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🌐 Body mass index, mammographic density and post-menopausal breast cancer risk: a mediation analysis V.1

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Keywords: Breast cancer, Body mass index, BMI, Mammographic density, Breast density, Mediation analysis, adult bmi on cancer risk, determinants of breast cancer risk, breast cancer risk, breast cancer prevention, greater bmi in early adulthood, body mass index, uk nhs breast screening programme, greater bmi, adult bmi, course strategies for breast cancer prevention, linking adiposity, cancer risk, higher bmi, mammographic density, mediation analysis within the predicting risk, counterfactual mediation models with bootstrap confidence interval, bmi, breast cancer, cancer at screening, breast tissue composition, using counterfactual mediation model, national cancer registry linkage, protective association, predicting risk, screening

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Abstract

Background: Body mass index (BMI) and mammographic density (MD) are established but inversely correlated determinants of breast cancer risk. While higher BMI in post-menopausal women increases risk, greater BMI in early adulthood appears protective. MD, reflecting the proportion of fibroglandular to fatty tissue, is a strong positive risk factor. The extent to which MD mediates the protective association of early-adult BMI with post-menopausal breast cancer remains unclear.

Methods: We will perform a mediation analysis within the *Predicting Risk of Cancer at Screening (PROCAS)* cohort (>57,000 women, UK NHS Breast Screening Programme). Early-adult BMI (age 18–21 years) is self-reported at recruitment; MD is measured at screening using visual analogue scales (VAS) and automated volumetric methods. Post-menopausal breast cancer incidence is determined through national cancer registry linkage. Analyses will estimate natural direct and indirect effects of early-adult BMI on cancer risk mediated by MD, using counterfactual mediation models with bootstrap confidence intervals, guided by an investigator-derived directed acyclic graph.

Expected Impact: This study will clarify causal pathways linking adiposity and breast tissue composition to post-menopausal breast cancer. Findings may refine mechanistic understanding, improve individualised risk prediction, and support life-course strategies for breast cancer prevention.

Keywords: Body mass index, mammographic density, breast cancer, mediation analysis, PROCAS

Image Attribution

Figure 1. Graphical representation of the primary hypothesis

Figure 2. Hypothesised causal diagram and potential intermediate confounding role of later adult obesity (at time of cohort entry).

Background and Rationale

- 1 The aetiology of breast cancer is multifactorial. Body mass index (BMI) is a well-established causal determinant, although this relationship is complex. Excess BMI increases risk in postmenopausal women but decreases risk in premenopausal women. In childhood and early adulthood (aged 18–21), however, excess BMI decreases risk of both pre- and post-menopausal breast cancer risk. The contrasting protective effects of childhood and early adulthood adiposity are posited to arise from various hormonal mechanisms that may influence later breast tissue development. Another increasingly appreciated mechanism is that mammographic density (MD) may mediate the effect between childhood and early adulthood adiposity on breast cancer risk. MD, which at a biological level, refers to the proportion of epithelial and stromal tissue relative to fat, is a strong, independent positive risk factor for breast cancer. This effect remains consistent even after adjustments for correlated risk factors, such as age, current BMI, and menopausal status. Additionally, women with raised or persistently increasing MD over time may have a higher risk of breast cancer.
- 2 There are several other mechanisms potentially underpinning the effect by which MD may promote mammary carcinogenesis. For example, the dense fibroglandular tissue is hormonally responsive and may be more susceptible to neoplastic transformation or it may independently provide a microenvironment by influencing cell proliferation, angiogenesis, and differentiation. Cumulative exposure to MD, linked to these tumorigenic processes, increases with age and may explain age-specific breast cancer incidence despite the expected breast density decline with age, particularly in post-menopausal women.
- 3 To our knowledge, there have been three studies that examined the mediating role of MD in the causal pathway for the effect of childhood adiposity or early adult BMI on breast cancer. Using a case-control design, and data from the Nurses' Health Studies (1290 invasive breast cancers; 3422 controls), Rice et al. reported that 26% of the effect of childhood adiposity on post-menopausal breast cancer risk was mediated through MD (measured in percent using Cumulus software). They additionally sub-divided MD and reported mediation of 16% through dense area and 10% through non-dense area. Vabistssevitits et al. performed a Mendelian randomization study using reported GWAS data on childhood body size from UK Biobank data (N = 246,511; female-only) and reported that 56% of the effect of childhood body size and breast cancer was mediated by dense area (based on GWAS data from two independent studies). Recently, Pedersen et al. studied 33,939 Danish women undergoing mammographic screening and linked early life anthropometry information from the Copenhagen School Health Records Register. There were 883 post-menopausal breast cancers and MD determined by BI-RADS categorised as low and high. They reported that MD mediated 37% of this effect, but with very wide confidence intervals (17–170%).

- 4 Here, we propose to extend this literature and use data from the population-based PROCAS-1 study of more than 57,000 women undergoing routine mammographic screening in the National Health Service Breast Screening Programme (NHSBSP), Manchester, North-West England and where it is known that early adulthood BMI reduces the risk of post-menopausal breast cancer, and raised MD at time of screening increases the risk of developing breast cancer across various density measures and following adjustment for BMI. The PROCAS data will add to the literature as (i) the numbers of breast cancers (n=1700) are greater than previously published studies; and (ii) there are several different MD measures including subjective assessment by clinicians using Visual Analogue scales (VAS), and fully-automated methods (Densitas, Quantra, Volpara, pVAS).

Justification

- 5 Early adulthood represents a critical transition between adolescent growth and adult metabolic function and may interfere with breast development. Mediation analysis of early adult BMI and mammographic density could help refine current mechanistic understandings and target early preventive interventions, aligning with an emerging perspective that breast cancer prevention initiatives should encompass a lifetime approach. Therefore, this study aims to test the hypothesis that MD mediates the effect of early adult BMI on breast cancer risk in postmenopausal women, using a large UK cohort.
 - 5.1 Public Health Impact: Breast cancer incidence continues to rise, and understanding modifiable risk factors is crucial for prevention strategies.
 - 5.2 Scientific Contribution: Further evidence to help clarify whether early adult BMI and MD have independent effects on breast cancer risk or if MD acts as a mediator.
 - 5.3 Use of Advanced Methods: Mediation analysis provides a robust counterfactual statistical framework to quantify indirect and direct effects, improving causal inference.
 - 5.4 Data Availability: The PROCAS cohort provides a large, well-characterised dataset with BMI, weight, MD measurements, and breast cancer incidence.

Objectives

- 6 Primary Objective: To determine the proportion that mammographic density (visually assessed and recorded on Visual Analogue Scales at the time of screening) mediates the effect of early adult BMI on post-menopausal breast cancer risk in the PROCAS cohort.

- 7 Secondary Objectives: To investigate correlations/associations between early adult BMI and post-menopausal breast cancer risk, early adult BMI and MD, later adult BMI and MD, and independent associations for MD and post-menopausal breast cancer.

Hypothesis

- 8 Primary Hypothesis (see Fig. 1): Higher early adult BMI decreases post-menopausal breast cancer risk, and this effect is in part mediated through percentage MD.

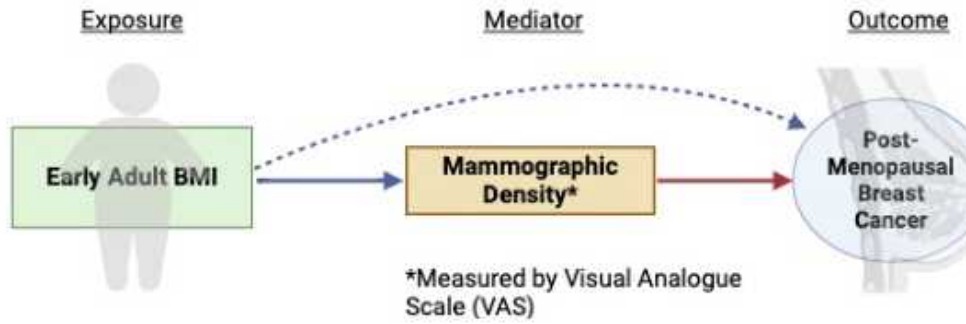


Figure 1. Graphical representation of the primary hypothesis

- 9 Secondary Hypothesis (see Fig. 2): Early adult BMI decreases percentage MD, increases fatty volume and decreases dense volume. Early adult BMI decreases post-menopausal breast cancer risk. *Later* adult BMI (at time of cohort entry) decreases percentage density and increases fatty and dense volumes. *Later* adult BMI (at time of cohort entry) increases post-menopausal breast cancer risk.

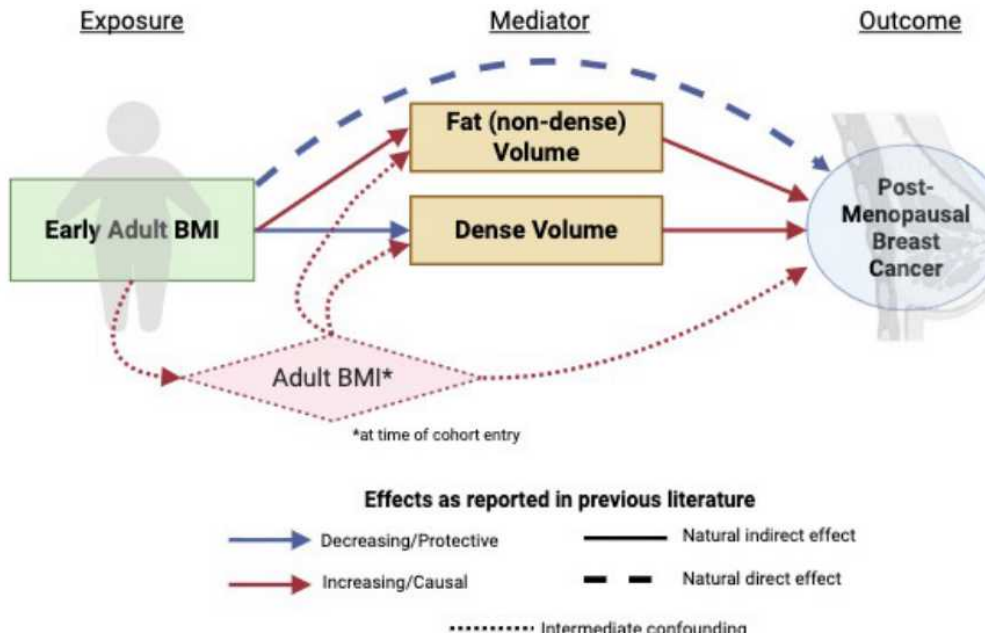


Figure 2. Hypothesised causal diagram and potential intermediate confounding role of later adult obesity (at time of cohort entry). Red arrows indicate an increasing effect from one variable to another, whereas blue arrows indicate a decreasing effect. The dashed lines represent the indirect and intermediate confounding pathways respectively.

Justifications for These Hypotheses and Mechanisms Using the Current Literature

- 10 Dense breast tissue, in contrast to non-dense fatty breast tissue, contains a higher proportion of fibroglandular components such as epithelial and stromal cells, connective tissue, and intercellular matrix (20). This tissue is at risk of carcinogenic transformation and is a well-established risk factor for developing breast cancer (15). In comparison, the role of non-dense tissue in breast carcinogenesis is more complex.
- 11 The impact of fat accumulation, including non-dense breast tissue, and the metabolic environment varies across different stages of breast development. In early adulthood, higher BMI and adipose tissue decrease breast cancer risk, partly mediated through BMI's role in lowering mammographic density and total dense volume (17,18). Genetically predicted higher BMI is also strongly associated with increased non-dense area and decreased proportional dense area, and may also decrease risk of breast cancer (21,22). Greater adiposity during early adulthood promotes fatty over glandular tissue and may alter hormonal pathways, including estrogen exposure, with cancer-protective implications for future breast structure (6,23).
- 12 In later adulthood, dense fibroglandular tissue remains the primary tissue at risk of carcinogenic transformation. However, adipose tissue, as part of the microenvironment surrounding the fibroglandular zone, is thought to influence epithelial differentiation and proliferation through mechanisms such as promoting inflammation and increasing estrogen levels via peripheral conversion, especially in postmenopausal women (24,25).

It is recognised that in a healthy population, breast density generally decreases with age, and this is particularly reduced in the menopausal transition (26). An important consideration is that, in later adulthood, women with a higher BMI tend to have greater overall breast fat, reducing percent density; however, unlike early adulthood, heavier post-menopausal women are also likely to have higher absolute breast density volume and fibroglandular tissue (24,25).

- 13 Weight changes mainly modify the proportion of density in the breasts. For example, in premenopausal women, losing weight has little to no effect on reducing the fibroglandular dense tissue, but it may have a small reducing effect in women after menopause (27,28). However, weight loss can lead to a noticeable reduction in breast fat in all age groups, leading to an increase in the relative percentage of breast density. This could be falsely ascribed to a perceived increased risk of breast cancer. The mediating role of breast density, as well as the quantity of adipose and fibroglandular tissue, in the observed reduction of breast cancer risk following weight loss remains to be thoroughly elucidated.

Methods

- 14 Study design: We will perform a mediation analysis within the Predicting Risk of Cancer at Screening (PROCAS) cohort study. The PROCAS study is a UK-based cohort that recruited over 57,900 women aged 46–73 attending triennial routine mammography screening between October 2009 and June 2015. The study has been described in detail elsewhere (8, 19), and following written consent and questionnaire forms collected detailed information on lifestyle factors, reproductive history, family history of breast cancer, and mammographic density measurements.
- 15 Participants: Study group(s): Described in further detail in PROCAS (8, 19), the Greater Manchester NHSBSP covers five main areas of Greater Manchester: Tameside, Oldham, Salford, Manchester and Trafford. Recruitment was carried out in two phases: for the first 3 years of recruitment, all women invited for breast screening were sent an invitation to participate in the PROCAS study; the second phase of recruitment involved inviting only those who had not previously attended screening in the area. As screening is triennial, all women attending screening during the recruitment period were invited once during this time.
- 16 Eligibility Criteria: Inclusion Criteria: Women enrolled in PROCAS. Available BMI and mammographic density data (we may consider sensitivity analyses imputing for missing data). Exclusion Criteria: Prior history of breast cancer. Technical issues with MD measurement. Development of premenopausal breast cancer during study period.
- 17 **Ascertainment of exposures**
- Exposure (Independent Variable): Early adult BMI (continuous, kg/m²) at 18–21 years. (*We have a noisy measure of this through the participant's recall of its value at study recruitment*).

- Mediator: Mammographic Density (MD) measured via:
 - o Visual Assessment Scores at time of screening - visual assessment of density recorded by two independent readers averaged across views and readers. VAS was chosen because it has the strongest correlation with cancer risk. Within PROCAS, Astley et al. (29) have previously shown that VAS is the strongest predictor for breast cancer risk compared with fully automated methods (Densitas, Quantra, Volpara).
 - o Quantify DV and NDV (fat tissue volume) as potential mediators. We have data for the density area (VAS) and the density volume and will use both separately.
- Covariates: Ethnicity, age at cohort entry, BMI at time of cohort entry, other history of cancer, benign breast disease, HRT, age of menarche, age of first pregnancy, parity, menopausal status, age of menopause family history of breast cancer, physical activity, and alcohol consumption.
 - o *Menopausal status: Women were considered post-menopausal if they reported one or more of the following criteria: natural menopause, surgical menopause involving bilateral oophorectomy; or current use of HRT; pre-menopausal if they self-reported continued menstrual periods or current use of hormonal birth control; and peri-menopausal if they did not meet these criteria and were unsure whether their periods had stopped.*

18

Outcome measures

The primary outcome measure is post-menopausal breast cancer incidence. The follow-up period extends from 2009 to 2022, linked to national cancer registries.

Measures to minimise bias

- We will ensure compliance with **AGReMA guidelines** for mediation analyses (30):
- Drawing a DAG and deriving the correct adjustment set to estimate the effect(s) of interest. We will use a consensus meeting approach.
- Adjustment for confounders through variety of causally grounded techniques.
- We will test the data for assumptions of mediation analysis – for example, testing for intermediate confounding.
- Sensitivity analyses: adjusting the main models for assumptions.

- o Determine the proportion that MD, using dense volume and fatty (non-dense) volume independently, mediates the effect of early adult BMI on post-menopausal breast cancer risk.

19 **Sample size**

Given the large PROCAS cohort (>57,000 women), statistical power is expected to be sufficient to detect mediation effects. Based on recent manuscript from Lee Malcomson, there are 48,417 women recruited with baseline BMI at median age 57 and recall BMI at age 20. There are 1,702 new breast cancers, the vast majority of which were post-menopausal.

20 **Statistical methods**

1. Descriptive and Preliminary Analyses

- Generate summary statistics for early adult BMI, MD (VAS, DV and NDV) and breast cancer incidence.
- Stratify descriptive statistics for modifying variables (**figures 1 and 2**) determined by construction of a DAG via a consensus group meeting.
- Preliminary correlations and associations to test and validate assumptions from the DAG (pwcorr e.g. BMI and MD, regression e.g. BMI and PM BCa, Chi Squared e.g. alcohol and PM BCa) prior to mediation analysis.

2. Mediation analysis

2.1. Associations – based on reports from previous research

- Examine early adult BMI → MD associations using linear regression.
- Examine early adult BMI → breast cancer association using logistic regression.
- Examine MD → breast cancer associations using logistic regression.
- Examine adult BMI at time of cohort entry ◇ breast cancer using logistic regression.
- Examine adult BMI at time of cohort entry ◇ MD associations using linear regression.
- Examine early adult BMI ◇ adult BMI at time of cohort entry association using linear regression.

2.2.

Confounder Adjustment

- Repeat all regressions with multivariable approach adjusting for confounders: e.g., alcohol etc. ...
- Stratify by modifying variables – for example, HRT.

2.3. Comparison of MD Measures - visual analogue scale (VAS), dense volume and fatty (non-dense) volume.

2.4. Mediation Analysis

- Using the paramed command in Stata.
- Run separate mediation analyses for dense volume and fatty (non-dense) volume, rather than considering them as additive or parallel mediators.
- **Counterfactual approach** needed: lots of confounders to account for (figures 2 and 3). Binary outcome, continuous exposure and mediator.
- Report natural direct effect (NDE), natural indirect effect (NIE), total effect, and proportion mediated with bootstrap confidence intervals.
- Ensure moderated mediation to account for exposure-mediator interaction in the paramed function.

- A preliminary DAG is shown in **Figure 3**.

Problem 1: selection bias (aka collider bias)

Problem 2: intermediate confounding

Problem 3: information bias (aka measurement error)

Problem 4: specific to linear models

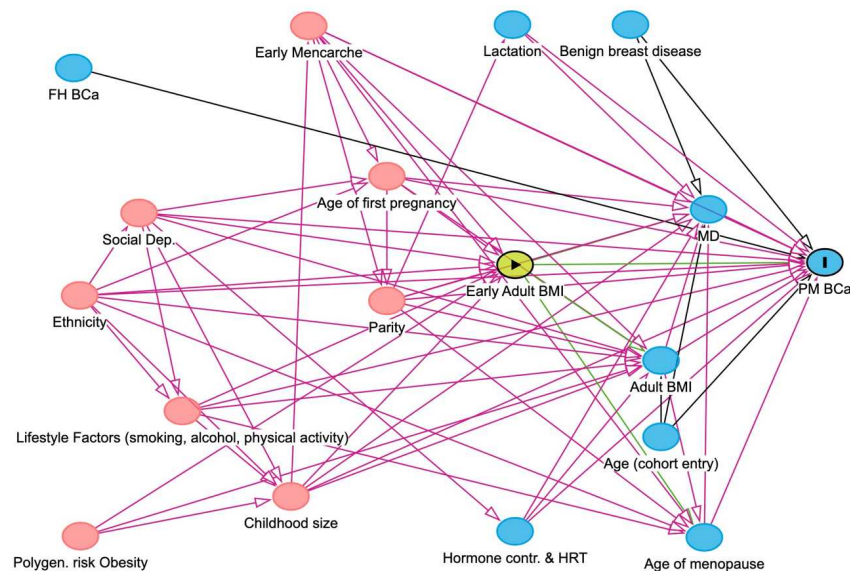


Figure 3. Preliminary detailed DAG (drawn in DAGitty). The grey nodes are covariates for discussion

and insertion into the DAG with the appropriate arcs to associated variables. We will establish a final DAG through a consensus group meeting including the listed investigators. Potential cycles are raised by the DAGitty platform and will be addressed if so, be expanding out over time.

To arrive at the final DAG, we will consider potential confounders of the exposure, mediator and outcome, as listed in **Figure 4**. The consensus may identify or remove additional factors.

2.5. Sensitivity analyses and Intermediate confounding

Sensitivity analyses (testing assumptions) will be conducted to assess the robustness of findings to potential violations, particularly if any intermediate confounding decided in DAG consensus. A sensitivity analysis will be conducted to analyse the group of women who were pre- or peri-menopausal at the time of screening.

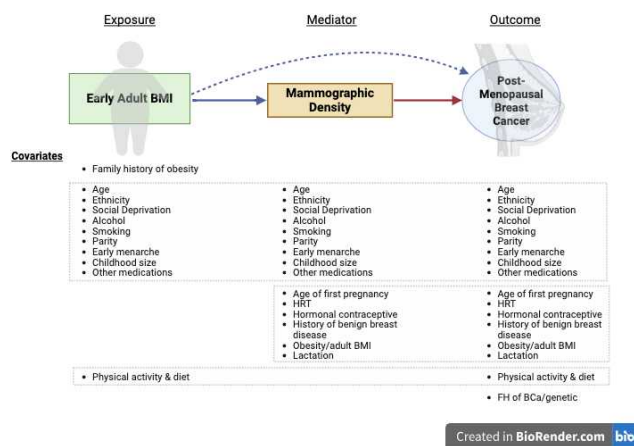


Figure 4. Summary of the potential factors that might influence exposure, mediator and outcome.

- Mediation models will be re-run with different covariates to test the stability of the natural direct and indirect effect estimates. This will help identify particularly influential confounders.
- Specific models will include/exclude variables such as alcohol use.
- Repeat mediation models stratified by intermediary confounder (*later* adult BMI). We can't adjust for it because on causal pathway so adjusting will bias the direct effect.
- Potentially:* We will apply a gformula computation sensitivity check will be explored as an alternative estimation strategy for mediation effects under the counterfactual framework.

3. Visualisation and Interpretation

- Create path diagrams for conceptual and statistical models.
- Interpret results in terms of consistency with biological expectations.
- Highlight if natural direct and indirect effects are opposing (e.g., suppressor mediation), and discuss implications.

Anticipated results/outcomes of the study

- 21 This study aims to clarify the role of mammographic density in the early adult BMI–breast cancer relationship, with potential implications for personalised breast cancer risk assessment and screening strategies.

Form part of a PhD proposal and grant proposal to investigate biological and tissue-level mechanisms linking childhood and early adult BMI to mammographic density and breast cancer risk.

The PROCAS study has already led to the development of the BC-Predict risk assessment tool.

This study could help improve early preventive intervention strategies by incorporating early adult BMI and MD as interacting factors in the risk prediction algorithm.

Study limitations and mitigations

- 22
- Ensure compliance with **AGReMA guidelines** for mediation analyses (21):
 - Residual Confounding: While we will adjust for known confounders (e.g., age, parity), unmeasured confounding may still exist.
 - Missing confounding variables: childhood size, smoking, HRT status...
 - There is known variance in the radiographer's user quality of measurement to determine MD. We will aim to approach this by conducting a sensitivity analysis using pVAS (an AI-trained method on VAS) where the data are available to do so.
 - Generalizability: The PROCAS cohort mainly consists of women from the UK screening population, which may limit its applicability to other populations.
 - Time Lag Between BMI Measurement and Breast Cancer Diagnosis: Although BMI at screening is a strong predictor, changes in weight over time could influence risk estimates.

Ethical considerations

- 23 The study was approved for the secondary analysis of PROCAS data by Central Manchester Research Ethics Committee (reference: 09/H1008/81). All participants consented to partake in the study. This study was performed in accordance with the



Declaration of Helsinki. This study will use de-identified data from the PROCAS cohort, ensuring patient confidentiality and compliance with data protection regulations, Good Clinical Practice (GCP) principles, and UK legislation. The study protocol will be prospectively included in the <https://www.protocols.io/> registry.

Public and patient involvement and risks to participants

- 24 There are no direct risks, as this is a retrospective study.

Potential Benefits to Subjects

- 25 There are no direct benefits to participants, but findings may improve breast cancer risk early preventive interventions.

ADDITIONAL INFORMATION

26 Data availability

The datasets supporting this article's conclusions are stored in a secured research database and may be available upon presentation of formal approval by contacting the corresponding author. The paper is not based on a previous communication to a society or meeting.

Competing interests

The authors declare no competing interests.

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Dissemination of findings

The findings of this study will be submitted for presentation at a relevant National meeting. The results may be submitted to an appropriate peer-reviewed journal. Authorship will be determined by mutual agreement.

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