Hormone therapy and coronary heart disease risk by vasomotor menopausal symptoms.

Gast, Gerrie-Cor M; Pop, Victor J M; Samsioe, Göran; Grobbee, Diederick E; Nilsson, Peter M; Keyzer, Jules J; Wijnands-van Gent, Colette J M; van der Schouw, Yvonne T

Link to publication

Total number of authors:
8

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Hormone therapy and risk of coronary heart disease by vasomotor menopausal symptoms

Gerrie-Cor M. Gast [1,2]**, Victor J.M. Pop** [3]**, Göran N. Samsioe** [2]**, Diederick E. Grobbee** [1]**, Peter M. Nilsson** [4]**, Jules J. Keyzer** [5]**, Colette J.M. Wijnands-van Gent** [6]*, **Yvonne T. van der Schouw** [1]

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, STR 6.131, PO Box 85500, 3508 GA Utrecht, The Netherlands

[2] Department of Obstetrics & Gynaecology, Lund University Hospital, Lund, Sweden

[3] Department of Clinical Health Psychology, University of Tilburg, Tilburg, The Netherlands

[4] Department of Clinical Sciences Medicine, University Hospital, University of Lund, Malmö, Sweden

[5] Research Unit of The Diagnostic Center Eindhoven, The Netherlands

[6] Research Unit of Praktijkondersteuning Zuidoost-Brabant, The Netherlands

Please address correspondence to:

Prof. Yvonne T. van der Schouw, PhD

Julius Center for Health Sciences and Primary Care

University Medical Center Utrecht

STR 6.131, PO Box 85500, 3508 GA Utrecht

The Netherlands

Phone +31 88 755 9360/9301 (secr)

Fax + 31 88 756 8099

E-mail: y.t.vanderschouw@umcutrecht.nl

Word count: 2,998

ABSTRACT

Objective: The presence of vasomotor menopausal symptoms (VMS) may be a marker for susceptibility to cardiovascular preventive effects of postmenopausal hormone therapy (HT). We examined whether the association between HT use and coronary heart disease (CHD) risk differed between women with and without VMS.

Design: Prospective cohort study.

Setting: A Dutch and a Swedish population-based sample of women enrolled between 1995-2000.

Patients: 8,865 women aged 46-64 years and free of CHD, stroke, venous thrombosis /pulmonary embolism or cancer at baseline.

Measurements: Data on VMS and HT, collected by questionnaires.

Main outcome measures: CHD endpoints, obtained via registries.

Results: 252 CHD cases occurred during 10.3 y of follow-up. Neither for women with nor for women without flushing or (night) sweats ever HT use was associated with CHD risk, compared with never HT use (P for interaction: flushing: 0.65; (night) sweats: 0.15). Among women with intense VMS, ever HT use borderline significantly decreased CHD risk compared with never HT use (HR 0.49 [95% CI 0.20-1.03]). Among women without intense VMS, ever HT use was associated with a borderline significant increased CHD risk (HR 1.28 [95% CI 0.96-1.70]; P for interaction = 0.03). However, after multivariate adjustment, as compared to never HT use, ever HT use was not associated with risk of CHD among women with or without intense VMS.

Conclusions: Our findings do not support the view that HT use increases the CHD risk for women with the proper indication, i.e. VMS, but this needs to be confirmed in specifically designed studies.

INTRODUCTION

The disparity among findings from the observational studies [1-3] and randomized trials [4, 5] on the effects of postmenopausal hormone therapy (HT) on the risk of coronary heart disease (CHD) has created considerable debate. The most common indications for which HT is prescribed are hot flushes and night sweats, the classical vasomotor menopausal symptoms (VMS) [6]. These symptoms are experienced by as many as 80% of women during the menopausal transition [7], of which nearly one third seeks medical care [8]. After the report of the main findings of the Million Women's Study [9] and Women's Health Initiative (WHI) trial [5], there has been a substantial decline in HT prescriptions [10]. Also for women with VMS, physicians as well as the women themselves are in doubt whether HT is safe, and guidelines are conflicting [11, 12].

We recently hypothesized that the presence of VMS may be a marker for susceptibility to cardiovascular preventive effects of HT [13]. Our previous findings of an adverse cardiovascular risk profile [14, 15] and an increased CHD risk [16] among women with VMS indeed indicate that these women differ from those without VMS and may therefore experience particular benefits.

However, recent findings of the WHI and Heart and Estrogen/Progestin Replacement Study (HERS) trial seem to suggest the opposite; in these studies the increased CHD risk associated with HT was particularly concentrated among women reporting VMS at baseline [17, 18]. However, women in these trials were preferentially selected on the absence of VMS and the small group of older women on which these findings are based are unlikely to be representative of the sizable number of women requiring HT because of VMS in the population at large [19, 20].

In the current study, we examined whether the association between HT use and CHD risk differed between women with and without VMS in a population-based cohort of 8,865 peri- and postmenopausal women who were followed for 10 years.

METHODS

Population

The Eindhoven Perimenopausal Osteoporosis Study (EPOS) is a prospective cohort study among 6,700 Dutch women aged 46-57 who participated in a screening program, established to assess determinants of low bone mineral density, between 1994 and 1995 [21]. The Women's Health in the Lund Area (WHILA) Study consists of 6,917 Swedish women aged 50-64 who participated in a health screening procedure, which took place between 1996 and 2000 [22]. For the present study, we pooled these two cohorts into one study population.

For the present analysis 13,617 women were eligible. Informed consent was obtained and both study protocols were approved by institutional review boards. Women were excluded if they did not consent to linkage with vital status registries, could not be traced in these registries (n=966), had an unknown date of inclusion or death (n=6) or did not provide information on VMS (n=1,721), or HT use (n=45). We also excluded the premenopausal women (n=986), since these women were by definition no current HT users. Furthermore, we excluded prevalent cases of CHD (n=131), stroke (n=101), venous thrombosis (n=306) or pulmonary embolism (n=34) or cancer (n=456), other than a basal cell carcinoma, being identified through linkage to the Swedish registries (1973-1996), Eindhoven Cancer Registry (1976-1994) and by self-report using the baseline general questionnaire (both cohorts). The final study population consisted of 8,865 women (3,921 from EPOS and 4,944 from WHILA).

Baseline measurements

At baseline, a general questionnaire containing questions on demographics, presence of chronic diseases and related potential risk factors was administered. Coding of this information was standardized and merged into one database. Smoking behavior was categorized as never, past or current smoking. The highest attained level of education of the women was classified into three categories: low (primary education up to completing intermediate vocational education), medium (up to higher secondary education) and high (those with higher vocational education and university). Physical activity during leisure time was categorized as inactive or active. HT and oral contraceptives (OCs) use was categorized as never or ever. Menopausal status at enrolment was defined as follows: women were 'perimenopausal' if they experienced irregular menses compared with their usual menstruation pattern and 'postmenopausal' if they reported not having had menses over the past 12 months or longer. Women with incomplete questionnaire data, or who reported current use of OCs or HT, were classified as perimenopausal if they were between 46 and 55 years of age, and postmenopausal if they were older than 55 years [23].

Physical examination

In EPOS, systolic and diastolic blood pressure was measured in a standardized manner using a mercury manometer. One measurement was taken after the participant had been seated quietly in a comfortable posture, with feet flat on the floor, with the back supported and the arm supported at or as close to the level of the heart as possible. In WHILA, systolic and diastolic blood pressure was measured twice at the right arm after 15 minutes rest in seated position, using a mercury manometer with a cuff size adjusted to the circumference of the arm. The average of two recordings was calculated. For both cohorts, body weight was measured in

light indoor clothing without shoes. Body mass index (BMI) was calculated as weight divided by height squared.

Vasomotor menopausal symptoms

To assess the presence of flushing in EPOS women, three questions were asked, one on the number of days a participant experienced hot flushes in the previous week; one on the average frequency of hot flushes during one day; and one on the highest number of hot flushes at one day. Two questions were asked to assess the frequency of night sweats, one on the number of nights a participant woke up in the previous week because of night sweats; and one on the frequency of waking up per night due to night sweats. From these questions, a dichotomous variable was created for the absence or presence of flushing or night sweats. Flushing or night sweats were present if women reported to have had at least one symptom during the previous week or if they answered 'yes' to one of the questions. In addition, four categories of flushing were constructed: absent, low, moderate and high [24].

To assess the presence of VMS in WHILA, two questions were asked. The first question was "Do you have problems with sweats/hot flushes?" and was asked in the reproductive history section of the questionnaire. The second question was "Did you experience symptoms of sweats during the preceding three months?" and was asked in a general section concerning somatic symptoms. This section also contained questions on other menopausal symptoms such as feeling cold, headache and sleeping problems. Both questions were answered by yes or no. Women were defined as suffering from flushing or sweats if they answered 'yes' to one of the questions. Symptom severity of flushing was graded on a visual analogue scale (0-10 cm). Absence of symptoms was marked at 0, and scoring 10 indicated a symptom at the highest level. Based on these scores, symptom severity was further classified into three degrees, i.e. mild (grade 1-2), moderate (grade 3-6) and severe (grade 7-10).

In the present analysis, presence of hot flushes and of (night) sweats was defined from these questions and classified as yes or no. In addition, to get an impression of the burden of the symptoms, we created the variable intensity of VMS, defined as intense when women reported symptoms of (night) sweats as well as a high frequency of flushing in EPOS or a severely graded flushing in WHILA.

Follow-up

For the EPOS women, data on morbidity were obtained from the PHARMO Record Linkage system [25], which includes the drug-dispensing records from community pharmacies and hospital discharge records of more than 2 million inhabitants of over 40 demographically defined areas in The Netherlands, including Eindhoven. The hospital records include detailed information concerning the primary discharge diagnoses and dates of hospital admission and discharge. All diagnoses are coded according to ICD-9. The database was linked to the cohort on the basis of birth date, sex, postal code, and general practitioner with a validated probabilistic method [25]. Information on vital status was obtained through linkage with the municipal administration registries. Causes of death were obtained from the Dutch Central Bureau of Statistics, coded according to ICD-10.

For the WHILA women, follow-up information was obtained via linkage with registries. Mortality is registered in the Swedish Cause of Death Registry, and morbidity, based on hospital discharge summaries, is registered in the Hospital Discharge Registries, a validated alternative to revised hospital discharge and death certificates [26, 27]. In these registries, also ICD-9 and ICD-10 codes were used. Using personal 10-digit numbers, individual cohort members were linked to registries [28].

For our analyses, CHD events (ICD-9; 410-414, and 427.5 or ICD-10 I20, and I23-I25) were the endpoints of interest. Whenever multiple CHD events occurred, the first clinical

diagnosis was taken as endpoint. For all women who had a CHD event, follow up ended at the date of diagnosis or, when hospital admission had not occurred, at the date of death. Mortality from non-cardiovascular causes and withdrawn alive were considered censoring events. Non-fatal events from the women participating in the EPOS study were registered from January 1st 1998 until July 27th 2007, and fatal events were registered from January 1st 1995 until July 27th 2007. Women participating in the WHILA study were followed from their cohort entry date until January 1st 2007.

Data analysis

Baseline characteristics are described for all women and for never, and ever HT users by means and standard deviations for normally distributed continuous variables and frequencies and percentages for categorical variables. The person-time for each woman was calculated from the month of return of the baseline questionnaire to the month of diagnosis of CHD, the month of death from other causes, or the end of follow-up. The association between HT use and incident CHD, stratified for baseline presence of hot flushes, (night) sweats, and intense VMS was investigated using Cox regression models. In all analyses, never HT users were considered as the reference category. Models were stratified by center to control for differences in questionnaire design and other center effects. We started with fitting a crude model (model 1). Subsequently, we fitted models adjusted for age at enrollment, physical activity, smoking, educational level, menopausal status, and OCs use (model 2). We formally tested the effect modification by adding an interaction between HT use and VMS to the models containing the individual variables and the confounders. Results were considered statistically significant at 2-sided $P \leq 0.05$. All statistical analyses were performed using the Statistical Analysis System, version 9.1 (SAS Institute, Inc.).

RESULTS

The analyses are based on a mean follow-up time of 10.3 ± 2.1 years and comprised 91,310 person-years. In total, 46% of the women reported ever HT use, 51% reported symptoms of flushing, of which 13% had the highest frequency/severity, 36% reported symptoms of (night) sweats, 29% reported to have both symptoms, and 9% were classified as having intense VMS. The mean age at baseline was 53.8 ± 4.1 y (range 46 to 64) and was higher in the ever HT users than in the never users. While reports of flushing, hysterectomy and ovariectomy were higher in the ever HT users, the prevalence of (night) sweats, postmenopausal status and current OCs use was slightly lower among the ever HT users. The prevalences of current smoking, physically active, and low education was similar for never and ever HT users. During follow-up, a total of 252 women experienced an incident CHD event, of which 8 were fatal. (Table 1)

Compared with never HT use, ever HT use was neither associated with risk of CHD for women with symptoms of flushing (HR 1.14 [95% CI 0.78 to 1.66]) or (night) sweats (HR 0.97 [95% CI 0.65 to 1.44]) nor for women without symptoms of flushing (HR 1.12 [95% CI 0.76 to 1.64]) or (night) sweats (HR 1.25 [95% CI 0.87 to 1.81]). These results did not change when confounders were added to the model. The interactions between VMS and HT use were not statistically significant (P-values flushing: 0.65; (night) sweats: 0.15). (Table 2)

Next, we studied the role of intensity of VMS. In the crude analysis, ever HT use was associated with a borderline significant decreased CHD risk for women with intense VMS, with an HR of 0.48 [95% CI 0.22 to 1.03], as compared to never HT use. However, after adding potential confounders to the model the estimate remained similar, but the CI became wider: HR 0.49 [95% CI 0.20 to 1.19]. Among women without intense VMS, ever HT use was associated with a borderline significant increased CHD risk compared to never use (HR 1.28 [95% CI 0.96 to 1.70]). However, after multivariate adjustment, as compared to never

HT use, this association attenuated to non-significant (HR 1.23 [95% CI 0.90 to 1.67]). The test for interaction between ever HT use and intense VMS was statistically significant (P-value 0.03).

DISCUSSION

Our results suggest that in both groups of women with and without VMS, HT use does not seem to be associated with the risk of CHD.

The strengths of this study are its large size and the fact that it is population-based. Importantly, women in this study did not start HT to prevent CHD, but rather because the presence and severity of VMS provided a clinical indication for use. For the present analysis we pooled data from two cohorts and some differences in baseline characteristics were present. Although these differences were likely to be caused by the higher age in the WHILA population, we stratified our analyses for study center, in order to compensate for possible errors generated by differences in the questionnaire design and other study specific characteristics. There are a few issues regarding the measurement of VMS and HT that should be considered. First, VMS and HT were self reported and measured on only a single occasion. However, any misclassification is most likely to be non-differential with regard to CHD, most likely leading to a dilution of the true effect. Second, in WHILA, it is not entirely clear whether symptoms of sweats reflect menopausal sweats. However, the prevalence of sweats in WHILA was very similar to that of night sweats in EPOS, and sleep problems were more common among women with (night) sweats than in asymptomatic women. Therefore, it is probable that the women have truly reported menopausal sweats. Finally, the EPOS study obtained data on frequency of flushing and the WHILA study obtained data on severity of flushing. However, frequency and severity of hot flushes have earlier been found to be highly

correlated (r=0.97, p<0.001) [29]. Therefore, we expect frequency and severity of flushing to have similar associations with CHD.

We observed that HT use does at least not seem to be associated with an increased risk for CHD among women with VMS. This is not in agreement with the findings from the further exploratory analyses of the WHI [17] and the HERS [18] trials, where HT appeared to be associated with an increased risk of CHD among women with VMS [17, 18]. The different selection criteria for both studies could be an explanation for the differences in results. Our observational study reflected common clinical practice where women typically initiated HT because they experienced VMS. However, women recruited for randomization into the clinical trials had to be willing to start HT at the flip of a coin, and hence their decision was not driven by the presence and severity of VMS. Conceivably, the study populations in the clinical trials included only a small proportion of the symptomatic women, with most of them having a mild to moderate severity of symptoms. This is likely to have occurred by either the exclusion of women with severe VMS, since these symptoms could reduce adherence to placebo treatment; through self-selection, as the presence of severe VMS could be a reason for women not to participate in the trials because of the risk receiving a placebo. Another explanation could be the higher age of the women enrolled in the trials and the inclusion of women with a current history of CHD increasing the likelihood of an already existent (advanced) atherosclerosis. It has been suggested that a healthy endothelium is necessary for the beneficial effects of HT [30].

It could be argued that a healthy user bias could have explained our findings. However, frequency of treatment for hypertension, current smokers, physically active, and low educated women were similar for women using and not using HT. In addition, we adjusted for education, therefore, we consider this an unlikely explanation for our findings.

We should be very cautious not drawing too firm conclusions from our findings. Although they were obtained in a large population-based study, they are based on small numbers of cases, with sub-optimal collection of determinant information. However, the same holds for the opposite HERS findings, that were based on small numbers of women being classified as having "clinically significant" hot flushes, and a small number of incident CHD events in the 1st year of follow-up among those who had VMS (n=11), with only one event recorded among those in the placebo group [31]. Furthermore, the presence of VMS was ascertained at only one point in time (current VMS at baseline), while we describe HT use as 'ever use', and therefore the nexus in time between VMS and HT is uncertain. As a consequence, it remains difficult to disentangle the independent effects of HT use from VMS. On the other hand, a previous small study demonstrated that pre-treatment intolerable hot flushes were associated with a tendency toward more beneficial vascular responses in 70% of the markers [32]. Clearly, larger studies with more events and better determinant information collection will be needed to help elucidate the complex interplay of VMS, HT, and CHD.

In conclusion, our findings do not support the view that HT use increases the CHD risk for women with an indication, i.e. VMS. The conflicting findings between posthoc randomized controlled trials analyses and this observational study urgently calls for further research.

had no role in the design, management, data collection, analyses, or interpretation of the data or in the writing of the manuscript or the decision to submit for publication.

1. Grodstein F, Stampfer M. The epidemiology of coronary heart disease and estrogen replacement in postmenopausal women. *Prog Cardiovasc Dis*. 1995;**38**:199-210.

2. Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. *Ann Intern Med*. 2000;**133**:933-941.

3. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med*. 1991;**20**:47-63.

4. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA*. 1998;**280**:605-613.

5. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;**288**:321-333.

6. Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG. A prospective population-based study of menopausal symptoms. *Obstet Gynecol*. 2000;**96**:351-358.

7. Stearns V, Ullmer L, Lopez JF, Smith Y, Isaacs C, Hayes D. Hot flushes. *Lancet*. 2002;**360**:1851-1861.

8. Guthrie JR, Dennerstein L, Taffe JR, Donnelly V. Health care-seeking for menopausal problems. *Climacteric*. 2003;**6**:112-117.

9. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*. 2003;**362**:419-427.

10. Majumdar SR, Almasi EA, Stafford RS. Promotion and prescribing of hormone therapy after report of harm by the Women's Health Initiative. *JAMA*. 2004;**292**:1983-1988.

11. Treatment of menopause-associated vasomotor symptoms: position statement of The North American Menopause Society. *Menopause*. 2004;**11**:11-33.

12. Estrogen and progestogen use in peri- and postmenopausal women: March 2007 position statement of The North American Menopause Society. *Menopause*. 2007;**14**:168-182.

13. van der Schouw YT, Grobbee DE. Menopausal complaints, oestrogens, and heart disease risk: an explanation for discrepant findings on the benefits of post-menopausal hormone therapy. *Eur Heart J*. 2005;**26**:1358-1361.

14. Gast GC, Grobbee DE, Pop VJ, Keyzer JJ, Wijnands-van Gent CJ, Samsioe GN, Nilsson PM, van der Schouw YT. Menopausal complaints are associated with cardiovascular risk factors. *Hypertension*. 2008;**51**:1492-1498.

15. Gast GC, Samsioe GN, Grobbee DE, Nilsson PM, van der Schouw YT. Vasomotor symptoms, estradiol levels and cardiovascular risk profile in women. *Maturitas*. 2010.

16. Gast GC, Pop VJ, Samsioe GN, Grobbee DE, Nilsson PM, Keyzer JJ, Wijnands-van Gent CJ, van der Schouw YT. Vasomotor menopausal symptoms are associated with increased risk of coronary heart disease. *Menopause*. 2010.

17. Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, Ko M, LaCroix AZ, Margolis KL, Stefanick ML. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA*. 2007;**297**:1465-1477.

18. Huang AJ, Sawaya GF, Vittinghoff E, Lin F, Grady D. Hot flushes, coronary heart disease, and hormone therapy in postmenopausal women. *Menopause*. 2009;**16**:639-643.

19. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials*. 1998;**19**:61-109.

20. Grady D, Applegate W, Bush T, Furberg C, Riggs B, Hulley SB. Heart and Estrogen/progestin Replacement Study (HERS): design, methods, and baseline characteristics. *Control Clin Trials*. 1998;**19**:314-335.

21. Smeets-Goevaers CG, Lesusink GL, Papapoulos SE, Maartens LW, Keyzer JJ, Weerdenburg JP, Beijers LM, Zwinderman AH, Knottnerus JA, Pols HA, Pop VJ. The prevalence of low bone mineral density in Dutch perimenopausal women: the Eindhoven perimenopausal osteoporosis study. *Osteoporos Int*. 1998;**8**:404-409.

22. Samsioe G, Lidfeldt J, Nerbrand C, Nilsson P. The Women's Health in the Lund Area (WHILA) study - an overview. *Maturitas*. 2009;**doi:10.1016/j.maturitas.2009.11.009**.

23. Friedenreich C, Cust A, Lahmann PH, Steindorf K, Boutron-Ruault MC, Clavel-Chapelon F, Mesrine S, Linseisen J, Rohrmann S, Boeing H, Pischon T, Tjonneland A, Halkjaer J, Overvad K, Mendez M, Redondo ML, Garcia CM, Larranaga N, Tormo MJ, Gurrea AB, Bingham S, Khaw KT, Allen N, Key T, Trichopoulou A, Vasilopoulou E, Trichopoulos D, Pala V, Palli D, Tumino R, Mattiello A, Vineis P, Bueno-de-Mesquita HB, Peeters PH, Berglund G, Manjer J, Lundin E, Lukanova A, Slimani N, Jenab M, Kaaks R, Riboli E. Anthropometric factors and risk of endometrial cancer: the European prospective investigation into cancer and nutrition. *Cancer Causes Control*. 2007;**18**:399-413.

24. Gast GC, Grobbee DE, Pop VJ, Keyzer JJ, Wijnands-van Gent CJ, Samsioe GN, Nilsson PM, van der Schouw YT. Vasomotor symptoms are associated with a lower bone mineral density. *Menopause*. 2009;**16**:231-238.

25. Herings RM, Bakker A, Stricker BH, Nap G. Pharmaco-morbidity linkage: a feasibility study comparing morbidity in two pharmacy based exposure cohorts. *J Epidemiol Community Health*. 1992;**46**:136-140.

26. Merlo J, Lindblad U, Pessah-Rasmussen H, Hedblad B, Rastam J, Isacsson SO, Janzon L, Rastam L. Comparison of different procedures to identify probable cases of myocardial infarction and stroke in two Swedish prospective cohort studies using local and national routine registers. *Eur J Epidemiol*. 2000;**16**:235-243.

27. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation*. 1994;**90**:583-612.

28. Calltorp J, Adami HO, Astrom H, Fryklund L, Rossner S, Trolle Y, Giesecke J. Country profile: Sweden. *Lancet*. 1996;**347**:587-594.

29. Freeman EW, Sammel MD, Grisso JA, Battistini M, Garcia-Espagna B, Hollander L. Hot flashes in the late reproductive years: risk factors for Africa American and Caucasian women. *J Womens Health Gend Based Med*. 2001;**10**:67-76.

30. Holm P, Stender S, Andersen HO, Hansen BF, Nordestgaard BG. Antiatherogenic effect of estrogen abolished by balloon catheter injury in cholesterol-clamped rabbits. *Arterioscler Thromb Vasc Biol*. 1997;**17**:1504-1511.

31. Allison MA, Manson JE. The complex interplay of vasomotor symptoms, hormone therapy, and cardiovascular risk. *Menopause*. 2009;**16**:619-620.

32. Tuomikoski P, Mikkola TS, Tikkanen MJ, Ylikorkala O. Hot flushes and biochemical markers for cardiovascular disease: a randomized trial on hormone therapy. *Climacteric*. 2010;**13**:457-466.