


Physical fitness, hormonal profile, nutritional and psychological aspects assessment of transgender women volleyball players submitted to physical tests: protocol paper of a prospective cohort

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ABSTRACT

To evaluate aerobic capacity, strength and other physiological, nutritional, and psychological variables which may influence the performance of transgender women (TW) athletes and compare them to cisgender women (CW) and cisgender men (CM) athletes, as well as changes in TW performance over the course of a year. Prospective cohort study including three groups: TW, CW and CM volleyball athletes. Subjects will be comprehensively assessed at two different moments: baseline and after 6–12 months of adequate hormonal therapy. Evaluation will comprise clinical, medical, nutritional and psychological interviews, incremental treadmill cardiopulmonary exercise testing, hand grip strength test, vertical jump test, analysis of sleep quality (Pittsburgh Sleep Quality Index), hormonal profile, echocardiogram, analysis of resting energy expenditure, assessment of bone mass and body composition through dual-energy X-ray absorptiometry scans, and untargeted metabolomic analysis. CW and CM matched by age, body mass index and level of physical activity will undergo a similar evaluation. The assessment of the strength, aerobic capacity, haematological, nutritional and psychological status of TW using gold-standard tests will contribute to understanding the impact of oestrogen therapy on the exercise performance of these athletes and how they compare with CW and CM.

INTRODUCTION

Participation of transgender women (TW) previously exposed to male puberty in competitive sports has raised questions about their possible advantages compared with cisgender women (CW) since the effects of testosterone on body composition (BC) and physical performance are well known.¹

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ There are little data about the exercise performance of transgender women athletes.

WHAT THIS STUDY ADDS

⇒ The assessment of the physical fitness of transgender women using gold-standard tests for the evaluation of cardiorespiratory parameters, muscle strength, body composition, and nutritional and psychological aspects will contribute to the understanding of their exercise performance. Moreover, these data will be compared with cisgender women and cisgender men athletes' data.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Hopefully, our study will contribute to evidence-based decision-making, allowing science to overcome speculation.

The objectives of gender-affirming hormone therapy (GAHT) using oestrogen in TW are to reduce the levels of endogenous testosterone and to promote the development of female characteristics^{2–3} using different pharmaceutical forms of oestrogen alone or combined with antiandrogen drugs.^{3–4} However, the main question in sports concerns the degree to which previous effects of testosterone TW are reversible on androgen deprivation.^{5–6}

Furthermore, the mental health of TW has also been a topic of recent attention. It has been reported that most transgender individuals have a negative experience when engaging in competitive sports-related physical activities due to non-acceptance by some



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groups or even difficulty using clothes or toilets during training.⁶

Despite all the uncertainties described above, we have witnessed an increase in TW athletes participating in high-performance sports, but few data about related aspects,⁷ which makes scientific analysis relevant.

OBJECTIVES

The primary objective of this study is to evaluate the aerobic capacity, strength and other physiological, nutritional, and psychological variables which may influence the performance of TW volleyball players undergoing hormone therapy and compare to CW and cisgender men (CM) volleyball players, as well as changes in performance of TW over the course of a year.

Secondarily, we aim to compare among these groups: (1) BC, (2) resting energy expenditure (REE), (3) nutritional aspects including eating pattern and dietary practices, (4) bone density, (5) cardiac chambers and

function, (6) haematological/hormonal status, (7) psychological aspects, including cognition and mood, (8) sleep quality and (9) metabolomic analysis.

METHODS

Study design and population

This is a prospective cohort study with up to 12 months of follow-up of TW volleyball athletes (figure 1). The enrolment started in February 2022 and is expected to finalise in September 2023 in São Paulo/Brazil.

Individuals will be deemed eligible if they have the following characteristics: (1) classified as gender incongruent according to the International Disease Code 11; (2) aged 18–50 years; (3) body mass index (BMI) between 18.0 and 34.9 kg/m²; (4) classified as having a very high level of physical activity, according to the International Physical Activity Questionnaire score^{8,9} and (5) if they are engaged in regular volleyball training. Conversely, participants will be excluded if they report or present with signs

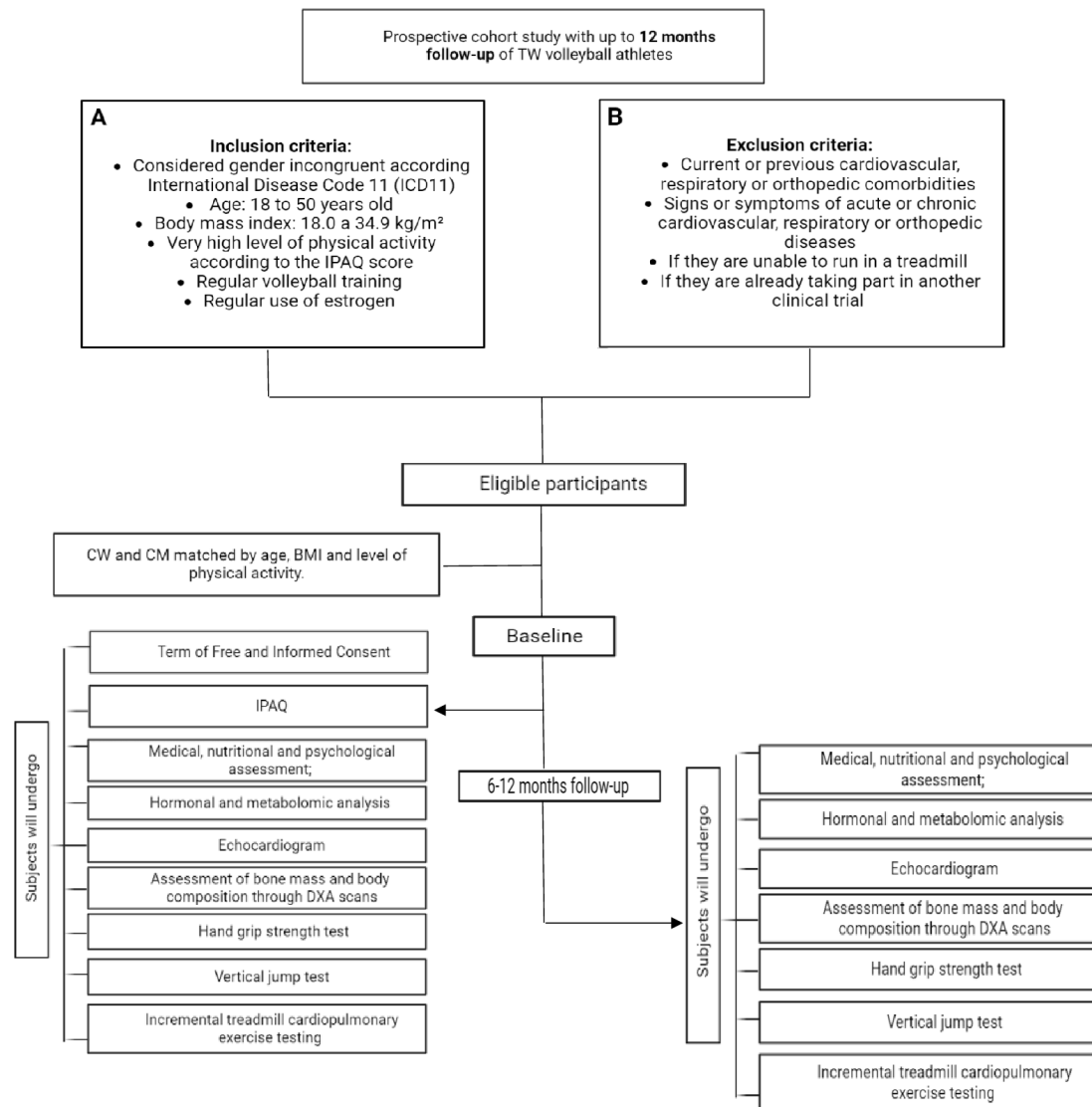


Figure 1 Selection of transgender women (TW). (A) Inclusion criteria; (B) exclusion criteria and the control group subjects. BMI, body mass index; CM, cisgender men; CW, cisgender women; DXA, dual-energy X-ray absorptiometry; IPAQ, International Physical Activity Questionnaire.

of acute or chronic cardiovascular, respiratory or orthopaedic comorbidities, which could potentially interfere with exercise capacity if they are pregnant or already taking part in another clinical trial.

CW and CM matched by age, BMI and level of physical activity will undergo a similar evaluation.

Participants are not allowed to change groups throughout the study.

The study will comprise two phases for the TW group: baseline and 6–12 months following regular use of oestrogen and blocked testosterone (<10 nmol/L). At each phase, participants will be required to attend three study visits, during which a full assessment of medical, mental and nutritional health, BC, strength and aerobic capacity will be performed. Several test dates will be made available to facilitate the participant's choice. The athlete's participation will be discontinued if requested or if any clinical condition arises, that may interfere with the athlete's performance or examination findings.

The researchers will not be blinded during the physical tests due to the phenotypic characteristics of the participants.

Equity, diversity and inclusion

Principles are integrated into our study design, allowing people of all biological sexes to participate and feel free to mention their gender identity.

The research group is composed of CM and CW alike.

Patient and public partnership

Volleyball team organisers made up of TW sought out our research centre and suggested an assessment of the sports capabilities of this population and made available the possibility of disseminating the research to volunteers.

The study was designed by specialist researchers from various areas of knowledge (two endocrinologists, one sports doctor, one exercise physiologist, one sports nutritionist, one pneumologist, one physiotherapist, two medical undergraduate students and one molecular biologist).

Research instruments and procedures

All of the following procedures will happen at baseline and at the follow-up phase (6–12 months later).

Basic laboratory tests

Participants will undergo a full haematological and hormonal assessment. The whole metabolic panel will include fasting glucose, glycated haemoglobin (HbA1c), total cholesterol, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), triglycerides. Hormonal profile will include total and free testosterone, estradiol, prolactin, sex hormone binding globulin (SHBG), parathyroid hormone (PTH), 25-hydroxyvitamin D, thyroid stimulating hormone (TSH) and free thyroxine (FT4). Total blood count, renal function (urea, creatinine) and electrolytes (P, Ca²⁺, Mg²⁺) will also be measured.

Maximal incremental exercise test

Participants will perform a maximal incremental exercise test to exhaustion on a treadmill (Super ATL, Inbrasport, Porto Alegre, RS, Brazil) to determine peak oxygen uptake (VO₂peak), peak heart rate (HRpeak) and other submaximal parameters (such as anaerobic threshold and respiratory compensation point). These parameters will be assessed using a metabolic system (K5, COSMED, Rome, Italy). Following an initial 5 min warm-up, the treadmill incline will be kept at 1%. At the same time, the speed will be increased by 1 km/hour every minute until exhaustion,¹⁰ aiming for a total incremental test duration of 8–12 min. Heart rate will be continuously recorded throughout the test with a Garmin heart rate sensor. Blood pressure will be measured at rest, immediately after maximal effort, and 1, 2 and 3 min after exercising by the same examiner. A calibrated upper limb manometer with manual auscultation through a stethoscope will be used.

Vertical jump tests

A 10 min dynamic stretching and warm-up period will be performed, followed by self-administered squatting exercises and submaximal vertical jumps for familiarisation. Before the testing session, the participants will be given instructions on the execution of the squat jump (SQJ) and the countermovement jump (CMJ) without arm swings. At the starting position, for the execution of each type of jump, the arms will be placed on the hips, with feet in full contact with the jump mat (Jump System Pro, Cefise, Nova Odessa, SP, Brazil), and knee angle at approximately 90° (when performing SQJ). The arms will be placed on the hips throughout the jump, flight and landing. Three SQJ attempts will be performed, separated by 10 s. After a rest period of 10 min, three CMJ attempts will be performed, also separated by 10 s.

All jumps will be performed barefoot, and participants will be instructed to jump as high and fast as possible (without a downward movement when performing SQJ). The best attempt of the three trials of each type of jump, using the criterion of maximum jump height achieved, will be selected for further analysis.

Hand grip strength test

The Jamar hydraulic hand dynamometer (Patterson Medical, Warrenville, Illinois, USA) will measure handgrip strength.

Participants will comfortably sit on a chair without armrests, with their feet flat on the floor, hips and knees at approximately 90° of flexion. The shoulder of the tested limb will be adducted and neutrally rotated, the elbow flexed at 90°, the forearm in a neutral position, and the wrist in 0°–30° dorsiflexion and 0°–15° adduction. The untested hand will be placed on the thigh. All participants will be evaluated individually.

Before the test, participants will perform submaximal handgrip movements and rest for 1 min. During the test, they cannot look at the dynamometer display to avoid

any visual feedback. No command will be given during the test, and the instructions for test execution will be standardised. The volume of the verbal command will remain constant to avoid any influence on the magnitude of muscle contraction.

Three attempts will be performed with both the dominant and non-dominant hands. A maximum contraction lasting 3s will be performed at each attempt, followed by a 30s rest period. The mean values of the three attempts of each hand will be used for data analysis.

Echocardiogram

A standard transthoracic Doppler echocardiogram will be performed with the participant lying supine and lateral. Participants' hearts will be examined for anatomical and functional aspects, including dimensions of the cardiac chambers, the interventricular septum and the left ventricular ejection fraction.

Food intake assessment

Food intake will be verified by 24-hour recalls (R24h) of non-consecutive days applied by trained professionals. Data collection will occur according to the Multiple-Pass Method, which consists of five steps: (1) quick listing of foods and beverages consumed, (2) commonly forgotten foods, (3) time and occasion of consumption, (4) detailing cycle and (5) final review.¹¹

Data collected from the R24h will be converted into standard measurement units (grams) based on tables and reference materials. Subsequently, macronutrients and micronutrients will be calculated in the DietBox. After that, the regular food intake components of interest (eg, carbohydrates, proteins and lipids) will be estimated using the Multiple Source Method software programs and the corrected intraindividual variability.

Dietary practices

The Dietary Practices Measurement Scale will be applied to assess dietary practices according to the Food Guide for the Brazilian Population (GAPB, in Portuguese).¹² This tool comprises 24 items that exemplify dietary practices, agreeing or disagreeing with the GAPB recommendations. Possible answers are 'strongly disagree, disagree, agree and strongly agree', which express the participant's level of agreement or accordance with this practice in their daily lives. The score on the scale is computed by the simple sum of the answers (values from 0 to 3), which can vary from 0 to 72 (the maximum). For classification purposes, two cut-off points were defined based on scoring percentiles, generating three score ranges: <P25 (<32 points), P25–P75 (32–41 points) and >P75 (>41 points). The higher the score, the greater the adherence to the GAPB recommendations.

Analysis of REE

REE will be assessed by indirect calorimetry (IC) using the metabolic system K5 (Cosmed, Rome, Italy). The test will be conducted for 20min in absolute resting conditions in the morning. The IC will be the first test of the

day, considering that REE is widely affected by external factors. Thus, on arriving at the laboratory, participants will rest for 10min in the supine position. Immediately after this, they will be connected to the equipment for REE assessment.

For improved IC reliability, participants will be instructed not to practice physical exercise or consume any food that could modify the resting metabolic rate (eg, coffee, food containing caffeine, high-protein meals) 24 hours before the test and will be asked to fast for 10 hours before the test. Moreover, the laboratory room will remain closed and temperature-controlled to avoid thermal changes that could modify energy expenditure (eg, thermoregulation).

BC and bone mineral density

Measurements of bone mineral density will be taken with dual-energy X-ray absorptiometry (Hologic QDR 4500, Waltham, Massachusetts, USA) at the lumbar spine L1–L4 (LS), total hip (TH) and femoral neck. The analysis of BC will be performed to assess the following parameters: fat mass, body fat percentage, skeletal muscle mass and fat-free mass. To minimise the interference of height, the following indices will be calculated: lean mass/height² and the Baumgartner Index (appendicular muscle mass/height²). To evaluate the total body fat mass, the fat mass index will be used, which represents the total body fat mass divided by the height squared.

Metabolomic analysis

The untargeted metabolomic analysis will be performed as previously described.^{13 14} Briefly, blood samples from each participant will be collected and packed in tubes containing EDTA. The extraction of plasma metabolites will be performed by adding 400µL of methanol and isopropanol (1:1 v/v) and 6µL of internal standard (3mg/mL of d27-myristic acid) to 100µL of plasma. The mixture will be subjected to ultrasound for 5min, homogenised for 20min at 4°C and centrifuged at 15 800×g at 4°C for 10min. Then, the supernatant will be transferred to a microcentrifuge tube and dried for 16hours in a SpeedVac. The sediment will be derivatised in two steps, beginning with protecting carbonyl functional groups using 50µL of 40mg/mL of methoxyamine hydrochloride in pyridine at 25°C for 16hours. Then, the samples will be derivatised using 100µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane at 25°C for 60min. The supernatant will be collected by centrifugation at 15 800×g at 4°C for 10min.

Gas chromatography-mass spectrometry (GC-MS) will analyse the metabolites using a 29m long (0.25mm×0.25µm) DB-5 MS column with a 10m DuraGuard precolumn. The Agilent Fiehn GC/MS Metabolomics RTL library will be employed for raw data processing and metabolite identification.^{13 14}

Sleep quality analysis

The Portuguese version of the Pittsburgh Sleep Quality Index (PSQI) questionnaire will be used to assess

sleep quality.¹⁵ The PSQI consists of four open and six multiple-choice questions to assess sleep quality over the last month. It consists of 24 items, 19 of which are self-reported, and their partner or roommate classifies 5. These five items include sleep duration estimates, latency and frequency, and the severity of specific sleep-related problems. Scores are added to generate an overall PSQI score, ranging from 0 to 21 points. Higher scores indicate poorer sleep quality.

Psychological and psychiatric assessments

We will employ the semistructured interview methodology,¹⁶ which combines the spontaneity of the interviewee and clarity of the interviewer's objective with the need for reliable and trustworthy records.

The semistructured interview guide will be as follows:

- ▶ Sports activity record: talk about the main sports activities linked to you throughout your life, situating them over time; that is, at what age you started practising it and at what age you stopped practising it.
- ▶ Is volleyball your main sport activity today? Yes/no. Please explain. If yes, can you comment on the reasons that led you to this sport and what sensations and feelings are involved in the choice and the practice?
- ▶ Up to now, have you always been able to practice the sports activities you want? If not, what were they and why/for what reasons were you unable to practice them? Do you intend, at any point, to try to practice them? Please explain.
- ▶ Do you believe your gender identity can be associated in some way with your sports choices, whether in terms of access or the possibility of practising it in a pleasurable way? If yes, do you understand this association more properly as an incentive, obstacle or other type of association?

In addition, the Five Digits Test¹⁷ will be applied to look at mental processing speed and the ability to direct and switch their attentional control. A formal assessment of depression and suicidal ideation will also be performed using the Patient Health Questionnaire-9.

Data management

Study data will be collected and managed using REDCap (Research Electronic Data Capture) hosted at Centro Universitário São Camilo (São Paulo/Brazil). REDCap^{18 19} is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages and (4) procedures for data integration and interoperability with external sources.

Interim access will be allowed to all researchers to evaluate the research's progress in their respective areas of expertise and provide feedback to the ethics committee that approved the research.

Statistical analysis

Due to the scarcity of potential subjects (TW volleyball athletes) and lack of previous data to guide the sample size estimation, a convenience sample will be used. We intend to evaluate 20 athletes in each group. Data will be analysed with respect to normality using the Shapiro-Wilk test. Normally distributed variables will be compared between groups using variance analysis (analysis of variance) and Tukey's post hoc test. In contrast, the Kruskal-Wallis post hoc test of Dunn will be used for non-parametrical variables. Given that muscle strength and peak oxygen uptake (VO_{2peak}) are dependent variables, linear regression models will be applied to verify the relationship between independent variables and the outcomes. The regression models will be elaborated considering biological plausibility criteria, $p < 0.20$, tolerance and multicollinearity. Independent variables are gender, BC, training status and covariables: age, dietary intake and sleep pattern. Thus, first, we will apply the univariate regression model to verify the association between independent variables and outcomes and variables with $p < 0.20$ will be entered into the multiple models.

Statistical analysis for untargeted metabolomics experiments will be performed using MetaboAnalyst V.5.0 software.²⁰ Data will be normalised and scaled for multivariate statistical analyses. Principal component analysis and supervised partial least squares-discriminant analysis will be performed to visualise the differences between groups.

Paired-samples t-test or Wilcoxon test will compare quantitative variables before and after adequate hormonal therapy among TW. All analyses will be performed using the free software R.

DISSEMINATION

Results will be presented in peer-reviewed journals and at international conferences. We aim to publish a main paper with the principal data of each area of knowledge. Since it is a huge protocol and there are scarce data on this topic, others specific papers of each area will be published.

Auditing

The Ethical Committee of Centro Universitário São Camilo may select the study for auditing at any time. The audit process is independent of investigators and sponsors.

DISCUSSION

The literature has described data about BC, haemoglobin and strength in TW mainly in the first or second year of GAHT. They have shown increases in gross weight, FM and %FM as well as decrease in skeletal muscle mass.²¹ However, the participants' level of physical activity has not been considered. Regarding VO_{2peak}, a study with non-athletes TW showed that its value is intermediate between CM and CW after long-term oestrogen therapy.²² Thus, our study will contribute to the understanding of the impact of GAHT on the performance of TW athletes.

We will be able to compare aerobic capacity and strength of TW to CW and CM, as well as other valuable

aspects such as BC, REE, eating pattern, dietary practices, bone density, cardiac chambers and function, haematological/hormonal status, psychological aspects, sleep quality, and untargeted metabolomic analysis. For the first time in the literature, these TW's parameters will be analysed together, not to mention that some of them have never been analysed before.

However, it should be noted that our study will probably be limited by a small sample size, given that TW in volleyball teams are scarce. Nevertheless, it could influence the decision to carry out similar studies worldwide.

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Contributors Study conception and design: LAMA and RESF. Data acquisition and analysis: LAMA, FPN, LMS, RESF, LMS, FR, LHD-C, MVQDS, RCCB and FML. Manuscript drafting: LAMA, LHD-C, FPN, LSN and GSN. Critical revision: LMA, LHD-C and FPN.

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Competing interests None declared.

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

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Ethical Committee of Centro Universitário São Camilo (CAAE 52428321.2.0000.0062). Written informed consent will be obtained from all participants, and the study will be conducted following the Declaration of Helsinki (SLAVICEK; FORSDAHL, 2009) and all revisions and amendments after that. Any protocol modification will be proposed to the Ethical Committee as amendments and requested approval.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

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