BMJ Open

Serum oestradiol levels and risk of adverse cardiovascular events associated with gender-affirming oestrogen therapy: a protocol for a systematic review and meta-analysis

Chantal L Rytz ,[1,2] Keila Turino Miranda,[1,2] Paul E Ronksley,[2] Sandra M Dumanski,[1,2,3] Nathalie Saad,[1,2] Satish R Raj,[1,2] Ranjani Somayaji,[2,3] Heather Ganshorn,[4] Amelia M Newbert,[5] Lindsay Peace,[5] Sofia B Ahmed [1,2,3]

To cite: Rytz CL, Turino Miranda K, Ronksley PE, *et al*. Serum oestradiol levels and risk of adverse cardiovascular events associated with gender-affirming oestrogen therapy: a protocol for a systematic review and meta-analysis. *BMJ Open* 2022;**12**:e064961. doi:10.1136/bmjopen-2022-064961

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-064961).

Received 19 May 2022
Accepted 13 October 2022

For numbered affiliations see end of article.

Correspondence to
Dr Sofia B Ahmed;
sofia.ahmed@albertahealthservices.ca

ABSTRACT

Introduction The use of gender-affirming oestrogen therapy (GAOT) is an integral part of the gender-affirming transition process for transgender women (assigned male at birth who identify as women) and gender-diverse individuals. However, its use may present significant cardiovascular implications, which may be influenced by systemic oestradiol levels. Therefore, we aim to establish the association between serum oestradiol levels and incidence of adverse cardiovascular events in individuals using GAOT.

Methods and analysis We will conduct a systematic review addressing the association between serum oestradiol levels and risk of adverse cardiovascular events in individuals using GAOT. Our primary outcome is the incidence of adverse cardiovascular events, our secondary outcome is the incidence of cardiovascular-related mortality and our tertiary outcome is cardiovascular-related risk factors. Electronic databases (Cochrane Central Register of Controlled Trials, Embase, MEDLINE and Web of Science) will be searched from inception until September 2022. Two investigators will independently complete screening to determine appropriateness of inclusion. Extracted data will include information on serum sex hormone levels (oestradiol and testosterone), participants, GAOT (route of administration, formulations, dosages and duration of exposure), incidence of cardiovascular outcomes, study quality and risk of bias. Inter-reviewer reliability will be calculated at both phases. Data will be presented both descriptively and meta-analysed using a random effects model, if appropriate. Heterogeneity will be explored and meta-regressed if noted.

Ethics and dissemination Ethics approval is not needed. We will disseminate findings through international conferences, distributions to transgender and gender-diverse support organisations, decision-makers and key stakeholders. The final systematic review will be published in a peer-reviewed journal.

Trial registration number CRD42021247717.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This qualitative systematic review will be conducted according to the Joanna Briggs Institute methodology for systematic reviews of association and will follow the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P), ensuring high quality and rigour.
⇒ This review will enhance the understanding of the association between serum oestradiol concentration and cardiovascular risk in the transgender and gender-diverse population who uses gender-affirming oestrogen therapy.
⇒ Heterogeneity in hormone therapy modalities (e.g., route of administration and dosages) as well as research and person-level factors (e.g., time of measurement, compliance and oestrogen dose titration) may limit generalisability.
⇒ Our results may be confounded by study-level data that contribute to cardiovascular risk, such as socioeconomic position or lifestyle habits (e.g., smoking).

INTRODUCTION

Transgender individuals (whose gender identity does not align with sex assigned at birth) individuals represent 0.4%–0.6% of the population worldwide[1] yet bear a disproportionate cardiovascular burden including greater adverse cardiovascular events,[2 3] mortality[4] and associated risks[5] as compared with their cisgender counterparts. This increased cardiovascular risk is most pronounced in transgender women and gender-diverse individuals (TGD)[3] and may be partially due to the use of gender-affirming oestrogen therapy (GAOT), a commonly used hormonal regimen as part of the feminisation transition process.[6–8]

GAOT can promote the development of desired secondary female sexual characteristics independently or in conjunction with other gender-affirming hormone therapy (GAHT; e.g., antiandrogens) aimed to minimise and even abolish undesired secondary male sexual characteristics.[6 8] Specifically,

GAOT can increase serum oestradiol levels in TGD to those in the premenopausal cisgender woman range[6–8] through exogenous oral and non-oral formulations of bioidentical estrogens (e.g., 17-β oestradiol). However, factors associated with the use of GAOT, including the dosage, formulation, route of GAOT administration—which may all influence serum oestradiol levels—can pose a significant risk to the cardiovascular system of TGD individuals.[9]

Epidemiological data show that serum oestradiol levels are higher in men with coronary artery disease[10] and in those who experience sudden cardiac arrest,[11] suggesting that a hyperestrogenic state is associated with a greater cardiovascular risk for this population. However, reports on the cardiovascular effects of GAOT in TGD individuals are limited and conflicting. Studies have shown a detrimental association between GAOT use and cardiovascular risk factor,[12 13] although effects may differ by route of GAOT administration.[14] While a lower prevalence of cardiovascular disease was observed in transgender women compared with age-matched and cisgender-matched counterparts,[15] other studies have reported increased rates of venous thromboembolism (VTE),[3 16] myocardial infarction (MI)[17] and cardiovascular-related mortality.[4] Similarly, GAOT has shown conflicting effects on traditional cardiovascular disease risk factors. Previous reports have shown positive[18] or insignificant[19] changes in lipid profiles, increased body fat mass,[18] and both improvements[18] and undesirable[20] changes in blood pressure. However, the association between serum oestradiol levels resulting from GAOT use and cardiovascular risk is unknown, highlighting the importance of exploring this relationship.

The number of TGD individuals is increasing globally.[21] The high cardiovascular risk in this population may be due to the increased serum oestradiol levels resulting from GAOT use, with risks potentially differing by route of administration. Furthermore, the benefits and risks of targeting cisgender women oestradiol levels on cardiovascular health outcomes in TGD individuals are poorly understood. Furthermore, the paucity of the current literature surrounding GAOT use presents challenges regarding current methodology, lack of control for confounding factor and unclear underlying mechanisms. To date, no systematic review (published or in progress) has explored this association. Therefore, our study aims to characterise the association between serum oestradiol levels and risk of adverse cardiovascular events, which has the potential to inform the care of TGD individuals who use GAOT.

METHODS

Study design

To assess the association between serum oestradiol levels and risk of adverse cardiovascular events in TGD individuals using GAOT, we developed a systematic review protocol in accordance with the Preferred Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines[22] (see online supplemental appendix 1: PRISMA-P checklist).[23] The final study protocol was registered with PROSPERO.[24]

Table 1 PEO framework for systematic review search

	PEO framework
Population	Transgender women, transfeminine, gender-diverse and/or non-binary individuals
Exposure of Interest	Serum oestradiol levels resulting from GAOT use
Outcome	1°: incidence of adverse cardiovascular events (e.g., MI)
	2°: incidence of cardiovascular-related mortality
	3°: changes in cardiovascular risk factors (e.g., blood pressure)

GAOT, gender-affirming oestrogen therapy; MI, myocardial infarction ; PEO, population, exposure of interest, outcome.

Patient and public involvement

Valuable feedback from patient partners with lived experience was received and implemented, specifically with respect to designing the research question. Patient partners will be consulted to assist with the narrative of study results, and their feedback will be sought to appropriately summarise and disseminate findings to relevant patient and provider groups.

Review question

What is the association between serum oestradiol levels, resulting for GAOT use, and risk of adverse cardiovascular events in TGD individuals?

Inclusion/exclusion criteria

Participants

The population, exposure of interest and outcomes (PEO)[25] used to determine study eligibility are presented in table 1. Included studies for this systematic review will report on transgender women, transfeminine, gender-diverse and/or non-binary individuals. Studies reporting only cisgender individuals, transgender men and/or transmasculine individuals will be excluded.

Exposure of interest

This review will consider studies that report serum oestradiol levels resulting from GAOT use in transgender women, transfeminine, gender-diverse and/or non-binary individuals. Any route of administration, formulations, dosages and duration of exposure of GAOT will be included.

Outcomes

This review will consider studies that include the following outcomes: 1°: incidence of adverse cardiovascular events (e.g., MI, stroke, cardiovascular disease and VTE), 2°:

incidence of cardiovascular-related mortality and 3°: cardiovascular-related risk factors (e.g., blood pressure).

Type of studies

This review will consider experimental and quasiexperimental study designs including randomised controlled trials and non-randomised controlled trials. Additionally, analytical observational studies including retrospective and prospective cohort studies, case–control studies and cross-sectional studies will be considered. Reviews, editorials, comments, opinions and conference proceedings will not be included.

Search methods

The purpose of this search is to identify published articles that assess the association between oestradiol levels and risk of adverse cardiovascular events in TGD individuals who use GAOT. An initial limited search of MEDLINE (PubMed) was undertaken to identify articles on the topic. Text words and index terms described in the articles were used to develop a full search strategy for Cochrane Central Register of Controlled Trials (CENTRAL), Embase, MEDLINE (Ovid) and Web of Science (see online supplemental appendix 2: MEDLINE search strategy). The search strategy, including all identified keywords and index terms, will be adapted for each included information source. The MEDLINE search will be peer reviewed.[26] Reference lists from eligible articles, reviews and clinical guidelines will be searched by the two reviewers independently to ensure inclusion of all appropriate studies. Studies published in all languages will be included. Studies published from online databases including CENTRAL (1996–April 2022), Embase (1980–April 2022), MEDLINE (1950–June 2021) and Web of Science (1997–April 2022) will be included. Unpublished studies will not be included.

Study selection

Following the search, all identified citations will be collated and uploaded into Covidence (Cochrane Technology, Melbourne, Victoria, Australia), and duplicates will be removed. A calibration exercise between the two reviewers will be completed on the first 100 abstracts returned from the search strategies. Independently, two reviewers will use the predetermined PEO framework and eligibility criteria to deem articles as either eligible, ineligible or of uncertain eligibility. After completing this exercise, a list of discrepant inclusion/exclusion results between the two reviewers will be constructed and discussed in depth to create an improved understanding of the interpretation of abstracts, the eligibility of articles and/or the classification of the study. This exercise may mitigate the level of discrepancy among the remaining abstracts. After completing this exercise, each reviewer will independently screen the remaining abstracts. Abstracts that are deemed eligible or have uncertain eligibility, as per the above eligibility criteria, will be selected for a full-text review. Each full text will be independently reviewed by the same reviewers. Each reviewer's eligibility assessment of an article at both stages (abstract and full-text review) will be recorded and quantification of agreement between reviewers will be calculated using the kappa statistic. Reasons for exclusion of full-text studies will be recorded and reported in the systematic review. Any disagreement between reviewers will be resolved by consensus. If a consensus is not achieved, a third independent reviewer will serve as a final adjudicator. The data will be managed through Excel V.16.16.2 (Microsoft Corporation, Redmond, Washington, USA) and Mendeley V1.19.8 (Elsevier, Amsterdam, The Netherlands). The results of the search and study selection and inclusion process will be reported in full in the final systematic review and presented in a PRISMA flow diagram.[22]

Quality appraisal

Eligible studies will be critically appraised by both reviewers independently. Non-randomised studies will be assessed using the National Institute of Health Study Quality Assessment Tools,[27] and randomised trials will be assessed using the Cochrane risk of bias tool.[28] Authors of eligible articles will be contacted for missing data or clarification, where required. Any disagreement between reviewers will be resolved by consensus. If a consensus is not achieved, a third independent reviewer will serve as a final adjudicator. The results of this assessment will be reported in a table with accompanying narrative. All studies, regardless of the results of their methodological quality, will undergo independent data extraction from the two reviewers and synthesised (where possible).

Data extraction

Data from included studies will be extracted by two reviewers independently using a pregenerated data extraction form within the Covidence platform. Extracted data will include specific details about study identifiers (authors, location of publication and year of publication), study design characteristics (sample size, inclusion and exclusion criteria, type, dose, route and frequency of and duration of time on GAOT administration), serum hormone levels (oestradiol and testosterone), participant characteristics (age, comorbidities, other GAHT used and additional medications), cardiovascular-based outcome data (incidence of adverse cardiovascular events (e.g., MI), incidence of cardiovascular-related mortality and cardiovascular risk factors (e.g., blood pressure)). Any disagreements between reviewers will be resolved by consensus. If consensus is not achieved, a third independent reviewer will serve as a final adjudicator.

Data synthesis

Data extracted from eligible studies will be summarised using descriptive statistics. Outcome measures, where possible, will be meta-analysed using DerSimonian Laird random effects model,[29] and separate analyses will be completed for controlled trials, cohort and observational studies. Reported average serum oestradiol levels will be

used to stratify and categorise articles into subphysiological, physiological and supraphysiological serum oestradiol reference ranges using target serum oestradiol levels of premenopausal cisgender women as the referent.[30] Measures of association for dichotomous variables will be reported as risk ratios, and measures of effect for continuous outcomes will be reported using weighted or standardised mean differences when common or different measurement instruments are used, respectively. If three or more studies reporting on the same outcome are included in the analysis, measures of heterogeneity (Cochrane Q and I^2 statistics) will be assessed to determine whether pooled analyses are appropriate to report. If significant heterogeneity is noted, stratified analysis will be employed to determine the effect of the following variables on the cardiovascular risk estimates: (1) population statistics (age, pre-existing comorbidities); (2) GAOT-related (route of administration, dose, duration and concomitant GAHT use); and (3) sample-size related (small-study effects). Planned sensitivity analyses include determining if the associations between serum oestradiol levels and risk of adverse cardiovascular events differ by high and low risk of bias, route of GAOT administration (ie, oral vs non-oral), with or without a concomitant antiandrogen, timing of initiation (ie, <15 years or ≥15 years of age), age of study participants (i.e., <18 years or ≥18 years old) and within those <18 years old, a sensitivity analysis of with or without concomitant puberty blocker use. Where statistical pooling is not possible, the findings will be presented in narrative form including tables and figures to aid in data presentation, where appropriate. A funnel plot will be generated in STATA (V.16.1, StataCorp LLC) to visually assess publication bias if there are ≥10 studies included in the meta-analysis. Statistical tests for publication bias will be assessed using Beggs test.

Confidence in the synthesised findings of the review

The Grading of Recommendations Assessment, Development and Evaluation approach for grading the certainty of evidence will be followed.[31] The quality of evidence will be classified into four grades: high, moderate, low or very low.

ETHICS AND DISSEMINATION

Ethical approval is not required for this protocol nor the systematic review. After completion of the systematic review, findings will be shared through presentations at national and international conferences and will be available and distributed to organisations, foundations, decision makers and key stakeholders. The final systematic review will be submitted and published in a peer-reviewed journal. Plain language summaries will be provided to relevant patient and provider groups.

Author affiliations
[1]Libin Cardiovascular Institute, University of Calgary, Calgary, Alberta, Canada
[2]Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
[3]O'Brien Institute of Public Health, University of Calgary, Calgary, Alberta, Canada
[4]Libraries and Cultural Resources, University of Calgary, Calgary, Alberta, Canada
[5]Skipping Stone Foundation, Calgary, Alberta, Canada

Twitter Sofia B Ahmed @SofiaAhmedMD

Contributors CR conceived and designed the study, registered the protocol in PROSPERO, wrote the first version of the protocol and provided feedback on the search strategy. KTM, PER, SMD, SRR, NS, RS, HG, AMN and LP provided input into the design of the study, search strategy and edited the draft protocol. HG generated the search strategy. SA conceived and designed the study and helped in writing the draft protocol. All authors read and approved the final protocol.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

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

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Chantal L Rytz http://orcid.org/0000-0001-5174-6708
Sofia B Ahmed http://orcid.org/0000-0003-3000-2229

REFERENCES

1 Meerwijk EL, Sevelius JM. Transgender population size in the United States: a meta-regression of population-based probability samples. *Am J Public Health* 2017;107:e1–8.
2 Streed CG, Harfouch O, Marvel F, *et al*. Cardiovascular disease among transgender adults receiving hormone therapy: a narrative review. *Ann Intern Med* 2017;167:256–67.
3 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;169:205–13.
4 Asscheman H, Giltay EJ, Megens JAJ, *et al*. A long-term follow-up study of mortality in transsexuals receiving treatment with cross-sex hormones. *Eur J Endocrinol* 2011;164:635–42.
5 Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, *et al*. Sex steroids and cardiovascular outcomes in transgender individuals: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2017;102:3914–23.
6 The World Professional Association for Transgender Health. *Standards of care for the health of transsexual, transgender and gender Nonconforming people*. 7th Version, 2011.
7 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;102:3869–903.
8 Feldman J, Safer J. Hormone therapy in adults: suggested revisions to the sixth version of the *standards of care*. *Int J Transgend* 2009;11:146–82.
9 Seal LJ. Cardiovascular disease in transgendered people: a review of the literature and discussion of risk. *JRSM Cardiovasc Dis* 2019;8:204800401988074.

10 Zumoff B, Phillips GB, Castelli WP. Association of hyperestrogenemia and coronary heart disease in men in the framingham cohort. *Am J Med* 1985;78:863–9.
11 Narayanan K, Havmoeller R, Reinier K, *et al*. Sex hormone levels in patients with sudden cardiac arrest. *Heart Rhythm* 2014;11:2267–72.
12 Giltay EJ, Lambert J, Gooren LJ, *et al*. Sex steroids, insulin, and arterial stiffness in women and men. *Hypertension* 1999;34:590–7.
13 Giltay EJ, Elbers JM, Gooren LJ, *et al*. Visceral fat accumulation is an important determinant of PAI-1 levels in young, nonobese men and women: modulation by cross-sex hormone administration. *Arterioscler Thromb Vasc Biol* 1998;18:1716–22.
14 Turino Miranda K, Kalenga CZ, Saad N, *et al*. Gender-affirming estrogen therapy route of administration and cardiovascular risk: a systematic review and narrative synthesis. *Am J Physiol Heart Circ Physiol* 2022;323:H861–8.
15 MÁB C, Sievers C, Fulda S. Comorbidities in transsexual patients under hormonal treatment compared to age- and gender-matched primary care comparison groups. *Reprod Syst Sex Disord* 2012;01:1–4.
16 Van Kesteren PJ, Asscheman H, Megens JA, *et al*. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin Endocrinol* 1997;47:337–43.
17 Asscheman H, Gooren LJ, Eklund PL. Mortality and morbidity in transsexual patients with cross-gender hormone treatment. *Metabolism* 1989;38:869–73.
18 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.
19 SoRelle JA, Jiao R, Gao E, *et al*. Impact of hormone therapy on laboratory values in transgender patients. *Clin Chem* 2019;65:170–9.
20 Elbers JMH, Giltay EJ, Teerlink T, *et al*. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol* 2003;58:562–71.
21 Goodman M, Adams N, Corneil T, *et al*. Size and distribution of transgender and gender nonconforming populations: a narrative review. *Endocrinol Metab Clin North Am* 2019;48:303–21.
22 Page MJ, Moher D, Bossuyt PM, *et al*. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ* 2021;372:n160.
23 Shamseer L, Moher D, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;349:g7647–25.
24 National Institute of Health Research. Prospero center for reviews and dissemination. Available: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016050651 [Accessed 11 Jun 2021].
25 Moola S, Munn Z, Tufanaru C. Chapter 7: Systematic reviews of etiology and risk. In: Aromataris E, Munn Z, eds, 2020. https://synthesismanual.jbi.global
26 Sampson M, McGowan J, Cogo E, *et al*. An evidence-based practice guideline for the peer review of electronic search strategies. *J Clin Epidemiol* 2009;62:944–52.
27 National Institute of Health (NIH). Study quality assessment tools. Available: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools [Accessed 11 Jun 2021].
28 Higgins JPT, Altman DG, Gøtzsche PC, *et al*. The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928–9.
29 DerSimonian R, Kacker R. Random-effects model for meta-analysis of clinical trials: an update. *Contemp Clin Trials* 2007;28:105–14.
30 Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, *et al*. Endocrine treatment of transsexual persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2009;94:3132–54.
31 Guyatt GH, Oxman AD, Vist GE, *et al*. Grade: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924–6.