Original research article – Special issue: Cardiovascular Prevention

Changes in cardiovascular risk profile in women after menopause (Prague Pre and Post Menopausal Female study)

Jan Pitha a,*, Ondřej Auzký a, Jan Kovář a, Magdaléna Lejsková b, Silvia Adámková c, Eva Babková d, Tomáš Adámek d, Petr Stávek a, Romana Dembovská a, Jolana Mrázková a

a Laboratory for Atherosclerosis Research, Institute of Clinical & Experimental Medicine, Prague, Czech Republic
b Institute for Postgraduate Medical Education, Prague, Czech Republic
Transplant Surgery Department, Institute of Clinical & Experimental Medicine, Prague, Czech Republic
d Department of Medicine I, Thomayer’s Hospital, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form
17 January 2014
Accepted 20 January 2014
Available online 7 March 2014

Keywords:
Menopausal transition
Cardiovascular risk factors
Plasma lipids
Longitudinal study

ABSTRACT

Introduction: Atherosclerosis is the main cause of mortality in the Czech Republic. In our previous cross-sectional studies, we detected a high prevalence of metabolic cardiovascular risk factors in women before and after menopause and found menopausal transition to be critical period for atherosclerosis acceleration. In the present longitudinal study, we studied changes of main cardiovascular risk factors in women after transition to menopause.

Methods: We analyzed data of 195 women who became menopausal and 292 women who stayed in menopause during 6-year period. The cardiovascular risk factors under study were as follows: smoking, body mass index, waist circumference, blood pressure, plasma lipids including apolipoprotein B and A1 and fasting glyceria.

Results: The most striking differences between newly and steadily menopausal women were found in changes of plasma lipids. With the exception of HDL cholesterol all changes were less favorable in newly menopausal women and were not associated with treatment with statins. No significant differences between both groups were found for changes in body mass index, waist circumference, blood pressure and fasting glyceria.

Conclusions: In longitudinal study we confirmed that time around menopausal transition is one of the most dynamic periods regarding changes of cardiovascular risk factors, mainly plasma lipids.

© 2014 The Czech Society of Cardiology. Published by Elsevier Urban & Partner Sp.z.o.o. All rights reserved.

* Corresponding author: Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21 Praha 4, Prague, Czech Republic. Tel.: +420 261 363 069; fax: +420 241 271 574.
E-mail address: japi@ikem.cz (J. Pitha).
http://dx.doi.org/10.1016/j.crvasa.2014.01.004
0010-8650/© 2014 The Czech Society of Cardiology. Published by Elsevier Urban & Partner Sp.z.o.o. All rights reserved.
Introduction

Cardiovascular disease caused by atherosclerosis is the main cause of mortality among men and women in developed countries including the Czech Republic [1]. Although cardiovascular disease caused by atherosclerosis is rare in premenopausal women, the incidence steeply increases after menopause. Unresolved issue remains if these changes are caused rather by chronological aging or by menopause accompanied by decrease in the estrogen concentration. If the latter is correct, the change of cardiovascular risk factors around menopause could be critical for the development of atherosclerosis and its complications [2,3]. Despite increasing interest in menopausal transition and evidence that it is really atherogenic [4,5], only general recommendations were recently published focused on this period in woman’s life [6,7] and only sparse data from reliable longitudinal studies are available [2,3].

In our previous work, we detected a high prevalence of metabolic cardiovascular risk factors in women before and after menopause [8,9]. In addition, we found menopausal status as a risk factor for the development of hypertension, though potentially mediated through increased body mass index [10]. We also found that menopausal transition could be a critical period for atherosclerosis acceleration under certain conditions, namely smoking [11]. The impact of menopausal transition could be mediated through several mechanisms including impaired vascular protection, impaired reverse cholesterol transport, and impaired balance of sex hormones [11]. In a recent longitudinal study, we studied changes of main cardiovascular risk factors after transition to menopause.

Materials and methods

Population

The study was already described in detail elsewhere [8–11]. In short, the Prague Pre and Post Menopausal Female (3PMFs) study is based on a 5% representative and random sample of women aged 45–54 years living in Prague recruited in 2003. In 2004–2006, 908 women underwent baseline examination. All women throughout the study reported their final menstrual period (FMP) on a monthly basis. For definition of reproductive status, we used criteria proposed by the “Stages of Reproductive Aging Workshop” (STRAW) [12] together with levels of follicle stimulating hormone (FSH). According to this, women were divided into 3 groups: premenopausal, when the FMP was reported in within 61 days before the interview; perimenopausal, if the FMP was reported 61–365 days before the interview; and postmenopausal, when the FMP was reported more than 365 days before the interview. The second examination was performed after 5.7 ± 0.8 years. Complete data were obtained in 676 women. In this analysis we focused on 303 newly menopausal women and on 292 steadily menopausal women as controls.

The definition of newly menopausal women was as follows: reported FMP within 61 days before baseline examination with level of FSH lower than 40 IU/L and reported FMP more than 365 days before the second examination with levels of FSH more than 40 IU/L. The definition of steadily menopausal women was as follows: reported FMP more than 365 days before the first and second examination with levels of FSH more than 40 IU/L at both examinations. After exclusion of women after hysterectomy/ovariectomy and of women using hormone replacement therapy the study group comprised 193 newly menopausal women and control group comprised 292 steadily menopausal women. The ethics committee of the Institute a priori approved the whole study, and all participants provided their signed informed consent for the second examination.

Anthropometric and laboratory variables

All participants were interviewed about their medical history and main cardiovascular risk factors. Height, weight, waist circumference and blood pressure were measured according to the WHO MONICA (“monitoring trends in cardiovascular disease”) protocol [13,14]. Body mass index was calculated as weight in kg divided by squared height in meters. Women with a history of current and past regular smoking were defined as smokers. Systolic and diastolic blood pressures were measured in the right arm with the subject in the sitting position after at least ten minutes at rest. Three blood pressure measurements were obtained, and the mean value of the last two measurements was used for further analyses. Blood samples were drawn after overnight fasting. Serum total cholesterol and triglycerides were measured using the fully automated (HITACHI 911 Auto Analyzer, Japan) enzymatic method (reagents from Hoffmann, La Roche, Basel, Switzerland). HDL-cholesterol was determined using the same method after precipitation of serum lipoproteins with sodium phosphotungstate and magnesium chloride kits. Serum LDL cholesterol was measured using an automated method with direct determination using an LDL-C plus kit from Hoffmann-LaRoche (Basel, Switzerland). FSH was measured using IRMA kits (ImmunoTech, Prague, Czech Republic). Fasting glycemia was determined enzymatically (Lachema, Brno, Czech Republic).

Data analysis

Data are presented as percentages for categorical variables and means for continuous variables. Between-group comparison of continuous variables was performed using unpaired t-test. Paired t-test was used for the detection of changes of cardiovascular risk factors in the case of continuous variables. The $\chi^2$ test was applied for discrete variables. Between-group comparisons of mean change of particular covariates were performed using unpaired t-test.

Results

At the baseline examination age and FSH levels were significantly lower in newly menopausal women than in steadily menopausal women; the same was found for waist circumference, LDL cholesterol, and apolipoprotein B. No significant differences were found in body mass index, blood
Changes of other cardiovascular risk factors under study are presented in Table 3. Body mass index and waist circumference significantly increased both in newly and steadily menopausal women; the increase was moderately higher in newly menopausal women, especially waist circumference; however, no statistically significant differences between groups were observed. Systolic blood pressure increased to a similar extent in pressure, triglycerides, HDL cholesterol, apolipoprotein A1, and glycemia (Table 1).

Regarding changes of smoking habits at the second examination in both groups of women, there was moderate decrease of current smokers. However, these differences were not significant both within each group and between groups (Table 2).

### Table 1 – Baseline values of cardiovascular risk factors in Prague Pre and Post Menopausal Female study.

<table>
<thead>
<tr>
<th></th>
<th>Women newly menopausal</th>
<th>Women steadily menopausal</th>
<th>p for difference between newly and steadily menopausal women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.87 (2.51)</td>
<td>51.66 (2.17)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Follicle stimulating hormone (IU/L)</td>
<td>18.02 (19.38)</td>
<td>73.42 (38.00)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>25.24 (4.18)</td>
<td>25.79 (4.77)</td>
<td>0.19</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.55 (11.05)</td>
<td>87.51 (12.90)</td>
<td>0.009</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>118.32 (15.68)</td>
<td>118.01 (15.69)</td>
<td>0.831</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78.66 (10.05)</td>
<td>78.44 (9.65)</td>
<td>0.809</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.31 (0.82)</td>
<td>3.62 (0.79)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.61 (0.38)</td>
<td>1.64 (0.38)</td>
<td>0.394</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.29 (0.60)</td>
<td>1.34 (0.72)</td>
<td>0.423</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>1.00 (0.23)</td>
<td>1.08 (0.25)</td>
<td>0.002</td>
</tr>
<tr>
<td>Apolipoprotein A1 (g/L)</td>
<td>1.64 (0.268)</td>
<td>1.64 (0.25)</td>
<td>0.933</td>
</tr>
<tr>
<td>Fasting glyceria (mmol/L)</td>
<td>5.11 (0.49)</td>
<td>5.16 (0.65)</td>
<td>0.361</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD).

### Table 2 – Change in smoking habits after 6 years in Prague Pre and Post Menopausal Female study.

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers</th>
<th>Past-smokers</th>
<th>Current smokers</th>
<th>p for difference between first and second examination (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline examination</td>
<td>107 (54.9)</td>
<td>36 (18.5)</td>
<td>46 (23.6)</td>
<td></td>
</tr>
<tr>
<td>Follow-up examination</td>
<td>110 (56.4)</td>
<td>44 (22.6)</td>
<td>37 (19.0)</td>
<td>0.412</td>
</tr>
<tr>
<td>Women newly menopausal</td>
<td>142 (48.8)</td>
<td>51 (17.5)</td>
<td>89 (26.6)</td>
<td></td>
</tr>
<tr>
<td>Women steadily menopausal</td>
<td>141 (48.3)</td>
<td>66 (22.6)</td>
<td>76 (26.0)</td>
<td>0.212</td>
</tr>
</tbody>
</table>

Values are expressed as numbers (%); in newly menopausal women two intermittent smokers were not included at the baseline visit in the analysis, in steadily menopausal women one intermittent smoker was not included.

### Table 3 – Changes in cardiovascular risk factors under study in Prague Pre and Post Menopausal Female study.

<table>
<thead>
<tr>
<th></th>
<th>Women newly menopausal</th>
<th>Women steadily menopausal</th>
<th>p for difference between newly and steadily menopausal women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle stimulating hormone (IU/L)</td>
<td>60.14 (37.5)</td>
<td>8.04 (34.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>1.36 (2.19)</td>
<td>1.12 (2.08)</td>
<td>0.228</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>2.51 (7.37)</td>
<td>1.28 (8.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>9.78 (15.71)</td>
<td>7.34 (16.08)</td>
<td>0.10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>2.06 (9.29)</td>
<td>0.79 (10.40)</td>
<td>0.17</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>0.08 (0.88)</td>
<td>--0.300 (0.90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.07 (0.29)</td>
<td>--0.001 (0.29)</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.13 (0.67)</td>
<td>0.04 (0.63)</td>
<td>0.004</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.04 (0.26)</td>
<td>--0.03 (0.27)</td>
<td>0.002</td>
</tr>
<tr>
<td>Apolipoprotein A1 (g/L)</td>
<td>--0.04 (0.27)</td>
<td>--0.08 (0.24)</td>
<td>0.09</td>
</tr>
<tr>
<td>Fasting glyceria (mmol/L)</td>
<td>0.278 (0.58)</td>
<td>0.28 (0.99)</td>
<td>0.971</td>
</tr>
<tr>
<td>Treatment with statins, n (%)</td>
<td>10 (5.0)</td>
<td>29 (9.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Antihypertensive treatment, n (%)</td>
<td>49 (25.1)</td>
<td>93 (31.6)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values are expressed as mean of difference (SD) if not stated differently; change from the baseline values inside each group.

[¹] p < 0.05.
[²] p < 0.01.
[³] p < 0.001.
[¹] Data from the second visit.
both groups, diastolic blood pressure increased significantly in newly, but not in steadily menopausal women; however, the difference of changes of blood pressure was not significant between both groups. The most divergent results in both groups were observed in changes of lipid parameters. In newly menopausal women no significant changes of LDL, HDL cholesterol, apolipoprotein B and apolipoprotein A1 were detected; however, levels of plasma triglycerides significantly increased. In contrast, in steadily menopausal women, LDL cholesterol and apolipoprotein B significantly decreased, while no significant changes of plasma HDL cholesterol and plasma triglycerides were observed. Changes of all lipid parameters were significantly different between newly and steadily menopausal women with the exception of apolipoprotein A1, in which case this difference was only of borderline significance. Fasting glycemia increased significantly and almost identically in both groups of women. Five percent of newly menopausal and ten percent of steadily menopausal women were treated by statins. This difference was not statistically significant. When women treated with statins were excluded from the analysis no substantial changes in results were detected. Significantly higher prevalence in antihypertensive treatment was found in steadily menopausal women. Exclusion of women with antihypertensive treatment did not significantly modify differences in changes of factors under study between newly and steadily menopausal women.

### Discussion

In the population-based longitudinal study we detected that after transition to menopause the most robust increase was observed in body mass index, waist circumference, fasting glycemia and plasma triglycerides. In addition, the most striking differences between newly menopausal women and steadily menopausal women were found for changes in LDL cholesterol, apolipoprotein B, plasma triglycerides and HDL cholesterol after 6 years. With the exception of HDL cholesterol, all detected plasma lipid changes were less favorable in newly menopausal women and were not associated with treatment with statins. No significant differences between groups were observed for body mass index, waist circumference, blood pressure, and fasting glycemia.

Although many studies investigated reproductive status and the impact of various hormones on atherosclerosis, many controversies remain. On one hand, the age at menopause was not detected as an additional predictor of subclinical atherosclerosis and/or coronary events [15,16]. On the other hand there is evidence that menopause and rate of changes during menopausal transition could be of importance in cardiovascular risk burden and atherosclerosis development [17,18]. However, the changes during menopausal transition could be complex and could mirror actual disequilibrium between deleterious and protective mechanisms. It had been known that natural menopause is associated with increase in all cardiovascular risk factors. Nevertheless, it is difficult to differentiate if changes of cardiovascular risk factors are caused by chronological aging or by menopause. Even from longitudinal studies the answer is not obvious, because there is problem to find appropriately matched controls. In our study we used as a control group steadily menopausal women from the same population in contrast to other studies, where premenopausal women of the same age were included as a control population. In addition, we measured changes of follicle stimulating hormone in both visits to differentiate between chronological and ovarian aging.

The most interesting results were observed in plasma lipids. Changes in plasma lipids were already described in longitudinal studies including thousands of participants. One of the most reliable is longitudinal SWAN study [2,3]. In this study, in 541 healthy and initially premenopausal women changes in lipid factors were established after 2–3 years. Age-matched premenopausal women comprised control group. In women with history of natural menopause and did not receive hormone-replacement therapy, serum levels of high-density lipoprotein (HDL) cholesterol declined more than in controls, and plasma LDL cholesterol increased. In contrast, natural menopause did not have any impact on blood pressure, plasma glucose, insulin levels, and body weight.

In our study we observed quite similar findings, with the exception of non-significant increase of HDL cholesterol in newly menopausal women, and its decrease in steadily menopausal women. These differences could be caused by different control group which comprised steadily menopausal women and also by longer time interval after menopause in our study compared to SWAN (5.7 vs. 2.5 years). For significant and robust decline in LDL cholesterol in steadily menopausal women we have no reliable explanation. When looking at crude available data in this study regarding changes in lifestyle (smoking, physical activity, basic dietary habits) we have not found any significant difference between newly and steadily menopausal women. One of the speculative explanations is the overall trend for decrease of LDL cholesterol in the whole Czech population [19], which was reflected in “stable” population of steadily menopausal women, but not in “dynamically changing” population of newly menopausal women.

Regarding another robust independent cardiovascular risk factor – blood pressure – we observed increase in systolic and diastolic blood pressures in both groups. While in newly menopausal women this increase was significant for both – systolic and diastolic pressure, in steadily menopausal women only systolic blood pressure increased significantly. Nevertheless, the difference in change in diastolic blood pressure was not significant between both groups and could be attributed to more frequent antihypertensive treatment in steadily postmenopausal women or to chance finding. In general, we observed the same situation as in already published studies – after menopause the values of systolic and diastolic blood pressure are increasing and are associated with increased prevalence of hypertension in women at older age.

Limitations of our study are only two examinations completed in period of almost 6 years with the lack of more frequent follow-up up and thus the absence of more detailed description of transition to menopause. However, striking difference in the increase of FSH between newly and steadily menopausal women reflects rather ovarian than chronological aging in the study group.

The strengths of our study are population-based approach with a well-defined reproductive status and its changes
and the single-center longitudinal study with prospective design. In addition, we believe, that steadily menopausal women are more relevant control group when detecting changes of cardiovascular risk factors in menopausal transition.

In conclusion, our results suggest that natural menopause has an unfavorable effect on lipid metabolism, which may contribute to robust increase in the risk of coronary disease after this period. In addition, we confirmed our previous findings, that menopausal transition is unique dynamic period in woman’s life accompanied by robust changes of cardiovascular risk factors, mainly plasma lipids. If these results are confirmed, transition to menopause could be the best period for complex intervention of cardiovascular risk factors including plasma lipids.

**Conflict of interest**

No conflict of interest.

**Funding**

Supported by Ministry of Health, Czech Republic – conceptual development of research organization (“Institute for Clinical and Experimental Medicine – IKEM, IN 00023001”) and by grant NT 14008-3/2013 (Internal Grant Agency of the Ministry of Health of the Czech Republic).

**Ethical statement**

All persons included in this study were recruited and examined according to ethical standards. The ethics committee of the Institute a priori approved the whole study, and all participants provided their signed informed consent for the examination and all procedures.

**Informed consent**

All persons included in this study signed informed consent.

**References**

5. F.A. Wild, Atherosclerotic burden during the menopausal transition is a wakeup call, Menopause 19 (2012) 1–2