VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

Uncovering the effects of gender affirming hormone therapy on skeletal muscle and epigenetics: protocol for a prospective matched cohort study in transgender individuals (the GAME study)

BMJ Open

Uncovering the effects of gender affirming hormone therapy on skeletal muscle and epigenetics: protocol for a prospective matched cohort study in transgender individuals (the GAME study)

Patrice R Jones [ID] ,[1] Sarah Voisin,[1] Brendan J Nolan [ID] ,[2,3] Shanie Landen,[1] Macsue Jacques,[1] Beau Newell,[4] Sav Zwickl,[2] Teddy Cook,[4] Alex Wong [ID] ,[2] Ariel Ginger,[2] Andrew Palmer,[1] Andrew Garnham,[1] Javier Alvarez-Romero,[1] Namitha Mohandas,[1] Kirsten Seale,[1] Ada Cheung,[2,3] Nir Eynon[1]

ABSTRACT

Introduction Gender affirming hormone therapy (GAHT) is increasingly used by transgender individuals and leads to shifts in sex hormone levels. Skeletal muscle is highly responsive to hormone activity, with limited data on the effects of GAHT on different human tissues. Here, we present the protocol for the GAME study (the effects of Gender Affirming hormone therapy on skeletal Muscle training and Epigenetics), which aims to uncover the effects of GAHT on skeletal muscle 'omic' profiles (methylomics, transcriptomics, proteomics, metabolomics) and markers of skeletal muscle health and fitness.

Methods and analysis This study is a prospective age-matched cohort study in transgender adults commencing GAHT (n=80) and age-matched individuals not commencing GAHT (n=80), conducted at Austin Health and Victoria University in Victoria, Australia. Assessments will take place prior to beginning GAHT and 6 and 12 months into therapies in adults commencing GAHT. Age-matched individuals will be assessed at the same time points. Assessments will be divided over three examination days, involving (1) aerobic fitness tests, (2) muscle strength assessments and (3) collection of blood and muscle samples, as well as body composition measurements. Standardised diets, fitness watches and questionnaires will be used to control for key confounders in analyses. Primary outcomes are changes in aerobic fitness and muscle strength, as well as changes in skeletal muscle DNA methylation and gene expression profiles. Secondary outcomes include changes in skeletal muscle characteristics, proteomics, body composition and blood markers. Linear mixed models will be used to assess changes in outcomes, while accounting for repeated measures within participants and adjusting for known confounders.

Ethics and dissemination The Austin Health Human Research Ethics Committee (HREC) and Victoria University HREC granted approval for this study (HREC/77146/Austin-2021). Findings from this project will be published in open-access, peer-reviewed journals and presented to scientific and public audiences.

Trial registration number ACTRN12621001415897; Pre-results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The GAME study will comprehensively study the effects of gender affirming hormone therapy (GAHT) on skeletal muscle 'omic profiles, muscle health and performance.
⇒ Age-matched male and female comparator groups will allow the differing effects of masculinising and feminising GAHT in skeletal muscle to be distinguished from other potential confounders.
⇒ This study will be rigorous in using standardised diets, fitness watches and questionnaires to further limit variability introduced from confounders and unmeasured variables.
⇒ The study is limited to one geographical region that may influence the generalisability of findings, and involves extended follow-up periods, which may influence study attrition.

INTRODUCTION

Transgender (or trans) individuals have a gender that is different from what was presumed for them at birth, including (but not limited to) trans, gender diverse and non-binary gender identities, or have a cultural gender identity different to man or woman. Gender affirming hormone therapy (GAHT) is an important component of care for many trans people and results in distinct changes in sex hormone milieu.[1] These hormone therapies are medical forms of gender affirmation,

acknowledging that a person's gender affirming experience may not involve medical interventions, and may also include social and legal gender affirming actions. For some, masculinising therapies with testosterone may be sought, which leads to serum total testosterone concentrations increasing to the typical male reference range. Comparatively, feminising therapies often involves oestrogen estradiol and anti-androgens, which lower serum testosterone concentrations to the typical female reference range while increasing estradiol concentrations. The subsequent physiological effects of shifting sex hormone levels in different tissues is unclear. Skeletal muscle has vital roles in driving exercise and metabolic responses and is highly responsive to hormonal changes,[2 3] and therefore represents a target tissue requiring comprehensive study in this context.

There are sex differences in skeletal muscle physiology, metabolism and responses to exercise,[4–6] with sex hormones partially contributing to these differences.[7–10] For example, females tend to oxidise more fats during endurance exercise whereas males oxidise more carbohydrates, with this in part modulated by estradiol.[4 10] However, the influence of these hormones is confounded by the presence of biological (eg, sex chromosomes) and environmental factors (eg, exercise, diet), that also influence sex variability in muscle phenotypes and exercise responses. Studies in individuals commencing GAHT provides a unique opportunity to directly assess in humans, how sex hormones shape skeletal muscle health and functioning, irrespective of other factors. Such studies may provide insights into the tissue-specific impacts of GAHT in trans people, and shed light on the fundamental mechanisms by which sex hormones drive sex-specific differences in all humans.

Prior studies link GAHT to substantial changes in muscle mass, with masculinising therapies increasing muscle mass (~5%–10%),[11] and feminising therapies reducing muscle mass (~3%–5%).[11 12] Whether these body composition changes reflect changes in skeletal muscle functioning is unclear. Feminising and masculinising GAHT leads to shifts in metabolic markers, such as blood lipid profiles[1] and insulin resistance,[13 14] which may reflect altered metabolic roles of skeletal muscle. A recent systematic review found consistent reductions in circulating haemoglobin and haematocrit levels following feminising treatments,[12] with an opposite trend shown for masculinising treatments.[15] This suggests GAHT affects oxygen transport and uptake, with possible impacts on aerobic exercise performance. However, no studies have yet evaluated aerobic fitness before and following commencement of GAHT. Muscle strength is further shown to be impacted by GAHT, with feminising therapies decreasing muscle strength,[12] and increases shown in masculinising therapies.[16 17] However, most of these studies have been limited to hand-grip strength assessments, given their availability in clinical settings. A comprehensive battery of aerobic, strength and body composition tests are required to truly uncover the effects of GAHT on muscle performance.

Such studies may inform the development of trans and gender diverse participation guidelines for sports with traditional single-sex sporting competitions.

Epigenetic changes may underpin the physiological and metabolic changes observed in different tissues following GAHT. Epigenetics refers to DNA modifications that regulate gene expression and function without changing the DNA code.[18 19] The epigenome is highly sensitive to stimuli such as exercise, diet and hormones,[4 20] with the best characterised epigenetic modification being DNA methylation. Prior studies show GAHT leads to shifts in the transcriptome (gene expression) of rectal mucosa,[18] and the methylome and transcriptome of blood.[19 21] A study of trans men and women (n=12/6) found correlations between changes in blood *ESR1* (oestrogen receptor 1) and *AR* (androgen receptor) methylation patterns and several metabolic and anthropometric parameters following 12 months of GAHT.[19] For example, positive correlations were shown between *ESR1* methylation patterns and body mass index changes in trans men, and *AR* methylation patterns and high-density lipoprotein cholesterol levels in trans women.[19] Skeletal muscle is highly responsive to sex hormones, which may drive epigenetic changes, however, the epigenetic effects of GAHT have not yet been explored in this tissue.

Given the growing use of GAHT by trans individuals,[22] well-designed studies are needed to advance our understanding of the effects of GAHT on skeletal muscle health and function. Research in this area may improve outcomes, personalise care for trans people and promote the safe and fair inclusion and participation of trans people into sports. Here, we present the study protocol for the GAME study (the effects of <u>G</u>ender <u>A</u>ffirming hormone therapy on skeletal <u>M</u>uscle training and <u>E</u>pigenetics). This study aims to uncover the impacts of GAHT on the 'omic' profiles (epigenomics, transcriptomics, proteomics, metabolomics) of skeletal muscle in trans individuals. We will assess how these effects correlate with changes in markers of skeletal muscle health and fitness, providing new insight into the influence of sex hormones on skeletal muscle and the potential health implications of GAHT.

METHODS
Overview of study design
The GAME study is a prospective age-matched cohort study in adults (18–65 years of age) commencing GAHT and age-matched individuals not commencing GAHT (comparator group). This is a multicentre study, with assessments conducted over two sites in Victoria, Australia: Austin Health, and Victoria University, Melbourne, from early 2022 until project completion (expected 2025). In adults commencing GAHT, assessments will take place at three time points; prior to beginning GAHT (baseline), and 6 and 12 months into treatment. Age-matched individuals will be assessed at the same time points. The use of both baseline data and age-matched comparator groups
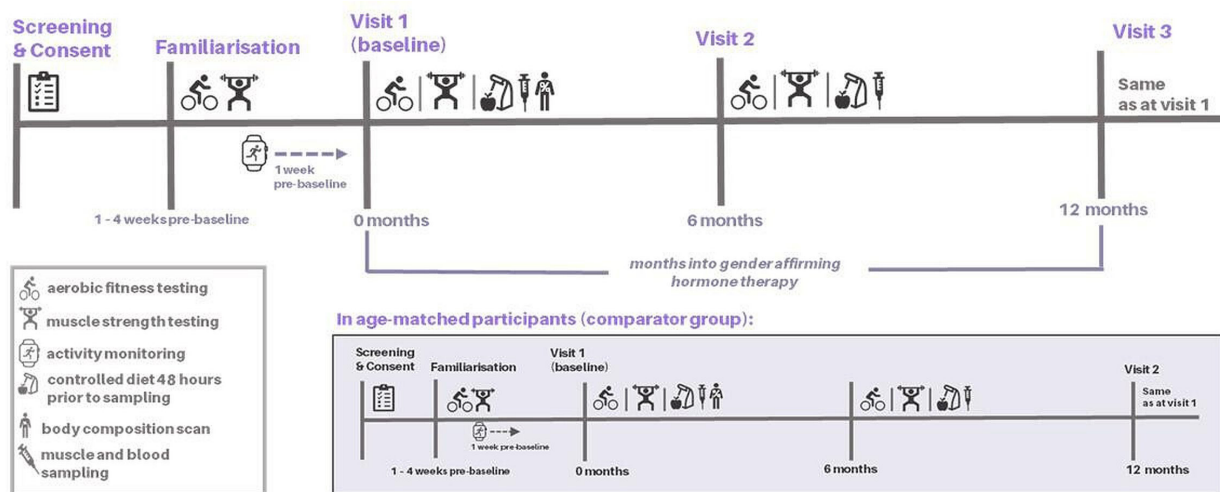
Figure 1 Timeline of the GAME study.

will allow for a comprehensive assessment of the differing effects of masculinising and feminising GAHT on molecular and physiological changes in skeletal muscle and related blood markers.

The timeline of the GAME study is outlined in figure 1. Potential participants will first be invited to discuss the study design, commitment and potential benefits and risks with a researcher at Victoria University. Eligibility will be assessed using a medical history questionnaire (see table 1: study inclusion/exclusion criteria) and all participants will provide written consent before study commencement.

Following recruitment but prior to baseline assessments, participants will complete several questionnaires (demographic, dietary, medical/exercise history) and wear a fitness watch for 7 days to monitor habitual physical activity. During the first session (~1–2 hours), participants will be familiarised with the aerobic fitness and muscle strength tests, which will be performed at each time point throughout the study.

The baseline assessment will be then divided over three examination days of 1–2 hours each, separated by at least 48 hours (72 hours for muscle biopsies) to minimise the effects of tests on each other; day 1; aerobic fitness tests, day 2; muscle strength assessments, day 3; collection of blood and muscle samples at rest and body composition measurements. Following the baseline assessments, adults commencing GAHT will complete two further assessments at 6 and 12 months into therapies. These assessments will be the same as those conducted at baseline, with the exception that body composition scans will only be undertaken again at 12 months. In the age-matched comparator groups, assessments will also be completed again following 6 and 12 months. Participants will be invited to perform another familiarisation exercise session before each time point, given each time point will be separated by at least 6 months. Participants will also be asked to refrain from strenuous exercise for 24 hours before each examination day.

Outcomes and analyses
Primary outcomes:
▶ Aerobic fitness and muscle strength measures.
▶ DNA methylation and gene (messenger RNA) expression profiles in skeletal muscle.
Secondary outcomes:

Table 1	Inclusion and exclusion criteria for the GAME study
Inclusion criteria	**Exclusion criteria**
▶ Adults aged 18 years and over. ▶ Willingness to comply with study requirements. ▶ Individuals about to commence gender affirming hormone therapy. ▶ Comparator women and men will be age-matched to recruited individuals commencing GAHT.	▶ Prior use of GAHT or prior hypogonadism including previous oophorectomy or orchidectomy. ▶ Medical history of cardiovascular conditions, dizziness or fainting during exertion, significant chronic or recurrent respiratory conditions, neuromuscular and major musculoskeletal problems interfering with ability to cycle, uncontrolled endocrine and metabolic disorders or diabetes requiring insulin and other therapies, current pregnancy, infectious blood borne disease, disorders or use of medications that will affect blood clotting and allergies to anaesthetic. ▶ Active nicotine (cigarette/vapes) use. ▶ Inability to provide written informed consent.
GAHT, gender affirming hormone therapy .	

3

- Sex hormone concentrations by immunoassay.
- Body composition, as assessed by dual-energy X-ray absorptiometry (DEXA).
- Blood lipid profiles.
- Levels of haemoglobin, and haematocrit.
- Insulin resistance (measured by homeostatic model assessment for insulin resistance (HOMA-IR); fasting glucose × fasting insulin/22.5).
- Skeletal muscle physiological and molecular markers (muscle fibre characteristics, proteomics, metabolomics, mitochondrial function and microRNA content).
- Genetic variants related to exercise response (in mitochondrial and nuclear DNA).

Medical, demographic and quality of life questionnaires

Questionnaires will be used to screen participants (see table 1: study inclusion/exclusion criteria) on their medical history current and past medication use, current hormonal therapy (dose/mode of administration/duration of therapy), demographics and lifestyle habits such as smoking and alcohol consumption. The medical history questionnaires will be completed with assistance from researchers at Victoria University. In the case that adequate details on medication use and current hormonal therapy cannot be gathered directly from participants, this information will be obtained via their medical records following consent. During the study, participants will also be asked to complete a short 5-item quality of life questionnaire (5-level EuroQol 5-dimension questionnaire (EQ-5D-5L[23])) to monitor changes in quality of life.

Physical activity monitoring

To control for potential differences in habitual physical activity between participants, participant's activity level will be monitored for seven consecutive days prior to baseline assessments. Monitoring participants' activity level will be performed using a Polar Unite fitness watch (Polar Electro Oy, Kempele, Finland), which provides objective measurement of human activity (eg, active time, steps, estimated calories expended). These Polar Unite devices are water resistant, can be programmed for 130 types of physical activity (eg, swimming, cycling), and will be worn on participant's wrist while they are awake. Polar watches estimate energy expenditure using both acceleration and heart rate signals, with prior studies showing that devices with heart rate sensors provide improved estimates of energy expenditure compared with accelerometers alone[24] and polar devices perform well compared with other wrist worn devices.[24 25] To identify changes in participants physical activity throughout the study, participants will also be asked complete a physical activity and exercise history questionnaire (adapted from Active Australia Survey[26]) prior to each time point.

Controlled diet and dietary questionnaires

To minimise the confounding effects of diet on outcomes, the diets of participants will be standardised before muscle and blood collection. At each time point, participants will be provided with an individualised pre-packaged diet for the 48 hours prior to muscle and blood sampling. The energy content of the provided meals will be calculated using the Mifflin-St Jeor equation using the participant's body mass, height, age and physical activity levels.[27] This equation differs by sex, with formula most aligned to the participant's gender identity and/or hormone therapy (ie, testosterone or oestrogen based) to be used, as recommended in prior case studies.[28] The content of the diets will be based on the current Australian National Health and Medical Research Council guidelines (15%–20% protein, 50%–55% carbohydrates <30% fat and <10% of saturated fat) with dietary restrictions and preferences considered.

Participants will be asked to fast for a minimum of 8 hours prior to the collection of muscle and blood samples, and refrain from caffeine and alcohol consumption before all tests. To identify any significant changes in participants dietary habits throughout the study, participants will also be asked complete a web-based 24-hour dietary recall (the Automated Self-Administered 24-Hour Dietary Assessment Australia Tool[29]) before all time points. This recall will take ~10–30 min to complete and will be self-administered, with assistance from a nutritionist in the study team if required. Paper versions of 24-hour dietary recalls will also available.

Aerobic fitness

At each time point, participants will perform a graded-exercise test (GXT) followed by a verification exhaustive bout, to determine maximal oxygen uptake (VO_2max) and participants' peak aerobic power (W_{peak}), which are gold standard measures of aerobic fitness.[30] Both tests involve cycling on a stationary cycle ergometer (Velotron, RacerMate, Seattle, Washington, USA). GXT and verification exhaustive bout protocols will first be derived using age, height, weight, self-reported physical activity rating and and sex presumed at birth. This information will be used to estimate VO_2max and maximum output using the formula derived by Jamnick *et al*.[31] An initial GXT protocol for each individual will then be created by dividing the maximum power output into ten 1-min intensities as previously described.[31] The GXT test will then require participants to cycle at these derived 1-min intensities until exhaustion. Personalised GXTs protocols will be tested on each participant during their familiarisation session at each time point, and adjusted appropriately if VO_2max is not reached between 8 and 12 min. Following the GXT, participants will rest for 5 min and commence a verification bout. For this test, participants will cycle for 3 min to warm-up and then continuously, until exhaustion, at 90% of the maximal watts attained in the preceding GXT. Throughout this test, the participant will wear a mouthpiece, connected to a calibrated Quark cardiopulmonary exercise testing (CPET) metabolic system (COSMED, Rome, Italy), which analyses the air being breathed out, allowing for

the determination of gas exchanges and VO_2max. In cases that VO_2max values from the GXT and verification exhaustion bout stages differ by a coefficient of variation (CV) of 3%, participants will be asked to return to repeat tests.

Lower body muscle strength

Knee isokinetic and isometric muscle strength (peak torque (Newtons)) will be assessed through isokinetic dynamometry and knee extension/flexion (hamstring and quadriceps muscle) tests. These tests will be performed with participants seated on a Cybex Isokinetic Dynomometer (Cybex International, Ronkonkoma, New York), with their trunk, hip and thigh stabilised with straps. Isokinetic dynamometry is considered the 'gold standard' for examining muscle strength in various settings (elite sport, rehabilitation, general population), with knee strength commonly used as an indicator of lower body strength.[32 33] After a 5-min warm up on a stationary cycle ergometer, maximal isokinetic strength will be assessed by performing knee extension–flexions at three concentric velocities, 60°/s, 120°/s and 240°/s (ie, slow to fast) at a range of motion of 90°. A total of 5, 15 and 20 repetitions will be performed at 60°/s, 120°/s and 240°/s velocities, respectively. Isometric tests will then be conducted at a fixed angle of 60°, where participants apply as much force as possible for 5 s during extension and flexion movements. Following isometric tests, isokinetic fatigue will be performed. Here, participants will perform 30 consecutive maximal flexor–extensor movements at 120°/s.[34] A fatigue index will then be derived for each leg, computed by the Cybex Dynomometer as the % change from the first three and last three repetitions. All sets will be separated by 30 s rests and will each be proceeded by two trial repetitions, to familiarise participants with each speed and/or movement. In addition to peak torque values and fatigue indices, several other torque and time parameters will be recorded from isokinetic and isometric tests (torque to body ratios, agonist/antagonist torque ratios, time to peak torque, work per repetition, average power per repetition).

Body composition assessments

At baseline and after 12 months, body composition will be assessed via a DEXA scan. DEXA scans will be used to determine the distribution fat, lean and total mass of the whole body and at specific regions (arm, leg, trunk, android and gynoid). The DEXA scan will be performed in a fasted state, on the same examination day as muscle and blood sampling. Technical variability in DEXA scans will be minimised as all scans will be performed by the same technician at Victoria University. At all time points, standard anthropometric measures (body weight, height, waist and hip circumference) will also be obtained for calculation of body mass index and measures of fat distribution (waist-to-hip ratio).

Muscle biopsies and analyses

At each time point, a muscle biopsy will be taken from the vastus lateralis muscle of participants. A medical doctor will first administer a subcutaneous and perifascial injection of local anaesthetic (2–3 mL, 1% lidocaine) to minimise muscle injury and sample contamination. Following this, a 5 mm incision will be made, and a 5 mm Bergstrom percutaneous muscle biopsy needle then inserted. Samples will be collected via manual suction and the collected sample (50–200 mg) will be immediately blotted on filter paper to remove excess blood, with approximately 10 mg then embedded in Tissue-Tek O.C.T. Compound for muscle structure and fibre typing analysis, with the remaining muscle snap frozen in liquid nitrogen and stored at −80°C for subsequent analyses. Primary outcomes are changes in DNA methylation and gene expression profiles, which will be assessed using the Infinium Human MethylationEPIC arrays (850K) and quantitative PCR (qPCR) and/or RNA sequencing methods. Secondary outcomes are alterations in muscle fibre characteristics (via histology methods), protein content (via Western blotting and mass spectrometry-based methods), microRNA (via TaqMan microRNA array Cards), mitochondrial respiration (via an Oroboros instrument), metabolomics (via nuclear magnetic resonance spectroscopy and/or high-resolution mass spectrometry) and intramuscular levels of sex hormones (via standard biochemical assays).

Blood sampling and analyses

At each time point, ~15 mL of venous blood will be collected to facilitate the analyses of biochemical, epigenetic and genetic markers. From this sample 5 mL will be stored in EDTA blood collection tubes and the resulted supernatant plasma samples will be stored. The residual blood sample will be saved for DNA extraction and analyses. Another 5 mL will be collected with BD Vacutainer serum separator tubes (SST) tubes and the resulting supernatant serum samples will be collected. Again another 3 mL blood will be stored in Tempus Blood RNA tubes (Applied Biosystems, USA) and shaken vigorously for 30 s, and then stored at −20°C and then −80°C for RNA extraction. Whole blood, serum and plasma samples will be appropriately used to assess levels of sex hormones and other biochemical markers (blood lipids, glucose, insulin, haemoglobin, haematocrit) via standardised assays, and to undertake genotyping and mitochondrial genome sequencing (via qPCR methods and an Ion Proton Sequencing Platform (Life Technologies, Thermo Scientific)). Changes in blood DNA methylation and gene expression profiles following GAHT may also be assessed.

In adults commencing GAHT, biochemical data (eg, blood lipid profiles, glucose and insulin levels) will be generated through pathology tests routinely performed as part of standard care at Austin Health and other gender clinics. In these cases, biochemical data for matched

individuals not commencing GAHT will be generated using the same assays used by clinical pathology services.

Recruitment and sample size

The study will involve a total of 160 participants (age range; 18–65 years), divided over four groups. Forty participants commencing feminising GAHT and 40 commencing masculinising GAHT will be recruited over 18 months through Austin Health and other local clinics specialising in transgender medicine. A further 40 male and 40 female participants not commencing GAHT (comparator groups) will be recruited via online and print advertisements circulated to staff and the general community by the two sites. The inclusion/exclusion criteria for this study is shown in table 1 and is based on criteria which may impact outcomes or the participant's ability to safely perform exercise tests and/or provide blood and muscle samples.

Sample size calculations were based on available data on primary aerobic fitness outcomes, peak aerobic power (W_{peak}) and peak oxygen consumption (VO_2max), using sex-stratified data for these outcomes in the Gene SMART (Skeletal Muscle Adaptive Response to Training) cohort (n=120).[35] Supposing individuals commencing feminising GAHT may decrease their W_{peak} from the mean W_{peak} of those presumed men at birth in the Gene SMART study (mean; 3.66, SD; 0.783) to the mean W_{peak} of those presumed women at birth (mean; 3.08, SD; 0.905), a sample size of 27 per group would be required to detect this difference (power 0.9 and level of significance 0.05). For VO_2max, a sample size of 32 per group will be required to detect a difference at 90% power and a 0.05 significance level, based on VO_2max data for men (mean 48.04, SD; 8.189) and women (mean 42.29, SD; 10.476) in the Gene SMART study. Considering a conservative drop-out rate of 25%, a target of 40 participants per group (160 total) was set.

Statistical analyses

Changes in primary and secondary outcomes will be investigated with linear mixed models that account for repeated measures within the same individuals (*limma* and *lmerTest* Bioconductor R packages). We will adjust the models for known confounders (eg, age) and add an interaction between group and time to ensure that the changes in outcomes are specific to the feminising or masculinising GAHT group. A q value will be considered as significant at a false discovery rate <0.005.

Data management

Victoria University provides enterprise-grade, secure, storage and backup for safe storage during research and for long-term retention. Each participant's data will be identified by a study number only and will be stored on a distributed data entry system (R-Drive) already established by investigators at Victoria University and password protected. Investigators based at the Austin Health site will be able to enter the data into this system from their own computer and this will be accessible by the data coordinating centre at Victoria University.

Patient and public involvement

Pride in Sport is an initiative of the Australian Human Rights Commission and the Australian Sports Commission and sits with the Pride Inclusion Programs division of ACON Health, Australia's largest LGBTQ community health organisation. This is a national not-for-profit sporting inclusion programme specifically designed to assist sporting organisations at all levels with the inclusion of employees, athletes, coaches, officials, volunteers, fans and spectators with diverse sexualities and genders. The Pride in Sport team were involved in the co-design of the GAME study to ensure the project addresses important questions relevant to the needs of the transgender community and related sporting communities. The design of the study was further guided by trans researchers within the Trans Health Research Group at Austin Health, University of Melbourne.

ETHICS AND DISSEMINATION

Written informed consent will be obtained from each participant prior to any part of the study being undertaken. The Austin Health Human Research Ethics Committee (HREC) and Victoria University HREC granted approval for this study (HREC/77146/Austin-2021). The GAME study has also been prospectively registered in the Australian New Zealand Clinical Trials Registry. It is anticipated that the results of this project will be presented in a variety of forums, including as peer-reviewed publications and conference and seminar presentations. In any publication and/or presentation, information will be provided in such a way that participants cannot be identified. Where possible, researchers will distribute project outcomes to public and organisational discussions that may enhance the health and well-being of trans people, informing patient care approaches and governance development within sporting bodies that aim to encourage regular physical activity in trans communities.

DISCUSSION

The GAME study will comprehensively explore the effects of GAHT on physiological and 'omic' changes in skeletal muscle. This study design has clear strengths in using both baseline data and age-matched comparator groups to examine the influences of GAHT on a multitude of molecular and physiological markers of skeletal muscle health, function and performance. The study design also considers several confounders and sources of variability, employing standardised diets pre-sampling, fitness watches and repeated questionnaires to control and/or consider known confounders in analyses. Aerobic fitness, muscle strength and blood/muscle collection procedures are purposely separated by at least 48 hours to minimise the effects of these tests on each other. Technical variability

in DEXA scans will be minimised by employing the same technician and batch effects in molecular measurements will be minimised by limiting the number of batches and randomly distributing samples across batches.

Trans people are at high risk of poor mental and physical health outcomes[36 37] and are significantly underrepresented in health research. A recent survey reports high rates of self-reported mental health conditions in transgender Australians (n=928), including anxiety (67%) and depression (73%).[36] Furthermore, GAHT results in changes in clinical outcomes such as blood lipids[1] and insulin resistance[13 14] in trans individuals, with implications for chronic disease risk. Lifestyle interventions, such as exercise, represent an important and feasible means to improving the physical and mental health of trans people. However, transgender individuals engage less in physical activity compared with cisgender individuals,[38] and are particularly hindered from participating in activities and sports typically separated by single-sex competitions.[39 40] In Australia, it is unlawful to discriminate on the grounds of sex or gender identity but a 'competitive sporting activity' exemption may apply if competition would be uneven because of differences in 'strength', 'stamina' and 'physique' between male and female competitors[41]. However, limited data and questions remain around the degree to which physical performance changes with gender affirming hormones. The GAME study aims to address these questions, by comprehensively examining measures of stamina (aerobic fitness), strength, physique (body composition) and molecular characteristics of skeletal muscle. Outcomes of this study may therefore be of particular interest to sporting and related organisations and may inform more inclusive and evidence-based sports policies that promote physical activity.

Potential study limitations are in planned time points being at least 6 months apart, which may make the study prone to dropout and may limit our ability to detect changes in confounding lifestyle factors (eg, habitual physical activity). Our sample size calculations consider this, and account for 25% attrition. Further, prior studies indicate gender affirming hormone therapies have profound impacts on muscle mass and blood,[11–13 15 16] and we therefore anticipate that smaller shifts in lifestyle habits will not mask overall effects. The use of controlled diets and comparator groups will further limit the influence of confounders on study conclusions. Although participant recruitment is limited to one geographical region, this study will examine hormone regimes (eg, oral estradiol valerate, intramuscular testosterone undecanoate[42 43]) that are routinely implemented in other settings, such as Europe[44] and the USA,[45] with outcomes therefore of relevance to broader populations. Lastly, this study will involve performing exercise assessments at several time points, however will not examine the effects of an exercise intervention or programme (eg, 4 weeks of high-intensity training). The inclusion of an exercise intervention may aid in further understanding the influence of GAHT in trained individuals, with implications for

competitive sports. However, before exploring the effects of training interventions in trans people commencing GAHT, it is essential to first characterise the functional effects of GAHT in isolation. In the future, we will explore substudies that address added research questions and international collaborations that increase sample size and cohort representation, including non-binary individuals, individuals who may be using lower doses of GAHT and individuals with varying levels of sports participation (eg, elite vs community sports).

Author affiliations
[1]Institute for Health and Sport, Victoria University, Footscray, Victoria, Australia
[2]Trans Health Research Group, Department of Medicine (Austin Health), The University of Melbourne, Heidelberg, Victoria, Australia
[3]Department of Endocrinology (Austin Health), The University of Melbourne, Heidelberg, Victoria, Australia
[4]Pride in Sport, ACON Health, Surry Hills, New South Wales, Australia

ORCID iDs
Patrice R Jones http://orcid.org/0000-0002-4948-1445
Brendan J Nolan http://orcid.org/0000-0001-8836-165X
Alex Wong http://orcid.org/0000-0002-3666-5261

REFERENCES
1. Moore E, Wisniewski A, Dobs A. Endocrine treatment of transsexual people: a review of treatment regimens, outcomes, and adverse effects. *J Clin Endocrinol Metab* 2003;88:3467–73.
2. McLeod M, Breen L, Hamilton DL, *et al*. Live strong and prosper: the importance of skeletal muscle strength for healthy ageing. *Biogerontology* 2016;17:497–510.
3. Mukund K, Subramaniam S. Skeletal muscle: a review of molecular structure and function, in health and disease. *Wiley Interdiscip Rev Syst Biol Med* 2020;12:e1462.
4. Landen S, Voisin S, Craig JM, *et al*. Genetic and epigenetic sex-specific adaptations to endurance exercise. *Epigenetics* 2019;14:523–35.

5 Bredella MA. Sex differences in body composition. *Adv Exp Med Biol* 2017;1043:9–27.

6 Tarnopolsky MA. Gender differences in substrate metabolism during endurance exercise. *Can J Appl Physiol* 2000;25:312–27.

7 Handelsman DJ, Hirschberg AL, Bermon S. Circulating testosterone as the hormonal basis of sex differences in athletic performance. *Endocr Rev* 2018;39:803–29.

8 Alexander SE, Pollock AC, Lamon S. The effect of sex hormones on skeletal muscle adaptation in females. *Eur J Sport Sci* 2021:1–11.

9 Spangenburg EE, Geiger PC, Leinwand LA, *et al*. Regulation of physiological and metabolic function of muscle by female sex steroids. *Med Sci Sports Exerc* 2012;44:1653–62.

10 Tarnopolsky MA. Sex differences in exercise metabolism and the role of 17-beta estradiol. *Med Sci Sports Exerc* 2008;40:648–54.

11 Spanos C, Bretherton I, Zajac JD, *et al*. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: a systematic review. *World J Diabetes* 2020;11:66–77.

12 Harper J, O'Donnell E, Sorouri Khorashad B, *et al*. How does hormone transition in transgender women change body composition, muscle strength and haemoglobin? systematic review with a focus on the implications for sport participation. *Br J Sports Med* 2021;55:865–72.

13 Bretherton I, Spanos C, Leemaqz SY, *et al*. Insulin resistance in transgender individuals correlates with android fat mass. *Ther Adv Endocrinol Metab* 2021;12:2042018820985681.

14 Shadid S, Abosi-Appeadu K, De Maertelaere A-S, *et al*. Effects of Gender-Affirming hormone therapy on insulin sensitivity and incretin responses in transgender people. *Diabetes Care* 2020;43:411–7.

15 Antun A, Zhang Q, Bhasin S, *et al*. Longitudinal changes in hematologic parameters among transgender people receiving hormone therapy. *J Endocr Soc* 2020;4:bvaa119.

16 Wiik A, Lundberg TR, Rullman E, *et al*. Muscle strength, size, and composition following 12 months of Gender-affirming treatment in transgender individuals. *J Clin Endocrinol Metab* 2020;10510.1210/clinem/dgz247. [Epub ahead of print: 01 03 2020].

17 Van Caenegem E, Wierckx K, Taes Y, *et al*. Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). *Eur J Endocrinol* 2015;172:163–71.

18 Ackerley CG, Billingsley JM, Tharp GK, *et al*. Short Communication:Transgender Women on Feminizing Hormone Therapy Demonstrate a Distinct Rectal Mucosal Transcriptome from Cisgender Men. *AIDS Res Hum Retroviruses* 2020;36:771–4.

19 Aranda G, Fernández-Rebollo E, Pradas-Juni M, *et al*. Effects of sex steroids on the pattern of methylation and expression of the promoter region of estrogen and androgen receptors in people with gender dysphoria under cross-sex hormone treatment. *J Steroid Biochem Mol Biol* 2017;172:20–8.

20 Hiam D, Simar D, Laker R, *et al*. Epigenetic reprogramming of immune cells in women with PCOS impact genes controlling reproductive function. *J Clin Endocrinol Metab* 2019;104:6155–70.

21 Shepherd R, Bretherton I, Pang K. Gender Affirming hormone therapy induces specific DNA methylation changes in blood. *medRxiv* 2021.

22 Cheung AS, Ooi O, Leemaqz S, *et al*. Sociodemographic and clinical characteristics of transgender adults in Australia. *Transgend Health* 2018;3:229–38.

23 Janssen MF, Pickard AS, Golicki D, *et al*. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. *Qual Life Res* 2013;22:1717–27.

24 O'Driscoll R, Turicchi J, Beaulieu K, *et al*. How well do activity monitors estimate energy expenditure? A systematic review and meta-analysis of the validity of current technologies. *Br J Sports Med* 2020;54:332–40.

25 Gilgen-Ammann R, Schweizer T, Wyss T. Accuracy of the multisensory Wristwatch polar Vantage's estimation of energy expenditure in various activities: instrument validation study. *JMIR Mhealth Uhealth* 2019;7:e14534.

26 Australian Institute of Health Welfare,. *The active Australia survey: a guide and manual for implementation, analysis and reporting*. Canberra: AIHW, 2003.

27 Mifflin MD, St Jeor ST, Hill LA, *et al*. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* 1990;51:241–7.

28 Linsenmeyer W, Drallmeier T, Thomure M. Towards gender-affirming nutrition assessment: a case series of adult transgender men with distinct nutrition considerations. *Nutr J* 2020;19:74.

29 National Cancer Institute. Epidemiology and Genomics Research Program - ASA24-Australia, 2016. Available: https://epi.grants.cancer.gov/asa24/respondent/australia.html

30 Williams CJ, Gurd BJ, Bonafiglia JT, *et al*. A Multi-Center Comparison of O$_{2peak}$ Trainability Between Interval Training and Moderate Intensity Continuous Training. *Front Physiol* 2019;10:19.

31 Jamnick NA, Botella J, Pyne DB, *et al*. Manipulating graded exercise test variables affects the validity of the lactate threshold and [Formula: see text]. *PLoS One* 2018;13:e0199794.

32 Chamorro C, Armijo-Olivo S, De la Fuente C, *et al*. Absolute reliability and concurrent validity of hand held dynamometry and isokinetic dynamometry in the hip, knee and ankle joint: systematic review and meta-analysis. *Open Med* 2017;12:359–75.

33 Toonstra J, Mattacola CG. Test-Retest reliability and validity of isometric knee-flexion and -extension measurement using 3 methods of assessing muscle strength. *J Sport Rehabil* 2013;22. doi:10.1123/jsr.2013.TR7. [Epub ahead of print: 01 02 2013].

34 Bosquet L, Maquet D, Forthomme B, *et al*. Effect of the lengthening of the protocol on the reliability of muscle fatigue indicators. *Int J Sports Med* 2010;31:82–8.

35 Yan X, Eynon N, Papadimitriou ID, *et al*. The gene smart study: method, study design, and preliminary findings. *BMC Genomics* 2017;18:821.

36 Bretherton I, Thrower E, Zwickl S, *et al*. The health and well-being of transgender Australians: a national community survey. *LGBT Health* 2021;8:42–9.

37 Cicero EC, Reisner SL, Merwin EI, *et al*. The health status of transgender and gender nonbinary adults in the United States. *PLoS One* 2020;15:e0228765.

38 Jones BA, Haycraft E, Bouman WP, *et al*. The levels and predictors of physical activity engagement within the treatment-seeking transgender population: a matched control study. *J Phys Act Health* 2018;15:99–107.

39 López-Cañada E, Devís-Devís J, Valencia-Peris A, *et al*. Physical activity and sport in trans persons before and after gender disclosure: prevalence, frequency, and type of activities. *J Phys Act Health* 2020;17:650–6.

40 Braumüller B, Menzel T, Hartmann-Tews I. Gender identities in organized Sports-Athletes' experiences and organizational strategies of inclusion. *Front Sociol* 2020;5:578213.

41 Australian Human Rights Commission. Guidelines for the inclusion of transgender and gender diverse people in sport, 2019. Available: https://humanrights.gov.au/our-work/lgbti/publications/guidelines-inclusion-transgender-and-gender-diverse-people-sport-2019

42 Cheung AS, Wynne K, Erasmus J, *et al*. Position statement on the hormonal management of adult transgender and gender diverse individuals. *Med J Aust* 2019;211:127–33.

43 Bretherton I, Thrower E, Grossmann M, *et al*. Cross-sex hormone therapy in Australia: the prescription patterns of clinicians experienced in adult transgender healthcare. *Intern Med J* 2019;49:182–8.

44 Dekker MJHJ, Wierckx K, Van Caenegem E, *et al*. A European network for the investigation of gender incongruence: endocrine part. *J Sex Med* 2016;13:994–9.

45 Unger CA. Hormone therapy for transgender patients. *Transl Androl Urol* 2016;5:877–84.