Sporting Performance of Athletes of the Gender Spectrum: A Cross-sectional Comparison Study Protocol.

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Abstract

The question of integrating transgender athletes into their affirmed gender categories is becoming more prominent with sport's governing bodies portraying mixed messaging when it comes to answering this question. Testosterone is beneficial to baseline sports performance, and it has been suggested that the differences in circulating testosterone concentrations between cisgender men and cisgender women explain most of the baseline differences in sports performance between the two groups. However, a secondary factor relative to sports performance is the physiological re-distribution of fat mass driven by gender-affirming hormone treatment (GAHT) and the loss of/gain of muscle mass with GAHT in both trans women and trans men and their effects on transgender sporting performance. Previous studies lack data on sports performance measures outside muscular strength and performance measures such as aerobic capacity, power and strength should be studied in tandem within an athletic cohort of trans women and trans men and compared with cisgender women and cisgender male athletes to ascertain whether any lasting advantages are present. New sports performance data on transgender athletes must be generated to inform a decision-making process to inform if the current policies in place are accurate or inexact to maintain fairness and the integrity of sport. Accordingly, this manuscript aims to further provide sports performance and anthropometrical data from a cross-sectional analysis of athletes from 4 groups, trans men (n = ≥6) and trans women (n = ≥6) who have undergone ≥ 1 year of GAHT, cis men (n = ≥6) and cis women (n = ≥6) to provide further evidence and consequently, guidance to sport's governing bodies for the eligibility of transgender athletes

1. Background

Transgender athletes experience conflict between the gender that they were assigned at birth and their experienced gender[1-3]. Some [3-5] but not all [2, 3, 6] will choose to undergo gender-affirming hormone therapy (GAHT) and patients accessing transgender health services have increased considerably in recent years in many European countries [7-9]. The question of integrating transgender athletes into their affirmed gender categories is becoming more prominent with sport's governing bodies portraying mixed messaging when it comes to answering this question, with some opting for blanket bans on trans women in the female category [10, 11], some opting to ask for the reduction of testosterone in the female category for a period of time [12, 13] and the IOC opting for the premise of self-identification into the athletes chosen category [14].

Testosterone is beneficial to baseline sports performance, and it has been suggested that the differences in circulating testosterone concentrations between cisgender men and cisgender women explain most of the baseline differences in sports performance between the two groups [15] before an athletes skill or opportunities in the sport are considered. Circulating testosterone is greatly correlated with fat free mass ($R = 0.73$, $p < 0.0001$),), thigh ($R = 0.66$, $p < 0.0001$) and quadricep ($R = 0.73$, $p < 0.0001$) muscle volume, while being moderately correlated with leg strength ($R = 0.48$, $p < 0.0005$) [15]. The argument against trans women competing in the female category of sport assumes that trans women have benefitted from high testosterone concentrations from the onset of puberty until the administration of GAHT, that this assumed benefit cannot be mitigated [16], and those cisgender female competitors cannot naturally possess this benefit of high testosterone concentrations [17]. This argument is not present in trans men, as serum testosterone concentrations are recommended to be maintained in the mid-normal range for healthy young men from the onset of GAHT [18], hypothetically not giving trans men the competitive advantages of exogenous testosterone concentrations over cisgender men and this viewpoint is reflected in the current inclusion sports policies for trans men [10-12], although this hypothesis is as yet unconfirmed. It should also be noted, the presence of high circulating testosterone concentrations does not guarantee increased performance, but the way an individual's body physiologically responds to testosterone does [15].

Loss of muscle mass has been reported in trans women following GAHT [19-24] and gains in muscle mass have been shown in trans men [20, 22-24]. Studies have shown that testosterone suppression in cisgender men resulted in decreased muscular strength [25, 26] although this result has been disputed in trans women, with some studies showing increases of 0.5-2% in muscle strength [22, 27, 28] and others showing decreases between 4.3-25% [29-31]. Trans men's muscular strength has been shown to improve by 12-26% during GAHT [22, 31]. However, a secondary factor relative to sports performance is the physiological distribution of fat mass driven by GAHT in both trans women and trans men. As a result of testosterone suppression and oestradiol (E2) supplementation, total body fat has been shown to consistently increase in trans women by 20 - 30% [23, 24, 32, 33] and testosterone

administration decreases body fat in trans men by 11 - 20% [23, 24, 32, 33]. The data above shows trans women reducing their percentage of fat-free mass and trans men increasing their percentage of fat-free mass. This data is intriguing as the differences in percentages of fat-free mass are suggested as the cause of the difference in sports performance between cisgender males, and cisgender females [34, 35]. Cisgender men and women have been shown to have similar relative muscular strength and trans women have also been shown to have 33.8 % weaker relative muscular strength than cisgender men and women while showing a similar absolute strength to cisgender women (31.9 kg ± 2.4 vs. 29.2 kg ± 4.4, [35]).

Previous studies lack data on sports performance measures outside muscular strength. Performance measures such as aerobic capacity, power and strength should be studied in tandem within a cohort of trans women and trans men and compared with cisgender women and cisgender male athletes to ascertain whether any lasting advantages are present. To the author's knowledge, only one study assessed $\dot{V}O_{2max}$ in trans women (n = 8) after GAHT (~15 years), discovering that trans women's absolute $\dot{V}O_{2max}$ sat between cisgender men and cisgender women's $\dot{V}O_{2max}$ [36]. Like muscular strength discussed above [35], dividing absolute $\dot{V}O_{2max}$ by their fat-free mass, trans women again came out below both cisgender men and women, showing relatively, trans women's maximum O_2 uptake is inferior to cisgender men and women. No $\dot{V}O_{2max}$ data has been gained from athletic trans men. It is a well-accepted concept that Hb and Hct concentrations of trans women drop to cisgender female levels after 3-6 months [16, 37, 38] and that trans men's Hb and Hct rise to cisgender male concentrations [38]. It is also well-accepted that reductions in Hb are generally associated with a reduced aerobic capacity [16, 39, 40]. Therefore, lung function should be measured independently and in conjunction with Hb concentrations to understand if any changes in Hb concentrations cause any effect on the VO_{2max} of transgender athletes independent or dependent of lung size.

In contrast to the growing amounts of data highlighting the effects of GAHT in non-athletic transgender populations, sports performance data on transgender athletes is scarce. Roberts *et al* [41], retrospectively found in an athletically trained transgender population that was compared against am athletically trained cisgender population, the upper body strength (37.09%) and core strength (15.94%) baseline advantages of trans women over cisgender women had been reduced (upper body 6.26%, core -1.99%) after 2 years of GAHT while running performance over 1.5-miles remained 12% (baseline 18.81%) faster after 2 years of GAHT [41]. Trans men's upper body strength (-35.42%) and core strength disadvantages (-3.89%) over cisgender men had been overturned into an advantage (upper body 8.55%, core 10.66%) after 2 years of GAHT while trans men's running performance over 1.5-miles was 1.26% (baseline -16.70%) faster than cisgender men after 2 years of GAHT [41]. These findings would suggest a different rate and extent of mitigation of any potential sporting performance advantage conferred by pubertal high testosterone concentrations of trans women given that strength advantages, but not cardiovascular advantages of trans women were mitigated after 2 years of GAHT.

The data above also highlights the performance-enhancing effect of trans men's exogenous testosterone administration and highlights the need to investigate the effects of GAHT on their sporting performance. Particularly, as all 3 metrics had baseline disadvantages under cisgender men ranging from -3.89% to -35.42%, overturned into advantages over cisgender men ranging from 1.66% to 10.66% after 2 years of GAHT. Due to the retrospective and uncontrolled nature of this research, this data requires replication in trained trans women and trans men athletes before any firm conclusions can be drawn.

As a consequence of the little performance data for sport's governing bodies to centre their decisions on, new sports performance data on transgender athletes must be generated to inform a decision-making process, as previously illustrated [42], to inform if the current policies in place are accurate or inexact to maintain fairness and the integrity of sport. Accordingly, this manuscript aims to further provide sports performance and anthropometrical data from a cross-sectional analysis of athletes from 4 groups of the gender spectrum to provide further evidence and consequently, recommendations to sports governing bodies for the eligibility of transgender athletes.

2. Methods

2.1. Study design

This cross-sectional study involves one visit to the laboratory at the School of Applied Sciences, University of Brighton, UK. Each participant will arrive at ~9 am after an overnight fast and depart from testing at ~3 pm. The full study design and the order of tests that will be undertaken by the participants can be found in Figure 1.

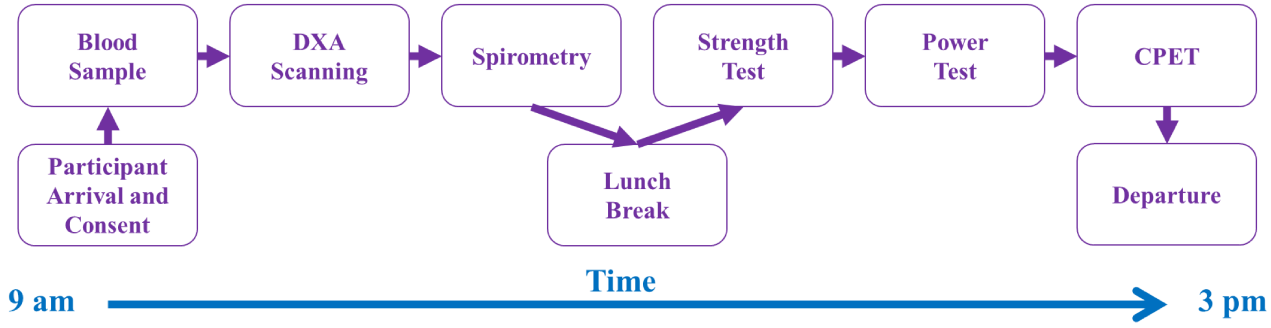

Figure 1. Schedule of activities during participant testing in the laboratory. *DXA, Dual-energy X-ray Absorptiometry; CPET, Cardiopulmonary Exercise Testing.*

2.2. Recruitment

Participants will be recruited through social media advertising on Facebook (Meta Platforms, Inc, California, USA), Instagram (Meta Platforms, Inc, California, USA), and Twitter (Twitter, Inc, California, USA) with the recruitment poster that is supplied in the supplementary materials. All participants will contact the first author (BH) through the email provided in the advert. After the participant responds to the advert, the first author (BH) will email the participant information sheet in return, on the reception of the participant information sheet the participant will have a minimum of 1 week to consider their participation before being invited to travel to the laboratory in Brighton. Before participation, all participants will be orally informed of the study procedures and their written informed consent will be obtained.

2.3. Participants and eligibility criteria

24 participants (6 trans men, 6 trans women, 6 cisgender men, and 6 cisgender women) will be sought that participate in a sport at a competitive level or undergo physical training three times per week. Trans men and trans women athletes must have completed ≥1 year of GAHT, which will be voluntarily disclosed during consent and verified during blood test analysis. The full inclusion/exclusion criteria can be found in Table 1.

Table 1: Inclusion/exclusion criteria for a study participants

Primary Inclusion Criteria

Trans Men and Trans Women	Cisgender Men and Cisgender Women
GAHT for +1 year.	-
Play a Competitive Sport	Play a Competitive Sport
or	or
Physically train 3x per week	Physically train 3x per week
Secondary Exclusion Criteria	
The Physical Activity Readiness Questionnaire highlights health/fitness concerns	
Exclude from DXA scanning only if the participant is: 1. pregnant 2. has a total annual radiation dose above 1mSV	

Notes: *GAHT, Gender-Affirming Hormone Therapy; DXA, Dual-energy X-ray absorptiometry; mSV, millisievert.*

2.4. Laboratory assessments

2.4.1.Blood sampling

Capillary blood samples will be collected from the ring finger of the non-dominant hand via a Unistik® 3 Comfort lancet (Owen Mumford Ltd, Woodstock, UK) due to capillary samples yielding a higher Hb value than venous samples [43]. Venous blood samples will be collected by the first author (BH) or the third author (FG) from an antecubital vein utilizing a closed vacuette system. Two 10 mL whole blood samples will be collected into a BD® serum tube (Becton, Dickinson, and Company, Wokingham, Berkshire, UK) for serum extraction. Once collected the tubes will be left at room temperature (18 ± 5°C) for 1 hour and then stored in a fridge (3 ± 2°C) for up to 4 hours maximum before being centrifuged by the first (BH) or second (CC) author at 1300G for 10 min at 4°C.

2.4.2. Body composition and Bone Mass

The participants will undertake a pre-DXA health questionnaire to screen for previous radiation exposure and a Physical Activity Readiness Questionnaire for Everyone to screen the participant's ability to undertake exercise. Body composition and Bone Mass will then be measured by DXA (Horizon W, Hologic Inc., Massachusetts, USA). Each participant will undergo a whole-body scan, a femoral neck scan and a lumbar spine scan in succession. The participant will be asked to lie on the scan bed and all participant placement for the three scans will be done by the first author (BH).

2.4.3. Lung Function

Lung function will be measured using a Vitalograph Alpha spirometer (Vitalograph Inc, Kansas, USA) with an antibacterial filter placed on the spirometer and a nose clip placed on the bridge of the participant’s nose. Each participant will be asked to perform 2 tests, the Vital Capacity (VC), and the Forced Vital Capacity (FVC) test. The participant will be seated in a slightly reclined chair so as not to close the lungs. For the VC test, the participant will be asked to breathe maximally and then exhale into the spirometer as forcefully and as long as possible. For the FVC test, the participant will perform a flow-volume loop manoeuvre with the nose clip on by inhaling as deeply as possible with their lips tightly over the tube followed by exhaling as forcefully as the participant can, repeating this manoeuvre twice. The two tests will be repeated until a trend of declining performance occurs for each test. The best result for each test will then be recorded.

2.4.4. Strength

Testing of strength will be measured with a handgrip dynamometer (TAKEI 5401, TAKEI Scientific Instruments Co., Ltd, Japan). The participant's hand size will be measured around the metacarpophalangeal joints of both hands before the testing begins, afterwards, the participants will be seated in a chair with their ankles placed against the legs of the chair and their backs against to back of the chair. The participants will then place their non-testing hand on their closest thigh, while

their testing arm is flexed to a 90° angle, with the palm facing medially. If the participants feet or hands move from the protocol during their attempt this will render that attempt void and this data will be excluded. The dynamometer will then be placed in their hand by the first (BH) or second author (CC) and the participant will be asked to squeeze for 10 seconds. Each hand will be tested 3 times in sequential order of left-right to allow each hand to rest, for a total of 6 repetitions and the mean scores will be taken for each hand.

2.4.5. Lower Body Power

Lower body power will be measured with the counter-movement jump (CMJ) manoeuvre on a JUM001 Jump Mat (Probotics Inc, Alabama, USA). Before testing, the participants will be asked to cycle on a cycle ergometer (Monark Exercise AB, Vansbro, Sweden) for 20 minutes at 60 revolutions per minute to warm up the muscle groups of the legs. During this cycle, the participants will be shown the technique of the CMJ procedure by the first (BH) or second author (CC). The test will be controlled to ~45° of counter-movement and hands must be placed on hips to prevent arm swing. The participant will then be allowed a period of sub-maximal familiarization and coaching by the first (BH) or second author (CC). During the test, if the participant went beyond 45° of counter-movement, or the hands came off the hips the test would be declared void for that attempt. After the recording of 3 legal maximal attempts, the mean scores will be recorded.

2.4.6. Cardiopulmonary Exercise Testing (CPET)

CPET will be performed using a 95T Engage Treadmill ergometer (Life Fitness, Illinois, USA). A landing crash mat was placed behind the treadmill for participant safety in case of falls. To ensure test accuracy, the metabolic gas will be calibrated before each test with COSMED™ certified reference gas of 16% O_2 and 5% CO_2 and the turbine flowmeter will be calibrated before each test using a certified 3L calibration syringe (Hans Rudolph, Kansas, USA) according to manufacturer instructions. The participants will wear a mask (Hans Rudolph, Kansas, USA) that will be strapped to the participant's head via Velcro™ straps and the participants' inspiration and expiration will be measured by O_2 and CO_2 analysers in the flowmeter sample line via breath-by-breath analysis (Omnia, Quark CPET, COSMED™ Srl, Rome, Italy). Heart Rate (HR and HR_{Max} will be recorded using Huawei Watch GT smartwatch HR technology (Huawei Technologies Co., Ltd, Shenzhen, China) secured to the participant's right wrist. The participants will be monitored under relaxed conditions for ~5 minutes to make sure that their resting Respiratory Quotient (RQ) will be ~0.80 and the participant's resting $\dot{V}O_2$ is ~ 500ml/min to prevent a false positive test. During the test, the participant's HR was monitored every minute and recorded after 2 minutes of each elevation of workload and the participant was then also asked to point to or signal a Rating of Perceived Exertion (RPE) scale (revised Borg 10-grade scale), allowing for compensation of each workload to occur. The participant will be considered to have reached their $\dot{V}O_{2max}$ if three out of four of the following occurred: a plateau or 'peaking over' in oxygen uptake, an RQ of ≥1.1, maximal HR is reached, and/or volitional

exhaustion. If three out of the four do not occur, then the test will not be considered for analysis. All $\dot{V}O_{2max}$ tests will be conducted and analysed by the first author (BH) to avoid any inter-investigator variability. The ramp protocol of Badawy and Muaidi [44] treadmill $\dot{V}O2max$ testing will be used for each $\dot{V}O_{2max}$ test.

2.5. Outcome measures

2.5.1. Blood measurements

Capillary Hb will be analysed by a HemoCue® 201+ (HemoCue AB, Ängelholm, Sweden) reported in grams per litre (g/l), Oestradiol (E2) concentrations in serum will be measured using tandem mass spectrometry (Model to be confirmed) and will be reported in picograms per millilitre (pg/mL), and testosterone in serum concentrations will also be measured using tandem mass spectrometry and will be reported in nanomoles per litre (nmol/L).

2.5.2. Body composition and Bone Mass

All analysis will be completed immediately after the 3 scans using Apex v5.6.0.5 software (Hologic, Connecticut, USA) by the first author (BH). Due to the in-built analysis assumptions regarding the measurement of head fat mass and percentage fat of 17%, subtotal data (whole-body less head) data will be used to report anthropometric data, which will also be reported regionally. Anthropometric data will be reported as fat mass (Kg), lean mass (Kg), and Fat-free mass (Kg). Bone measures will be reported as bone area (cm^{-3}), bone mineral content (BMC, g) and bone mineral density (BMD, $g \cdot cm^3$). A report of the DXA analysis will be given to each participant.

2.5.3. Lung function

All lung function tests will be analysed by the inbuilt analysis hardware of the Vitalograph Alpha spirometer. Lung function data will be presented as vital capacity (VC) in litres (L), to determine the maximum amount of air exhaled in a relaxed state; forced vital capacity (FVC) in L to determine the maximum volume of air exhaled forcefully; forced expiratory volume in 1 second (FEV^1) in L to determine the volume of air that the participant can forcibly expire in the first 1 second; $FEV^{1\%}$ as a percentage (%), to determine the proportion of the participants VC that they can expire in the FEV^1 to the full, FVC; peak expiratory flow (PEF) in L per minute (L/min), to determine the maximum speed of maximally forced expiration initiated at full inspiration. Lastly, forced expiratory flow (FEF^{25-75}) in L to determine the mean flow of expired air between 25-75% of FVC. A report of the lung function analysis will be given to each participant.

2.5.4. Muscular Strength

Hand size for both the participant's right hand and the left hand will be presented in centimetres (cm). Absolute handgrip scores will be presented in Kg, relative handgrip scores will be presented as the

absolute handgrip score divided by hand size (kg/cm) and the absolute hand grip score divided by FFM reported by DXA (kg/kg).

2.5.5. Lower Body Power

Counter movement jump height in inches (in) and airtime will be reported in seconds (s) and analysed by the inbuilt hardware of the JUM001 Jump Mat. Jump height will be converted to cm by multiplying the result in inches by 2.54. Peak power in watts (W) and Average power in W will be determined by the equations developed by Johnson and Bahamonde [45] shown below:

$$Peak\ power\ (W) = \left((78.6 \times Jump\ Height[cm]) + (60.3 \times mass[kg])\right) - \left((15.3 \times height\ [cm]) - 1308\right)$$

$$Average\ Power\ (W) = \left((43.8 \times Jump\ Height[cm]) + (32.7 \times mass[kg])\right) - \left((16.8 \times height\ [cm]) + 431\right)$$

2.5.6. Maximal Oxygen Uptake ($\dot{V}O_{2max}$)

Each $\dot{V}O_{2max}$ test will be analysed immediately using COSMED Omnia software (COSMED Srl, Rome, Italy) while the participant is in a cool-down period and the results will be immediately emailed to the participant. Absolute $\dot{V}O_{2max}$ and absolute anaerobic threshold (AT) will be reported in millilitres per min (ml/min). Relative $\dot{V}O_{2max}$ and AT will be presented by dividing ml/min by body mass (ml/min/kg) and FFM reported by DXA (ml/min/kg). The respiratory quotient (RQ) at $\dot{V}O_{2max}$, defined as the volume of carbon dioxide released over the volume of oxygen absorbed during respiration, will be reported alongside the HR_{max} recorded at the participant's $\dot{V}O_{2max}$.

2.6.Statistical analyses

Statistical analysis was performed using Jamovi [46]. The mean and standard deviations (SD) will be presented to compare the differences between the means. The mean scores for the measures of, testosterone, E2, and Hb will be compared using a two-way ANOVA with the two factors of gender and length of GAHT. The measures of body composition and bone mass will be compared with a three-way ANOVA with three factors being gender, testosterone and E2. Lung function, muscular strength, lower body power and maximal oxygen uptake will be compared with a four-way ANOVA with the four factors being gender, testosterone, E2 and FFM. All ANOVAs will be combined with a Turkey post-hoc correction. Pearson's correlation coefficient will be used to assess the relationship between the blood measures of testosterone, E2 and Hb, in addition to the DXA measure of FFM, with the measures of muscular strength, lower body power, and maximal oxygen uptake. Due to the large number of comparisons expected, a Bonferroni correction will be applied for the correlation

analysis which will be determined by $\alpha = \frac{\alpha}{m}$ where α is the desired alpha level of 0.05 and m is the number of hypotheses tested. This will be calculated manually.

2.7.Ethical Approval and Funding

Ethical Approval for this study has been granted by the School of Applied Sciences Research Ethics Committee of the University of Brighton, Brighton, UK (Ref: 9496). The study has also been funded through a combination of the School of Applied Sciences, University of Brighton, UK, and the International Olympic Committee (IOC Grant 2018), Lausanne, Switzerland.

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Competing interests

All authors declare no competing interests in this research.

Author Contributions

Conceptualization, BH and FG; Methodology, BH; Data Collection: BH and CC; writing–original draft preparation: BH, CC and FG Writing–review and editing: ALL.

Data Availability

An Open Science Framework project titled Sporting Performance of Athletes of the Gender Spectrum: A Cross-sectional Comparison Study with all materials can be found here: (insert link)

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