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Masculinizing testosterone treatment and effects on preclinical cardiovascular disease, muscle strength and power, aggression, physical fitness and respiratory function in transgender men: Protocol on a ten-years, prospective, observational cohort study at the Body Identity Clinic (BIC).

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Title

Masculinizing testosterone treatment and effects on preclinical cardiovascular disease, muscle strength and power, aggression, physical fitness and respiratory function in transgender men: Protocol on a ten-years, prospective, observational cohort study at the Body Identity Clinic (BIC).

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Ethical approval

The Regional Ethics Committee, Region of Southern Denmark approved the study.

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Abstract

Introduction: The number of individuals with gender dysphoria seeking gender-affirming treatment is increasing. The short- and long-term effects of masculinizing treatment with testosterone are debated as serum testosterone increase up to 20-fold compared to cisgender women. We hypothesize that, testosterone treatment is associated with non-calcified coronary plaque (NCP) development in transgender men.

Methods and analyses: Prospective, single-center, observational cohort study at the Body Identity Clinic (BIC), Odense University Hospital, Denmark, where all investigations are performed at inclusion and after 1, 3, 5 and 10 years of testosterone therapy.

NCP volume and calcium score are estimated by coronary CT angiography (CCTA) and upper body muscle strength and power are measured by a "Low Row" weight stack resisted exercise machine. Evaluation of aggression and quality of life are assessed by questionnaires, VO_2max by maximal testing on bike ergometer, and cardiac and respiratory function are measured by echocardiography and spirometry, respectively. Markers of cardiovascular risk and inflammation and also cortisol and cortisone are assessed in blood, diurnal urine and/or hair samples. National registry data, regarding International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic codes, prescriptions, socioeconomics and causes of death will be available also in patients lost to follow-up in the clinical study.

Ethics and dissemination: The Regional Committees on Health Research Ethics for Southern Denmark (S-20190108) and the Danish Data Protection Agency approved the study (19/27572). Signed informed consent will be obtained from all participants. All findings will be published in peer-reviewed journals or at scientific conferences.

Trial registration: Clinicaltrials.gov ID: NCT04254354

Article Summary

Strengths and limitations of this study

- Body Identity Clinic (BIC) is a research clinic, where this prospective longitudinal 10 years cohort study in transgender men will assess preclinical coronary disease by estimating NCP volume and calcium score using CCTA. These results will provide important knowledge regarding cardiovascular risk in transgender men on testosterone therapy.
- Scientific evidence will be obtained regarding upper body muscle strength and power, aggression, VO_2max, cardiac and respiratory function in transgender men, during short- and long-term testosterone treatment. This data is required in transgender men, regarding health as well as sports.
- Access to registry data in all included persons, also those lost to follow-up in the clinical study, is a great advantage, but a proper control group is missing. However, it is unethical to perform a placebo-controlled randomized trial when gender dysphoria is present.
- Information will only be available on effects of testosterone therapy in transgender individuals, who are transgender men or non-binary individuals. However, a parallel study has been planned in transgender women and non-binary persons on feminizing treatment.

Introduction

Recent European studies report that 0.6-0.8 % of individuals assigned female at birth have incongruent gender identity or gender dysphoria[1 2]. Masculinizing treatment is testosterone treatment and is initiated both in transgender men and if requested by non-binary individuals. Testosterone is an important androgenic and anabolic hormone. The androgen effects of testosterone are terminal hair growth and deepening of the voice, whereas the anabolic effects include muscle growth. Body shape changes from feminine to masculine during testosterone treatment, as subcutaneous fat is reduced[3] and redistributed[4 5]. The changes in sex hormones are quite dramatic in transgender men, as circulating testosterone concentrations increase up to 20-fold into the normal range for cisgender men[6], while estradiol levels are supressed.

Clinical coronary disease is rare in young persons[7], however, preclinical coronary disease is observed and testosterone treatment may accelerate non-calcified coronary plaque (NCP) formation. Both NCP and calcified plaques may be detected by the sensitive coronary CT angiography (CCTA)[8] in combination with a semi-automated computer program[9]. In a large cardiac CT registry study in younger (mean age 39 years) participants, 22 % of cisgender men and 15 % of cisgender women, without cardiovascular symptoms, had coronary artery disease defined as any plaque formation (non-calcified or calcified)[8]. The association between testosterone and NCP formation has been reported in ageing cisgender men after 1 year of testosterone treatment[10], with increased waist-to-hip ratio at baseline as the strongest predictor of NCP formation[11], but there is a lack of knowledge regarding NCP formation, in relatively young men on masculinizing testosterone therapy. We know that lipid status is deteriorated during masculinizing testosterone treatment, with an increase in low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) cholesterol[12] and blood pressure may be elevated[13 14]. However, studies on cardiovascular endpoints are limited and conflicting. A four-

fold increase in odds ratio for myocardial infarction in transgender men has been found, after adjusting for age, diabetes mellitus, hypertension, hypercholesterolemia, chronic kidney disease, smoking and exercise levels[15]. The study design was, however, cross-sectional, hence it is not possible to conclude on cause and effect. A retrospective study in transgender men reported no cases of myocardial infarction after 10 years of testosterone treatment[16], but the retrospective design increased the risk of selection bias due to loss to follow-up before 10 years.

Testosterone treatment is associated with dose-dependent changes in muscle mass[17 18] and strength in cisgender men[5 17]. Muscle mass also increases in transgender men[19], but there is only limited data on muscle strength before and during masculinizing testosterone treatment. Due to higher number of androgen receptors in the upper body muscles[20], it would be of interest to characterize muscle strength and power in the large muscles of the arms and upper back. It has been shown, previously, that grip strength was higher in transgender men compared to cisgender women[21]. The study was, however, cross-sectional, and hand grip strength only tests the small muscles of the hand and lower arm. Furthermore, an interesting study has, recently, reported an increase in lower body muscle strength[22].

The World Professional Association for Transgender Health (WPATH)[23] recommends to warn of increased aggression as a psychological adverse effect of masculinizing testosterone treatment, however, this presumption was not supported by recent data[24 25]. The term aggression covers a wide range of inter-correlated behaviors, thoughts and emotions. There is no uniform definition of aggression[26] and there is severe methodological problems and high risk of bias in studies reporting increased aggression during testosterone treatment[27]. To obtain valid information on aggression constructs[27] during short- and long-term testosterone therapy, we apply a questionnaire, which incorporates all dimensions of aggression[28] with concomitant information on anxiety, depression, testosterone dose, duration of treatment and physical health.

Low cardiorespiratory fitness has been associated with an increased risk of premature death from all causes, but primarily cardiovascular disease, in cisgender individuals[29]. The maximum oxygen uptake (VO_2max), determined from a graded maximal exercise test, is an approved way of classifying cardiorespiratory fitness level[30]. VO_2max is generally higher in cisgender men compared to cisgender women[31], presumably due to differences in muscle mass, cardiac output, haematocrit and lung size. There is no prospective data on VO_2max during masculinizing testosterone treatment, but VO_2max is expected to increase, as testosterone has marked effects on both muscle mass[22] and haematocrit[14]. Some data exists on physical fitness and exogenous testosterone administration in cisgender women. Aerobic running time increased in young physically active cisgender women during 10 weeks of 10 mg testosterone gel per day, this testosterone dose equals 20 % of masculinizing testosterone treatment[32]. Reference ranges for spirometry vary with gender assigned at birth, weight, height and age. Obstructive and restrictive estimates of respiratory function are estimated by spirometry and asthma is characterized by obstructively decreased respiratory function with airway hyper-responsiveness and inflammation. Cisgender women have a much higher prevalence of asthma compared to cisgender men[33], but there are no reference ranges for respiratory function in transgender men[34]. Masculinizing testosterone treatment is for life and currently no prospective data is available on long-term morbidity; but we may acquire information on important preclinical health issues regarding cardiovascular and respiratory health and upper body muscle strength and power. Testosterone therapy may activate aggression, but the temporal relation has not been clarified. The combination of long-term clinical observational data and access to national registry data will provide further knowledge on benefits and risks of masculinizing testosterone treatment.

Aim

We will investigate short- and long-term effects of masculinizing testosterone treatment on pre-clinical and clinical coronary disease, muscle strength and power, VO_2max, cardiac and respiratory function, and quality of life including aggression.

We hypothesize that:

Masculinizing testosterone treatment is associated with NCP development and progression.

Testosterone treatment will be associated with long-term increase in muscle strength and power, VO_2max, cardiac and respiratory function.

Aggression scores will be significantly higher during initiation of testosterone treatment, but the aggression scores will return to baseline during long-term testosterone treatment.

Methods and analysis

Study population and recruitment

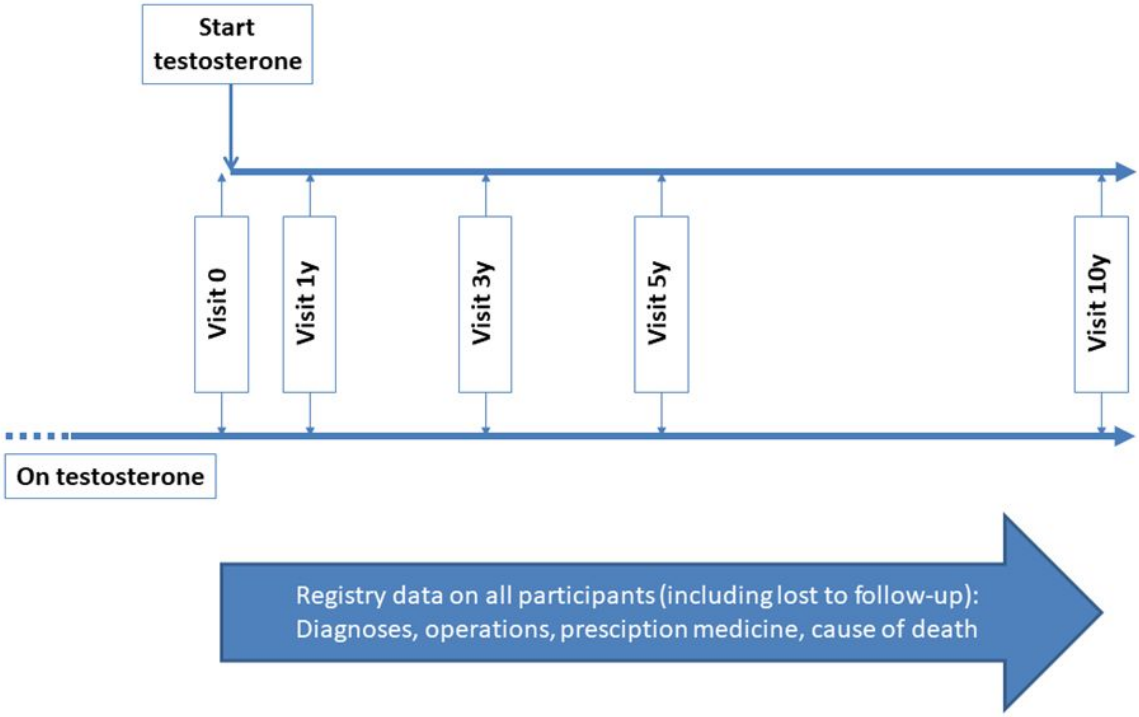
Participants are individuals assigned female at birth with a diagnosis of gender dysphoria treated with testosterone or approved to start treatment with testosterone. We include only individuals associated with one of the three centers of gender identity in Denmark: Odense, Aalborg and Copenhagen. No individual will be included, with known use of 'self-prescribed' sex hormones.

The inclusion period for the cohort (N=200) is estimated to last for 2 years.

Study design

For study outline, please see figure 1. The study is a prospective single-center observational cohort study at BIC, Odense University Hospital, Oden se, Denmark with duration of ten years. Participants (Age 18 year and older) are invited for five visits (Baseline, 1, 3, 5, and 10 years), after informed

consent has been given. The participants in the study will spend one day in BIC per visit and all examinations use the same equipment and protocols (table 1). Ethical permission has been obtained for the use of registry data regarding International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic codes, medical treatment, socioeconomics and causes of death in all participants including those, who are lost to follow-up. The participants accept by written informed consent. All individuals in Denmark have a civil registration number (CPR) reflecting binary gender. During legal transitioning individuals may change CPR and data from the two CPR numbers is merged. We will be able to compare study participants with participants lost to follow-up by combining the clinical outcomes with registry data (table 1, figure 1).



Legend figure 1: Study design

Patient and Public Involvement statement

An advisory board of transgender men has been established. The participant advisory board meetings were arranged prior to application for ethical approval of the study to secure inputs in terms of relevance of research questions, recruitment, outcomes and participant time consumption. The participant advisory board has read and commented on the study material and will be contacted for continuous sparring.

Endpoints

Primary endpoint

NCP volume

Secondary endpoints

Calcium score

Upper body muscle strength and power

Aggression and quality of life

VO_2max

Left ventricular muscle mass and function

Respiratory function

Serum levels of testosterone, estradiol and cortisol.

Circulating markers of cardiovascular risk and inflammation

Diurnal urine and hair samples for assessment of cortisol and cortisone

Outcomes

Outcomes and visits are outlined in table 1.

Investigations	Inclusion	1 yr.	3 yr.	5 yr.	10 yr.
Coronary CT angiography (CTTA)	X	X			X
Muscle strength and power	X	X	X	X	X
Aggression, quality of life questionnaires	X	X	X	X	X
VO_2max	X	X	X	X	X
Echocardiography	X	X	X	X	X
Spirometry	X	X	X	X	X
Blood, urine and hair samples	X	X	X	X	X
DXA whole-body and bone density	X	X	X	X	X
Medical history	X	X	X	X	X
Physical examination	X	X	X	X	X

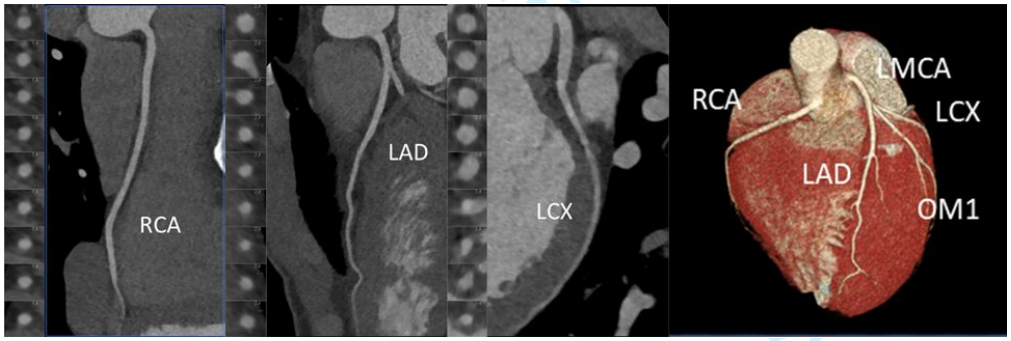
Legend table 1: Overview of study outcomes and visits

Coronary CT angiography (CCTA)

CCTA (high-end CT scanner) is conducted at the first visit, after 1 year and at 10 years follow-up (table 1) to examine the presence of NCP and calcified coronary plaques (figure 2). The scanning protocol depends on the patient heart rate. In patients with a stable heart rate above 60 beats per minute, orally or intravenously β-blocker are administered until the heart rate is appropriate (if possible below 60), and a prospectively gated protocol is used. Participants with a heart rate > 70 bpm despite β-

blocker pretreatment are subjected to a retrospectively gated scan with dose modulation. Additionally, sublingual nitrates are administered prior to the scan. Administrating of β-blocker and nitrates are in accordance to daily clinical practice. An experienced cardiologist performs data analysis of NCPs, calcium score/stenosis and pericardial fat using a semi-automatic program[9]. Radiation amounts to 1.6 mSv per CCTA. We have ethical permission to perform three CCTAs during the study period of 10 years. Radiation from CCTA is lower than expected and we are applying for an additional CCTA at five years.

Figure 2



Legend figure 2: The figure displays a coronary CT angiography (CCTA). The presence of NCP is detected by a semi-automatic program. RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery; OM, obtuse marginal branch 1.

Upper body muscle strength and power

A "Low Row" weight stack resisted exercise machine (Technogym, Italy) tests arm and upper back muscles. The machine is adjusted to fit the individual participant relative to body height and arm length. Body weight, training history and age estimates initial loading. Warm-up is 3 x 10 and 1 x 5

repetitions at the estimated test load, with 1 minute of rest in between sets. Subsequently maximal effort (1 repetitions maximum, 1RM) is obtained using single repetitions with increasing load until task failure (the participant is unable to complete the full range of motion) with 2 minutes of rest in between sets. Hereafter participants are instructed to perform at least 3 sets of one repetition at forceful and fast as possible to evaluate muscle power with a load corresponding to 80 % of 1RM and 2 minutes of rest in between sets. During each repetition, peak and mean power is measured. To evaluate muscle power we attach a PUSH 2.0 inertial motion device (PUSH, Toronto, Canada) to the weight stack. The PUSH device includes a 3-axis accelerometer and gyroscope, enabling measurements of human movement kinematics at a sampling rate of 1000 Hz. Previously, PUSH has been validated to evaluate power using resistance exercises[35]. Peak and mean power (Watt), respectively, are calculated based on kinematic data derived by the PUSH software.

Questionnaires

Buss-Perry Aggression Questionnaire, Quality of life: SF-36®, Inventory of Interpersonal Problems ®, Gender Q (Development in progress), GAD7 (General Anxiety Disorder-7) and PHQ9 (Patient Health Questionnaire-8).

VO_2max

Measurement of VO_2max is performed on a bike ergometer and Vyntus®CPS system. The resistance will start low and increase gradually until maximum capacity or exhaustion. At maximum capacity lactate is measured.

Echocardiography

A comprehensive transthoracic echocardiography is performed by a medical doctor. The recordings are stored digitally for blinded analysis. The following are included: Size and mass of all cardiac chambers, left ventricle ejection fraction (LVEF), global longitudinal strain (GLS), left ventricle diastolic function and heart valve function.

Respiratory function

Respiratory function is tested by a spirometer (Vyntus®CPS system) measuring forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and peak flow.

Blood, urine and hair samples

Testosterone, estradiol and cortisol levels and cardiovascular risk markers in blood and serum (HbA1c, lipids, hematocrit, adiponectin, suPAR) are analyzed along with inflammation markers in blood and serum (CRP, IL6). Hormone levels are measured after an overnight fast between 8 and 9 am by liquid chromatography tandem mass spectrometry (LC-MS/MS), which is calibrated by in-house prepared calibrators, and the relative standard deviation (SD) is < 10 %. Quality for steroid hormones is assured by monthly participation in the external quality control program for steroid hormones from The United Kingdom National External Quality Assessment Service (UKNEQAS). Sex hormone binding globulin is determined on serum samples by a Roche assay on Cobas e602 with a precision of 1.8 %-4.0 % (14.9-21.9 nmol/L). Free testosterone levels are calculated assuming a plasma albumin concentration of 4.3 g/dL[36].

Hemoglobin is measured using a photometric analyzer with a coefficient of variation (CV) of 2.8 %. Plasma total cholesterol and HDL cholesterol are analyzed by enzymatic colorimetric reactions (Modular P, Roche), and LDL cholesterol is calculated using the Friedewald equation[37]. HbA1c is

measured by high-performance liquid chromatography using Tosoh G8 (Medinor, Broendby, Denmark); the analytical CV is 0.9 %. Adiponectin is determined by an in-house timeresolved immunofluorometric assay[38], with intra- and inter-assay CV averaging 5 and 10 % respectively. Plasma suPAR is measured with the suPARnostic® ELISA (ViroGates A/S, Birkerød, Denmark) with a mean CV of 4 %. CRP is analyzed using latex based immunoanalysis (CRP Ultra, Sentinel Diagnostics, Milan, Italy) by an Architect c8000 instrument (Abbott). The intra-assay and inter-assay CVs are 0.8 % and 1.9 % for normal levels of CRP, respectively. Plasma IL-6 is measured with Quantikine® HS-IL-6 ELISA (R&D Systems, Minneapolis, USA) with a mean CV of 8 %.

Diurnal urine samples: Participants are instructed to note the time for voiding in the morning before starting sample collection, and all urine collected until the morning of the second day. Urine samples are kept frozen at −20°C until analysis. Cortisol and cortisone are analyzed by LC-MS/MS. Urine samples are solid phase extracted on an Oasis HLB 96-well plate after addition of deuterated internal standards; the analysis is calibrated by in-house prepared calibrators, and the relative SD is <10%. Quality is assured by monthly participation with satisfactory results in the external quality control program for steroid hormones from the UKNEQAS.

Hair samples: A section of hair strands approximately 3 mm in diameter is cut as close to the scalp as possible from the posterior vertex area. Hair samples are stored in aluminum foil as previously described[39], the 4-cm hair segment closest to the scalp is used for analyses to represent cortisol secretion over the most recent 4-month period[40]. The analyses will be carried out at Department of Psychology, Technical University of Dresden, Germany using LC-MS/MS[41].

Whole body dual x-ray absorptiometry and bone mineral density

Dual x-ray absorptiometry, Horizon A Discovery is used to measure whole body lean body mass, fat mass and bone density. Radiation amounts to 0.1 mSv per scan.

Medical history

Chronic diseases, medication and supplements, alcohol, tobacco and abuse, gynecological history, previous treatment with testosterone, information on diet and physical activity are recorded.

Physical examination

Height, weight, body mass index (BMI, kg/m^2), blood pressure, waist and hip circumference and face and body hair (Ferriman-Gallwey Score) are recorded.

Sample size and statistics.

As no valid data exists for a proper power calculation regarding the primary endpoint, NCP, we have estimated the sample size. There are around 300 referrals for masculinizing therapy in Denmark per year and patients are treated for life within the three centers of gender identity in Denmark. We aim to include 200 participants, including 50 who are approved to start testosterone treatment based on the power calculation of the main secondary endpoint, upper body muscle strength and power. Sample size estimation for upper extremity muscle strength and power was based on data (mean baseline/standard deviation) from a comparable cohort[42]. The estimation was based on a 10 % within-participant difference (deemed as functional relevant) with an alpha level of 0.05 and a statistical power of 0.80. On the basis of this data, a sample size of 39 was sufficient to detect within-participants differences

with two-tailed comparison. To account for potential dropouts (estimated to 20 %) 50 individuals approved to start testosterone treatment will be recruited for the trial.

Normally and non-normally distributed data will be analyzed using parametric and non-parametric statistics, respectively. Associations between testosterone and endpoints will be investigated with multiple linear regression modeling. Random mixed-effects linear regression models will be applied to investigate associations between testosterone and longitudinal repeated markers of assessed outcomes. We will collaborate with a statistician regarding random mixed-effects linear regression modeling.

A directed acyclic graph (DAG) will be depicted in order to transparently identify a priori assumptions of causal relations between exposure, outcome, potential confounders, intermediate factors, and selection bias. Missing data will be handled according to type. Depending on data, analyses will be performed based on either complete case analyses or, if appropriate, by imputing missing data in collaboration with the statistical department. Analyses will be conducted using STATA 14 (StataCorp 2015). Post regression diagnostics/model validation will be performed. Assumptions of linearity between predictors and the outcome variable will be inspected by using scatter plots and augmented component-plus-residual plots. Normality of predicted residuals will be checked with quantile-normal and probability-normal plots. Homogeneity of variance (homoscedasticity) of the residuals will be investigated by plotting residuals against the fitted (predicted) values. Multicollinearity will be assessed using variance inflation factors. Open-ended questions will not be included. Data is anonymized according to Danish law and regulations (The Regional Committees on Health Research Ethics for Southern Denmark, Project-ID: S-20190108, The Danish Data Protection Agency, journal no. 19/27572), and therefore analyses will be performed through a remote VPN access to Statistics Denmark.

Data management

Data is stored and analyzed electronically and unauthorized access denied. Original data is filed according to one participant number. Research Electronic Data Capture (REDCap®) (www.project-redcap.org), hosted by Open Patient data Explorative Network (OPEN), will be used for registration of data[43 44]. REDCap® meets the safety requirements set by the Danish Data Protection Agency for storage of person-sensible data. OPEN Analyse, a secure remote desktop solution hosted by OPEN, is used for storage and analyses of the pseudo-anonymized data.

Ethics and dissemination

All participants give written informed consent. The study results will be published in peer-reviewed journals, publication will be according to the International Committee of Medical Journal Editors (ICMJE) recommendations and the investigators oblige themselves to publish both positive and negative findings. The study is in accordance with the Helsinki II declaration and the regulations of the General Data Protection Regulation. It is approved by the Danish Data Protection Agency (journal no. 19/27572), the Regional Committees on Health Research Ethics for Southern Denmark (project-ID: S-20190108) and registered at ClinicalTrials.gov (NCT04254354).

Discussion

Body Identity Clinic is a research clinic, where this prospective longitudinal 10 years cohort study in transgender men will assess preclinical coronary disease by determining NCP volume and calcium score. NCP volume is assessed by state-of-the-art CCTA and we use the lowest detectable change in NCP formation (1 mm^3) as the clinically relevant difference[45]. However, we cannot perform a proper power calculation regarding our primary endpoint, because, no previous data has been published, thus

the distribution of data is unknown and the standard deviation cannot be calculated. The possible lack of NCP development during 10 years is acknowledged, but this potential negative result would be reassuring and clinically relevant. In a large cardiac CT registry study in relatively young men (mean age 39 ± 6 years) 424 out of 1143 were registered as having no cardiovascular symptoms[8]. Any coronary plaque, was detected in 22 % of the 424 men by experienced readers. In our study, we intend to increase the sensitivity of CCTA by using a semi-automatic program, which is comparable to Autoplaque[9].

We also aim at providing new scientific evidence regarding upper body muscle strength and power in men on masculinizing therapy; these muscles are of special interest as the number of androgen receptors are higher in the upper body muscles[20] compared to lower body. Also, our study will elaborate on interesting data on the possible activating effect of testosterone therapy on aggression and the temporal relation between masculinizing testosterone treatment and levels of aggression, a very relevant clinical issue with respect to current WPATH warning regarding aggression on testosterone therapy[23]. We will report new data on VO_2max and cardiac function as improvements in cardiorespiratory fitness are associated with considerably reduced mortality risk and adverse cardiovascular event rates[29]; furthermore, data on respiratory function will add new knowledge and all the above-mentioned results are important for transgender men and non-binary individuals on testosterone therapy in health and sports.

It is a great advantage that we have access to registry data on all included persons, also those lost to follow-up in the clinical study, but a proper control group is still missing. However, it is unethical to perform a placebo-controlled randomized trial when gender dysphoria is present. We address this issue in two ways: Firstly, participants will be their own control for within subject comparison, prospectively, and secondly study participants will be compared to participants from our Odense

Androgen Study (OAS)[6 46-48]. The OAS was a large cross-sectional study in 783 young healthy men (20-29 years old). We plan a reinvestigation in 2021 (follow-up time 14 years), however, we do not know if it is feasible to use them as controls regarding the effects of ageing. Our study is a non-representative cohort study with risk of selection bias regarding age, BMI, smoking, ethnicity, fitness level and morbidity. However, we are performing a national registry study, including all transgender men in Denmark, which enables comparison between included individuals and data from the national register-based cohort of transgender men. We have, previously used this embedded design in women with polycystic ovarian syndrome (PCOS)[49-54]. In addition, we have obtained ethical permission to access registry data from all participants including those, who leaves the clinical study part. Registry data will provide knowledge, regarding national ICD-10 diagnostic codes, medical treatment, socioeconomics and causes of death. Hence, registry data will enable a thorough characterization of drop-outs and we can compare these individuals to those who stay in the clinical study. Data on health consequences regarding all aspects of gender affirming treatment is warranted, however, this study will only provide information on transgender men and non-binary persons on testosterone therapy. To counteract this unbalance, a parallel study has been planned in transgender women and non-binary persons on feminizing treatment.

Competing interests' statement

The authors declare that they have no competing interests.

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Authors' contributions

MA, JF: Conception and design of the study. LC, MA, DG, TTK, AD, JF and GT contributed in writing the protocol. All Authors read and approved the final manuscript.

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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 ten-year, prospective, observational cohort study in Denmark at the Body Identity Clinic (BIC)

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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 ten-year, prospective, observational cohort study in Denmark at the Body Identity Clinic (BIC)

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Ethical approval

The Regional Ethics Committee, Region of Southern Denmark approved the study.

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Abstract

Introduction: The number of individuals with gender dysphoria seeking gender-affirming treatment is increasing. The short- and long-term effects of masculinizing treatment with testosterone are debated as serum testosterone increases up to twenty-fold compared to cisgender women. We will investigate short- and long-term effects of masculinizing testosterone treatment on preclinical and clinical coronary disease, muscle strength and power, VO_2 max, cardiac and respiratory function and quality of life including aggression in transgender men.

Methods and analyses: Prospective, single-center, observational cohort study at the Body Identity Clinic, Odense University Hospital, Denmark. Investigations are performed at inclusion and following one, three, five and ten years of testosterone therapy.

Non-calcified coronary plaque volume and calcium score are estimated by coronary computed tomography angiography. CT is only performed at inclusion and following one and ten years. Upper body muscle strength and power are measured by a "Low Row" weight stack resisted exercise machine. Evaluation of aggression and quality of life is assessed by questionnaires, VO_2 max is estimated by maximal testing on bike ergometer, and cardiac and respiratory functions are measured by echocardiography and spirometry, respectively. Markers of cardiovascular risk and inflammation and also cortisol and cortisone are assessed in blood, diurnal urine and/or hair samples. Our cohort (BIC), including dropouts, will be an embedded sub-cohort in a future national registry study in all individuals with gender dysphoria and controls. Data are available on International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic codes, prescriptions, socioeconomics and causes of death.

Ethics and dissemination: The Regional Committee on Health Research Ethics for Southern Denmark (S-20190108) and the Danish Data Protection Agency approved the study (19/27572). Signed informed consent will be obtained from all participants. All findings will be published in peer-reviewed journals or at scientific conferences.

Trial registration: Clinicaltrials.gov ID: NCT04254354

Article Summary

Strengths and limitations of this study

- Body Identity Clinic is a research clinic where this prospective longitudinal ten-year cohort study in transgender men will assess preclinical coronary disease by estimating non- calcified plaque volume and calcium score using coronary computed tomography angiography.
- The applied methods ensure important health- and sport-related data on upper body muscle strength and power, aggression, VO_2 max, cardiac and respiratory function in transgender men, during short- and long-term testosterone treatment.
- A proper control group is missing, but it is unethical to perform a placebo-controlled randomized trial on long-term testosterone therapy in persons with gender dysphoria.
- Results will only be available in transgender and non-binary individuals, treated with testosterone, however, a parallel study in transgender women and non-binary persons on feminizing treatment is planned.

Introduction

Recent European studies report that 0.6-0.8 % of individuals assigned female at birth have incongruent gender identity or gender dysphoria[1 2]. Testosterone treatment is the cornerstone of masculinizing treatment and testosterone treatment initiated in transgender men and by request in non-binary individuals. Testosterone is an important androgenic and anabolic hormone. The androgen effects of testosterone include terminal hair growth and deepening of the voice, whereas the anabolic effects include muscle growth. Body shape changes from feminine to masculine during testosterone treatment, as subcutaneous fat is reduced[3] and redistributed[4 5]. The changes in sex hormones are quite dramatic in transgender men, as circulating testosterone concentrations increase up to 20-fold into the normal range for cisgender men[6], while estradiol levels are partly supressed.

Clinical coronary disease is rare in young persons[7], however, preclinical coronary disease is observed and testosterone treatment may accelerate non-calcified coronary plaque (NCP) formation. Both NCP and calcified plaques may be detected by the sensitive coronary CT angiography (CCTA)[8] in combination with a semi-automated computer program[9]. In a large cardiac CT registry study in younger (mean age 39 years) participants, 22 % of cisgender men and 15 % of cisgender women without cardiovascular symptoms, had coronary artery disease defined as any plaque formation (non-calcified or calcified)[8]. The association between testosterone treatment and NCP formation has been reported in ageing cisgender men after 1 year of testosterone treatment[10], with increased waist-to-hip ratio at baseline as the strongest predictor of NCP formation[11]. There is a lack of knowledge regarding NCP formation in relatively young transgender men on masculinizing testosterone therapy. We know that lipid status is deteriorated during masculinizing testosterone treatment in transgender men, with an increase in low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) cholesterol[12] and blood pressure may increase [13 14]. However, studies on cardiovascular endpoints in

transgender men are limited and conflicting. A four-fold increased odds ratio for myocardial infarction in transgender men was reported, which was significant after adjusting for age, diabetes mellitus, hypertension, hypercholesterolemia, chronic kidney disease, smoking and exercise levels[15]. The study design was, however, cross-sectional, hence it was not possible to conclude on cause and effect. A retrospective study in transgender men reported no cases of myocardial infarction after ten years of testosterone treatment[16], but the retrospective study design increased risk of selection bias due to loss to follow-up before ten years.

Testosterone treatment is associated with dose-dependent increases in muscle mass[17 18] and muscle strength[5 17] in cisgender men. Muscle mass also increases during masculinizing treatment with testosterone in transgender men[19], but there is limited data on changes in muscle strength ~~before and~~ during masculinizing testosterone treatment. One study reported higher lower body muscle strength during gender affirming treatment with testosterone[20]. Upper body muscles have higher number of androgen receptors than lower body muscles[21] and characterizing muscle strength and power in the large muscles of the arms and upper back during testosterone treatment in transgender men would be of interest. Grip strength was higher in transgender men compared to cisgender women[22]. However, the study was cross-sectional, and hand grip strength only tests the small muscles of the hand and lower arm.

The World Professional Association for Transgender Health (WPATH)[23] recommends to warn against elevated aggression levels as a psychological adverse effect of masculinizing testosterone treatment in transgender men, however, this presumption was not supported by recent data[24 25]. The term aggression covers a wide range of inter-correlated behaviors, thoughts and emotions. There is no uniform definition of aggression[26] and studies reporting increased aggression scores during testosterone treatment[27] had severe methodological problems and high risk of bias. Valid information on aggression

constructs[27] during short- and long-term testosterone therapy is ensured in the present study by a questionnaire, which incorporates all dimensions of aggression[28] and questionnaires containing information on anxiety, depression, testosterone dose, duration of treatment and physical health.

Low cardiorespiratory fitness has been associated with an increased risk of premature death from all causes, but cardiovascular disease is the most common cause of death in cisgender individuals[29]. The maximum oxygen uptake (VO_2 max), determined from a graded maximal exercise test, is an approved way of classifying cardiorespiratory fitness levels[30]. VO_2 max is generally higher in cisgender men compared to cisgender women[31], presumably due to differences in muscle mass, cardiac output, haematocrit and lung size. There are no prospective data on VO_2 max during masculinizing testosterone treatment in transgender men, but VO_2 max is expected to increase, as testosterone increased muscle mass[20] and haematocrit[14]. Exogenous testosterone increased aerobic running time in young physically active cisgender women[32]; testosterone gel, 10 mg per day for ten weeks, was used, the dose equals 20 % of masculinizing testosterone treatment.

Obstructive and restrictive estimates of respiratory function are estimated by spirometry and reference ranges for spirometry vary with gender assigned at birth, weight, height and age. Asthma is characterized by obstructively decreased respiratory function with airway hyper-responsiveness and inflammation. Asthma is much more prevalent in cisgender women compared to cisgender men[33]; however we have no reference ranges for respiratory function in transgender men[34].

Masculinizing testosterone treatment in transgender men is for life and currently no prospective data are available on long-term morbidity. This study will provide important information on health issues regarding cardiovascular and respiratory health and upper body muscle strength and power. Aggression levels may be elevated during testosterone therapy, but the temporal relation has not been clarified. The

combination of long-term clinical observational data and access to national registry data will provide further knowledge on benefits and risks of masculinizing testosterone treatment.

Aim

To investigate short- and long-term effects of masculinizing testosterone treatment on preclinical and clinical coronary disease, muscle strength and power, VO_2 max, cardiac and respiratory function and quality of life including aggression in transgender men.

We hypothesize that, in transgender men:

Masculinizing testosterone treatment accelerate NCP development and progression.

Testosterone treatment will be associated with long-term increase in upper body muscle strength and power, VO_2 max, cardiac and respiratory function.

Aggression scores will increase during initiation of testosterone treatment, but the aggression scores will return to baseline during long-term testosterone treatment.

Methods and analysis

Study population and recruitment

Participants are individuals assigned female at birth with a diagnosis of gender dysphoria treated with testosterone or approved to start treatment with testosterone. Only individuals associated with one of the three centers of gender identity in Denmark: Odense, Aalborg and Copenhagen are included. No individual with known use of 'self-prescribed' sex hormones is included. The inclusion period for the cohort (N=200) is estimated to last 2 years.

Study design

For study outline, please see figure 1. The study is a prospective single-center observational cohort study at BIC, Odense University Hospital, Odense, Denmark of ten years duration. Participants (aged 18 years and older) are invited for five visits (baseline, one, three, five, and ten years) after informed consent has been given. The participants will spend one day in BIC per visit and all examinations use the same equipment and study protocols (table 1). Ethical permission has been obtained for the use of registry data regarding International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic codes, medical treatment, socioeconomics and causes of death in all participants including participants lost to follow-up. The participants accept study participation by written informed consent. All individuals in Denmark have a civil registration number (CPR) reflecting binary gender. During legal transitioning individuals may change CPR and data from the two CPR numbers is merged. We will be able to compare study participants with participants lost to follow-up by combining the clinical outcomes with registry data (table 1, figure 1).

Patient and Public Involvement statement

An advisory board of transgender men has been established. The participant advisory board meetings were arranged prior to application for ethical approval of the study to secure inputs in terms of relevance of research questions, recruitment, outcomes and participant time consumption. The participant advisory board has read and commented on the study material and will be contacted for continuous sparring.

Endpoints

Primary endpoint

NCP volume

Secondary endpoints

Calcium score

Upper body muscle strength and power

Aggression and quality of life

VO_2 max

Left ventricular muscle mass and function

Respiratory function

Serum levels of testosterone, estradiol and cortisol.

Circulating markers of cardiovascular risk and inflammation

Diurnal urine and hair samples for assessment of cortisol and cortisone

Outcomes

Outcomes and visits are outlined in table 1.

Investigations	Inclusion	1 yr.	3 yr.	5 yr.	10 yr.
Coronary CT angiography (CTTA)	X	X			X
Muscle strength and power	X	X	X	X	X
Aggression, quality of life questionnaires	X	X	X	X	X
VO_2 max	X	X	X	X	X
Echocardiography	X	X	X	X	X
Spirometry	X	X	X	X	X
Blood, urine and hair samples	X	X	X	X	X

DXA whole-body and bone density	X	X	X	X	X
Medical history	X	X	X	X	X
Physical examination	X	X	X	X	X

Legend table 1: Overview of study outcomes and visits

Coronary CT angiography (CCTA)

CCTA (high-end CT scanner) is conducted at the first visit, after one year and at ten years follow-up (table 1) to examine the presence of NCP and calcified coronary plaques (figure 2). The scanning protocol depends on the patient heart rate. In patients with a stable heart rate above 60 beats per minute, orally or intravenously β-blocker are administered until the heart rate is appropriate (if possible below 60), and a prospectively gated protocol is used. Participants with a heart rate > 70 bpm despite β-blocker pretreatment are subjected to a retrospectively gated scan with dose modulation. Additionally, sublingual nitrates are administered prior to the scan. Administrating of β-blocker and nitrates are in accordance to daily clinical practice. An experienced cardiologist performs data analyses of NCPs, calcium score/stenosis and pericardial fat using a semi-automatic program[9]. Radiation amounts to 1.6 mSv per CCTA. We have ethical permission to perform three CCTAs during the study period of ten years. Radiation from CCTA is lower than expected and we are applying for an additional CCTA at five years.

Upper body muscle strength and power

A “Low Row” weight stack resisted exercise machine (Technogym, Italy) tests arm and upper back muscles. The machine is adjusted to fit the individual participant relative to body height and arm

length. Body weight, training history and age estimates initial loading. Warm-up is 3 x 10 and 1 x 5 repetitions at the estimated test load, with 1 minute of rest in between sets. Subsequently maximal effort (1 repetitions maximum, 1RM) is obtained using single repetitions with increasing load until task failure (the participant is unable to complete the full range of motion) with 2 minutes of rest in between sets. Hereafter participants are instructed to perform at least 3 sets of one repetition as forceful and fast as possible to evaluate muscle power with a load corresponding to 80 % of 1RM and 2 minutes of rest in between sets. During each repetition, peak and mean power is measured. To evaluate muscle power we attach a PUSH 2.0 inertial motion device (PUSH, Toronto, Canada) to the weight stack. The PUSH device includes a 3-axis accelerometer and gyroscope, enabling measurements of human movement kinematics at a sampling rate of 1000 Hz. Previously, PUSH has been validated to evaluate power using resistance exercises[35]. Peak and mean power (Watt), respectively, are calculated based on kinematic data derived by the PUSH software.

Questionnaires

Buss-Perry Aggression Questionnaire, Quality of life: SF-36®, Inventory of Interpersonal Problems ®, Gender Q (Development in progress), GAD7 (General Anxiety Disorder-7) and PHQ9 (Patient Health Questionnaire-8).

VO_2 max

Measurement of VO_2 max is performed on a bike ergometer and Vyntus®CPS system. The resistance will start low and increase gradually until maximum capacity or exhaustion. At maximum capacity lactate is measured.

Echocardiography

A comprehensive transthoracic echocardiography is performed by a medical doctor. The recordings are stored digitally for blinded analysis. The following are included: Size and mass of all cardiac chambers, left ventricle ejection fraction (LVEF), global longitudinal strain (GLS), left ventricle diastolic function and heart valve function.

Respiratory function

Respiratory function is tested by a spirometer (Vyntus®CPS system) measuring forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and peak flow.

Blood, urine and hair samples

Testosterone, estradiol and cortisol levels and cardiovascular risk markers in blood and serum (HbA1c, lipids, hematocrit, adiponectin, soluble urokinase-type plasminogen activator receptor (suPAR)) are analyzed along with inflammation markers in blood and serum (c-reactive protein (CRP), interleukin 6 (IL-6)). Hormone levels are measured after an overnight fast between 8 and 9 am by liquid chromatography tandem mass spectrometry (LC-MS/MS), which is calibrated by in-house prepared calibrators, and the relative standard deviation (SD) is < 10 %. Quality for steroid hormones is assured by monthly participation in the external quality control program for steroid hormones from The United Kingdom National External Quality Assessment Service (UKNEQAS). Sex hormone binding globulin is determined on serum samples by a Roche assay on Cobas e602 with a precision of 1.8 %-4.0 % (14.9-21.9 nmol/L). Free testosterone levels are calculated assuming a plasma albumin concentration of 43 g/L[36].

Hemoglobin is measured using a photometric analyzer with a coefficient of variation (CV) of 2.8 %. Plasma total cholesterol and HDL cholesterol are analyzed by enzymatic colorimetric reactions (Modular P, Roche), and LDL cholesterol is calculated using the Friedewald equation[37]. HbA1c is measured by high-performance liquid chromatography using Tosoh G8 (Medinor, Broendby, Denmark); the analytical CV is 0.9 %. Adiponectin is determined by an in-house timeresolved immunofluorometric assay[38], with intra- and inter-assay CV averaging 5 and 10 % respectively. Plasma suPAR is measured with the suPARnostic® ELISA (ViroGates A/S, Birkerød, Denmark) with a mean CV of 4 %. CRP is analyzed using latex based immunoanalysis (CRP Ultra, Sentinel Diagnostics, Milan, Italy) by an Architect c8000 instrument (Abbott). The intra-assay and inter-assay CVs are 0.8 % and 1.9 % for normal levels of CRP, respectively. Plasma IL-6 is measured with Quantikine® HS-IL-6 ELISA (R&D Systems, Minneapolis, USA) with a mean CV of 8 %.

Diurnal urine samples: Participants are instructed to note the time for voiding in the morning before starting sample collection, and all urine collected until the morning of the second day. Urine samples are kept frozen at −20°C until analysis. Cortisol and cortisone are analyzed by LC-MS/MS. Urine samples are solid phase extracted on an Oasis HLB 96-well plate after addition of deuterated internal standards; the analysis is calibrated by in-house prepared calibrators, and the relative SD is <10%. Quality is assured by monthly participation with satisfactory results in the external quality control program for steroid hormones from the UKNEQAS.

Hair samples: A section of hair strands approximately 3 mm in diameter is cut as close to the scalp as possible from the posterior vertex area. Hair samples are stored in aluminum foil as previously described[39], the 4-cm hair segment closest to the scalp is used for analyses to represent cortisol secretion over the most recent 4-month period[40]. The analyses will be carried out at Department of Psychology, Technical University of Dresden, Germany using LC-MS/MS[41].

Whole body dual x-ray absorptiometry and bone mineral density

Dual x-ray absorptiometry, Horizon A Discovery is used to measure whole body lean body mass, fat mass and bone density. Radiation amounts to 0.1 mSv per scan.

Medical history

Chronic diseases, medication and supplements, alcohol, tobacco and abuse, gynecological history, previous treatment with testosterone, information on diet and physical activity are recorded.

Physical examination

Height, weight, body mass index (BMI, kg/m^2), blood pressure, waist and hip circumference and face and body hair (Ferriman-Gallwey Score) are recorded.

Sample size and statistics.

The primary study endpoint is NCP. We have estimated the sample size, as no valid data exist for a proper power calculation regarding the primary endpoint, NCP. We aim to include 200 participants, including 50 men who are approved to start testosterone treatment based on the power calculation of the main secondary endpoint, upper body muscle strength and power. Sample size estimation for upper extremity muscle strength and power was based on data (mean baseline/standard deviation) from a comparable cohort[42]. The estimation was based on a 10 % within-participant difference (deemed as functional relevant) with an alpha level of 0.05 and a statistical power of 0.80. On the basis of this data, a sample size of 39 was sufficient to detect within-participants differences with two-tailed comparison.

To account for potential dropouts (estimated to 20 %) 50 individuals approved to start testosterone treatment will be recruited for the trial.

Normally and non-normally distributed data will be analyzed using parametric and non-parametric statistics, respectively. Associations between testosterone and endpoints will be investigated with multiple linear regression modeling. Random mixed-effects linear regression models will be applied to investigate associations between testosterone and longitudinal repeated markers of assessed outcomes. We will collaborate with a statistician regarding random mixed-effects linear regression modeling.

A directed acyclic graph (DAG) will be depicted in order to transparently identify a priori assumptions of causal relations between exposure, outcome, potential confounders, intermediate factors, and selection bias. Missing data will be handled according to type. Depending on data, analyses will be performed based on either complete case analyses or, if appropriate, by imputing missing data in collaboration with the statistical department. Analyses will be conducted using STATA 14 (StataCorp 2015). Post regression diagnostics/model validation will be performed. Assumptions of linearity between predictors and the outcome variable will be inspected by using scatter plots and augmented component-plus-residual plots. Normality of predicted residuals will be checked with quantile-normal and probability-normal plots. Homogeneity of variance (homoscedasticity) of the residuals will be investigated by plotting residuals against the fitted (predicted) values. Multicollinearity will be assessed using variance inflation factors. Open-ended questions will not be included. Data are anonymized according to Danish law and regulations (The Regional Committees on Health Research Ethics for Southern Denmark, Project-ID: S-20190108, The Danish Data Protection Agency, journal no. 19/27572), and therefore analyses will be performed through a remote VPN access to Statistics Denmark.

Data management

Data are stored and analyzed electronically and unauthorized access denied. Original data are filed according to one participant number. Research Electronic Data Capture (REDCap®) (www.project-redcap.org), hosted by Open Patient data Explorative Network (OPEN), is used for registration of data[43 44]. REDCap® meets the safety requirements set by the Danish Data Protection Agency for storage of person-sensible data. OPEN Analyse, a secure remote desktop solution hosted by OPEN, is used for storage and analyses of the pseudo-anonymized data.

Ethics and dissemination

All participants give written informed consent. The study results will be published in peer-reviewed journals, publication will be according to the International Committee of Medical Journal Editors (ICMJE) recommendations and the investigators oblige themselves to publish both positive and negative findings. The study is performed in accordance with the Helsinki II declaration and regulations of the General Data Protection Regulation. It is approved by the Danish Data Protection Agency (journal no. 19/27572), the Regional Committees on Health Research Ethics for Southern Denmark (project-ID: S-20190108) and registered at ClinicalTrials.gov (NCT04254354).

Discussion

Preclinical coronary disease is determined by NCP volume and calcium score in transgender men during this ten-year prospective cohort study at BIC. NCP volume is assessed by state-of-the-art CCTA and we use the lowest detectable change in NCP formation (1 mm^3) as the clinically relevant difference[45]. However, a proper power calculation regarding our primary endpoint cannot be performed as no previous data has been published. Therefore, distribution of data is unknown and the standard

deviation cannot be calculated. The possible lack of NCP development during ten years is acknowledged, but this potential negative result would be reassuring and clinical relevant. In a large cardiac CT registry study in relatively young cisgender men (mean age 39 ± 6 years) 424 out of 1143 participants did not have any cardiovascular symptoms[8]. Experienced cardiologists assessed the CCTAs, reporting coronary plaque of any kind in 22 % of asymptomatic 424 cisgender men[8]. In our study, we increase the sensitivity of detecting NCPs by using a semi-automatic program, which is comparable to Autoplaque[9].

We also aim at providing new scientific evidence regarding upper body muscle strength and power in men on masculinizing therapy. Upper body muscles are of special interest as the number of androgen receptors are higher in the upper body muscles compared to lower body[21]. Also, our study will elaborate on interesting data on possible increased levels of aggression during testosterone therapy and the temporal relation between masculinizing testosterone treatment and levels of aggression[27]. Aggression is very relevant clinical issue in the context of the WPATH warning regarding aggression on testosterone therapy[23]. We will report new data on VO_2 max and cardiac function as improvements in cardiorespiratory fitness are associated with considerably reduced mortality risk and adverse cardiovascular event rates[29]; furthermore, data on respiratory function will add new knowledge and all the above-mentioned results are important for transgender men and non-binary individuals on testosterone therapy in health and sports.

Ethical permission is granted to access registry data from all participants including those, who leaves the clinical study part. Registry data will provide knowledge, regarding national ICD-10 diagnostic codes, medical treatment, socioeconomics and causes of death. Future access to national registry data in all individuals with registered gender dysphoria and controls will allow us to study our cohort (BIC) as an embedded sub-cohort including individuals, who remain in the ten-year study as well as dropouts.

We have previously used this design in women with polycystic ovary syndrome (PCOS)[46]. The gold standard research design to assess benefits and risks of testosterone therapy in transgender persons is a long-term placebo-controlled randomized trial on masculinizing therapy, however, it is deeply unethical to perform such a study, when gender dysphoria is present. Apart from the future registry study we will compare data from our cohort to results from Odense Androgen Study (OAS)[6 47-49]. OAS is a large cross-sectional study in 783 young healthy men (20-29 years old); data are available on BMI, waist circumference, muscle mass, fat mass, blood pressure, Hba1c, testosterone and estradiol levels, lipid status and VO_2 max and a follow-up is planned (Ethical Committee Journal number, S-20130097). Our study is a non-representative cohort study with risk of selection bias regarding age, BMI, smoking, ethnicity, fitness level and morbidity. Data on health consequences regarding all aspects of gender affirming treatment is warranted, however, this study will only provide information on transgender men and non-binary persons on testosterone therapy. To counteract this unbalance, a parallel study has been planned in transgender women and non-binary persons on feminizing treatment.

Competing interests' statement

The authors declare that they have no competing interests.

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Authors' contributions

All authors meet the ICMJE criteria for authorship. MA, JF: Conception and design of the study. LC, MA, DG, TTK, AD, JF and GT contributed in writing the protocol. All Authors read and approved the final manuscript.

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Legend figure 1: Study design.

Legend figure 2: The figure displays a coronary CT angiography (CCTA). A semi-automatic program detects NCP. RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery; OM, obtuse marginal branch 1.

Legend table 1: Overview of study outcomes and visits.

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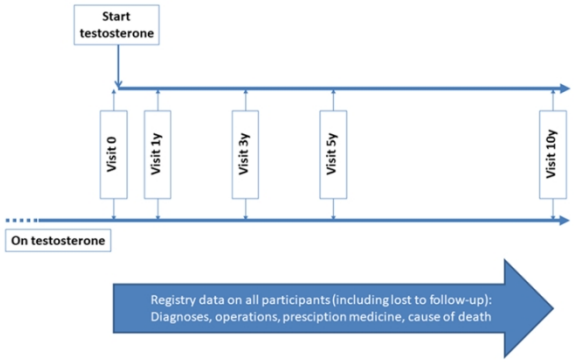

Legend figure 1: Study design

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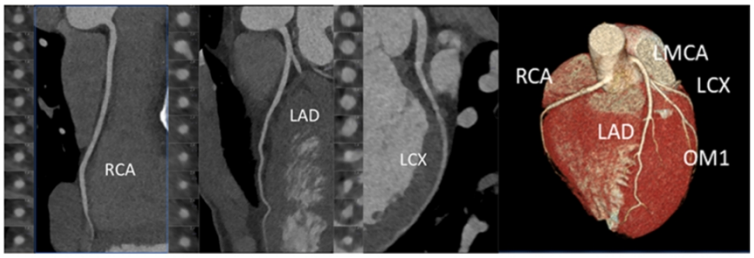

Legend figure 2: The figure displays a coronary CT angiography (CCTA). A semi-automatic program detects NCP. RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery; OM, obtuse marginal branch 1.

338x190mm (96 x 96 DPI)