

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

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33

1 Abstract

2 The question of integrating transgender athletes into their affirmed gender categories is becoming
3 more prominent with sport's governing bodies portraying mixed messaging when it comes to
4 answering this question. Testosterone is beneficial to baseline sports performance, and it has been
5 suggested that the differences in circulating testosterone concentrations between cisgender men and
6 cisgender women explain most of the baseline differences in sports performance between the two
7 groups. However, a secondary factor relative to sports performance is the physiological re-distribution
8 of fat mass driven by gender-affirming hormone treatment (GAHT) and the loss of/gain of muscle
9 mass with GAHT in both trans women and trans men and their effects on transgender sporting
10 performance. Previous studies lack data on sports performance measures outside muscular strength
11 and performance measures such as aerobic capacity, power and strength should be studied in tandem
12 within an athletic cohort of trans women and trans men and compared with cisgender women and
13 cisgender male athletes to ascertain whether any lasting advantages are present. New sports
14 performance data on transgender athletes must be generated to inform a decision-making process to
15 inform if the current policies in place are accurate or inexact to maintain fairness and the integrity of
16 sport. Accordingly, this manuscript aims to further provide sports performance and anthropometrical
17 data from a cross-sectional analysis of athletes from 4 groups, trans men ($n = \geq 6$) and trans women (n
18 $= \geq 6$) who have undergone ≥ 1 year of GAHT, cis men ($n = \geq 6$) and cis women ($n = \geq 6$) to provide
19 further evidence and consequently, guidance to sport's governing bodies for the eligibility of
20 transgender athletes

1 1. Background

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

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

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

1 administration decreases body fat in trans men by 11 - 20% [23, 24, 32, 33]. The data above shows
2 trans women reducing their percentage of fat-free mass and trans men increasing their percentage of
3 fat-free mass. This data is intriguing as the differences in percentages of fat-free mass are suggested as
4 the cause of the difference in sports performance between cisgender males, and cisgender females [34,
5 35]. Cisgender men and women have been shown to have similar relative muscular strength and trans
6 women have also been shown to have 33.8 % weaker relative muscular strength than cisgender men
7 and women while showing a similar absolute strength to cisgender women ($31.9 \text{ kg} \pm 2.4$ vs. 29.2 kg
8 ± 4.4 , [35]).

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

24 In contrast to the growing amounts of data highlighting the effects of GAHT in non-athletic
25 transgender populations, sports performance data on transgender athletes is scarce. Roberts *et al* [41],
26 retrospectively found in an athletically trained transgender population that was compared against an
27 athletically trained cisgender population, the upper body strength (37.09%) and core strength
28 (15.94%) baseline advantages of trans women over cisgender women had been reduced (upper body
29 6.26%, core -1.99%) after 2 years of GAHT while running performance over 1.5-miles remained 12%
30 (baseline 18.81%) faster after 2 years of GAHT [41]. Trans men's upper body strength (-35.42%) and
31 core strength disadvantages (-3.89%) over cisgender men had been overturned into an advantage
32 (upper body 8.55%, core 10.66%) after 2 years of GAHT while trans men's running performance over
33 1.5-miles was 1.26% (baseline -16.70%) faster than cisgender men after 2 years of GAHT [41]. These
34 findings would suggest a different rate and extent of mitigation of any potential sporting performance
35 advantage conferred by pubertal high testosterone concentrations of trans women given that strength
36 advantages, but not cardiovascular advantages of trans women were mitigated after 2 years of GAHT.

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

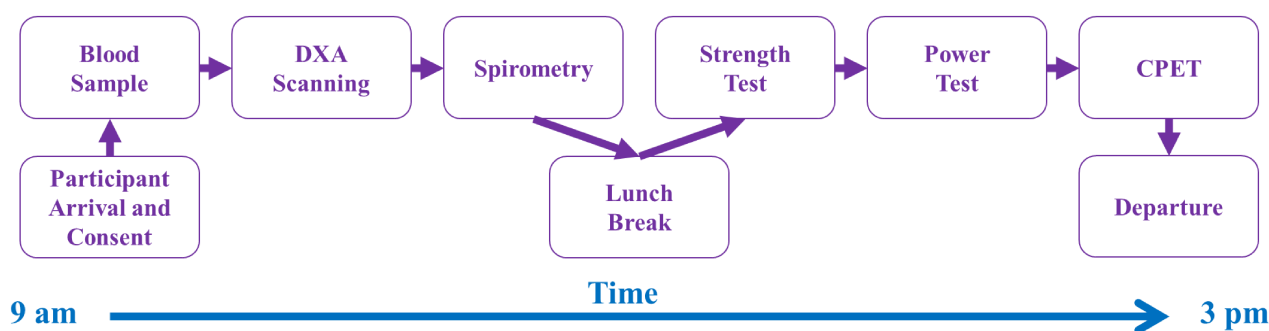
8 As a consequence of the little performance data for sport's governing bodies to centre their decisions
9 on, new sports performance data on transgender athletes must be generated to inform a decision-
10 making process, as previously illustrated [42], to inform if the current policies in place are accurate or
11 inexact to maintain fairness and the integrity of sport. Accordingly, this manuscript aims to further
12 provide sports performance and anthropometrical data from a cross-sectional analysis of athletes from
13 4 groups of the gender spectrum to provide further evidence and consequently, recommendations to
14 sports governing bodies for the eligibility of transgender athletes.

15 2. Methods

16 2.1. Study design

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

21



22

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

1 2.2. Recruitment

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

11 2.3. Participants and eligibility criteria

12 24 participants (6 trans men, 6 trans women, 6 cisgender men, and 6 cisgender women) will be
13 sought that participate in a sport at a competitive level or undergo physical training three times per
14 week. Trans men and trans women athletes must have completed ≥ 1 year of GAHT, which will be
15 voluntarily disclosed during consent and verified during blood test analysis. The full
16 inclusion/exclusion criteria can be found in Table 1.

17 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.

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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 ($18 \pm 5^\circ\text{C}$) for 1 hour and then stored in a fridge ($3 \pm 2^\circ\text{C}$) 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

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

7 2.4.5. Lower Body Power

8 Lower body power will be measured with the counter-movement jump (CMJ) manoeuvre on a
9 JUM001 Jump Mat (Probotics Inc, Alabama, USA). Before testing, the participants will be asked to
10 cycle on a cycle ergometer (Monark Exercise AB, Vansbro, Sweden) for 20 minutes at 60 revolutions
11 per minute to warm up the muscle groups of the legs. During this cycle, the participants will be shown
12 the technique of the CMJ procedure by the first (BH) or second author (CC). The test will be
13 controlled to ~45° of counter-movement and hands must be placed on hips to prevent arm swing. The
14 participant will then be allowed a period of sub-maximal familiarization and coaching by the first
15 (BH) or second author (CC). During the test, if the participant went beyond 45° of counter-movement,
16 or the hands came off the hips the test would be declared void for that attempt. After the recording of
17 3 legal maximal attempts, the mean scores will be recorded.

18 2.4.6. Cardiopulmonary Exercise Testing (CPET)

19 CPET will be performed using a 95T Engage Treadmill ergometer (Life Fitness, Illinois, USA). A
20 landing crash mat was placed behind the treadmill for participant safety in case of falls. To ensure test
21 accuracy, the metabolic gas will be calibrated before each test with COSMED™ certified reference
22 gas of 16% O₂ and 5% CO₂ and the turbine flowmeter will be calibrated before each test using a
23 certified 3L calibration syringe (Hans Rudolph, Kansas, USA) according to manufacturer instructions.
24 The participants will wear a mask (Hans Rudolph, Kansas, USA) that will be strapped to the
25 participant's head via Velcro™ straps and the participants' inspiration and expiration will be measured
26 by O₂ and CO₂ analysers in the flowmeter sample line via breath-by-breath analysis (Omnia, Quark
27 CPET, COSMED™ Srl, Rome, Italy). Heart Rate (HR and HR_{Max} will be recorded using Huawei
28 Watch GT smartwatch HR technology (Huawei Technologies Co., Ltd, Shenzhen, China) secured to
29 the participant's right wrist. The participants will be monitored under relaxed conditions for ~5
30 minutes to make sure that their resting Respiratory Quotient (RQ) will be ~0.80 and the participant's
31 resting $\dot{V}O_2$ is ~ 500ml/min to prevent a false positive test. During the test, the participant's HR was
32 monitored every minute and recorded after 2 minutes of each elevation of workload and the
33 participant was then also asked to point to or signal a Rating of Perceived Exertion (RPE) scale
34 (revised Borg 10-grade scale), allowing for compensation of each workload to occur. The participant
35 will be considered to have reached their $\dot{V}O_{2max}$ if three out of four of the following occurred: a
36 plateau or 'peaking over' in oxygen uptake, an RQ of ≥ 1.1 , maximal HR is reached, and/or volitional

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

5 **2.5.Outcome measures**

6 2.5.1. Blood measurements

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

12 2.5.2. Body composition and Bone Mass

13 All analysis will be completed immediately after the 3 scans using Apex v5.6.0.5 software (Hologic,
14 Connecticut, USA) by the first author (BH). Due to the in-built analysis assumptions regarding the
15 measurement of head fat mass and percentage fat of 17%, subtotal data (whole-body less head) data
16 will be used to report anthropometric data, which will also be reported regionally. Anthropometric
17 data will be reported as fat mass (Kg), lean mass (Kg), and Fat-free mass (Kg). Bone measures will be
18 reported as bone area (cm^2), bone mineral content (BMC, g) and bone mineral density (BMD,
19 $\text{g}\cdot\text{cm}^{-3}$). A report of the DXA analysis will be given to each participant.

20 2.5.3. Lung function

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

31 2.5.4. Muscular Strength

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

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

3 2.5.5. Lower Body Power

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

$$\begin{aligned} 8 \quad & \text{Peak power (W)} \\ 9 \quad & = ((78.6 \times \text{Jump Height[cm]}) + (60.3 \times \text{mass[kg]})) \\ 10 \quad & - ((15.3 \times \text{height [cm]}) - 1308) \end{aligned}$$

$$\begin{aligned} 11 \quad & \text{Average Power (W)} \\ 12 \quad & = ((43.8 \times \text{Jump Height[cm]}) + (32.7 \times \text{mass[kg]})) \\ 13 \quad & - ((16.8 \times \text{height [cm]}) + 431) \end{aligned}$$

14 2.5.6. Maximal Oxygen Uptake ($\dot{V}O_{2\max}$)

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

22 2.6. Statistical analyses

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

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

3 **2.7.Ethical Approval and Funding**

4 Ethical Approval for this study has been granted by the School of Applied Sciences Research Ethics
5 Committee of the University of Brighton, Brighton, UK (Ref: 9496). The study has also been funded
6 through a combination of the School of Applied Sciences, University of Brighton, UK, and the
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11 **Competing interests**

12 All authors declare no competing interests in this research.

13 **Author Contributions**

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

16 **Data Availability**

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

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