

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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).

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-045714
Article Type:	Protocol
Date Submitted by the Author:	09-Oct-2020
Complete List of Authors:	Lehmann Christensen, Louise; Odense University Hospital Department of Endocrinology, Body Identity Clinic Glintborg, Dorte ; Odense University Hospital Department of Endocrinology Taulbjerg Kristensen, Tine; Odense University Hospital Department of Endocrinology Diederichsen, Axel; Odense University Hospital, Cardiology T'Sjoen, Guy; University Hospital Ghent, Department of Endocrinology Frystyk, Jan; Odense University Hospital Department of Endocrinology Skovsager Andersen, Marianne; Odense University Hospital, Body Identity Clinic, Department of Endocrinology
Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, Coronary heart disease < CARDIOLOGY, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Resubmission bmjopen-2020-042607

Clean version, October 8th 2020**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).

Authors

Louise Lehmann Christensen^{1,4}, Dorte Glintborg¹, Tine Taulbjerg Kristensen¹, Axel Cosmus Pyndt Diederichsen², Guy T'Sjoen³, Jan Frystyk^{1,4}, Marianne Skovsager Andersen¹

¹Body Identity Clinic, Department of Endocrinology Odense University Hospital, Odense, Denmark

²Department of Cardiology, Odense University Hospital, Odense, Denmark

³Department of Endocrinology, Ghent University Hospital, Ghent, Belgium

⁴Open Patient data Explorative Network (OPEN), Odense University Hospital, Region of Southern Denmark, Odense, Denmark

Corresponding author

Louise Lehmann Christensen, louise.lehmann.christensen@rsyd.dk

Ethical approval

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

Journal number: S-20190108.

Inclusion start

February 2020

Word count: 3914

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

24 **Abstract**

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

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

33 NCP volume and calcium score are estimated by coronary CT angiography (CCTA) and upper body
34 muscle strength and power are measured by a “Low Row” weight stack resisted exercise machine.
35 Evaluation of aggression and quality of life are assessed by questionnaires, VO₂max by maximal
36 testing on bike ergometer, and cardiac and respiratory function are measured by echocardiography and
37 spirometry, respectively. Markers of cardiovascular risk and inflammation and also cortisol and
38 cortisone are assessed in blood, diurnal urine and/or hair samples. National registry data, regarding
39 International Statistical Classification of Diseases and Related Health Problems (ICD-10) diagnostic
40 codes, prescriptions, socioeconomics and causes of death will be available also in patients lost to
41 follow-up in the clinical study.

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

1
2
3
4 47 **Trial registration:** Clinicaltrials.gov ID: NCT04254354
5
6
7 48

8
9 49 **Article Summary**

10
11 50 **Strengths and limitations of this study**

- 12
13 51
- 14 • Body Identity Clinic (BIC) is a research clinic, where this prospective longitudinal 10 years
15 cohort study in transgender men will assess preclinical coronary disease by estimating NCP
16 52 volume and calcium score using CCTA. These results will provide important knowledge
17 53 regarding cardiovascular risk in transgender men on testosterone therapy.
18 54
 - 19 • Scientific evidence will be obtained regarding upper body muscle strength and power,
20 55 aggression, VO₂max, cardiac and respiratory function in transgender men, during short- and
21 56 long-term testosterone treatment. This data is required in transgender men, regarding health as
22 57 well as sports.
23 58
 - 24 • Access to registry data in all included persons, also those lost to follow-up in the clinical study,
25 59 is a great advantage, but a proper control group is missing. However, it is unethical to perform a
26 60 placebo-controlled randomized trial when gender dysphoria is present.
27 61
 - 28 • Information will only be available on effects of testosterone therapy in transgender individuals,
29 62 who are transgender men or non-binary individuals. However, a parallel study has been planned
30 63 in transgender women and non-binary persons on feminizing treatment.
31 64
- 32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

65 Introduction

66 Recent European studies report that 0.6-0.8 % of individuals assigned female at birth have incongruent
67 gender identity or gender dysphoria^{1 2}. Masculinizing treatment is testosterone treatment and is initiated
68 both in transgender men and if requested by non-binary individuals. Testosterone is an important
69 androgenic and anabolic hormone. The androgen effects of testosterone are terminal hair growth and
70 deepening of the voice, whereas the anabolic effects include muscle growth. Body shape changes from
71 feminine to masculine during testosterone treatment, as subcutaneous fat is reduced³ and redistributed⁴
72 ⁵. The changes in sex hormones are quite dramatic in transgender men, as circulating testosterone
73 concentrations increase up to 20-fold into the normal range for cisgender men⁶, while estradiol levels
74 are suppressed.

75 Clinical coronary disease is rare in young persons⁷, however, preclinical coronary disease is observed
76 and testosterone treatment may accelerate non-calcified coronary plaque (NCP) formation. Both NCP
77 and calcified plaques may be detected by the sensitive coronary CT angiography (CCTA)⁸ in
78 combination with a semi-automated computer program⁹. In a large cardiac CT registry study in
79 younger (mean age 39 years) participants, 22 % of cisgender men and 15 % of cisgender women,
80 without cardiovascular symptoms, had coronary artery disease defined as any plaque formation (non-
81 calcified or calcified)⁸. The association between testosterone and NCP formation has been reported in
82 ageing cisgender men after 1 year of testosterone treatment¹⁰, with increased waist-to-hip ratio at
83 baseline as the strongest predictor of NCP formation¹¹, but there is a lack of knowledge regarding NCP
84 formation, in relatively young men on masculinizing testosterone therapy. We know that lipid status is
85 deteriorated during masculinizing testosterone treatment, with an increase in low-density lipoprotein
86 (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) cholesterol¹² and blood pressure
87 may be elevated^{13 14}. However, studies on cardiovascular endpoints are limited and conflicting. A four-

1
2
3
4 88 fold increase in odds ratio for myocardial infarction in transgender men has been found, after adjusting
5
6
7 89 for age, diabetes mellitus, hypertension, hypercholesterolemia, chronic kidney disease, smoking and
8
9 90 exercise levels¹⁵. The study design was, however, cross-sectional, hence it is not possible to conclude
10
11 91 on cause and effect. A retrospective study in transgender men reported no cases of myocardial
12
13 92 infarction after 10 years of testosterone treatment¹⁶, but the retrospective design increased the risk of
14
15
16 93 selection bias due to loss to follow-up before 10 years.

17
18 94 Testosterone treatment is associated with dose-dependent changes in muscle mass^{17 18} and strength in
19
20 95 cisgender men^{5 17}. Muscle mass also increases in transgender men¹⁹, but there is only limited data on
21
22 96 muscle strength before and during masculinizing testosterone treatment. Due to higher number of
23
24
25 97 androgen receptors in the upper body muscles²⁰, it would be of interest to characterize muscle strength
26
27 98 and power in the large muscles of the arms and upper back. It has been shown, previously, that grip
28
29
30 99 strength was higher in transgender men compared to cisgender women²¹. The study was, however,
31
32 100 cross-sectional, and hand grip strength only tests the small muscles of the hand and lower arm.

33
34 101 Furthermore, an interesting study has, recently, reported an increase in lower body muscle strength²².
35
36 102 The World Professional Association for Transgender Health (WPATH)²³ recommends to warn of
37
38
39 103 increased aggression as a psychological adverse effect of masculinizing testosterone treatment,
40
41 104 however, this presumption was not supported by recent data^{24 25}. The term aggression covers a wide
42
43
44 105 range of inter-correlated behaviors, thoughts and emotions. There is no uniform definition of
45
46 106 aggression²⁶ and there is severe methodological problems and high risk of bias in studies reporting
47
48 107 increased aggression during testosterone treatment²⁷. To obtain valid information on aggression
49
50
51 108 constructs²⁷ during short- and long-term testosterone therapy, we apply a questionnaire, which
52
53 109 incorporates all dimensions of aggression²⁸ with concomitant information on anxiety, depression,
54
55 110 testosterone dose, duration of treatment and physical health.

1
2
3
4 111 Low cardiorespiratory fitness has been associated with an increased risk of premature death from all
5
6 112 causes, but primarily cardiovascular disease, in cisgender individuals²⁹. The maximum oxygen uptake
7
8
9 113 (VO_2max), determined from a graded maximal exercise test, is an approved way of classifying
10
11 114 cardiorespiratory fitness level³⁰. VO_2max is generally higher in cisgender men compared to cisgender
12
13 115 women³¹, presumably due to differences in muscle mass, cardiac output, haematocrit and lung size.
14
15
16 116 There is no prospective data on VO_2max during masculinizing testosterone treatment, but VO_2max is
17
18 117 expected to increase, as testosterone has marked effects on both muscle mass²² and haematocrit¹⁴.
19
20 118 Some data exists on physical fitness and exogenous testosterone administration in cisgender women.
21
22
23 119 Aerobic running time increased in young physically active cisgender women during 10 weeks of 10 mg
24
25 120 testosterone gel per day, this testosterone dose equals 20 % of masculinizing testosterone treatment³².
26
27 121 Reference ranges for spirometry vary with gender assigned at birth, weight, height and age. Obstructive
28
29 122 and restrictive estimates of respiratory function are estimated by spirometry and asthma is
30
31
32 123 characterized by obstructively decreased respiratory function with airway hyper-responsiveness and
33
34 124 inflammation. Cisgender women have a much higher prevalence of asthma compared to cisgender
35
36 125 men³³, but there are no reference ranges for respiratory function in transgender men³⁴.
37
38
39 126 Masculinizing testosterone treatment is for life and currently no prospective data is available on long-
40
41 127 term morbidity; but we may acquire information on important preclinical health issues regarding
42
43 128 cardiovascular and respiratory health and upper body muscle strength and power. Testosterone therapy
44
45 129 may activate aggression, but the temporal relation has not been clarified. The combination of long-term
46
47
48 130 clinical observational data and access to national registry data will provide further knowledge on
49
50 131 benefits and risks of masculinizing testosterone treatment.
51
52
53 132
54
55 133

1
2
3
4 134 *Aim*

5
6
7 135 We will investigate short- and long-term effects of masculinizing testosterone treatment on pre-clinical
8
9 136 and clinical coronary disease, muscle strength and power, VO₂max, cardiac and respiratory function,
10
11 137 and quality of life including aggression.

12
13
14 138 We hypothesize that:

15
16 139 Masculinizing testosterone treatment is associated with NCP development and progression.

17
18 140 Testosterone treatment will be associated with long-term increase in muscle strength and power,
19
20 141 VO₂max, cardiac and respiratory function.

21
22
23 142 Aggression scores will be significantly higher during initiation of testosterone treatment, but the
24
25 143 aggression scores will return to baseline during long-term testosterone treatment.

26
27 144
28
29
30 145 **Methods and analysis**

31
32 146 *Study population and recruitment*

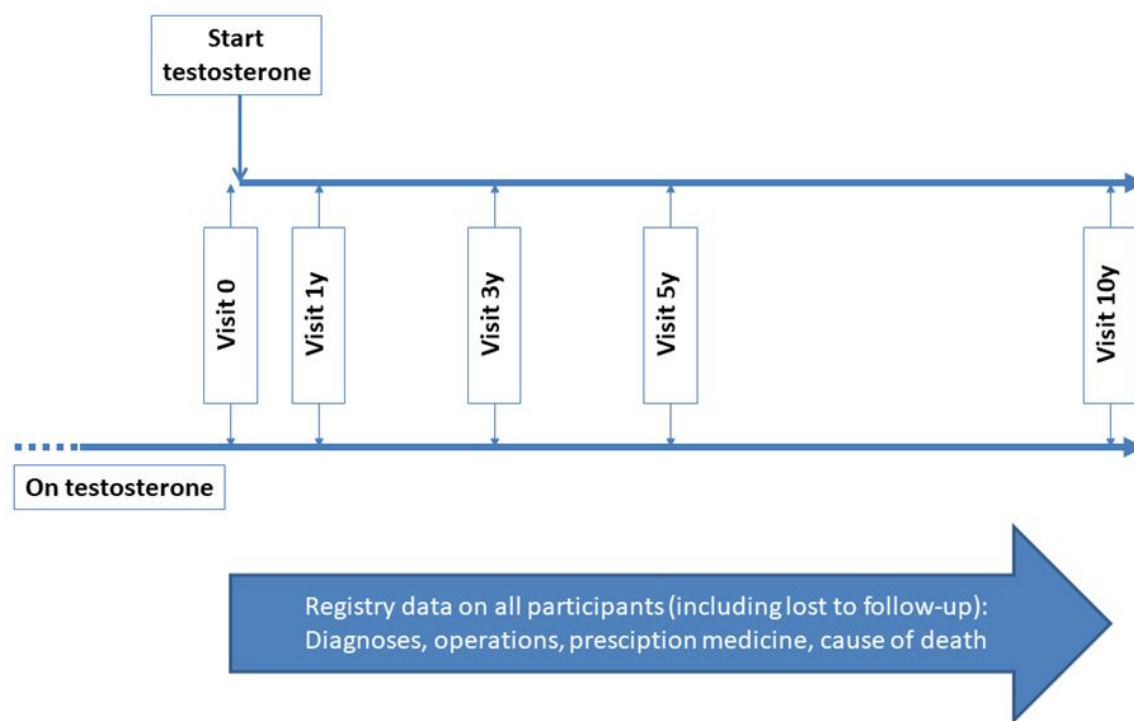
33
34 147 Participants are individuals assigned female at birth with a diagnosis of gender dysphoria treated with
35
36 148 testosterone or approved to start treatment with testosterone. We include only individuals associated
37
38
39 149 with one of the three centers of gender identity in Denmark: Odense, Aalborg and Copenhagen. No
40
41 150 individual will be included, with known use of ‘self-prescribed’ sex hormones.

42
43 151 The inclusion period for the cohort (N=200) is estimated to last for 2 years.

44
45
46 152
47
48 153 *Study design*

49
50 154 For study outline, please see figure 1. The study is a prospective single-center observational cohort
51
52 155 study at BIC, Odense University Hospital, Odense, Denmark with duration of ten years. Participants
53
54
55 156 (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

Resubmission bmjopen-2020-042607

Clean version, October 8th 2020

1
2
3
4 170 *Patient and Public Involvement statement*

5
6 171 An advisory board of transgender men has been established. The participant advisory board meetings
7
8
9 172 were arranged prior to application for ethical approval of the study to secure inputs in terms of
10
11 173 relevance of research questions, recruitment, outcomes and participant time consumption. The
12
13 174 participant advisory board has read and commented on the study material and will be contacted for
14
15
16 175 continuous sparring.
17

18 176
19
20 177 *Endpoints*

21
22
23 178 **Primary endpoint**

24
25 179 NCP volume
26

27 180 **Secondary endpoints**

28
29
30 181 Calcium score
31

32 182 Upper body muscle strength and power
33

34 183 Aggression and quality of life
35

36 184 VO₂max
37

38
39 185 Left ventricular muscle mass and function
40

41 186 Respiratory function
42

43 187 Serum levels of testosterone, estradiol and cortisol.
44

45
46 188 Circulating markers of cardiovascular risk and inflammation
47

48 189 Diurnal urine and hair samples for assessment of cortisol and cortisone
49

50 190
51

52 191
53

54 192
55
56
57

1

2

3

4 193 *Outcomes*

5

6

7 194 Outcomes and visits are outlined in table 1.

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

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

37 195 *Legend table 1: Overview of study outcomes and visits*

38

39

40 196

41

42 197 Coronary CT angiography (CCTA)

43

44 198 CCTA (high-end CT scanner) is conducted at the first visit, after 1 year and at 10 years follow-up

45

46 199 (table 1) to examine the presence of NCP and calcified coronary plaques (figure 2). The scanning

47

48

49 200 protocol depends on the patient heart rate. In patients with a stable heart rate above 60 beats per minute,

50

51 201 orally or intravenously β -blocker are administered until the heart rate is appropriate (if possible below

52

53 202 60), and a prospectively gated protocol is used. Participants with a heart rate > 70 bpm despite β -

54

55

56

57

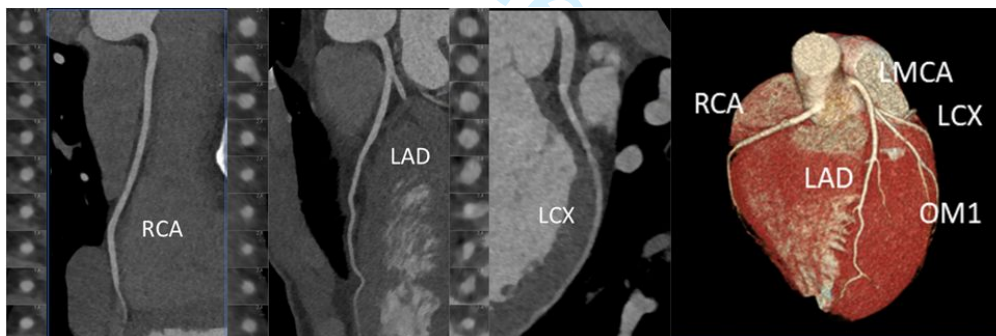
58

59

60

blocker pretreatment are subjected to a retrospectively gated scan with dose modulation. Additionally, sublingual nitrates are administered prior to the scan. Administering 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 program⁹. 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

1
2
3
4 221 repetitions at the estimated test load, with 1 minute of rest in between sets. Subsequently maximal
5
6 222 effort (1 repetitions maximum, 1RM) is obtained using single repetitions with increasing load until task
7
8
9 223 failure (the participant is unable to complete the full range of motion) with 2 minutes of rest in between
10
11 224 sets. Hereafter participants are instructed to perform at least 3 sets of one repetition at forceful and fast
12
13 225 as possible to evaluate muscle power with a load corresponding to 80 % of 1RM and 2 minutes of rest
14
15
16 226 in between sets. During each repetition, peak and mean power is measured. To evaluate muscle power
17
18 227 we attach a PUSH 2.0 inertial motion device (PUSH, Toronto, Canada) to the weight stack. The PUSH
19
20 228 device includes a 3-axis accelerometer and gyroscope, enabling measurements of human movement
21
22
23 229 kinematics at a sampling rate of 1000 Hz. Previously, PUSH has been validated to evaluate power
24
25 230 using resistance exercises³⁵. Peak and mean power (Watt), respectively, are calculated based on
26
27 231 kinematic data derived by the PUSH software.
28
29
30 232

31 32 233 Questionnaires

33
34 234 Buss-Perry Aggression Questionnaire, Quality of life: SF-36®, Inventory of Interpersonal Problems®,
35
36 235 Gender Q (Development in progress), GAD7 (General Anxiety Disorder-7) and PHQ9 (Patient Health
37
38
39 236 Questionnaire-8).
40

41 237 42 43 238 VO₂max

44
45 239 Measurement of VO₂max is performed on a bike ergometer and Vyntus®CPS system. The resistance
46
47
48 240 will start low and increase gradually until maximum capacity or exhaustion. At maximum capacity
49
50 241 lactate is measured.
51

52 242 53 54 243 Echocardiography

1
2
3
4 244 A comprehensive transthoracic echocardiography is performed by a medical doctor. The recordings are
5
6
7 245 stored digitally for blinded analysis. The following are included: Size and mass of all cardiac chambers,
8
9 246 left ventricle ejection fraction (LVEF), global longitudinal strain (GLS), left ventricle diastolic function
10
11 247 and heart valve function.
12

13 248 14 15 16 249 Respiratory function

17
18 250 Respiratory function is tested by a spirometer (Vyntus[®]CPS system) measuring forced expiratory
19
20
21 251 volume in one second (FEV1), forced vital capacity (FVC) and peak flow.
22
23 252

24 25 253 Blood, urine and hair samples

26
27 254 Testosterone, estradiol and cortisol levels and cardiovascular risk markers in blood and serum (HbA1c,
28
29
30 255 lipids, hematocrit, adiponectin, suPAR) are analyzed along with inflammation markers in blood and
31
32 256 serum (CRP, IL6). Hormone levels are measured after an overnight fast between 8 and 9 am by liquid
33
34 257 chromatography tandem mass spectrometry (LC-MS/MS), which is calibrated by in-house prepared
35
36
37 258 calibrators, and the relative standard deviation (SD) is < 10 %. Quality for steroid hormones is assured
38
39 259 by monthly participation in the external quality control program for steroid hormones from The United
40
41 260 Kingdom National External Quality Assessment Service (UKNEQAS). Sex hormone binding globulin
42
43
44 261 is determined on serum samples by a Roche assay on Cobas e602 with a precision of 1.8 %-4.0 %
45
46 262 (14.9-21.9 nmol/L). Free testosterone levels are calculated assuming a plasma albumin concentration of
47
48 263 4.3 g/dL³⁶.

49
50 264 Hemoglobin is measured using a photometric analyzer with a coefficient of variation (CV) of 2.8 %.

51
52
53 265 Plasma total cholesterol and HDL cholesterol are analyzed by enzymatic colorimetric reactions
54
55 266 (Modular P, Roche), and LDL cholesterol is calculated using the Friedewald equation³⁷. HbA1c is

1
2
3
4 267 measured by high-performance liquid chromatography using Tosoh G8 (Medinor, Broendby,
5
6
7 268 Denmark); the analytical CV is 0.9 %. Adiponectin is determined by an in-house timeresolved
8
9 269 immunofluorometric assay³⁸, with intra- and inter-assay CV averaging 5 and 10 % respectively.
10
11 270 Plasma suPAR is measured with the suPARnostic® ELISA (ViroGates A/S, Birkerød, Denmark) with
12
13
14 271 a mean CV of 4 %. CRP is analyzed using latex based immunoanalysis (CRP Ultra, Sentinel
15
16 272 Diagnostics, Milan, Italy) by an Architect c8000 instrument (Abbott). The intra-assay and inter-assay
17
18 273 CVs are 0.8 % and 1.9 % for normal levels of CRP, respectively. Plasma IL-6 is measured with
19
20
21 274 Quantikine® HS-IL-6 ELISA (R&D Systems, Minneapolis, USA) with a mean CV of 8 %.
22
23 275 Diurnal urine samples: Participants are instructed to note the time for voiding in the morning before
24
25 276 starting sample collection, and all urine collected until the morning of the second day. Urine samples
26
27 277 are kept frozen at -20°C until analysis. Cortisol and cortisone are analyzed by LC-MS/MS. Urine
28
29
30 278 samples are solid phase extracted on an Oasis HLB 96-well plate after addition of deuterated internal
31
32 279 standards; the analysis is calibrated by in-house prepared calibrators, and the relative SD is <10%.
33
34 280 Quality is assured by monthly participation with satisfactory results in the external quality control
35
36
37 281 program for steroid hormones from the UKNEQAS.
38
39 282 Hair samples: A section of hair strands approximately 3 mm in diameter is cut as close to the scalp as
40
41 283 possible from the posterior vertex area. Hair samples are stored in aluminum foil as previously
42
43
44 284 described³⁹, the 4-cm hair segment closest to the scalp is used for analyses to represent cortisol
45
46 285 secretion over the most recent 4-month period⁴⁰. The analyses will be carried out at Department of
47
48 286 Psychology, Technical University of Dresden, Germany using LC-MS/MS⁴¹.
49
50
51 287
52
53 288
54
55 289
56
57
58
59
60

1
2
3
4 290 Whole body dual x-ray absorptiometry and bone mineral density

5
6
7 291 Dual x-ray absorptiometry, Horizon A Discovery is used to measure whole body lean body mass, fat
8
9 292 mass and bone density. Radiation amounts to 0.1 mSv per scan.
10

11 293
12
13 294 Medical history

14
15
16 295 Chronic diseases, medication and supplements, alcohol, tobacco and abuse, gynecological history,
17
18 296 previous treatment with testosterone, information on diet and physical activity are recorded.
19

20 297
21
22
23 298 Physical examination

24
25 299 Height, weight, body mass index (BMI, kg/m²), blood pressure, waist and hip circumference and face
26
27 300 and body hair (Ferriman-Gallwey Score) are recorded.
28

29
30 301
31
32 302 *Sample size and statistics.*

33
34 303 As no valid data exists for a proper power calculation regarding the primary endpoint, NCP, we have
35
36 304 estimated the sample size. There are around 300 referrals for masculinizing therapy in Denmark per
37
38 305 year and patients are treated for life within the three centers of gender identity in Denmark. We aim to
39
40 306 include 200 participants, including 50 who are approved to start testosterone treatment based on the
41
42 307 power calculation of the main secondary endpoint, upper body muscle strength and power. Sample size
43
44 308 estimation for upper extremity muscle strength and power was based on data (mean baseline/standard
45
46 309 deviation) from a comparable cohort⁴². The estimation was based on a 10 % within-participant
47
48 310 difference (deemed as functional relevant) with an alpha level of 0.05 and a statistical power of 0.80.
49
50 311 On the basis of this data, a sample size of 39 was sufficient to detect within-participants differences
51
52
53
54
55
56
57
58
59
60

1
2
3
4 312 with two-tailed comparison. To account for potential dropouts (estimated to 20 %) 50 individuals

5
6 313 approved to start testosterone treatment will be recruited for the trial.

7
8
9 314 Normally and non-normally distributed data will be analyzed using parametric and non-parametric

10
11 315 statistics, respectively. Associations between testosterone and endpoints will be investigated with

12
13 316 multiple linear regression modeling. Random mixed-effects linear regression models will be applied to

14
15
16 317 investigate associations between testosterone and longitudinal repeated markers of assessed outcomes.

17
18 318 We will collaborate with a statistician regarding random mixed-effects linear regression modeling.

19
20 319 A directed acyclic graph (DAG) will be depicted in order to transparently identify a priori assumptions

21
22 320 of causal relations between exposure, outcome, potential confounders, intermediate factors, and

23
24 321 selection bias. Missing data will be handled according to type. Depending on data, analyses will be

25
26 322 performed based on either complete case analyses or, if appropriate, by imputing missing data in

27
28 323 collaboration with the statistical department. Analyses will be conducted using STATA 14 (StataCorp

29
30 324 2015). Post regression diagnostics/model validation will be performed. Assumptions of linearity

31
32 325 between predictors and the outcome variable will be inspected by using scatter plots and augmented

33
34 326 component-plus-residual plots. Normality of predicted residuals will be checked with quantile-normal

35
36 327 and probability-normal plots. Homogeneity of variance (homoscedasticity) of the residuals will be

37
38 328 investigated by plotting residuals against the fitted (predicted) values. Multicollinearity will be assessed

39
40 329 using variance inflation factors. Open-ended questions will not be included. Data is anonymized

41
42 330 according to Danish law and regulations (The Regional Committees on Health Research Ethics for

43
44 331 Southern Denmark, Project-ID: S-20190108, The Danish Data Protection Agency, journal no.

45
46 332 19/27572), and therefore analyses will be performed through a remote VPN access to Statistics

47
48 333 Denmark.

49
50 334

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 mm³) as the clinically relevant difference⁴⁵. However, we cannot perform a proper power calculation regarding our primary endpoint, because, no previous data has been published, thus

1
2
3
4 358 the distribution of data is unknown and the standard deviation cannot be calculated. The possible lack
5
6
7 359 of NCP development during 10 years is acknowledged, but this potential negative result would be
8
9 360 reassuring and clinically relevant. In a large cardiac CT registry study in relatively young men (mean
10
11 361 age 39 ± 6 years) 424 out of 1143 were registered as having no cardiovascular symptoms⁸. Any
12
13 362 coronary plaque, was detected in 22 % of the 424 men by experienced readers. In our study, we intend
14
15
16 363 to increase the sensitivity of CCTA by using a semi-automatic program, which is comparable to
17
18 364 Autoplaque⁹.

19
20 365 We also aim at providing new scientific evidence regarding upper body muscle strength and power in
21
22
23 366 men on masculinizing therapy; these muscles are of special interest as the number of androgen
24
25 367 receptors are higher in the upper body muscles²⁰ compared to lower body. Also, our study will
26
27 368 elaborate on interesting data on the possible activating effect of testosterone therapy on aggression and
28
29
30 369 the temporal relation between masculinizing testosterone treatment and levels of aggression, a very
31
32 370 relevant clinical issue with respect to current WPATH warning regarding aggression on testosterone
33
34 371 therapy²³. We will report new data on VO₂max and cardiac function as improvements in
35
36
37 372 cardiorespiratory fitness are associated with considerably reduced mortality risk and adverse
38
39 373 cardiovascular event rates²⁹; furthermore, data on respiratory function will add new knowledge and all
40
41 374 the above-mentioned results are important for transgender men and non-binary individuals on
42
43
44 375 testosterone therapy in health and sports.

45
46 376 It is a great advantage that we have access to registry data on all included persons, also those lost to
47
48 377 follow-up in the clinical study, but a proper control group is still missing. However, it is unethical to
49
50
51 378 perform a placebo-controlled randomized trial when gender dysphoria is present. We address this issue
52
53 379 in two ways: Firstly, participants will be their own control for within subject comparison,
54
55 380 prospectively, and secondly study participants will be compared to participants from our Odense
56
57

1
2
3
4 381 Androgen Study (OAS)^{6 46-48}. The OAS was a large cross-sectional study in 783 young healthy men
5
6
7 382 (20-29 years old). We plan a reinvestigation in 2021 (follow-up time 14 years), however, we do not
8
9 383 know if it is feasible to use them as controls regarding the effects of ageing. Our study is a non-
10
11 384 representative cohort study with risk of selection bias regarding age, BMI, smoking, ethnicity, fitness
12
13 385 level and morbidity. However, we are performing a national registry study, including all transgender
14
15 386 men in Denmark, which enables comparison between included individuals and data from the national
16
17
18 387 register-based cohort of transgender men. We have, previously used this embedded design in women
19
20 388 with polycystic ovarian syndrome (PCOS)⁴⁹⁻⁵⁴. In addition, we have obtained ethical permission to
21
22 389 access registry data from all participants including those, who leaves the clinical study part. Registry
23
24
25 390 data will provide knowledge, regarding national ICD-10 diagnostic codes, medical treatment,
26
27 391 socioeconomics and causes of death. Hence, registry data will enable a thorough characterization of
28
29 392 drop-outs and we can compare these individuals to those who stay in the clinical study. Data on health
30
31 393 consequences regarding all aspects of gender affirming treatment is warranted, however, this study will
32
33 394 only provide information on transgender men and non-binary persons on testosterone therapy. To
34
35 395 counteract this unbalance, a parallel study has been planned in transgender women and non-binary
36
37 396 persons on feminizing treatment.
38
39
40
41 397
42

43 398 **Competing interests' statement**

44
45 399 The authors declare that they have no competing interests.
46
47
48 400
49

50 401 **Funding statement**

51
52 402 Study funded by Odense University Hospital (Frontlinjepuljen).
53
54
55 403
56
57
58
59
60

404 **Authors' contributions**

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

408 **Acknowledgements**

409 We would like to thank our collaborators:

410 Per Aagaard, Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark,
411 Odense, Denmark

412 Kaya Rössler, Department of Psychology, University of Southern Denmark, Odense, Denmark

413 Malene Hilden, Center of Gender Identity, Rigshospitalet, Copenhagen, Denmark

414 Astrid Højgaard, Center of Gender Identity, Aalborg University Hospital, Aalborg Denmark

416 **References**

- 417 1. Kuyper L, Wijzen C. Gender identities and gender dysphoria in the Netherlands. *Archives of sexual behavior*
418 2014;43(2):377-85. doi: 10.1007/s10508-013-0140-y [published Online First: 2013/07/17]
- 419 2. Van Caenegem E, Wierckx K, Elaut E, et al. Prevalence of Gender Nonconformity in Flanders, Belgium.
420 *Archives of sexual behavior* 2015;44(5):1281-7. doi: 10.1007/s10508-014-0452-6 [published Online
421 First: 2015/01/16]
- 422 3. Frederiksen L, Hojlund K, Hougaard DM, et al. Testosterone therapy decreases subcutaneous fat and
423 adiponectin in aging men. *European journal of endocrinology* 2012;166(3):469-76. doi: 10.1530/eje-11-
424 0565 [published Online First: 2011/12/23]
- 425 4. Magnussen LV, Andersen PE, Diaz A, et al. MR spectroscopy of hepatic fat and adiponectin and leptin levels
426 during testosterone therapy in type 2 diabetes: a randomized, double-blinded, placebo-controlled trial.

Resubmission bmjopen-2020-042607

Clean version, October 8th 2020

- 1
2
3
4 427 *European journal of endocrinology* 2017;177(2):157-68. doi: 10.1530/eje-17-0071 [published Online
5
6 428 First: 2017/05/20]
- 8
9 429 5. Magnussen LV, Hvid LG, Hermann AP, et al. Testosterone therapy preserves muscle strength and power in
10
11 430 aging men with type 2 diabetes-a randomized controlled trial. *Andrology* 2017;5(5):946-53. doi:
12
13 431 10.1111/andr.12396 [published Online First: 2017/09/16]
- 15 432 6. Nielsen TL, Hagen C, Wraae K, et al. Visceral and subcutaneous adipose tissue assessed by magnetic
17
18 433 resonance imaging in relation to circulating androgens, sex hormone-binding globulin, and luteinizing
19
20 434 hormone in young men. *The Journal of clinical endocrinology and metabolism* 2007;92(7):2696-705.
21
22 435 doi: 10.1210/jc.2006-1847 [published Online First: 2007/04/12]
- 24 436 7. Rubin JB, Borden WB. Coronary heart disease in young adults. *Current atherosclerosis reports*
26
27 437 2012;14(2):140-9. doi: 10.1007/s11883-012-0226-3 [published Online First: 2012/01/18]
- 29 438 8. Otaki Y, Gransar H, Cheng VY, et al. Gender differences in the prevalence, severity, and composition of
31
32 439 coronary artery disease in the young: a study of 1635 individuals undergoing coronary CT angiography
33
34 440 from the prospective, multinational confirm registry. *European heart journal cardiovascular Imaging*
35
36 441 2015;16(5):490-9. doi: 10.1093/ehjci/jeu281 [published Online First: 2014/12/30]
- 38 442 9. Dey D, Gaur S, Ovrehus KA, et al. Integrated prediction of lesion-specific ischaemia from quantitative
39
40 443 coronary CT angiography using machine learning: a multicentre study. *European radiology*
41
42 444 2018;28(6):2655-64. doi: 10.1007/s00330-017-5223-z [published Online First: 2018/01/21]
- 44
45 445 10. Snyder PJ, Bhasin S, Cunningham GR, et al. Lessons From the Testosterone Trials. *Endocr Rev*
46
47 446 2018;39(3):369-86. doi: 10.1210/er.2017-00234 [published Online First: 2018/03/10]
- 49 447 11. Shaikh K, Ellenberg SS, Nakanishi R, et al. Biomarkers and Noncalcified Coronary Artery Plaque Progression
50
51 448 in Older Men Treated With Testosterone. *The Journal of clinical endocrinology and metabolism*
52
53 449 2020;105(7):2142-9. doi: 10.1210/clinem/dgz242 [published Online First: 2019/12/01]
- 55
56
57

- 1
2
3
4 450 12. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, et al. Sex Steroids and Cardiovascular Outcomes in
5
6 451 Transgender Individuals: A Systematic Review and Meta-Analysis. *The Journal of clinical endocrinology*
7
8 *and metabolism* 2017;102(11):3914-23. doi: 10.1210/jc.2017-01643 [published Online First:
9 452 2017/09/26]
10
11 453
12
13 454 13. Emi Y, Adachi M, Sasaki A, et al. Increased arterial stiffness in female-to-male transsexuals treated with
14
15 455 androgen. *The journal of obstetrics and gynaecology research* 2008;34(5):890-7. doi: 10.1111/j.1447-
16
17 456 0756.2008.00857.x [published Online First: 2008/10/07]
18
19
20 457 14. Wierckx K, Van Caenegem E, Schreiner T, et al. Cross-sex hormone therapy in trans persons is safe and
21
22 458 effective at short-time follow-up: results from the European network for the investigation of gender
23
24 459 incongruence. *The journal of sexual medicine* 2014;11(8):1999-2011. doi: 10.1111/jsm.12571
25
26
27 460 [published Online First: 2014/05/16]
28
29 461 15. Alzahrani T, Nguyen T, Ryan A, et al. Cardiovascular Disease Risk Factors and Myocardial Infarction in the
30
31 462 Transgender Population. *Circulation Cardiovascular quality and outcomes* 2019;12(4):e005597. doi:
32
33 463 10.1161/circoutcomes.119.005597 [published Online First: 2019/04/06]
34
35
36 464 16. Wierckx K, Mueller S, Weyers S, et al. Long-term evaluation of cross-sex hormone treatment in transsexual
37
38 465 persons. *The journal of sexual medicine* 2012;9(10):2641-51. doi: 10.1111/j.1743-6109.2012.02876.x
39
40 466 [published Online First: 2012/08/22]
41
42 467 17. Bhasin S, Woodhouse L, Casaburi R, et al. Testosterone dose-response relationships in healthy young men.
43
44 468 *American journal of physiology Endocrinology and metabolism* 2001;281(6):E1172-81. doi:
45
46 469 10.1152/ajpendo.2001.281.6.E1172 [published Online First: 2001/11/10]
47
48
49 470 18. Frederiksen L, Hojlund K, Hougaard DM, et al. Testosterone therapy increased muscle mass and lipid
50
51 471 oxidation in aging men. *Age (Dordrecht, Netherlands)* 2012;34(1):145-56. doi: 10.1007/s11357-011-
52
53 472 9213-9 [published Online First: 2011/02/25]
54
55
56
57
58
59
60

- 1
2
3
4 473 19. T'Sjoen G, Arcelus J, Gooren L, et al. Endocrinology of Transgender Medicine. *Endocr Rev* 2019;40(1):97-
5
6 474 117. doi: 10.1210/er.2018-00011 [published Online First: 2018/10/12]
7
8
9 475 20. Kadi F, Bonnerud P, Eriksson A, et al. The expression of androgen receptors in human neck and limb
10
11 476 muscles: effects of training and self-administration of androgenic-anabolic steroids. *Histochemistry and*
12
13 477 *cell biology* 2000;113(1):25-9. doi: 10.1007/s004180050003 [published Online First: 2000/02/09]
14
15 478 21. Van Caenegem E, Wierckx K, Taes Y, et al. Bone mass, bone geometry, and body composition in female-to-
16
17 male transsexual persons after long-term cross-sex hormonal therapy. *The Journal of clinical*
18 479 *endocrinology and metabolism* 2012;97(7):2503-11. doi: 10.1210/jc.2012-1187 [published Online First:
19
20 480 2012/05/09]
21
22 481
23
24 482 22. Wiik A, Lundberg TR, Rullman E, et al. Muscle Strength, Size, and Composition Following 12 Months of
25
26 Gender-affirming Treatment in Transgender Individuals. *The Journal of clinical endocrinology and*
27 483 *metabolism* 2020;105(3) doi: 10.1210/clinem/dgz247 [published Online First: 2019/12/04]
28
29 484
30
31 485 23. Coleman E, Bockting W, Botzer M, et al. Standards of Care for the Health of Transsexual, Transgender, and
32
33 486 Gender-Nonconforming People, Version 7. *International Journal of Transgenderism* 2012;13(4):165-
34
35 487 232. doi: 10.1080/15532739.2011.700873
36
37
38 488 24. Defreyne J, Kreukels B, T'Sjoen G, et al. No correlation between serum testosterone levels and state-level
39
40 489 anger intensity in transgender people: Results from the European Network for the Investigation of
41
42 490 Gender Incongruence. *Hormones and behavior* 2019;110:29-39. doi: 10.1016/j.yhbeh.2019.02.016
43
44
45 491 [published Online First: 2019/03/02]
46
47 492 25. Defreyne J, T'Sjoen G, Bouman WP, et al. Prospective Evaluation of Self-Reported Aggression in
48
49 493 Transgender Persons. *The journal of sexual medicine* 2018;15(5):768-76. doi:
50
51 494 10.1016/j.jsxm.2018.03.079 [published Online First: 2018/04/28]
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

26. A C. Sex differences in direct aggression: What are the psychological mediators? *Aggression and Violent Behavior* 2006;11(3):237-64.
27. Kristensen TT, Christensen, L.L., Frystyk, J., Glintborg, D., T'Sjoen, G. Roessler, K.K., Andersen, M. . The effect of testosterone treatment on aggression in transgender men: A systematic review. *Horm Behav in review* 2019
28. Buss AH, Perry M. The aggression questionnaire. *Journal of personality and social psychology* 1992;63(3):452-9. doi: 10.1037//0022-3514.63.3.452 [published Online First: 1992/09/01]
29. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama* 2009;301(19):2024-35. doi: 10.1001/jama.2009.681 [published Online First: 2009/05/21]
30. Chen L, Kuang J, Pei JH, et al. Predictors of cardiorespiratory fitness in female and male adults with different body mass index: National Health and Nutrition Examination Survey 1999-2004 dataset. *Annals of medicine* 2017;49(1):83-92. doi: 10.1080/07853890.2016.1252056 [published Online First: 2016/10/22]
31. Myers J, Kaminsky LA, Lima R, et al. A Reference Equation for Normal Standards for VO(2) Max: Analysis from the Fitness Registry and the Importance of Exercise National Database (FRIEND Registry). *Progress in cardiovascular diseases* 2017;60(1):21-29. doi: 10.1016/j.pcad.2017.03.002 [published Online First: 2017/04/06]
32. Hirschberg AL, Elings Knutsson J, Helge T, et al. Effects of moderately increased testosterone concentration on physical performance in young women: a double blind, randomised, placebo controlled study. *British journal of sports medicine* 2020;54(10):599-604. doi: 10.1136/bjsports-2018-100525 [published Online First: 2019/10/17]
33. Shah R, Newcomb DC. Sex Bias in Asthma Prevalence and Pathogenesis. *Frontiers in immunology* 2018;9:2997. doi: 10.3389/fimmu.2018.02997 [published Online First: 2019/01/09]

Resubmission bmjopen-2020-042607

Clean version, October 8th 2020

- 1
2
3
4 518 34. Haynes JM, Stumbo RW. The Impact of Using Non-Birth Sex on the Interpretation of Spirometry Data in
5
6 519 Subjects With Air-Flow Obstruction. *Respiratory care* 2018;63(2):215-18. doi: 10.4187/respcare.05586
7
8
9 520 [published Online First: 2017/12/01]
10
11 521 35. Hughes LJ, Peiffer JJ, Scott BR. Reliability and Validity of Using the Push Band v2.0 to Measure Repetition
12
13 522 Velocity in Free-Weight and Smith Machine Exercises. *Journal of strength and conditioning research*
14
15 523 2019 doi: 10.1519/jsc.0000000000003436 [published Online First: 2019/12/21]
16
17
18 524 36. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free
19
20 525 testosterone in serum. *The Journal of clinical endocrinology and metabolism* 1999;84(10):3666-72. doi:
21
22 526 10.1210/jcem.84.10.6079 [published Online First: 1999/10/16]
23
24 527 37. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein
25
26 528 cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*
27
28 529 1972;18(6):499-502. [published Online First: 1972/06/01]
29
30
31 530 38. Frystyk J, Tarnow L, Hansen TK, et al. Increased serum adiponectin levels in type 1 diabetic patients with
32
33 531 microvascular complications. *Diabetologia* 2005;48(9):1911-8. doi: 10.1007/s00125-005-1850-z
34
35 532 [published Online First: 2005/08/04]
36
37
38 533 39. D'Anna-Hernandez KL, Ross RG, Natvig CL, et al. Hair cortisol levels as a retrospective marker of
39
40 534 hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. *Physiology*
41
42 535 & *behavior* 2011;104(2):348-53. doi: 10.1016/j.physbeh.2011.02.041 [published Online First:
43
44 536 2011/03/15]
45
46
47 537 40. Stalder T, Kirschbaum C. Analysis of cortisol in hair--state of the art and future directions. *Brain, behavior,*
48
49 538 *and immunity* 2012;26(7):1019-29. doi: 10.1016/j.bbi.2012.02.002 [published Online First: 2012/03/01]
50
51 539 41. Gao W, Stalder T, Foley P, et al. Quantitative analysis of steroid hormones in human hair using a column-
52
53 540 switching LC-APCI-MS/MS assay. *Journal of chromatography B, Analytical technologies in the*
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 541 *biomedical and life sciences* 2013;928:1-8. doi: 10.1016/j.jchromb.2013.03.008 [published Online First:
- 542 2013/04/16]
- 543 42. Seo DI, Kim E, Fahs CA, et al. Reliability of the one-repetition maximum test based on muscle group and
- 544 gender. *Journal of sports science & medicine* 2012;11(2):221-5. [published Online First: 2012/01/01]
- 545 43. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of
- 546 software platform partners. *Journal of biomedical informatics* 2019;95:103208. doi:
- 547 10.1016/j.jbi.2019.103208 [published Online First: 2019/05/13]
- 548 44. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven
- 549 methodology and workflow process for providing translational research informatics support. *Journal of*
- 550 *biomedical informatics* 2009;42(2):377-81. doi: 10.1016/j.jbi.2008.08.010 [published Online First:
- 551 2008/10/22]
- 552 45. DG A. Practical statistics for medical research: Chapman and Hall/CRC 1991:458.
- 553 46. Nielsen TL, Wraae K, Brixen K, et al. Prevalence of overweight, obesity and physical inactivity in 20- to 29-
- 554 year-old, Danish men. Relation to sociodemography, physical dysfunction and low socioeconomic
- 555 status: the Odense Androgen Study. *International journal of obesity (2005)* 2006;30(5):805-15. doi:
- 556 10.1038/sj.ijo.0803197 [published Online First: 2006/01/19]
- 557 47. Christensen L.L NTL, Hermann P, Glintborg D, Andersen M,. Body composition and testosterone determined
- 558 VO2max in 780 young men - results from the Odense Androgen Study. European Conference of
- 559 Endocrinology Lisboa, 2017.
- 560 48. Nielsen TL, Hagen C, Wraae K, et al. The impact of the CAG repeat polymorphism of the androgen receptor
- 561 gene on muscle and adipose tissues in 20-29-year-old Danish men: Odense Androgen Study. *European*
- 562 *journal of endocrinology* 2010;162(4):795-804. doi: 10.1530/eje-09-0763 [published Online First:
- 563 2010/02/06]

- 1
2
3
4 564 49. Glintborg D, Rubin KH, Nybo M, et al. Cardiovascular disease in a nationwide population of Danish women
5
6 565 with polycystic ovary syndrome. *Cardiovascular diabetology* 2018;17(1):37. doi: 10.1186/s12933-018-
7
8 566 0680-5 [published Online First: 2018/03/10]
9
10
11 567 50. Glintborg D, Rubin KH, Abrahamsen B, et al. Response to Letter to the Editor: "Development and Risk
12
13 568 Factors of Type 2 Diabetes in a Nationwide Population of Women With Polycystic Ovary Syndrome".
14
15 569 *The Journal of clinical endocrinology and metabolism* 2018;103(1):362-63. doi: 10.1210/jc.2017-02123
16
17 [published Online First: 2017/11/11]
18 570
19
20 571 51. Rubin KH, Andersen MS, Abrahamsen B, et al. Socioeconomic status in Danish women with polycystic ovary
21
22 572 syndrome: A register-based cohort study. *Acta obstetricia et gynecologica Scandinavica*
23
24 573 2019;98(4):440-50. doi: 10.1111/aogs.13514 [published Online First: 2018/12/06]
25
26
27 574 52. Glintborg D, Hass Rubin K, Nybo M, et al. Morbidity and medicine prescriptions in a nationwide Danish
28
29 575 population of patients diagnosed with polycystic ovary syndrome. *European journal of endocrinology*
30
31 576 2015;172(5):627-38. doi: 10.1530/eje-14-1108 [published Online First: 2015/02/07]
32
33 577 53. Rubin KH, Glintborg D, Nybo M, et al. Fracture Risk Is Decreased in Women With Polycystic Ovary
34
35 578 Syndrome: A Register-Based and Population-Based Cohort Study. *Journal of bone and mineral research*
36
37 : the official journal of the American Society for Bone and Mineral Research 2016;31(4):709-17. doi:
38 579 10.1002/jbmr.2737 [published Online First: 2015/11/07]
39
40 580
41
42 581 54. Rubin KH, Glintborg D, Nybo M, et al. Development and Risk Factors of Type 2 Diabetes in a Nationwide
43
44 582 Population of Women With Polycystic Ovary Syndrome. *The Journal of clinical endocrinology and*
45
46 583 *metabolism* 2017;102(10):3848-57. doi: 10.1210/jc.2017-01354 [published Online First: 2017/09/25]
47
48
49 584
50
51
52
53
54
55
56
57
58
59
60

BMJ Open

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)

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-045714.R1
Article Type:	Protocol
Date Submitted by the Author:	25-Nov-2020
Complete List of Authors:	Lehmann Christensen, Louise; Odense University Hospital Department of Endocrinology, Body Identity Clinic Glintborg, Dorte ; Odense University Hospital Department of Endocrinology Taulbjerg Kristensen, Tine; Odense University Hospital Department of Endocrinology Diederichsen, Axel; Odense University Hospital, Cardiology T'Sjoen, Guy; University Hospital Ghent, Department of Endocrinology Frystyk, Jan; Odense University Hospital Department of Endocrinology Skovsager Andersen, Marianne; Odense University Hospital, Body Identity Clinic, Department of Endocrinology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, Coronary heart disease < CARDIOLOGY, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3
4 **1 Title**

5
6
7 2 Masculinizing testosterone treatment and effects on preclinical cardiovascular disease, muscle strength
8
9 3 and power, aggression, physical fitness and respiratory function in transgender men: Protocol for a ten-
10
11 4 year, prospective, observational cohort study in Denmark at the Body Identity Clinic (BIC)
12
13
14 5

15
16 6 *Authors*

17
18 7 Louise Lehmann Christensen^{1,4}, Dorte Glintborg¹, Tine Taulbjerg Kristensen¹, Axel Cosmus Pyndt
19
20 8 Diederichsen², Guy T'Sjoen³, Jan Frystyk^{1,4}, Marianne Skovsager Andersen¹
21
22

23 9 ¹Body Identity Clinic, Department of Endocrinology Odense University Hospital, Odense, Denmark
24

25 10 ²Department of Cardiology, Odense University Hospital, Odense, Denmark
26

27 11 ³Department of Endocrinology, Ghent University Hospital, Ghent, Belgium
28

29 12 ⁴Open Patient data Explorative Network (OPEN), Odense University Hospital, Region of Southern
30
31
32 13 Denmark, Odense, Denmark
33

34 14
35
36 15 *Corresponding author*

37
38
39 16 Louise Lehmann Christensen, louise.lehmann.christensen@rsyd.dk
40

41 17 *Ethical approval*

42
43 18 The Regional Ethics Committee, Region of Southern Denmark approved the study.
44

45
46 19 Journal number: S-20190108.
47

48 20 *Inclusion start*

49
50 21 February 2020
51
52
53 22

54
55 23 *Word count: 3842*
56
57

1
2
3
4 **24 Abstract**

5
6 **25 Introduction:** The number of individuals with gender dysphoria seeking gender-affirming treatment is
7
8
9 **26** increasing. The short- and long-term effects of masculinizing treatment with testosterone are debated as
10
11 **27** serum testosterone increases up to twenty-fold compared to cisgender women. We will investigate
12
13 **28** short- and long-term effects of masculinizing testosterone treatment on preclinical and clinical coronary
14
15
16 **29** disease, muscle strength and power, VO₂ max, cardiac and respiratory function and quality of life
17
18 **30** including aggression in transgender men.

19
20 **31 Methods and analyses:** Prospective, single-center, observational cohort study at the Body Identity
21
22 **32** Clinic, Odense University Hospital, Denmark. Investigations are performed at inclusion and following
23
24
25 **33** one, three, five and ten years of testosterone therapy.
26
27 **34** Non-calcified coronary plaque volume and calcium score are estimated by coronary computed
28
29
30 **35** tomography angiography. CT is only performed at inclusion and following one and ten years. Upper
31
32 **36** body muscle strength and power are measured by a “Low Row” weight stack resisted exercise
33
34 **37** machine. Evaluation of aggression and quality of life is assessed by questionnaires, VO₂ max is
35
36 **38** estimated by maximal testing on bike ergometer, and cardiac and respiratory functions are measured by
37
38
39 **39** echocardiography and spirometry, respectively. Markers of cardiovascular risk and inflammation and
40
41 **40** also cortisol and cortisone are assessed in blood, diurnal urine and/or hair samples. Our cohort (BIC),
42
43 **41** including dropouts, will be an embedded sub-cohort in a future national registry study in all individuals
44
45
46 **42** with gender dysphoria and controls. Data are available on International Statistical Classification of
47
48 **43** Diseases and Related Health Problems (ICD-10) diagnostic codes, prescriptions, socioeconomics and
49
50 **44** causes of death.
51
52
53 **45**

1
2
3
4 46 **Ethics and dissemination:** The Regional Committee on Health Research Ethics for Southern Denmark
5
6
7 47 (S-20190108) and the Danish Data Protection Agency approved the study (19/27572). Signed informed
8
9 48 consent will be obtained from all participants. All findings will be published in peer-reviewed journals
10
11 49 or at scientific conferences.
12

13 50
14
15 51 **Trial registration:** Clinicaltrials.gov ID: NCT04254354
16
17
18 52

19 53 **Article Summary**

20 54 **Strengths and limitations of this study**

- 25 55 • Body Identity Clinic is a research clinic where this prospective longitudinal ten-year cohort
26
27 56 study in transgender men will assess preclinical coronary disease by estimating non- calcified
28
29
30 57 plaque volume and calcium score using coronary computed tomography angiography.
31
- 32 58 • The applied methods ensure important health- and sport-related data on upper body muscle
33
34 59 strength and power, aggression, VO₂ max, cardiac and respiratory function in transgender men,
35
36 60 during short- and long-term testosterone treatment.
37
38
- 39 61 • A proper control group is missing, but it is unethical to perform a placebo-controlled
40
41 62 randomized trial on long-term testosterone therapy in persons with gender dysphoria.
42
43
44 63 • Results will only be available in transgender and non-binary individuals, treated with
45
46 64 testosterone, however, a parallel study in transgender women and non-binary persons on
47
48 65 feminizing treatment is planned.
49
50
51
52
53
54
55
56
57

66 Introduction

67 Recent European studies report that 0.6-0.8 % of individuals assigned female at birth have incongruent
68 gender identity or gender dysphoria^{1 2}. Testosterone treatment is the cornerstone of masculinizing
69 treatment and testosterone treatment initiated in transgender men and by request in non-binary
70 individuals. Testosterone is an important androgenic and anabolic hormone. The androgen effects of
71 testosterone include terminal hair growth and deepening of the voice, whereas the anabolic effects
72 include muscle growth. Body shape changes from feminine to masculine during testosterone treatment,
73 as subcutaneous fat is reduced³ and redistributed^{4 5}. The changes in sex hormones are quite dramatic in
74 transgender men, as circulating testosterone concentrations increase up to 20-fold into the normal range
75 for cisgender men⁶, while estradiol levels are partly suppressed.

76 Clinical coronary disease is rare in young persons⁷, however, preclinical coronary disease is observed
77 and testosterone treatment may accelerate non-calcified coronary plaque (NCP) formation. Both NCP
78 and calcified plaques may be detected by the sensitive coronary CT angiography (CCTA)⁸ in
79 combination with a semi-automated computer program⁹. In a large cardiac CT registry study in
80 younger (mean age 39 years) participants, 22 % of cisgender men and 15 % of cisgender women
81 without cardiovascular symptoms, had coronary artery disease defined as any plaque formation (non-
82 calcified or calcified)⁸. The association between testosterone treatment and NCP formation has been
83 reported in ageing cisgender men after 1 year of testosterone treatment¹⁰, with increased waist-to-hip
84 ratio at baseline as the strongest predictor of NCP formation¹¹. There is a lack of knowledge regarding
85 NCP formation in relatively young transgender men on masculinizing testosterone therapy. We know
86 that lipid status is deteriorated during masculinizing testosterone treatment in transgender men, with an
87 increase in low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL)
88 cholesterol¹² and blood pressure may increase^{13 14}. However, studies on cardiovascular endpoints in

1
2
3
4 89 transgender men are limited and conflicting. A four-fold increased odds ratio for myocardial infarction
5
6
7 90 in transgender men was reported, which was significant after adjusting for age, diabetes mellitus,
8
9 91 hypertension, hypercholesterolemia, chronic kidney disease, smoking and exercise levels¹⁵. The study
10
11 92 design was, however, cross-sectional, hence it was not possible to conclude on cause and effect. A
12
13 93 retrospective study in transgender men reported no cases of myocardial infarction after ten years of
14
15
16 94 testosterone treatment¹⁶, but the retrospective study design increased risk of selection bias due to loss to
17
18 95 follow-up before ten years.

19
20 96 Testosterone treatment is associated with dose-dependent increases in muscle mass^{17 18} and muscle
21
22 97 strength^{5 17} in cisgender men. Muscle mass also increases during masculinizing treatment with
23
24
25 98 testosterone in transgender men¹⁹, but there is limited data on changes in muscle strength ~~before and~~
26
27 99 during masculinizing testosterone treatment. One study reported higher lower body muscle strength
28
29
30 100 during gender affirming treatment with testosterone²⁰. Upper body muscles have higher number of
31
32 101 androgen receptors than lower body muscles²¹ and characterizing muscle strength and power in the
33
34 102 large muscles of the arms and upper back during testosterone treatment in transgender men would be of
35
36 103 interest. Grip strength was higher in transgender men compared to cisgender women²². However, the
37
38
39 104 study was cross-sectional, and hand grip strength only tests the small muscles of the hand and lower
40
41 105 arm.

42
43 106 The World Professional Association for Transgender Health (WPATH)²³ recommends to warn against
44
45 107 elevated aggression levels as a psychological adverse effect of masculinizing testosterone treatment in
46
47
48 108 transgender men, however, this presumption was not supported by recent data^{24 25}. The term aggression
49
50 109 covers a wide range of inter-correlated behaviors, thoughts and emotions. There is no uniform
51
52 110 definition of aggression²⁶ and studies reporting increased aggression scores during testosterone
53
54
55 111 treatment²⁷ had severe methodological problems and high risk of bias. Valid information on aggression

1
2
3
4 112 constructs²⁷ during short- and long-term testosterone therapy is ensured in the present study by a
5
6 113 questionnaire, which incorporates all dimensions of aggression²⁸ and questionnaires containing
7
8
9 114 information on anxiety, depression, testosterone dose, duration of treatment and physical health.
10

11 115 Low cardiorespiratory fitness has been associated with an increased risk of premature death from all
12
13 116 causes, but cardiovascular disease is the most common cause of death in cisgender individuals²⁹. The
14
15
16 117 maximum oxygen uptake (VO₂ max), determined from a graded maximal exercise test, is an approved
17
18 118 way of classifying cardiorespiratory fitness levels³⁰. VO₂ max is generally higher in cisgender men
19
20 119 compared to cisgender women³¹, presumably due to differences in muscle mass, cardiac output,
21
22
23 120 haematocrit and lung size. There are no prospective data on VO₂ max during masculinizing testosterone
24
25 121 treatment in transgender men, but VO₂ max is expected to increase, as testosterone increased muscle
26
27 122 mass²⁰ and haematocrit¹⁴. Exogenous testosterone increased aerobic running time in young physically
28
29
30 123 active cisgender women³²; testosterone gel, 10 mg per day for ten weeks, was used, the dose equals 20
31
32 124 % of masculinizing testosterone treatment.
33

34 125 Obstructive and restrictive estimates of respiratory function are estimated by spirometry and reference
35
36 126 ranges for spirometry vary with gender assigned at birth, weight, height and age. Asthma is
37
38
39 127 characterized by obstructively decreased respiratory function with airway hyper-responsiveness and
40
41 128 inflammation. Asthma is much more prevalent in cisgender women compared to cisgender men³³;
42
43 129 however we have no reference ranges for respiratory function in transgender men³⁴.
44

45
46 130 Masculinizing testosterone treatment in transgender men is for life and currently no prospective data
47
48 131 are available on long-term morbidity. This study will provide important information on health issues
49
50 132 regarding cardiovascular and respiratory health and upper body muscle strength and power. Aggression
51
52
53 133 levels may be elevated during testosterone therapy, but the temporal relation has not been clarified. The
54
55
56
57

1
2
3
4 134 combination of long-term clinical observational data and access to national registry data will provide
5
6
7 135 further knowledge on benefits and risks of masculinizing testosterone treatment.
8

9 136
10
11 137 *Aim*

12
13 138 To investigate short- and long-term effects of masculinizing testosterone treatment on preclinical and
14
15
16 139 clinical coronary disease, muscle strength and power, VO₂ max, cardiac and respiratory function and
17
18 140 quality of life including aggression in transgender men.
19

20 141 We hypothesize that, in transgender men:

21
22
23 142 Masculinizing testosterone treatment accelerate NCP development and progression.

24
25 143 Testosterone treatment will be associated with long-term increase in upper body muscle strength and
26
27 144 power, VO₂ max, cardiac and respiratory function.

28
29
30 145 Aggression scores will increase during initiation of testosterone treatment, but the aggression scores
31
32 146 will return to baseline during long-term testosterone treatment.
33

34 147
35
36 148 **Methods and analysis**

37
38
39 149 *Study population and recruitment*

40
41 150 Participants are individuals assigned female at birth with a diagnosis of gender dysphoria treated with
42
43 151 testosterone or approved to start treatment with testosterone. Only individuals associated with one of
44
45
46 152 the three centers of gender identity in Denmark: Odense, Aalborg and Copenhagen are included. No
47
48 153 individual with known use of 'self-prescribed' sex hormones is included. The inclusion period for the
49
50 154 cohort (N=200) is estimated to last 2 years.
51

52
53 155
54
55 156 *Study design*

1
2
3
4 157 For study outline, please see figure 1. The study is a prospective single-center observational cohort
5
6 158 study at BIC, Odense University Hospital, Odense, Denmark of ten years duration. Participants (aged
7
8
9 159 18 years and older) are invited for five visits (baseline, one, three, five, and ten years) after informed
10
11 160 consent has been given. The participants will spend one day in BIC per visit and all examinations use
12
13 161 the same equipment and study protocols (table 1). Ethical permission has been obtained for the use of
14
15
16 162 registry data regarding International Statistical Classification of Diseases and Related Health Problems
17
18 163 (ICD-10) diagnostic codes, medical treatment, socioeconomics and causes of death in all participants
19
20 164 including participants lost to follow-up. The participants accept study participation by written informed
21
22
23 165 consent. All individuals in Denmark have a civil registration number (CPR) reflecting binary gender.
24
25 166 During legal transitioning individuals may change CPR and data from the two CPR numbers is merged.
26
27 167 We will be able to compare study participants with participants lost to follow-up by combining the
28
29
30 168 clinical outcomes with registry data (table 1, figure 1).
31

32 169

33

34 170 *Patient and Public Involvement statement*

35

36 171 An advisory board of transgender men has been established. The participant advisory board meetings
37
38
39 172 were arranged prior to application for ethical approval of the study to secure inputs in terms of
40
41 173 relevance of research questions, recruitment, outcomes and participant time consumption. The
42
43 174 participant advisory board has read and commented on the study material and will be contacted for
44
45
46 175 continuous sparring.
47

48 176

49

50 177 *Endpoints*

51

52 178 **Primary endpoint**

53

54
55 179 NCP volume
56
57

1
2
3
4 180 **Secondary endpoints**

5
6 181 Calcium score

7
8
9 182 Upper body muscle strength and power

10
11 183 Aggression and quality of life

12
13 184 VO₂ max

14
15 185 Left ventricular muscle mass and function

16
17 186 Respiratory function

18
19 187 Serum levels of testosterone, estradiol and cortisol.

20
21 188 Circulating markers of cardiovascular risk and inflammation

22
23 189 Diurnal urine and hair samples for assessment of cortisol and cortisone

24
25
26
27 190
28
29
30 191 *Outcomes*

31
32 192 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 program⁹. 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 exercises³⁵. 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).

VO₂ max

Measurement of VO₂ 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/L³⁶.

1
2
3
4 257 Hemoglobin is measured using a photometric analyzer with a coefficient of variation (CV) of 2.8 %.

5
6
7 258 Plasma total cholesterol and HDL cholesterol are analyzed by enzymatic colorimetric reactions

8
9 259 (Modular P, Roche), and LDL cholesterol is calculated using the Friedewald equation³⁷. HbA1c is

10
11 260 measured by high-performance liquid chromatography using Tosoh G8 (Medinor, Broendby,

12
13 261 Denmark); the analytical CV is 0.9 %. Adiponectin is determined by an in-house timeresolved

14
15 262 immunofluorometric assay³⁸, with intra- and inter-assay CV averaging 5 and 10 % respectively.

16
17
18 263 Plasma suPAR is measured with the suPARnostic® ELISA (ViroGates A/S, Birkerød, Denmark) with

19
20 264 a mean CV of 4 %. CRP is analyzed using latex based immunoanalysis (CRP Ultra, Sentinel

21
22 265 Diagnostics, Milan, Italy) by an Architect c8000 instrument (Abbott). The intra-assay and inter-assay

23
24 266 CVs are 0.8 % and 1.9 % for normal levels of CRP, respectively. Plasma IL-6 is measured with

25
26 267 Quantikine® HS-IL-6 ELISA (R&D Systems, Minneapolis, USA) with a mean CV of 8 %.

27
28 268 Diurnal urine samples: Participants are instructed to note the time for voiding in the morning before

29
30 269 starting sample collection, and all urine collected until the morning of the second day. Urine samples

31
32 270 are kept frozen at -20°C until analysis. Cortisol and cortisone are analyzed by LC-MS/MS. Urine

33
34 271 samples are solid phase extracted on an Oasis HLB 96-well plate after addition of deuterated internal

35
36 272 standards; the analysis is calibrated by in-house prepared calibrators, and the relative SD is <10%.

37
38 273 Quality is assured by monthly participation with satisfactory results in the external quality control

39
40 274 program for steroid hormones from the UKNEQAS.

41
42 275 Hair samples: A section of hair strands approximately 3 mm in diameter is cut as close to the scalp as

43
44 276 possible from the posterior vertex area. Hair samples are stored in aluminum foil as previously

45
46 277 described³⁹, the 4-cm hair segment closest to the scalp is used for analyses to represent cortisol

47
48 278 secretion over the most recent 4-month period⁴⁰. The analyses will be carried out at Department of

49
50 279 Psychology, Technical University of Dresden, Germany using LC-MS/MS⁴¹.

1
2
3
4 280
5
6
7 281 Whole body dual x-ray absorptiometry and bone mineral density

8
9 282 Dual x-ray absorptiometry, Horizon A Discovery is used to measure whole body lean body mass, fat
10
11 283 mass and bone density. Radiation amounts to 0.1 mSv per scan.
12

13 284
14
15
16 285 Medical history

17
18 286 Chronic diseases, medication and supplements, alcohol, tobacco and abuse, gynecological history,
19
20 287 previous treatment with testosterone, information on diet and physical activity are recorded.
21
22

23 288
24
25 289 Physical examination

26
27 290 Height, weight, body mass index (BMI, kg/m²), blood pressure, waist and hip circumference and face
28
29 291 and body hair (Ferriman-Gallwey Score) are recorded.
30
31

32 292
33
34 293 *Sample size and statistics.*

35
36 294 The primary study endpoint is NCP. We have estimated the sample size, as no valid data exist for a
37
38 295 proper power calculation regarding the primary endpoint, NCP. We aim to include 200 participants,
39
40 296 including 50 men who are approved to start testosterone treatment based on the power calculation of
41
42 297 the main secondary endpoint, upper body muscle strength and power. Sample size estimation for upper
43
44 298 extremity muscle strength and power was based on data (mean baseline/standard deviation) from a
45
46 299 comparable cohort⁴². The estimation was based on a 10 % within-participant difference (deemed as
47
48 300 functional relevant) with an alpha level of 0.05 and a statistical power of 0.80. On the basis of this data,
49
50 301 a sample size of 39 was sufficient to detect within-participants differences with two-tailed comparison.
51
52
53
54
55
56
57

1
2
3
4 302 To account for potential dropouts (estimated to 20 %) 50 individuals approved to start testosterone
5
6
7 303 treatment will be recruited for the trial.

8
9 304 Normally and non-normally distributed data will be analyzed using parametric and non-parametric
10
11 305 statistics, respectively. Associations between testosterone and endpoints will be investigated with
12
13 306 multiple linear regression modeling. Random mixed-effects linear regression models will be applied to
14
15
16 307 investigate associations between testosterone and longitudinal repeated markers of assessed outcomes.

17
18 308 We will collaborate with a statistician regarding random mixed-effects linear regression modeling.

19
20 309 A directed acyclic graph (DAG) will be depicted in order to transparently identify a priori assumptions
21
22
23 310 of causal relations between exposure, outcome, potential confounders, intermediate factors, and
24
25 311 selection bias. Missing data will be handled according to type. Depending on data, analyses will be
26
27 312 performed based on either complete case analyses or, if appropriate, by imputing missing data in
28
29
30 313 collaboration with the statistical department. Analyses will be conducted using STATA 14 (StataCorp
31
32 314 2015). Post regression diagnostics/model validation will be performed. Assumptions of linearity
33
34 315 between predictors and the outcome variable will be inspected by using scatter plots and augmented
35
36 316 component-plus-residual plots. Normality of predicted residuals will be checked with quantile-normal
37
38
39 317 and probability-normal plots. Homogeneity of variance (homoscedasticity) of the residuals will be
40
41 318 investigated by plotting residuals against the fitted (predicted) values. Multicollinearity will be assessed
42
43
44 319 using variance inflation factors. Open-ended questions will not be included. Data are anonymized
45
46 320 according to Danish law and regulations (The Regional Committees on Health Research Ethics for
47
48 321 Southern Denmark, Project-ID: S-20190108, The Danish Data Protection Agency, journal no.
49
50 322 19/27572), and therefore analyses will be performed through a remote VPN access to Statistics
51
52
53 323 Denmark.

54
55 324

325 *Data management*

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

333 **Ethics and dissemination**

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

342 **Discussion**

343 Preclinical coronary disease is determined by NCP volume and calcium score in transgender men
344 during this ten-year prospective cohort study at BIC. NCP volume is assessed by state-of-the-art CCTA
345 and we use the lowest detectable change in NCP formation (1 mm³) as the clinically relevant
346 difference⁴⁵. However, a proper power calculation regarding our primary endpoint cannot be performed
347 as no previous data has been published. Therefore, distribution of data is unknown and the standard

1
2
3
4 348 deviation cannot be calculated. The possible lack of NCP development during ten years is
5
6
7 349 acknowledged, but this potential negative result would be reassuring and clinical relevant. In a large
8
9 350 cardiac CT registry study in relatively young cisgender men (mean age 39 ± 6 years) 424 out of 1143
10
11 351 participants did not have any cardiovascular symptoms⁸. Experienced cardiologists assessed the
12
13 352 CCTAs, reporting coronary plaque of any kind in 22 % of asymptomatic 424 cisgender men⁸. In our
14
15
16 353 study, we increase the sensitivity of detecting NCPs by using a semi-automatic program, which is
17
18 354 comparable to Autoplaque⁹.
19
20 355 We also aim at providing new scientific evidence regarding upper body muscle strength and power in
21
22 356 men on masculinizing therapy. Upper body muscles are of special interest as the number of androgen
23
24
25 357 receptors are higher in the upper body muscles compared to lower body²¹. Also, our study will
26
27 358 elaborate on interesting data on possible increased levels of aggression during testosterone therapy and
28
29
30 359 the temporal relation between masculinizing testosterone treatment and levels of aggression²⁷.
31
32 360 Aggression is very relevant clinical issue in the context of the WPATH warning regarding aggression
33
34 361 on testosterone therapy²³. We will report new data on VO₂ max and cardiac function as improvements
35
36 362 in cardiorespiratory fitness are associated with considerably reduced mortality risk and adverse
37
38
39 363 cardiovascular event rates²⁹; furthermore, data on respiratory function will add new knowledge and all
40
41 364 the above-mentioned results are important for transgender men and non-binary individuals on
42
43 365 testosterone therapy in health and sports.
44
45
46 366 Ethical permission is granted to access registry data from all participants including those, who leaves
47
48 367 the clinical study part. Registry data will provide knowledge, regarding national ICD-10 diagnostic
49
50 368 codes, medical treatment, socioeconomics and causes of death. Future access to national registry data
51
52
53 369 in all individuals with registered gender dysphoria and controls will allow us to study our cohort (BIC)
54
55 370 as an embedded sub-cohort including individuals, who remain in the ten-year study as well as dropouts.
56
57
58
59
60

1
2
3
4 371 We have previously used this design in women with polycystic ovary syndrome (PCOS)⁴⁶. The gold
5
6 372 standard research design to assess benefits and risks of testosterone therapy in transgender persons is a
7
8
9 373 long-term placebo-controlled randomized trial on masculinizing therapy, however, it is deeply
10
11 374 unethical to perform such a study, when gender dysphoria is present. Apart from the future registry
12
13 375 study we will compare data from our cohort to results from Odense Androgen Study (OAS)^{6 47-49}. OAS
14
15
16 376 is a large cross-sectional study in 783 young healthy men (20-29 years old); data are available on BMI,
17
18 377 waist circumference, muscle mass, fat mass, blood pressure, HbA1c, testosterone and estradiol levels,
19
20 378 lipid status and VO₂ max and a follow-up is planned (Ethical Committee Journal number, S-20130097).
21
22
23 379 Our study is a non-representative cohort study with risk of selection bias regarding age, BMI, smoking,
24
25 380 ethnicity, fitness level and morbidity. Data on health consequences regarding all aspects of gender
26
27 381 affirming treatment is warranted, however, this study will only provide information on transgender men
28
29
30 382 and non-binary persons on testosterone therapy. To counteract this unbalance, a parallel study has been
31
32 383 planned in transgender women and non-binary persons on feminizing treatment.
33

34 384

36 385 **Competing interests' statement**

37
38
39 386 The authors declare that they have no competing interests.
40
41 387

42 43 388 **Funding statement**

44
45
46 389 Study funded by Odense University Hospital (Frontlinjepuljen). Award/grant number: N/A
47
48 390
49

50 391 **Authors' contributions**

51
52
53
54
55
56
57

1
2
3
4 392 All authors meet the ICMJE criteria for authorship. MA, JF: Conception and design of the study. LC,
5
6 393 MA, DG, TTK, AD, JF and GT contributed in writing the protocol. All Authors read and approved the
7
8
9 394 final manuscript.
10

11 395 12 13 396 **Acknowledgements**

14
15
16 397 We would like to thank our collaborators:

17
18 398 Per Aagaard, Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark,
19
20 399 Odense, Denmark

21
22
23 400 Kaya Rössler, Department of Psychology, University of Southern Denmark, Odense, Denmark

24
25 401 Malene Hilden, Center of Gender Identity, Rigshospitalet, Copenhagen, Denmark

26
27 402 Astrid Højgaard, Center of Gender Identity, Aalborg University Hospital, Aalborg Denmark
28
29

30 403
31
32 404 Legend figure 1: Study design.
33

34 405
35
36 406 Legend figure 2: The figure displays a coronary CT angiography (CCTA). A semi-automatic program
37
38
39 407 detects NCP. RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior
40
41 408 descending artery; LCX, left circumflex artery; OM, obtuse marginal branch 1.
42

43 409
44
45
46 410 Legend table 1: Overview of study outcomes and visits.
47

48 411 **References**

- 49
50 412 1. Kuyper L, Wijzen C. Gender identities and gender dysphoria in the Netherlands. *Archives of sexual behavior*
51 413 2014;43(2):377-85. doi: 10.1007/s10508-013-0140-y [published Online First: 2013/07/17]
52 414 2. Van Caenegem E, Wierckx K, Elaut E, et al. Prevalence of Gender Nonconformity in Flanders, Belgium.
53 415 *Archives of sexual behavior* 2015;44(5):1281-7. doi: 10.1007/s10508-014-0452-6 [published Online
54 416 First: 2015/01/16]
55
56
57
58
59
60

3. Frederiksen L, Hojlund K, Hougaard DM, et al. Testosterone therapy decreases subcutaneous fat and adiponectin in aging men. *European journal of endocrinology* 2012;166(3):469-76. doi: 10.1530/eje-11-0565 [published Online First: 2011/12/23]
4. Magnussen LV, Andersen PE, Diaz A, et al. MR spectroscopy of hepatic fat and adiponectin and leptin levels during testosterone therapy in type 2 diabetes: a randomized, double-blinded, placebo-controlled trial. *European journal of endocrinology* 2017;177(2):157-68. doi: 10.1530/eje-17-0071 [published Online First: 2017/05/20]
5. Magnussen LV, Hvid LG, Hermann AP, et al. Testosterone therapy preserves muscle strength and power in aging men with type 2 diabetes-a randomized controlled trial. *Andrology* 2017;5(5):946-53. doi: 10.1111/andr.12396 [published Online First: 2017/09/16]
6. Nielsen TL, Hagen C, Wraae K, et al. Visceral and subcutaneous adipose tissue assessed by magnetic resonance imaging in relation to circulating androgens, sex hormone-binding globulin, and luteinizing hormone in young men. *The Journal of clinical endocrinology and metabolism* 2007;92(7):2696-705. doi: 10.1210/jc.2006-1847 [published Online First: 2007/04/12]
7. Rubin JB, Borden WB. Coronary heart disease in young adults. *Current atherosclerosis reports* 2012;14(2):140-9. doi: 10.1007/s11883-012-0226-3 [published Online First: 2012/01/18]
8. Otaki Y, Gransar H, Cheng VY, et al. Gender differences in the prevalence, severity, and composition of coronary artery disease in the young: a study of 1635 individuals undergoing coronary CT angiography from the prospective, multinational confirm registry. *European heart journal cardiovascular Imaging* 2015;16(5):490-9. doi: 10.1093/ehjci/jeu281 [published Online First: 2014/12/30]
9. Dey D, Gaur S, Ovrehus KA, et al. Integrated prediction of lesion-specific ischaemia from quantitative coronary CT angiography using machine learning: a multicentre study. *European radiology* 2018;28(6):2655-64. doi: 10.1007/s00330-017-5223-z [published Online First: 2018/01/21]
10. Snyder PJ, Bhasin S, Cunningham GR, et al. Lessons From the Testosterone Trials. *Endocr Rev* 2018;39(3):369-86. doi: 10.1210/er.2017-00234 [published Online First: 2018/03/10]
11. Shaikh K, Ellenberg SS, Nakanishi R, et al. Biomarkers and Noncalcified Coronary Artery Plaque Progression in Older Men Treated With Testosterone. *The Journal of clinical endocrinology and metabolism* 2020;105(7):2142-9. doi: 10.1210/clinem/dgz242 [published Online First: 2019/12/01]
12. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, et al. Sex Steroids and Cardiovascular Outcomes in Transgender Individuals: A Systematic Review and Meta-Analysis. *The Journal of clinical endocrinology and metabolism* 2017;102(11):3914-23. doi: 10.1210/jc.2017-01643 [published Online First: 2017/09/26]
13. Emi Y, Adachi M, Sasaki A, et al. Increased arterial stiffness in female-to-male transsexuals treated with androgen. *The journal of obstetrics and gynaecology research* 2008;34(5):890-7. doi: 10.1111/j.1447-0756.2008.00857.x [published Online First: 2008/10/07]
14. Wierckx K, Van Caenegem E, Schreiner T, et al. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. *The journal of sexual medicine* 2014;11(8):1999-2011. doi: 10.1111/jsm.12571 [published Online First: 2014/05/16]
15. Alzahrani T, Nguyen T, Ryan A, et al. Cardiovascular Disease Risk Factors and Myocardial Infarction in the Transgender Population. *Circulation Cardiovascular quality and outcomes* 2019;12(4):e005597. doi: 10.1161/circoutcomes.119.005597 [published Online First: 2019/04/06]
16. Wierckx K, Mueller S, Weyers S, et al. Long-term evaluation of cross-sex hormone treatment in transsexual persons. *The journal of sexual medicine* 2012;9(10):2641-51. doi: 10.1111/j.1743-6109.2012.02876.x [published Online First: 2012/08/22]

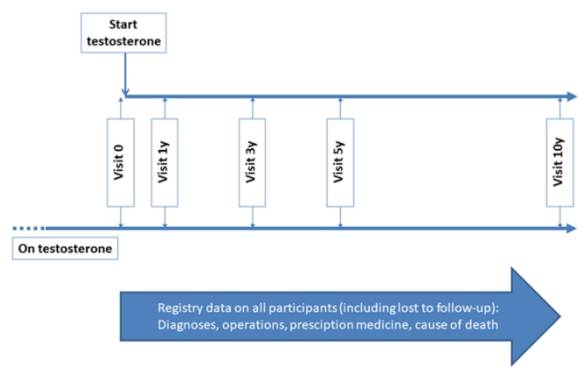
- 1
2
3
4 462 17. Bhasin S, Woodhouse L, Casaburi R, et al. Testosterone dose-response relationships in healthy young men.
5 463 *American journal of physiology Endocrinology and metabolism* 2001;281(6):E1172-81. doi:
6 464 10.1152/ajpendo.2001.281.6.E1172 [published Online First: 2001/11/10]
7
8 465 18. Frederiksen L, Hojlund K, Hougaard DM, et al. Testosterone therapy increased muscle mass and lipid
9 466 oxidation in aging men. *Age (Dordrecht, Netherlands)* 2012;34(1):145-56. doi: 10.1007/s11357-011-
10 467 9213-9 [published Online First: 2011/02/25]
11 468 19. T'Sjoen G, Arcelus J, Gooren L, et al. Endocrinology of Transgender Medicine. *Endocr Rev* 2019;40(1):97-
12 469 117. doi: 10.1210/er.2018-00011 [published Online First: 2018/10/12]
13 470 20. Wiik A, Lundberg TR, Rullman E, et al. Muscle Strength, Size, and Composition Following 12 Months of
14 471 Gender-affirming Treatment in Transgender Individuals. *The Journal of clinical endocrinology and*
15 472 *metabolism* 2020;105(3) doi: 10.1210/clinem/dgz247 [published Online First: 2019/12/04]
16 473 21. Kadi F, Bonnerud P, Eriksson A, et al. The expression of androgen receptors in human neck and limb
17 474 muscles: effects of training and self-administration of androgenic-anabolic steroids. *Histochemistry and*
18 475 *cell biology* 2000;113(1):25-9. doi: 10.1007/s004180050003 [published Online First: 2000/02/09]
19 476 22. Van Caenegem E, Wierckx K, Taes Y, et al. Bone mass, bone geometry, and body composition in female-to-
20 477 male transsexual persons after long-term cross-sex hormonal therapy. *The Journal of clinical*
21 478 *endocrinology and metabolism* 2012;97(7):2503-11. doi: 10.1210/jc.2012-1187 [published Online First:
22 479 2012/05/09]
23 480 23. Coleman E, Bockting W, Botzer M, et al. Standards of Care for the Health of Transsexual, Transgender, and
24 481 Gender-Nonconforming People, Version 7. *International Journal of Transgenderism* 2012;13(4):165-
25 482 232. doi: 10.1080/15532739.2011.700873
26 483 24. Defreyne J, Kreukels B, T'Sjoen G, et al. No correlation between serum testosterone levels and state-level
27 484 anger intensity in transgender people: Results from the European Network for the Investigation of
28 485 Gender Incongruence. *Hormones and behavior* 2019;110:29-39. doi: 10.1016/j.yhbeh.2019.02.016
29 486 [published Online First: 2019/03/02]
30 487 25. Defreyne J, T'Sjoen G, Bouman WP, et al. Prospective Evaluation of Self-Reported Aggression in
31 488 Transgender Persons. *The journal of sexual medicine* 2018;15(5):768-76. doi:
32 489 10.1016/j.jsxm.2018.03.079 [published Online First: 2018/04/28]
33 490 26. A C. Sex differences in direct aggression: What are the psychological mediators? *Aggression and Violent*
34 491 *Behavior* 2006;11(3):237-64.
35 492 27. Kristensen T, Christensen LL, Frystyk J, et al. Effects of testosterone therapy on constructs related to
36 493 aggression in transgender men: A systematic review. *Hormones and behavior, accepted for publication*
37 494 2020
38 495 28. Buss AH, Perry M. The aggression questionnaire. *Journal of personality and social psychology*
39 496 1992;63(3):452-9. doi: 10.1037//0022-3514.63.3.452 [published Online First: 1992/09/01]
40 497 29. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause
41 498 mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama*
42 499 2009;301(19):2024-35. doi: 10.1001/jama.2009.681 [published Online First: 2009/05/21]
43 500 30. Chen L, Kuang J, Pei JH, et al. Predictors of cardiorespiratory fitness in female and male adults with different
44 501 body mass index: National Health and Nutrition Examination Survey 1999-2004 dataset. *Annals of*
45 502 *medicine* 2017;49(1):83-92. doi: 10.1080/07853890.2016.1252056 [published Online First: 2016/10/22]
46 503 31. Myers J, Kaminsky LA, Lima R, et al. A Reference Equation for Normal Standards for VO(2) Max: Analysis
47 504 from the Fitness Registry and the Importance of Exercise National Database (FRIEND Registry). *Progress*
48 505 *in cardiovascular diseases* 2017;60(1):21-29. doi: 10.1016/j.pcad.2017.03.002 [published Online First:
49 506 2017/04/06]
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 507 32. Hirschberg AL, Elings Knutsson J, Helge T, et al. Effects of moderately increased testosterone concentration
5 508 on physical performance in young women: a double blind, randomised, placebo controlled study.
6 509 *British journal of sports medicine* 2020;54(10):599-604. doi: 10.1136/bjsports-2018-100525 [published
7 510 Online First: 2019/10/17]
- 8 511 33. Shah R, Newcomb DC. Sex Bias in Asthma Prevalence and Pathogenesis. *Frontiers in immunology*
9 512 2018;9:2997. doi: 10.3389/fimmu.2018.02997 [published Online First: 2019/01/09]
- 10 513 34. Haynes JM, Stumbo RW. The Impact of Using Non-Birth Sex on the Interpretation of Spirometry Data in
11 514 Subjects With Air-Flow Obstruction. *Respiratory care* 2018;63(2):215-18. doi: 10.4187/respcare.05586
12 515 [published Online First: 2017/12/01]
- 13 516 35. Hughes LJ, Peiffer JJ, Scott BR. Reliability and Validity of Using the Push Band v2.0 to Measure Repetition
14 517 Velocity in Free-Weight and Smith Machine Exercises. *Journal of strength and conditioning research*
15 518 2019 doi: 10.1519/jsc.0000000000003436 [published Online First: 2019/12/21]
- 16 519 36. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free
17 520 testosterone in serum. *The Journal of clinical endocrinology and metabolism* 1999;84(10):3666-72. doi:
18 521 10.1210/jcem.84.10.6079 [published Online First: 1999/10/16]
- 19 522 37. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein
20 523 cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*
21 524 1972;18(6):499-502. [published Online First: 1972/06/01]
- 22 525 38. Frystyk J, Tarnow L, Hansen TK, et al. Increased serum adiponectin levels in type 1 diabetic patients with
23 526 microvascular complications. *Diabetologia* 2005;48(9):1911-8. doi: 10.1007/s00125-005-1850-z
24 527 [published Online First: 2005/08/04]
- 25 528 39. D'Anna-Hernandez KL, Ross RG, Natvig CL, et al. Hair cortisol levels as a retrospective marker of
26 529 hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. *Physiology*
27 530 & behavior 2011;104(2):348-53. doi: 10.1016/j.physbeh.2011.02.041 [published Online First:
28 531 2011/03/15]
- 29 532 40. Stalder T, Kirschbaum C. Analysis of cortisol in hair--state of the art and future directions. *Brain, behavior,*
30 533 *and immunity* 2012;26(7):1019-29. doi: 10.1016/j.bbi.2012.02.002 [published Online First: 2012/03/01]
- 31 534 41. Gao W, Stalder T, Foley P, et al. Quantitative analysis of steroid hormones in human hair using a column-
32 535 switching LC-APCI-MS/MS assay. *Journal of chromatography B, Analytical technologies in the*
33 536 *biomedical and life sciences* 2013;928:1-8. doi: 10.1016/j.jchromb.2013.03.008 [published Online First:
34 537 2013/04/16]
- 35 538 42. Seo DI, Kim E, Fahs CA, et al. Reliability of the one-repetition maximum test based on muscle group and
36 539 gender. *Journal of sports science & medicine* 2012;11(2):221-5. [published Online First: 2012/01/01]
- 37 540 43. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of
38 541 software platform partners. *Journal of biomedical informatics* 2019;95:103208. doi:
39 542 10.1016/j.jbi.2019.103208 [published Online First: 2019/05/13]
- 40 543 44. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven
41 544 methodology and workflow process for providing translational research informatics support. *Journal of*
42 545 *biomedical informatics* 2009;42(2):377-81. doi: 10.1016/j.jbi.2008.08.010 [published Online First:
43 546 2008/10/22]
- 44 547 45. DG A. Practical statistics for medical research: Chapman and Hall/CRC 1991:458.
- 45 548 46. Rubin KH, Glintborg D, Nybo M, et al. Development and Risk Factors of Type 2 Diabetes in a Nationwide
46 549 Population of Women With Polycystic Ovary Syndrome. *The Journal of clinical endocrinology and*
47 550 *metabolism* 2017;102(10):3848-57. doi: 10.1210/jc.2017-01354 [published Online First: 2017/09/25]
- 48 551 47. Nielsen TL, Wraae K, Brixen K, et al. Prevalence of overweight, obesity and physical inactivity in 20- to 29-
49 552 year-old, Danish men. Relation to sociodemography, physical dysfunction and low socioeconomic

- 1
2
3
4 553 status: the Odense Androgen Study. *International journal of obesity (2005)* 2006;30(5):805-15. doi:
5 554 10.1038/sj.ijo.0803197 [published Online First: 2006/01/19]
6 555 48. Christensen L.L NTL, Hermann P, Glintborg D, Andersen M. Body composition and testosterone determined
7 556 VO2max in 780 young men - results from the Odense Androgen Study. European Conference of
8 557 Endocrinology Lisboa, 2017.
9 558 49. Nielsen TL, Hagen C, Wraae K, et al. The impact of the CAG repeat polymorphism of the androgen receptor
10 559 gene on muscle and adipose tissues in 20-29-year-old Danish men: Odense Androgen Study. *European*
11 560 *journal of endocrinology* 2010;162(4):795-804. doi: 10.1530/eje-09-0763 [published Online First:
12 561 2010/02/06]
13 562
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

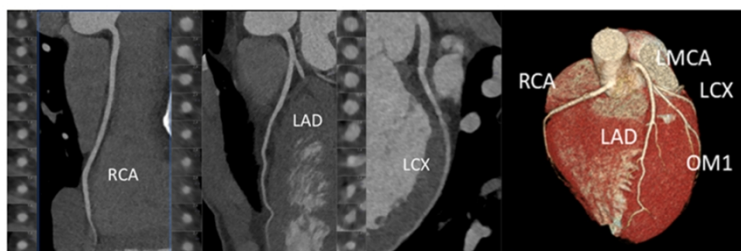
For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Legend figure 1: Study design

338x190mm (96 x 96 DPI)



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)