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Effect of menopause on cardiovascular disease and its risk factors: a 9-year follow-up study

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ABSTRACT

Objectives To explore the cardiovascular risk attributable to menopausal status in a 9-year follow-up, population-based study.

Method All middle-aged women who met our eligibility criteria were selected from the Tehran Lipid and Glucose Study cohort. Data were collected by face-to-face interviews, physical examination and biochemical assessments at 3-year intervals. The World Health Organization classification was used to define menopausal status. Cardiovascular events that occurred in the cohort were investigated by a panel of medical specialists.

Results Based on menopausal status, there were no significant differences in cardiovascular disease after adjustment for age, body mass index and other confounders; however, significant relationships between serum concentrations of low density cholesterol and total cholesterol and menopausal status were observed.

Conclusions Menopause, independent of other cardiovascular disease risk factors, incurred cardiometabolic risk.

INTRODUCTION

Women of reproductive age display substantially lower cardiovascular disease (CVD) mortality than similarly aged postmenopausal women[1]; a gender difference, however, is generally attenuated after menopause[2–4]. The exact effect of menopause on increasing CVD risk is still a controversy; some studies suggest that estrogens may confer a cardiovascular protective effect in premenopausal women[4–7]. Age is considered as the main confounder for reliable interpretation of the effect of menopause on cardiometabolic disturbances. It is not clear whether the increased rate of CVD after menopause is directly due to estrogen deficiency, or indirectly because of an increase in CVD risk factors with aging[2].

There is a serious gap in this area in our existing literature. The majority of studies available that explore the association between menopause and CVD have a cross-sectional design[8] and only a limited number of cohort studies investigate the association after further adjustment by age, body mass index (BMI), physical activity and smoking habits. These studies report contradictory results which are possibly a limitation of the studies' designs. Casiglia and colleagues reported that the cardiovascular effects attributed to menopause are possibly the consequence of aging of menopausal women[9], whereas Lukaszewicz and colleagues suggested that a combination of risk factors can be found in premenopausal women, who are traditionally considered to be at low cardiovascular risk[10]. Environmental factors such as nutrition and culture have been demonstrated to be associated with the development of CVD[11,12]; this reduces the generalizability of current data to other nations with differences in cultures and lifestyles.

Considering that the influence of menopause on CVD is still a contentious question, we aim to explore the cardiovascular risk attributable in healthy middle-aged women to their menopausal status in a 9-year, follow-up, population-based study.

METHOD

Study population

Our subjects were selected from the Tehran Lipid and Glucose Study (TLGS), an ongoing prospective, population-based, cohort study that began in 1998 to explore the prevalence and

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risk factors of non-communicable diseases[13–15]. After consenting to participate, 15 005 ethnic Iranian residents aged >3 years, living in district 13 of the capital Tehran, were recruited. The comprehensive interview and physical examination were performed at the time of recruitment (T0) and three times after that (T1, T2, T3).

Figure 1 presents the study flowchart. All women, aged 35 years and over who met our eligibility criteria at the initiation of the study were included. Those with uncertain menopausal status, history of endocrine disorders, hysterectomy, oophorectomy or any other kind of ovarian surgery, users of hormone replacement therapy prior to or during the follow-up period, and those with a family history of premature CVD were excluded. We also excluded those with incomplete data. Ultimately, 675 women were subdivided into two groups according to their menopausal status as follows: group 1 was comprised of premenopausal women having regular menstrual cycles during the last 12 months, and group 2 was comprised of postmenopausal women whose menstruation had naturally ceased for at least 12 months. Those who were in menopausal transition were also excluded as there were not enough reproductive and endocrinological data to classify them appropriately. Moreover, recently postmenopausal women were defined as those who became menopausal during the most recent follow-up.

Data collection

Questionnaire

All women were visited and interviewed in person by trained investigators (four times at approximately 3-year intervals) to obtain information on demographic characteristics (age, marital status, educational and employment status), menstruation history, lifestyle (smoking habits and physical activity), medical history of CVD, stroke, diabetes and hypertension, and family history of CVD, using a structured questionnaire. Current smokers were defined as having smoked and still smoking during the last month; past smokers were those who had smoked previously but who had not smoked during the last month. Passive smoking was defined as involuntary inhalation of smoke from cigarettes smoked by other people. Physical activity was defined using the Lipid Research Clinic (LRC) questionnaire[16] and was categorized into light, moderate or strenuous physical activity levels. As part of the TLGS cohort, trained nurses contacted study participants every year and documented all the medical events experienced by the cohort member during the past year. Any event reported was followed at a home visit by a trained physician and by the collection of medical data (diagnostic or treatment) from the hospital or other service providers;

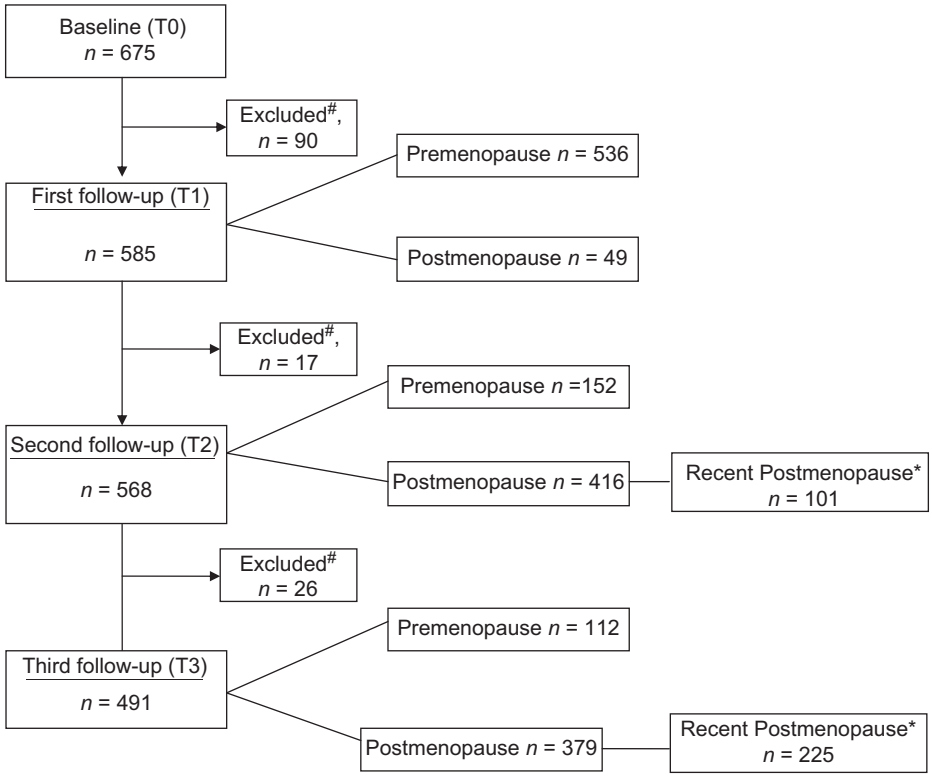

Figure 1 An overview of the study cohort. *, Recent postmenopause was defined as those women who become menopausal during the recent follow-up; #, those with incomplete data or those without eligibility criteria to be classified as one of the study subgroups (pre- or postmenopause)

follow-up of the outcomes of the TLGS has been published in detail elsewhere[17].

Physical examination

At each follow-up phase, participants underwent clinical examinations, where body weight, height, waist and hip circumferences and blood pressure were measured by trained staff. Height and weight were measured with subjects wearing light clothes and no shoes, using standard apparatus. Weight was measured to the nearest 0.1 kg on a calibrated beam scale. Height and waist circumference were measured to the nearest 0.5 cm with a measuring tape. Waist circumference was measured, with patients standing relaxed and in light clothing, at midway between the lower rib margin and the iliac crest at the end of a gentle expiration, and hip circumference was measured at the level of maximum extension of the buttocks. The waist-to-hip ratio was calculated as waist circumference divided by hip circumference. Body mass index (BMI) was calculated as weight in kilograms, divided by the height in meters squared (kg/m^2). Overweight was defined as BMI $\geq$ 25 kg/m^2 and obesity as BMI $\geq$ 30 kg/m^2. Blood pressure was measured twice at the right brachial artery after the participant had been resting for 15 min in a seated position before each measurement. Two measurements of systolic and diastolic blood pressures were taken using a standardized mercury sphygmomanometer (calibrated by the Iranian Institute of Standards and Industrial Researches); there was at least a 30-s interval between these two separate measurements, and the mean of the two measurements was considered as the participant's blood pressure.

Biochemical measures

After 12–14 h overnight fasting, venous blood samples were obtained to measure lipids and glucose. All the blood analyses were conducted at the TLGS research laboratory on the day of blood collection. Plasma glucose was measured by an enzymatic colorimetric method using a glucose oxidase kit (Pars Azmoon, Tehran, Iran); inter- and intra-assay coefficients of variation were both 2.2%. Serum concentrations of total cholesterol and triglycerides were determined using enzymatic colorimetric assays (Pars Azmoun, Iran) at all four visits. High density lipoprotein (HDL) cholesterol was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungistic acid. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula and was not done if the triglyceride concentration was $>$4.52 mmol/l. Lipid standard (C.f.a.s., Boehringer Mannheim, Germany; Cat. no. 759350) was used to calibrate the selectra 2 autoanalyzer for each day of laboratory analyses. All samples were analyzed when the internal quality control met the acceptable criteria. Inter- and intra-assay coefficients of variation were 2% and 0.5% for total cholesterol and 1.6% and 0.6% for triglycerides, respectively.

Definition of terms

Participants with fasting plasma glucose $\geq$ 126 mg/dl, or 2-h postchallenge plasma glucose $\geq$ 200 mg/dl or those who used anti-diabetic drugs were defined as diabetic[18], and prediabetes was defined as fasting blood glucose $\geq$ 100 and $<$ 126 mg/dl. Hypertension was defined as any regular or irregular usage of any blood pressure-lowering drug or systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg[19]. Based on the ATP III criteria, dyslipidemia was defined as total cholesterol $\geq$ 240 mg/dl or LDL cholesterol $\geq$ 160 mg/dl or triglycerides $\geq$ 200 mg/dl or HDL cholesterol $<$ 35 mg/dl[20]. Cardiovascular events that occurred in the cohort were identified by the panel of medical specialists, as reported in detail before[17].

Statistical analyses

Continuous measures are shown as mean $\pm$ standard deviation (SD), and categorical variables were expressed as percentages. The *T*-test, paired *t*-test, and χ^2 test were used for unadjusted comparison of demographic, physical and biochemical characteristics of postmenopausal vs. premenopausal women, while the general liner model was performed to compare age-adjusted means. Age- and BMI-adjusted and multivariate logistic/liner regression models were used to compare CVD and its risk factors after adjustment for multiple confounders. Age, BMI, physical activity and smoking and lipid-lowering therapy, blood pressure-lowering therapy, diabetes-lowering therapy and time elapsed since menopause were assumed as potential confounding factors; the results were further adjusted for the baseline characteristics of the study participants. The odds ratios (OR) and their 95% confidence intervals (CIs) were calculated for CVD and its risk factors after adjustments for potential confounders. Statistical tests were two-sided and $p < 0.05$ was considered as statistically significant. Data analyses were conducted using the Statistical Package for the Social Sciences version 11 (SPSS Inc., Chicago, IL, USA).

Ethical consideration

The ethical review board of the Research Institute for Endocrine Sciences approved the study proposal and written informed consent was obtained from all subjects during the face-to-face interviews.

RESULTS

The mean age of the women at baseline was 38.6 years (SD 4.6 years). Most of them were housewives (77%), had light physical activity (60%) and never smoked cigarettes (95%); however, one-quarter were passive smokers (Table 1).

Unadjusted comparisons of physical and biochemical characteristics according to menopausal status are presented in Table 2. At the end of the cohort study, there were 8, 15, 12, 2 and 2 events of diabetes, pre-diabetes, hypertension, stroke and CVD, respectively in premenopausal women and 35, 52, 45, 3 and 12 events, respectively in the postmenopausal group. The number of these outcomes in the postmenopausal group was higher than in the premenopausal group, but this difference was not statistically significant, due to the relatively low number of events.

Also, the means of BMI, systolic and diastolic blood pressures, waist-to-hip ratio, waist circumference, total cholesterol, triglycerides, LDL cholesterol and serum glucose, at each follow-up among the postmenopausal women were statistically higher than those women in the premenopausal group. However, serum glucose was slightly increased and HDL cholesterol levels were decreased in postmenopausal women, but were not statistically significant. Comparisons within groups at different phases of the study demonstrated that with aging the means of waist circumference, waist-to-hip ratio, diastolic blood pressure, fasting blood sugar, total cholesterol and LDL cholesterol were significantly increased (Table 2).

Also, statistical significance was presented for age-adjusted BMI, waist circumference, waist-to-hip ratio, systolic and diastolic blood pressures, and lipid profiles between the premenopause and postmenopause groups. After further adjustment for age and BMI, diastolic blood pressure, total cholesterol, LDL cholesterol and triglycerides, there were statistically significant differences according to menopausal status. However, when adjusted for age, or age and BMI, or multiple confounders, including physical activity and smoking history, lipid-lowering therapy, blood pressure-lowering therapy, diabetes-lowering therapy and time elapsed since menopause, only total cholesterol and LDL cholesterol were significantly associated with menopause (Table 3).

Table 1 Demographic characteristics of the study participants at baseline ($n = 675$). Data are given as mean $\pm$ standard deviation or *n* (%)

Age (years)	40.58 ± 4.5
Married	612 (90.6%)
High school education	245 (36.2%)
Housewife	525 (77.7%)
Smoking history	
Never	643 (95.2%)
Past	12 (1.7%)
Current	20 (2.9%)
<10 cigarettes per day	8 (40%)
≥ 10 cigarettes per day	12 (60%)
Passive smoker	174 (25.7%)
Physical activity	
Light	407 (60.3%)
Moderate	195 (28.8%)
Strenuous	73 (10.8%)

DISCUSSION

The aim of this study was to investigate the effect of menopausal status on CVD and its risk factors in healthy middle-aged women. Despite there being no differences in the prevalence of cardiovascular disorders and diabetes between pre- and postmenopausal women, serum total cholesterol and LDL cholesterol levels had significant associations with menopausal status in Iranian women, even after adjustment for age, BMI, physical activity, smoking history and treatment including lipid-lowering therapy, blood pressure- and diabetes-lowering therapy. Furthermore, higher age-adjusted levels of BMI, waist-to-hip ratio, systolic and diastolic blood pressures, fasting blood sugar, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol were found in the postmenopausal group; the higher levels of diastolic blood pressure and total cholesterol, LDL cholesterol and triglycerides remained after further adjustment for BMI. It seems that menopause exacerbates CVD risk factors independent of aging and obesity[21,22].

Menopause is associated with significant declines in estrogen levels that could be linked with the increases of triglyceride and LDL cholesterol levels[4]; several mechanisms have been suggested for this linkage. Estrogen could inhibit oxygen free radical-mediated LDL oxidation[5]; moreover, estrogen induces an early elevation of LDL receptors that are responsible for the uptake of plasma lipoproteins, and decreases 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) activity[23]. It has been shown that oral hormone replacement therapy in postmenopausal women could enhance the lipid profile and, as a result, may protect them against atherosclerosis[6,24].

Previous researchers have suggested that dyslipidemia is the most important risk factor for developing CVD[25–27]. Chen and colleagues[28] showed that an increasing level of serum cholesterol was independently associated with excess risk of death from coronary heart disease (CHD), adjusted for age, sex, diastolic blood pressure, smoking and alcohol consumption. In agreement with our results, it has been shown that serum total cholesterol levels and serum LDL cholesterol levels increase after menopause[4,29]. Agrinier and colleagues[2] studied the effect of the menopause on various CHD risk factors on the global risk of CHD in a population-based sample of women, differentiating between menopause and age-related effects, and found that postmenopausal women had significantly higher levels of total cholesterol and LDL cholesterol, independently of age, BMI, and co-morbidities. In contrast to our study, natural menopause resulted in no differences in HDL cholesterol, triglycerides, fasting blood or blood pressure in a prospective study conducted by Poehlman and colleagues[30]. In our study, total cholesterol levels were increased following menopause even after adjusting for age and BMI, whereas decreases in the HDL cholesterol level disappeared after further adjustment for other confounders.

Despite the raised serum concentrations of total cholesterol and LDL cholesterol after menopause in the present

Table 2 Characteristics of cardiovascular disease and its risk factors in participants after 9 years follow-up. Data are given as mean ± standard deviation or *n* (%). Diabetes was defined as fasting plasma glucose ≥ 126 mg/dl, or 2-h post-challenge plasma glucose ≥ 200 mg/dl or those who used anti-diabetic drugs; pre-diabetes was defined as fasting blood glucose ≥ 100 and < 126 mg/dl; hypertension was defined as regular or irregular usage of any blood pressure-lowering drug or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg; dyslipidemia was defined as total cholesterol ≥ 240 mg/dl or low density lipoprotein cholesterol ≥ 160 mg/dl or triglycerides ≥ 200 mg/dl or high density lipoprotein cholesterol < 35 mg/dl; waist-to-hip ratio was calculated as waist circumference divided by hip circumference; overweight was defined as body mass index ≥ 25 kg/m^2; obesity as body mass index ≥ 30 kg/m^2

		Premenopause			*Postmenopause*				
	T0 (*n* = 675)	*T1* (*n* = 536)	*T2* (*n* = 416)	*T3* (*n* = 112)	*T1* (*n* = 49)	*T2* (*n* = 152)	*Recent in T2** (*n* = 101)	*T3* (*n* = 379)	*Recent in T3** (*n* = 225)
Body mass index (kg/m^2)	27.19 ± 4.5	28.30 ± 4.1[a]	28.53 ± 4.2[a]	29.21 ± 4.6[a]	29.62 ± 4.4[b]	29.98 ± 4.2[bd]	29.80 ± 4.04[bd]	30.28 ± 4.8[be]	30.12 ± 3.2[b]
Waist circumference (cm)	85.23 ± 10.8	88.26 ± 10.8[a]	89 ± 10.4[a]	92.97 ± 9.9[agh]	92.76 ± 11.1[b]	97.86 ± 9.5[bdi]	93.77 ± 12.6[bd]	97.73 ± 11.01[bej]	95.35 ± 10.2[be]
Waist-to-hip ratio	0.81 ± 0.06	0.84 ± 0.07[a]	0.85 ± 0.06[a]	0.90 ± 0.06[ag]	0.87 ± 0.07[bc]	0.89 ± 0.05[bd]	0.88 ± 0.03[bd]	0.94 ± 0.06[bej]	0.92 ± 0.04[be]
Systolic blood pressure (mmHg)	110.23 ± 11.7	110.67 ± 11.04	111.97 ± 17.06[a]	112.73 ± 14[a]	119.26 ± 15.01[bc]	120.59 ± 14.5[bd]	119.60 ± 13.08[bd]	123.80 ± 17.3[be]	120.13 ± 14.3[be]
Diastolic blood pressure (mmHg)	71.88 ± 8.6	72.77 ± 8.9	74.63 ± 7.6[af]	75.34 ± 9.08[ag]	75.25 ± 9.7[b]	77.70 ± 8.9[bd]	75.23 ± 7.4[bd]	79.01 ± 10.2[bej]	77.13 ± 8.9[be]
Fasting glycemia (mg/dl)	86.48 ± 10.7	88.02 ± 9.6[a]	91.89 ± 10.3[af]	93.73 ± 19.7[ag]	90.84 ± 18.8[b]	91.88 ± 11.4[b]	90.23 ± 52.3[b]	96.22 ± 19.7[bj]	93.22 ± 82.1[b]
Serum lipids (mg/dl)									
total cholesterol	181.07 ± 27.2	181.04 ± 28.6	186.76 ± 25.5[af]	191.13 ± 26.01[ag]	195.98 ± 31.7[bc]	198.75 ± 28.9[bd]	194.90 ± 13.03[bd]	197.05 ± 30.8[bj]	195.14 ± 32.1[b]
LDL cholesterol	114.63 ± 24.5	115.20 ± 24.7	117.83 ± 23.1[a]	120.79 ± 27.06[ag]	123.65 ± 29.6[b]	124.83 ± 26.5[bd]	123.15 ± 33.02[b]	128.05 ± 24.05[be]	125.65 ± 22.01[be]
HDL cholesterol	47.09 ± 9.1	45.48 ± 9.5[a]	43.81 ± 10.4[a]	42.03 ± 10.8[ag]	42.28 ± 9.8[bc]	42.12 ± 10.3[b]	43.35 ± 9.01[b]	41.70 ± 10.9[b]	42.01 ± 90.3[b]
triglycerides	115.14 ± 38.1	119.11 ± 57.3	122.34 ± 53.8[a]	125.89 ± 47.4[a]	131.71 ± 50.1[b]	136.01 ± 83.3[bd]	134.15 ± 12.6[bd]	137.18 ± 59.7[be]	135.03 ± 9.5[be]
Blood pressure-lowering therapy	0	28 (5.2)	25 (6)	18 (16.07)	4 (8.1)	16 (10.5)	12 (11.8)	83 (22.1)	43 (19.1)
Lipid-lowering therapy	0	12 (2.2)	24 (5.7)	8 (8.9)	4 (8.1)	16 (10.5)	8 (7.9)	49 (12.9)	19 (8.4)
Sugar-lowering therapy	0	2 (0.3)	3 (0.7)	4 (3.5)	2 (4.08)	7 (4.6)	5 (4.9)	27 (7.1)	12 (5.3)
Diabetes	0	24 (4.4)	25 (6)	8 (7.1)	4 (8.1)	14 (9.2)	10 (9.9)	35 (9.2)	21 (9.3)
Pre-diabetes	0	43 (8.02)	44 (10.5)	15 (13.3)	5 (10.2)	17 (11.1)	10 (9.9)	52 (13.7)	25 (11.1)
Hypertension	0	43 (8.02)	38 (9.1)	12 (10.7)	6 (10.8)	20 (13.1)	10 (9.9)	45 (11.8)	23 (10.2)
Diabetes and hypertension	0	0	2 (0.4)	1 (0.8)	0	2 (1.3)	1 (0.9)	8 (2.1)	3 (1.3)
Dyslipidemia	0	82 (15.2)	70 (16.8)	22 (19.6)	9 (18.3)	28 (18.4)	18 (17.8)	80 (21.1)	44 (19.5)
Coronary heart disease	0	0	3 (0.7)	5 (4.4)	0	5 (3.2)	1 (0.9)	6 (1.5)	2 (0.8)
Stroke	0	0	1 (0.2)	2 (1.7)	0	2 (1.3)	0	3 (0.7)	1 (0.4)
Cardiovascular disease	0	3 (0.5)	6 (1.4)	2 (1.7)	1 (2.04)	4 (2.6)	1 (0.9)	12 (3.1)	4 (1.7)

T0, time of recruitment; T1, 1st follow-up; T2, 2nd follow-up; T3, 3rd follow-up; LDL, low density lipoprotein; HDL, high density lipoprotein

*, Women with recent postmenopause were those who become menopausal in this phase; [a], unadjusted $p < 0.05$ for T0 vs. same women in premenopausal and postmenopausal T1; [b], unadjusted $p < 0.05$ for T0 vs. same women in premenopausal and postmenopausal T2; [c], unadjusted $p < 0.05$ for premenopausal T1 vs. postmenopausal T1; [d], unadjusted $p < 0.05$ for premenopausal T2 vs. postmenopausal T2; [e], unadjusted $p < 0.05$ for premenopausal T3 vs. postmenopausal T3; [f], unadjusted $p < 0.05$ for premenopausal T1 vs. premenopausal T2; [g], unadjusted $p < 0.05$ for premenopausal T1 vs. premenopausal T3; [h], unadjusted $p < 0.05$ for premenopausal T2 vs. premenopausal T3; [i], unadjusted $p < 0.05$ for postmenopausal T1 vs. postmenopausal T2; [i], unadjusted $p < 0.05$ for postmenopausal T1 vs. postmenopausal T3; [k], unadjusted $p < 0.05$ for postmenopausal T2 vs. postmenopausal T3

Table 3 Comparisons of age-adjusted, age- and body mass index (BMI)-adjusted and multiple adjusted *p* values for cardiovascular disease and its risk factors. Multiple-adjustments were made for physical activity and smoking history, lipid-lowering therapy, blood pressure-lowering therapy, diabetes-lowering therapy, and time elapsed since menopause

	Age-adjusted p value							*Age- and BMI-adjusted p value*							*Multiple adjusted p value*						
Risk factors for cardiovascular disease	*Premenopause*			*Postmenopause*			*Overall comparison OR (95% CI)*	*Premenopause*			*Postmenopause*			*Overall comparison OR (95% CI)*	*Premenopause*			*Postmenopause*			*Overall comparison OR (95% CI)*
	T1	*T2*	*T3*	*T1*	*T2*	*T3*		*T1*	*T2*	*T3*	*T1*	*T2*	*T3*		*T1*	*T2*	*T3*	*T1*	*T2*	*T3*	
Body mass index	ns	ns	ns	ns	bd	be	_	_	_	_	_	_	_	_	_	_	_	_	_	_	
Waist circumference	ns	ns	ns	ns	bd	be	_	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	0.14 (0.05–1.08)
Waist-to-hip ratio	ns	ns	ns	ns	bd	be	_	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	0.93 (0.50–1.68)
Systolic blood pressure	ns	ns	ns	ns	ns	be	_	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	0.24 (0.01–1.11)
Diastolic blood pressure	ns	ns	ns	ns	ns	be	_	ns	ns	ns	ns	ns	b	_	ns	ns	ns	ns	ns	ns	1.02 (0.15–1.63)
Fasting glycemia	ns	ns	ns	ns	b	be	_	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	1.80 (0.29–2.15)
Serum lipids																					
total cholesterol	ns	ns	a	bc	bd	beg	_	ns	ns	ns	ns	ns	be	_	ns	ns	ns	ns	ns	e	3.61 (2.77–5.02)
LDL cholesterol	ns	ns	a	ns	bd	be	_	ns	ns	ns	ns	ns	be	_	ns	ns	ns	ns	b	be	1.37 (1.15–1.86)
HDL cholesterol	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	_	ns	ns	ns	ns	ns	ns	0.05 (0.05–1.24)
triglycerides	ns	ns	a	ns	bd	be	_	ns	ns	ns	ns	b	be	_	ns	ns	ns	ns	ns	ns	0.02 (0.01–1.43)
Diabetes	ns	ns	ns	ns	ns	ns	1.47 (0.82–2.36)	ns	ns	ns	ns	ns	ns	1.14 (0.80–2.08)	ns	ns	ns	ns	ns	ns	0.99 (0.75–1.94
Pre-diabetes	ns	ns	ns	ns	ns	ns	1.04 (0.04–2.74)	ns	ns	ns	ns	ns	ns	0.52 (0.04–2.44)	ns	ns	ns	ns	ns	ns	0.23 (0.01–1.98)
Hypertension	ns	ns	ns	ns	ns	ns	1.23 (0.94–2.37)	ns	ns	ns	ns	ns	ns	1.17 (0.80–1.96)	ns	ns	ns	ns	ns	ns	1.12 (0.76–1.81)
Diabetes and hypertension	ns	ns	ns	ns	ns	ns	1.70 (0.81–2.81)	ns	ns	ns	ns	ns	ns	1.64 (0.72–2.52)	ns	ns	ns	ns	ns	ns	1.54 (0.65–2.03)
Coronary heart disease	ns	ns	ns	ns	ns	ns	1.12 (0.68–1.77)	ns	ns	ns	ns	ns	ns	1.09 (0.65–1.69)	ns	ns	ns	ns	ns	ns	1.01 (0.61–1.63)
Stroke	ns	ns	ns	ns	ns	ns	1.80 (0.57–5.08)	ns	ns	ns	ns	ns	ns	1.76 (0.49–4.63)	ns	ns	ns	ns	ns	ns	1.47 (0.48–4.25)
Cardiovascular disease	ns	ns	ns	ns	ns	ns	1.32 (0.94–2.80)	ns	ns	ns	ns	ns	ns	1.25 (0.83–1.68)	ns	ns	ns	ns	ns	ns	1.09 (0.71–1.53)

T0, time of recruitment; T1, 1st follow-up; T2, 2nd follow-up; T3, 3rd follow-up; LDL, low density lipoprotein; HDL, high density lipoprotein; OR, odds ratio; CI, confidence interval; ns, non significant

a, unadjusted $p < 0.05$ for T0 vs. same women in premenopausal and postmenopausal T1; b, unadjusted $p < 0.05$ for T0 vs. same women in premenopausal and postmenopausal T2; c, unadjusted $p < 0.05$ for premenopausal T1 vs. postmenopausal T1; d, unadjusted $p < 0.05$ for premenopausal T2 vs. postmenopausal T2; e, unadjusted $p < 0.05$ for premenopausal T3 vs. postmenopausal T3; f, unadjusted $p < 0.05$ for premenopausal T1 vs. premenopausal T2; g, unadjusted $p < 0.05$ for premenopausal T1 vs. premenopausal T3; h, unadjusted $p < 0.05$ for premenopausal T2 vs. premenopausal T3; i, unadjusted $p < 0.05$ for postmenopausal T1 vs. postmenopausal T2; j, unadjusted $p < 0.05$ for postmenopausal T1 vs. postmenopausal T3; k, unadjusted $p < 0.05$ for postmenopausal T2 vs. postmenopausal T3

study, the number of cardiovascular events was not increased. The most common interpretation of these findings is that CVD is one of the multi-factorial complications and menopause is one of the most important steps in a chain of causations leading to CVD due to changes in lipid profile mechanisms. Furthermore, our cohort population included healthy middle-aged women without any risk factors and the time elapsed from menopause was short, as a result of which the number of cardiovascular events in our study was limited. In our study, BMI, waist circumference and waist-to-hip ration were increased by aging regardless of menopausal status; this trend of obesity by aging could possibly dilute the impact of menopausal status *per se* on CVD. Menopause is associated with a significant decline in estrogen levels that could have adverse effect on anthropometric parameters; it has been shown that changes in body composition and fat distribution at menopause may be caused by decreases in circulating estrogen. For fat distribution shifts, the relative increase in the androgen–estrogen ratio may be important[31,32]; it may induce a more android distribution of body fat, and thus contribute to the increase in cardiovascular disease after the menopause[33]. Poehlman and colleagues stated that the natural menopause transition might be mitigated by deleterious changes in obesity and body fat distribution and it could possibly lead to further enhancement of CVD[30].

However, in agreement with our results, Sternfeld and colleagues[34] demonstrated that weight gain is due to aging rather than to menopause itself; it seems that aging, *per se*, is associated with obesity and fat body mass. Decreases in physical activity level in the domains of both sports and exercise among older women is related to aging obesity[3]. Also, aging exacerbates obesity-induced oxidative stress and inflammation and significantly increases macrophage infiltration in periaortic adipose tissue[35].

The association between hypertension, diabetes and dyslipidemia is well established[36-38]; furthermore, abnormal serum lipid or blood sugar levels are believed to be independent risk factors for essential hypertension and the initiation therefore could increase the individual's susceptibility to coronary heart disease[39,40]. Some studies suggested that dyslipidemia could be the fundamental step for CVD[41] and, as a result, could be the only parameter observed in cohort studies without a long enough follow-up study.

Our study has the advantage of having a population-based cohort of women with a 9-year follow-up, enabling us to assess risk factors prospectively. Furthermore, our design enables us to assess the impact of menopausal status on CVD risk factors after adjustment for those attributed to aging. Diabetes, prediabetes and dyslipidemia were identified precisely, as the intra- and inter-assay variabilities were minimal; all biochemical assays were performed in the same laboratory. Cardiovascular events occurring in the cohort were investigated by a panel of medical specialists using clear definitions.

Our study has limitations as well. The menopausal age was self-reported; however, we confirmed the reliability of the data on menopausal age by asking the women the same questions 3 years later; therefore the effect of recall error is minimal. In order to reduce misclassification bias, we included only those women with regular menstrual cycles at the initiation of the study. Women who were on hormone replacement therapy or those affected by hysterectomy or oophorectomies were not enrolled in our study, as their metabolic complications could differ from those of naturally menopausal women[42]. We also excluded those women who were in the menopausal transition as there were not enough reproductive and endocrinological data to classify them appropriately; perimenopause should include the period immediately prior to menopause (when the endocrinological, biological, and clinical features of approaching menopause commence) and the first year after menopause[43].

Our study was not long enough to have an adequate number of CVD events .However, the ongoing characteristics of our study enable us to further evaluate our study participants. We did not measure other proxy variables such as intima thickness in our study.

CONCLUSION

In summary, this population-based cohort study did not find any statistically significant association between menopause and CVD in an Iranian population during a 9-year follow-up. However, in an adjusted comparison with premenopausal women, postmenopausal women had higher levels of LDL cholesterol and total cholesterol, two of the fundamental proxy variables for the CVD cascade. Further longitudinal studies of sufficient duration and including hormonal profiles are recommended.

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