Menopause: The Journal of The North American Menopause Society
Vol. 30, No. 6, pp. 599-606
DOI: 10.1097/GME.0000000000002184
Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The North American Menopause Society.

OPEN

ORIGINAL STUDY

Early menopause and cardiovascular risk factors: a cross-sectional and longitudinal study

Zayne Milena Roa-Díaz, PhD,[1,2] Faina Wehrli, PhD,[1] Irene Lambrinoudaki, PhD,[3] Catherine Gebhard, PhD,[4] Iris Baumgartner, PhD,[5] Pedro Marques-Vidal, PhD,[6] Arjola Bano, PhD,[1,7] Peter Francis Raguindin, PhD,[1,2,8] and Taulant Muka, PhD[1,9]

Abstract

Objective: The aim of the study is to evaluate the cross-sectional and longitudinal association of early natural menopause with changes in cardiovascular risk factors (CVRFs).

Methods: Postmenopausal women from the Swiss CoLaus study, reporting age at natural menopause (ANM) and having CVRFs measurements (blood lipids, blood pressure, glucose, homeostatic model assessment for insulin resistance [HOMA-IR], and inflammatory markers) at baseline (2003-2006) and first follow-up (2009-2012) were eligible for analysis. Age at natural menopause was analyzed as a continuous variable and in categories (ANM <45 and ≥45 y old). Linear regression analysis and linear mixed models were used to assess whether ANM is associated cross-sectionally and longitudinally with changes in CVRFs. Models were adjusted for demographic characteristics, lifestyle-related factors, time since menopause, medication, and clinical conditions.

Results: We analyzed 981 postmenopausal women. The cross-sectional analysis showed that women with ANM younger than 45 years had lower diastolic blood pressure ($\beta = -3.76$ mm Hg; 95% confidence interval [CI] = −5.86 to −1.65) compared with women whose ANM was 45 years or older. In the longitudinal analysis, ANM younger than 45 years was associated with changes in log insulin ($\beta = 0.26$; 95% CI = 0.08 to 0.45) and log homeostatic model assessment for insulin resistance levels ($\beta = 0.28$; 95% CI = 0.08 to 0.48). No associations were found between ANM and other CVRFs.

Received November 24, 2022; revised and accepted February 6, 2023.

From the [1]Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland; [2]Graduate School for Health Sciences, University of Bern, Bern, Switzerland; [3]Department of Obstetrics and Gynecology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [4]Department of Nuclear Medicine, University Hospital Zurich, Zurich, Switzerland; [5]Division of Angiology, Swiss Cardiovascular Center, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [6]Department of Medicine, Internal Medicine, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland; [7]Department of Cardiology Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [8]Swiss Paraplegic Research, Nottwil, Switzerland; and [9]Epistudia, Bern, Switzerland.

Funding/Support: Z.M.R.-D. and P.F.R. are PhD research fellows and received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 801076, through the SSPH+ Global PhD Fellowship Program in Public Health Sciences (GlobalP3HS) of the Swiss School of Public Health. TM is the founder of Epistudia. Epistudia has received no funding for this project. The CoLaus study was supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, Switzerland, and the Swiss National Science Foundation (grants 3200B0-105993, 3200B0-118308, 33CSCO-122661, 33CS30-139468, 33CS30-148401, 33CS30_177535 and 3247730_204523).

Financial disclosures/conflicts of interest: P.M.-V. received financing from Leading House South America to conduct a study on nutrition and COVID. The other authors have nothing to disclose.

Author contributions: T.M., I.B., and P.M.-V. contributed to the conception and design, the analysis and interpretation, and critical revision of the manuscript. Z.M.R.-D. contributed to the analysis and interpretation and drafting and critical revision of the manuscript. F.W. participated in the analysis and critical revision of the manuscript. I.L., C.G., A.B., and P.F.R. contributed to the analysis and critical revision of the manuscript. P.M.-V. contributed to data acquisition. All authors approved the final version of the manuscript and agreed to the published version of the manuscript

Availability of data and materials: Data supporting the conclusions of this study are available under research application to the CoLaus|PsyCoLaus data manager.

Declarations: Ethics approval and consent to participate: Institutional Ethics Committee approved CoLaus study. The study was performed in agreement with the Helsinki Declaration and its former amendments and in accordance with the applicable Swiss legislation.

Supplemental digital content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's Website (www.menopause.org).

Address correspondence to: Zayne Milena Roa-Díaz, RN, PhD, Institute of Social and Preventive Medicine (ISPM), University of Bern, Mittelstrasse 43, 3012, Bern, Switzerland. E-mail: zayne.roadiaz@unibe.ch

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

source: https://doi.org/10.48350/182263 | downloaded: 12.10.2024

Conclusions: Early menopause may be associated with changes in glucose metabolism, while it may have little to no impact on other CVRFs. Larger longitudinal studies are needed to replicate our findings.

Key Words: Blood pressure – Diabetes – Inflammation – Menopause – Prospective study.

Menopause indicates the end of the ovulation process.[1] Twelve consecutive months of amenorrhea without obvious intervention are the criteria for defining natural menopause, a phenomenon related to changes in multiple physiological systems, including the cardiovascular system and glucose metabolism. Recent literature suggests an association of menopause with a detrimental cardiometabolic profile in women.[1,2] In particular, early menopause (<45 y) has been described as a risk factor for type 2 diabetes,[3] ischemic heart disease, and all-cause mortality.[4]

However, the association of age at natural menopause (ANM) with cardiovascular risk factors (CVRFs) (ie, blood pressure, blood lipids, glucose metabolism markers) is not fully understood. Current knowledge is mainly derived from cross-sectional studies, and a temporal association is not fully explored.[5] Some cross-sectional studies reported no association of ANM with dyslipidemia and carotid atherosclerosis,[6,7] while others, including longitudinal studies, showed positive associations.[4,8,9] A study combining data from two population-based cohorts showed that a 1-year delay in menopause onset was associated with a 2% increased odds of having hypertension. However, Mendelian randomization (MR) studies failed to show this association is casual.[10,11]

Evidence on the association of ANM and markers of glucose metabolism is scarce.[12] While MR studies have shown conflicting results on whether ANM has a causal effect on glucose metabolism,[11,13] the largest study to date using the most comprehensive set of genes demonstrated ANM to be causally related to the risk of type 2 diabetes.[13] Finally, few studies have evaluated the role of ANM in inflammatory biomarkers, showing no association.[11,12]

Understanding whether ANM is associated with postmenopausal changes in CVRFs, such as lipids, inflammatory biomarkers, and glucose metabolism, might help understand factors that could accelerate cardiovascular aging in women. Therefore, in this study, using data from a population-based cohort study in Lausanne, we aimed to determine whether early ANM is cross-sectionally and longitudinally associated with changes in CVRFs, including total cholesterol, triglycerides, low- and high-density lipoprotein, apolipoprotein B (ApoB), fasting glucose, insulin levels, homeostatic model assessment for insulin resistance (HOMA-IR), systolic and diastolic blood pressure, and high-sensitivity C-reactive protein (hsCRP).

MATERIALS AND METHODS

Study population

The CoLaus study is a population-based cohort study including participants aged 35-75 years living in Lausanne, Switzerland. The baseline recruitment was conducted between 2003 and 2006, and the first follow-up was between 2009 and 2012. The design of the study has been described elsewhere.[14]

Age at natural menopause

Women participating in the CoLaus cohort who reported menopause also reported age at menopause and whether their menopause was natural or due to other causes. Age at natural menopause was defined as self-reported age at the last menstrual period. Additional verification of menopausal status was performed through self-reporting of present or absent menstruation at each of the study measurements

Cardiovascular risk factors

In our study, the CVRFs included were fasting lipid profile (total cholesterol, triglycerides, low- and high-density lipoprotein), ApoB, fasting glucose, insulin levels, HOMA-IR,[15] systolic and diastolic blood pressure, and hsCRP. Apolipoprotein B was not evaluated in the longitudinal analysis. The laboratory Centre Hospitalier Universitaire Vaudois Clinical Laboratory measured all biomarkers in fasting serum (see Supplemental Digital Content 1, http://links.lww.com/MENO/B111, which list the characteristics of the laboratory test used for measuring the biomarkers).[14,16] Low-density lipoprotein was estimated with the Friedewald formula,[17] and HOMA-IR was estimated with the formula: fasting glucose (in millimoles per liter) × fasting insulin (in milliunits per liter) / 22.5.[18,19]

Blood pressure was measured thrice after a rest of at least 10 minutes in a seated position, with an appropriately sized cuff using an Omron HEM-907 automatic sphygmomanometer. The average of the last two measurements was used in our analysis.

Assessment of covariates

Demographic, lifestyle, and medication use (statins, antihypertensive, hormone therapy [HT]) data were recorded during the interview. Hormone therapy was established through the question, "have you ever taken a hormone replacement therapy?" (yes/no). Education was classified as low (primary education), intermediate (general secondary or vocational education), or high (higher vocational education or university). Smoking status was defined as never, current, or former. Alcohol consumption was categorized as drinkers (>0 per/wk) and nondrinkers (0 per/wk). Physical activity was established with the question, "How many times a week do you engage in physical activity for at least 20 minutes?" (none, once a week, twice a week, three or more times per week).

Body weight and height measurements were taken with calibrated instruments under standardized procedures during the study interviews.[20] Body mass index (BMI) was calculated as weight divided by height squared (in kilograms per meter squared). Clinical characteristics such as the history of cardiovascular disease were confirmed with medical records. Diabetes was defined as fasting plasma glucose ≥7 mmol/L and/or the use of antidiabetic treatment.

 © 2023 The Author(s)

Inclusion and exclusion criteria

All postmenopausal women at baseline were included. Exclusion criteria were as follows: no information on ANM or reporting an age of menopause younger than 40 years or older than 60 years, surgical menopause (oophorectomy or hysterectomy), missing outcome information at baseline, or conflicting reproductive information (older age at menarche than the age at menopause or older age at menopause than chronological age).

Statistical analysis

Age at natural menopause was analyzed as a continuous variable and in categories (ANM <45 and ≥45 y old). Menopause onset before 45 years old was considered early menopause. The distribution of the variables was evaluated with histograms and the Shapiro-Wilk test. We described the characteristics of the participants with median and interquartile range (IQR) or absolute and relative frequencies; medians across ANM categories were compared with Mann-Whitney test, and categories were compared with χ^2 and Fisher exact test. Variables that did not follow an average distribution were natural log-transformed.

Cross-sectional analyses were conducted using ordinary least-squares linear regression to evaluate the association of ANM with CVRFs at baseline. Age at natural menopause was evaluated both as a continuous variable and by categories. To account for the confounding effect of factors such as anthropometric, clinical, and lifestyle characteristics on the association of ANM and CVRF,[3,21-23] the following three iterative models were used: model 1 being the unadjusted model; model 2 adjusted for chronological age, drinking (yes, no), smoking status (never, former, current), physical activity (once, twice, three times per week, and none), history of cardiovascular diseases (yes vs. no), diabetes (yes vs. no); model 3 additionally adjusted for age of menarche, use of antihypertensive medication, HT, statins (yes vs. no), and BMI. In addition, we evaluated the interaction between time since menopause and ANM, the presence of outliers, linearity, and normality in the residuals of model 3.

Prospective analyses were conducted using multilevel linear mixed models to evaluate the longitudinal association between ANM and changes in CVRFs. The association of ANM with CVRFs changes over time was calculated by the interaction term between reported ANM and time from baseline to first follow-up. The models were constructed following the same models implemented in the cross-sectional analysis.

We performed several sensitivity analyses. First, we evaluated the cross-sectional association of four categories of ANM (early, 40-44 y; intermediate, 45-49 y; average, 50-54 y [reference]; and late, ≥55 y) with CVRFs. Second, linear trends were tested by examining whether the β estimates changed monotonically with increasing ANM categories. Third, we performed a stratified analysis according to categories of time since menopause (early postmenopause, ≤5 y; and late postmenopause, >5 y). Fourth, we excluded women who received HT and reported smoking. Fifth, we built an ordinary least-squares linear regression model using restricted cubic splines with three knots to explore nonlinearity; furthermore, each model was adjusted for the same confounders as model 3. Sixth, to explore the potential of selection bias, we repeated the cross-sectional analysis in the excluded women (those with at least one exclusion criteria) with information on CVRFs as baseline (n = 925). Finally, we analyzed a subgroup of 365 women still having menses at baseline and who reported menopause in the first follow-up; within this group, we studied the association of ANM (continuous and categories) with CVRFs, adjusting for the same variables included in model 3.

Longitudinal analysis

We performed sensitivity analyses by evaluating the association of ANM by early, intermediate, average, and late age at menopause categories and restricting the analysis to women without comorbidities, those without HT, and women in early and late postmenopausal stage.

Finally, to account for multiple comparisons, we applied a Bonferroni correction, which considers the correlation among the outcomes evaluated. Therefore, cross-sectional and longitudinal findings were interpreted with an adjusted *P* value of 0.007.[24] Analyses were performed on Stata V.15.1 (Stata Corp, College Station, TX) and R software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

The institutional ethics committee of the University of Lausanne, which afterward became the Ethics Commission of Canton Vaud (http://www.cer-vd.ch), approved the baseline CoLaus study (reference 16/03). The approval was renewed for the first follow-up (reference 33/09). The study was performed in agreement with the Helsinki Declaration and its former amendments and in accordance with the applicable Swiss legislation. All participants gave their signed informed consent before entering the study.

RESULTS

Selection and characteristics of participants

One thousand nine hundred six women were eligible for inclusion in our study. Of these, 461 were excluded because of surgical menopause, 71 reported menopause out of the age range 40-60 years, and 33 did not have data on age at menopause. Three hundred twenty-four were excluded because of missing information on the first follow-up, and 36 because of inconsistent or missing information on reproductive factors at baseline and follow-up. Thus, 981 women were included in our cross-sectional and longitudinal analysis (see Supplemental Digital Content 2, http://links.lww.com/MENO/B112, which illustrates the participant flowchart and Supplemental Digital Content 3, http://links.lww.com/MENO/B113, where the characteristics of excluded women are described).

The clinical and sociodemographic characteristics of the 981 women included in our analysis were summarized in Table 1 according to menopause onset categories (ANM <45 vs. ANM ≥45 y old). Regarding the timing of menopause, 10.0% (n = 99) of women reported early ANM (<45 y old). Women in the early ANM category reported the highest smoking frequency, 27.3% (n = 27), and had lived longer with menopause (median of 14.1 y since last menses). In addition, women who reported early ANM had the lowest systolic and diastolic blood

TABLE 1. *Baseline characteristics of the participants by categories of age at natural menopause*

	Age at natural menopause		
	<45 y old	≥45 y old	
	n = 99	n = 882	P^a
	Median (IQR)	Median (IQR)	
Age	56.3 (51.0–61.6)	60.6 (56.2–65.3)	<0.01
Age at menarche, y[b]	13 (12–14)	13 (12–14)	0.19
Time since menopause, y	14.1 (9.5–20.2)	9.2 (5.0–15.3)	<0.01[c]
Body mass index, kg/m^2	24.9 (22.1–27.9)	24.6 (22.1–27.8)	0.33
Total cholesterol, nmol/L	5.7 (5.3–6.3)	5.9 (5.2–6.6)	0.23
Low-density lipoprotein, mmol/L	3.6 (3.2–4.4)	3.8 (3.2–4.5)	0.48
High-density lipoprotein, mmol/L	1.7 (1.5–2.1)	1.8 (1.5–2.1)	0.29
Triglycerides, mmol/L	1.0 (0.8–1.4)	1 (0.8–1.4)	0.68
Apolipoprotein B, mg/dL[d]	143.0 (98.6–221.0)	141.6 (101.2–196.0)	0.77
High sensitivity C-reactive protein, μg/mL[e]	1.7 (0.7–3.5)	1.5 (0.7–2.9)	0.74
Glucose, mmol/L	5.2 (4.9–5.6)	5.3 (4.9–5.7)	0.23
Insulin, microIU/mL[f]	7.4 (4.6–11.1)	6.8 (4.6–10)	0.36
HOMA-IR	1.7 (1.1–2.8)	1.6 (1.1–2.5)	0.67
Systolic blood pressure, mm Hg[g]	121 (110.0–134.0)	129 (117.0–141.0)	<0.01[h]
Diastolic blood pressure, mm Hg[g]	76.0 (68.0–83.0)	79 (72.0–85.0)	<0.01[i]
	n (%)	n (%)	
Smoking categories			
Never	41 (41.4)	444 (50.3)	0.17
Former	31 (31.3)	259 (29.4)	
Current	27 (27.3)	179 (20.3)	
Alcohol drinker (yes)	59 (59.6)	585 (66.3)	0.25
Educational level			
High	9 (9.1)	113 (12.8)	0.37
Middle	31 (31.3)	232 (26.3)	
Low	58 (59.6)	537 (60.1)	
Physical activity (20 min)[j]			
None	36 (36.4)	288 (32.7)	0.14
Once a week	9 (9.1)	48 (5.4)	
Twice a week	52 (52.5)	539 (61.1)	
≥Thrice a week	2 (2.0)	7 (0.8)	
Diabetes, n (%)[j]	5 (5.1)	31 (3.5)	0.40
Hormone therapy (yes)	54 (54.5)	511 (57.9)	0.74
Prevalence of cardiovascular diseases (yes)	10 (10.1)	64 (7.3)	0.28
Statin therapy (yes)	12 (12.2)	101 (11.5)	0.76
Antihypertensive medication (yes)	16 (16.2)	195 (22.1)	0.14

HOMA-IR, homeostatic model assessment for insulin resistance; IQR, interquartile range; n, number of women.
[a]Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables.
[b]Three missing values.
[c]P value = 0.001.
[d]Sixty-one missing values.
[e]One missing value.
[f]One hundred sixty-eight missing values.
[g]Two missing values.
[h]P value = 0.0001.
[i]P value = 0.0037.
[j]Fisher exact test.

pressure median levels and the lowest percentage of high education levels; however, differences between educational groups were not significant. More than 50% of women in both ANM categories received HT. Finally, six outcomes had missing values; the variable insulin had the highest number of missing data (n = 168; Table 1).

Cross-sectional association of ANM and CVRFs

We found no association between ANM and cardiovascular risk factors, except for diastolic blood pressure in the fully adjusted model (Table 2). In model 3, women in the early ANM category had on average 3.76 mm Hg less in diastolic blood pressure levels than women with ANM 45 years or older (see Supplemental Digital Content 4, http://links.lww.com/MENO/B114, where estimates for models 1 and 2 can be found). The normality assumption was met in the evaluation of the residuals of the third model. The exclusion of outliers did not change the estimates or their significance. No interaction was found between time since menopause and ANM (data not shown).

Longitudinal association of ANM and changes in CVRFs

Women were followed for a median (IQR) of 5.6 years (4.6–8.4 y). In model 3, we found increased levels of log insulin ($\beta = 0.264$) and log HOMA-IR ($\beta = 0.280$) in women in early menopause compared with women with ANM 45 years or older (see Supplemental Digital Content 5, http://links.lww.com/MENO/B115, where estimates for longitudinal models 1 and 2 can be found). Aside from insulin and HOMA-IR, none of the other models showed an association between ANM and longitudinal CVRFs changes in fully adjusted models (Table 3, Figure 1).

Sensitivity analysis

Cross-sectional analysis

The characteristics of the women according to four ANM categories (early, intermediate, average, and late) are presented in Table S5, http://links.lww.com/MENO/B115. When ANM was analyzed in four categories, no association with CVRFs was observed (see Supplemental Digital Content 6, http://links.lww.com/MENO/B116, which shows the cross-sectional analysis of the four categories of ANMs and CVRFs, and Supplemental Digital Content 7, http://links.lww.com/MENO/B117, which presents the baseline characteristics of the women in the four categories analyzed).

Stratified analyses, in general, showed similar results to the main analysis (see Supplemental Digital Content 8, http://links.lww.com/MENO/B118, which presents estimates from the analysis restricted to women without HT, nonsmokers, women within the first 5 years after experiencing menopause).

None of the restricted cubic spline models assessing associations of ANM and CVRFs at baseline was significant (see Supplemental Digital Content 9, http://links.lww.com/MENO/B119, which presents the nonlinear associations of ANM and CVRFs). In the analysis of the 925 menopausal women excluded from the analysis, no association between ANM and CVRFs was observed (see Supplemental Digital Content 10, http://links.lww.com/MENO/B120, where the cross-sectional analysis estimates for excluded women are presented). In the evaluation of 365 women who reported experiencing menopause at first follow-up, each year of later ANM was associated with 0.801 mm Hg increase in systolic blood pressure $P < 0.001$ (See Supplemental Digital Content 11, http://links.lww.com/MENO/B121, where cross-sectional analysis estimates for these newly menopausal women are presented and see Supplemental

© 2023 The Author(s)

TABLE 2. *Cross-sectional association of age at natural menopause and cardiovascular risk factors at baseline*[a]

	Age at natural menopause			
	Continuous		<45 y old vs ≥45 y old (Ref)	
	β (95% CI)	*P*	β (95% CI)	*P*
Total cholesterol, mmol/L	0.001 (−0.014 to 0.016)	0.929	−0.046 (−0.248 to 0.157)	0.659
Log high-density lipoprotein	−0.002 (−0.006 to 0.001)	0.166	−0.006 (−0.053 to 0.040)	0.790
Low-density lipoprotein, mmol/L	0.003 (−0.011 to 0.018)	0.669	−0.027 (−0.222 to 0.169)	0.790
Log triglycerides	0.005 (−0.001 to 0.011)	0.116	−0.028 (−0.112 to 0.056)	0.514
Log apolipoprotein B	0.001 (−0.008 to 0.010)	0.838	0.035 (−0.095 to 0.165)	0.596
Log glucose	0.002 (0.0008 to 0.003)	0.033	−0.020 (−0.042 to 0.003)	0.083
Log insulin	0.0002 (−0.008 to 0.008)	0.944	−0.027 (−0.140 to 0.086)	0.644
Log HOMA-IR	0.002 (−0.007 to 0.011)	0.631	−0.047 (−0.167 to 0.072)	0.438
Log high sensitivity C-reactive protein	−0.004 (−0.018 to 0.011)	0.617	0.027 (−0.171 to 0.225)	0.789
Systolic blood pressure, mm Hg	0.076 (−0.183 to 0.336)	0.564	−1.890 (−5.418 to 1.638)	0.293
Diastolic blood pressure, mm Hg	0.157 (0.001 to 0.312)	0.048	−3.759 (−5.865 to −1.652)	<0.001

HOMA-IR, homeostatic model assessment for insulin resistance; <45 Years, menopausal onset before 45 years; ≥45 Years menopause onset at 45 years or older.
[a]The reported results correspond to model 3: adjusted for chronological age, body mass index, drinking status (yes vs. no), smoking (never, current, former), physical activity (once, twice, thrice per week, and none), history of cardiovascular diseases (yes vs. no), diabetes (yes vs. no), age at menarche, use of antihypertensive medication, hormone therapy, and statins (yes vs. no).
Log apolipoprotein B n = 917; Log glucose n = 978; Log insulin n = 811, Log HOMA-IR n = 811; Log high sensitivity C-reactive protein n = 977; systolic blood pressure n = 976; diastolic blood pressure n = 976.

Digital Content 12, http://links.lww.com/MENO/B122, where characteristics of newly menopausal women are available).

Longitudinal analysis

In the longitudinal sensitivity analyses, no association between ANM categories and CVRFs was found (see Supplemental Digital Content 13, http://links.lww.com/MENO/B123, where estimates of the longitudinal association of ANM in four categories and CVRFs are presented). Restricting the analysis to women without HT and in the late postmenopausal stage did not change the trend of the main findings, early ANM was still associated with changes in log insulin and log HOMA-IR; however, *P* values increased and could not be considered significant under the Bonferroni-corrected *P* value of 0.007. In the analysis of nonsmokers, an association of later ANM and changes in low-density lipoprotein (β = 0.10 mmol/L, *P* = 0.006) were observed (see Supplemental Digital Content 14, http://links.lww.com/MENO/B124, which presents the finding for the longitudinal stratified analysis).

DISCUSSION

In general, our study showed no consistent associations between ANM and changes in CVRFs. However, our results indicated potential adverse changes in glucose metabolism related to early ANM. Our study found an association between early menopause and changes in insulin and HOMA-IR levels. Most evidence has evaluated the association between early menopause and the risk of type 2 diabetes, but few studies have evaluated its association with biomarkers of glucose metabolism as was done in the present work.[4] Early menopause has been inconsistently associated with an increased risk of type 2 diabetes.[3,4,25] Prospective cohort studies and genetic studies showed that women with early ANM had a 1.4-2.4 higher risk of developing type 2 diabetes than women with average ANM.[3,26] Likewise, the most recent MR analysis using 290 genetic variants related to ANM found that genetically mediated later ANM has beneficial effects on type 2 diabetes.[13] However, another MR study using 56 genetic variants related to ANM did not find an association with glucose or glycated hemoglobin HbA1C.[11]

TABLE 3. *Longitudinal analysis association of age at natural menopause with changes in cardiovascular risk factors*

	Age at natural menopause			
	Continuous		<45 y old vs ≥45 y old (Ref)	
	β (95% CI)	*P*	β (95% CI)	*P*
Total cholesterol, mmol/L	0.017 (−0.008 to 0.043)	0.191	0.010 (−0.310 to 0.330)	0.951
Log high-density lipoprotein	0.002 (−0.005 to 0.008)	0.593	−0.041 (−0.122 to 0.041)	0.328
Low-density lipoprotein, mmol/L	0.019 (−0.006 to 0.043)	0.135	0.062 (−0.244 to 0.367)	0.692
Log triglycerides	−0.004 (−0.015 to 0.007)	0.504	0.080 (−0.058 to 0.219)	0.256
Log glucose	0.001 (−0.002 to 0.004)	0.609	0.015 (−0.023 to 0.052)	0.444
Log insulin	−0.004 (−0.018 to 0.010)	0.566	0.264 (0.076 to 0.451)	0.006
Log HOMA-IR	−0.003 (−0.018 to 0.012)	0.713	0.280 (0.079 to 0.482)	0.006
Log high sensitivity C-reactive protein	0.002 (−0.024 to 0.028)	0.893	−0.023 (−0.349 to 0.303)	0.889
Systolic blood pressure, mm Hg	−0.123 (−0.599 to 0.354)	0.614	2.420 (−3.549 to 8.389)	0.427
Diastolic blood pressure, mm Hg	0.080 (−0.207 to 0.366)	0.585	−0.468 (−4.051 to 3.115)	0.798

ANM, age at natural menopause; HOMA-IR, homeostatic model assessment for insulin resistance.
Model 3: using linear mixed models' regression, adjusted for chronological age, drinking status (yes vs. no), smoking (never, current, former), physical activity (once, twice, three times per week and none), history of cardiovascular diseases (yes vs. no), diabetes (yes vs. no), age at menarche, use of antihypertensive medication, diabetes medication, hormone therapy and statins (yes vs. no), body mass index, and interaction term ANM and follow-up time in years.

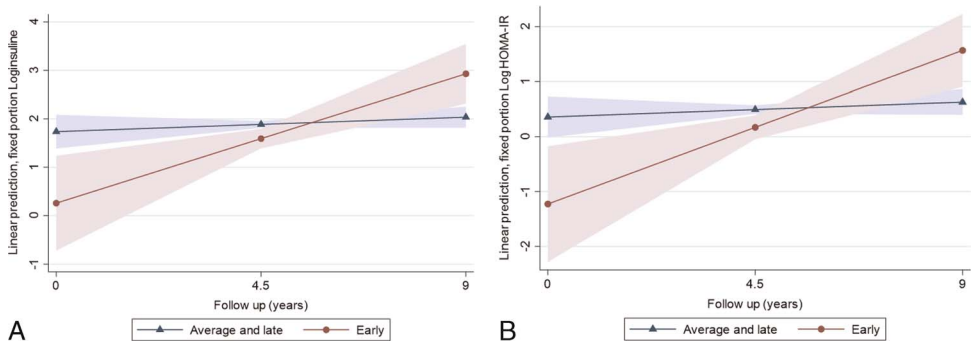

FIG 1. Longitudinal changes in insulin and HOMA-IR. (**A**) Changes in log insulin in women with early menopause compared with women with menopause at average or late menopause. (**B**) Changes in log HOMA-IR in women with early menopause compared with women with average or late menopause. Model 3: using linear mixed models' regression, adjusted for chronological age, drinking status (yes vs. no), smoking (never, current, former), physical activity (once, twice, three times per week and none), history of cardiovascular diseases (yes vs. no), diabetes (yes vs. no), age at menarche, use of antihypertensive medication, diabetes medication, hormone therapy and statins (yes vs. no), body mass index, and interaction term ANM and follow-up time in years. Early menopause <45 years old, average and late ≥45 years old (Ref). ANM, age at natural menopause; HOMA-IR, homeostatic model assessment for insulin resistance.

Possible mechanisms behind the association of early menopause with changes in insulin and HOMA-IR could be the insulin resistance profile associated with the drastic decrease in estrogen and estrogen receptor α activity. In addition, androgen secretion and decreased sex hormone binding globulin (SHBG), common in postmenopausal women, are also associated with increased insulin resistance.[27,28] Iron metabolism could be another mechanism underlying the association of early menopause with insulin. Previous studies have shown that iron metabolism changes after menopause.[29,30] Similarly, an association between iron biomarkers and insulin resistance has been reported,[31] and the SWAN study found that elevated iron was associated with insulin resistance in women with the largest increase in iron levels after menopause.[29]

Few studies have evaluated the association of ANM with CVRFs, and none have assessed the role of early ANM on lipid profile. However, similar to our study, a longitudinal study of 915 postmenopausal women from the UK Medical Research Council National Survey of Health and Development found no evidence of an association between ANM and changes in trajectories of blood lipids in postmenopausal women.[22] However, the Study of Women's Health Across the Nation showed that 1–3 years after menopause, later ANM was associated with less adverse changes in triglycerides, total cholesterol, and low-density lipoprotein. These effects did not translate into a reduced risk for subclinical carotid disease.[32]

Regarding blood pressure traits, the present cross-sectional analysis found that early ANM was associated with lower diastolic blood pressure levels, but this association was not observed in the longitudinal analysis. Similarly, in the SWAN study, during the transition to menopause, a subgroup of women showed a decrease in systolic, diastolic, and mean arterial blood pressure after menopause; however, age of menopausal onset was not associated with these changes.[33] These results are in line with a recent MR study our group has published showing no causal association between ANM and blood pressure traits.[10]

Other studies have reported adverse cardiovascular profiles after menopause,[23,34] especially among women with early menopause.[4] The physiological mechanisms behind these associations are unclear; hypotheses on estrogen anti-inflammatory and antioxidative activity changes and the role of inflammatory states related to the aging process are part of the possible explanations. Likewise, the concurrence of changes in estrogen receptors and secretion patterns and age-associated vascular changes may explain these changes.[35] Estrogens' effects depend on the state of the cardiovascular system so that in the presence of small fatty plaques (common in women going through menopause), estrogens may not have their recognized protective effect and instead be associated with a higher risk of cardiovascular events.[35]

Strengths and limitations

Our study has several strengths, including the prospective, detailed, and longitudinal collection of data performed in the CoLaus Cohort. We evaluated exclusively women with natural and early menopause (ANM between 40 and before 45 y old). We have used multilevel models that account for the clustering of repeated measures and adjusted for various confounders. In addition, we performed several sensitivity analyses to evaluate the robustness of our findings, including the analysis of women who reported going through menopause during the study follow-up, which addresses the selection bias of choosing women based on their menopausal status.

However, our analysis has some limitations that should be considered in interpreting our findings. These include self-reporting of menopause status and ANM, as well as HT. Self-report ANM has shown to be inaccurate, for instance, women who have early menopause overestimate age at menopause, while women who have late menopause underestimate age at menopause.[36] Furthermore, women on HT experiencing periods may not be aware of their ANM. Nevertheless, there is evidence for the reliability of self-reports of all these reproductive variables among postmenopausal women.[37] In addition, there is no gold standard for measuring ANM,[5] and in our study, we implemented a strict algorithm to identify inconsistencies about reported biological menopausal age. In case our study incurred misclassification it was nondifferential between the main two groups of comparison, which could bias the estimates to the null. Furthermore, more

 © 2023 The Author(s)

than 55% of the women reported receiving HT, which despite being included in the models as a confounding factor, may still be generating some residual confounding, making this finding worth interpreting with caution. To note, when women receiving HT were excluded, the longitudinal associations of early ANM with insulin and HOMA-IR were no longer significant under Bonferroni-corrected threshold.

Moreover, more than 50% of included women had lived 9 or more years with menopause, especially women with early menopause, where more than 75% have lived more than 9 years with menopause, which affects the generalization of our findings and could attenuate the association between ANM and CVRFs. Finally, although our study has repeated measures, we have just two data points for analysis, which limits the analysis of the trajectories of CVRF changes in postmenopausal stages. Dietary information was also not included in this analysis. Although diet can affect CVRFs, its association with ANM is inconsistent and, thus, should not affect our main analysis. Only alcohol consumption has a consistent association[38] and therefore was included in the current analysis.

CONCLUSIONS

Our finding suggests that ANM is not consistently associated with changes in cardiovascular risk factors in the postmenopausal years. However, early ANM may be associated with adverse changes in insulin and HOMA-IR postmenopause. Larger studies with validated menopause status and ANM are needed to replicate our findings and to explore further whether ANM has short or long-term effects on CVRFs.

REFERENCES

1. El Khoudary SR, Aggarwal B, Beckie TM, et al. Menopause transition and cardiovascular disease risk: implications for timing of early prevention: a scientific statement from the American Heart Association. *Circulation* 2020;142:e506-e532. doi: 10.1161/CIR.0000000000000912
2. Vogel B, Acevedo M, Appelman Y, et al. The Lancet women and cardiovascular disease commission: reducing the global burden by 2030. *Lancet* 2021;397: 2385-2438. doi: 10.1016/S0140-6736(21)00684-X
3. Muka T, Asllanaj E, Avazverdi N, et al. Age at natural menopause and risk of type 2 diabetes: a prospective cohort study. *Diabetologia* 2017;60:1951-1960. doi: 10.1007/s00125-017-4346-8
4. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: a systematic review and meta-analysis. *JAMA Cardiol* 2016;1:767-776. doi: 10.1001/jamacardio.2016.2415
5. Roa-Díaz ZM, Raguindin PF, Bano A, Laine JE, Muka T, Glisic M. Menopause and cardiometabolic diseases: what we (don't) know and why it matters. *Maturitas* 2021;152:48-56. doi: 10.1016/j.maturitas.2021.06.013
6. Stöckl D, Peters A, Thorand B, et al. Reproductive factors, intima media thickness and carotid plaques in a cross-sectional study of postmenopausal women enrolled in the population-based KORA F4 study. *BMC Womens Health* 2014;14:17. doi: 10.1186/1472-6874-14-17
7. He L, Tang X, Li N, et al. Menopause with cardiovascular disease and its risk factors among rural Chinese women in Beijing: a population-based study. *Maturitas* 2012;72:132-138. doi: 10.1016/j.maturitas.2012.02.013
8. Lee JS, Hayashi K, Mishra G, Yasui T, Kubota T, Mizunuma H. Independent association between age at natural menopause and hypercholesterolemia, hypertension, and diabetes mellitus: Japan nurses' health study. *J Atheroscler Thromb* 2012;14746:161-169. doi: 10.5551/jat.14746
9. Joakimsen O, Bønaa KH, Stensland-Bugge E, Jacobsen BK. Population-based study of age at menopause and ultrasound assessed carotid atherosclerosis: the Tromsø Study. *J Clin Epidemiol* 2000;53:525-530. doi: 10.1016/s0895-4356(99)00197-3
10. Roa-Díaz ZM, Asllanaj E, Amin HA, et al. Age at natural menopause and blood pressure traits: Mendelian randomization study. *J Clin Med* 2021; 10:4299. doi: 10.3390/jcm10194299
11. Dam V, Onland-Moret NC, Burgess S, et al. Genetically determined reproductive aging and coronary heart disease: a bidirectional 2-sample Mendelian randomization. *J Clin Endocrinol Metab* 2022;107:e2952-e2961. doi: 10.1210/clinem/dgac171
12. Matthews KA, Crawford SL, Chae CU, et al. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? *J Am Coll Cardiol* 2009;54:2366-2373. doi: 10.1016/j.jacc.2009.10.009
13. Ruth KS, Day FR, Hussain J, et al. Genetic insights into biological mechanisms governing human ovarian ageing. *Nature* 2021;596:393-397. doi: 10.1038/s41586-021-03779-7
14. Firmann M, Mayor V, Vidal PM, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord* 2008;8:6. doi: 10.1186/1471-2261-8-6
15. Gast KB, Tjeerdema N, Stijnen T, Smit JW, Dekkers OM. Insulin resistance and risk of incident cardiovascular events in adults without diabetes: meta-analysis. *PloS One* 2012;7:e52036. doi: 10.1371/journal.pone.0052036
16. Kilani N, Vollenweider P, Waeber G, Marques-Vidal P. Iron metabolism and incidence of metabolic syndrome. *Nutr Metab Cardiovasc Dis* 2015;25: 1025-1032. doi: 10.1016/j.numecd.2015.07.005
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502. doi: 10.1093/clinchem/18.6.499
18. Katsuki A, Sumida Y, Gabazza EC, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. *Diabetes Care* 2001;24:362-365. doi: 10.2337/diacare.24.2.362
19. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-1495. doi: 10.2337/diacare.27.6.1487
20. Marques-Vidal P, Bochud M, Bastardot F, et al. Association between inflammatory and obesity markers in a Swiss population-based sample (CoLaus Study). *Obes Facts* 2012;5:734-744. doi: 10.1159/000345045
21. Zhu D, Chung H-F, Dobson AJ, et al. Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data. *Lancet Public Health* 2019;4:e553-e564. doi: 10.1016/S2468-2667(19)30155-0
22. O'Keeffe LM, Kuh D, Fraser A, Howe LD, Lawlor D, Hardy R. Age at period cessation and trajectories of cardiovascular risk factors across mid and later life. *Heart* 2020;106:499-505. doi: 10.1136/heartjnl-2019-315754
23. De Kat AC, Dam V, Onland-Moret NC, Eijkemans MJ, Broekmans FJ, Van Der Schouw YT. Unraveling the associations of age and menopause with cardiovascular risk factors in a large population-based study. *BMC Med* 2017;15:2. doi: 10.1186/s12916-016-0762-8
24. Vickerstaff V, Omar RZ, Ambler G. Methods to adjust for multiple comparisons in the analysis and sample size calculation of randomised controlled trials with multiple primary outcomes. *BMC Med Res Methodol* 2019;19:129. doi: 10.1186/s12874-019-0754-4
25. Brand JS, Van Der Schouw YT, Onland-Moret NC, et al. Age at menopause, reproductive life span, and type 2 diabetes risk: results from the EPIC-InterAct study. *Diabetes Care* 2013;36:1012-1019. doi: 10.2337/dc12-1020
26. LeBlanc ES, Kapphahn K, Hedlin H, et al. Reproductive history and risk of type 2 diabetes mellitus in postmenopausal women: findings from the Women's Health Initiative. *Menopause* 2017;24:64-72. doi: 10.1097/GME.0000000000000714
27. Haffner SM, Dunn JF, Katz MS. Relationship of sex hormone-binding globulin to lipid, lipoprotein, glucose, and insulin concentrations in postmenopausal women. *Metabolism* 1992;41:278-284. doi: 10.1016/0026-0495(92)90271-b
28. Sutton-Tyrrell K, Wildman RP, Matthews KA, et al. Sex hormone–binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation* 2005;111: 1242-1249. doi: 10.1161/01.CIR.0000157697.54255.CE
29. Kim C, Nan B, Kong S, Harlow S. Changes in iron measures over menopause and associations with insulin resistance. *J Womens Health* 2012;21:872-877. doi: 10.1089/jwh.2012.3549

30. Milman N, Byg KE, Ovesen L, Kirchhoff M, Jürgensen KSL. Iron status in Danish women, 1984-1994: a cohort comparison of changes in iron stores and the prevalence of iron deficiency and iron overload. *Eur J Haematol* 2003;71:51-61. doi: 10.1034/j.1600-0609.2003.00090.x
31. Wlazlo N, Van Greevenbroek MM, Ferreira I, et al. Iron metabolism is prospectively associated with insulin resistance and glucose intolerance over a 7-year follow-up period: the CODAM study. *Acta Diabetol* 2015; 52:337-348. doi: 10.1007/s00592-014-0646-3
32. Matthews KA, Chen X, Barinas-Mitchell E, et al. Age at menopause in relationship to lipid changes and subclinical carotid disease across 20 years: study of women's health across the nation. *J Am Heart Assoc* 2021;10: e021362. doi: 10.1161/JAHA.121.021362
33. Samargandy S, Matthews KA, Brooks MM, et al. Trajectories of blood pressure in midlife women: does menopause matter? *Circ Res* 2022;130: 312-322. doi: 10.1161/CIRCRESAHA.121.319424
34. Karvinen S, Jergenson MJ, Hyvärinen M, et al. Menopausal status and physical activity are independently associated with cardiovascular risk factors of healthy middle-aged women: cross-sectional and longitudinal evidence. *Front Endocrinol* 2019;10:589. doi: 10.3389/fendo.2019.00589
35. Glisic M, Mujaj B, Rueda-Ochoa OL, et al. Associations of endogenous estradiol and testosterone levels with plaque composition and risk of stroke in subjects with carotid atherosclerosis. *Circ Res* 2018;122:97-105. doi: 10.1161/CIRCRESAHA.117.311681
36. Rödström K, Bengtsson C, Lissner L, Björkelund C. Reproducibility of self-reported menopause age at the 24-year follow-up of a population study of women in Göteborg, Sweden. *Menopause* 2005;12:275-280. doi: 10.1097/01.gme.0000135247.11972.b3
37. Lucas R, Azevedo A, Barros H. Self-reported data on reproductive variables were reliable among postmenopausal women. *J Clin Epidemiol* 2008;61: 945-950. doi: 10.1016/j.jclinepi.2007.11.001
38. Freeman JR, Whitcomb BW, Purdue-Smithe AC, et al. Is alcohol consumption associated with risk of early menopause? *Am J Epidemiol* 2021;190:2612-2617. doi: 10.1093/aje/kwab182

© 2023 The Author(s)