Final Report – Endocrine Society of Australia 2020 Ken Wynne Postdoctoral Award

Awardee: Dr Ada Cheung

Administering institution: The University of Melbourne

Project title: A randomised double-blind placebo-controlled trial of low-dose transdermal estrogen in transgender women during surgery.

I am incredibly thankful to the ESA for awarding the Ken Wynne Postdoctoral Award in 2020 to support my project. This has provided critical seed funding to enable the commencement of this project and significantly contributed to my subsequent successful RACP Foundation grant enabling me to successfully fund this project to completion. Ethics and governance approvals have been now finalised over the last 12 months and the trial is registered with the ANZCTR. We have successfully negotiated clinical trials agreements with collaborating hospitals (Ramsay Healthcare), collaborators (surgeons and haematologists) as well as the pharmaceutical supplier of the research trial medications (Besins Healthcare). We have taken delivery of the research trial medication and have commenced recruitment. Recruitment numbers are feasible and we will complete this project as planned. During my tenure of the 2020 ESA Ken Wynne Award, I have published the following publications which have gratefully acknowledged the ESA Ken Wynne/Postdoctoral Award for funding support and the publications are attached:

1. Cheung AS, Lim HY, Cook T, Zwickl S, Ginger A, Chiang C, Zajac JD. Approach to interpreting common laboratory pathology tests in transgender individuals. *J Clin Endocrinol Metab* 2020 https://doi.org/10.1210/clinem/dgaa546
2. Nolan BJ, Liang B, Cheung AS. Efficacy of micronised progesterone for sleep: a systematic review and meta-analysis of randomised controlled trial data. *J Clin Endocrinol Metab* 2020 [accepted 21st November 2020]
3. Spanos C, Bretherton I, Zajac JD, Cheung AS. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: A systematic review. *World J Diabetes* 2020 Mar 15;11(3):66-77. doi: 10.4239/wjd.v11.i3.66.
4. Bretherton I, Spanos C, Leemaqz SY, Premaratne G, Grossmann M, Zajac JD, Cheung AS. Insulin resistance in transgender individuals correlates with android fat mass. *Ther Adv Endocrinol Metab* 2020 [Accepted 13/12/20]
5. Lim HY, Leemaqz SY, Torkamani N, Grossmann M, Zajac JD, Nandurkar H, Ho P, Cheung AS. Global coagulation assays in transgender women on oral and transdermal estradiol therapy. *J Clin Endocrinol Metab*. 2020 Jul 1;105(7):dgaa262. doi: 10.1210/clinem/dgaa262.
6. Nolan BJ, Brownhill A, Bretherton I, Wong P, Fox S, Locke P, Russell N, Grossmann M, Zajac JD, Cheung AS. Relationships between body mass index with oral estradiol dose and serum estradiol concentration in transgender adults undergoing feminising hormone therapy. *Ther Adv Endocrinol Metab* 2020;11:1-7. https://doi.org/10.1177/2042018820924543

Approach to interpreting common laboratory pathology tests in transgender individuals

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ABSTRACT

Context: As the number of transgender (trans) people (including those who are binary and/or non-binary identified) seeking gender-affirming hormone therapy rises, endocrinologists are increasingly asked to assist with interpretation of laboratory tests. Many common laboratory tests such as hemoglobin, iron studies, cardiac troponin and creatinine are affected by sex steroids or body size. We seek to provide a summary of the impact of feminizing and masculinizing hormone therapy on common laboratory tests and an approach to interpretation.

Cases: Case scenarios discussed include 1) hemoglobin and hematocrit in a non-binary person undergoing masculinizing hormone therapy; 2) estimation of glomerular filtration rate in a trans woman at risk of contrast-induced nephropathy; 3) prostate-specific antigen (PSA) in a trans woman; and 4) chest pain in a trans man with a cardiac troponin concentration in-between the reported male and female reference ranges.

Conclusions: The influence of exogenous gender-affirming hormone therapy on fat and muscle distribution and other physiological changes determines interpretation of laboratory tests which have sex-specific differences. In addition to affirmative practice to ensure a patient's name, gender and pronoun are used appropriately, we propose that once individuals have commenced gender-affirming hormone therapy, the reference range of the affirmed gender be reported (and specified by treating clinicians) except for PSA or cardiac troponin which is dependent on organ size. Whilst suggestions may be challenging to implement, they also represent an opportunity to lead best practice to improve the quality of care and experiences of healthcare for all trans people.

Introduction

As the number of transgender (trans) people (including those who are binary and/or non-binary identified) seeking gender-affirming hormone therapy rises in society (1), endocrinologists are increasingly asked to assist with interpretation of laboratory test results. Many common laboratory tests such as hemoglobin, iron studies, cardiac troponin and creatinine are affected by sex steroids or body size determined during pubertal growth and as such, have specific reference ranges (2). The difference between sex-specific reference ranges for these analytes are most marked during puberty, as the pubertal spikes of testosterone occur later in those presumed male at birth (3). We seek to provide a summary of the impact of feminizing and masculinizing hormone therapy on common clinical laboratory tests and an approach to interpretation.

Trans people are individuals whose gender is different to that presumed for them at birth and includes people with a binary and/or non-binary gender identity. Estimates worldwide suggest that between 0.6 – 1.2% of the general population are trans or gender diverse (4,5). Due to often intense feelings of incongruence between ones gender and body (termed dysphoria), many trans individuals undergo gender-affirming hormone therapy to align their physical characteristics with their gender to improve psychological, social and cultural functioning (6). Masculinizing hormone therapy is typically testosterone alone, which induces significant gains in muscle mass, decrease in fat mass and fat redistribution as well as deepening of the voice, facial and body hair growth (7,8). Feminizing hormone therapy is usually estradiol and an anti-androgen such as spironolactone or cyproterone acetate which will induce body fat redistribution to a more gynoid pattern with increases in fat mass, decreases in muscle mass as well as skin softening, decrease in libido and breast growth (7,9). Target sex steroid reference ranges on gender-affirming hormone therapy are generally the normal reference ranges of an individual's affirmed gender, rather than the gender presumed for them at birth (10-12). Notably, people with non-binary gender identities who seek medical affirmation, may undergo partial masculinization with low-dose testosterone therapy to target sex steroid reference ranges that may be

lower than the typical 'male' reference range (or conversely, partial feminization). Alternatively, some may desire a slower rate of physical change and hence use low-dose or micro-dose gender-affirming hormone therapy(13). While many trans people will seek to update their legal name and gender marker to align with their identity, costs to do so are prohibitive for many (14). Using a former pronoun, gender marker or name, even if still listed on identity documents, can cause intense distress and lead to, or exacerbate dysphoria for an individual (15). Due to current limitations of electronic medical records, laboratory information systems and health professionals' understanding of gender identity, interactions with laboratory services and healthcare providers can often magnify severe distress and affect the mental health of an individual. This may result in the individual's reluctance to return to care.

Whilst there are publications describing the effects of feminizing or masculinizing hormone therapy on gender-specific laboratory tests, there is a lack of long-term outcome data on the safety of using a particular reference range. As such, we base most of this discussion on physiological principles. Furthermore, it is unclear if; 1) the reference ranges are a direct switch to the affirmed gender identity for analytes which are immediately affected by sex hormones (e.g. growth hormone); 2) whether analytes which are determined during pubertal growth but are not affected by sex hormones in later life (e.g. troponin which reflects cardiac size) should continue with the reference range of the gender presumed at birth or; 3) whether trans-specific reference ranges are required for a period after gender-affirming hormones are commenced to monitor analytes which are gradually affected by sex hormones (e.g. creatinine which reflects muscle mass). The pivotal question is how we can have a "one size fits all" solution to cater for a heterogenous group who have sex steroid concentrations and resultant body composition changes that increase or decrease at different velocities and magnitudes.

Sex steroid concentrations

Whilst guidelines recommend targeting sex steroid reference ranges of the affirmed gender for people seeking full masculinization or feminization, this is based predominantly upon expert opinion and in some instances, there is debate whether measurement of sex steroids are indeed clinically useful at all. One example of this is measurement of serum estradiol concentrations in those on feminizing hormone therapy, with some clinicians adjusting therapy to estradiol concentrations whereas others adjust therapy based on clinical response (10-12). No studies have evaluated the optimal estradiol concentration for feminization in people presumed male at birth. In clinical practice, several clinical cohorts of trans individuals in Europe, USA and Australia have reported the sex steroid concentrations achieved in specialised gender clinics. These studies report that individuals undergoing feminizing hormone therapy for at least 6 months typically achieve estradiol concentrations in the range of 211 – 400 pmol/L (57 – 109 pg/mL) with testosterone concentrations of 2 – 4 nmol/L (0.57 – 1.15 ng/mL)(7,16-19). These changes in sex steroid concentrations induce some shifts from lean mass to fat mass which impacts upon laboratory tests such as creatinine (20).

For trans people presumed female at birth using masculinizing hormone therapy, testosterone concentrations rise from the female reference range of < 2 nmol/L (<0.57 ng/mL) up to the male reference range of 10 – 35 nmol/L (2.88 – 10.09 ng/mL) (7,18,21). It has been noted that despite masculinizing hormone therapy increasing testosterone concentrations, serum estradiol concentrations do not fall dramatically. A retrospective chart review in a US gender affirmation clinic, found that mean estradiol only decreased by 26 pmol/L, from 217 to 191 pmol/L (59 to 52 pg/mL) after 6 months on masculinizing hormone therapy (16). Consistent with this, a European cohort observed that after commencing testosterone therapy, serum estradiol concentrations in trans men decreased by approximately 50 to 60 pmol/L (13.6 to 17.1 pg/mL) from baseline (18,22). In the setting of high testosterone concentrations, the estradiol concentration per se does not affect masculinization, with significant gains in muscle mass and strength of approximately 10% and loss of fat mass (8). In addition to muscle mass gains, high testosterone concentrations also significantly impact upon hematopoiesis and interpretation of hematology laboratory tests.

Case 1

A 28-year-old non-binary individual presumed female at birth has recently commenced full masculinizing hormone therapy with transdermal testosterone gel. You receive a referral from their primary care physician concerned about polycythemia. Their hemoglobin is 168 g/L with hematocrit 0.49 which has been flagged in the laboratory report as high (reported with female reference range of 115 – 155g/L and 0.33-0.45 compared to the male reference interval of 120 -170g/L and 0.36 – 0.50).

Hematology

Androgens are known to stimulate erythropoiesis whilst the impact of estrogens are not as well understood. In trans people who have been on established and full-dose feminizing hormone therapy (estradiol and anti-androgen) for at least 6 months, there is a significant decrease in hemoglobin, hematocrit and red blood cell count to the female reference range (16,23-25). Conversely after 6 months of masculinizing testosterone therapy, trans people demonstrate an increase in hemoglobin, hematocrit and red blood cell count to the male reference range (16,20,23,26). Serum hematocrit in the range of the affirmed gender is evident from 3 months after commencing gender-affirming hormone therapy (27). Of note, there are association studies suggesting higher hematocrit is associated with a higher risk of cardiovascular disease (28,29). This is probably a consideration for people using masculinizing hormone therapy more so than those using feminizing hormone therapy. As smoking may additionally increase hematocrit, smoking cessation should be emphasised in those with elevated hematocrit. Whilst the long-term cardiovascular implications of using a different reference range for hemoglobin or hematocrit are unclear in general, reference ranges of the affirmed gender should be used. Female reference ranges should be used for someone taking gender-affirming feminizing hormone therapy and male reference ranges should be used for people using masculinizing hormone therapy.

In trans women, there is a small statistically significant but clinically insignificant rise in platelet count (which remains within the normal reference range) shown in several cohort studies after 6 – 12 months of feminizing hormone treatment (16,19) while white blood cells do not change significantly. No apparent changes are observed in either platelet count or white blood cells with masculinizing hormone therapy(16,19).

Case 1 outlines a non-binary individual presumed female at birth receiving full-dose masculinizing hormone therapy. In this case, the male reference range for hemoglobin and hematocrit would be most appropriate and this should be shared with the non-binary individual so they are aware and can expect to be misgendered when reviewing their own results. As such, their hemoglobin of 168 g/L and hematocrit of 0.49 would fall within the expected reference range and no change in management needs to occur.

Iron studies

Reference ranges for serum ferritin, a common indicator of body iron status, vary depending on age and sex (30). Ferritin reference ranges are typically lowest in premenopausal people presumed female at birth, followed by postmenopausal people and are highest in people presumed male at birth, with lower limits of the female reference range approximately 10 to 20 ug/L below that of the male reference range (30 ug/L) (31). This may be partially attributed to increased iron utilisation in menstruating individuals resulting in lower ferritin, as well as a multitude of factors which have been shown to impact upon adult serum ferritin levels including age, body mass index, waist to hip ratio, and liver function (30,32). Animal studies suggest that iron is distributed differently in males and females associated with differences in hepatic hepcidin expression rather than sex steroid

concentrations (33,34). No studies have evaluated if ferritin or other iron indicators change with gender-affirming hormone therapy.

From a practical perspective, the main reason to evaluate for iron deficiency is anemia. In individuals who have a ferritin below the 'male' reference range, we suggest interpreting the iron studies in the context of red cell indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) to guide management rather than on the use of gender-affirming hormone therapy. If the trans individual is menstruating or pregnant, it would be most practical to use the premenopausal female reference range for interpretation of iron studies.

For evaluation of possible iron overload, in situations of borderline results which fall in between the female and male reference ranges, relying on the absolute ferritin level or transferrin saturation will be difficult. It is pertinent to assess for concurrent inflammatory disease, liver disease or iron overload states such as hemochromatosis which may further guide clinical management.

Case 2

A cardiologist calls as they are planning a coronary angiogram for a 68-year-old trans woman and are concerned because the estimated glomerular filtration rate (eGFR) is unknown. They are uncertain how to risk stratify her for potential contrast-induced nephropathy. She has a history of longstanding hypertension and hypercholesterolaemia, vaginoplasty and has been on various formulations of estradiol therapy for over 20 years. On review of her investigations, her serum creatinine is 109 umol/L (1.23 mg/dL) but her eGFR has not been reported for the last 18 months. Laboratory providers cannot report eGFR if a male or female marker is not provided on the request form, as this is required along with age to estimate eGFR. Using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, if classified as female, the eGFR would be 45

mL/min/1.73m^2 classed as Stage 3 Chronic Kidney Disease and would meet the guidelines for intravenous hydration prior to procedure. However, if classified male, the patient would have an eGFR of 60 mL/min/1.73m^2 which would be classed as Stage 2 Chronic Kidney Disease and would not require pre-hydration. Which is the most appropriate eGFR to use?

Renal function

Accurately assessing renal function is essential for not only assessment of renal diseases, but also clinical situations which may potentially affect renal function (such as diabetes or radioiodine contrast administration) as well as considerations for medication dosing of renally cleared drugs. The most commonly used marker of renal function in clinical pathology laboratories is eGFR which is calculated based upon an individual's serum creatinine level, age, sex(35). Typically, people presumed male at birth have a higher eGFR than people presumed female at birth at the same level of serum creatinine because the formula assumes a higher muscle mass in men contributing to the serum creatinine independent of renal function. The difference between these groups (given the same age and weight) is more marked at higher levels (with a difference of approximately 30 when eGFR > 90 mL/min/1.73m^2), becoming much more similar as eGFR declines (difference of approximately 4 when eGFR < 30 mL/min/1.73m^2). In clinical situations where accurate assessment of renal function is necessary such as in the transplant setting, it may be more appropriate to use 24 hour urine creatinine clearance, urinary inulin clearance (36) or serum cystatin c which are less affected by sex and not affected by muscle mass in contrast to serum creatinine (37). Inulin clearance and cystatin c are more expensive and less readily available. Creatinine clearance can be calculated on paired 24-hour urine and serum creatinine concentration and is independent of muscle mass and sex steroids. This can provide a baseline estimation for renal function and cumulative serum creatinine results can then be used to monitor decline in renal function with aging.

From a practical perspective, laboratory reports will need to make an assessment on how to report the eGFR for trans individuals. For individuals receiving masculinizing or feminizing hormone therapy, changes in body composition appear to be maximal in the early period after commencement, evident within the first 3 months of treatment (38,39). For those receiving masculinizing hormone therapy with testosterone, given higher muscle mass and lower fat mass compared to females, the male CKD-Epidemiology Collaboration (CKD-EPI) formula would be more appropriate. Conversely if a person has been on feminizing hormone therapy which typically induces gain in fat and decrease in muscle mass from 3 months of use, then the female equations should be used. It would be a challenging task to expect pathology laboratories to provide the "right" eGFR given limited access to clinical information.

We recommend that the treating clinician specify the sex-specific reference interval desired for reporting on the laboratory request (i.e. female for a trans person using feminizing hormone therapy). Using current laboratory information systems, the gender marker can be used as a field to specify the reference range desired for reporting. Whilst the binary female or male gender may not necessarily reflect the individual's gender, this will allow for the appropriate reference range to be reported and the trans patient informed so they can prepare to be misgendered. For laboratory providers, if the gender marker is unknown, then treating clinicians should be contacted to specify the sex-specific reference interval desired.

For the trans woman described in Case 2 who was on long-standing feminizing hormone therapy with female body composition, the female reference range for renal function would be most appropriate triggering appropriate renoprotection prior to administration of radioiodine contrast for her angiogram. From a harm reduction approach, given the absence of data in the field, if either the male or female calculated eGFR suggests renoprotective strategies, then this can be implemented. A 24-hour urine creatinine clearance can also be performed to more accurately assess renal function.

Case 3

A 70-year-old trans woman who had been on feminizing hormone therapy for 6 months had a PSA performed as part of a routine health check. She was taking transdermal estradiol 100mcg/24hr patches twice weekly and cyproterone acetate 12.5mg daily. Her total testosterone was 1.5 nmol/L (43 ng/dl) and PSA was 2 ng/mL. She had mild lower urinary tract symptoms with reduced urinary flow over a number of years but had no family history of prostate cancer. How should she be managed?

Prostate-Specific Antigen

There are no studies examining the effect of feminizing hormone therapy on prostate-specific antigen (PSA). It is known that androgen deprivation as part of feminizing hormone therapy is associated with a substantially lower risk for prostate cancer than the general male population(40). All published case reports of prostate cancer in trans people using feminizing hormone therapy have had histology showing high risk adenocarcinoma with PSA concentrations at diagnosis ranging from 5 to 1722 ng/mL (ng/mL equivalent to ug/L) (40,41). Physiologically, in the setting of androgen deprivation in people with a prostate gland, it would be expected that PSA should be lower than the age-specific reference interval. There is insufficient data to recommend a specific cut-off for trans people using feminizing hormone therapy. Individualized decisions based upon clinical history and examination should inform need for serial monitoring for PSA velocity or imaging.

Case 3 had a digital rectal examination which showed a smooth but mildly enlarged prostate gland. She had an ultrasound of her prostate which showed a mildly enlarged prostate volume of 35 mL (35 cc). Repeat PSA monitoring revealed progressive lowering of her PSA concentration with ongoing feminizing hormone therapy and an improvement in her urinary flow.

Case 4

A 49-year-old trans man who had been on testosterone therapy for 10 years presented to the emergency department with central chest pain. His high-sensitivity cardiac troponin was 24 ng/L (female reference range <16 ng/L, male reference range <26 ng/L). How should he be managed?

High sensitivity cardiac troponin

Cardiac troponin is released from damaged cardiomyocytes and is one of the most common biomarkers used in the prediction of myocardial infarction. There is considerable debate regarding the use of sex-specific reference ranges for high sensitivity cardiac troponin (hs-cTn) as there is uncertainty whether the use of sex-specific reference limits impact upon clinical management or outcome prediction (42). However, as upper reference limits based on sex-specific 99th percentiles for hs-cTn are subtly higher for people recorded as males than those recorded females in population studies (43), use of sex-specific cut-offs for hs-cTn assays have been endorsed by the International Federation of Clinical Chemistry and Laboratory Medicine (44). The difference has been attributed to people presumed male at birth having a larger cardiac mass as well as subclinical coronary artery disease (45). No studies have been performed to examine cardiac mass changes that may occur with masculinizing hormone therapy in people presumed female at birth. There are however data in polycystic ovary syndrome (PCOS) in which high testosterone concentrations are a clinical feature (albeit far lower than testosterone concentrations seen in transgender men). PCOS has been associated with higher left ventricular mass index and larger left atrial diameter over 5 years of follow-up even after adjustment for blood pressure, body mass index, glucose and lipids (46). Large population-based studies have also shown that left ventricular mass correlates with body weight, lean body mass and fat mass (47). There is currently insufficient data to draw an inference regarding the appropriate reference range in people using gender-affirming hormone therapy, and emphasis must be placed on clinical

history, electrocardiogram (ECG) changes and serial trajectory of hs-cTn levels if the hs-cTn falls in between the male and female-specific reference ranges.

Despite the fact that Case 3 had been on established testosterone therapy for 10 years with resultant male body composition, there is insufficient data to suggest that cardiac remodelling or change in cardiac size occurs with high (or low) testosterone concentrations. Despite the risk of being oversensitive, in order to minimise the risk of missing an acute coronary event, we suggest that the reference range of the sex presumed at birth (female) should be used to interpret hs-cTn, provided the patient is informed of this rationale in addition to monitoring with serial troponin to ensure there is no rise. Case 3's subsequent hs-cTn was elevated above the male reference range and his ECG revealed anterior ST-segment depression consistent with acute coronary syndrome.

Recommendations

Given that changes in sex steroid concentrations, body composition and common laboratory values begin to occur within 3 months of gender-affirming hormone therapy, we recommend that once an individual has commenced gender-affirming hormone therapy, the reference range of the individual's affirmed gender (either female or male – see below for non-binary recommendations) should be used for tests with sex-specific reference ranges except for tests dependent on organ size for which the reference range for the sex presumed at birth should be used (cardiac troponin, PSA). An overview of recommended reference ranges for common laboratory tests is provided in the Table. Individualized interpretation and decision-making will still need to occur, particularly for individuals early in the course of gender-affirming therapy and for individuals on low-doses of gender-affirming hormones, non-standard regimens or concurrent medical conditions.

In the absence of non-binary reference ranges, for people who may be using low dose masculinizing hormone therapy as part of non-binary gender affirmation, the appropriate reference range is typically somewhere between the male and female reference ranges. This poses challenges for reporting, and from a practical perspective, we recommend that similar to people using standard doses of gender-affirming hormones, for someone using masculinizing hormones, the male reference range be used, and for someone using feminizing hormones, the female reference range be used, except for laboratory tests dependent on organ size such as cardiac troponin. We acknowledge that there is no "one size fits all" and interpretation ideally should be individualised by the treating clinician based on the clinical information. Similarly, in the early period after newly commencing gender-affirming hormones, the optimal range should be individualised as the serum sex steroid concentrations of the affirmed gender gradually increase to their target concentrations.

Treating clinicians should clearly specify on the laboratory request whether the female or male reference range should be reported. This may be easily satisfied within current constraints of laboratory information systems by specifying the corresponding gender marker for the patient on the laboratory request. In instances where the gender marker is unknown or not specified, clinicians should be contacted to obtain the desired reference interval to be reported (i.e. the female reference range for someone on feminizing hormone therapy). Laboratories should always report a reference range to ensure that critical result notifications are triggered and appropriately actioned. Dual reporting of both male and female reference ranges is another potential option, however current laboratory information system barriers exist. Dual reporting and identifying a person as trans in the gender marker field has the potential to lead to confusion and potentially open the opportunity for discrimination which is feared by many trans people.

The challenge for medical record systems and laboratory information systems will be ensuring that affirming practices are in place to provide quality care for trans people. Whilst our recommendations

propose a simplified approach for laboratories, this may pose challenges when more than one test and reference range is desired in an episode (such as renal function and cardiac troponin). Ideally, system fields that encompass legal name, preferred name, presumed gender at birth, actual gender and pronoun which is particularly relevant for treating clinicians and laboratory test collection staff, should be incorporated. Signage or posters indicating to patients the need to obtain sensitive information such as presumed gender at birth (which may induce dysphoria for some but is of clinical importance for tests such as cardiac troponin), may help convey a safe, affirming space for trans people.

Conclusions

There is increasing visibility of trans individuals globally and it is likely that endocrinologists will be asked to interpret common laboratory results for people receiving gender-affirming hormone therapy. We propose that once individuals have commenced gender-affirming hormone therapy, that the reference range of the affirmed gender be reported other than for PSA and cardiac troponin which is dependent upon organ size. The influence of exogenous gender-affirming hormone therapy on fat and muscle distribution and other physiological changes determines the interpretation of most laboratory tests which have sex-specific differences. This vital piece of clinical information should be provided by clinicians to laboratory staff and the desired reference range can be reflected in current laboratory information systems by using the corresponding gender marker on the laboratory request. As the binary male or female marker may not always reflect a trans person's gender, communication to explain the limitations are essential. Whilst these suggestions may be challenging to implement, they also represent an opportunity to lead best practice to improve the quality of care and experiences of health and healthcare for all trans people.

References

1. Cheung AS, Ooi O, Leemaqz S, Cundill P, Silberstein N, Bretherton I, Thrower E, Locke P, Grossmann M, Zajac JD. Sociodemographic and Clinical Characteristics of Transgender Adults in Australia. *Transgender health*. 2018;3(1):229-238.
2. Hannemann A, Friedrich N, Dittmann K, Spielhagen C, Wallaschofski H, Völzke H, Rettig R, Endlich K, Lendeckel U, Stracke S, Nauck M. Age- and sex-specific reference limits for creatinine, cystatin C and the estimated glomerular filtration rate. *Clinical Chemistry and Laboratory Medicine (CCLM)*. 2012;50(5):919.
3. Hoq M, Matthews S, Karlaftis V, Burgess J, Cowley J, Donath S, Carlin J, Yen T, Ignjatovic V, Monagle P. Reference Values for 30 Common Biochemistry Analytes Across 5 Different Analyzers in Neonates and Children 30 Days to 18 Years of Age. *Clin Chem*. 2019;65(10):1317-1326.
4. Zucker KJ. Epidemiology of gender dysphoria and transgender identity. *Sex Health*. 2017.
5. Ahs JW, Dhejne C, Magnusson C, Dal H, Lundin A, Arver S, Dalman C, Kosidou K. Proportion of adults in the general population of Stockholm County who want gender-affirming medical treatment. *PloS one*. 2018;13(10):e0204606.
6. White Hughto JM, Reisner SL. A Systematic Review of the Effects of Hormone Therapy on Psychological Functioning and Quality of Life in Transgender Individuals. *Transgender health*. 2016;1(1):21-31.
7. Klaver M, de Blok CJM, Wiepjes CM, Nota NM, Dekker M, de Mutsert R, Schreiner T, Fisher AD, T'Sjoen G, den Heijer M. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *Eur J Endocrinol*. 2018;178(2):165-173.
8. Van Caenegem E, Wierckx K, Taes Y, Schreiner T, Vandewalle S, Toye K, Lapauw B, Kaufman JM, T'Sjoen G. 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(2):163-171.
9. Fighera TM, da Silva E, Lindenau JD, Spritzer PM. Impact of cross-sex hormone therapy on bone mineral density and body composition in transwomen. *Clin Endocrinol (Oxf)*. 2018;88(6):856-862.
10. T'Sjoen G, Arcelus J, De Vries ALC, Fisher AD, Nieder TO, Özer M, Motmans J. European Society for Sexual Medicine Position Statement "Assessment and Hormonal Management in Adolescent and Adult Trans People, With Attention for Sexual Function and Satisfaction". *J Sex Med*. 2020;17(4):570-584.
11. Cheung AS, Wynne K, Erasmus J, Murray S, Zajac JD. Position statement on the hormonal management of adult transgender and gender diverse individuals. *Medical Journal of Australia*. 2019;211(3):127-133.
12. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, Rosenthal SM, Safer JD, Tangpricha V, T'Sjoen GG. Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society* Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2017;102(11):3869-3903.
13. Cocchetti C, Ristori J, Romani A, Maggi M, Fisher AD. Hormonal Treatment Strategies Tailored to Non-Binary Transgender Individuals. *J Clin Med*. 2020;9(6).
14. Scheim AI, Perez-Brumer AG, Bauer GR. Gender-concordant identity documents and mental health among transgender adults in the USA: a cross-sectional study. *Lancet Public Health*. 2020;5(4):e196-e203.
15. Restar A, Jin H, Breslow A, Reisner SL, Mimiaga M, Cahill S, Hughto JMW. Legal gender marker and name change is associated with lower negative emotional response

to gender-based mistreatment and improve mental health outcomes among trans populations. *SSM Popul Health*. 2020;11:100595.
16. SoRelle JA, Jiao R, Gao E, Veazey J, Frame I, Quinn AM, Day P, Pagels P, Gimpel N, Patel K. Impact of Hormone Therapy on Laboratory Values in Transgender Patients. *Clin Chem*. 2019;65(1):170-179.
17. Nolan BJ, Brownhill A, Bretherton I, Wong P, Fox S, Locke P, Russell N, Grossmann M, Zajac JD, Cheung AS. Relationships between body mass index with oral estradiol dose and serum estradiol concentration in transgender adults undergoing feminising hormone therapy. *Ther Adv Endocrinol Metab*. 2020;11:2042018820924543.
18. Auer MK, Ebert T, Pietzner M, Defreyne J, Fuss J, Stalla GK, T'Sjoen G. Effects of Sex Hormone Treatment on the Metabolic Syndrome in Transgender Individuals: Focus on Metabolic Cytokines. *J Clin Endocrinol Metab*. 2018;103(2):790-802.
19. Humble RM, Imborek KL, Nisly N, Greene DN, Krasowski MD. Common Hormone Therapies Used to Care for Transgender Patients Influence Laboratory Results. *J Appl Lab Med*. 2019;3(5):799-814.
20. Mueller A, Zollver H, Kronawitter D, Oppelt PG, Claassen T, Hoffmann I, Beckmann MW, Dittrich R. Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*. 2011;119(2):95-100.
21. Nolan BJ, Leemaqz SY, Ooi O, Cundill P, Silberstein N, Locke P, Grossmann M, Zajac JD, Cheung AS. Prevalence of polycythaemia with different formulations of testosterone therapy in transmasculine individuals. *Internal medicine journal*. 2020.
22. Defreyne J, Aers XP, Collet SM, Wiepjes CM, Fisher AD, Schreiner T, Den Heijer M, Kaufman JM, T'Sjoen GGR. Lower Serum Estradiol Levels in Assigned Female at Birth Transgender People with Initiation of Testosterone Therapy: Results from the European Network for the Investigation of Gender Incongruence. *LGBT health*. 2020;7(2):71-81.
23. Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher AD, Toye K, Kaufman JM, T'Sjoen G. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. *J Sex Med*. 2014;11(8):1999-2011.
24. Colizzi M, Costa R, Scaramuzzi F, Palumbo C, Tyropani M, Pace V, Quagliarella L, Brescia F, Natilla LC, Loverro G, Todarello O. Concomitant psychiatric problems and hormonal treatment induced metabolic syndrome in gender dysphoria individuals: a 2 year follow-up study. *J Psychosom Res*. 2015;78(4):399-406.
25. Roberts TK, Kraft CS, French D, Ji W, Wu AH, Tangpricha V, Fantz CR. Interpreting laboratory results in transgender patients on hormone therapy. *Am J Med*. 2014;127(2):159-162.
26. Fernandez JD, Tannock LR. METABOLIC EFFECTS OF HORMONE THERAPY IN TRANSGENDER PATIENTS. *Endocr Pract*. 2016;22(4):383-388.
27. Defreyne J, Vantomme B, Van Caenegem E, Wierckx K, De Blok CJM, Klaver M, Nota NM, Van Dijk D, Wiepjes CM, Den Heijer M, T'Sjoen G. Prospective evaluation of hematocrit in gender-affirming hormone treatment: results from European Network for the Investigation of Gender Incongruence. *Andrology*. 2018;6(3):446-454.
28. Erikssen G, Thaulow E, Sandvik L, Stormorken H, Erikssen J. Haematocrit: a predictor of cardiovascular mortality? *J Intern Med*. 1993;234(5):493-499.

29. Paul L, Jeemon P, Hewitt J, McCallum L, Higgins P, Walters M, McClure J, Dawson J, Meredith P, Jones GC, Muir S, Dominiczak AF, Lowe G, McInnes GT, Padmanabhan S. Hematocrit predicts long-term mortality in a nonlinear and sex-specific manner in hypertensive adults. *Hypertension*. 2012;60(3):631-638.
30. McKinnon EJ, Rossi E, Beilby JP, Trinder D, Olynyk JK. Factors that affect serum levels of ferritin in Australian adults and implications for follow-up. *Clin Gastroenterol Hepatol*. 2014;12(1):101-108 e104.
31. Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, Roger SD, Savoia HF, Tampi R, Thomson AR, Wood EM, Robinson KL. Diagnosis and management of iron deficiency anaemia: a clinical update. *Med J Aust*. 2010;193(9):525-532.
32. Gillum RF. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men--the Third National Health and Nutrition Examination Survey. *Int J Obes Relat Metab Disord*. 2001;25(5):639-645.
33. Kong WN, Niu QM, Ge L, Zhang N, Yan SF, Chen WB, Chang YZ, Zhao SE. Sex differences in iron status and hepcidin expression in rats. *Biol Trace Elem Res*. 2014;160(2):258-267.
34. Dhindsa S, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Green K, Makdissi A, Chaudhuri A, Dandona P. Effect of testosterone on hepcidin, ferroportin, ferritin and iron binding capacity in patients with hypogonadotropic hypogonadism and type 2 diabetes. *Clin Endocrinol (Oxf)*. 2016;85(5):772-780.
35. Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. *Am J Kidney Dis*. 2014;63(5):820-834.
36. Berger EY, Farber SJ, Earle DP, Jr. Comparison of the constant infusion and urine collection techniques for the measurement of renal function. *The Journal of clinical investigation*. 1948;27(6):710-716.
37. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD, 3rd, Zhang YL, Greene T, Levey AS. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis*. 2008;51(3):395-406.
38. Haraldsen IR, Haug E, Falch J, Egeland T, Opjordsmoen S. Cross-sex pattern of bone mineral density in early onset gender identity disorder. *Horm Behav*. 2007;52(3):334-343.
39. Spanos C, Bretherton I, Zajac JD, Cheung AS. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: A systematic review. *World J Diabetes*. 2020;11(3):66-77.
40. de Nie I, de Blok CJM, van der Sluis TM, Barbé E, Pigot GLS, Wiepjes CM, Nota NM, van Mello NM, Valkenburg NE, Huirne J, Gooren LJG, van Moorselaar RJA, Dreijerink KMA, den Heijer M. Prostate Cancer Incidence under Androgen Deprivation: Nationwide Cohort Study in Trans Women Receiving Hormone Treatment. *J Clin Endocrinol Metab*. 2020;105(9).
41. McFarlane T, Zajac JD, Cheung AS. Gender-affirming hormone therapy and the risk of sex hormone-dependent tumours in transgender individuals-A systematic review. *Clin Endocrinol (Oxf)*. 2018;89(6):700-711.
42. Eggers KM, Lindahl B. Impact of Sex on Cardiac Troponin Concentrations-A Critical Appraisal. *Clin Chem*. 2017;63(9):1457-1464.
43. Christenson RH, Duh SH, Mullins KE, LeClair MM, Grigorov IL, Peacock WF. Analytical and clinical characterization of a novel high-sensitivity cardiac troponin assay in a United States population. *Clin Biochem*. 2020.

44. Apple FS, Sandoval Y, Jaffe AS, Ordonez-Llanos J, Bio-Markers ftITFoCAoC. Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. *Clinical Chemistry*. 2017;63(1):73-81.
45. Salton CJ, Chuang ML, O'Donnell CJ, Kupka MJ, Larson MG, Kissinger KV, Edelman RR, Levy D, Manning WJ. Gender differences and normal left ventricular anatomy in an adult population free of hypertension. A cardiovascular magnetic resonance study of the Framingham Heart Study Offspring cohort. *J Am Coll Cardiol*. 2002;39(6):1055-1060.
46. Wang ET, Ku IA, Shah SJ, Daviglus ML, Schreiner PJ, Konety SH, Williams OD, Siscovick D, Bibbins-Domingo K. Polycystic ovary syndrome is associated with higher left ventricular mass index: the CARDIA women's study. *J Clin Endocrinol Metab*. 2012;97(12):4656-4662.
47. Gardin JM, Wagenknecht LE, Anton-Culver H, Flack J, Gidding S, Kurosaki T, Wong ND, Manolio TA. Relationship of cardiovascular risk factors to echocardiographic left ventricular mass in healthy young black and white adult men and women. The CARDIA study. Coronary Artery Risk Development in Young Adults. *Circulation*. 1995;92(3):380-387.
48. Velho I, Fighera TM, Ziegelmann PK, Spritzer PM. Effects of testosterone therapy on BMI, blood pressure, and laboratory profile of transgender men: a systematic review. *Andrology*. 2017;5(5):881-888.
49. Deutsch MB, Bhakri V, Kubicek K. Effects of cross-sex hormone treatment on transgender women and men. *Obstet Gynecol*. 2015;125(3):605-610.
50. Pelusi C, Costantino A, Martelli V, Lambertini M, Bazzocchi A, Ponti F, Battista G, Venturoli S, Meriggiola MC. Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med*. 2014;11(12):3002-3011.
51. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, Davidge-Pitts CJ, Nippoldt TB, Prokop LJ, Murad MH. Sex Steroids and Cardiovascular Outcomes in Transgender Individuals: A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2017;102(11):3914-3923.
52. Smith GI, Reeds DN, Okunade AL, Patterson BW, Mittendorfer B. Systemic delivery of estradiol, but not testosterone or progesterone, alters very low density lipoprotein-triglyceride kinetics in postmenopausal women. *J Clin Endocrinol Metab*. 2014;99(7):E1306-1310.

Table. Recommendations for laboratory tests with sex-specific reference ranges in trans people using gender-affirming hormone therapy

Test	Recommended Reference Range for Interpretation		Comments
	Affirmed Gender	Presumed Sex at Birth	
Estradiol	✓		
Total Testosterone	✓		
Creatinine	✓		
Estimated GFR	✓		Alternatively perform a 24 hour urine creatinine clearance
Hemoglobin	✓		
Hematocrit	✓		
Iron studies	✓		Insufficient data. Premenopausal female reference range should be used for menstruating or pregnant individuals regardless of gender.
Electrolytes	✓		No sex-specific reference ranges. Minor changes in sodium observed in small retrospective uncontrolled studies; sodium reduced with feminizing hormone therapy and increased with masculinizing hormone therapy.
Liver Function	✓		No sex-specific reference ranges. There is no clear evidence to suggest clinically significant changes occur with gender-affirming hormone therapy (16,19,23,25,48).
Lipid Profile	✓		No sex-specific reference ranges. Masculinizing hormone therapy associated with decreases in HDL-c (19,20,23,24,49,50). Feminizing hormone therapy associated with inconsistent lipid effects (19,23-25,51). If raised triglycerides observed, consider use of transdermal rather than oral estradiol formulations(52).
Prostate Specific Antigen (PSA)		✓	Valid only for people with a prostate. The prostate remains insitu even after orchiectomy, vaginoplasty or labioplasty surgery. PSA is expected to be low in the setting of low testosterone concentrations.
High Sensitivity Cardiac Troponin		✓	Cardiac troponin is based upon organ size which is not expected to change with gender-affirming hormone therapy.

Note that consideration should be made as to the duration and dose of feminizing or masculinizing hormone therapy used in interpretation of laboratory tests.

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SYSTEMATIC REVIEWS

Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: A systematic review

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Abstract

BACKGROUND

Transgender individuals receiving masculinising or feminising gender-affirming hormone therapy with testosterone or estradiol respectively, are at increased risk of adverse cardiovascular outcomes, including myocardial infarction and stroke. This may be related to the effects of testosterone or estradiol therapy on body composition, fat distribution, and insulin resistance but the effect of gender-affirming hormone therapy on these cardiovascular risk factors has not been extensively examined.

AIM

To evaluate the impact of gender-affirming hormone therapy on body composition and insulin resistance in transgender individuals, to guide clinicians in minimising cardiovascular risk.

METHODS

We performed a review of the literature based on PRISMA guidelines. MEDLINE, Embase and PsycINFO databases were searched for studies examining body composition, insulin resistance or body fat distribution in transgender individuals aged over 18 years on established gender-affirming hormone therapy. Studies were selected for full-text analysis if they investigated transgender individuals on any type of gender-affirming hormone therapy and reported effects on lean mass, fat mass or insulin resistance.

RESULTS

The search strategy identified 221 studies. After exclusion of studies that did not meet inclusion criteria, 26 were included (2 cross-sectional, 21 prospective-uncontrolled and 3 prospective-controlled). Evidence in transgender men suggests that testosterone therapy increases lean mass, decreases fat mass and has no impact on insulin resistance. Evidence in transgender women suggests

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that feminising hormone therapy (estradiol, with or without anti-androgen agents) decreases lean mass, increases fat mass, and may worsen insulin resistance. Changes to body composition were consistent across almost all studies: Transgender men on testosterone gained lean mass and lost fat mass, and transgender women on oestrogen experienced the reverse. No study directly contradicted these trends, though several small studies of short duration reported no changes. Results for insulin resistance are less consistent and uncertain. There is a paucity of prospective controlled research, and existing prospective evidence is limited by small sample sizes, short follow up periods, and young cohorts of participants.

CONCLUSION
Further research is required to further characterise the impact of gender-affirming hormone therapy on body composition and insulin resistance in the medium-long term. Until further evidence is available, clinicians should aim to minimise risk by monitoring cardiovascular risk markers regularly in their patients and encouraging healthy lifestyle modifications.

Key words: Transgender persons; Insulin resistance; Body composition; Gender dysphoria; Metabolic syndrome

Core tip: Evidence in transgender men suggests that testosterone therapy increases lean mass, decreases fat mass and has no impact on insulin resistance. Evidence in transgender women suggests that feminising hormone therapy (estradiol, with or without anti-androgen agents) decreases lean mass, increases fat mass, and may worsen insulin resistance. There is a paucity of prospective controlled research, and existing prospective evidence is limited by small sample sizes, short follow up periods, and young cohorts of participants. Until further evidence is available, clinicians should aim to minimise risk by monitoring cardiovascular risk markers regularly in their patients and encouraging healthy lifestyle modifications.

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INTRODUCTION

Transgender healthcare is a rapidly expanding field of medicine and transgender individuals are presenting in increasing numbers for advice and assistance in transitioning[1]. Research indicates that many physicians are unsure how to optimally manage gender dysphoria and support individuals through the process of transition. Further research and education in this area is vital to meet the increasing demand for treatment[2,3].

The term transgender refers to an individual who has a gender identity which differs from their sex assigned at birth. Gender dysphoria denotes the distress that may accompany this incongruence, and the goal of therapy is to alleviate this distress[4]. The exact prevalence of transgender people and gender dysphoria is unknown and, for various reasons, including the lack of clear nomenclature, is likely to be underestimated[5]. Population-based studies have found a higher prevalence than clinical studies, ranging from 0.5% in the United States to 0.6%-1.1% in the Netherlands and Belgium[6]. The range of management options available is broad and encompasses many disciplines of medicine, including psychological, hormonal and surgical interventions. Whether or not an individual chooses to undertake any or all of these interventions is variable, and management is ideally tailored to the individual's goals.

Gender-affirming hormone therapy (GAHT) is commonly given to align an individual's physical characteristics to more closely match their gender identity with

the goal of improving mental health and reducing dysphoria. Masculinising hormonal therapy for individuals assigned female sex at birth (transgender men) most commonly involves administration of intramuscular or transdermal testosterone in doses similar to those used for hypogonadal men. We use the term transgender in this review to also include people with gender diverse or non-binary identities. Feminising hormonal therapy for individuals assigned male sex at birth (transgender women) involves oestrogen replacement usually with oral or transdermal formulations and concurrent suppression of endogenous testosterone with androgen antagonists[7].

A number of studies and reviews have demonstrated the safety of gender-affirming hormone therapy in the short and medium-term, however there is a paucity of long term clinical safety and efficacy data[6]. Notably, two recent large cohort studies have demonstrated that transgender individuals on hormone therapy have higher rates of adverse cardiovascular events, with transmen on testosterone at increased risk of myocardial infarction, and transwomen on oestrogen at increased risk of ischaemic stroke[8,9]. Given the sexually dimorphic nature of cardiovascular disease and its associated risk factors, manipulation of sex hormones may underlie this increase in cardiovascular risk[10-12]. However, there are few reviews looking at the effects of hormone therapy on cardiovascular risk. Specifically, there is a lack of reviews into the available evidence in the effect of GAHT on insulin resistance, and its relationship to the changes in body composition brought about by hormone therapy. Both oestrogen and testosterone are capable of altering insulin sensitivity in both cisgender women and men[11,13-16], however, the impact of gender-affirming hormone therapy on insulin resistance in transgender individuals is less clear.

Studies demonstrating that transgender people on hormone therapy are at increased risk of adverse cardiovascular events highlight the importance of investigating surrogate markers while awaiting further long-term research. We aimed to investigate the existing evidence of the effects of gender-affirming hormone therapy on insulin resistance and body composition, as this may provide insight into the long-term risks of hormone therapy in transgender individuals.

MATERIALS AND METHODS

A search of the MEDLINE and EMBASE databases using the Ovid platform was conducted in March 2019. Terms defining the participant population included "Transgender", "Gender dysphoria", "Transsexual", "Gender identity", "Gender variant", "Trans men", "Trans women", "Trans people", "Two-spirit", "FTM" and "MTF". Terms defining the intervention included "Cross-sex hormone therapy", "Gonadal steroid hormones", "Estrogen replacement therapy", "Estradiol/Oestradiol", "Estrogen/Oestrogen", "Testosterone", 'Gonadotropin-Releasing Hormone analogues", "Androgen antagonists", "Antiandrogen", "Spironolactone", "Hormone therapy", "Hormone treatment", "Sex steroid", "Progestin", and "Androgen". Terms used for the study outcomes included "Body Composition", "Lean mass", "Muscle mass", "Fat mass", "Adipose fat or tissue", "Intra-abdominal fat", "Central or abdominal obesity", "Body fat distribution", "Anthropometry", "Waist or body circumference", "Waist-hip ratio", "Insulin resistance", "Insulin sensitivity", "HbA1c", "Blood glucose", "Insulin-Secreting Cells", "beta cell" and "Diabetes mellitus, Type 2". The full search strategy is included in Supplementary Tables 1 and 2. After removal of conference abstracts and duplicates, this search yielded a total of 221 studies for title and abstract screening.

Screening was conducted using the Rayyan web application[17]. Studies were selected for full-text analysis if they investigated transgender individuals on any type of gender-affirming hormone therapy and reported impacts (or lack thereof) on lean mass, fat mass or insulin resistance. Studies were excluded if they did not meet these inclusion criteria, or if they only reported fasting insulin levels, fasting plasma glucose or HbA1c in isolation as these were not taken to be sufficiently representative of insulin sensitivity.

A summary of the study selection process is included (Figure 1). After exclusion (reasons listed in Figure 1), 26 studies were included in the final analysis.

RESULTS

Overall

Of the 26 studies evaluated, 21 examined transgender men and 16 examined transgender women[18-43]. In total, these studies included 751 transgender male and 689

Table 1 Summary of studies investigating body composition and insulin resistance in transgender individuals

Ref.	Country	Design	Time period	*n*	Average age (yr)	Number of controls	Methods	Lean mass	Fat mass	IR	WHR
Studies in Transgender men											
Van Caenegem *et al*[41]	Belgium	Prospective controlled	12 mo	23	27 (9)	23 age-matched	DXA and pQCT	↑10.4%	↓9.7%		↔
Haraldsen *et al*[31]	Norway	Prospective controlled	3 mo, 12 mo	21	25.1 (4.8)	45, not age-matched	DXA	↑	↔		
Cupisti *et al*[24]	Germany	Prospective controlled	12 mo	29	29.9 (18-40)	240 PCOS, age-matched	HOMA-IR			↔	
Aranda *et al*[19]	Spain	Prospective	6 mo, 12 mo	20	27.1 (8.0)		DXA and HOMA-IR	↑5.8%	↓6.3%	↔	↑A:G ratio
Auer *et al*[21]	Germany	Prospective	13 mo	45	27.5 (1.3)		DXA and HOMA-IR	↑	↓	↓	↔
Klaver *et al*[32]	the Netherlands and Belgium	Prospective	12 mo	162	24 (18-58)		DXA	↑10%	↓9%		↑
Gava *et al*[30]	Italy	Prospective	12 mo, 36 mo, 60 mo	50	30.1 (6.1)		DXA and HOMA-IR	↑	↔	↔	↔
Aranda *et al*[18]	Spain	Prospective	6 mo, 12 mo	12	27.1 (17-43)		HOMA-IR			↔	WC ↔
Auer *et al*[20]	Belgium and Norway	Prospective	12 mo	20	NR		DXA and HOMA-IR	↑	↔	↔	↔
Colizzi *et al*[23]	Italy	Prospective	12 mo, 24 mo	43	28.8 (5.6)		HOMA-IR			↔	WC ↑
Pelusi *et al*[37]	Italy	Prospective	30 wk, 54 wk	45	29.5		DXA and HOMA-IR	↑	↓	↔	↔
Wierckx *et al*[42]	Belgium	Prospective	12 mo	53	24.5 (7.0)		DXA	↑	↓		↑
Mueller *et al*[35]	Germany	Prospective	12 mo, 24 mo	45	NR		DXA	↑	↔		
Yahyaoui *et al*[43]	United States	Prospective	12 mo, 24 mo	47	25.7 (6.0)		HOMA-IR			↓	
Meriggiola *et al*[34]	Italy	Prospective	12 mo	15	35.7 (5.0)		DXA and HOMA-IR	↑	↔	↔	↑ (NS)
Berra *et al*[22]	Italy	Prospective	6 mo	16	NR		BIA and HOMA	↑	↓	↔	WC ↔
Elbers *et al*[27]	the Netherlands	Prospective	12 mo	17	23 (16-34)		MRI and EGC		↑ in VAT	↔	
Elbers *et al*[25]	the Netherlands	Prospective	4 mo, 12 mo	15	23 (16-34)		MRI and BIA		↓		
Elbers *et al*[26]	the Netherlands	Prospective	12 mo, 36 mo	10	24 (16-33)		MRI	↑ thigh muscle	↑ in VAT		
Polderman *et al*[38]	the Netherlands	Prospective	4 mo	13	23.1 (18-33)		BIA and EGC	↑		↑	
Van Caenegem *et al*[39]	Belgium	Cross-sectional	10 yr on GAHT (3-28)	50	37 (8)	50 age-matched	DXA and pQCT	9% more	30% less		Larger
Studies in Transgender women											
Haraldsen *et al*[31]	Norway	Prospective controlled	3 mo, 12 mo	12	29.3 (7.8)	77, not age-matched	DXA	↓	↑		
Auer *et al*[21]	Germany	Prospective	12 mo	24	34.8(1.4)		DXA and HOMA-IR	↔	↑	↑	↓
Klaver *et al*[32]	the Netherlands and Belgium	Prospective	12 mo	179	29 (18-66)		DXA	↓3%	↑28%		↓
Fighera *et al*[28]	Brazil	Prospective	31 mo	46	33.7 (10.3)		DXA	↓	↑		
Aranda *et al*[18]	Spain	Prospective	6 mo, 12 mo	6	18.8 (16-21)		HOMA-IR			↔	WC ↑

Auer *et al*[20]	Belgium and Norway	Prospective	12 mo	20	NR		DXA and HOMA-IR	↓	↑	↑	↔
Gava *et al*[29]	Italy	Prospective	12 mo	40	31.2 (9.8)		DXA and HOMA-IR	↓ (NS)	↑	↔	↔
Colizzi *et al*[23]	Italy	Prospective	12 mo, 24 mo	79	30.2 (9.6)		HOMA-IR			↑	WC ↑
Van Caenegem *et al*[40]	Belgium	Prospective	12 months, 24 months	46	33 (12)	49 (not followed prospective-ly)	DXA and pQCT	↓	↑		↓
Wierckx *et al*[42]	Belgium	Prospective	12 mo	53	30.3 (14.0)		DXA	↓	↑		↓
Mueller *et al*[36]	Germany	Prospective	12 mo, 24 mo	84	NR		DXA	↓	↑		
Yahyaoui *et al*[43]	United States	Prospective	12 mo, 24 mo	22	23.1 (9.4)		HOMA-IR			↔	
Elbers *et al*[27]	the Nether-lands	Prospective	12 mo	20	26 (18-36)		MRI and EGC		↑ VAT	↑	
Elbers *et al*[25]	the Nether-lands	Prospective	4 mo, 12 mo	17	26 (18-37)		MRI and BIA		↔		
Polderman *et al*[38]	the Nether-lands	Prospective	4 mo	18	26.5 (18-36)		BIA and EGC	↓ (NS)		↑	
Lapauw *et al*[33]	Belgium	Cross-sectional	8 yr on GAHT (4-20)	23	41 (7)	46, age-matched	DXA, pQCT	20% lower	30% higher		

A:G ratio: Android-Gynoid ratio; BIA: Bioelectrical Impedance Analysis; DXA: Dual-energy X-ray Absorptiometry; ECG: Euglycaemic clamp; HOMA-IR: Homeostasis model assessment of Insulin Resistance; IR: Insulin resistance; MRI: Magnetic Resonance Imaging; NR: Not reported; NS: Not significant; pQCT: Peripheral Quantitative Computed Tomography: VAT: Visceral adipose tissue; WC: Waist circumference; WHR: Waist-hip ratio. Ages given as mean (SD or range).

transgender female participants. A large majority of the research was conducted in the Netherlands and Belgium (11 studies) and other European countries (6 Italian, 4 German, 2 Spanish, 1 Norwegian). Two studies were conducted outside Europe; 1 in the United States and 1 in Brazil. Results are summarised in Table 1.

All studies managed transgender men with testosterone and transgender women with oestrogen. With the exception of one study[31] all transgender women also received antiandrogen therapy (cyproterone acetate 50-100 mg or, in one study, goserelin acetate[36]) unless they were post-orchiectomy. The majority of studies treated transgender men with testosterone undecanoate 1000 mg IM every 12 wk (n = 12), or testosterone esters 250 mg IM every 2-3 wk (n = 6). Regimens in transgender women were more variable, though most studies used ethinyl estradiol or estradiol valerate in dosages of 100 g or 1-4 mg, respectively.

Transgender men

Controlled prospective studies: There are few controlled prospective studies (Table 1). Only 3 studies in transgender men included cisgender female control participants[24,31,41]. An initial 2007 study assessed 21 transgender men at initiation of testosterone therapy and followed them for 12 mo[31]. Comparisons were made with controls at baseline only and notably were not age-matched, with female controls almost 10 years older than the transgender male participants. This study noted no differences between groups at baseline, and that transgender men gained lean mass and lost fat mass, becoming more similar in body composition to male references over the 12-mo time period. These changes were noted as early as 3 mo after commencing hormone therapy[31].

A Belgian study of 23 transgender men compared them with 23 age-matched control women over 12 mo and found that treated transgender men gained lean mass and lost fat mass while the control women gained fat and lost lean mass and muscle strength[41]. The researchers also used peripheral quantitative computed tomography (pQCT) to investigate cross-sectional muscle area, finding significant increases in transgender men, along with increased grip strength.

To examine insulin resistance, 29 transgender men before and after commencing testosterone therapy were compared to 240 cisgender women with polycystic ovary syndrome (PCOS)[24]. Whilst the control women with PCOS and had significantly higher insulin resistance than the transgender men at baseline, there did not appear to be any induction of insulin resistance with testosterone therapy. Further, there was no

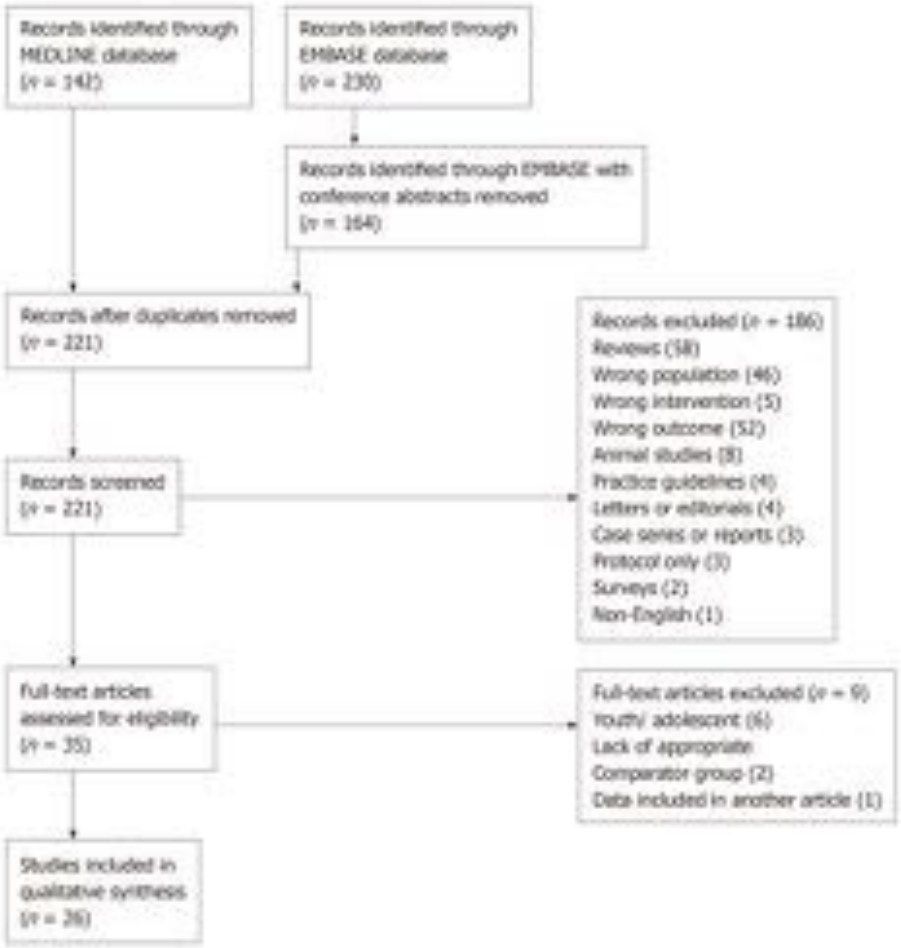

Figure 1 Study selection flowchart.

correlation between serum testosterone concentrations and insulin resistance.

Prospective studies without controls: All uncontrolled prospective studies examining body composition have found that testosterone was associated with increases in lean mass (Table 1). The majority also found an associated decrease in fat mass, but despite this two imaging studies showed that testosterone was associated with an increase in visceral fat area[26,27]. The magnitude of visceral adipose tissue (VAT) gain was as high as 47% and correlated with the relative amount of weight gained during therapy. Although more recent studies have failed to report on visceral fat accumulation, several report an increase in waist-to-hip ratio. Increases in android-to-gynoid (A:G) fat ratios[19] occur, driven primarily by decreases in gynoid region fat (-14%), without increases in the android region[32].

Prospective studies reporting on insulin resistance are somewhat conflicting. Testosterone treatment was associated with increased insulin resistance measured by the hyperinsulinaemic euglycaemic clamp technique in only one study, which followed 13 people over 4 mo[38]. In contrast, no changes were seen in insulin sensitivity over 1 year in 17 healthy, nonobese young transgender men[27]. Many studies summarised in Table 1 have demonstrated no change (n = 10), or in two cases, decreased resistance. The largest prospective study of 50 transgender men followed for 5 years found that testosterone had no impact on insulin resistance[30].

Cross-sectional evidence: All of the above studies assessed transgender men newly commencing testosterone therapy. However, when comparing transgender men on established therapy (mean treatment duration 10 years) compared with age-matched cisgender female controls, lean mass remained higher and fat mass remained lower suggesting persistence of changes over time[39].

Transgender women

Prospective studies with controls: Body composition changes are summarised in

Table 1. Whilst two studies made comparisons to controls at baseline[31,40], there were no studies that followed the control group over time. There were baseline differences between transgender women and control men; with transgender women having less lean mass and more fat mass than control males even before commencing any kind of hormone therapy. Over time oestrogen therapy induced a loss of lean mass and gain of fat mass in their participants, which is consistent across studies in this review.

Prospective studies without controls: As shown in Table 1, almost all prospective data in transgender women is consistent. In transgender women commencing estradiol therapy, lean mass decreases and fat mass increases. There is also a shift in body fat distribution with a decrease in waist-to-hip ratio seen in 4 studies, and rise in gynoid fat[21,32,40,42]. The largest study involving 179 transgender women, demonstrated a 42% increase in leg fat and 34% increase in gynoid fat, compared to a more modest 18% increase in android fat[32]. These changes persist over the first two years of hormone therapy[36]. Oestrogen therapy was additionally associated with an 18% increase in VAT over 12 mo, along with a 38% increase in subcutaneous fat[27].

Whether feminising hormone therapy is associated with insulin resistance is unclear. Five studies noted increases in insulin resistance and three others found no change (Table 1). Of the studies reporting increased resistance, the largest and longest-running is Colizzi *et al*[23], who followed 79 participants for two years. The participants, who were metabolically healthy at baseline, had an increase in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) values of 72% in the first year of treatment and by a further 9% over the second year, suggesting that the first 12 mo is an important period. A confounding factor was the presence of participants with psychiatric comorbidities who may have been on other medications capable of altering their insulin sensitivity, however there were only 12 participants in this category. Both of the studies employing the gold-standard euglycaemic clamp method found that GAHT in transgender women decreased peripheral glucose uptake, and simultaneously noted an increase in fasting insulin of up to 50%[27,38]. This would support the notion that oestrogen therapy has negative effects on insulin sensitivity. In addition, two of the three the studies reporting no change are limited by small participant numbers (n = 22 and n = 6)[19,43].

Cross sectional evidence: One cross-sectional study compared 23 transgender women who had been on GAHT for a mean of 8 years with 46 age-matched controls[33]. Fat mass was higher and lean mass was lower than controls including a 25% decrease in lower limb muscle area measured by pQCT.

DISCUSSION

There is evidence that hormone therapy can have a significant effect on body composition within 12 mo and these changes persist long-term. The effect on insulin resistance is uncertain. Changes to body composition were consistent across almost all studies: transgender men on testosterone gained lean mass and lost fat mass, and transgender women on oestrogen experienced the reverse. No study directly contradicted these trends, though several small studies of short duration reported no changes. These results are consistent with a recent meta-analysis investigating body composition in individuals on GAHT, which found that hormone therapy in transgender individuals affects lean and fat mass significantly, with an increase in fat mass and decrease in lean mass observed in transwomen and the reverse occurring in transmen[44]. Results for insulin resistance are less consistent and uncertain.

It would be unethical to conduct a randomised controlled trial using hormone therapy and placebo, therefore prospective cohort studies represent the highest available level of evidence. However, there is a paucity of prospective controlled data and research with a longer follow-up duration is needed. It should also be noted that most of the participants in these studies are of Caucasian ethnicity, which limits the generalisability of the findings to transgender individuals of other backgrounds.

Insulin resistance, body composition and cardiovascular risk

Insulin resistance has been shown to independently predict a variety of poor outcomes including hypertension, obesity, and dyslipidaemia, as well as cardiovascular and all-cause mortality, even in non-diabetic metabolically well individuals[45-47].

In 2015, increased body mass index contributed to 4.0 million deaths globally (7.1% of the total number of deaths from any cause). 39% of these deaths occurred in people with body mass index less than 30, indicating that this increased risk is not confined to people who are clinically obese, and that small changes in body fat have the

potential to influence long term mortality risk[48]. The distribution of body is also important, as central or "abdominal" obesity has been shown to confer a higher risk, and an elevated waist-hip ratio correlates with adverse cardiovascular outcomes more strongly than body mass index[49]. This is significant from the perspective of gender, as male "android" obesity which is more centrally distributed is therefore more predictive of poor outcomes than female "gynoid" obesity, which involves storage of fat around the hips, thighs and buttocks[12,50].

Body fat distribution and insulin resistance are interrelated[51]. Adipose tissue has been shown to decrease sensitivity to insulin through release of multiple mediators including free fatty acids, steroid hormones and proinflammatory cytokines[51]. There is some debate about whether visceral fat in particular has a greater influence over insulin sensitivity than subcutaneous fat. Visceral adipose tissue is more metabolically active, and its proximity to portal circulation may bestow a greater impact on hepatic lipid and glucose production[12]. However, some research suggests subcutaneous fat mass is more highly correlated with insulin resistance and attribute this to its greater contribution to circulating free fatty acid levels[50]. No association between fat distribution and insulin sensitivity is evident[27], suggesting that GAHT may influence insulin resistance by mechanisms other than body fat, including direct action on tissues. More research investigating the relationships between body fat distribution and insulin resistance in transgender people on hormone therapy is needed.

Impact of testosterone therapy in transgender men

Testosterone and body composition: Testosterone therapy in transgender men is associated with an increase in lean mass and decrease in fat mass. Data suggests that changes emerge within three months. Changes seen in the early years of hormone therapy are likely to persist in the longer term, with analysis of participants on hormone therapy for 10 years demonstrating consistent changes in body composition[39].

Testosterone and visceral fat: The evidence that testosterone decreases fat mass is strong however the effects on visceral fat specifically are less clear. Whilst testosterone appears to be associated with increased VAT; there are few studies, sample sizes are small with short follow-up, and the lack of controls do not allow for the comparison with normal visceral fat accumulation with time. More recent studies reporting an increase in waist-to-hip ratio in transgender men may indicate that increases in VAT are occurring. This is supported by evidence of raised android-to-gynoid fat ratios, suggestive of changes in body fat distribution[32]. Of note, Elbers *et al*[26] found no changes in VAT after 12 mo, but significant increases after 3 years, indicating these changes may take time to develop. Further controlled prospective studies are required to assess not only VAT but cardiovascular outcomes and mortality.

Testosterone and insulin resistance: The effects of testosterone on insulin resistance are unclear, even in cisgender populations. In cisgender men, testosterone deficiency is associated with insulin resistance, and this improves with testosterone replacement[15,16]. In men with both hypogonadism and established diabetes, testosterone replacement does not appear to improve glycaemic control[52]. Elevated levels of testosterone in cisgender women as occurs in PCOS, are associated with increased insulin resistance. In animal models, testosterone is associated with a decrease in insulin sensitivity[11]. This review suggests that testosterone therapy has no negative effect on insulin sensitivity and may even be associated with an improvement. This raises the possibility that the impairment in insulin sensitivity in PCOS is independent of hyperandrogenism.

Impact of estradiol therapy in transgender women

Estradiol and body composition: Estradiol therapy in transgender women appears to be associated with an increase in fat mass and decrease in lean mass, although prospectively controlled data is limited. Interestingly, the studies that were controlled demonstrate these differences at baseline[31,40]. Several studies have reported significantly less weekly physical activity in transgender women compared to control men[33,39] possibly as a way to intentional to minimise muscle mass and influence body shape.

The increase in body fat associated with oestrogen therapy raises questions about the effect on cardiovascular risk. Elbers *et al*[27]noted that oestrogen and antiandrogen therapy was associated with a visceral fat increase of 18%, and subcutaneous fat increase of 38% over 12 mo. This represents an increase in the absolute amount of visceral fat, but a reduction in the ratio of visceral to subcutaneous fat. The implications for long-term cardiovascular health are unclear.

Estradiol and insulin resistance: Five of the eight studies reporting on insulin

resistance in transwomen found that oestrogen therapy was associated with a worsening in insulin resistance measures. The remaining three studies were limited by small sample sizes. This is consistent with other high-oestrogen states such as women taking the oral contraceptive pill who have increased insulin resistance, as well as gestational diabetes mellitus during pregnancy[11]. Other research suggests that oestrogen has a protective role. Insulin sensitivity in cisgender women decreases after menopause and improves with menopausal hormone therapy[14]. Selective oestrogen receptor modulators have been shown to decrease insulin sensitivity, while Tamoxifen is associated with a 24% increase in diabetes risk in breast cancer survivors[13]. In cisgender men, decreased oestrogen production also induces insulin resistance[11]. The mechanism underlying this protective effect is largely unknown. Oestrogen has been shown to enhance peripheral insulin sensitivity *via* actions on skeletal muscle, liver and adipose tissue, and also has a role regulating energy intake and expenditure[13]. This review as well as other studies of high-oestrogen states may indicate that supra-physiological levels of oestrogen, in both men and women, can impair insulin sensitivity through a mechanism which is not entirely understood.

Gender-affirming hormone therapy has differing effects on body composition and insulin resistance in transgender men and women. Transgender men taking testosterone therapy have an associated gain in lean mass and decrease in fat mass. Transgender women taking oestrogen therapy have an associated decline in lean mass and gain in fat mass. The data examining the effect of hormone therapy on insulin resistance is so far inconsistent and limited. It is possible that oestrogen therapy in transgender women has a negative impact on insulin sensitivity. Both body fat and insulin resistance are known to impact cardiovascular risk and it is reasonable to conclude that gender-affirming hormone therapy may have an impact on long-term cardiovascular health.

The studies reviewed are limited by small sample sizes, young participants who may be yet to develop insulin resistance and short duration of follow-up. There is a paucity of controlled prospective research. These limitations make it difficult to draw conclusions about the long-term effects of gender-affirming hormone therapy on cardiovascular risk.

The long-term effects of hormone therapy on body composition and insulin resistance are unknown. Significant changes in body composition occur, most detrimentally in transgender women who gain fat mass and also have an increase in insulin resistance. Further research is therefore needed and given the long-term nature of cardiovascular disease progression, large prospectively controlled cohort studies are required. In the meantime, further research into the impact of hormone therapy on surrogate markers of cardiovascular risk is encouraged, and it would be prudent for clinicians to monitor markers of cardiovascular risk in patients taking gender-affirming hormone therapy.

ARTICLE HIGHLIGHTS

Research background

Transgender individuals receiving masculinising or feminising gender-affirming hormone therapy with testosterone or estradiol respectively, are at increased risk of heart disease and stroke. Testosterone and estradiol play important roles in regulating body fat and muscle and in the general population, males who have relatively high levels of testosterone compared with females are at higher risk for heart disease. The increased risk of heart disease may be related to the effects of testosterone or estradiol therapy on fat and muscle distribution, as well as insulin resistance, a measure of diabetes and heart disease risk. The effect of gender-affirming hormone therapy on these cardiovascular risk factors has not been extensively examined.

Research motivation

Studies demonstrating that transgender people on hormone therapy are at increased risk of adverse cardiovascular events highlight the importance of investigating surrogate markers while awaiting further long-term research. Both oestrogen and testosterone are capable of altering insulin resistance in both cisgender women and men, however, the impact of gender-affirming hormone therapy on insulin resistance in transgender individuals is less clear. This review was conducted to examine the relationship between insulin resistance and its relationship to the changes in body composition brought about by hormone therapy in order to enable clinicians to proactively lower risk factors which may contribute to heart disease and diabetes.

Research objectives

We aimed to investigate the existing evidence of the effects of gender-affirming hormone therapy on insulin resistance and body composition, as this may provide insight into the long-term risks of hormone therapy in transgender individuals.

Research methods

We performed a systematic review of the literature based on PRISMA guidelines. MEDLINE, Embase and PsycINFO databases were searched for studies examining body composition, insulin resistance or body fat distribution in transgender individuals aged over 18 years on established gender-affirming hormone therapy. Studies were selected for full-text analysis if they investigated transgender individuals on any type of gender-affirming hormone therapy and reported effects on lean mass, fat mass or insulin resistance.

Research results

The search strategy identified 221 studies. After exclusion of studies that did not meet inclusion criteria, 26 were included (2 cross-sectional, 21 prospective-uncontrolled and 3 prospective-controlled). Evidence in transgender men suggests that testosterone therapy increases lean mass, decreases fat mass and has no impact on insulin resistance. Evidence in transgender women suggests that feminising hormone therapy (estradiol, with or without anti-androgen agents) decreases lean mass, increases fat mass, and may worsen insulin resistance. Changes to body composition were consistent across almost all studies: transgender men on testosterone gained lean mass and lost fat mass, and transgender women on oestrogen experienced the reverse. No study directly contradicted these trends, though several small studies of short duration reported no changes. Results for insulin resistance are less consistent and uncertain. There is a paucity of prospective controlled research, and existing prospective evidence is limited by small sample sizes, short follow up periods, and young cohorts of participants.

Research conclusions

Masculinising gender-affirming hormone therapy increases lean mass, decreases fat mass and has no impact on insulin resistance. Feminising gender-affirming hormone therapy decreases lean mass, increases fat mass, and may worsen insulin resistance. Further research is required to further characterise the impact of gender-affirming hormone therapy on body composition and insulin resistance in the medium-long term. Until further evidence is available, clinicians should aim to minimise risk by monitoring cardiovascular risk markers regularly in their patients and encouraging healthy lifestyle modifications is paramount.

Research perspectives

Clinicians need to be aware of body composition changes and potential insulin resistance changes. Proactive lowering of cardiovascular risk factors such as optimising diet and physical activity as well as managing weight, lipids, blood pressure and glucose are important. Long-term prospective controlled studies will provide further insights in the future.

REFERENCES

1 **Telfer M**, Tollit M, Feldman D. Transformation of health-care and legal systems for the transgender population: The need for change in Australia. *J Paediatr Child Health* 2015; **51**: 1051-1053 [PMID: 26303157 DOI: 10.1111/jpc.12994]

2 **Davidge-Pitts C**, Nippoldt TB, Danoff A, Radziejewski L, Natt N. Transgender Health in Endocrinology: Current Status of Endocrinology Fellowship Programs and Practicing Clinicians. *J Clin Endocrinol Metab* 2017; **102**: 1286-1290 [PMID: 28324050 DOI: 10.1210/jc.2016-3007]

3 **Bretherton I**, Thrower E, Grossmann M, Zajac JD, Cheung AS. Cross-sex hormone therapy in Australia: the prescription patterns of clinicians experienced in adult transgender healthcare. *Intern Med J* 2019; **49**: 182-188 [PMID: 29992681 DOI: 10.1111/imj.14035]

4 **American Psychiatric Association**. Gender Dysphoria. In: Diagnostic and Statistical Manual of Mental Disorders. 5th ed. 2013 [DOI: 10.1176/appi.books.9780890425596.dsm14]

5 **Collin L**, Reisner SL, Tangpricha V, Goodman M. Prevalence of Transgender Depends on the "Case" Definition: A Systematic Review. *J Sex Med* 2016; **13**: 613-626 [PMID: 27045261 DOI: 10.1016/j.jsxm.2016.02.001]

6 **T'Sjoen G**, Arcelus J, Gooren L, Klink DT, Tangpricha V. Endocrinology of Transgender Medicine. *Endocr Rev* 2019; **40**: 97-117 [PMID: 30307546 DOI: 10.1210/er.2018-00011]

7 **Hembree WC**, Cohen-Kettenis P, Delemarre-van de Waal HA, Gooren LJ, Meyer WJ, Spack NP, Tangpricha V, Montori VM; Endocrine Society. Endocrine treatment of transsexual persons: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2009; **94**: 3132-3154 [PMID: 19509099 DOI: 10.1210/jc.2017-01658]

8 **Getahun D**, Nash R, Flanders WD, Baird TC, Becerra-Culqui TA, Cromwell L, Hunkeler E, Lash TL, Millman A, Quinn VP, Robinson B, Roblin D, Silverberg MJ, Safer J, Slovis J, Tangpricha V, Goodman M. Cross-sex Hormones and Acute Cardiovascular Events in Transgender Persons: A Cohort Study. *Ann Intern Med* 2018; **169**: 205-213 [PMID: 29987313 DOI: 10.7326/M17-2785]

9 **Nota NM**, Wiepjes CM, de Blok CJM, Gooren LJG, Kreukels BPC, den Heijer M. Occurrence of Acute Cardiovascular Events in Transgender Individuals Receiving Hormone Therapy. *Circulation* 2019; **139**: 1461-1462 [PMID: 30776252 DOI: 10.1161/circulationaha.118.038584]

10 **Regitz-Zagrosek V**, Lehmkuhl E, Weickert MO. Gender differences in the metabolic syndrome and their role for cardiovascular disease. *Clin Res Cardiol* 2006; **95**: 136-147 [PMID: 16598526 DOI: 10.1007/s00392-006-0351-5]

11 **Geer EB**, Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med* 2009; **6** Suppl 1: 60-75 [PMID: 19318219 DOI: 10.1016/j.genm.2009.02.002]

12 **Palmer BF**, Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol* 2015; **402**: 113-119 [PMID: 25578600 DOI: 10.1016/j.mce.2014.11.029]

13 **Mauvais-Jarvis F**, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* 2013; **34**: 309-338 [PMID: 23460719 DOI: 10.1210/er.2012-1055]

14 **Salpeter SR**, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women.

Diabetes Obes Metab 2006; **8**: 538-554 [PMID: 16918589 DOI: 10.1111/j.1463-1326.2005.00545.x]
15 **Dhindsa S**, Ghanim H, Batra M, Kuhadiya ND, Abuaysheh S, Sandhu S, Green K, Makdissi A, Hejna J, Chaudhuri A, Punyanitya M, Dandona P. Insulin Resistance and Inflammation in Hypogonadotropic Hypogonadism and Their Reduction After Testosterone Replacement in Men with Type 2 Diabetes. *Diabetes Care* 2016; **39**: 82-91 [PMID: 26622051 DOI: 10.2337/dc15-1518]
16 **Simon D**, Charles MA, Lahlou N, Nahoul K, Oppert JM, Gouault-Heilmann M, Lemort N, Thibult N, Joubert E, Balkau B, Eschwege E. Androgen therapy improves insulin sensitivity and decreases leptin level in healthy adult men with low plasma total testosterone: a 3-month randomized placebo-controlled trial. *Diabetes Care* 2001; **24**: 2149-2151 [PMID: 11723098 DOI: 10.2337/diacare.24.12.2149]
17 **Ouzzani M**, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016; **5**: 210 [PMID: 27919275 DOI: 10.1186/s13643-016-0384-4]
18 **Aranda G**, Fernández-Rebollo E, Pradas-Juni M, Hanzu FA, Kalko SG, Halperin I, Mora M. 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**: 20-28 [PMID: 28539237 DOI: 10.1016/j.jsbmb.2017.05.010]
19 **Aranda G**, Mora M, Hanzu FA, Vera J, Ortega E, Halperin I. Effects of sex steroids on cardiovascular risk profile in transgender men under gender affirming hormone therapy. *Endocrinol Diabetes Nutr* 2019; **66**: 385-392 [PMID: 30704917 DOI: 10.1016/j.endinu.2018.11.004]
20 **Auer MK**, Cecil A, Roepke Y, Bultynck C, Pas C, Fuss J, Prehn C, Wang-Sattler R, Adamski J, Stalla GK, T'Sjoen G. 12-months metabolic changes among gender dysphoric individuals under cross-sex hormone treatment: a targeted metabolomics study. *Sci Rep* 2016; **6**: 37005 [PMID: 27833161 DOI: 10.1038/srep37005]
21 **Auer MK**, Ebert T, Pietzner M, Defreyne J, Fuss J, Stalla GK, T'Sjoen G. Effects of Sex Hormone Treatment on the Metabolic Syndrome in Transgender Individuals: Focus on Metabolic Cytokines. *J Clin Endocrinol Metab* 2018; **103**: 790-802 [PMID: 29216353 DOI: 10.1210/jc.2017-01559]
22 **Berra M**, Armillotta F, D'Emidio L, Costantino A, Martorana G, Pelusi G, Meriggiola MC. Testosterone decreases adiponectin levels in female to male transsexuals. *Asian J Androl* 2006; **8**: 725-729 [PMID: 16855767 DOI: 10.1111/j.1745-7262.2006.00204.x]
23 **Colizzi M**, Costa R, Scaramuzzi F, Palumbo C, Tyropani M, Pace V, Quagliarella L, Brescia F, Natilla LC, Loverro G, Todarello O. Concomitant psychiatric problems and hormonal treatment induced metabolic syndrome in gender dysphoria individuals: a 2 years follow-up study. *J Psychosom Res* 2015; **78**: 399-406 [PMID: 25691225 DOI: 10.1016/j.jpsychores.2015.02.001]
24 **Cupisti S**, Giltay EJ, Gooren LJ, Kronawitter D, Oppelt PG, Beckmann MW, Dittrich R, Mueller A. The impact of testosterone administration to female-to-male transsexuals on insulin resistance and lipid parameters compared with women with polycystic ovary syndrome. *Fertil Steril* 2010; **94**: 2647-2653 [PMID: 20451188 DOI: 10.1016/j.fertnstert.2010.03.048]
25 **Elbers JM**, Asscheman H, Seidell JC, Frölich M, Meinders AE, Gooren LJ. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J Clin Endocrinol Metab* 1997; **82**: 3267-3270 [PMID: 9329351 DOI: 10.1210/jcem.82.10.4284]
26 **Elbers JM**, Asscheman H, Seidell JC, Megens JA, Gooren LJ. Long-term testosterone administration increases visceral fat in female to male transsexuals. *J Clin Endocrinol Metab* 1997; **82**: 2044-2047 [PMID: 9215270 DOI: 10.1210/jcem.82.7.4078]
27 **Elbers JM**, Giltay EJ, Teerlink T, Scheffer PG, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol (Oxf)* 2003; **58**: 562-571 [PMID: 12699437 DOI: 10.1046/j.1365-2265.2003.01753.x]
28 **Fighera TM**, da Silva E, Lindenau JD, Spritzer PM. Impact of cross-sex hormone therapy on bone mineral density and body composition in transwomen. *Clin Endocrinol (Oxf)* 2018; **88**: 856-862 [PMID: 29630732 DOI: 10.1111/cen.13607]
29 **Gava G**, Cerpolini S, Martelli V, Battista G, Seracchioli R, Meriggiola MC. Cyproterone acetate vs leuprolide acetate in combination with transdermal oestradiol in transwomen: a comparison of safety and effectiveness. *Clin Endocrinol (Oxf)* 2016; **85**: 239-246 [PMID: 26932202 DOI: 10.1111/cen.13050]
30 **Gava G**, Mancini I, Cerpolini S, Baldassarre M, Seracchioli R, Meriggiola MC. Testosterone undecanoate and testosterone enanthate injections are both effective and safe in transmen over 5 years of administration. *Clin Endocrinol (Oxf)* 2018; **89**: 878-886 [PMID: 30025172 DOI: 10.1111/cen.13821]
31 **Haraldsen IR**, Haug E, Falch J, Egeland T, Opjordsmoen S. Cross-sex pattern of bone mineral density in early onset gender identity disorder. *Horm Behav* 2007; **52**: 334-343 [PMID: 17604029 DOI: 10.1016/j.yhbeh.2007.05.012]
32 **Klaver M**, de Blok CJM, Wiepjes CM, Nota NM, Dekker MJHJ, de Mutsert R, Schreiner T, Fisher AD, T'Sjoen G, den Heijer M. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *Eur J Endocrinol* 2018; **178**: 163-171 [PMID: 29183889 DOI: 10.1530/EJE-17-0496]
33 **Lapauw B**, Taes Y, Simoens S, Van Caenegem E, Weyers S, Goemaere S, Toye K, Kaufman JM, T'Sjoen GG. Body composition, volumetric and areal bone parameters in male-to-female transsexual persons. *Bone* 2008; **43**: 1016-1021 [PMID: 18835591 DOI: 10.1016/j.bone.2008.09.001]
34 **Meriggiola MC**, Armillotta F, Costantino A, Altieri P, Saad F, Kalhorn T, Perrone AM, Ghi T, Pelusi C, Pelusi G. Effects of testosterone undecanoate administered alone or in combination with letrozole or dutasteride in female to male transsexuals. *J Sex Med* 2008; **5**: 2442-2453 [PMID: 18624972 DOI: 10.1111/j.1743-6109.2008.00909.x]
35 **Mueller A**, Haeberle L, Zollver H, Claassen T, Kronawitter D, Oppelt PG, Cupisti S, Beckmann MW, Dittrich R. Effects of intramuscular testosterone undecanoate on body composition and bone mineral density in female-to-male transsexuals. *J Sex Med* 2010; **7**: 3190-3198 [PMID: 20584125 DOI: 10.1111/j.1743-6109.2010.01912.x]
36 **Mueller A**, Zollver H, Kronawitter D, Oppelt PG, Claassen T, Hoffmann I, Beckmann MW, Dittrich R. Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. *Exp Clin Endocrinol Diabetes* 2011; **119**: 95-100 [PMID: 20625973 DOI: 10.1055/s-0030-1255074]
37 **Pelusi C**, Costantino A, Martelli V, Lambertini M, Bazzocchi A, Ponti F, Battista G, Venturoli S, Meriggiola MC. Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med* 2014; **11**: 3002-3011 [PMID: 25250780 DOI: 10.1111/jsm.12698]
38 **Polderman KH**, Gooren LJ, Asscheman H, Bakker A, Heine RJ. Induction of insulin resistance by androgens and estrogens. *J Clin Endocrinol Metab* 1994; **79**: 265-271 [PMID: 8027240 DOI:

10.1210/jcem.79.1.8027240]
39 **Van Caenegem E**, Wierckx K, Taes Y, Dedecker D, Van de Peer F, Toye K, Kaufman JM, T'Sjoen G. Bone mass, bone geometry, and body composition in female-to-male transsexual persons after long-term cross-sex hormonal therapy. *J Clin Endocrinol Metab* 2012; **97**: 2503-2511 [PMID: 22564669 DOI: 10.1210/jc.2012-1187]
40 **Van Caenegem E**, Wierckx K, Taes Y, Schreiner T, Vandewalle S, Toye K, Kaufman JM, T'Sjoen G. Preservation of volumetric bone density and geometry in trans women during cross-sex hormonal therapy: a prospective observational study. *Osteoporos Int* 2015; **26**: 35-47 [PMID: 25377496 DOI: 10.1007/s00198-014-2805-3]
41 **Van Caenegem E**, Wierckx K, Taes Y, Schreiner T, Vandewalle S, Toye K, Lapauw B, Kaufman JM, T'Sjoen G. 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**: 163-171 [PMID: 25550352 DOI: 10.1530/EJE-14-0586]
42 **Wierckx K**, Van Caenegem E, Schreiner T, Haraldsen I, Fisher AD, Toye K, Kaufman JM, T'Sjoen G. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. *J Sex Med* 2014; **11**: 1999-2011 [PMID: 24828032 DOI: 10.1111/jsm.12571]
43 **Yahyaoui R**, Esteva I, Haro-Mora JJ, Almaraz MC, Morcillo S, Rojo-Martínez G, Martínez J, Gómez-Zumaquero JM, González I, Hernando V, Soriguer F. Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. *J Clin Endocrinol Metab* 2008; **93**: 2230-2233 [PMID: 18349066 DOI: 10.1210/jc.2007-2467]
44 **Klaver M**, Dekker MJHJ, de Mutsert R, Twisk JWR, den Heijer M. Cross-sex hormone therapy in transgender persons affects total body weight, body fat and lean body mass: a meta-analysis. *Andrologia* 2017; 49 [PMID: 27572683 DOI: 10.1111/and.12660]
45 **Esteghamati A**, Khalilzadeh O, Abbasi M, Nakhjavani M, Novin L, Esteghamati AR. HOMA-estimated insulin resistance is associated with hypertension in Iranian diabetic and non-diabetic subjects. *Clin Exp Hypertens* 2008; **30**: 297-307 [PMID: 18633753 DOI: 10.1080/10641960802269919]
46 **Geloneze B**, Vasques AC, Stabe CF, Pareja JC, Rosado LE, Queiroz EC, Tambascia MA; BRAMS Investigators. HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). *Arq Bras Endocrinol Metabol* 2009; **53**: 281-287 [PMID: 19466221 DOI: 10.1590/s0004-27302009000200020]
47 **Zhang X**, Li J, Zheng S, Luo Q, Zhou C, Wang C. Fasting insulin, insulin resistance, and risk of cardiovascular or all-cause mortality in non-diabetic adults: a meta-analysis. *Biosci Rep* 2017; **37**: BSR20170947 [PMID: 28811358 DOI: 10.1042/BSR20170947]
48 **Afshin A**, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL; GBD 2015 Obesity Collaborators. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/nejmoa1614362]
49 **Després JP**. Body fat distribution and risk of cardiovascular disease: an update. *Circulation* 2012; **126**: 1301-1313 [PMID: 22949540 DOI: 10.1161/CIRCULATIONAHA.111.067264]
50 **Patel P**, Abate N. Body fat distribution and insulin resistance. *Nutrients* 2013; **5**: 2019-2027 [PMID: 23739143 DOI: 10.3390/nu5062019]
51 **Kahn BB**, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473-481 [PMID: 10953022 DOI: 10.1172/JCI10842]
52 **Gianatti EJ**, Dupuis P, Hoermann R, Strauss BJ, Wentworth JM, Zajac JD, Grossmann M. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. *Diabetes Care* 2014; **37**: 2098-2107 [PMID: 24804695 DOI: 10.2337/dc13-2845]

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Insulin resistance in transgender individuals correlates with android fat mass

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Abstract

Background: Transgender individuals receiving gender-affirming hormone therapy (GAHT) are at increased risk of adverse cardiovascular outcomes. This may be related to effects on body composition and insulin resistance.

Aims: To examine relationships between body fat distribution and insulin resistance in transgender individuals on established GAHT.

Methods: Comparisons of body composition (dual energy X-ray absorptiometry) and insulin resistance [Homeostasis Model of Insulin Resistance (HOMA2-IR)] were made between transgender individuals (43 trans men and 41 trans women) on established GAHT (>12 months) and age-matched cisgender controls (30 males and 48 females). Multiple linear regressions were used to examine the relationship between HOMA2-IR and fat mass with gender, adjusting for age and total duration of GAHT and Pearson correlation coefficients are reported.

Results: Compared with control cisgender women, trans men had mean difference of +7.8 kg (4.0, 11.5), $p < 0.001$ in lean mass and higher android:gynoid fat ratio [0.2 (0.1, 0.3), $p < 0.001$], but no difference in overall fat mass or insulin resistance. Compared with control cisgender men, trans women had median difference in lean mass of −6.9 kg (−10.6, −3.1), $p < 0.001$, fat mass of +9.8 kg (3.9, 14.5), $p = 0.001$, lower android:gynoid fat ratio −0.1 (−0.2,−0.0), $p < 0.05$), and higher insulin resistance 1.6 (1.3–1.9), $p < 0.001$). Higher HOMA2-IR correlated with higher android ($r^2 = 0.712$, $p < 0.001$) and gynoid ($r^2 = 0.572$, $p < 0.001$) fat mass in both trans men and trans women.

Conclusion: Android fat more strongly correlates with insulin resistance than gynoid fat in transgender individuals. Higher fat mass and insulin resistance in trans women may predispose to increased cardiovascular risk. Despite adverse fat distribution, insulin resistance was not higher in trans men.

Keywords: body composition, gender dysphoria, gender identity, insulin resistance, transgender persons, transsexualism

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Introduction

Background

It is estimated that 0.6–1.2% of the population identify as transgender (or trans)[1,2] and the number of individuals presenting to medical services for assistance with gender transition is rapidly rising.[3,4] Transgender individuals experience incongruence between the sex assigned to them at birth and their deeply held sense of gender identity. Gender-affirming hormone therapy (GAHT) is used by many transgender individuals to align physical characteristics with their gender identity. Masculinising hormone therapy with testosterone for trans men and feminising hormone therapy with oestradiol and anti-androgen agents for trans women are both associated with improvements in psychological outcomes and quality of life.[5]

GAHT is usually continued lifelong; however, little is known about the long-term effects. Two large cohort studies suggest higher rates of cardiovascular events in transgender individuals on hormone therapy compared with cisgender individuals. Trans men on testosterone had higher rates of myocardial infarction compared with cisgender women, and trans women on oestrogen had an increased risk of ischaemic stroke and venous thromboembolism when compared with both cisgender men and cisgender women.[6,7]

Regional fat distribution, in particular central adiposity, is an important contributor to cardiovascular risk and is heavily influenced by sex steroids.[8] A recent systematic review confirmed that feminising hormone therapy is consistently associated with increases in fat mass and decreases in lean mass, while trans men experience decreases in fat mass and increases in lean mass with masculinising hormone therapy.[9] In cisgender populations central abdominal fat, also referred to as android fat, is associated with high cardiovascular risk and one of its measures, the waist:hip ratio, is more strongly correlated with cardiovascular outcomes than is body mass index (BMI).[10] Trans men, but not trans women, have an increase in waist:hip ratio 12 months after commencing hormone therapy,[9] suggesting higher cardiovascular risk. Additionally, the route of administration of hormone therapy may influence fat mass. In postmenopausal women, oral conjugated equine oestrogen is associated with higher body fat and loss of lean tissue when compared with transdermal oestradiol, thought to be mediated *via* insulin-like growth factor 1 (IGF-1) production.[11–13]

Insulin resistance is also an important contributor to cardiovascular risk. Both oestrogen and testosterone are capable of altering insulin sensitivity *via* a direct effect on liver, muscle and endothelial tissues, as well indirect effects *via* changes in body fat distribution.[14–18] Although central to the pathogenesis of type 2 diabetes mellitus, insulin resistance also independently predicts a variety of poor outcomes in otherwise well non-diabetic individuals including hypertension, obesity and dyslipidaemia, as well as cardiovascular and all-cause mortality.[19–22]

Regional fat distribution and insulin resistance in cisgender individuals are well correlated. Adipose tissue, particularly central adiposity, has been shown to induce insulin resistance through release of multiple mediators including free fatty acids, steroid hormones and proinflammatory cytokines.[18] Given the significant body composition changes known to occur in transgender individuals with gain in fat mass and loss of lean mass with feminising hormone therapy and the reverse seen with masculinising hormone therapy,[9] determining whether a correlation exists between regional fat mass and insulin sensitivity is important, yet not previously described. Understanding this link will provide insights into whether GAHT affects insulin resistance predominantly through direct *versus* indirect (*via* changes in body composition) mechanisms, and guide clinicians in providing more accurate preventative strategies.

We aimed to further investigate the effect of GAHT on insulin resistance and body composition as surrogate markers of cardiovascular risk. We hypothesised that, first, trans men on testosterone therapy would have higher lean mass, lower fat mass and greater android:gynoid body fat distribution compared with control cisgender women and that opposite effects would be seen in trans women on oestradiol therapy compared with control cisgender men. Second, we hypothesised that insulin resistance would correlate with higher android fat and, therefore, would be higher in trans men compared with control cisgender women, with opposite effects seen in trans women.

Materials and methods

Study design and participants

We conducted a cross-sectional study between 1 April 2017 and 30 April 2018 in transgender individuals aged 18 years and over who had been on continuous GAHT for 12 months or more. Trans men on standard dose testosterone therapy were compared with individuals of the same sex assigned at birth; cisgender female controls. Trans women receiving standard doses of oestradiol-based therapy for feminisation were compared with cisgender male controls. Transgender participants were recruited from endocrinology outpatient clinics and from primary care general practice clinics specialising in transgender health in Melbourne, Australia. These participants were compared with age-matched cisgender control groups. Healthy control individuals were additionally recruited as control participants for a longitudinal study in bone health in transgender individuals and exclusion criteria included

diabetes, established osteoporosis, metabolic bone disease, glucocorticoid therapy, bisphosphonate therapy, antiepileptic medication, HIV pre-exposure prophylaxis, pregnancy, thromboembolic disease, liver disease, or any disease likely to lead to impairment in bone health. All participants provided written informed consent and the protocol was approved by the Austin Health Human Research Ethics Committee (approval no. HREC/17/Austin/74).

Data collection

All participants underwent fasting blood testing to measure oestradiol, testosterone, sex hormone binding globulin (SHBG), blood glucose, insulin, C-peptide and IGF-1 levels. Where possible blood testing was undertaken as a trough level for those on depot medications (such as testosterone undecanoate). In cisgender female participants blood testing was not able to be timed to a particular point in the menstrual cycle. Oestradiol was measured using immunoassay (Cobas E801, Roche Diagnostics, inter-assay variation 25% at level of 100 pnmol/L or less and 25% at a level of greater than 100 pmol/L). Those on unmeasurable forms of oestradiol (such as ethinyloestradiol) were not included in the calculation of median oestradiol levels. Testosterone was measured using immunoassay (Cobas E801, Roche Diagnostics, inter-assay variation 14.8% at level of 2.7 nmol/L or less and 15% at a level of greater than 2.7 nmol/L). SHBG was measured on immunoassay (Cobas E801, Roche Diagnostics, interassay variation 6% at a level of 21 nmol/L and 6% at a level of 40 nmol/L). Fasting plasma glucose was measured using hexokinase photometric assay (Cobas C8000, Roche Diagnostics, inter-assay variation 1.5 at levels of 4.8 and 15.5 mmol/L). Electrochemiluminescence immunoassay (Cobas C8000, Roche Diagnostics) was used to measure insulin (interassay variation 4% at 16.3 mIU/L and 5% at 154 mIU/L) and C-peptide (interassay variation 4.5% at a level of 2.5 nmol/L and 6.8% at 0.55 nmol/L). Fasting blood glucose and C-peptide were used to calculate insulin resistance using updated Homeostasis Model of Insulin Resistance (HOMA2-IR),[23] which is available for download from The Oxford Centre for Diabetes, Endocrinology and Metabolism.[24] This is a non-linear model, which accounts for variations in hepatic and peripheral glucose resistance. C-peptide can be used to model both beta-cell function and insulin resistance and compared with insulin is less likely to degrade if any haemolysis of the sample occurs.[25] IGF-1 was measured using chemiluminescence immunoassay (Liaison XL, DiaSorin); interassay at a level of 11.4 nmol/L is 10% and at 42.2 nmol/L is 8.5%. Body composition was measured using dual energy X-ray absorptiometry (DXA) (Prodigy Version 7.51 GE Lunar, Madison, WI, USA). Coefficient of variation was $<2\%$.[26]

Statistical analysis

Characteristics and body composition parameters of participants were summarised as median and interquartile ranges for each group. Multiple linear regressions were used to examine the relationship between HOMA2-IR and fat mass with sex, adjusting for age and total duration of GAHT. HOMA2-IR, android fat mass, gynoid fat mass and total fat mass were log-transformed to approximate normality, and results were back-transformed to estimate the ratio of geometric means with corresponding 95% confidence intervals (CIs). The mean difference with corresponding 95% CI (denoted in round brackets) were reported for fat mass measures that were not log-transformed. Separate analyses were done comparing females *versus* transgender men, and males *versus* transgender women. Further analysis of correlation between HOMA2-IR with fat mass was also performed using linear regression, and the Pearson correlation coefficients and *t*-tests for regression coefficient slope were reported. All statistical analyses were performed using R (version 3.6.0, R Foundation for Statistical Computing). A *p*-value of less than 0.05 was considered statistically significant. No adjustment for multiple comparisons was performed as the analysis is of an exploratory nature.

Results

Participant characteristics

This study recruited a total of 162 participants: 84 transgender individuals (41 trans women and 43 trans men) and 78 controls (30 cisgender females and 48 cisgender males). Participant characteristics are summarised in Table 1. Mean age in the cisgender male controls was younger than in trans women and as such analyses were adjusted for age.

All 43 trans men were receiving testosterone [intramuscular (IM) testosterone undecanoate

Table 1. Results (participant characteristics and effect sizes).

	Trans men ***n*** **= 43**	**Control cisgender women** ***n*** **= 48**	**Effect (95% CI)**
Age, years	28.8 (25.0–33.0)	28.1 (24.0–38.7)	–
BMI	25.2 (23.1–28.6)	22.7 (20.9–26.1)	–
Total duration of GAHT, months	44.0 (22.6–67.0)	–	–
Oestradiol, pmol/L	115.0 (93.0–164.0)	177.0 (32.5–359.2)	1.12 (0.57, 2.22)
Testosterone, nmol/L	15.6 (13.2–19.7)	0.9 (0.4–1.2)	**22.62 (15.73, 32.53)****
SHBG	31.5 (21.0–41.0)	98.5 (73.8–132.0)	**0.30 (0.21, 0.44)****
IGF-1	29.0 (22.0–33.5)	36.2 (28.1–40.2)	↓4.40 (–10.52, 1.73)
HOMA2-IR	1.2 (1.0–1.6)	1.1 (0.9–1.4)	0.99 (0.75, 1.30)
Total fat mass, kg	18.4 (14.3–28.0)	20.1 (14.6–25.4)	↑5.0 kg (–1.7, 11.8)
Android fat mass	2.0 (1.3–2.7)	1.4 (1.0–2.0)	1.4 (1.0, 2.1)
Gynoid fat mass	3.8 (2.9–5.4)	4.7 (3.5–5.5)	1.0 (0.8, 1.3)
Android:gynoid fat ratio	1.0 (0.9–1.1)	0.8 (0.7–0.9)	↑**0.2 (0.1, 0.3)****
Total lean mass, kg	48.1 (44.9–51.7)	40.7 (37.0–43.7)	↑**7.8 kg (4.0, 11.5)****
	Trans women ***n*** **= 41**	**Control cisgender men** ***n*** **= 30**	**Effect (95% CI)**
Age, years	41.1 (26.4–52.7)	32.0 (26.3–40.9)	–
BMI	23.6 (21.7–29.2)	23.8 (23.1–25.8)	–
Total duration of GAHT, months	39.0 (19.9–60.0)	–	–
Oestradiol, pmol/L	327.0 (147.2–460.5)+	72.5 (49.5–93.8)	**5.12 (3.44, 7.61)****
Testosterone, nmol/L	0.6 (0.4–0.9)	20.5 (16.0–24.1)	**0.04 (0.03, 0.06)****
SHBG	86.0 (59.5–116.8)	51.5 (39.0–75.2)	**1.37 (1.06, 1.76)***
IGF-1	22.5 (16.5–27.5)	28.3 (23.0–35.2)	↓1.86 (–6.57, 2.86)
HOMA2-IR	1.5 (1.3–2.2)	1.1 (0.8–1.3)	**1.6 (1.3, 1.9)****
Total fat mass, kg	22.5 (17.3–34.2)	15.7 (11.6–20.5)	↑**9.8 kg (3.9, 14.5)****
Android fat mass	2.1 (1.3–3.6)	1.5 (1.1–2.0)	**1.40 (1.05, 1.87)****
Gynoid fat mass	4.5 (3.9–6.4)	3.1 (2.5–4.1)	**1.53 (1.26, 1.85)****
Android:gynoid fat ratio	1.0 (0.8–1.0)	1.0 (0.9–1.2)	↓**0.1 (–0.2, 0.0)***
Total lean mass, kg	51.5 (47.0–55.7)	58.3 (54.2–64.0)	↓**6.9 kg (–10.6, –3.1)****

Results are presented as median (interquartile range).
Effect adjusted for age and total duration of GAHT is presented as a ratio of geometric means, or mean difference (where arrows are shown).
*$p < 0.05$.
**$p < 0.001$.
+Those on unmeasurable forms of oestradiol (such as ethinyloestradiol) were not included in the calculation of median oestradiol levels.
BMI, body mass index; CI, confidence interval; GAHT, gender-affirming hormone therapy; HOMA2-IR, Homeostasis Model of Insulin Resistance; IGF-1, insulin-like growth factor 1; SHBG, sex hormone binding globulin

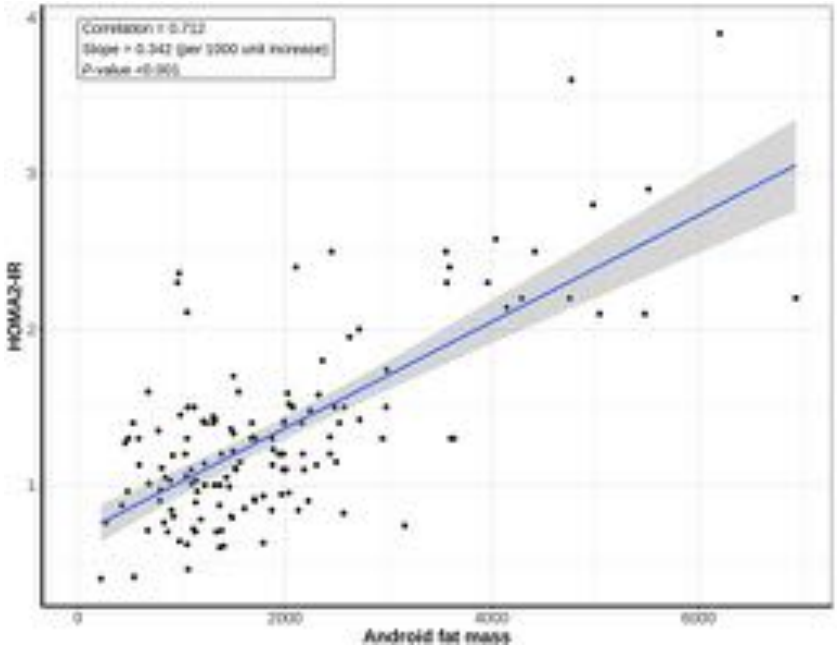

Figure 1. Correlation between android fat mass and Homeostasis Model of Insulin Resistance (HOMA2-IR).

$n=30$, IM testosterone enanthate $n=8$, topical testosterone gel 1% $n=5$] and all 41 trans women were receiving oestradiol (oral oestradiol valerate $n=34$, oral ethinyloestradiol $n=3$, transdermal oestradiol $n=4$). Seventy-eight per cent ($n=32$) of the trans women were taking anti-androgen therapy in addition to oestradiol therapy (cyproterone acetate $n=21$, spironolactone $n=5$, progestogens=5) (levonorgestrel $n=3$, medroxyprogesterone $n=1$, micronised progesterone $n=1$), gonadotropin releasing hormone analogue $n=1$. Twenty-seven per cent ($n=11$) of the trans women had undergone orchidectomy and 5% ($n=2$) of the trans men had undergone oophorectomy. In both trans men and trans women, the median oestradiol and testosterone levels were within the target reference range for their affirmed gender. In trans men, median oestradiol was 115.0 (93.0, 164.0) pmol/L (laboratory male reference range for oestradiol was <160 pmol/L) and median testosterone was 15.6 nmol/L (13.2, 19.7) (laboratory male reference range for testosterone was 9.9–27.8 nmol/L). In trans women, median oestradiol concentration was 327.0 (147.2, 460.5) pmol/L (laboratory female reference range for oestradiol during follicular phase was 46–607 pmol/L) and mean testosterone concentration was 0.6 (0.4, 0.9) nmol/L (laboratory female reference range for testosterone was <1.8 nmol/L).

Masculinising hormone therapy

Trans men had significantly higher lean mass than cisgender women with mean difference +7.8 kg 95% CI (4.0, 11.5), $p<0.001$) (Table 1). Other absolute body composition parameters (total fat mass, android and gynoid fat mass) were not significantly different from cisgender female controls; however, android:gynoid fat mass ratio was higher [mean difference +0.2 (0.1, 0.3), $p<0.001$]. Total fat mass was lower and android: gynoid fat mass was higher in trans men compared with cisgender female controls.

There was no difference in HOMA2-IR in trans men compared with cisgender female controls. Insulin resistance as estimated by HOMA2-IR was significantly correlated with android fat mass ($r^2=0.712$, $p<0.001$) and gynoid fat mass ($r^2=0.572$, $p<0.001$); see Figures 1 and 2. HOMA2-IR was also weakly correlated with android lean mass ($r^2=0.449$, $p<0.001$) and gynoid lean mass ($r^2=0.220$, $p=0.01$) (data not shown).

Whilst not the primary aim of our analyses, when comparing trans men with cisgender male controls, there was also no difference in HOMA2-IR (Supplemental Material Appendix 1 online). Although trans men were younger [trans men median 28.8 years (25.0–33.0)] compared with cisgender

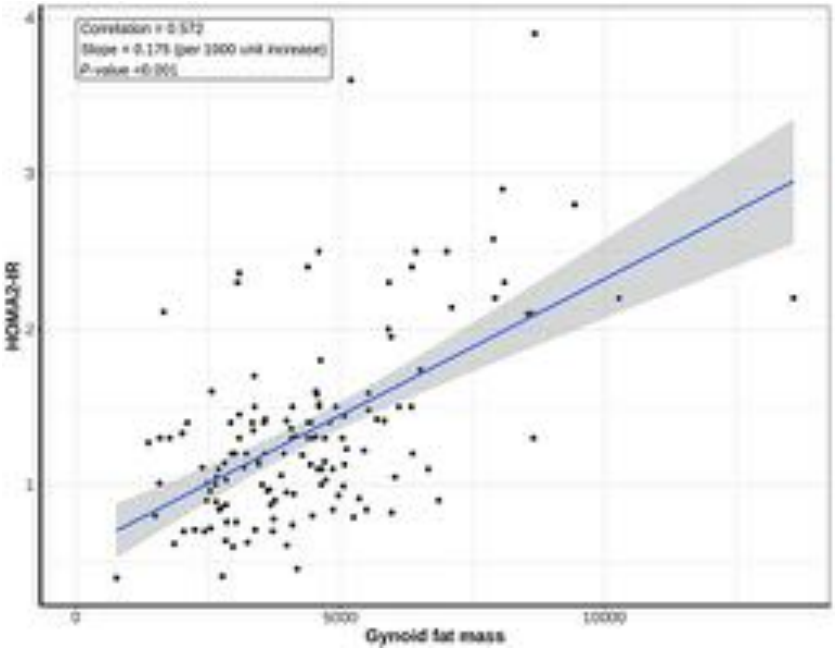

Figure 2. Correlation between gynoid fat mass and Homeostasis Model of Insulin Resistance (HOMA2-IR).

male controls [32.0 years (26.3–40.9)], they had a higher BMI and lower lean mass. Testosterone levels between groups were similar, but trans men had higher median oestradiol than cisgender male controls [115.0 pmol/L (93.0–164.0) *versus* 72.5 (49.5–93.8), $p < 0.01$] (Supplemental Appendix 1).

Feminising hormone therapy

Trans women had significantly lower lean mass [mean difference –6.8 kg (–10.6, –3.1), $p < 0.001$] and all fat mass parameters were significantly higher than cisgender male controls (Table 1). Android fat mass and gynoid fat mass were 40% and 53% higher respectively in trans women compared with cisgender male controls. There was a significantly lower android:gynoid fat ratio [mean difference –0.1 (–0.2, –0.0), $p < 0.05$].

Despite a lower android:gynoid fat mass, the total android fat mass was still high amongst trans women [median 2.1 kg (1.3–3.6)]. HOMA2-IR in trans women was 1.5; significantly higher than cisgender male controls ($p < 0.001$).

As with trans males, insulin resistance as estimated by HOMA2-IR was significantly correlated with android fat mass and, to a lesser degree, gynoid fat mass. See Figures 1 and 2.

As an exploratory analysis, when trans women were compared with cisgender female controls, HOMA2-IR was significantly higher in trans women [mean difference 1.47 (1.22, 1.77), $p < 0.01$] as was android fat mass [mean difference 1.39 kg (1.06, 1.83), $p < 0.001$]. Trans women were, however, significantly older [trans women median 41.1 years (26.4, 52.7)] compared with cisgender female controls [28.1 years (24.0, 38.)], had a significantly higher median oestradiol [327.0 pmol/L (147.2–460.5) *versus* 177.0 pmol/L (32.5–359.2), $p < 0.01$], but median testosterone concentrations and BMI were not different (Supplemental Appendix 1).

Trans women who had undergone orchidectomy ($n = 11$) had a significantly lower HOMA2-IR than trans women who had not ($n = 30$) [1.3 (1.1–1.5) *versus* 1.8 (1.4–2.3) $p < 0.03$]. This is despite trans women who had undergone orchidectomy being older in age [57.0 years (40.5, 68.4) compared with 33.5 (25.5, 48.9)] ($p < 0.03$), having longer duration of GAHT [115.1 months (41.7, 180.9) *versus* 27.0 months (15.3, 47.1)] ($p < 0.002$), yet similar body composition (no significant difference between BMI, fat mass, or lean mass). See Supplemental Appendix 2.

Discussion

This cross-sectional study showed a correlation between insulin resistance and fat mass in transgender individuals on established GAHT. Trans men had significantly higher lean mass as well as a higher android:gynoid fat ratio with no significant differences in insulin resistance or overall fat mass compared with control cisgender women. Trans women were more insulin resistant than control cisgender men and had lower lean mass, higher fat mass and a lower android:gynoid fat ratio. Insulin resistance correlated with android fat mass but, contrary to our original hypothesis, trans men did not have higher insulin resistance, most likely because higher lean mass may be protective in trans men.

Masculinising hormone therapy

Our findings of higher lean mass (median 7.8 kg) and a higher android:gynoid fat ratio are consistent with previous studies investigating masculinising hormone therapy in trans men.[27–39] Testosterone is known to increase the synthesis of muscle tissue by promoting differentiation of cells of the myogenic lineage and to inhibit the differentiation of adipocyte precursor cells.[40] Moreover, testosterone also inhibits lipoprotein lipase activity in adipocytes, an enzyme that increases fat deposition by decreasing adipose tissue lipolysis.[14]

We found no significant difference in insulin resistance between trans men and cisgender female controls, in keeping with all but one prior study in transgender men that showed either no change[27–29,31–37,41] or a decrease[30,39] in insulin resistance. All these studies were prospective longitudinal in design but only one had a control group.[33] The lack of change in insulin resistance is consistent with data that found no change in incretin (glucagon-like peptide-1 and gastric inhibitory polypeptide) responses in trans men before and after 12 months of GAHT.[41] The importance of body composition – which takes many months to change with masculinising hormone therapy – is highlighted by a small study demonstrating an increase in insulin resistance measured by hyperinsulinaemic euglycaemic clamps in 13 transgender men over the first 4 months,[38] but with follow-up over 12 months, no significant differences in insulin resistance over time emerged.[34]

It is important to note that the roles of sex steroids in insulin sensitivity in cisgender populations are not fully understood. Men with hypogonadism have increased insulin resistance;[15,42] however, exogenous testosterone replacement is associated only with a small, likely clinically insignificant, improvement in insulin sensitivity.[43] Elevated testosterone levels in women, such as in polycystic ovary syndrome, are associated with increased, rather than decreased, insulin resistance,[14] suggesting that the primary driver of insulin sensitivity may be due to the indirect, rather than direct, effects of testosterone.

The association observed between android fat mass and insulin resistance in trans men also supports the importance of body composition. Whilst there is also a significant correlation with gynoid fat mass and insulin resistance, it is stronger for android. This is in keeping with the predisposition to insulin resistance associated with abdominal adiposity in cisgender populations.[10]

Feminising hormone therapy

Trans women in our study had a significantly higher fat mass (median 9.8 kg) and lower lean mass (median 6.9 kg) compared with cisgender male controls. These results are in line with previous studies that have evaluated body composition using DXA in trans women.[29,30,41,44–50] Only four studies have also previously looked specifically at android and gynoid fat mass regions, either using DXA or magnetic resonance imaging, and, like this study, found an increase in fat mass in both regions.[41,34,45,51] These findings support the theory that activation of oestrogen receptors can lead to stimulation of adipocyte proliferation as well as lipoprotein lipase activity.[52,53] Oestrogen may also act indirectly *via* oestrogen receptors in the hypothalamus to regulate energy expenditure.[53,54]

We found that trans women have significantly higher levels of insulin resistance estimated by HOMA2-IR compared with cisgender male controls. Nine studies have previously looked at insulin resistance in trans women on feminising hormone therapy and, of these, six similarly showed worsening insulin resistance.[29,30,32,34,38,41] Three did not detect a significant change – one showed a trend towards increase insulin resistance but failed to reach statistical significance,[55] another had a sample size of only six participants[27] and the remaining study did not measure body composition changes so it is unclear whether changes to this occurred.[39] All but one study[55] was prospective longitudinal in design but none had a control group.

Only four studies have specifically looked at android and gynoid fat mass regions, and of these only one sought to correlate insulin resistance and regional fat mass. This 2003 case–control study found a small increase in visceral fat area in both trans males and trans females; however, it failed to find a correlation with insulin sensitivity or fasting insulin levels in either group, likely due to small sample size and lack of control group.[34]

It is important to note that our findings, and others', contradict existing theories and animal models suggesting that oestradiol has direct beneficial effects on insulin sensitivity.[14,16,56]

In studies of cisgender women, oestrogen has generally been associated with a favourable effect on insulin sensitivity.[57] Low oestrogen states such as menopause are associated with a decrease in insulin sensitivity, increased central adiposity and a higher risk of metabolic disease, and subsequent administration of oestrogen therapy in menopausal women is associated with an improvement in insulin sensitivity.[58]

One proposed explanation for the differences seen in trans women is the high amount of overall fat as well as retention of central fat, which may indirectly lead to increased insulin resistance. This may mitigate any potentially beneficial direct effect of oestrogen on the insulin receptor.[16]

The route of oestrogen administration may also be important, with the majority of trans women in this study taking oral oestradiol valerate. Oral, but not transdermal, oestrogen has been shown to impair the metabolic effect of growth hormone in the liver, resulting in lower IGF-1 production and fat oxidation with a subsequent gain of body fat and loss of lean tissue seen in postmenopausal women.[11–13] Our study showed that trans women had a lower IGF-1 than both cisgender male and cisgender female controls. This is in contrast to the only other study investigating IGF-1 and body composition in trans women, which found serum IGF-1 levels at 24 months were similar to baseline, and that any changes were independent of the route of administration of oestrogen.[59] All participants aged under 45 years ($n = 34$) were taking oral oestradiol and those aged 45 years and over ($n = 15$) were taking transdermal oestradiol, so participant age may have been a factor. Interestingly, another study showed that there was no difference in total regional fat mass between trans women on oral or transdermal oestrogen.[45] Oestrogen doses in GAHT are generally higher than for menopausal hormone therapy, so further prospective studies in the transgender population are warranted.

Our findings of lower HOMA2-IR in the subset of trans women who had undergone orchidectomy are in keeping with a small prospective study from 2016,[60] which hypothesised that orchidectomy in this context may be protective due to the ratio of circulating sex hormone levels; however, further research is needed to confirm this.

Limitations

Limitations of the study include its cross-sectional design. Characteristics were not assessed prior to GAHT and baseline differences may have existed. In fact, two previous studies suggest that trans women have lower muscle mass and higher fat mass than cisgender male controls at baseline and reported doing significantly less physical activity.[47,50] The trans women were slightly older than the cisgender male controls. For simplicity the data were presented uniformly using the median and interquartile range; however, the mean age between the two groups was more closely matched – trans women were aged 40.8 $\pm$15.7 years *versus* cisgender male controls aged 36.0 $\pm$ 14.2 years. Data were adjusted for age. Trans men had a higher median BMI than cisgender female controls; however, if anything, this should lead to an overestimation of insulin resistance in the trans male group. Whilst our participants had undertaken GAHT for several years (median 44 months in trans men and 39 months in trans women) it is possible that changes to body composition are still ongoing. Participants were not on standardised GAHT regimens and we cannot discount that different hormone formulations, particularly oral *versus* transdermal oestradiol, may have differential effects on body composition and insulin resistance. Many participants were on a progestogen and this may affect the outcomes measured in this study. Testosterone and oestradiol assays used measured *via* immunoassay rather than liquid chromatography mass spectrometry. Although this study focuses on body composition and insulin resistance there are other contributors to cardiovascular risk. Additional research is needed and a prospective longitudinal study with a cisgender control group is needed to further investigate the impact of GAHT on body composition and insulin resistance.

Conclusion

We highlight the importance of lean and fat mass and the correlation with insulin resistance among transgender individuals and the relatively stronger correlation of insulin resistance with android over gynoid fat. Significantly higher levels of fat mass and lower lean mass in trans women is associated with insulin resistance, and whilst there is some degree of higher fat mass in trans men on established GAHT, the significantly higher lean mass relative to fat mass appears to be protective. These findings provide insights into sex hormone action and suggest a predominantly indirect mechanism of action (*via* changes in body composition) in mediating insulin resistance. Longitudinal studies are needed to further investigate this correlation and to better guide clinical practice. Until then, a proactive clinical approach to mitigate gain in fat as well maintain or increase lean mass, particularly in trans women, should be strongly encouraged.

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Author contributions

IB was involved in the conception, design, analysis, interpretation of data and writing the manuscript. CS and SYL were involved in data analysis and revising the manuscript. GP, MG, JDZ were involved in revising the manuscript. ASC was involved in the conception, design, funding acquisition, analysis, interpretation of data, as well as revising the manuscript.

Conflict of interest statement

AC has received speaker's honoraria from Astra Zeneca and Merck Sharp & Dohme. MG has received research funding from Bayer, Weight Watchers, Lilly Otzuka, and speaker's honoraria from Besins Health Care and Novartis. All other authors have no conflicts of interest to declare.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Supplemental material

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References

1. Dhejne C, Lichtenstein P, Boman M, *et al.* Long-term follow-up of transsexual persons undergoing sex reassignment surgery: cohort study in Sweden. *PLoS One* 2011; 6: e16885.
2. Flores AR, Herman JL, Gates GJ, *et al. How many adults identify as transgender in the United States?* Los Angeles: The Williams Institute, 2016.
3. Telfer M, Tollit M and Feldman D. Transformation of health-care and legal systems for the transgender population: the need for change in Australia. *J Paediatr Child Health* 2015; 51: 1051–1053.
4. Cheung AS, Ooi O, Leemaqz S, *et al.* Sociodemographic and clinical characteristics of transgender adults in Australia. *Transgend Health* 2018; 3: 229–238.
5. White Hughto JM and Reisner SL. A systematic review of the effects of hormone therapy on psychological functioning and quality of life in transgender individuals. *Transgend Health* 2016; 1: 21–31.
6. Getahun D, Nash R, Flanders WD, *et al.* Cross-sex hormones and acute cardiovascular events in transgender persons: a cohort study. *Ann Intern Med* 2018; 169: 205–213.
7. Nota NM, Wiepjes CM, de Blok CJM, *et al.* Occurrence of acute cardiovascular events in transgender individuals receiving hormone therapy. *Circulation* 2019; 139: 1461–1462.

8. Palmer BF and Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol* 2015; 402: 113–119.
9. 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.
10. Despres JP. Body fat distribution and risk of cardiovascular disease: an update. *Circulation* 2012; 126: 1301–1313.
11. Moller N, Jorgensen JO, Abildgard N, *et al.* Effects of growth hormone on glucose metabolism. *Horm Res* 1991; 36(Suppl. 1): 32–35.
12. Leung KC, Johannsson G, Leong GM, *et al.* Estrogen regulation of growth hormone action. *Endocr Rev* 2004; 25: 693–721.
13. O'Sullivan AJ, Crampton LJ, Freund J, *et al.* The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. *J Clin Invest* 1998; 102: 1035–1040.
14. Geer EB and Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med* 2009; 6(Suppl. 1): 60–75.
15. Dhindsa S, Ghanim H, Batra M, *et al.* Insulin resistance and inflammation in hypogonadotropic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. *Diabetes Care* 2016; 39: 82–91.
16. Mauvais-Jarvis F, Clegg DJ and Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* 2013; 34: 309–338.
17. Salpeter SR, Walsh JM, Ormiston TM, *et al.* Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. *Diabetes Obes Metab* 2006; 8: 538–554.
18. Simon D, Charles MA, Lahlou N, *et al.* Androgen therapy improves insulin sensitivity and decreases leptin level in healthy adult men with low plasma total testosterone: a 3-month randomized placebo-controlled trial. *Diabetes Care* 2001; 24: 2149–2151.
19. Esteghamati A, Khalilzadeh O, Abbasi M, *et al.* HOMA-estimated insulin resistance is associated with hypertension in Iranian diabetic and non-diabetic subjects. *Clin Exp Hypertens* 2008; 30: 297–307.
20. Geloneze B, Vasques AC, Stabe CF, *et al.* HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). *Arq Bras Endocrinol Metabol* 2009; 53: 281–287.
21. Zhang X, Li J, Zheng S, *et al.* Fasting insulin, insulin resistance, and risk of cardiovascular or all-cause mortality in non-diabetic adults: a meta-analysis. *Biosci Rep* 2017; 37: BSR20170947.
22. Porte D Jr and Kahn SE. Beta-cell dysfunction and failure in type 2 diabetes: potential mechanisms. *Diabetes* 2001; 50(Suppl. 1): S160–S163.
23. Levy JC, Matthews DR and Hermans MP. Correct Homeostasis Model Assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998; 21: 2191–2192.
24. Diabetes Trials Unit. HOMA2 calculator, https://www.dtu.ox.ac.uk/homacalculator/. (2004, accessed 28 December 2020).
25. Wallace TM, Levy JC and Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27: 1487–1495.
26. Hamilton EJ, Gianatti E, Strauss BJ, *et al.* Increase in visceral and subcutaneous abdominal fat in men with prostate cancer treated with androgen deprivation therapy. *Clin Endocrinol (Oxf)* 2011; 74: 377–383.
27. Aranda G, Fernandez-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: 20–28.
28. Aranda G, Mora M, Hanzu FA, *et al.* Effects of sex steroids on cardiovascular risk profile in transgender men under gender affirming hormone therapy. *Endocrinol Diabetes Nutr* 2019; 66: 385–392.
29. Auer MK, Cecil A, Roepke Y, *et al.* 12-months metabolic changes among gender dysphoric individuals under cross-sex hormone treatment: a targeted metabolomics study. *Sci Rep* 2016; 6: 37005.
30. Auer MK, Ebert T, Pietzner M, *et al.* Effects of sex hormone treatment on the metabolic syndrome in transgender individuals: focus on metabolic cytokines. *J Clin Endocrinol Metab* 2018; 103: 790–802.
31. Berra M, Armillotta F, D'Emidio L, *et al.* Testosterone decreases adiponectin levels in female to male transsexuals. *Asian J Androl* 2006; 8: 725–729.

32. Colizzi M, Costa R, Scaramuzzi F, *et al.* Concomitant psychiatric problems and hormonal treatment induced metabolic syndrome in gender dysphoria individuals: a 2 year follow-up study. *J Psychosom Res* 2015; 78: 399–406.
33. Cupisti S, Giltay EJ, Gooren LJ, *et al.* The impact of testosterone administration to female-to-male transsexuals on insulin resistance and lipid parameters compared with women with polycystic ovary syndrome. *Fertil Steril* 2010; 94: 2647–2653.
34. Elbers JMH, Giltay EJ, Teerlink T, *et al.* Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol (Oxf)* 2003; 58: 562–571.
35. Gava G, Mancini I, Cerpolini S, *et al.* Testosterone undecanoate and testosterone enanthate injections are both effective and safe in transmen over 5 years of administration. *Clin Endocrinol (Oxf)* 2018; 89: 878–886.
36. Meriggiola MC, Armillotta F, Costantino A, *et al.* Effects of testosterone undecanoate administered alone or in combination with letrozole or dutasteride in female to male transsexuals. *J Sex Med* 2008; 5: 2442–2453.
37. Pelusi C, Costantino A, Martelli V, *et al.* Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med* 2014; 11: 3002–3011.
38. Polderman KH, Gooren LJG, Asscheman H, *et al.* Induction of insulin resistance by androgens and estrogens. *J Clin Endocrinol Metab* 1994; 79: 265–271.
39. Yahyaoui R, Esteva I, Haro-Mora JJ, *et al.* Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. *J Clin Endocrinol Metab* 2008; 93: 2230–2233.
40. Miller KK. Androgen deficiency: effects on body composition. *Pituitary* 2009; 12: 116–124.
41. Shadid S, Abosi-Appeadu K, De Maertelaere AS, *et al.* Effects of gender-affirming hormone therapy on insulin sensitivity and incretin responses in transgender people. *Diabetes Care* 2020; 43: 411–417.
42. Cheung AS, Hoermann R, Dupuis P, *et al.* Relationships between insulin resistance and frailty with body composition and testosterone in men undergoing androgen deprivation therapy for prostate cancer. *Eur J Endocrinol* 2016; 175: 229–237.
43. Mohler ER III, Ellenberg SS, Lewis CE, *et al.* The effect of testosterone on cardiovascular biomarkers in the testosterone trials. *J Clin Endocrinol Metab* 2018; 103: 681–688.
44. Haraldsen IR, Haug E, Falch J, *et al.* Cross-sex pattern of bone mineral density in early onset gender identity disorder. *Horm Behav* 2007; 52: 334–343.
45. Klaver M, de Blok CJM, Wiepjes CM, *et al.* Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *Eur J Endocrinol* 2018; 178: 163–171.
46. Fighera TM, da Silva E, Lindenau JD, *et al.* Impact of cross-sex hormone therapy on bone mineral density and body composition in transwomen. *Clin Endocrinol (Oxf)* 2018; 88: 856–862.
47. 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: 163–171.
48. Wierckx K, Van Caenegem E, Schreiner T, *et al.* Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. *J Sex Med* 2014; 11: 1999–2011.
49. Mueller A, Zollver H, Kronawitter D, *et al.* Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. *Exp Clin Endocrinol Diabetes* 2011; 119: 95–100.
50. Lapauw B, Taes Y, Simoens S, *et al.* Body composition, volumetric and areal bone parameters in male-to-female transsexual persons. *Bone* 2008; 43: 1016–1021.
51. Elbers JM, Asscheman H, Seidell JC, *et al.* Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J Clin Endocrinol Metab* 1997; 82: 3267–3270.
52. Karastergiou K, Smith SR, Greenberg AS, *et al.* Sex differences in human adipose tissues – the biology of pear shape. *Biol Sex Differ* 2012; 3: 13.
53. Lovejoy JC and Sainsbury A. Sex differences in obesity and the regulation of energy homeostasis. *Obes Rev* 2009; 10: 154–167.
54. Brown LM, Gent L, Davis K, *et al.* Metabolic impact of sex hormones on obesity. *Brain Res* 2010; 1350: 77–85.

55. Gava G, Cerpolini S, Martelli V, *et al.* Cyproterone acetate vs leuprolide acetate in combination with transdermal estradiol in transwomen: a comparison of safety and effectiveness. *Clin Endocrinol (Oxf)* 2016; 85: 239–246.

56. Nokoff NJ, Scarbro SL, Moreau KL, *et al.* Body composition and markers of cardiometabolic health in transgender youth compared with cisgender youth. *J Clin Endocrinol Metab* 2019; 105: e704–e714.

57. Meyer MR, Clegg DJ, Prossnitz ER, *et al.* Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiol (Oxf)* 2011; 203: 259–269.

58. D'Eon TM, Souza SC, Aronovitz M, *et al.* Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 2005; 280: 35983–35991.

59. Van Caenegem E, Wierckx K, Taes Y, *et al.* Preservation of volumetric bone density and geometry in trans women during cross-sex hormonal therapy: a prospective observational study. *Osteoporos Int* 2015; 26: 35–47.

60. Nelson MD, Szczepaniak LS, Wei J, *et al.* Transwomen and the metabolic syndrome: is orchiectomy protective? *Transgend Health* 2016; 1: 165–171.

Global Coagulation Assays in Transgender Women on Oral and Transdermal Estradiol Therapy

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Context: The thrombotic effects of estradiol therapy in transgender women are unclear. Global coagulation assays (GCA) may be better measures of hemostatic function compared with standard coagulation tests.

Objective: To assess the GCA profiles of transgender women in comparison to cisgender controls and to compare how GCA differ between routes of estradiol therapy in transgender women.

Design: Cross-sectional case-control study.

Setting: General community.

Participants: Transgender women, cisgender male and cisgender female controls.

Main outcome measures: Citrated blood samples were analyzed for (i) whole blood thromboelastography (TEG®5000), (ii) platelet-poor plasma thrombin generation (calibrated automated thrombogram); and (iii) platelet-poor plasma fibrin generation (overall hemostatic potential assay). Mean difference (95% confidence intervals) between groups are presented.

Results: Twenty-six transgender women (16 oral estradiol, 10 transdermal estradiol) were compared with 98 cisgender women and 55 cisgender men. There were no differences in serum estradiol concentration ($P = 0.929$) and duration of therapy ($P = 0.496$) between formulations. Transgender women demonstrated hypercoagulable parameters on both thromboelastography (maximum amplitude + 6.94 mm (3.55, 10.33); $P < 0.001$) and thrombin generation (endogenous thrombin potential + 192.62 nM.min (38.33, 326.91); $P = 0.009$; peak thrombin + 38.10 nM (2.27, 73.94); $P = 0.034$) but had increased overall fibrinolytic potential (+4.89% (0.52, 9.25); $P = 0.024$) compared with cisgender men. No significant changes were observed relative to cisgender women. Route of estradiol delivery or duration of use did not influence the GCA parameters.

Conclusion: Transgender women on estradiol therapy demonstrated hypercoagulable GCA parameters compared with cisgender men with a shift towards cisgender female parameters. Route of estradiol delivery did not influence the GCA parameters. **(*J Clin Endocrinol Metab* 105: e2369–e2377, 2020)**

Freeform/Key Words: global coagulation assays, coagulation, estrogen, thrombosis, transgender

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Abbreviations: APTT, activated partial thromboplastin time;CAT, calibrated automated thrombogram;ETP, endogenous thrombin potential;GCA, global coagulation assay;HbA1c, glycated hemoglobin A1c; OFP, overall fibrinolytic potential;OHP, overall hemostatic potential; PAI-1, plasminogen activator inhibitor-1;PT, prothrombin time;SHBG, sex hormone–binding globulin.

Rapid rises in the number of transgender individuals, including gender-diverse people seeking health services have been observed worldwide (1-4). Approximately 0.1% to 2% of the general population are estimated to be gender-diverse (5). Transgender women (assigned male at birth) are typically treated with estradiol as feminizing gender-affirming hormone therapy to increase their serum estradiol concentrations to the cisgender female reference range (6). Antiandrogens to lower testosterone concentrations are also commonly used in transgender women who have not had genital reassignment surgery (7). Most transgender women remain on estradiol therapy for life although the long-term effects are unclear.

One of the adverse effects of estradiol therapy is thrombosis and this risk is applicable to cisgender women on the oral contraceptive pill, postmenopausal women on oral estradiol therapy, as well as transgender women undergoing feminizing hormone therapy with estradiol (8-10). Recently, a large Dutch study of 2517 transgender women followed for a median of 6 years found that myocardial infarction and ischemic stroke was increased by more than 2-fold in transgender women compared with cisgender women. Moreover, this study reported a 5-fold increase in incidence of venous thrombosis in transgender women, compared with both cisgender women and men (11). Moreover, the impact of the mode of estradiol delivery on the thrombotic risk in transgender women remains unclear, with data in cisgender women demonstrating that transdermal estradiol use is associated with lower venous (12) and arterial thrombotic events compared with oral estradiol therapy (13). One of the remaining challenges is our ability to predict thrombotic risk in these individuals.

Current routine coagulation tests, such as activated partial thromboplastin time (APTT) or prothrombin time (PT), only measure the time to start of clot formation, which accounts for < 5% of total thrombin generation (14), making them poor indicators of a hypercoagulable state. Relatively newer global coagulation assays (GCA) may provide a more comprehensive assessment of global hemostatic profile which may aid in predicting venous and arterial thrombotic risks (15, 16). Thromboelastography, currently used to guide blood product replacement in trauma and massive transfusion settings (17), has recently been shown to have a potential role in predicting hypercoagulability (16). Furthermore, thrombin generation was found to be altered in individuals with coronary artery disease and may help to distinguish individuals with low, intermediate, or high risk of venous thromboembolism (18, 19). Fibrin generation using overall hemostatic potential assay is less well studied, but fibrin generation has been reported to be increased and fibrinolysis to be reduced in patients with arterial disease or venous thromboembolism (20).

This study aimed to use GCA to assess the hemostatic profiles of transgender women compared with cisgender controls. As a secondary objective, we also investigated the differences in GCA between transgender women on oral estradiol therapy compared with those on transdermal therapy. We hypothesized that transgender women on estradiol therapy would have more hypercoagulable GCA parameters than cisgender male and female controls and those on oral therapy would also have more hypercoagulable GCA parameters than those on transdermal therapy.

Material and Methods

This study was conducted at 2 major tertiary referral centers (Austin Health and Northern Health, Melbourne, Australia) and was approved by the Austin Health Human Research Ethics Committee (H2013/04977) and the Northern Health Human Research Ethics Committee (P5/13). This cross-sectional analysis involved transgender women on established estradiol therapy for at least 6 months and they were recruited from outpatient endocrine clinics and in the general community via advertisement on social media. Transgender women on anticoagulation or antiplatelet therapy, those who had previous venous or arterial thrombotic events, moderate renal or liver impairment (estimated glomerular filtration rate < 60ml/min/1.73m^2; liver function tests more than 3 times upper limit normal or known liver pathology), cardiac disease (ischemic heart disease or cardiac failure as reported by the individual or recorded on medical records), or current malignancy and active infection/inflammation were excluded. Basic demographic details such as age, body mass index, cardiovascular risk factors, duration and type of estradiol therapy (and/or anti-androgen therapy), as well as mode of delivery were recorded.

The transgender women were compared with previously recruited cisgender male and female controls. In addition to above exclusion criteria, the cisgender healthy controls were not known to have any modifiable cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes or smoking nor on any medications that may modify the coagulation parameters such as oral contraceptive pill and hormone replacement therapy. Written informed consent was obtained from all participants.

Fasting blood samples were obtained from participants. Electrochemiluminescence immunoassay assay using Cobas C8000, Roche Diagnostics (Basel, Switzerland) was used to detect serum total testosterone (minimum detection 0.4 nmol/l, inter-assay variation 5.0%-6.9%), and estradiol (interassay variation 3.5% at 330 pmol/L and 1.9% at 1800 pmol/L). Full blood examination, renal and liver function tests, coagulation studies and von Willebrand studies were performed using standard methodology for routine clinical care. The conventional tests were performed in 2 laboratories, both accredited

by the Royal College of Pathologists of Australasia (RCPA) and National Association of Testing Authorities (NATA) based on the international standard ISO 15189 Standard for Medical Laboratories. Both laboratories also participate in RCPA quality assurance programs. The experimental GCA assays were performed in 1 research laboratory by a single operator. Thromboelastography using TEG5000 was performed on whole blood citrated tubes and analyzed within 4 hours of sample collection. Other research bloods were collected in citrated tubes, double spun at 2500*g* within 2 hours to generate platelet-poor plasma and immediately stored at −80°C for thrombin and fibrin generation testing (16).

Thromboelastography assay

Thromboelastography measures the changes in the elastic properties of whole blood during clot formation, propagation, and dissolution (21). 1 mL of citrated whole blood was added to a kaolin tube and 340 µL of this mix was added to 20 µL of calcium chloride in the cup. All samples underwent computerized TEG analysis (TEG®5000; Haemonetics). Thromboelastography parameters generated include R-time (minutes), the reaction time to first clot formation; K-time (minutes), the achievement of certain clot firmness; maximum amplitude (MA), the maximum strength of clot (within-run variation 3.8%, between-run variation 3.4%); α-angle (°), kinetics of clot development; and LY30 (%), percent lysis 30 minutes after maximum amplitude.

Thrombin generation assay via calibrated automated thrombogram

The thrombin generation assay determined the rate and extent of thrombin generated from thawed platelet-poor plasma samples after tissue factor stimulus (5 pM), using calibrated automated thrombogram (CAT) (Diagnostica Stago) (22, 23). All samples were performed in triplicates, with the average values recorded. A dedicated software program, Thrombinoscope BV (Diagnostica Stago), was used to calculate thrombin activity against the calibrator to display thrombin activity versus time. Parameters of thrombin generation, including lag time (minutes), the initiation phase; endogenous thrombin potential (ETP; nM.min), amount of thrombin formed over 60 minutes (within-run variation 4.0%, between-run variation 9.3%); peak thrombin (nM), highest thrombin concentration that can be achieved; and velocity index (nM/min) were calculated.

Overall hemostatic potential assay

This assay is a spectrophotometric assessment of fibrin-aggregation (24, 25). For each sample, the overall coagulation potential (OCP) curve was generated from the addition of a buffer consisting of Tris/NaCl/$CaCl_2$/thrombin to 75 µL of platelet-poor plasma (within-run variation 3.5%, between-run variation 4.6%) and a corresponding overall hemostatic potential (OHP) curve was generated using a similar buffer with the addition of tissue plasminogen activator (tPA) (within-run variation 4.0%, between-run variation 6.8%). All samples were performed in triplicates. Using the FLUOstar Optima (BMG Labtech) plate reader operating at 37°C, the minute-by-minute absorbance at 405 nm was recorded to construct 2 fibrin-aggregation curves (OCP and OHP) with the difference between the 2 areas under the curve reflecting the overall fibrinolytic potential (OFP).

Statistical analyses

Baseline characteristics were reported as median and interquartile ranges, with associations examined using Student's *t*-test or Mann-Whitney U test as the non-parametric alternative. Analysis of GCAs were performed using linear regression, adjusted for age, with Tukey's post-hoc pairwise comparisons between cisgender male or cisgender female controls and transgender women. Further analysis was performed amongst transgender women, where data was available, using linear regression adjusted for age to examine the relationship between GCAs and testosterone concentration, estradiol concentration, estradiol treatment duration and anti-androgen treatment. All analyses were performed using R, version 3.6.1 (R Foundation for Statistical Computing).

Results

Demographics and baseline characteristics

Twenty-six transgender women were compared with 98 healthy cisgender female and 55 cisgender male controls (Table 1). Only 2 transgender women had associated cardiovascular risk factors (1 with stage 2 hypertension and the other with stage 1 hypertension, hyperlipidemia (total cholesterol 2.7 mmol/L; low-density lipoprotein [LDL] 1.0 mmol/L) and type 2 diabetes (glycated hemoglobin A1c [HbA1c] 7.1%), all on treatment). Two transgender women reported being current smokers. From a cardiovascular risk perspective, the median body mass index of the transgender women was 25.6 kg/m^2 and they had lower HbA1c and total cholesterol compared with both groups of controls.

Sixteen transgender women were taking oral estradiol (all using estradiol valerate, median dose 6 mg [range, 4-8]) with the remaining 10 women using transdermal estradiol (median dose 100 mcg/24 hours [100-137.5]). The overall duration of estradiol therapy was 25.5 months (22.5-31.2). Estradiol concentrations achieved and estradiol treatment duration as well as median testosterone levels were no different between transdermal and oral groups (Table 2). Nine individuals were taking estradiol therapy alone (5 of these women had had previous genital reassignment surgery), 14 were taking estradiol plus cyproterone acetate (median daily dose 12.5 mg [12.5-18.8]) and 3 were taking estradiol plus spironolactone (median daily dose 100 mg [100-200]).

In comparison to cisgender male controls, transgender women had lower hemoglobin levels but largely comparable routine coagulation parameters (Table 1). There were no differences in APTT, PT, factor VIII, and von Willebrand factor antigen. Transgender women

Table 1. Characteristics of Transgender Women on Estradiol Therapy Compared With Cisgender Male and Female Controls

	Transgender Women	Cisgender Male Controls	*P* Value*	Cisgender Female Controls	*P* Value**
N	26	55		98	
Age (years)	32.8 (26.7-44.7)	28.7 (24.9-57.3)	0.867	44.9 (25.5- 58.3)	0.072
Hemoglobin (g/L)	138.5 (133.0-143.8)	154.5 (150.0-161.0)	**<0.001**	139.5 (134.2-146.0)	0.643
Platelet count (× 10^9/L)	272.5 (229.0-297.0)	230.5 (202.0-257.8)	**0.004**	258.0 (222.0-289.0)	0.210
Prothrombin time (s)	12.0 (11.0-12.0)	11.4 (10.8-13.0)	0.662	11.0 (10.3-11.7)	**0.001**
Activated partial thromboplastin time (s)	29.0 (27.0-30.0)	29.2 (27.0-31.2)	0.473	27.5 (25.6-30.0)	0.052
Factor VIII (%)	103.0 (81.0-120.0)	100.5 (83.2-127.5)	0.798	112.0 (91.0-149.5)	**0.029**
von Willebrand factor antigen (%)	115.5 (96.8-135.5)	100.0 (81.8-136.0)	0.091	108.0 (89.0-141.5)	0.327
D-dimer (ng/mL FEU)	282.0 (215.0-393.0)	240.0 (150.0-270.0)	**0.001**	250.0 (150.0-342.5)	**0.022**
Fibrinogen (g/L)	3.1 (2.7-3.4)	2.5 (2.3-3.2)	**0.009**	3.1 (2.7-3.6)	0.620
Total cholesterol (mmol/L)	4.6 (3.7-4.9)	5.2 (4.1-5.9)	**0.004**	5.1 (4.4-6.1)	**<0.001**
HbA1c (%)	5.0 (4.9-5.3)	5.2 (5.1-5.5)	**0.020**	5.4 (5.1-5.6)	**0.001**
Thromboelastography (TEG)					
R time (min)	6.2 (5.2-6.8)	6.7 (5.5-8.2)	0.193	6.2 (5.2-7.2)	0.608
K time (min)	1.8 (1.6-1.9)	2.5 (2.2-3.1)	**<0.001**	2.0 (1.6-2.3)	0.143
Alpha-angle (°)	47.6 (43.0-52.0)	55.4 (50.4-58.9)	0.154	61.3 (49.5-66.0)	**0.001**
Maximum amplitude (mm)	64.2 (60.4-67.4)	57.7 (54.0-61.8)	**<0.001**	61.1 (58.2-65.0)	0.065
Lysis 30 (%)	0.5 (0.1-1.5)	0.1 (0.0-1.1)	0.870	0.6 (0.1-1.4)	0.483
Calibrated automated thrombogram (CAT)					
Lag time (min)	3.3 (3.0-3.7)	3.3 (3.0-3.7)	0.695	3.0 (2.7-3.7)	0.092
Endogenous thrombin potential (nM.min)	1380.2 (1214.2-1582.2)	1215.4 (1080.4-1376.0)	**0.009**	1375.5 (1193.2-1585.8)	0.823
Peak thrombin (nM)	207.5 (192.5-276.4)	188.1 (146.8-230.2)	**0.034**	240.9 (186.3-283.5)	0.832
Velocity index (nM/min)	56.9 (44.9-93.1)	52.0 (31.0-72.9)	0.185	75.7 (47.2-105.2)	0.304
Overall Hemostatic Potential (OHP)					
Overall coagulation potential (U)	50.8 (46.3-55.7)	51.7 (43.0-63.5)	0.776	58.1 (52.2-65.4)	**0.022**
Overall hemostatic potential (U)	23.3 (21.1-26.1)	25.8 (21.4-30.1)	0.079	27.2 (24.0-31.3)	**0.042**
Overall fibrinolytic potential (%)	53.5 (50.0-55.2)	49.5 (42.6-54.3)	**0.024**	52.7 (49.0-55.9)	0.549

Median and (interquartile range) are reported. Post hoc (Tukey's) *P* value from models adjusted for age. **P* value of transgender women vs cisgender men; ***P* value of transgender women vs cisgender women

had higher D-dimer and fibrinogen ($P < 0.05$) compared with cisgender male controls, although the levels remained within the normal reference range. When compared with cisgender female controls, transgender women had slightly more prolonged PT but comparable APTT and fibrinogen levels. D-dimer was higher in the transgender women while factor VIII levels were higher in cisgender female controls, with no differences seen in the von Willebrand factor antigen levels.

Global coagulation assays in transgender women on estradiol therapy compared with cisgender male controls

Thromboelastography. Transgender women demonstrated shortened median K-time (1.8 vs 2.5 minutes; $P < 0.001$) representing faster clot amplification and increased clot strength or maximum amplitude (64.2 vs 57.7 mm; $P < 0.001$) compared with cisgender male controls (Table 1).

Thrombin generation. Thrombin generation was significantly increased in transgender women compared with cisgender male controls (ETP 1380.2 vs 1215.4 nM.min; peak thrombin (207.5 vs 188.1 mm; $P = 0.034$).

Fibrin generation. There were no differences in the fibrin generation parameters but the overall fibrinolytic potential was increased in the transgender women (53.5% vs 49.5%; $P = 0.024$) compared with cisgender male controls.

Global coagulation assays in transgender women on estradiol therapy compared with cisgender female controls

Thromboelastography. The rate of clot formation (alpha angle) was reduced in transgender women compared with cisgender female controls (47.6 vs 61.3°; $P = 0.001$) while other parameters were not significant

Table 2. Characteristics of Transgender Women on Oral Estradiol Therapy Compared With Those on Transdermal Therapy

	Oral Estradiol Therapy	Transdermal Estradiol Therapy	*P* Value (oral vs transdermal)
N	16	10	
Age (years)	29.0 (25.4-42.7)	36.7 (31.9-59.1)	0.165
Body mass index (kg/m^2)	25.2 (21.0-29.7)	27.7 (24.0-35.5)	0.159
Duration of estradiol therapy (months)	28.5 (15.5-34.2)	14.0 (10.8-62.5)	0.496
Dose of estradiol therapy	6 mg (4-8)	100 mcg/24h (100-137.5)	
Serum estradiol (pmol/L)	362.0 (263.2-451.0)	311.5 (220.2-459.2)	0.929
Serum testosterone (nmol/L)	0.6 (0.4-1.0)	0.9 (0.7-1.6)	0.549
Hemoglobin (g/L)	140.0 (132.8-143.2)	138.5 (133.5-143.0)	0.833
Platelet count (× 10^9/L)	291.0 (232.0-305.5)	263.5 (229.2-285.2)	0.493
Prothrombin time (s)	11.5 (11.0-12.0)	12.0 (12.0-12.0)	0.274
Activated partial thromboplastin time (s)	29.0 (27.0-30.0)	29.6 (27.0-31.0)	0.537
Factor VIII (%)	97.0 (84.0-118.0)	106.0 (78.5-120.8)	1.000
von Willebrand factor antigen (%)	121.0 (94.5-132.5)	110.0 (103.0-185.2)	0.921
D-dimer (ng/mL FEU)	268.0 (221.0-329.0)	350.5 (231.8-420.8)	0.315
Fibrinogen (g/L)	3.0 (2.7-3.3)	3.3 (2.8-3.5)	0.577
Total cholesterol (mmol/L)	4.6 (3.6-4.8)	4.3 (3.9-5.0)	0.812
HbA1c (%)	5.0 (4.9-5.1)	5.1 (5.0-5.4)	0.221
Thromboelastography (TEG)			
R time (min)	6.0 (5.2-6.6)	6.5 (5.2-6.8)	1.000
K time (min)	1.8 (1.5-2.0)	1.8 (1.7-1.9)	0.997
Alpha-angle (°)	47.2 (42.5-51.4)	49.2 (46.8-62.6)	0.627
Maximum amplitude (mm)	65.0 (60.4-67.8)	63.7 (60.6-66.0)	0.909
Lysis 30 (%)	0.5 (0.1-1.4)	0.7 (0.1-1.4)	1.000
Calibrated automated thrombogram (CAT)			
Lag time (min)	3.2 (3.0-3.4)	3.3 (3.0-3.9)	0.961
Endogenous thrombin potential (nM. min)	1396.7 (1198.8-1580.1)	1380.2 (1251.5-1564.5)	1.000
Peak thrombin (nM)	203.1 (194.1-278.9)	241.9 (193.0-254.6)	1.000
Velocity index (nM/min)	55.8 (43.9-94.0)	70.0 (48.6-90.0)	1.000
Overall Hemostatic Potential (OHP)			
Overall coagulation potential (U)	50.1 (46.2-55.5)	53.0 (47.2-59.5)	1.000
Overall hemostatic potential (U)	23.3 (21.0-26.2)	23.3 (21.3-25.3)	0.992
Overall fibrinolytic potential (%)	52.8 (49.4-54.5)	54.8 (53.5-55.2)	0.954

Median and (interquartile range) are reported. Post hoc (Tukey's) *P* value from models adjusted for age.

including maximum amplitude (64.2 vs 41.1 mm; $P = 0.065$).

Thrombin generation. No significant differences were observed compared with cisgender female controls.

Fibrin generation. Compared with cisgender female controls, fibrin generation was reduced in transgender women while there was no significant difference in OFP ($P = 0.549$).

Global coagulation assays in transgender women receiving oral and transdermal estradiol therapy

Impact of mode of estrogen delivery. The method of estrogen delivery did not influence the GCA parameters (Table 2). The maximum amplitude was comparable between oral and transdermal delivery ($P = 0.91$), with similar clot lysis. There were also no significant differences seen in the thrombin generation parameters or fibrin generation and OFP. The duration of estradiol replacement also did not impact GCA parameters (maximum amplitude slope coefficient = 0.03; $P = 0.35$, ETP slope coefficient = 1.34; $P = 0.45$, OHP slope coefficient = 0.06; $P = 0.008$).

Impact of serum estradiol and testosterone concentration in transgender women. The GCA parameters did not have any significant relationship with serum estradiol concentration, (maximum amplitude slope coefficient = 0.004; $P = 0.42$, ETP slope coefficient = –0.01; $P = 0.98$, OHP slope coefficient = –0.002; $P = 0.51$), or testosterone concentration (maximum amplitude slope coefficient = –0.12; $P = 0.65$, ETP slope coefficient = –16.00; $P = 0.31$, OHP slope coefficient = 0.04; $P = 0.85$).

Impact of anti-androgen therapy. No significant differences were seen in the key GCA parameters in those on anti-androgen therapy compared with those without.

Discussion

This is the first study to evaluate global coagulation assays in transgender women. The combination of 3 GCAs offers a comprehensive assessment of the hemostatic status, compared with routine coagulation tests. Transgender women demonstrated hypercoagulable parameters on thromboelastography with faster clot amplification and increased clot strength as measured by maximal amplitude, as well as increased thrombin generation (peak thrombin and ETP) when compared with cisgender male controls. In contrast, transgender women demonstrated increased overall fibrinolytic potential in comparison with cisgender male controls. Of note, these differences were not observed when transgender women were compared with cisgender female controls, suggesting a shift of their GCA parameters towards typically cisgender female parameters. Our study, however, was not designed to determine whether these changes influence clinical thrombotic risks.

Global coagulation assays in transgender women

To the best of our knowledge, there are no studies investigating the use of viscoelastic testing (thromboelastography) in transgender women or cisgender postmenopausal women on menopausal hormone therapy. Thromboelastography has been investigated in healthy young cisgender women before and 3 months after using the low-dose oral contraceptive pill (<35 mcg ethinyl estradiol) and there was no overall significant trend toward hypercoagulability (26). Oral contraceptives in cisgender women have however, been associated with increased maximum clot firmness (27) on rotational thromboelastography, a parameter similar to maximum amplitude on thromboelastography. Of note, the transgender women in our study were on relatively higher doses of estradiol therapy which may explain their more marked hypercoagulable parameters observed on thromboelastography compared with cisgender women on oral contraceptive pills.

In thrombin generation studies, transgender women demonstrated increased ETP when compared with cisgender male controls. In contrast, there was no difference in ETP between transgender women and cisgender female controls. Previous studies have shown that ETP is elevated in cisgender women taking the oral contraceptive pill compared with cisgender women not on the pill (28). A study comparing cisgender men, cisgender women not using oral contraceptive pill, and cisgender women using the oral contraceptive pill similarly found that the latter group exhibited the highest peak height and ETP on CAT (29).

Of note, in contrast to the hypercoagulable thromboelastography and thrombin generation parameters, the overall fibrinolytic potential in transgender women was increased compared with cisgender male controls. The significance of this finding is uncertain, as estrogen therapy is generally thought to induce hypercoagulability. Teede et al measured the hemostatic parameters in 42 postmenopausal cisgender women receiving either menopausal hormone therapy or placebo for 6 weeks and found increased prothrombin fragments 1 + 2 (F1 + 2), another method of thrombin generation measurement. Interestingly, they also observed reduced plasma fibrinolytic inhibitor activity (plasminogen activator inhibitor-1 [PAI-1]) and increased fibrinolysis (D-dimer) in those receiving hormone therapy compared with placebo (30). PAI-1 is the principal inhibitor of tPA, leading to downregulation of plasminogen conversion to plasmin, which is required for fibrinolysis. Reduced PAI-1 levels may be a surrogate for increased in endogenous fibrinolytic activity as a result of estrogen use, and have been reported in several other studies (31-33). The transgender women in our cohort also demonstrated increased D-dimer compared with both cisgender male and female controls. Further understanding of the pathophysiology and clinical significance of the increased fibrinolytic capacity is required.

Route of estradiol administration

Most studies suggest that oral but not transdermal estradiol therapy is associated with increased venous and arterial thrombosis (12, 13, 34, 35). One exception is a population-based nested case-control study which suggested an increase in stroke in postmenopausal women using high dose transdermal estradiol at doses of > 50 mcg/24 hours but not with low doses (36). Similarly, postmenopausal cisgender women on HRT demonstrated increased thrombin generation parameters with oral estradiol ($P < 0.001$), but not with transdermal formulations (37). Based on these data demonstrating no increased risk of thrombosis with transdermal routes of administration, some treatment protocols recommend transdermal estradiol for older transgender women over 45 years of age due to the probable lower risk of thrombosis (38).

While data in transgender women are limited, several retrospective studies suggest that the rate of venous thrombosis is highest with oral estrogen, particularly ethinyl estradiol and in the first year after commencement, and lowest with transdermal estrogen (39), with more uncertainty with regards to arterial cardiovascular effects. Our pilot study did not observe a difference in any GCA parameter in those using oral estradiol

compared with transdermal, but we acknowledge that larger numbers are needed for a definitive comparison. It is also possible that the lack of a difference in GCA parameter between oral and transdermal estradiol in our study may be related to the relatively high dose transdermal estradiol used in transgender women compared with the menopausal hormone therapy setting.

Oral estradiol is metabolized via the hepatic first-pass effect and in exposing the liver to high concentrations of estradiol, increases production of hepatic proteins such as coagulation factors and sex-hormone binding globulin (SHBG) in a dose-dependent manner in postmenopausal women (40-42). Transdermal estradiol used in postmenopausal women, (50 mcg/24h) avoids the first-pass effect and hepatic SHBG and coagulation factors are typically not induced (43). The difference with transgender women is that the doses of transdermal estradiol used are higher than that used in the postmenopausal setting in order to achieve serum estradiol concentrations in the cisgender female reference range. The median dose of transdermal estradiol patch used in our study was 100 mcg/24h and median serum estradiol concentration was 330 pmol/L. Hence, we postulate that these higher circulating serum concentrations of estradiol may expose the liver to relatively high-dose estradiol and as such, may induce coagulation factors. However, given that global coagulation assay parameters were similarly hypercoagulable in our transgender women on transdermal therapy, our data suggest that the recommendation to use transdermal estradiol therapy in older transgender women may not limit hypercoagulability if high doses of transdermal therapy are used.

There are several limitations to this study. We acknowledge that it is a pilot study with small numbers, and hence, our ability to assess the impact of the mode of delivery, different doses of estrogen therapy and anti-androgen therapy was limited. In addition, the transgender women were relatively young (median age 32.8 years), so we were not able to comment on the hypercoagulability risk of older transgender women on oral versus transdermal therapy. This pilot study was not designed to study clinical thrombotic risks; however, the findings are hypothesis-generating and prompt further investigation, particularly the thrombotic potential of high-dose transdermal estradiol formulations as used in transgender women. Our study only looked at a single time point and hence, we were unable to follow patients over time to map the potential change in their hemostatic profile. While the standard coagulation testing demonstrated some significant differences between groups, the results were within the normal reference ranges. Given that GCAs are currently still only used in the research setting, the effect of the observed differences in the parameters on the actual thrombotic risk is unclear. Nonetheless, this is the first study to evaluate GCAs in transgender women, a population where thrombosis is a significant clinical concern, and uses 3 different GCA assays to evaluate different aspects of hemostasis. Our proof-of-principle study demonstrates that GCAs may detect abnormalities in the hemostatic process not captured by routine coagulation assays. Further validation of these findings in larger studies are needed, in addition to ultimately testing their utility to predict clinical thrombotic events.

Conclusions

Transgender women on estradiol therapy demonstrated a hypercoagulable thromboelastography and thrombin generation profile compared with cisgender male controls with a shift towards the parameters of cisgender female controls. In contrast, there was evidence of increased overall fibrinolytic potential compared with cisgender male controls. Transdermal formulations in the transgender women in this study did not appear to confer any advantage in thrombotic potential over oral formulations. A larger prospective study is required with larger sample sizes and older transgender women to assess how these GCA parameter differences may influence clinical thrombotic risks, the mechanisms for induction of hypercoagulability and to evaluate fibrinolytic pathways.

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Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to

preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References

1. Cheung AS, Ooi O, Leemaqz S, et al. Sociodemographic and clinical characteristics of transgender adults in Australia. *Transgend Health*. 2018;**3**(1):229-238.
2. Delahunt JW, Denison HJ, Sim DA, Bullock JJ, Krebs JD. Increasing rates of people identifying as transgender presenting to Endocrine Services in the Wellington region. *N Z Med J*. 2018;**131**(1468):33-42.
3. Åhs JW, Dhejne C, Magnusson C, et al. Proportion of adults in the general population of Stockholm County who want gender-affirming medical treatment. *PLoS One*. 2018;**13**(10):e0204606.
4. Ewald ER, Guerino P, Dragon C, Laffan AM, Goldstein Z, Streed C Jr. Identifying medicare beneficiaries accessing transgender-related care in the era of ICD-10. *LGBT Health*. 2019;**6**(4):166-173.
5. Goodman M, Adams N, Corneil T, Kreukels B, Motmans J, Coleman E. Size and distribution of transgender and gender nonconforming populations: a narrative review. *Endocrinol Metab Clin North Am*. 2019;**48**(2):303-321.
6. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;**102**(11):3869-3903.
7. Cheung AS, Wynne K, Erasmus J, Murray S, Zajac JD. Position statement on the hormonal management of adult transgender and gender diverse individuals. *Med J Aust*. 2019;**211**(3):127-133.
8. de Bastos M, Stegeman BH, Rosendaal FR, Van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Combined oral contraceptives: venous thrombosis. *Cochrane Database Syst Rev*. 2014;(3):CD010813.
9. Olié V, Canonico M, Scarabin PY. Risk of venous thrombosis with oral versus transdermal estrogen therapy among postmenopausal women. *Curr Opin Hematol*. 2010;**17**(5):457-463.
10. Getahun D, Nash R, Flanders WD, et al. Cross-sex hormones and acute cardiovascular events in transgender persons: a cohort study. *Ann Intern Med*. 2018;**169**(4):205-213.
11. Nota NM, Wiepjes CM, de Blok CJM, Gooren LJG, Kreukels BPC, den Heijer M. Occurrence of acute cardiovascular events in transgender individuals receiving hormone therapy. *Circulation*. 2019;**139**(11):1461-1462.
12. Laliberté F, Dea K, Duh MS, Kahler KH, Rolli M, Lefebvre P. Does the route of administration for estrogen hormone therapy impact the risk of venous thromboembolism? Estradiol transdermal system versus oral estrogen-only hormone therapy. *Menopause*. 2018;**25**(11):1297-1305.
13. Simon JA, Laliberté F, Duh MS, et al. Venous thromboembolism and cardiovascular disease complications in menopausal women using transdermal versus oral estrogen therapy. *Menopause*. 2016;**23**(6):600-610.
14. Mann KG, Brummel K, Butenas S. What is all that thrombin for? *J Thromb Haemost*. 2003;**1**(7):1504-1514.
15. Lance MD. A general review of major global coagulation assays: thrombelastography, thrombin generation test and clot waveform analysis. *Thromb J*. 2015;**13**(1):1-6.
16. Lim HY, O'Malley C, Donnan G, Nandurkar H, Ho P. A review of global coagulation assays - is there a role in thrombosis risk prediction? *Thromb Res*. 2019;**179**:45-55.
17. Srivastava A, Kelleher A. Point-of-care coagulation testing. *Contin Educ Anaesth Crit Care Pain*. 2012;**13**(1):12-16.
18. ten Cate H, Hemker HC. Thrombin generation and atherothrombosis: what does the evidence indicate? *J Am Heart Assoc*. 2016;**5**(8):e003553.
19. Tripodi A, Martinelli I, Chantarangkul V, Battaglioli T, Clerici M, Mannucci PM. The endogenous thrombin potential and the risk of venous thromboembolism. *Thromb Res*. 2007;**121**(3):353-359.
20. Curnow JL, Morel-Kopp MC, Roddie C, Aboud M, Ward CM. Reduced fibrinolysis and increased fibrin generation can be detected in hypercoagulable patients using the overall hemostatic potential assay. *J Thromb Haemost*. 2007;**5**(3):528-534.
21. Bowbrick VA, Mikhailidis DP, Stansby G. The use of citrated whole blood in thromboelastography. *Anesth Analg*. 2000;**90**(5):1086-1088.
22. Hemker HC, Giesen P, AlDieri R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. *Pathophysiol Haemost Thromb*. 2002;**32**(5-6):249-253.
23. Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost*. 2006;**96**(5):553-561.
24. Antovic A. The overall hemostasis potential: a laboratory tool for the investigation of global hemostasis. *Semin Thromb Hemost*. 2010;**36**(7):772-779.
25. He S, Antovic A, Blombäck M. A simple and rapid laboratory method for determination of haemostasis potential in plasma. II. Modifications for use in routine laboratories and research work. *Thromb Res*. 2001;**103**(5):355-361.
26. Zahn CM, Gonzalez DI Jr, Suto C, Kennedy S, Hines JF. Low-dose oral contraceptive effects on thromboelastogram criteria and relationship to hypercoagulability. *Am J Obstet Gynecol*. 2003;**189**(1):43-47.
27. Sucker C, Tharra K, Litmathe J, Scharf RE, Zotz RB. Rotation thromboelastography (ROTEM) parameters are influenced by age, gender, and oral contraception. *Perfusion*. 2011;**26**(4):334-340.
28. Mohamed ABO, Kelchtermans H, Konings J, et al. The effects of oral contraceptive usage on thrombin generation and activated protein C resistance in Saudi women, with a possible impact of the body mass index. *PLoS One*. 2018;**13**(10):e0206376.
29. Tchaikovski SN, van Vliet HA, Thomassen MC, et al. Effect of oral contraceptives on thrombin generation measured via calibrated automated thrombography. *Thromb Haemost*. 2007;**98**(6):1350-1356.
30. Teede HJ, McGrath BP, Smolich JJ, et al. Postmenopausal hormone replacement therapy increases coagulation activity and fibrinolysis. *Arterioscler Thromb Vasc Biol*. 2000;**20**(5):1404-1409.
31. Meijers JC, Middeldorp S, Tekelenburg W, et al. Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives. *Thromb Haemost*. 2000;**84**(1):9-14.
32. Koh KK. Effects of hormone replacement therapy on coagulation and fibrinolysis in postmenopausal women. *Int J Hematol*. 2002;**76 Suppl 2**:44-46.
33. Douketis JD, Gordon M, Johnston M, Julian JA, Adachi JR, Ginsberg JS. The effects of hormone replacement therapy on thrombin generation, fibrinolysis inhibition, and resistance to activated protein C: prospective cohort study and review of literature. *Thromb Res*. 2000;**99**(1):25-34.
34. Cobin RH, Goodman NF; AACE Reproductive Endocrinology Scientific Committee. American association of clinical endocrinologists and American college of endocrinology position statement on menopause-2017 update. *Endocr Pract*. 2017;**23**(7):869-880.
35. Olié V, Canonico M, Scarabin PY. Risk of venous thrombosis with oral versus transdermal estrogen therapy among postmenopausal women. *Curr Opin Hematol*. 2010;**17**(5):457-463.

36. Renoux C, Dell'aniello S, Garbe E, Suissa S. Transdermal and oral hormone replacement therapy and the risk of stroke: a nested case-control study. *Bmj*. 2010;**340**:c2519.
37. Bagot CN, Marsh MS, Whitehead M, et al. The effect of estrone on thrombin generation may explain the different thrombotic risk between oral and transdermal hormone replacement therapy. *J Thromb Haemost*. 2010;**8**(8):1736-1744.
38. Dekker MJ, Wierckx K, Van Caenegem E, et al. A European network for the investigation of gender incongruence: endocrine part. *J Sex Med*. 2016;**13**(6):994-999.
39. Shatzel JJ, Connelly KJ, DeLoughery TG. Thrombotic issues in transgender medicine: A review. *Am J Hematol*. 2017;**92**(2):204-208.
40. Shifren JL, Rifai N, Desindes S, McIlwain M, Doros G, Mazer NA. A comparison of the short-term effects of oral conjugated equine estrogens versus transdermal estradiol on C-reactive protein, other serum markers of inflammation, and other hepatic proteins in naturally menopausal women. *J Clin Endocrinol Metab*. 2008;**93**(5):1702-1710.
41. Casson PR, Elkind-Hirsch KE, Buster JE, Hornsby PJ, Carson SA, Snabes MC. Effect of postmenopausal estrogen replacement on circulating androgens. *Obstet Gynecol*. 1997;**90**(6):995-998.
42. Scarabin PY, Alhenc-Gelas M, Plu-Bureau G, Taisne P, Agher R, Aiach M. Effects of oral and transdermal estrogen/progesterone regimens on blood coagulation and fibrinolysis in postmenopausal women. A randomized controlled trial. *Arterioscler Thromb Vasc Biol*. 1997;**17**(11):3071-3078.
43. Matsui S, Yasui T, Kasai K, et al. Sex hormone-binding globulin and antithrombin III activity in women with oral ultra-low-dose estradiol. *J Obstet Gynaecol*. 2017;**37**(5):627-632.

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Relationships between body mass index with oral estradiol dose and serum estradiol concentration in transgender adults undergoing feminising hormone therapy

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Abstract

Aim: Feminising hormone therapy with estradiol is used to align an individual's physical characteristics with their gender identity. Given considerable variations in doses of estradiol therapy administered as gender-affirming hormone therapy (GAHT), we aimed to assess if body mass index (BMI) correlated with estradiol dose/concentration and assess the correlation between estradiol dose and estradiol concentrations.

Methods: In a retrospective cross-sectional study, we analysed transgender individuals attending a primary or secondary care clinic in Melbourne, Australia who were prescribed oral estradiol valerate for at least 6 months and had estradiol dose and concentration available. Estradiol concentration was measured by immunoassay. Outcomes were the correlation between estradiol dose and BMI, and estradiol dose and estradiol concentration.

Results: Data were available for 259 individuals {median age 25.8 [interquartile range (IQR) 21.9, 33.5] years}. Median duration of estradiol therapy was 24 (15, 33) months. Median estradiol concentration was 328 (238, 434) pmol/l [89 (65, 118) pg/ml] on 6 (4, 8) mg estradiol valerate. Median BMI was 24.7 (21.8, 28.6) kg/m^2. There was a weak positive correlation between estradiol dose and estradiol concentration ($r = 0.156$, $p = 0.012$). There was no correlation between BMI and estradiol concentration achieved ($r = -0.063$, $p = 0.413$) or BMI and estradiol dose ($r = 0.048$, $p = 0.536$). Estradiol concentrations were within the target range recommended in consensus guidelines in 172 (66%) individuals.

Conclusion: Estradiol dose was only weakly correlated with estradiol concentration, suggesting significant interindividual variability. Prescription of estradiol dose should not be based upon an individual's BMI, which did not correlate with estradiol concentration achieved. In all, 66% of individuals achieved estradiol concentrations recommended in Australian consensus guidelines with a relatively high oral estradiol dose.

Keywords: estradiol, gender dysphoria, gender identity, transgender

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Introduction

Transgender, including gender diverse and non-binary, individuals who seek feminisation of physical characteristics (hereafter termed transfeminine individuals) are typically treated with estradiol with or without anti-androgen to increase serum estradiol concentration and decrease serum testosterone concentration into the respective female reference ranges. This results in development of feminine physical characteristics, including softening of skin, a decrease in facial and body hair growth, breast development, and changes in body composition manifested by body fat redistribution and decreased muscle mass.[1,2]

Estradiol is most commonly administered *via* the oral or transdermal route,[3] and oral estradiol valerate is the most common first-line feminising treatment in clinicians experienced in transgender healthcare in Australia.[4] Several consensus guidelines give recommendations for estradiol concentrations to allow titration of estradiol therapy.[1,5] The Australian 'Position statement on the hormonal management of adult transgender and gender diverse individuals' recommends targeting estradiol concentrations of 250–600 pmol/l (68–163 pg/ml) (GRADE 2D recommendation) based on local cross-sectional data.[5] Notably, given the lack of data, this is an approximate guide and the position statement states that the value of biochemical testing in addition to clinical assessment is unclear. It is also unclear if body mass index (BMI) should be a consideration during estradiol prescribing.

As such, the aim of this retrospective study in adult transfeminine individuals on established estradiol therapy was to assess, firstly, the correlation between BMI and serum estradiol concentration, and secondly the relationship between estradiol dose and serum estradiol concentration. In order to assess implementation of current guidelines in clinical practice, we also aimed to assess the proportion of individuals achieving estradiol concentrations within the range now recommended by consensus guidelines.

Methods

A retrospective cross-sectional analysis was undertaken of consultations for gender-affirming hormone therapy (GAHT) at Equinox Gender Diverse Clinic, a primary care clinic specialising in transgender health, and an Endocrine Specialist Centre, a secondary care clinic, in Melbourne, Australia. Data were obtained from consultations between 1 January 2011 and 21 October 2019. The audit was approved by the Austin Health Human Research Ethics Committee (LNR/17/Austin/102) and Thorne Harbour Health (THH/CREP 19/015), who waived the need for informed consent.

Transfeminine individuals treated with oral estradiol for at least 6 months who had estradiol dose and estradiol concentration documented in their medical records were included in this retrospective cross-sectional analysis. For individuals with multiple estradiol concentrations available, the most recent prior to sex reassignment surgery was included. The primary outcome of interest was to establish the correlation between BMI and estradiol concentration, and estradiol dose and estradiol concentration. We also aimed to establish the proportion of individuals achieving estradiol concentrations in consensus guidelines.

As data were obtained retrospectively, sex steroid concentrations were performed using immunoassay available at several laboratories used as standard care in clinical practice. Multiple National Association of Testing Authorities (NATA, the national accreditation body for Australia) accredited laboratories were used. Time of blood sampling with respect to estradiol dosing was not systematically recorded in clinical notes and was therefore unavailable for analysis in this study.

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA). As data were not normally distributed, values are reported as median [interquartile range (IQR)]. Differences between groups were tested using the Mann–Whitney *U* test or Kruskal–Wallis test followed by Dunn's *post hoc* comparisons. Spearman's rank correlation coefficient was used to assess the correlation between variables. For all analyses, the level of significance was set at $p < 0.05$.

Results

A total of 390 transfeminine individuals were included in our analysis. After excluding individuals treated with concurrent transdermal estradiol ($n = 34$), ethinyl estradiol ($n = 12$), estradiol implant ($n = 4$), anti-androgen monotherapy ($n = 3$) or gonadotropin-releasing hormone (GnRH) subcutaneous implant ($n = 3$), 334 individuals treated with oral estradiol valerate were left for analysis. Of these, 259 were treated with oral estradiol valerate for at least 6 months and had estradiol concentration available prior to sex reassignment surgery.

In all, 73 individuals were treated with oral estradiol valerate without anti-androgen, 95 with spironolactone, 87 with cyproterone acetate, 2 with both cyproterone acetate and spironolactone, and 2 with bicalutamide. Concurrent progesterone was prescribed in 80 individuals, and concurrent finasteride in 12 individuals.

Median age of individuals was 25.8 (21.9, 33.5) years and duration of estradiol therapy was 24

Table 1. Characteristics by treatment group.

	Estradiol without anti-androgen (*n* = 73)	Estradiol with spironolactone (*n* = 95)	Estradiol with cyproterone acetate (*n* = 87)	*p* value
Age (years)	27.9 (22.8, 38.6)	25.4 (20.9, 30.2)	25.4 (21.8, 30.2)	0.058
Duration of GAHT (months)	18.1 (12.9, 29.7)	24.4 (15.0, 32.4)	24.3 (15.5, 38.3)	0.130
Estradiol valerate dose (mg)	6 (4, 6)	6 (6, 8)	6 (4, 8)	0.015
Estradiol concentration (pmol/l)	332 (225, 416)	355 (271, 456)	304 (230, 417)	0.264
BMI (kg/m^2)	25.3 (22.7, 28.7)	24.3 (21.6, 28.2)	24.7 (20.7, 28.9)	0.353

(15, 33) months. Median BMI was 24.7 (21.8, 28.6) kg/m^2. Median estradiol concentration achieved was 328 (238, 434) pmol/l [89 (65, 118) pg/ml] on 6 (4, 8) mg estradiol valerate.

There was no difference in the estradiol concentrations achieved between those treated with estradiol without anti-androgen, estradiol with spironolactone, or estradiol with cyproterone acetate ($p = 0.264$) (Table 1). There was no difference in the estradiol concentrations achieved between groups at each estradiol valerate dose (data not shown). There was no difference in the estradiol concentrations achieved between those treated with [331 (259, 453) pmol/l] or without [325 (238, 423) pmol/l] concurrent progesterone therapy ($p = 0.354$).

There was a weak positive correlation between estradiol dose and estradiol concentration ($r = 0.156$, $p = 0.012$) (Figure 1). There was no correlation between BMI and estradiol concentration achieved ($r = -0.063$, $p = 0.413$) (Figure 2) or BMI and estradiol dose ($r = 0.048$, $p = 0.536$).

Table 2 demonstrates the estradiol concentrations achieved and the proportion of individuals achieving recommended estradiol concentrations in consensus guidelines for each estradiol valerate dose. A total of 172 (66%) individuals had estradiol concentrations within the target range of 250–600 pmol/l[5]; 70 (27%) individuals had estradiol concentrations below target, and 17 (7%) above target. However, if using the Endocrine Society Clinical Practice Guidelines target of 367–734 pmol/l (100–200 pg/ml),[1] 95 (35%) individuals reached target concentrations with 158 (61%) below target and 6 (2%) above target.

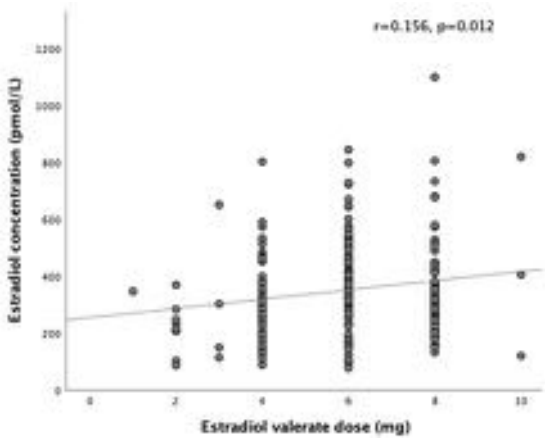

Figure 1. Correlation between estradiol valerate dose and estradiol concentration.
BMI, body mass index.

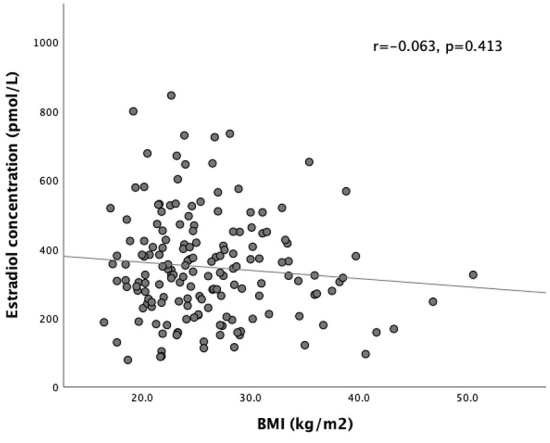

Figure 2. Lack of correlation between BMI and estradiol concentration.
BMI, body mass index.

Table 2. Estradiol concentration by oral estradiol valerate dose.

Estradiol valerate dose (mg)	Number of individuals	Estradiol concentration (pmol/l)	Number of individuals reaching target in Australian guidelines	Number of individuals reaching target in Endocrine Society guidelines
1	2	246 (196, 297)	1 (50%)	0 (0%)
2	9	217 (207, 248)	2 (22%)	1 (11%)
3	4	226 (141, 390)	1 (25%)	1 (25%)
4	63	293 (207, 381)	39 (62%)	20 (32%)
6	111	362 (299, 470)	83 (75%)	51 (46%)
8	67	319 (249, 423)	45 (67%)	25 (37%)
10	3	406 (263, 613)	1 (33%)	2 (66%)

Median (IQR) are presented.
IQR, interquartile range.

Discussion

In this retrospective cross-sectional analysis of transfeminine individuals treated with oral estradiol valerate, there was no correlation between BMI and estradiol concentration. There was a weak positive correlation between estradiol valerate dose and estradiol concentration with significant interindividual variability. In all, 66% of individuals achieved estradiol concentrations within Australian consensus guideline recommendations of 250–600 pmol/l, with small numbers achieving supraphysiological concentrations.

Association between BMI and estradiol concentration

Observational studies have demonstrated discrepant results with respect to the impact of BMI on estradiol concentration in women treated with menopausal hormone therapy. BMI was found to be positively associated with estradiol concentration in a nested case-control study evaluating the influence of sex steroids on breast cancer risk,[6] whereas increasing BMI was associated with a lower odds of achieving an estradiol concentration greater than 165 pmol/l in a cross-sectional analysis of 309 post-menopausal women.[7] Other studies have found no association.[8,9]

In a previous retrospective analysis of 184 transfeminine individuals, BMI was positively associated with estradiol concentration, but the variance attributable to BMI was small.[10] BMI did not influence estradiol dose.[10] In another retrospective cohort of 84 individuals, there was no statistically significant correlation between BMI and the estradiol concentration achieved though there was a trend toward a positive correlation.[11] However, of the individuals who achieved estradiol concentration within the target range, there was a significant negative correlation between estradiol dose and BMI.[11] The median BMI reported in both studies was significantly higher than our cohort, which could contribute to the discrepancy between previous studies and the current findings.

Association between estradiol dose and estradiol concentration

The association between estradiol dose and estradiol concentration in transfeminine individuals was evaluated in two previous retrospective analyses. Both studies reported a positive correlation between estradiol dose and the estradiol concentration achieved.[10,11] Spironolactone was reported to reduce the effectiveness of estradiol dosing achieving desired estradiol concentrations.[10] However, our study did not find a difference in estradiol concentration achieved between anti-androgen groups, and further prospective studies are required. The weak association between estradiol dose and serum estradiol concentration highlights significant interindividual variability, where different individuals achieve similar serum estradiol concentrations on wide estradiol doses.

Estradiol concentrations and consensus guidelines

No studies have examined the optimal estradiol concentrations in transfeminine individuals. Based on cross-sectional data,[12] Australian consensus guidelines recommend targeting trough estradiol concentrations of 250–600 pmol/l,[5] whereas the Endocrine Society Clinical Practice Guidelines recommend a target range of 367–734 pmol/l based on the physiological range of pre-menopausal women.[1] The estradiol assay used and timing of blood testing in relation to the estradiol dose are other important considerations when interpreting the estradiol concentration.

Estradiol concentrations are best measured *via* liquid chromatography–mass spectrometry (LC-MS) given that immunoassays lack selectivity and precision, particularly at low estradiol concentrations.[13] The clinician must also consider the estradiol concentration in relation to the timing of the estradiol dose, given that peak estradiol concentration is generally 4–5 hours post-dose with an elimination half-life of 14–22 h.[14] In one study in which transfeminine individuals were treated with 2 mg oral estradiol valerate, there was a peak median estradiol concentration of 189 (99) pmol/l with 24-h post-dose concentration of 56 (154) pmol/l.[15] No large study has evaluated the pharmacokinetic parameters at doses used in feminising hormone therapy regimens.

Various clinical guidelines also give recommendations for estradiol dosing. The Endocrine Society Clinical Practice Guidelines recommend estradiol dosing of 2–6 mg daily,[1] whereas the European Network for Investigation of Gender Incongruence (ENIGI) use an oral estradiol valerate dose of 2 mg twice daily.[16] Despite a median estradiol valerate dose higher than that used in ENIGI, only one-third of individuals in our cohort achieved estradiol concentrations recommended in international guidelines.

Retrospective analyses have documented the proportion of individuals achieving estradiol concentrations in consensus guidelines; 77/136 (55.7%) achieved an estradiol concentration in the Endocrine Society Clinical Practice Guidelines target range on 4–8 mg/day oral estradiol valerate.[10] Only 21/136 (15.4%) achieved target estradiol concentrations on 4 mg/day; 13 individuals (9.6%) did not achieve target estradiol concentration at 8 mg oral estradiol valerate. Similarly, 35 (41%) individuals were able to achieve estradiol concentrations within the Endocrine Society Clinical Practice Guidelines in a separate analysis.[11] The estradiol valerate doses that achieved target estradiol concentrations were not reported, but the entire cohort had a dose range 1–10 mg/day.

Clinical implications

The value of biochemical monitoring is currently unclear, and no prospective study has been designed to compare the effectiveness of different routes of estradiol administration or varying estradiol concentrations. Prospective studies from the ENIGI cohort have reported that estradiol concentration did not predict breast development or more feminine changes in body composition in transfeminine individuals.[2,17] However, higher estradiol concentrations have been associated with a higher lumbar spine bone mineral density.[18] Ultimately, the best assessment of hormonal efficacy is the clinical response.[19]

Ensuring adequate feminisation also needs to be weighed against the potential risks of escalating estradiol doses. There is evidence in post-menopausal women that higher oral estradiol doses are associated with an increased prevalence of venous thromboembolic (VTE) events.[20] However, recent evidence in transfeminine individuals has demonstrated a low incidence of VTE.[21]

No prospective studies have assessed the effectiveness of different estradiol concentration targets on clinical features of feminisation or potential adverse events. Until further data are available, current guidelines provide clinicians with target ranges that allow suppression of testosterone while avoiding supraphysiological estradiol concentrations. Further research is required.

Limitations

This study has several limitations. The most critical of which is the lack of patient-reported outcomes or objective measures of feminisation such as breast development. Although most clinicians monitor trough estradiol concentrations, the concentrations reported represent a single time point as part of routine clinical care so are not strictly collected in a standardized manner, and we do not have data on compliance with therapy. We cannot be certain that an individual would have

achieved stable dosing by 6 months but the clinical practice of clinicians at these clinics is to uptitrate over a period of approximately 3–6 months so the 6 month cut-off was an arbitrary timepoint to ensure that the gradual titration often seen at initiation of feminising hormone therapy was not captured. Data on medications that could influence estradiol metabolism such as cytochrome P450 3A4 (CYP3A4) modulators were not collected.[22] Given that bloods were taken as standard care, estradiol was measured *via* immunoassay and not liquid chromatography tandem mass spectrometry (LCMS/MS). However, all were performed using NATA-accredited laboratories.

Conclusion

No correlation between BMI and serum estradiol concentration is apparent, and a weak positive correlation between estradiol dose and serum estradiol concentration exists; 66% of transfeminine individuals treated with oral estradiol valerate had a serum estradiol concentration within the target range recommended by the Australian consensus guidelines. Reassuringly, few individuals had supraphysiological estradiol concentrations. Given the limited evidence base, it remains reasonable for clinicians to consider other factors, such as clinical feminisation, patient satisfaction, and potential risks of escalating estradiol dose, in making dosing decisions. Further prospective longitudinal studies comparing various estradiol concentration targets or doses and clinical features of feminisation are required.

Author contribution(s)

Brendan J Nolan: Conceptualization; Formal analysis; Methodology; Writing-original draft; Writing-review & editing.

Adam Brownhill: Conceptualization; Investigation; Resources; Writing-review & editing.

Ingrid Bretherton: Investigation; Resources; writing-review & editing.

Peggy Wong: Investigation; Resources; Writing-review & editing.

Susan Fox: Investigation; Resources; Writing-review & editing.

Peter Locke: Investigation; Resources; Writing-review & editing.

Nicholas D Russell: Investigation; Resources; Writing-review & editing.

Mathis Grossmann: Methodology; Supervision; Writing-review & editing.

Jeffrey D Zajac: Methodology; Supervision; Writing-review & editing.

Ada S. Cheung: Conceptualization; Methodology; Supervision; Writing-review & editing.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

1. Hembree WC, Cohen-Kettenis PT, Gooren L, *et al*. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2017; 102: 3869–3903.
2. Klaver M, de Blok CJM, Wiepjes CM, *et al*. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. *Eur J Endocrinol* 2018; 178: 163–171.
3. Cheung AS, Ooi O, Leemaqz S, *et al*. Sociodemographic and clinical characteristics of transgender adults in Australia. *Transgend Health* 2018; 3: 229–238.
4. 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: 182–188.

5. 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: 127–133.
6. Tworoger SS, Missmer SA, Barbieri RL, *et al*. Plasma sex hormone concentrations and subsequent risk of breast cancer among women using postmenopausal hormones. *J Natl Cancer Inst* 2005; 97: 595–602.
7. Gavaler JS. Oral hormone replacement therapy: factors that influence the estradiol concentrations achieved in a multiracial study population. *J Clin Pharmacol* 2002; 42: 137–144.
8. Lambrinoudaki I, Armeni E, Rizos D, *et al*. Sex hormones in postmenopausal women receiving low-dose hormone therapy: the effect of BMI. *Obesity (Silver Spring)* 2011; 19: 988–993.
9. Waaseth M, Bakken K, Dumeaux V, *et al*. Hormone replacement therapy use and plasma levels of sex hormones in the Norwegian Women and Cancer postgenome cohort - a cross-sectional analysis. *BMC Womens Health* 2008; 8: 1.
10. Leinung MC, Feustel PJ and Joseph J. Hormonal treatment of transgender women with oral estradiol. *Transgend Health* 2018; 3: 74–81.
11. Fernandez JD, Kendjorsky K, Narla A, *et al*. Assessment of gender-affirming hormone therapy requirements. *LGBT Health* 2019; 6: 101–106.
12. Angus L, Leemaqz SY, Ooi O, *et al*. Cyproterone acetate or spironolactone in lowering testosterone concentrations for transgender individuals receiving oestradiol therapy. *Endocr Connect* 2019; 8: 935–940.
13. Denver N, Khan S, Homer NZM, *et al*. Current strategies for quantification of estrogens in clinical research. *J Steroid Biochem Mol Biol* 2019; 192: 105373.
14. Jarvinen A, Kainulainen P, Nissila M, *et al*. Pharmacokinetics of estradiol valerate and medroxyprogesterone acetate in different age groups of postmenopausal women. *Maturitas* 2004; 47: 209–217.
15. Hiransuthikul A, Janamnuaysook R, Himmad K, *et al*. Drug-drug interactions between feminizing hormone therapy and pre-exposure prophylaxis among transgender women: the iFACT study. *J Int AIDS Soc* 2019; 22: e25338.
16. Dekker MJ, Wierckx K, Van Caenegem E, *et al*. A European network for the investigation of gender incongruence: endocrine part. *J Sex Med* 2016; 13: 994–999.
17. de Blok CJM, Klaver M, Wiepjes CM, *et al*. Breast development in transwomen after 1 year of cross-sex hormone therapy: results of a prospective multicenter study. *J Clin Endocrinol Metab* 2018; 103: 532–538.
18. Wiepjes CM, de Jongh RT, de Blok CJ, *et al*. Bone safety during the first ten years of gender-affirming hormonal treatment in transwomen and transmen. *J Bone Miner Res* 2019; 34: 447–454.
19. Coleman E, Bockting W, Botzer M, *et al*. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgend* 2012; 13: 165–232.
20. Vinogradova Y, Coupland C and Hippisley-Cox J. Use of hormone replacement therapy and risk of venous thromboembolism: nested case-control studies using the QResearch and CPRD databases. *BMJ* 2019; 364: k4810.
21. Arnold JD, Sarkodie EP, Coleman ME, *et al*. Incidence of venous thromboembolism in transgender women receiving oral estradiol. *J Sex Med* 2016; 13: 1773–1777.
22. Blode H, Zeun S, Parke S, *et al*. Evaluation of the effects of rifampicin, ketoconazole and erythromycin on the steady-state pharmacokinetics of the components of a novel oral contraceptive containing estradiol valerate and dienogest in healthy postmenopausal women. *Contraception* 2012; 86: 337–344.