Natural Postmenopause Is Associated With an Increase in Combined Cardiovascular Risk Factors

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Summary

Cardiovascular disease, while rare in women of reproductive age, is the main cause of mortality in menopause. The purpose of our study was to determine the association of natural menopause with cardiovascular risk factors, including their clustering into metabolic syndrome (MS). A random 5 % representative population sample of women aged 45-54 years was examined. In 575 women, we were able to determine their natural reproductive aging status. Multiple regression analysis was used to calculate the association between age, menopausal status, and risk factors under study. After adjustment for age, there was an increase in the odds ratio of developing MS, as defined by NCEP (OR=2.0; 95 % CI [1.1; 3.7]), and an increase in plasma lipid ratios (total cholesterol/HDL-C, LDL-C/HDL-C, apolipoprotein-B/ apolipoprotein-A1; $p<0.05$ for all) in postmenopausal women. Age, but not menopausal status, was associated with some single components of MS; only waist circumference significantly increased after menopause, independently of age. Clustering of risk factors in MS and lipid ratios (combined factors) was strongly associated with menopause whereas worsening of single components of MS was strongly associated with age. In conclusion, based on our results, the menopause may pose a risk to women through clustering of cardiovascular risk factors beyond simple aging.

Key words

Menopause • Metabolic syndrome • Dyslipidemia • Cardiovascular disease • Central obesity

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Introduction

Compared with age-matched men, women of reproductive age have a low rate of cardiovascular events and low levels of most cardiovascular risk factors. Despite this, the total rate of death from cardiovascular disease as well as from an unhealthy lifestyle, along with the long life expectancy in developed countries, is higher in women than in men (Roger *et al.* 2011). It is a well-known fact that both artificially induced and natural menopause associated with reduced ovarian function result in an increase in cholesterol levels. There has been emerging evidence showing an increase in obesity and in the prevalence of the metabolic syndrome (MS) (Teede *et al.* 2010), which may affect only the part of the population at higher risk (Lejsková *et al.* 2011) and metabolic effects of menopause may not be unique (Carr 2003, Feng *et al.* 2008), with single MS components not necessarily being identical in the period of their postmenopausal rise (Cho *et al.* 2008). Another problem is separating the effect of menopause from that of aging (Mesch *et al.* 2006). The results of studies assessing menopausal changes of the single components of MS are inconsistent: studies of peri- and postmenopausal women

of different ethnics may have a different prevalence of MS and a different ratio between its main components (Feng *et al.* 2008, Inhavivadhana *et al.* 2011).

Prevention of cardiovascular disease is important in middle-aged individuals, even in those at low 10-year cardiovascular risk, yet high lifelong risk (Berry *et al.* 2009). Screening of women entering menopausal transition with slightly increased risk of cardiovascular disease might be helpful in providing early and effective intervention while reducing the lifelong cardiovascular risk in this particular population.

In our previous study (Lejsková *et al.* 2011) we found that the acceleration of MS incidence at the onset of menopause may be accompanied by an increase in insulin resistance only in the part of population at highest risk. The aim of our recent epidemiological study was to assess the effect of menopause on the metabolic risk factors of atherosclerosis, particularly to evaluate clustering of risk factors for cardiovascular disease (RFCVD) in MS and changes in lipid ratios in relation to age and menopausal status.

Methods

Participants

The study was conducted using a random 5 % representative population sample of women aged 45 to 54 years (10-year age range). The median age range was consistent with the mean age of women in perimenopause (McKinlay *et al.* 2008). The age range was selected to include large enough and approximately identical proportions of those still in premenopause and those already postmenopausal, in addition to women in perimenopause lasting less than 4 years. The limitation of the age range to 10 years also made it possible to limit the difference in the age means between fertile and postmenopausal women to an extent necessary to achieve the study goals, that is, to compare some characteristics of women close to menopausal transition with those close after menopause.

The women were selected from the General Health Company registry which keeps a database of all insured individuals. In the Czech Republic, all citizens are required by law to be insured; therefore, the database of insured individuals is a continuously updated population registry. Randomly selected individuals were contacted by mail, and invited to visit a specialist clinic within the same district. A total of 909 women (response rate, 64 %) attended the examination. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all participants.

Data sources

A physician-completed questionnaire including each participant's medical history, treatment of hypertension, hyperlipidemia, and diabetes was obtained including the date of therapy initiation. To rule out any potential bias, the physician carefully entered details regarding all prescribed treatments, a thorough gynecological history, and the interval since the last menstrual bleeding.

Body weight, height, and waist circumference were measured with an accuracy of 0.1 kg and 0.5 cm, respectively, according to the World Health Organization MONICA study protocol (Meisinger *et al.* 2002). The waist-to-hip ratio and body mass index (BMI; kg/m^2) were calculated. Systolic and diastolic blood pressures (SBP, DBP) were measured in the sitting position on the right arm after at least 20 minutes' rest in an outpatient clinic. The mean of the second and third measurements of three consecutive readings was used for analyses.

Blood samples were taken after an overnight fast. Serum total cholesterol and triglyceride (TG) levels were measured using a fully automated (HITACHI 911 autoanalyzer, Tokyo, Japan) enzymatic method (reagents from Boehringer Mannheim, Germany, and Hoffmann-La Roche, Basel, Switzerland). High-density lipoprotein cholesterol (HDL-C) was determined by the same method after precipitation of serum lipoproteins with sodium phosphotungstate and magnesium chloride kits. Serum low-density lipoprotein cholesterol (LDL-C) was measured by an automated method with direct determination using an LDL-C kit from Hoffmann-LaRoche (Basel, Switzerland). All methods of lipoprotein analysis in the laboratory are under permanent control by the Center for Disease Control (CDC), Atlanta, GA, USA. Follicle-stimulating hormone (FSH) was measured using IRMA kits (Immunotech, Prague, Czech Republic). Additional details regarding the methods used in the study are available in the paper by Cífková *et al.* (2008).

Study design

For the purpose of our study, only women with natural reproductive aging were selected from among all those examined. Excluded were women with surgical menopause (including those with unilateral ovariectomy), women with hormone replacement therapy, and women with ambiguous data. All women had their levels of

cardiovascular risk, both "single risk factors" and "combined risk factors", determined. The latter included more complicated factors: three lipid ratios and MS as determined using 6 variants of two definitions.

At the end of the study, multiple regression analysis was used to determine the effects of age and menopausal status on each of the cardiovascular risk factors studied.

Definitions: menopausal status, combined risk factors

Of the 909 women examined in the study, there were 575 women with unambiguous data about natural reproductive aging. Preliminary characteristics were used to divide these women into three subgroups using the Stages Reproductive Aging Workshop (STRAW) (Soules *et al.* 2001) criteria by the time since their last menstrual period (LMP): a subgroup of women still in premenopause ("Premenopause" – 351 women: less than 33 postmenstrual days), a menopausal transition subgroup ("Perimenopause" – 95 women: 33-365 postmenstrual days), and a postmenopausal one ("Postmenopause" – 129 women: more than 365 postmenstrual days).

Traditional cardiovascular risk factors were established in all groups. Fasting glucose and insulin were used to calculate the insulin resistance index [HOMA-IR, HOmeostasis Model Assessment of Insulin Resistance = (fasting plasma glucose; mmol/l) × (fasting plasma insulin; mU/l)/22.5)] and the insulin sensitivity index [QUICKI, QUantitative Insulin sensitivity ChecK Index = 1/(log(fasting plasma insulin, mU/l) + log(fasting plasma glucose, mmol/l) + log18.01] (Katz *et al.* 2000).

Lipid ratios were calculated by dividing the plasma levels of proatherogenic and antiatherogenic lipoproteins. The total cholesterol/HDL-C and the LDL-C/HDL-C ratios are the two longest-used atherogenic lipid ratios shown to be superior to total or LDL-C levels in predicting cardiovascular events (Kinosian *et al.* 1994). In addition, recent studies have suggested that the apolipoprotein B/apolipoprotein A1 ratio is comparable or superior to the above ratios in predicting the development of coronary heart disease (Ingelsson *et al.* 2007).

The study used two definitions of MS: first, the National Cholesterol Education Program Adult Treatment Panel III (NCEP) definition created in 2001 (Grundy *et al.* 2001) and, second, the definition published in 2009 (Alberti *et al.* 2009) and developed by several major organizations in order to unify criteria (Harmonizing the

MS). According to both definitions, MS was diagnosed using the original one and variants proposed in the literature. The variants using the NCEP definition were as follows: first, one calculated without pharmacotherapy (NCEP-2001), second, with treatment of diabetes and hypertension (G + BP therapy), and a third one with pharmacotherapy of any of the components (G + BP + LIP therapy). When using the recent definition, Harmonizing the MS, the variants were as follows: first based on the original definition, second with the addition of an increased limit for waist circumference (waist ≥88cm), and a third one with the addition of an increased limit for glycemia (glycemia ≥6.1 mmol/l).

Statistical methods

Distributions of all continuous variables were assessed prior to performing statistical analyses. In two cases (variables HOMA-IR and triglycerides), the corresponding distributions were determined as log-normal rather than normal. Therefore, in the two respective cases, the log-transformed rather than original values were entered into the linear regression models which assume a normal error structure. Results of the multiple regressions and multiple logistic regression modeling, including analysis of variance of the modeling terms, are presented in figures with data tables. Age and indicators of menopausal status were entered into all the models as explanatory variables. The effect of menopause on each investigated variable (e.g., total cholesterol, non-HDL cholesterol, etc.) was assessed using tests with the premenopausal period serving as baseline. We studied the effects of age, perimenopause, and postmenopause separately. Results of MS, its parameters, and related factors are presented in Figure 1 while Figure 2 presents results related to the cholesterol lipid spectrum and related ratios. Summaries of effect sizes for binary variables are presented as odds ratios with 95 % confidence intervals.

Results

Characteristics

Among the 575 women with unambiguous natural reproductive aging examined in our study, only 95 women were classified as perimenopausal (33-365 postmenstrual days; Table 1). The majority, 351 women, were no longer than 33 days since their last menstruation ("Premenopause"). The remaining 129 women were more than 365 days since their last menstruation

Table 1. Main characteristics of women according to menopausal status (mean ± S.D. or %).

	Premenopause n = 351	Perimenopause n = 95	Postmenopause n = 129
Last menstrual period/subgroup interval (days)	[0;33)	[33;366)	[366;inf)
a) Continuous variables			
Age (years)	48.6 ± 2.4	50.4 ± 2.3	52.2 ± 2.0*
Age at last menstrual period (years)	48.6 ± 2.4	50.1 ± 2.3	47.9 ± 3.6
Time since last menstrual period (days)	13 ± 8	119 ± 84	1574 ± 1129*
Follicle stimulated hormone (IU/l)	18.5 ± 20.6	55.0 ± 43.4	79.3 ± 34.1*
Total cholesterol (mmol/l)	5.44 ± 0.91	5.57 ± 0.90	5.88 ± 0.80*
Non-HDL cholesterol (mmol/l)	3.82 ± 0.95	3.88 ± 0.92	4.31 ± 0.93*
LDL cholesterol (mmol/l)	3.30 ± 0.82	3.39 ± 0.83	3.68 ± 0.82*
HDL cholesterol (mmol/l)	1.63 ± 0.38	1.69 ± 0.39	1.57 ± 0.37
Triglycerides (mmol/l)	1.31 ± 0.75	1.20 ± 0.49	1.57 ± 0.98*
Apolipoprotein B (g/l)	1.02 ± 0.25	0.99 ± 0.26	1.12 ± 0.29*
Apolipoprotein A1 (g/l)	1.65 ± 0.30	1.64 ± 0.25	1.61 ± 0.26
Apolipoprotein B/Apolipoprotein A1	0.63 ± 0.18	0.62 ± 0.20	0.72 ± 0.21*
LDL cholesterol/HDL cholesterol	2.18 ± 0.86	2.16 ± 0.89	2.52 ± 0.93*
Total cholesterol/HDL cholesterol	3.53 ± 1.02	3.47 ± 1.03	3.98 ± 1.18*
Waist circumference (cm)	85.1 ± 11.6	88.8 ± 14.9	91.4 ± 14.1*
Waist-to-hip ratio	0.83 ± 0.07	0.85 ± 0.07	0.86 ± 0.08*
Body mass index (kg/m^{-2})	25.7 ± 4.4	26.5 ± 6.1	27.5 ± 6.0*
Hip circumference (cm)	102.1 ± 9.2	104.1 ± 11.9	105.9 ± 10.9*
SBP (mm Hg)	119 ± 16	120 ± 16	121 ± 17
DBP (mm Hg)	79 ± 10	80 ± 11	80 ± 10
Fasting plasma glucose (mmol/l)	5.2 ± 0.9	5.2 ± 0.7	5.4 ± 1.6
Fasting plasma insulin (IU/ml)	6.6 ± 4.0	7.1 ± 4.2	6.6 ± 3.7
HOMA-IR	1.54 ± 1.03	1.69 ± 1.09	1.72 ± 1.85
QUICKI	0.37 ± 0.04	0.37 ± 0.03	0.37 ± 0.04
b) Categorical variables			
MS NCEP-2001 (without therapy)	11.40 %	11.58 %	24.81 %*
MS NCEP-2001 (G + BP therapy)	13.11 %	13.68 %	26.36 %*
MS NCEP-2001 (G + BP + LIP therapy)	13.68 %	13.68 %	27.91 %*
MS NCEP-2001 (G + BP + LIP therapy), non-smokers	11.11 %	13.04 %	19.61 %*
Harmonizing MS (fasting glucose cut point ≥6.1 mmol/l)	18.23 %	18.95 %	31.78 %*
Harmonizing MS (waist circumference cut point ≥88 cm)	16.81 %	23.16 %	33.33 %*
Harmonizing MS 2009	23.08 %	29.47 %	37.21 %*
Harmonizing MS 2009, non-smokers	21.21 %	23.91 %	33.33 %*
Waist circumference ≥88 cm	35.61 %	43.16 %	51.16 %*
Waist circumference ≥80 cm	63.53 %	71.58 %	79.84 %*
Triglycerides ≥1.7 mmol/l	20.51 %	20.00 %	26.36 %
HDL cholesterol <1.3 mmol/l	19.43 %	12.63 %	27.13 %
Fasting glucose ≥6.1 mmol/l or a history of diabetes	4.30 %	3.16 %	10.08 %*
Fasting glucose ≥5.6 mmol/l or a history of diabetes	19.20 %	25.26 %	31.78 %*

	Premenopause n = 351	Perimenopause n = 95	Postmenopause n = 129
Last menstrual period/subgroup interval (days)	[0;33)	[33;366)	[366;inf)
SBP ≥130 mm Hg	22.22 %	25.26 %	31.78 %*
DBP ≥85 mm Hg	24.50 %	27.37 %	28.68 %
SBP ≥130mm Hg or DBP ≥85mm Hg	31.05 %	32.63 %	36.43 %
SBP ≥130mm Hg or DBP ≥85mm Hg or BP-lowering ther.	37.04 %	41.05 %	43.41 %
Blood pressure-lowering therapy	12.82 %	12.63 %	18.60 %
Cholesterol-lowering therapy (statins)	3.42 %	9.47 %	7.81 %
Triglyceride-lowering therapy (fibrates)	0.28 %	1.05 %	3.88 %*
Smoking	43.6 %	51.6 %	60.2 %*

* $p < 0.01$ for the difference between the Premenopause and Postmenopause groups. SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure, HOMA-IR, HOmeostasis Model Assessment of Insulin Resistance = (fasting plasma glucose; mmol/l) × (fasting plasma insulin; mU/l)/22.5), QUICKI, QUantitative Insulin sensitivity ChecK Index = 1/(log(fasting plasma insulin, mU/l) + log(fasting plasma glucose, mmol/l) + log18.01 (Katz *et al.* 2000).

("Postmenopause"). Women in natural postmenopause had high FSH levels consistent with their postmenopausal status: FSH >30 IU/l (>40 IU/l) was seen in 93.3 % (90.0 %) of women in this subgroup, and the interval since the final menstruation of the eight women with FSH <30 IU/l was 3.2±1.8 years, consistent with the postmenstrual interval of all women in natural postmenopause (4.3±3.1 years; median 3.5 years).

Postmenopausal women were older and showed higher levels of atherogenic lipid parameters, BMI, and both markers of abdominal obesity while not differing in age at their last menstrual period. The prevalence of MS using the NCEP definition variants for all study populations together was much lower (14.4 % without pharmacotherapy; 16.8 % with pharmacotherapy) compared with the Harmonizing MS definition variants (21.6 % with waist ≥88 cm; 27.3 % original definition with waist ≥80 cm).

Main results

When studying the age-adjusted effect of peri- and postmenopause separately (with premenopause representing the baseline), the odds ratio of MS NCEP (Grundy *et al.* 2001) increased significantly only in postmenopause with an OR ~ 2, p<0.05 for all variants (Fig. 1). The effect of age was consistently non-significant. By contrast, when using any of the variants of the more recent definition Harmonizing the MS (Alberti *et al.* 2009) with lower cut points for waist circumference and/or glycemia, the age-adjusted effect of postmenopause did not reach the level of statistical significance. Nevertheless, the

age-adjusted OR in the range of 1.269 to 1.598 with quite wide 95 % CI suggest a trend similar to MS-NCEP (although weaker), and the association could be possibly significant with a larger sample size.

Among all the 12 single parameters related to MS, the age-adjusted effect of postmenopausal status was evident only on the waist circumference. The age-adjusted increase in waist circumference reached an average of 2.6 cm (p=0.091) in perimenopause and 4.0 cm in postmenopause (p=0.010). With the other eleven MS-related parameters, the age-adjusted effect of postmenopause did not reach the level of statistical significance. At the same time, the effect of age was significant in only seven parameters (BMI, log-triglycerides, apolipoprotein B, glycemia, systolic blood pressure, and both types of conditions defining hypertension in MS).

A significant effect of age and postmenopause was observed with atherogenic lipid cholesterol fractions (Fig. 2). After adjusting for the effect of age and comparing peri- and postmenopause against premenopause separately, the lipid ratios increased significantly only in postmenopause (LDL-C/HDL-C, p=0.029; total CHOL/HDL-C, p=0.012; Apo-B/Apo-A1, p=0.013). Thus, the lipid ratios were shown to reach levels of statistical significance similar to those observed for the incidence of MS using the NCEP definition variants. Furthermore, the effect of age appeared non-significant in all respective models of combined markers that were examined.

Interactions between age and menopausal status were non-significant in any of the monitored variables.
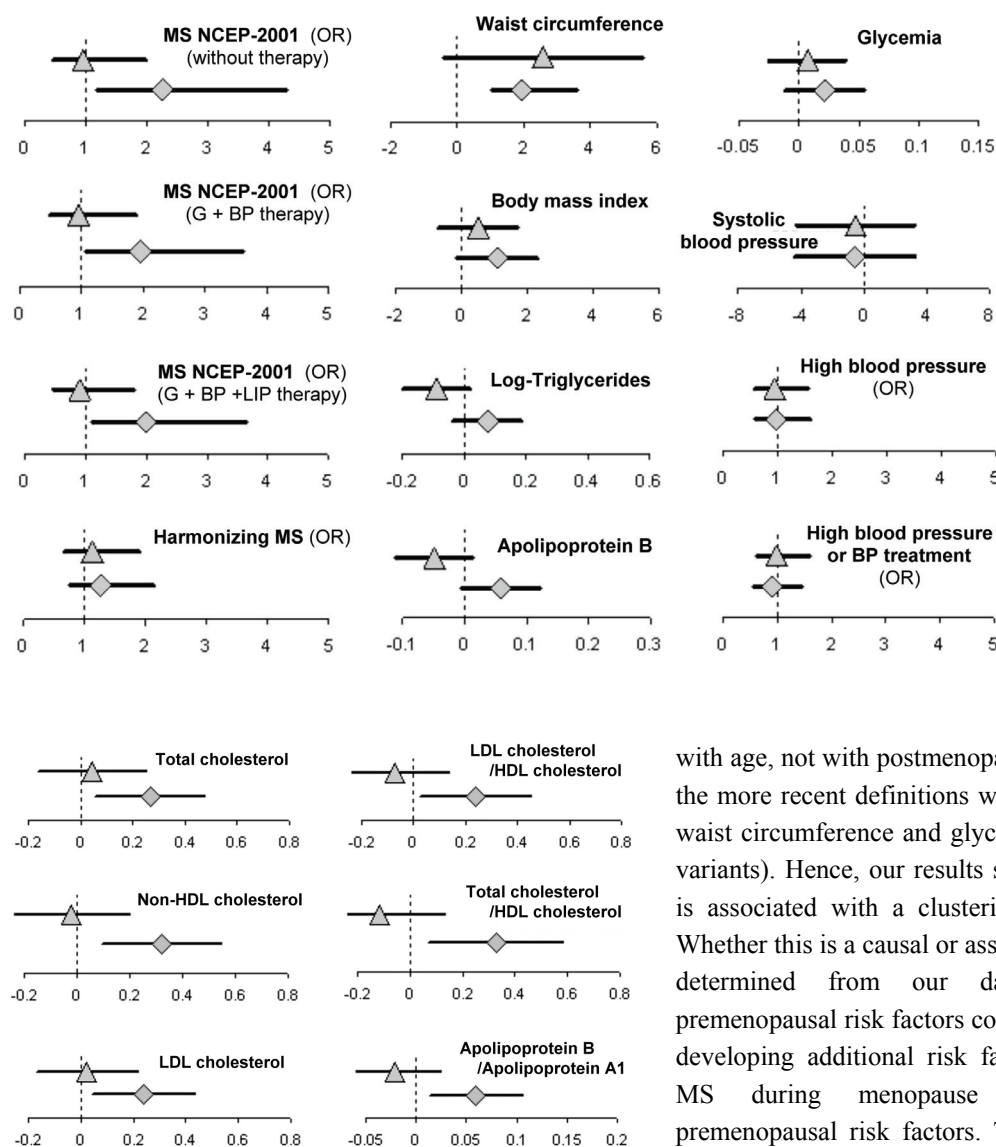
Fig. 1. Metabolic syndrome, metabolic syndrome components and other factors. Age-adjusted effect sizes with 95 % confidence intervals. Summaries of effect sizes for categorical variables are presented as odds ratios with 95 % confidence intervals. △ Perimenopause (menopausal transition); ◇ Postmenopause.

Fig. 2. Cholesterol parameters and lipid ratios. Age-adjusted effect sizes with 95 % confidence intervals. △ Perimenopause (menopausal transition); ◇ Postmenopause.

Discussion

Clustering of cardiovascular risk factors during menopause

The main goal of this study was to assess the effect of menopause on metabolic cardiovascular risk factors. The results of multiple regression analysis showed it is combined risk markers (lipid atherogenic ratios and MS with higher cut points – NCEP variants) whose increases contribute significantly to the increase in cardiovascular risk seen in postmenopause after adjustment for age. By contrast, single risk factors of MS (except for waist circumference) were associated exclusively with age – and a similar association of MS with age, not with postmenopause, was found when using the more recent definitions with decreased cut points for waist circumference and glycemia (Harmonizing the MS variants). Hence, our results suggest that postmenopause is associated with a clustering of risk factors of MS. Whether this is a causal or associational finding cannot be determined from our data. Women with 1-2 premenopausal risk factors could be more likely at risk of developing additional risk factors and "diagnosticable" MS during menopause than women without premenopausal risk factors. This issue warrants further research, preferably using a prospective study. Logically, NCEP-MS definitions with higher limits associated with higher risk do identify smaller numbers of individuals at higher risk (Moebus *et al.* 2007, Cameron 2010). Our finding that postmenopause, when adjusted for age, is associated with the prevalence of MS-NCEP is another piece of evidence that the most adverse metabolic changes may occur in women entering menopause with a predisposition to MS.

Our results have a biologically plausible explanation: the android body structure with abdominal obesity is associated with an increase in cardiovascular risk levels: increased circulating adhesion molecules in patients with abdominal obesity play an important role in the development of endothelial dysfunction/ atherosclerosis (Bošanská *et al.* 2010). There is even experimental evidence that obesity increased the size of myocardial infarction in male, but not in female Wistar rats (Clark *et al.* 2011). Menopause is known to be

Table 2. Table of data for Figure 1 (previous page).

	Age		**Perimenopause**		**Postmenopause**	
Categorical variables						
MS NCEP-2001	1.036 [0.931;1.151]	ns	0.957 [0.459;1.995]	ns	2.264 [1.191;4.301]	<0.05
MS NCEP-2001 (G + BP therapy)	1.055 [0.954;1.168]	ns	0.955 [0.481;1.897]	ns	1.954 [1.057;3.613]	<0.05
MS NCEP-2001 (G + BP + LIP therapy)	1.058 [0.957;1.168]	ns	0.907 [0.458;1.795]	ns	2.002 [1.094;3.660]	<0.05
Harmonizing MS; glycemia 6.1 cut point	1.078 [0.985;1.180]	<0.01	0.918 [0.502;1.677]	ns	1.598 [0.915;2.794]	ns
Harmonizing MS; waist 88 cm cut point	1.138 [1.038;1.248]	<0.01	1.192 [0.670;2.125]	ns	1.573 [0.901;2.747]	ns
Harmonizing MS	1.133 [1.042;1.232]	<0.01	1.121 [0.660;1.903]	ns	1.269 [0.751;2.143]	ns
High blood pressure	1.081 [1.000;1.169]	=0.05	0.936 [0.565;1.552]	ns	0.960 [0.577;1.597]	ns
High BP or BP therapy	1.111 [1.030;1.197]	<0.01	0.984 [0.606;1.597]	ns	0.894 [0.546;1.463]	ns
Continuous variables						
Waist circumference	0.635 [0.175;1.095]	<0.01	2.591 [-0.410;5.592]	ns	4.022 [0.953;7.091]	<0.05
Body mass index	0.188 [0.004;0.373]	<0.05	0.519 [-0.685;1.723]	ns	1.113 [-0.118;2.344]	ns
Log-Triglycerides	0.020 [0.003;0.037]	<0.05	-0.089 [-0.199;0.020]	ns	0.076 [-0.036;0.187]	ns
Apolipoprotein B	0.012 [0.002;0.021]	<0.05	-0.049 [-0.111;0.013]	ns	0.058 [-0.005;0.122]	ns
Glycemia	0.006 [0.001;0.011]	<0.05	0.007 [-0.026;0.039]	ns	0.021 [-0.012;0.055]	ns
Systolic blood pressure	0.758 [0.179;1.336]	<0.05	-0.518 [-4.296;3.260]	ns	-0.548 [-4.412;3.315]	ns
HDL cholesterol	0.001 [-0.013;0.014]	ns	0.064 [-0.026;0.154]	ns	-0.057 [-0.149;0.035]	ns
Insulin	0.030 [-0.115;0.174]	ns	0.466 [-0.475;1.406]	ns	-0.127 [-1.100;0.846]	ns
Log-HOMA-IR	0.003 [-0.020;0.026]	ns	0.109 [-0.040;0.258]	ns	0.055 [-0.099;0.210]	ns
Diastolic blood pressure	0.168 [-0.185;0.521]	ns	0.001 [-2.305;2.307]	ns	-0.272 [-2.630;2.085]	ns

Table 3. Table of data for Figure 2 (previous page).

	Age		**Perimenopause**		**Postmenopause**	
Cholesterol parameters						
Total cholesterol	0.047, [0.015;0.079]	<0.01	0.043, [-0.164;0.250]	ns	0.266, [0.054;0.478]	<0.05
Non-HDL cholesterol	0.047, [0.013;0.081]	<0.01	-0.023, [-0.244;0.198]	ns	0.320, [0.094;0.547]	<0.01
LDL cholesterol	0.041, [0.011;0.070]	<0.01	0.024, [-0.168;0.216]	ns	0.238, [0.041;0.435]	<0.05
Lipid ratios						
LDL cholesterol/ HDL cholesterol	0.029, [-0.003;0.061]	ns	-0.072, [-0.280;0.136]	ns	0.237, [0.025;0.450]	<0.05
Total cholesterol/HDL cholesterol	0.035, [-0.003;0.073]	ns	-0.117, [-0.366;0.132]	ns	0.327, [0.072;0.582]	<0.05
Apolipoprotein B/ A1	0.006, [-0.001;0.013]	ns	-0.021, [-0.067;0.025]	ns	0.059, [0.013;0.106]	<0.05

associated with a relative increase in android hormonal effects resulting in an increased prevalence of MS (Janssen *et al.* 2008). In a relatively non-risk population, the adverse effects of menopause, even in the presence of an increase in android obesity (larger waist circumference), may not be present (Feng *et al.* 2008). These facts suggest that the level of cardiovascular risk factors may increase, particularly in a (sub)population at higher risk, i.e., in premenopausal women with an a priori any factor(s) of MS or in those with a priori mild dyslipidemia. Provided that the adverse effects are more appreciable in the part of population at risk, a change in the mean values of the index parameter during menopause may not be statistically significant (Lejsková *et al.* 2011).

Confounding effect of treated individual risk factors

While the proportion of women with antihypertensive therapy ("Blood pressure-lowering therapy" in Table 1) in postmenopause was higher by almost half, this fact virtually did not get reflected in an increase in the prevalence of defined hypertension, which rose by an approx. 6 % regardless of the presence/absence of antihypertensive therapy ("SBP ≥130 mm Hg or DBP ≥85 mm Hg" rose by 5.4 %, with "SBP ≥130 mm Hg or DBP ≥85 mm Hg or BP-lowering therapy" increasing by 6.4 %). The increase in MS prevalence in postmenopause was unaffected by antihypertensive therapy at all ("NCEP-2001 without therapy" increased by 13.4 % and "NCEP-2001 G+BP therapy" rose by 13.3 %). Similarly, the increase in the prevalence of defined MS in postmenopause was not markedly affected even after inclusion of the criterion fibrate and/or hypoglycemic therapy ("MS NCEP-2001 G+BP+LIP therapy" rose by 14 %). In addition, each of the above definitions was assessed separately to detect even a small potential effect of pharmacotherapy in statistical analysis.

Strengths and limitations of the study

To the best of our knowledge, ours is the first study examining in more detail a potential effect of menopause on cardiovascular risk factor clustering. While the aims of our study were achieved using a random 5 % representative population sample without major ethnic differences and within a narrow age range, the cross-sectional design of the study was a limitation to interpretation of its results.

Conclusion

The cluster of cardiovascular risk factors referred to as MS, as defined by the NCEP-ATPIII in 2001, and combined lipid markers of cardiovascular risk – lipid atherogenic ratios – was predicted by menopause, not by age. The results of our epidemiological study support the notion that women with lost ovarian function in natural menopause are at risk of developing not only atherogenic dyslipidemia but, also, of clustering of additional cardiovascular risk factors. In this population, it would be particularly women entering menopause with one or two components of MS and/or mild dyslipidemia, on whom efforts to prevent cardiovascular risk factors from clustering should be focused to reduce their lifelong cardiovascular risk.

Conflict of Interest
There is no conflict of interest.

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