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Menopausal transition enhances the atherogenic risk of smoking in middle aged women ☆

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ABSTRACT

Background: The presence of cardiovascular risk factors during the menopausal transition could be critical in the development of atherosclerosis. In the present study, we evaluated whether the menopausal transition has impact on traditional and newly discussed risk factors.
Methods: Six hundred ninety nine women from population-based study underwent ultrasound measurement of the intima-media thickness of the common carotid arteries (CIMT) — Prague Pre and Postmenopausal Females study (3PMFs). In addition, 40 women selected according to reproductive and smoking status were examined with regard to number of circulating endothelial progenitor cells, markers of reverse cholesterol transport and sex hormones, including their fluctuation — Hormone Variability study (HVs).
Results: Age, smoking, body mass index, systolic blood pressure and HDL cholesterol were independently associated with the CIMT in 3PMFs group. The increase in the CIMT with age was markedly steeper in current/past smokers than in non-smokers among perimenopausal women (p for equality of slopes = 0.005). This difference was not observed in premenopausal and menopausal women. In the HVs group, endothelial progenitor cells and reverse cholesterol transport were substantially higher while triglycerides and fluctuation of free testosterone were lower in non-smokers than in smokers in menopausal transition. In contrast, in menopausal women, the fluctuation of free testosterone was higher in non-smokers; no other differences between smokers and non-smokers were detected.
Conclusions: These results suggest that atherogenic effect of smoking may be enhanced during menopausal transition. The mechanism could be impaired reparative vascular processes, impaired reverse cholesterol transport and rapidly changing status of sex hormones.

1. Introduction

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 increases after menopause. Despite still unresolved issue is if these changes are really caused by menopause, or if they are only of ageing, this increase is believed to be caused by a decrease in the oestrogen concentration, with consequently decreasing protection against traditional cardiovascular risk factors. Therefore, the presence of cardiovascular risk factors during the menopausal transition and before this period could be critical for further development of atherosclerosis and its complications [2]. Despite increasing interest about menopausal transition and evidence, that it is really atherogenic [3,4], only general recommendations are recently available focusing on this vulnerable period in a woman's life [5–7].

Although many studies investigated reproductive status and the impact of various hormones on atherosclerosis, many controversies remain. On the one hand, the age at menopause was not detected as an additional predictor of subclinical atherosclerosis and/or coronary

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events [8–10]. On the other hand there is evidence available, that menopause and rapidity of changes during menopausal transition could be of importance in changes of cardiovascular risk factors and atherosclerosis development [11–13]. However, the changes during menopausal transition could be complex and could mirror (dis)equilibrium between deleterious and protective mechanisms. One of them is potentially decreased vascular protection represented by circulating endothelial progenitor cells detected during the preovulatory and mid-luteal phases [14].

In our previous work, we detected a high prevalence of metabolic cardiovascular risk factors in women before and after menopause (aged 45–54 years) [15]. In addition, we found menopausal status as a risk factor for development of hypertension, though potentially mediated through increased body mass index [16].

In the present study, we studied if menopausal transition has any impact on cardiovascular risk expressed as traditional cardiovascular risk factors in population-based study focused on marker of preclinical atherosclerosis in carotid arteries — intima media thickness measured by ultrasound. In addition, based on findings from the population-based study we studied in detail smaller subgroup of women in menopausal transition and in menopause. In this subgroup we focused on the potential impact of menopausal transition and smoking on reverse cholesterol transport, circulating endothelial progenitor cells and fluctuations of sex hormones.

2. Materials and methods

2.1. Population

For the present study we used the data from ongoing population-based study. The methodology was already described [15–17]. The Prague Pre and Post Menopausal Females study (3PMFs) is based on a 5% representative and random sample of 29,440 women aged 45–54 years living in Prague selected from the registers of health insurance companies. From a random sample of 1472 women, 908 agreed and came for examination. After excluding women not fulfilling criteria for reproductive status or incomplete data, 699 women were evaluated. In addition, to evaluate potential impact of the menopausal transition and smoking on fluctuations of female sex hormones, 40 women were examined in Hormone Variability study (HVs). Women from the latter group were invited for three examinations in 4 weeks interval — during the first visit women filled up protocol of 3PMFs and in addition to all measurements done in 3PMFs reverse cholesterol transport (cholesterol efflux) and number of circulating endothelial progenitor cells were measured. In addition sex hormones were measured in 4 weeks intervals (estradiol, total, free testosterone and anti-Mullerian hormone). The reason for this study was to assess hormonal variability according to reproductive status and smoking. We hypothesised that menopausal transition and smoking could also substantially increase hormonal fluctuation. In HVs group only women without serious cardiovascular disease including hypertension and diabetes mellitus, not treated by antihypertensive or hypolipemic drugs and without any hormonal therapy were included. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the institution's human research committee. In addition, the authors of this manuscript have certified that they have complied with the Principles of Ethical Publishing in the International Journal of Cardiology. The ethics committee of the Institute a priori approved the whole study, and all participants provided their signed informed consent.

2.2. Anthropometric and clinical variables

All participants were interviewed about their medical history, focused on the presence of cardiovascular disease and main cardiovascular risk factor including history of hysterectomy and/or ovariectomy. Women from population-based study and women selected for more detailed metabolic studies were examined according to identical protocol. Height, weight, waist circumference and blood pressure were measured according to the WHO MONICA ("Monitoring trends and determinants in cardiovascular disease") protocol [18,19]. 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. Irregular smokers (less than 1 cigarette per day) were excluded from the analysis. 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 BP readings 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). Follicle-stimulating hormone (FSH), was measured using IRMA kits (Immunotech, Prague, Czech Republic).

2.3. Definition of categorical risk factors

Hypertension was defined as a systolic blood pressure equal to or higher than 140 mm Hg, a diastolic blood pressure equal to or higher than 90 mm Hg, or the use of antihypertensive drugs. Dyslipidaemia was defined as a total serum cholesterol level higher than 5.0 mmol/L, serum triglycerides higher than 1.7 mmol/L, serum HDL cholesterol less than 1.2 mmol/L or the use of hypolipidaemic drugs. Glucose intolerance and/or diabetes mellitus was defined as fasting glycaemia equal to or higher than 5.6 mmol/L or use of antidiabetic drugs including insulin. Overweight was defined as a BMI equal to or greater than 25 $kg \cdot m^{-2}$. Central obesity was defined as a waist circumference equal to or greater than 88 cm.

2.4. Definition of reproductive status

All women reported their final menstrual period (FMP) on a monthly basis. To assess the impact of reproductive status, we used the definitions proposed by the "Stages of Reproductive Aging Workshop" (STRAW) [20] combined with plasma FSH levels. According to this information, women were divided into 3 groups: premenopausal, when the FMP was reported in within 61 days before the interview (STRAW stages −5 to −2) and FSH was equal to or less than 40 IU/L; perimenopausal, when the FMP was reported 61–365 days before the interview (stages −1 to +1a) and FSH was higher than 40 IU/L; and postmenopausal, when the FMP was reported more than 365 days before the interview (stages +1b to +2) and FSH was higher than 40 IU/L. Women who did not fulfil these criteria and/or had not all data available were excluded from further analyses (n = 209). The final number of women was 699.

2.5. Measurements of subclinical atherosclerosis

The mean intima-media thickness of the common carotid arteries (CIMT) was measured using the Acuson 128/4 ultrasound system (Mountain View, CA, USA) with a 7.0 MHz probe with all participants in the supine position. Longitudinal images from lateral approaches to the left and right common carotid arteries were recorded on videotapes (S-VHS). All measurements were performed on a computer, off-line, using Image Measure PC Vision MicroScience software (Imaging Technology Inc., Bedford, MA, USA). Digitised images were used to trace the media-adventitial and intima-lumen interfaces and for calculations of the mean CIMT according to a previously published method [21]. A mean of four measurements in the far wall of a distal (10-mm) segment of both common carotid arteries (two in the right common carotid artery and two in the left common carotid artery) was used as an outcome for statistical analyses. All measurements were performed by the same physician (JP). All off-line readings were taken by a technician blinded to the status of the participants. Variability was assessed by repeated examination in 10 randomly chosen women. The coefficient of variation of the measurement was 4.9%.

2.6. Measurements of reverse cholesterol transport/cholesterol efflux

Measurements were done by the already established method at our department and described previously [22]. In short, human serum from each subject was used as an acceptor of cholesterol released from cells for the determination of cholesterol efflux using THP-1 human monocytes (human monocytic leukemia cells, ECACC 88087201), which were induced to differentiate into macrophages. To measure cholesterol efflux, prelabeled cells were incubated for 240 min in RPMI medium containing 5% serum of study subjects. Percent cholesterol efflux was expressed as radioactivity (cpm) in the efflux media divided by the total radioactivity of the sample (media plus cell) and multiplied by 100. Cholesterol efflux was measured between 15 and 240 min. The efflux phase was ended at 240 min and this measurement was used for further analyses. The coefficient of variation for the cholesterol efflux assay was 9.95%.

2.7. Detection of endothelial progenitor cells

Human endothelial progenitor cells were analysed for the expression of surface antigens as previously reported [23]. Briefly, before staining with specific monoclonal antibodies, cells were treated with 40 μl of fetal serum for 15 min. Then 200 μl of peripheral blood was stained with 40 μl of phycoerytrin conjugated anti CD 34 (Beckman Coulter), 20 μl of fluorescein isothiocyanate conjugated CD 45 (Beckman Coulter) and 10 μl of Alexa Fluor 647 conjugated anti-KDR (e-Bioscience). Analysis was performed with an automated fluorescence-activated cell counter (CyAn, Beckman Coulter) and 1 million events for each analysis were counted. Based on population human studies stem cells were defined as mononuclear $CD34^{+}/CD45^{low+}$ cells and endothelial progenitor cells as mononuclear $CD34^{+}/CD45^{low+}/KDR^{+}$ cells.

2.8. Sex hormones

In HVs group in addition to FSH, additional sex hormones were measured three times in four weeks interval: estradiol, total and free testosterone and anti-Müllerian hormone. Estradiol was measured by ELISA access immunoassay systems (Beckman Coulter), anti-Müllerian hormone by Immunotech AMH/MIS enzyme immunoassay

(A16507, Beckman Coulter Company), and total and free testosterone by solid-phase enzyme immunoassay (InterMedical, Italy).

2.9. Data analysis

Data are presented as percentages for categorical variables and means for continuous variables. Between-group comparison of continuous variables was performed using t-test or analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The χ^2 test was applied for discrete variables. Partial correlation analyses were performed to detect variables significantly associated with subclinical atherosclerosis after adjusting for age. Variables were selected a priori as follows: age, history of hysterectomy/ovariectomy, hormone replacement therapy, antihypertensive/hypolipidaemic treatment, cigarette smoking, body mass index, waist circumference, systolic and diastolic blood pressures, plasma cholesterol, triglycerides, and HDL and LDL cholesterol. The final models for the associations between risk factors and the CIMT were identified by stepwise multiple regression. The interactions between reproductive status and risk factors in predicting the CIMT were examined by ANCOVA. In HVs group between-group comparisons of mean values were performed using t-test (Mann–Whitney) and were, if appropriate, standardised for age and body mass index; between-group comparisons of variability in hormone levels were performed using standard deviations of 3 consequent measurements, evaluating the differences by t-test. Regarding data of sex hormones — estradiol, anti-Müllerian hormone, total and free testosterone, their mean values are average of 3 measurements in 4 weeks intervals.

3. Results

3.1. 3PMFs group

The clinical characteristics of women, stratified by reproductive status, are summarised in Table 1. Postmenopausal women were older and had the highest prevalence/values of almost all cardiovascular risk factors under investigation. The only exception was the prevalence of current smoking, which was the highest in perimenopausal women. On the other hand, the number of cigarettes smoked per day was similar in all three groups of women. It was 11.8 ± 7.8 in premenopausal women, 10.7 ± 7.7 in women in menopausal transition and 12.9 ± 7.6 in menopausal women.

Simultaneously, the perimenopausal women had more favourable values of plasma triglycerides and HDL cholesterol than premenopausal and postmenopausal women. Perimenopausal and postmenopausal women were more frequently treated with hypolipidaemic and antihypertensive drugs. The mean CIMT was slightly greater among postmenopausal as compared to premenopausal patients. No statistically significant differences between the groups were observed with respect to past smoking, body weight, or blood pressure.

We used stepwise multiple regression analysis to identify the cardiovascular risk factors that were independently associated with the CIMT. Age, smoking, body mass index, systolic blood pressure and HDL cholesterol were independently associated with the CIMT in the group of all women. Because almost identical results were found for current and past smokers, these groups were merged for the benefit of statistical analysis.

To determine whether the association of cardiovascular risk factors with the CIMT is altered by reproductive status, we stratified women according to reproductive status and the cardiovascular risk factors under study (smoking, diabetes mellitus, overweight, hypertension and dyslipidaemia). Subsequently, we tested the interactions between particular risk factors and reproductive status as determined by the effects on the CIMT.

The increase in the CIMT with age was markedly steeper in current/past smokers than in non-smokers among perimenopausal women (p for equality of slopes = 0.005), while no such difference was observed in premenopausal and postmenopausal women (Fig. 1).

Using multiple linear regression, the difference in change in CIMT per year of age between smokers and non-smokers was significantly higher in perimenopausal than in premenopausal and postmenopausal women (Table 2).

The modification of the effect of smoking on the CIMT by reproductive age was further analysed again with adjustment for age, body mass index, systolic blood pressure and HDL cholesterol (Table 3). The interaction between reproductive age and smoking was found to be significant ($p = 0.035$).

No significant interaction between reproductive status and any other risk factor under investigation was observed. These findings

Table 1
Characteristics of the middle-aged women included in the study, stratified by reproductive status. (Values are expressed as percentages or means with 95% confidence intervals.)

Variable	Premenopause (n = 397)	Perimenopause (n = 61)	Postmenopause (n = 241)	p-value (Global test of equality)
Age (years)	48. 7 (48.4, 49.0)	51.1 (50.5, 51.7)	52.0 (51.7, 52.3)	p<0.0001
Hysterectomy and bilateral ovariectomy	-	8.2% (1.3%, 15.1%)	13.8% (9.4%, 18.2%)	p<0.0001
Hormonal therapy at any time	15.9% (12.3%, 19.5%)	23.0% (12.4%, 33.6%)	30.4% (24.6%, 36.2%)	p<0.0001
Current smokers	29.5% (25.0%, 34.0%)	42.6% (30.2%, 55.0%)	39.6% (33.4%, 45.8%)	p = 0.0113
Past smokers	16.1% (12.5%, 19.7%)	13.1% (4.6%, 21.6%)	15.4% (10.8%, 20.0%)	p = 0.8296
Hypolipidaemic or antihypertensive therapy	16.9% (13.2%, 20.6%)	24.6% (13.8%, 35.4%)	27.0% (21.4%, 32.6%)	p = 0.0078
Body mass index ($kg \cdot m^{-2}$)	25.7 (25.2, 26.2)	25.0 (23.3, 26.7)	26.2 (25.5, 26.9)	p = 0.1524
Waist circumference (cm)	85.4 (84,.1, 86.7)	85.9 (81.6, 90.2)	88.3 (86.5, 90.1)	p = 0.0110
Systolic blood pressure (mm Hg)	119.1 (117.4 120.8)	119.9 (115.0, 124.8)	120.2 (117.7, 122.5)	p = 0.6902
Diastolic blood pressure (mm Hg)	79.1 (78.1, 79.6)	79.7 (76.5, 82.9)	79.3 (77.9, 80.7)	p = 0.8696
Plasma triglycerides (mmol/L)	1.27 (1.19, 1.35)	1.21 (1.03, 1.37)	1.45 (1.32, 1.58)	p = 0.0093
Plasma HDL cholesterol (mmol/L)	1.62 (1.58, 1.66)	1.76 (1.64, 1.88)	1.65 (1.59, 1.71)	p = 0.0253
Plasma LDL cholesterol (mmol/L)	3.27 (3.18, 3.36)	3.32 (3.08, 3.56)	3.69 (3.56, 3.82)	p<0.0001
Fasting glycaemia (mmol/L)	5.16 (5.07–5.25)	5.41 (5.12–5.70)	5.26 (5.15–5.37)	p = 0.0889
Common carotid intima-media thickness (mm)	0.569 (0.562, 0.576)	0.574 (0.556, 0.592)	0.599 (0.588, 0.610)	p<0.0001

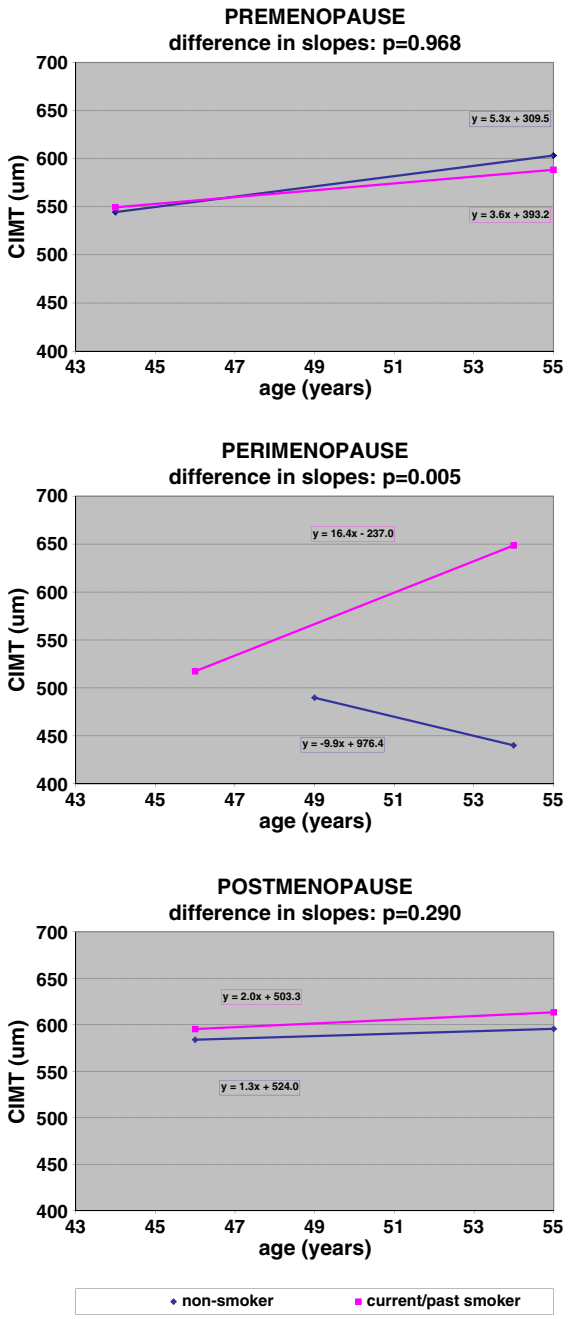

Fig. 1. Association of intima-media thickness of the common carotid arteries (CIMT) with age, stratified by smoking and reproductive ageing status. Regression lines were standardised for body mass index (26 $kg \cdot m^{-2}$), systolic blood pressure (120 mm Hg) and HDL cholesterol (1.60 mmol/L).

Table 2
The change in carotid intima-media thickness (mm) per year of age according to smoking and reproductive ageing status, standardised for age, body mass index, systolic blood pressure and HDL cholesterol. Multiple linear regression coefficient (95% CI).

	Premenopause	Perimenopause	Postmenopause
Smokers	n = 179	n = 34	n = 132
	0.0035	0.0164	0.002
	(−0.002; 0.0073)	(0.0092; 0.0236)	(−0.0052;0.0092)
Non-smokers	n = 214	n = 27	n = 107
	0.0053	−0.0099	0.0013
	(0.0019; 0.0088)	(−0.0301; 0.0102)	(−0.0055; 0.0080)

Premenopause: final menstrual period (FMP) less than 60 days before the interview and follicle stimulating hormone (FSH) levels equal to or less than 40 IU/L; perimenopause: FMP 60–365 days before the interview and FSH levels higher than 40 IU/L; postmenopause: FMP more than 365 days before the interview and FSH levels higher than 40 IU/L.

Table 3
Effect of cardiovascular risk factors on carotid intima-media thickness including the interaction between reproductive ageing and smoking status (ANCOVA results).

Covariates	Regression coefficients	p
Age	3.801	0.0011
Body mass index	2.19	0.0003
Systolic blood pressure	0.71	<0.0001
HDL cholesterol	−16.257	0.0183
Effects	F-statistics (df1, df2)	p
Smoking	9.05 (1, 683)	0.0026
Reproductive ageing status	3.69 (2, 683)	0.0255
Interaction		
Reproductive ageing and smoking status	3.36 (1, 683)	0.0352

were not substantially changed when the history of antihypertensive or hypolipidaemic therapy, hormonal therapy or bilateral ovariectomy or hysterectomy was included in the statistical models.

3.2. HVs group

The comparison of cardiovascular risk and protective factors and sex hormones between groups under study are shown in Table 4. In the group of women in menopausal transition, non-smoking women were older ($p=0.04$), had higher HDL cholesterol ($p=0.006$), apolipoprotein A1 ($p=0.018$), cholesterol efflux ($p=0.048$) higher number of circulating stem cells ($p=0.047$) and endothelial progenitor cells ($p=0.010$), lower triglycerides ($p=0.018$) and lower pulse frequency ($p=0.040$) than smoking women. After standardisation for age and body mass index, non-smoking women had higher number of the circulating endothelial progenitor cells ($p=0.023$), higher HDL cholesterol ($p=0.011$) and values of cholesterol efflux, the latter of borderline significance ($p=0.065$), and lower triglycerides ($p=0.021$).

In menopausal women fasting glycaemia and the level of free testosterone were significantly higher in non-smoking than in smoking women ($p=0.034$). After standardisation for age and body mass index these differences were no more significant.

No other significant differences between groups under study were observed according to effect of reproductive status and smoking.

Regarding variation in female sex hormones, expressed as standard deviations from three subsequent measurements, significant differences between non-smoking and smoking women were observed in fluctuation of free testosterone. These differences were in opposite directions in women in menopausal transition compared to menopausal women. In women in menopausal transition smoking women had higher variability of free testosterone than non-smoking women (0.09 ± 0.14 vs. 0.24 ± 0.17, $p=0.051$). In contrast, in menopausal women smoking women had significantly lower variability of free testosterone than non-smoking women (0.24 ± 0.09 vs. 0.43 ± 0.49, $p=0.026$). No other significant differences between groups under study were observed according to effect of reproductive status and smoking on variations of estradiol, anti-Müllerian hormone and total testosterone.

4. Discussion

According to our data, reproductive status strongly modified the effect of smoking on carotid arteries, circulating endothelial progenitor cells, reverse cholesterol transport, and fluctuation of free testosterone. The differences of risk factors under study between smoking and non-smoking women were detected almost uniformly only in women undergoing menopausal transition. The finding that reproductive status and smoking could interact in such complex manner is, to our knowledge, rather novel because this question has not been systematically examined in previous studies. The importance of this finding is further supported by recently published data showing that smoking could be even more deleterious in women than in men [24,25]. Our data show, that in addition to gender, also reproductive status could be of importance.

The potential biological explanation for increased sensitivity to atherosclerosis in women in menopausal transition could be really complex and we detected three potential areas of interest. First, the negative impact of smoking on protection of the vessel wall could be more deleterious in menopausal transition than in menopause. The hypothesis of "menopausal atherosclerotic transition" [3,4] is further supported by findings of significantly higher number of circulating endothelial progenitor cells in non-smoking than in smoking women but only in menopausal transition, and not in menopause. This finding, not affected by potential differences in age and body mass index, is considered very important, because in almost all studies the attention is mainly focused on risk factors and not on protective factors. From that point of view, however, evidence already exists proving an association between endothelial progenitor cells and coronary artery disease, mainly regarding collateral coronary circulation; moreover, these associations were present despite no

Table 4
Characteristics of the middle-aged women, stratified by reproductive and smoking status. (Values are expressed as means, with 95% CI.)

	Menopausal transition		Menopause	
	Non-smokers n = 13	Smokers n = 10	Non-smokers n = 9	Smokers n = 8
Age (years)	50.6 (48.6, 52.6)	47.0 (44.8, 49.2) *	53.8 (53.0, 54.6)	54.3 (53.4, 55.2)
No. of cigarettes per day	–	11.8 (6.4, 17.2)	–	10.9 (6.9,14.9)
Body mass index ($kg\cdot m^{-2}$)	26.0 (23.1, 28.9)	26.1 (22.9, 29.3)	26.4 (24.4, 28.4)	22.8 (20.4, 25.2) *
Systolic blood pressure (mm Hg)	114.3 (107.1, 121.5)	116.9 (111.4, 122.4)	117.5 (112.5, 122.5)	116 (108.4, 123.6)
Diastolic blood pressure (mm Hg)	70.3 (66.2, 74.4)	72.8 (69.0, 76.6)	70.3 (66.9,73.7)	72.1 (68.3, 75.9)
Pulse frequency (beats/minute)	62.6 (58.7, 66.5)	68.5 (64.0, 73.0) *	65.6 (59.0, 72.2)	65.9 (58.5, 73.3)
Adjusted	62.9 (58.8, 67)	68.0 (63.1, 72.9)		
Triglycerides (mmol/L)	0.97 (0.81, 1.13)	1.60 (1.09, 2.11)*	1.21 (0.82, 1.60)	2.22 (−0.11, 4.55)
Adjusted	0.98 (0.67, 1.29)	1.63 (1.28, 1.98)*		
LDL cholesterol (mmol/L)	3.04 (2.67, 3.41)	3.01 (1.36, 4.66)	3.57 (2.96, 4.18)	3.30 (2.65, 3.95)
HDL cholesterol (mmol/L)	2.00 (1.82, 2.18)	1.50 (1.26, 1.74)**	1.97 (1.72, 2.22)	1.65 (1.45, 1.85)
Adjusted	1.98 (1.78, 2.18)	1.53 (1.29, 1.77)*		
Apolipoprotein A1 (g/L)	1.76 (1.60, 1.92)	1.50 (1.34, 1.66)*	1.67 (1.53, 1.81)	1.72 (1.50, 1.94)
Adjusted	1.74 (1.58, 1.90)	1.52 (1.32, 1.72)		
Cholesterol efflux (%)	21.4 (20.4, 22.4)	19.9 (19.1, 20.7)*	20.7 (19.7, 21.7)	20.7 (19.1, 22.3)
Adjusted	21.4 (20.6, 22.2)	20.1 (19.1, 21.1)		
Glycemia (mmol/L)	5.4 (5.1, 5.7)	5.4 (5.2, 5.6)	6.0 (5.8, 6.2)	5.3 (4.6, 6.0) *
Adjusted	–	–	5.8 (5.4, 6.2)	5.5 (5.1, 5.9)
Circulating stem cells (No/mL)	2044 (1440, 2648)	1128 (946, 1310)*	1263 (925, 1,601)	1376 (692, 2060)
Adjusted	1925 (1455, 2395)	1284 (739, 1,829)		
Endothelial progenitor cells (No/mL)	286 (189, 383)	116 (52, 180) **	90 (63, 117)	78 (33, 123)
Adjusted	289 (−191, 769)	112 (10, 214) *		
Anti-Müllerian hormone (pmol/L)	5.70 (3.56, 7.84)	12.30 (5.48, 19.12)	5.25 (4.64, 5.86)	4.49 (3.22, 5.76)
Estradiol (pmol/L)	455 (332, 578)	316 (200, 432)	98 (60, 136)	72 (57, 87)
Total testosterone (ng/mL)	0.37 (0.21, 0.53)	0.50 (0.36, 0.64)	0.41 (0.27, 0.55)	0.64 (0.39, 0.89)
Free testosterone (pg/mL)	0.52 (0.09, 0.95)	1.05 (0.62, 1.48)	1.23 (0.64, 1.82)	0.56 (0.15, 0.97) *
Adjusted	–	–	1.03 (0.54, 1.52)	0.78 (0.25, 1.31)

Adjusted — adjusted for age and body mass index; *p<0.05; ** p<0.01 non-smokers vs. smokers.

substantial differences between traditional risk factors [26]. In addition, another finding indicates, that smoking could negatively affect number of circulating endothelial cells [27]. However, this study was done in younger premenopausal women and no data are to our knowledge available regarding interaction between smoking and reproductive status on this parameter. Overall risk profile regarding blood pressure and metabolic factors in women in menopausal transition was very favourable including smoking women, with the exception of markers of reverse cholesterol transport. The consistent and very impressive difference between smoking and non-smoking women in circulating endothelial progenitor cells really indicates potential critical role also of reparative processes in this period of women life. Second, the reversed cholesterol transport could be more altered by smoking in women in menopausal transition than in menopausal women. We found plasma HDL cholesterol and reverse cholesterol transport to be substantially lower and triglycerides to be substantially higher in smoking women in menopausal transition, but not in menopausal women. These factors were not substantially affected by age and body mass index. This also supports the importance of reverse cholesterol transport as found in population part of this study also in context of low HDL cholesterol and high triglyceride levels. Third interesting finding is higher variability of the level of free testosterone in smoking women in menopausal transition in contrast to menopause when higher variability was detected in non-smokers. The changes of sex hormones caused by smoking were already described in cross-sectional studies including large ones [28–30], but as to our knowledge no study was focused on the variability of sex hormone levels. Therefore, these data support the hypothesis that the rate of hormonal changes could be more important than absolute magnitude of changes and could be strongly modified by smoking. However, this finding should be confirmed in larger study.

As found almost uniformly in other studies, age, systolic blood pressure and the body mass index were strongly associated with subclinical atherosclerosis in women from population-based study, independently of reproductive status. Regarding plasma lipids, only HDL cholesterol seemed to be an independent predictor of subclinical atherosclerosis in a population based study, which confirms the already discussed importance of reverse cholesterol transport. The effect of reproductive age/status on the composition and function of HDL particles has been already described [31]. Nevertheless, in our study we detected the impact of reproductive status and smoking not only on HDL cholesterol but also on reverse cholesterol transport expressed independently as cholesterol efflux.

One limitation of this study stems from its cross-sectional design. The question of whether menopausal transition really represents a more vulnerable period for atherosclerosis progression in women with a history of smoking needs to be definitely confirmed in prospective studies. Another limitation is the relatively low number of perimenopausal women included. Despite that we were able to identify strong interaction between reproductive ageing and smoking with regard to cardiovascular risk, we still cannot exclude the possibility that reproductive ageing could also modify the impact of other risk factors that we were not able to detect in both populations under study.

In contrast, a strength of this study is population-based approach with a well-defined and established outcome (subclinical atherosclerosis in carotid arteries detected by ultrasound) and the single-centre study design. Another strength is the use of well-established criteria for defining reproductive status. In addition, the hypothesis regarding menopausal transition as very sensitive period especially for smokers was further supported by results from the study conducted in smaller subgroup of women, in whom more detailed studies of circulating endothelial progenitor cells, reverse cholesterol transport and hormonal status including variability of hormone levels during 3 months were performed.

In summary, in a population-based group of middle-aged women, the atherogenic impact of smoking appeared to be significantly modified by reproductive status. We found that menopausal transition especially in the presence of smoking could be more sensitive period than premenopause or menopause for atherosclerosis process in carotid arteries. This impact could be mediated through several mechanisms including impaired vascular protection, impaired reverse cholesterol transport, and impaired balance of sex hormones.

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