Title: Changes in cardiometabolic health in the menopause transition: a longitudinal study

**Updated title:** Cardiovascular health in the menopause transition: a longitudinal study of up to 3,892 women with up to 4 repeat measures of risk factors

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# Aims

Women may experience changes in cardiovascular health in mid-life not only due to the effects of ageing, but potentially also due to the changes occurring around the menopausal transition. The aim of this study is to examine changes in measures of cardiovascular risk factors in women in mid-life and compare the contributions of chronological age and reproductive age to any change over time. The analysis is based on women drawn from a population-based sample who have up to four repeat cardiovascular measures within a five-year follow-up period. With the longitudinal data, we study the relationship between cardiovascular risk factors while adjusting for the underlying effects of ageing and a range of potentially important confounders.

# Objectives

* With the longitudinal data, we study the relationship between chronological age and reproductive age (time centered around final menstrual period) and compare the goodness of fit of the data with each of these individually and with both combined.
* For any factors where we see change in risk factors we will explore the extent to which they may mediate differences in carotid intermediate thickness (CIMT) between the first and last data collection.

# Sample

This study uses information from women participating in ALSPAC mothers’ clinics (there were 4 clinics) who had up to four repeat reproductive and cardiovascular measures and experienced a natural menopause. Observations for women who reported using HRT or hormonal contraception in the follow-up are also censored at the last point before reported use. Women who had experienced surgical menopause, defined by hysterectomy, oophorectomy, endometrial ablation, or radio- or chemotherapy related to reproductive organs, are also excluded.

# Exposures

* Reproductive age: for each woman, reproductive age was calculated in years before and after the date of their menopause, hence 0 is the date of their FMP, -1 would be one year before that date and +1, one year after that date.
* *Chronological age:* age at each clinic assessment, based on difference between dob and clinic data

# Outcomes

Cardiovascular outcomes available, excluding CIMT were collected at each of the 4 clinics, with CIMT collected at first and last clinic:

## Primary outcome: CIMT

CIMT was collected at the first and last clinic: include absolute and the change between the 2 time points - look if the RF change mediated any change in CIMT (or if there was any evidence of change)

*Anthropometry*

* Body mass index (BMI): Weight and height were measured according to standard protocols, with women wearing light clothes and unshod. Weight was measured using an electronic scale with accuracy of 100g (Tanita TBF-401) and height was measured using a Harpenden stadiometer and recorded to the nearest 1mm.
* Total fat and lean mass: these measures were derived from whole body dual energy X-ray absorptiometry (DXA) scans using a Lunar prodigy narrow fan beam densitometer. These were height-adjusted in the analyses by dividing by height squared.

*Blood pressure and pulse rate*

* Systolic blood pressure (SBP): measured using an Omron M6 upper arm BP/Pulse monitor in mmHg. Two measures of blood pressure were available for each arm. We take the average of these two measurements across arms at each time point.
* Diastolic blood pressure (DBP): measured using an Omron M6 upper arm BP/Pulse monitor in mmHg. Two measures of blood pressure were available for each arm. We take the average of these two measurements across arms at each time point.
* Pulse rate: measured using an Omron M6 upper arm BP/Pulse monitor in beats per minute (bpm). Two measures of pulse rate were available for each arm. We take the average of these two measurements across arms at each time point.

*Blood based biomarkers*

The following blood samples were all fasted:

* High-lipoprotein density (HDL) Cholesterol: measured in mmol/l.
* Triglycerides: measured in mmol/l.
* Glucose: measured in mmol/l.
* Non-HDL: derived by taking HDL away from total cholesterol (in mmol/l).
* C-Reactive Protein (CRP): measured in mg/l.
* Insulin: measured in IU/ml.

# Confounders

By definition, a confounder should be known to or plausibly influence both the exposure (change reproductive age) and outcome (cardiovascular risk factors and CIMT). Directed acyclic graphs (DAGs), informed by knowledge from the literature are used to decide what to include as potential confounders. We used measurements of confounders obtained before the exposure and outcome assessments:

* *Chronological age:* age at hormones and cardiometabolic measure assessment, in years
* *Pre-pregnancy BMI*: BMI (normal/underweight (≤24.9 kg/m2), overweight (25.0-29.9 kg/m2), obese (≥30.0 kg/m2)). Based on pre-pregnancy self-reported weight and height reported at cohort enrolment (pregnancy of index child). Continuous measure as we assume a linear relationship between pre-pregnancy BMI and each of the cardiometabolic risk factors.
* *Smoking status:* never, past, current, assessed before the first clinic
* *Parity*: 1, 2, 3, ≥4 pregnancies, based on self-reported parity at recruitment and updated until first clinic
* *Alcohol intake:* never or ≤4 times/month, 2-3 times/week, ≥ 4 times/week, assessed before the first clinic
* *Education:* highest attained qualification (i) Certificate of Secondary Education (CSE), ordinary- (O-) level or vocational certificate (qualifications usually obtained at age 16, the UK minimum school leaving age when these women were at school), (ii) Advanced A-level (usually taken at 18 years) or (iii) university degree, assessed at recruitment
* *Age at menarche:* (early (≤11 years), average (12-14y), late (≥15y)), assessed at recruitment

Information on age at menarche, number of previous pregnancies and maternal education were obtained around the time of recruitment to the study (mean age 28.3, SD 4.8), with information from subsequent questionnaires used to update parity (last data obtained at similar time of the first mid-life clinic assessment when women were mean 48.4 (SD 4.4) years old).

Visual representation of potential causal relationships between reproductive age (exposure) and cardiovascular risk factors (CVD RF=outcome), whilst accounting for potential confounders (chronological age, pre-pregnancy BMI, smoking, parity, alcohol and social economic position):

Reproductive age (x)

CVD RF (y)

1. Chronological age
2. Pre preg BMI
3. Smoking status
4. Parity
5. Alcohol
6. SES (measured by education)
7. Age at menarche

# Other variables of interest

* Stages of Reproductive Aging Workshop (STRAW) criteria are used to categorise women into one of three mutually exclusive reproductive stages at each clinic: (i) pre-menopausal (late reproductive age); (ii) menopause transition; and (iii) post menopause (irrespective of the years since menopause)

# Statistical analyses

* We use multilevel models to examine associations of each cardiovascular outcome with chronological and reproductive ageing, allowing for repeated measures within women.
* We allow the relationship between cardiovascular outcomes and chronological age and reproductive age to be non-linear, separately.
* We use fractional polynomials to assess non-linear associations and fit to the middle 95% of the data – i.e not assessing model fit on the lower 2.5 and upper 97.5th percentile.
* The multilevel models include all women with at least one hormone measure, under the missing at random assumption.

## Reproductive/chronological age models

* (1) reproductive age as time scale,
* (2) chronological age as time scale,
* (3) reproductive and chronological age mutually adjusted,
* (4) model (3) adjusted for pre-pregnancy BMI, smoking status, parity, age at menarche, alcohol intake and education

## Mediation analysis

For cardiovascular factors where we see evidence of a change across reproductive or chronological age, we will explore the extent to which they may mediate differences in CIMT between the first and the last data collection. Mediation will be assessed with the cardiovascular health measures at clinic assessments 2 and 3 explored as potential mediators (1, 2) . These timepoints will be used because they occur after the first CIMT and before the second CIMT measure, and hence could be plausibly influenced by the first and influence the last CIMT measures. The total effect of reproductive or chronological age (at the first clinic assessment) on CIMT (at the last clinic assessment) is estimated by regressing CIMT on reproductive or chronological age, adjusting for chronological or reproductive age respectively, CIMT (at the first clinic assessment) and baseline confounders. The direct effect (i.e., effect of reproductive or chronological age on CIMT not via the mediator) is estimated in the same way as the total effect but additionally including the mediator. We also regress the mediator on reproductive or chronological age, adjusted for chronological or reproductive age, CIMT (at the first clinic assessment) and baseline confounders. The indirect effect (i.e., effect of reproductive or chronological age via the mediator) is estimated by multiplying the coefficient for age in this latter regression and the coefficient for the mediator in the previous regression model. Standard errors obtained via bootstrapping and will be verified using *paramed* in Stata.

Mediator at clinic 2/3

Reproductive age/chronological age at clinic 1

CIMT at clinic 4

1. Pre preg BMI
2. Smoking status
3. Parity
4. Alcohol
5. SES (measured by education)
6. Age at menarche
7. Chronological age/reproductive age at clinic 1
8. CIMT at clinic 1

## Model fit

To assess model fit, we compare models with respect to: Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). AIC and BIC are likelihood-based fit statistics that penalise for model complexity.

# Missing data

Missing data on potential confounders (smoking, pre-pregnancy BMI, parity, alcohol, education, age at menarche) are imputed using multivariable multiple imputation with chained equations, performed using the mice command in Stata. We use 50 imputed data sets and include all variables included in any models (including the time-varying hormone measures) in the imputation models. Data on smoking status and alcohol intake from questionnaires completed up to 7 years prior to the first mid-life clinic are also used in the prediction models for missing confounders. Outcome variables are also used in the imputation model: all have less than 5% missing but are included and imputed themselves.

# Sensitivity analyses

* Assessing the impact of outliers are planned.
* To circumvent the possibility of influential outliers in time since menopause, we perform sensitivity analysis restricting the data at the 5th and 95th centiles of time since FMP
* To explore the sensitivity of our results to including all women with at least one cardiovascular measure we also repeat analyses only in women with 3 or 4 repeat measures
* We compare our main analyses (using multivariable imputation for confounder values) to analyses including only those with complete data on confounders.
* Women on anti-hypertensive or statin medication or medication for diabetes will be censored at the last point before reported use in a sensitivity analysis.
* We repeat our main analysis using general estimating equations (GEE) to check the robustness of the results, as the GEE assumptions are different than MLM. GEE assume missing completely at random (compared to MAR in multilevel models) and are more robust to the misspecification of the covariance structure of the random effects.
* Our main analyses where time since FMP is the key exposure can only include women who are known to have gone through the menopause, as this is required to calculate reproductive age. This might introduce selection bias, and to explore this, we compared relationships in cardiovascular outcomes by chronological age according to menopausal stages (pre-menopause, peri-menopause and post-menopause) in all women, irrespective of whether or not they had gone through the menopause (N= 3,892 women with 9,841 observations) [this was added 11/2020 following reviewer comments on another paper: (3)]

# Multiple testing

No formal adjustment will be made for multiple testing. Consideration will be taken in interpretation of results to reflect the number of statistical tests performed and the consistency, magnitude and direction of effect estimates for different outcomes.

# References

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3. Soares AG, Kilpi F, Fraser A, Nelson SM, Sattar N, Welsh PI, et al. Longitudinal changes in reproductive hormones through the menopause transition in the Avon Longitudinal Study of Parents and Children (ALSPAC). Scientific Reports. 2020;10(1):21258.

# Model formulae

## Random intercept on individual and random slope on chronological age:

allows chronological age to have a different effect on the outcome for each woman (i.e. we allow age to vary randomly across women)

Or

with a covariance matrix where the variances can be correlated:

*Fixed effects parameters*

Interpretation of a fixed effect: Fixed effect parameters describe the population average change in outcome value

* Intercept mean (β0)
* reproductive age (β1)
* chronological age (β2) - for each 1-year increase in age, increases/decreases by

*Random effects parameters*

* – between individual variance, i.e. variance at level 2- allows for individual variation in level of y (intercept) - is the individual departure about the intercept of this line
* Intercept variance () - between-individual variance in the mean of y at baseline
* Intercept intercept-chronological age slope covariance ()
* Chronological age slope variance () - between-individual variance in the change in chronological age
* Residual variance (σ2e)

## Interpretation

“Change in exposure/outcome” e.g. models for reproductive age show “at any chronological age, difference in outcome between two women with one year difference in reproductive age.” See Table 1

# Changes between the analysis plan and the manuscript submitted for review/publication (22/11/2021):

* Insulin was not included as checks on the data revealed inconsistencies.

## Table 1: interpretation of reproductive and chronological age from multilevel model

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Woman** | **Chronological age** | **Reproductive age** | **Prediction** | **Interpretation** |
| Example 1 |  |  |  |  |
| 1 | 40 | Constant (or the same) | Coefficient for chronological age | At the same reproductive age, difference in outcome between two women with year difference in chronological age. |
| 2 | 41 | Constant (or the same) |
| Example 2 |  |  |  |  |
| 1 | 40 | Constant (or the same) | Coefficient for chronological age | Difference in outcome in a woman with one year difference in chronological age. |
| 1 | 41 | Constant (or the same) |
| Example 3 |  |  |  |  |
| 1 | Constant (or the same) | 2 | Coefficient for reproductive age | At the same chronological age, difference in outcome between two women with one year difference in reproductive age. |
| 2 | Constant (or the same) | 3 |
| Example 4 |  |  |  |  |
| 1 | Constant (or the same) | 2 | Coefficient for reproductive age | Difference in outcome in a woman with one year difference in reproductive age. |
| 1 | Constant (or the same) | 3 |