematic large-scale confounding and mediation testing framework we previously have described. This unique investigation enables unprecedented possibilities to investigate the effects of sex hormones in humans in vivo while keeping many relevant factors, such as genetics, relatively constant, so far only possible in specialised animal models. It will help contextualise findings from preclinical systems in the human complex frame and/or generate further hypotheses on mechanistic underpinnings that could be explored in preclinical work.

One limitation should be noted, namely the 2-year duration. In a future follow-up study, we aim to extend duration (reflecting power calculations for a longer-term study) up to a full decade, thereby enabling in-depth exploration of CVD risk profile evolution over an even longer period. This long-term investigation aims to explore interactions between the ageing process, effects of received treatment, individual CVD baseline susceptibility and the dynamics of complex within-body adaptive systems such as the gut microbiome and immune repertoires.

We aim to assess whether patterns observed in these populations can be attributed to the influence of circulating sex hormones, potentially mediated by gut microbiome alterations induced by GAHT. We will also evaluate whether the strength of these associations varies predictably among individuals. These findings could provide a foundation for personalised medicine. In doing so, we aim to adapt cardiovascular gender medicine guidelines for transgender populations in a non-reductive, respectful and evidence-based approach.

Trial status

The article reflects the amended XCVD study protocol version V.4 of 7 September 2023. Recruitment started on 29 August 2022 and is expected to continue until December 2027.

Author affiliations

- ¹Experimental and Clinical Research Center, Charité – Universitätsmedizin Berlin and Max Delbrück Center for Molecular Medicine, Berlin, Germany
- ²Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
- ³German Centre for Cardiovascular Research (DZHK) partner site Berlin, DZHK, Berlin, Germany
- ⁴Deutsches Herzzentrum der Charité – Medical Heart Center of Charité and German Heart Institute Berlin, Department of Cardiology, Angiology and Intensive Care Medicine, Berlin, Germany
- ⁵Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
- ⁶Berlin Institute of Health (BIH), Berlin, Germany
- ⁷Medical Department III Endocrinology Nephrology Rheumatology, University of Leipzig Medical Center, Leipzig, Germany
- ⁸Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Gothenburg Wallenberg Laboratory for Cardiovascular and Metabolic Research, Goteborg, Sweden
- ⁹HI-MAG, Helmholtz Institute for Metabolic Obesity and Vascular Research, Leipzig, Germany
- ¹⁰Clinic for Cognitive Neurology, University of Leipzig Medical Center, and Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany
- ¹¹Digital Health - Connected Healthcare, Hasso Plattner Institute, University of Potsdam, Potsdam, Germany
- ¹²Helios Clinic Berlin-Buch, Berlin, Germany
- ¹³University of Toronto, Toronto, Ontario, Canada
- ¹⁴Center for Applied Transgender Studies (CATS), Chicago, Illinois, USA
- ¹⁵Transgender Professional Association for Transgender Health, TPATH, Toronto, Ontario, Canada
- ¹⁶Department of Endocrinology, Odense Universitetshospital, Odense, Denmark
- ¹⁷Institute of Clinical Research, University of Southern Denmark, Odense, Syddanmark, Denmark
- ¹⁸Body Identity Clinic, Odense Universitetshospital Endokrinologisk Afdeling M, Odense, Denmark
- ¹⁹ViRo, Berlin, Germany
- ²⁰European Molecular Biology Laboratory Structural and Computational Biology Unit, Heidelberg, Baden-Württemberg, Germany

Acknowledgements We would like to acknowledge all study participants, all researchers, study personnel and the voluntarily participating clinics and practices in Berlin and widely in Germany for the recruitment of participants: Dr Viehweger (ViroPraxis, Berlin), Professor Mai (Endocrinology Charité, Berlin), Dr Franz (MediCover, Berlin), Dr Herzig (Endocrinology, Berlin), Dr Buspavanich (Psychiatry and Neurosciences Charité, Berlin), Dr Dorn (AMEDES, Hamburg), Dr Stamm (Medicover, Saarbrücken), as well as psychologists (Dr Reininghaus; Dr Janssen-Faller; Dr Krausser; Dr Schneider and others, Berlin). We are also thankful for the support and exchange with leading associations in Berlin such as Berufsverband Trans* (Berlin), AG Queer Lichtenberg Berlin (Berlin), AG Queerite Charité (Berlin), Mission TRANS* e.V. (Berlin), Sonntagsclub e.V. (Berlin), AHA Berlin e.V. (Berlin) and Schwulenberatung Berlin (Berlin).

Contributors All authors meet the International Committee of Medical Journal Editors criteria for authorship. SKF is the principal investigator and supervisor of the design and implementation. SKF, LSB, AM, LM, RD, NS and KF drafted the conception and design of the study. SKF procured funding, and AM provided further resources for conducting the study. KF was responsible for data collection, management, analysis, interpretation of the data and yearly study reports. KF drafted the manuscript. LM, AM, RC, SH, HS, JS, NS, RD, NA, MA, DG, CF, MV, LSB and SKF provided critical intellectual content regarding the draft. KF, LM, LSB and SKF contributed to the revision process. SKF is the guarantor. All authors read and approved the final manuscript.

Funding The study is performed with the help of the highly efficient research infrastructure provided by the NCRC - Neuroscience Clinical Research Center (logistics and spaces) and Charité - Universitätsmedizin Berlin (university resources). This work is funded by the German Centre for Cardiovascular Research partner site in Berlin, Germany (DZHK, funding number 81Z0100113), with additional support from the German Research Foundation (DFG, HFpEF project, funding number SFB1470) and the European Union (Horizon, IMMEDIATE project, funding number 101095540). The financial support from the ECRC clinical study fund covered part of the expense allowances for the participants (university resources). The publication costs for this manuscript were financed by a publication fund of the Charité - Universitätsmedizin Berlin.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Kristina Franz <http://orcid.org/0000-0002-3819-9671>

Sofia Kirke Forslund-Startceva <http://orcid.org/0000-0003-4285-6993>

REFERENCES

- Benjamin EJ, Virani SS, Callaway CW, *et al*. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation* 2018;137:e67-492.
- GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018;392:1736-88.
- Virani SS, Alonso A, Aparicio HJ, *et al*. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. *Circulation* 2021;143:e254-743.
- Marquina C, Talic S, Vargas-Torres S, *et al*. Future burden of cardiovascular disease in Australia: impact on health and economic outcomes between 2020 and 2029. *Eur J Prev Cardiol* 2022;29:1212-9.
- Meyers J, Hoog M, Mody R, *et al*. The Health Care Resource Utilization and Costs Among Patients With Type 2 Diabetes and Either Cardiovascular Disease or Cardiovascular Risk Factors: An Analysis of a US Health Insurance Database. *Clin Ther* 2021;43:1827-42.
- Ventura-Clapier R, Dworatzek E, Seeland U, *et al*. Sex in basic research: concepts in the cardiovascular field. *Cardiovasc Res* 2017;113:711-24.
- Colafella KMM, Denton KM. Sex-specific differences in hypertension and associated cardiovascular disease. *Nat Rev Nephrol* 2018;14:185-201.
- Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovascular Disease. *Physiol Rev* 2017;97:1-37.
- Mosca L, Benjamin EJ, Berra K, *et al*. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American heart association. *Circulation* 2011;123:1243-62.
- Mozaffarian D, Benjamin EJ, Go AS, *et al*. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* 2015;131:e29-322.
- Ash-Bernal R, Peterson LR. The cardiometabolic syndrome and cardiovascular disease. *J Cardiometab Syndr* 2006;1:25-8.
- Govindarajan G, Whaley-Connell A, Mugo M, *et al*. The cardiometabolic syndrome as a cardiovascular risk factor. *Am J Med Sci* 2005;330:311-8.
- Kaar JL, Luberto CM, Campbell KA, *et al*. Sleep, health behaviors, and behavioral interventions: Reducing the risk of cardiovascular disease in adults. *World J Cardiol* 2017;9:396-406.
- Mensah GA, Collins PY. Understanding mental health for the prevention and control of cardiovascular diseases. *Glob Heart* 2015;10:221-4.
- Rahman MM, Islam F, Rashid MH, *et al*. The Gut Microbiota (Microbiome) in Cardiovascular Disease and Its Therapeutic Regulation. *Front Cell Infect Microbiol* 2022;12:903570.
- Gerdts E, Regitz-Zagrosek V. Sex differences in cardiometabolic disorders. *Nat Med* 2019;25:1657-66.
- Pedersen HK, Gudmundsdottir V, Nielsen HB, *et al*. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature New Biol* 2016;535:376-81.
- Wilck N, Matus MG, Kearney SM, *et al*. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature New Biol* 2017;551:585-9.
- Forslund K, Hildebrand F, Nielsen T, *et al*. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature New Biol* 2015;528:262-6.
- Cui C, Huang C, Liu K, *et al*. Large-scale in silico identification of drugs exerting sex-specific effects in the heart. *J Transl Med* 2018;16:236.
- Gaignebet L, Kararigas G. En route to precision medicine through the integration of biological sex into pharmacogenomics. *Clin Sci (Lond)* 2017;131:329-42.
- Regitz-Zagrosek V, Dworatzek E, Kintscher U, *et al*. Sex and sex hormone-dependent cardiovascular stress responses. *Hypertension* 2013;61:270-7.
- Humphries KH, Izadnegahdar M, Sedlak T, *et al*. Sex differences in cardiovascular disease - Impact on care and outcomes. *Front Neuroendocrinol* 2017;46:46-70.
- Miyagi M, Guthman EM, Sun SED-K. Transgender rights rely on inclusive language. *Science* 2021;374:1568-9.
- Murphy CN, Delles C, Davies E, *et al*. Cardiovascular disease in transgender individuals. *Atherosclerosis* 2023;384:S0021-9150(23)05203-6.
- Pei J, Harakalova M, Treibel TA, *et al*. H3K27ac acetylome signatures reveal the epigenomic reorganization in remodeled non-failing human hearts. *Clin Epigenetics* 2020;12:106.
- Ober C, Loisel DA, Gilad Y. Sex-specific genetic architecture of human disease. *Nat Rev Genet* 2008;9:911-22.
- Beikoghli Kalkhoran S, Kararigas G. Oestrogenic Regulation of Mitochondrial Dynamics. *Int J Mol Sci* 2022;23:1118.
- Sanchez-Ruderisch H, Queirós AM, Fliegner D, *et al*. Sex-specific regulation of cardiac microRNAs targeting mitochondrial proteins in pressure overload. *Biol Sex Differ* 2019;10:8.
- Gaignebet L, Kañdula MM, Lehmann D, *et al*. Sex-Specific Human Cardiomyocyte Gene Regulation in Left Ventricular Pressure Overload. *Mayo Clin Proc* 2020;95:688-97.
- Sabbatini AR, Kararigas G. Menopause-Related Estrogen Decrease and the Pathogenesis of HFpEF: JACC Review Topic of the Week. *J Am Coll Cardiol* 2020;75:1074-82.
- Sabbatini AR, Kararigas G. Estrogen-related mechanisms in sex differences of hypertension and target organ damage. *Biol Sex Differ* 2020;11:31.
- Mueller A, Haerberle L, Zollver H, *et al*. Effects of intramuscular testosterone undecanoate on body composition and bone mineral density in female-to-male transsexuals. *J Sex Med* 2010;7:3190-8.
- Costantino A, Cerpolini S, Alvisi S, *et al*. A prospective study on sexual function and mood in female-to-male transsexuals during testosterone administration and after sex reassignment surgery. *J Sex Marital Ther* 2013;39:321-35.
- Ohlsson C, Barrett-Connor E, Bhasin S, *et al*. High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. The MrOS (Osteoporotic Fractures in Men) study in Sweden. *J Am Coll Cardiol* 2011;58:1674-81.
- Boden WE, Miller MG, McBride R, *et al*. Testosterone concentrations and risk of cardiovascular events in androgen-deficient men with atherosclerotic cardiovascular disease. *Am Heart J* 2020;224:65-76.
- Gao A, Su J, Liu R, *et al*. Sexual dimorphism in glucose metabolism is shaped by androgen-driven gut microbiome. *Nat Commun* 2021;12:7080.
- Canonico M, Plu-Bureau G, Lowe GDO, *et al*. Hormone replacement therapy and risk of venous thromboembolism in postmenopausal women: systematic review and meta-analysis. *BMJ* 2008;336:1227-31.
- Chng KR, Ghosh TS, Tan YH, *et al*. Metagenome-wide association analysis identifies microbial determinants of post-antibiotic ecological recovery in the gut. *Nat Ecol Evol* 2020;4:1256-67.
- Jeffery IB, Lynch DB, O'Toole PW. Composition and temporal stability of the gut microbiota in older persons. *ISME J* 2016;10:170-82.
- Sanz Y, Olivares M, Moya-Pérez Á, *et al*. Understanding the role of gut microbiome in metabolic disease risk. *Pediatr Res* 2015;77:236-44.

- 42 Siokatas G, Papatheodorou I, Daiou A, *et al.* Sex-Related Effects on Cardiac Development and Disease. *J Cardiovasc Dev Dis* 2022;9:90.
- 43 Forslund SK, Chakaroun R, Zimmermann-Kogadeeva M, *et al.* Combinatorial, additive and dose-dependent drug-microbiome associations. *Nature New Biol* 2021;600:500–5.
- 44 Adlercreutz H, Martin F, Järvenpää P, *et al.* Steroid absorption and enterohepatic recycling. *Contraception* 1979;20:201–23.
- 45 Adlercreutz H, Martin F, Pulkkinen M, *et al.* Intestinal metabolism of estrogens. *J Clin Endocrinol Metab* 1976;43:497–505.
- 46 Kwa M, Plottel CS, Blaser MJ, *et al.* The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J Natl Cancer Inst* 2016;108:djw029.
- 47 Bartolomaeus H, Balogh A, Yakoub M, *et al.* Short-Chain Fatty Acid Propionate Protects From Hypertensive Cardiovascular Damage. *Circulation* 2019;139:1407–21.
- 48 Li S, Karargas G. Role of Biological Sex in the Cardiovascular-Gut Microbiome Axis. *Front Cardiovasc Med* 2021;8:759735.
- 49 Fromentin S, Forslund SK, Chechi K, *et al.* Microbiome and metabolome features of the cardiometabolic disease spectrum. *Nat Med* 2022;28:303–14.
- 50 Hou K, Wu Z-X, Chen X-Y, *et al.* Microbiota in health and diseases. *Signal Transduct Target Ther* 2022;7:135.
- 51 Hashimoto K. Emerging role of the host microbiome in neuropsychiatric disorders: overview and future directions. *Mol Psychiatry* 2023;28:3625–37.
- 52 Frasier K. The role of skin microbiota in cardiovascular health: exploring the gut-skin-heart axis. *Am J Prev Cardiol* 2024;19:100741.
- 53 DeStefano F, Anda RF, Kahn HS, *et al.* Dental disease and risk of coronary heart disease and mortality. *BMJ* 1993;306:688–91.
- 54 von Elm E, Altman DG, Egger M, *et al.* The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. *PLoS Med* 2007;4:e296.
- 55 Winter S, Diamond M, Green J, *et al.* Transgender people: health at the margins of society. *Lancet* 2016;388:390–400.
- 56 Ingraham N, Fox L, Gonzalez AL, *et al.* “I just felt supported”: Transgender and non-binary patient perspectives on receiving transition-related healthcare in family planning clinics. *PLoS One* 2022;17:e0271691.
- 57 Chan Swe N, Ahmed S, Eid M, *et al.* The effects of gender-affirming hormone therapy on cardiovascular and skeletal health: A literature review. *Metabol Open* 2022;13:100173.
- 58 Defreyne J, Van de Bruaene LDL, Rietzschel E, *et al.* Effects of Gender-Affirming Hormones on Lipid, Metabolic, and Cardiac Surrogate Blood Markers in Transgender Persons. *Clin Chem* 2019;65:119–34.
- 59 Bartolomaeus TUP, Birkner T, Bartolomaeus H, *et al.* Quantifying technical confounders in microbiome studies. *Cardiovasc Res* 2021;117:863–75.
- 60 Jahangiry L, Farhangi MA, Rezaei F. Framingham risk score for estimation of 10-years of cardiovascular diseases risk in patients with metabolic syndrome. *J Health Popul Nutr* 2017;36:36.
- 61 Graham IM, Di Angelantonio E, Visseren F, *et al.* Systematic Coronary Risk Evaluation (SCORE): JACC Focus Seminar 4/8. *J Am Coll Cardiol* 2021;77:3046–57.
- 62 Jones BA, Bouman WP, Haycraft E, *et al.* The Gender Congruence and Life Satisfaction Scale (GCLS): Development and validation of a scale to measure outcomes from transgender health services. *Int J Transgend* 2019;20:63–80.
- 63 Tran VD, Do VV, Pham NM, *et al.* Validity of the International Physical Activity Questionnaire-Short Form for Application in Asian Countries: A Study in Vietnam. *Eval Health Prof* 2020;43:105–9.
- 64 Spitzer RL, Kroenke K, Williams JBW, *et al.* A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006;166:1092–7.
- 65 Wang YP, Gorenstein C. Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. *Braz J Psychiatry* 2013;35:416–31.
- 66 Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001;16:606–13.
- 67 Fliege H, Rose M, Arck P, *et al.* The Perceived Stress Questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosom Med* 2005;67:78–88.
- 68 Cella D, Riley W, Stone A, *et al.* The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. *J Clin Epidemiol* 2010;63:1179–94.
- 69 Roenneberg T, Wirz-Justice A, Meroo M. Life between clocks: daily temporal patterns of human chronotypes. *J Biol Rhythms* 2003;18:80–90.
- 70 Haftenberger M, Heuer T, Heidemann C, *et al.* Relative validation of a food frequency questionnaire for national health and nutrition monitoring. *Nutr J* 2010;9:36.
- 71 Chen CY, Löber U, Forslund SK. LongDat: an R package for covariate-sensitive longitudinal analysis of high-dimensional data. *Bioinform Adv* 2023;3:vbad063.
- 72 Maifeld A, Bartolomaeus H, Löber U, *et al.* Fasting alters the gut microbiome reducing blood pressure and body weight in metabolic syndrome patients. *Nat Commun* 2021;12:1970.
- 73 Pedersen HK, Forslund SK, Gudmundsdottir V, *et al.* A computational framework to integrate high-throughput “-omics” datasets for the identification of potential mechanistic links. *Nat Protoc* 2018;13:2781–800.
- 74 Lehmann Christensen L, Glintborg D, Taulbjerg Kristensen T, *et al.* Masculinising testosterone treatment and effects on preclinical cardiovascular disease, muscle strength and power, aggression, physical fitness and respiratory function in transgender men: protocol for a 10-year, prospective, observational cohort study in Denmark at the Body Identity Clinic (BIC). *BMJ Open* 2020;10:e045714.
- 75 Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life: Scoping review. *SAGE Open Med* 2016;4:2050312116671725.
- 76 Kroenke K, Strine TW, Spitzer RL, *et al.* The PHQ-8 as a measure of current depression in the general population. *J Affect Disord* 2009;114:163–73.
- 77 Hamburger C, Sturup GK, Dahl-Iversen E. Transvestism; hormonal, psychiatric, and surgical treatment. *J Am Med Assoc* 1953;152:391–6.
- 78 Connelly PJ, Marie Freel E, Perry C, *et al.* Gender-Affirming Hormone Therapy, Vascular Health and Cardiovascular Disease in Transgender Adults. *Hypertension* 2019;74:1266–74.
- 79 Masumori N, Nakatsuka M. Cardiovascular Risk in Transgender People With Gender-Affirming Hormone Treatment. *Circ Rep* 2023;5:105–13.