Does climacteric status impact the regulation of the autonomic nervous system at the age of 46?

Authors: Satu Salin[1,2], Susanna Savukoski[1,2], Mikko Tulppo[3], Paula Pesonen[4], Juha Auvinen[2,5], Eila Suvanto[1,2], Katri Puukka[2,6,7], Maarit Niinimäki[1,2]

[1]Department of Obstetrics and Gynecology, PEDEGO Research Unit, Oulu University Hospital and University of Oulu, OYS, Finland; [2]Medical Research Centre Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland; [3]Research Unit of Biomedicine, Medical Research Center, Faculty of Medicine, University of Oulu and Oulu University Hospital, 90014 Oulu, Finland; [4]Infrastructure for Population Studies, Faculty of Medicine, University of Oulu, Oulu, Finland; [5]Centre for Life Course Health Research, University of Oulu, Oulu, Finland; [6]NordLab Oulu, Oulu University Hospital, Oulu, Finland; [7]Department of Clinical Chemistry, University of Oulu, Oulu, Finland.

Corresponding author: Satu Salin (satu.salin@student.oulu.fi). Address Kajaanintie 50, 90220 Oulu, Finland. Phone +358445699912.

Reprint request: to Satu Salin (satu.salin@student.oulu.fi).

Abstract

Objective

To investigate whether an earlier-onset climacteric phase is associated with autonomic imbalance at the age of 46.

Methods

This cross-sectional birth cohort study included 2,661 46-year-old women. Participants were divided into climacteric (n=359) and preclimacteric (n=2,302) groups based on menstrual history and follicle-stimulating hormone (FSH) values. Mean heart rate (HR), low frequency (LF) power, high frequency (HF) power, and the LF/HF ratio were analyzed from heart rate variability (HRV) recordings. The variables were compared between the groups using multivariable linear regression models, including body mass index (BMI), smoking, and physical activity (PA). The effects of hormone therapy (HT) and hot flashes on autonomic function were evaluated in subanalyses.

Results

Climacteric women had a lower mean HR in seated (71.9 ± 10.5 vs 72.6 ± 10.4 bpm, P = 0.015) and standing (81.2 ± 12.8 vs 83.6 ± 12.1 bpm, P = 0.002) positions compared to preclimacteric women, and the differences remained significant after the adjustments. In the subanalyses, more frequent hot flashes were associated with a lower LF power and LF/HF ratio in the sitting position.

Conclusions

The present study suggested an association between greater parasympathetic activation in women with more advanced climacteric status at the age of 46.

Keywords: autonomic function; heart rate variability; hot flashes; early menopause; menopause

Introduction

Climacterium is a normal phase of aging that starts from the decline in ovarian function and lasts until ovarian activity ends [1]. This phase includes the last menstrual period, called menopause, which occurs in Western countries at a median age of 50–51 years [2]. Early menopause (EM) has been defined as a condition in which menopause occurs at the age of 40–44 [3]. Menopause before the age of 40 is termed premature ovarian insufficiency (POI) [4]. After menopause, endogenous estrogen synthesis declines, which contributes to an increased risk for cardiovascular diseases (CVDs) [5,6]. Women with EM and POI have an increased CVD risk and mortality [7,8], which may be due to the early cessation of ovarian estrogen production [9].

Studies have shown that ovarian hormones, and estrogen especially, have a beneficial influence on the autonomic nervous system (ANS), which regulates cardiovascular function. Estrogen reduces the sympathetic drive and increases parasympathetic activity [10,11]. In menopausal transition, the autonomic balance is shifted toward sympathetic hyperactivity, which increases the risk for CVDs [12,13]. One of the non-invasive methods used to assess autonomic imbalance and risk of diseases and mortality is heart rate variability (HRV) [14,15]. Hormonal changes after the average age of menopause, the aging process, and physical inactivity are all associated with modifications in autonomic cardiovascular control, such as a reduction in HRV [13,16,17]. The evidence on the effects of hormone therapy (HT) on autonomic cardiovascular control is contradictory. In some studies, HT increased HRV [10,18], but in others there was no effect [19–21].

In previous studies, earlier climacterium has been associated with unfavorable metabolic changes and impaired insulin sensitivity [22,23]. However, the association between autonomic cardiovascular control and earlier menopausal transition has been less studied. In the current population-based study, we aimed to investigate whether an earlier-onset climacteric phase was associated with autonomic imbalance by analyzing HRV in study groups divided by climacteric status at the age of 46.

Material and methods

Study population

The Northern Finland Birth Cohort (NFBC1966) is a large, prospective, population-based birth cohort that comprises 12,058 live births in 1966 in the Northern Finnish provinces of Oulu and Lapland [24]. The cohort covers 96.3% of all live births of the mothers in the cohort with an estimated date of delivery during 1966 in the two provinces [25]. Members of the cohort have been followed since the antenatal period through age 46 with questionnaires and clinical examinations. During adulthood, the latest data collection point has been at the age of 46. Postal questionnaires and invitations to clinical examination were sent to every living cohort member with a known address. Menstrual history, smoking, level of education, marital status, and frequency of hot flashes were queried in the questionnaire. Clinical examinations at the age of 46 included the assessment of cardiovascular health status and the evaluation of HRV. Also, weight, height, and serum follicle-stimulating hormone (FSH) values were measured during the examinations. The NFBC1966 data have been linked to several Finnish registers and databases, and hence medication purchases, based on the Social Insurance

Institution of Finland's statistics on Reimbursements for Prescription Medicines, were available.

Study groups

The flow chart of the study is shown in Figure 1. Based on menstrual history and FSH values, the female cohort members who participated in the 46-year follow-up were divided into climacteric (late perimenopausal and postmenopausal women) and preclimacteric (premenopausal and early perimenopausal women) groups. The Stages of Reproductive Aging Workshop +10 staging system criteria for menopausal transition were used to divide the groups as there are no general criteria for climacterium [26]. The criteria for the climacteric group were as follows: (1) FSH value >25 IU/L, and (2) time since last menstrual period ≥60 days. The criteria for the preclimacteric group were the following: (1) FSH value ≤25 IU/L, and (2) time since last menstrual period <60 days. The immunochemiluminometric method (Advia Centaur XP; Siemens Healthcare Diagnostics Inc., Tarrytown, NY) was used to determine the FSH values.

Women were classified by only the FSH value if they were hysterectomized or had progestin-only treatment (peroral, capsule, intrauterine device). We excluded combined estrogen-progestin contraceptive pill or ring users and tamoxifen users as well as women with a discrepancy between FSH values and menstrual anamnesis. Systemic HT users were found using national registers for prescription medications, and they were included in the climacteric group. A participant was classified as an HT user if she purchased systemic HT (Anatomical Therapeutic Chemical [ATC] codes starting with G03C and G03F) during the year prior to the 46-year clinical examination. Women who did not give permission to use

their medication register data were excluded. Participants using beta-blockers (ATC codes starting with C07) were also identified from the medicine reimbursement register and were excluded from the HRV analysis.

[Figure 1 near here]

Evaluation of cardiac autonomic function

We evaluated cardiovascular autonomic function by means of HRV at the 46-year clinical examination. To assess HRV, an HR monitor (RS800CK; Polar Electro Oy, Kempele, Finland) was used to record R-R intervals (RRi) to an accuracy of 1 ms. After preparations, participants' HR was allowed to stabilize for 1 min in a seated position before the first 3-min recording was made, also while seated. Then, the participant actively stood up and the second 3-min recording was made while standing up.

HRV analysis

We analyzed the first 150 s of the sitting data and the last 150 s of the standing data. Based on visual inspections, the RRi data were edited, and artefacts and ectopic beats were removed and replaced by the local average (Hearts 1.2; University of Oulu, Oulu, Finland). Sequences with ≥10 consecutive beats of noise or ectopic beats were deleted. The RRi series with ≥80% accepted data were included in the analyses. Mean HR (bpm), low frequency (LF: 0.04–0.15 Hz, ms^2) power, and high frequency (HF: 0.15–0.40 Hz, ms^2) power, as well as their ratio (LF/HF), were analyzed from the data. HF power is considered as a marker of parasympathetic activity, whereas LF power is mainly considered as a marker of sympathetic

activity, but also parasympathetic activity and other factors can affect LF power [15,27,28]. The LF/HF ratio has been used to estimate the sympatho-vagal balance, but the complex origin of LF power makes conclusions limited [28].

Cardiorespiratory fitness

At the 46-year examination, the participants also performed a submaximal 4-min single-step test to measure their cardiorespiratory fitness (CRF), thereby permitting the evaluation of autonomic function. Stepping was performed without shoes on a 33-cm-high bench with a stepping rate of 23 ascents per min paced by a metronome [29]. HR was measured 90 s after (HR_{FINAL}) stepping in a seated position (RS800CX). Of the study groups, 251 climacteric women and 1,771 preclimacteric women successfully performed the test.

Covariates

In previous studies, higher physical activity (PA) [29,30], more optimal body mass index (BMI) [29,31], and non-smoking [32] have been associated with better autonomic function as measured by HRV. In the multivariable adjusted linear model, these potential contributing factors were taken into account in the analyses. PA was measured with a wrist-worn Polar Active device (Polar Electro Oy, Kempele, Finland), which participants were asked to wear continuously for at least 14 days on the nondominant wrist. PA was calculated as metabolic equivalent of task (MET) scores in min per day based on the frequency and duration of PA over the entire measured period. BMI values were divided into three classes: ≤25.0, 25.01–30.0, >30.01 kg/m^2. Based on the questionnaire, participants were classified as current non-smokers or smokers.

Statistical analyses

Pearson's chi-square test and a Mann-Whitney U-test were used to compare the distribution of background variables between climacteric and preclimacteric women. To compare HRV variables between the study groups, an independent-samples t-test was used for distributed continuous variables and a Mann-Whitney U-test for skewed continuous variables. We also calculated Benjamini-Hochberg adjusted P-values to evaluate the possibility that our significant findings occurred by chance. Multivariable generalized linear models were used to investigate the association between autonomic function and climacteric status at age 46. In the models, HRV variables were dependent variables and climacteric status, PA, BMI, and smoking were independent variables. For the analysis, a logarithmic transformation was conducted to normalize the distribution of HRV variables with a skewed distribution (HF, LF, LF/HF).

To evaluate the effects of HT and hot flashes on autonomic cardiovascular control, we performed subanalyses in which we included only the climacteric participants. All HRV variables were first compared between climacteric HT users and nonusers with an independent-samples t-test or a Mann-Whitney U-test. Two separate multivariable generalized linear models were also executed. In the first model, HRV variables were dependent variables, and the use of HT, PA, BMI, and smoking were independent variables. In the second model, HRV variables were dependent variables and frequency of hot flashes, PA, BMI, and smoking were independent variables.

Two-way interaction terms were tested in all of the generalized linear models, and only the significant interactions were included in the final models. IBM SPSS Statistics for Windows,

Version 24.0 (IBM, Armonk, NY) was used for analyses. P-values of <0.05 were considered statistically significant.

Results

The final study population consisted of 336 climacteric women and 2,146 preclimacteric women (Fig. 1). Background characteristics of the study population are shown in Table 1. Climacteric women were significantly more often current smokers (22.9% vs 16.5%, P = 0.006), and a higher proportion of them reported that they had hot flashes sometimes or more often (34.2% vs 10.1%, P < 0.001). Other baseline characteristics did not differ between the study groups.

[Table 1 near here]

The results of the HRV variables between the study groups are shown in Table 2. Climacteric women had a significantly lower mean HR in the seated position (HR_{SIT}, 71.9 ± 10.5 vs 72.6 ± 10.4 bpm, P = 0.015) and the standing position (HR_{STAND}, 81.2 ± 12.8 vs 83.6 ± 12.1 bpm, P = 0.002) compared to preclimacteric women. The Benjamini-Hochberg adjusted P-value was significant in the standing position (HR_{STAND}, P* = 0.018). Other variables did not differ between the groups.

[Table 2 near here]

In the multivariate linear regression model of HRV variables, climacteric status, smoking, BMI, and PA were used as independent variables. Being climacteric was associated with a

224 significantly lower mean HR in the seated position (HR_{SIT}, adjusted β coefficient = -1.56,
225 95% CI -2.83 to -0.288, P = 0.016) and the standing position (HR_{STAND}, adjusted β coefficient
226 = -2.43, 95% CI -3.94 to -0.917, P = 0.002) compared to preclimacteric women. A BMI
227 ≥30.01 was associated with higher HR_{SIT} and HR_{STAND} and higher PA with lower HR_{SIT} and
228 HR_{STAND}. The results are shown in Figure 2. Other HRV variables did not differ between the
229 groups and were not associated with climacteric status (data not shown).
230
231 [Figure 2 near here]
232
233 In the subanalyses, only the climacteric participants were included. HRV variables did not
234 significantly differ between climacteric HT users and nonusers (data not shown). In the first
235 multivariate linear regression model of HRV variables, use of HT, smoking, BMI, and PA
236 were used as independent variables. Use of HT was not significantly associated with any of
237 the HRV variables (data not shown). The second model consisted of hot flashes, smoking,
238 BMI, and PA as independent variables. More frequent hot flashes were associated with
239 significantly lower LF power in the seated position (LF_{SIT}, adjusted β coefficient = -0.565,
240 95% CI -1.10 to -0.026, P = 0.040) and LF/HF ratio in the seated position (LF/HF_{SIT}, adjusted
241 β coefficient = -0.625, 95% CI -1.10 to -0.147, P = 0.010) (Fig. 3).
242
243 [Figure 3 near here]
244
245 **Discussion**
246
247 In this population-based cohort study, the HRV variables were mainly similar between the
248 groups. However, women who were climacteric at the age of 46 had a lower mean HR in both

seated and standing positions, suggesting increased parasympathetic activity compared to preclimacteric women. The difference remained significant after adjusting for BMI, smoking, and physical activity. Earlier studies concerning the associations between menopause and ANS regulation were mainly performed among women women who had reached the natural age of menopause, in their 50s [13,16,33,34].

Autonomic imbalance has been suggested to play a role in menopausal hot flashes, but the relationship between ANS regulation and hot flashes is not totally understood [35]. Studies have reported decreases in parasympathetic activity during hot flashes [36,37], but increases in sympathetic activation have also been observed [38,39]. In the present study, more frequent hot flashes were associated with a lower LF power and LF/HF ratio in the sitting position in the climacteric group, suggesting decreased sympathetic activity and parasympathetic dominance. Greater parasympathetic activation with more frequent and severe hot flashes among perimenopausal and postmenopausal women have also been previously reported [40]. However, some studies have not found an association between resting autonomic function and hot flash frequency or severity [38,41].

Contrary to our findings, earlier studies have associated menopause at around the age of 50 with a decrease in HRV, which is related to sympathetic hyperactivity and a reduction in parasympathetic activity [13,33]. A decline in estrogen during climacterium has been suggested to be a contributing factor to these findings [10,11,34], and attenuated HRV is especially seen after surgical menopause when estrogen suddenly decreases [12]. However, the autonomic balance shifts toward sympathetic activity also during the physiological aging process [42], and the role of menopause remains unclear [16]. Some evidence even suggests that the aging process itself explains these findings rather than estrogen deprivation [43]. In

another cross-sectional study, there was no clinically important relationship between cardiovascular autonomic control and menopausal status [44]. Compared to our study, the women were older, and the age difference was significant between premenopausal and postmenopausal women.

In the subanalysis of the study, use of HT was not associated with HRV variables in the climacteric group. Some earlier studies have reported beneficial effects of HT on HRV [10,18,33], but other studies have reported no effect [19,20,45]. Different study methods and HT regimens could partly explain these conflicting findings. Also, some studies were placebo-controlled [45], whereas others were not [18]. The association of HT and ANS function was not the main focus of this study, and due to the low number of HT users, the comparison of different regimens and doses was not possible.

There were several strengths in our study. We had a large sample of climacteric and preclimacteric women who took part in the RRi recordings, and multiple HRV variables were analyzed. Climacteric status was determined with both FSH values and menstrual history, and the use of hormonal preparations was also taken into account. Reliable sources were used to retrieve information on HT and beta-blocker use, as we used nationwide registry data. It is known that HRV is affected by aging [16,42,46], PA [29,30,47], BMI [29,31], and smoking [32]. All of our study participants were at the same age, and PA, BMI, and smoking were included in the adjusted models. PA was comprehensively investigated with a wrist-worn device. Many earlier studies investigating the regulation of the ANS and menopause did not take all of these contributing factors into account [13,16,33,34].

A limitation of this study was the cross-sectional setting, as the evaluation of HRV was conducted only once, at the age of 46. Also, some female participants from the original cohort were lost to follow-up. However, we believe that participation was not affected by menopausal status. Changes in body functions during menopausal transition are mainly mediated by decreasing estrogen levels. Unfortunately, estrogen levels were not measured in this study. We suggest that estrogen levels may have been variable but mainly low in the climacteric group. As adverse changes in cardiovascular risk factors already occur during menopausal transition [48], we found it relevant to investigate ANS function in this study setting. In our study, HRV parameters were used to assess cardiac autonomic function, but it should be considered that other measures are also used to investigate dysfunction in the ANS [49]. Also, we did not study the regulation of peripheral vascular tone, which would have yielded additional information about autonomic function [50]. For the HRV analysis, a longer recording time would have been more optimal, although our 150-s recording period should be reliable [15]. In addition, there may be other contributing factors affecting HRV that could not all be taken into account in the analyses.

Conclusions

Unlike earlier studies investigating the association between the ANS and menopause, we found no association between sympathetic nervous system activity and climacteric status. Our study consisted of women in their mid-40s, while previous studies have investigated the ANS in women facing menopause around the age of 50. Despite the mainly similar findings between the groups, some findings suggested parasympathetic dominance in women who were perimenopausal or postmenopausal at the age of 46. Greater parasympathetic activation was also seen in climacteric women who had more frequent hot flashes. The connection between

ANS regulation and menopause is complex and affected by several confounding factors, also among women facing menopause in their mid-40s. The present study provides novel information about the association between early-onset climacteric transition and regulation of the ANS and enhances understanding of the complex physiology of menopausal symptoms.

Acknowledgments

We thank the late professor Paula Rantakallio, the NFBC1966 participants in the 46-year follow-up study, and the Northern Finland Birth Cohort (NFBC) Project Centre.

Declaration of interest

The authors declare no potential conflicts of interest.

Source of funding

Satu Salin, Mikko Tulppo, Paula Pesonen, Juha Auvinen, Eila Suvanto, Katri Puukka, and Maarit Niinimäki received no financial support for the research, authorship, and/or publication of this article. Susanna Savukoski received a grant from the Finnish Menopause Society, the Finnish Medical Foundation, and the Juho Vainio Foundation.

References

[1] Utian WH. Ovarian function, therapy-oriented definition of menopause and climacteric. Exp Gerontol. 1994;29:245–251.

[2] TeVelde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8:141–154.

[3] Mishra GD, Pandeya N, Dobson AJ, et al. Early menarche, nulliparity and the risk for premature and early natural menopause. Human Reprod. 2017;32:679–686.

[4] DeVos M, Devroey P, J M Fauser BC. Primary ovarian insufficiency. Lancet. 2010;376:911–921.

[5] de Kleijn MJJ, van der Schouw YT, Verbeek ALM, et al. Endogenous estrogen exposure and cardiovascular mortality risk in postmenopausal women. Am J Epidemiol. 2002;155:339–345.

[6] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999;340:1801–1811.

[7] Muka T, Oliver-Williams C, Kunutsor S, et al. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: A systematic review and meta-analysis. JAMA Cardiol. 2016;1:767–776.

[8] Zhu D, Chung HF, Dobson AJ, et al. Type of menopause, age of menopause and variations in the risk of incident cardiovascular disease: Pooled analysis of individual data from 10 international studies. Hum Reprod. 2020;35:1933–1943.

[9] Shuster LT, Rhodes DJ, Gostout BS, et al. Premature menopause or early menopause: Long-term health consequences. Maturitas. 2010;65:161–166.

[10] Huikuri H v, Pikkujämsä SM, Airaksinen KE, et al. Sex-Related Differences in Autonomic Modulation of Heart Rate in Middle-aged Subjects. Circulation. 1996;94:122–125.

[11] Dart AM, Du X-J, Kingwell BA. Gender, sex hormones and autonomic nervous control of the cardiovascular system. Cardiovasc Res. 2002;53:678–687.

[12] Mercuro G, Podda A, Pitzalis L, et al. Evidence of a Role of Endogenous Estrogen in the Modulation of Autonomic Nervous System. Am J Cardiol. 2000;85:787–789.

[13] Brockbank CL, Chatterjee F, Bruce SA, et al. Heart Rate and its Variability Change After the Menopause. Exp Physiol. 2000;85:327–330.

[14] Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141:122–131.

[15] Task force of the European Society of cardiology and the North American Society of pacing and electrophysiology. Heart Rate Variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93:1043–1065.

[16] Lavi S, Nevo O, Thaler I, et al. Effect of aging on the cardiovascular regulatory systems in healthy women. Am J Physiol Regul Integr Comp Physiol Physiol. 2007;292:R788–R793.

[17] Davy KP, Desouza CA, Jones PP, et al. Elevated Heart Rate Variability in Physically Active Young and Older Adult Women. Clin Sci. 1998;94:579–584.

[18] Yildirir A, Kabakci G, Yarali H, et al. Effects of Hormone Replacement Therapy on Heart Rate Variability in Postmenopausal Women. Ann Noninvasive Electrocardiol. 2001;6:280–284.

[19] Leo Niskanen L, Laitinen T, Tuppurainen M, et al. Does postmenopausal hormone replacement therapy affect cardiac autonomic regulation in osteoporotic women? Menopause. 2002;9:52–57.

[20] Carnethon MR, Anthony MS, Cascio WE, et al. Prospective association between hormone replacement therapy, heart rate, and heart rate variability: The Atherosclerosis Risk in Communities Study. J Clin Epidemiol. 2003;56:567–571.

[21] Lantto H, Haapalahti P, Tuomikoski P, et al. Vasomotor hot flashes and heart rate variability: A placebo-controlled trial of postmenopausal hormone therapy. Menopause. 2012;19:82–88.

[22] Savukoski S, Mäkelä H, Auvinen J, et al. Climacteric status at the age of 46: impact on metabolic outcomes in population-based study. J Clin Endocrinol Metab. 2019;104:2701–2711.

[23] Savukoski SM, J Suvanto ET, Auvinen JP, et al. Onset of the climacteric phase by the mid-forties associated with impaired insulin sensitivity: a birth cohort study. Menopause. 2020;28:70–79.

[24] University of Oulu. Northern Finland Birth Cohort 1966 [Internet]. [cited 2022 Feb 3]. Available from: http://urn.fi/urn:nbn:fi:att:bc1e5408-980e-4a62-b899-43bec3755243.

[25] Rantakallio P. Groups at risk in low birth weight infants and perinatal mortality. Acta Paediatr Scand. 1969;193:1–71.

[26] Harlow SD, Gass M, Hall JE, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: Addressing the unfinished agenda of staging reproductive aging. Menopause. 2012;19:387–395.

[27] Goldstein DS, Bentho O, Park MY, et al. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Exp Physiol. 2011;96:1255–1261.

[28] Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol. 2013;4:26.

[29] Teisala T, Mutikainen S, Tolvanen A, et al. Associations of physical activity, fitness, and body composition with heart rate variability-based indicators of stress and recovery on workdays: A cross-sectional study. J Occup Med Toxicol. 2014;9:16.

[30] Felber Dietrich D, Ackermann-Liebrich U, Schindler C, et al. Effect of physical activity on heart rate variability in normal weight, overweight and obese subjects: Results from the SAPALDIA study. Eur J Appl Physiol. 2008;104:557–565.

[31] Karason K, Mølgaard H, Wikstrand J, et al. Heart Rate Variability in Obesity and the Effect of Weight Loss. Am J Cardiol. 1999;83:1242–1247.

[32] Dinas PC, Koutedakis Y, Flouris AD. Effects of active and passive tobacco cigarette smoking on heart rate variability. Int J Cardiol. 2013;163:109–115.

[33] Rosano GMC, Patrizi R, Leonardo F, et al. Effect of Estrogen Replacement Therapy on Heart Rate Variability and Heart Rate in Healthy Postmenopausal Women. Am J Cardiol. 1997;80:815–817.

[34] Akiyoshi M, Kato K, Owa Y, et al. Relationship between estrogen, vasomotor symptoms, and heart rate variability in climacteric women. J Med Dent Sci. 2011;58:49–59.

[35] Hautamäki H, Piirilä P, Haapalahti P, et al. Cardiovascular autonomic responsiveness in postmenopausal women with and without hot flushes. Maturitas. 2011;68:368–373.

[36] Thurston RC, Christie IC, Matthews KA. Hot flashes and cardiac vagal control: A link to cardiovascular risk? Menopause. 2010;17:456–461.

[37] Thurston RC, Christie IC, Matthews KA. Hot flashes and cardiac vagal control during women's daily lives. Menopause. 2012;19:406–412.

[38] Hoikkala H, Haapalahti P, Viitasalo M, et al. Association between vasomotor hot flashes and heart rate variability in recently postmenopausal women. Menopause. 2010;17:315–320.

[39] Freedman RR, Kruger ML, Wasson SL. Heart rate variability in menopausal hot flashes during sleep. Menopause. 2011;18:897–900.

[40] Gibson CJ, Mendes WB, Schembri M, et al. Cardiac autonomic function and hot flashes among perimenopausal and postmenopausal women. Menopause. 2017;24:756–761.

[41] Jones SMW, Guthrie KA, LaCroix AZ, et al. Is heart rate variability associated with frequency and intensity of vasomotor symptoms among healthy perimenopausal and postmenopausal women? Clin Auton Res. 2016;26:7–13.

[42] Pikkujämsä SM, Mäkikallio TH, Sourander LB, et al. Cardiac Interbeat Interval Dynamics From Childhood to Senescence Comparison of Conventional and New Measures Based on Fractals and Chaos Theory. Circulation. 1999;100:393–399.

[43] Tezini GCSV, Becari C, Zanotto CZ, et al. Ageing is the main determinant of haemodynamics and autonomic cardiac changes observed in post-menopausal female rats. Auton Neurosci. 2013;174:36–41.

[44] Neufeld IW, Kiselev AR, Karavaev AS, et al. Autonomic control of cardiovascular system in pre- and postmenopausal women: a cross-sectional study. J Turk Ger Gynecol Assoc. 2015;16:11–20.

[45] Hautamäki H, Mikkola TS, Sovijärvi ARA, et al. Menopausal hot flushes do not associate with changes in heart rate variability in controlled testing: A randomized trial on hormone therapy. Acta Obstet Gynecol Scand. 2013;92:902–908.

[46] Stein PK, Kleiger RE, Rottman JN. Differing effects of age on heart rate variability in men and women. Am J Cardiol. 1997;80:302–305.

[47] Tulppo MP, Hautala AJ, Mäkikallio TH, et al. Effects of aerobic training on heart rate dynamics in sedentary subjects. J Appl Physiol. 2003;95:364–372.

[48] el Khoudary SR, Aggarwal B, Beckie TM, et al. Menopause Transition and Cardiovascular Disease Risk: Implications for Timing of Early Prevention: A Scientific Statement From the American Heart Association. Circulation. 2020;142:506–532.

[49] Bernardi L, Spallone V, Stevens M, et al. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab Res Rev. 2011;27:654–664.

[50] Allen J. Photoplethysmography and its application in clinical physiological measurement. Physiol Meas. 2007;28:R1–R39.

Table 1. Background characteristics of the study population.

	Climacteric	Preclimacteric	P-value
BMI (kg/m^2) (N, %)			0.786[a]
≤25.0	154 (48.3)	1,010 (47.2)	
25.01–30.0	106 (33.2)	698 (32.6)	
≥30.01	59 (18.5)	432 (20.2)	
Smoking (N, %)			0.006[a]
Non-smoker	249 (77.1)	1,729 (83.5)	
Current smoker	74 (22.9)	341 (16.5)	
Physical activity in MET (min/d) (Median, IQR)	56.1 (39.4–74.9)	57.4 (40.8–77.1)	0.233[b]
Education (N, %)			0.124[a]
Basic	24 (7.3)	105 (5.0)	
Secondary	216 (65.3)	1,328 (63.6)	
Tertiary	91 (27.5)	655 (31.4)	
Marital status (N, %)			0.892[a]
Unmarried	31(9.4)	200 (9.6)	
Married/domestic partnership	264 (79.8)	1,629 (78.2)	
Divorced	34 (10.3)	243 (11.7)	
Widow	2 (0.6)	11 (0.5)	
Frequency of hot flashes (N, %)			<0.001[a]
Never to rarely	221 (65.8)	1,929 (89.9)	
Sometimes to often	115 (34.2)	217 (10.1)	

IQR, interquartile range; MET, metabolic equivalent of task.

[a] Pearson's chi-square test. [b] Mann-Whitney U-test.

480 **Table 2.** Heart rate variability (HRV) variables in climacteric and preclimacteric women in
481 seated and standing positions.

Outcome		N	Mean ± SD/median [IQR]	P-value	P-value*
HR_{SIT} (bpm)	Climacteric	300	71.9 ± 10.5	0.015[a]	0.068
	Preclimacteric	2,030	72.6 ± 10.4		
HR_{STAND} (bpm)	Climacteric	300	81.2 ± 12.8	0.002[a]	0.018
	Preclimacteric	2,030	83.6 ± 12.1		
LF_{SIT} (ms^2)	Climacteric	300	301 [165–599]	0.682[b]	0.877
	Preclimacteric	2,030	301 [158–552]		
LF_{STAND} (ms^2)	Climacteric	300	200 [111–412]	0.459[b]	0.826
	Preclimacteric	2,030	205 [107–370]		
HF_{SIT} (ms^2)	Climacteric	300	241 [110–576]	0.930[b]	0.930
	Preclimacteric	2,030	258 [113–550]		
HF_{STAND} (ms^2)	Climacteric	300	95 [42.0–190]	0.178[b]	0.401
	Preclimacteric	2,030	78 [33.0–178]		
LF/HF_{SIT}	Climacteric	300	1.22 [0.74–2.27]	0.811[b]	0.912
	Preclimacteric	2,029	1.22 [0.70–2.29]		
LF/HF_{STAND}	Climacteric	300	2.47 [1.47–4.50]	0.150[b]	0.450
	Preclimacteric	2,029	2.70 [1.53–4.86]		
HR_{FINAL} (bpm)	Climacteric	251	149 ± 15.5	0.627[a]	0.941
	Preclimacteric	1,884	149 ± 14.7		

482 HF, high frequency (0.15–0.4 Hz) power; HR, heart rate; IQR, interquartile range; LF, low
483 frequency (0.04–0.15 Hz) power; SD, standard deviation.
484 [a] Independent-samples t-test. [b] Mann-Whitney U-test. *Benjamini-Hochberg adjusted P-value.

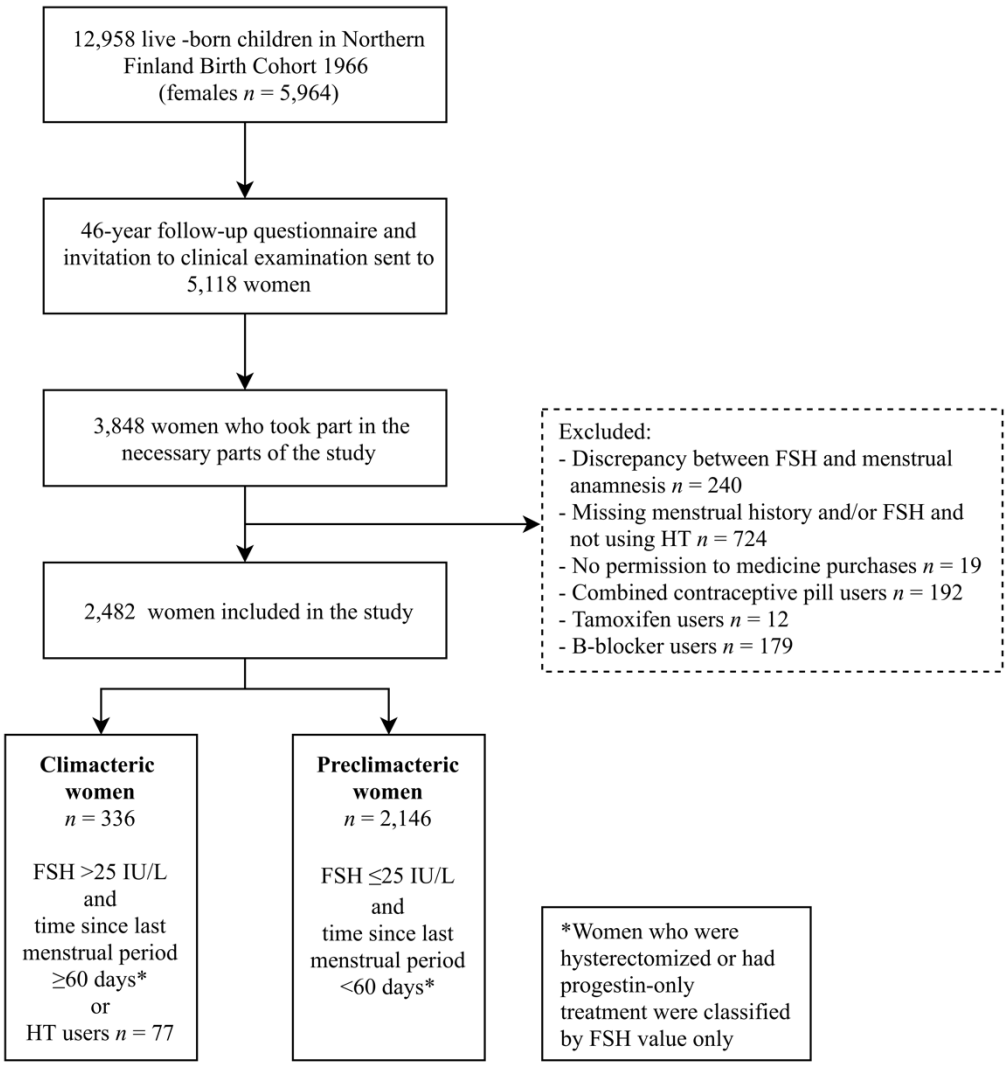

Figure 1. Flow chart of the study population. FSH, follicle-stimulating hormone; HT, hormone therapy.

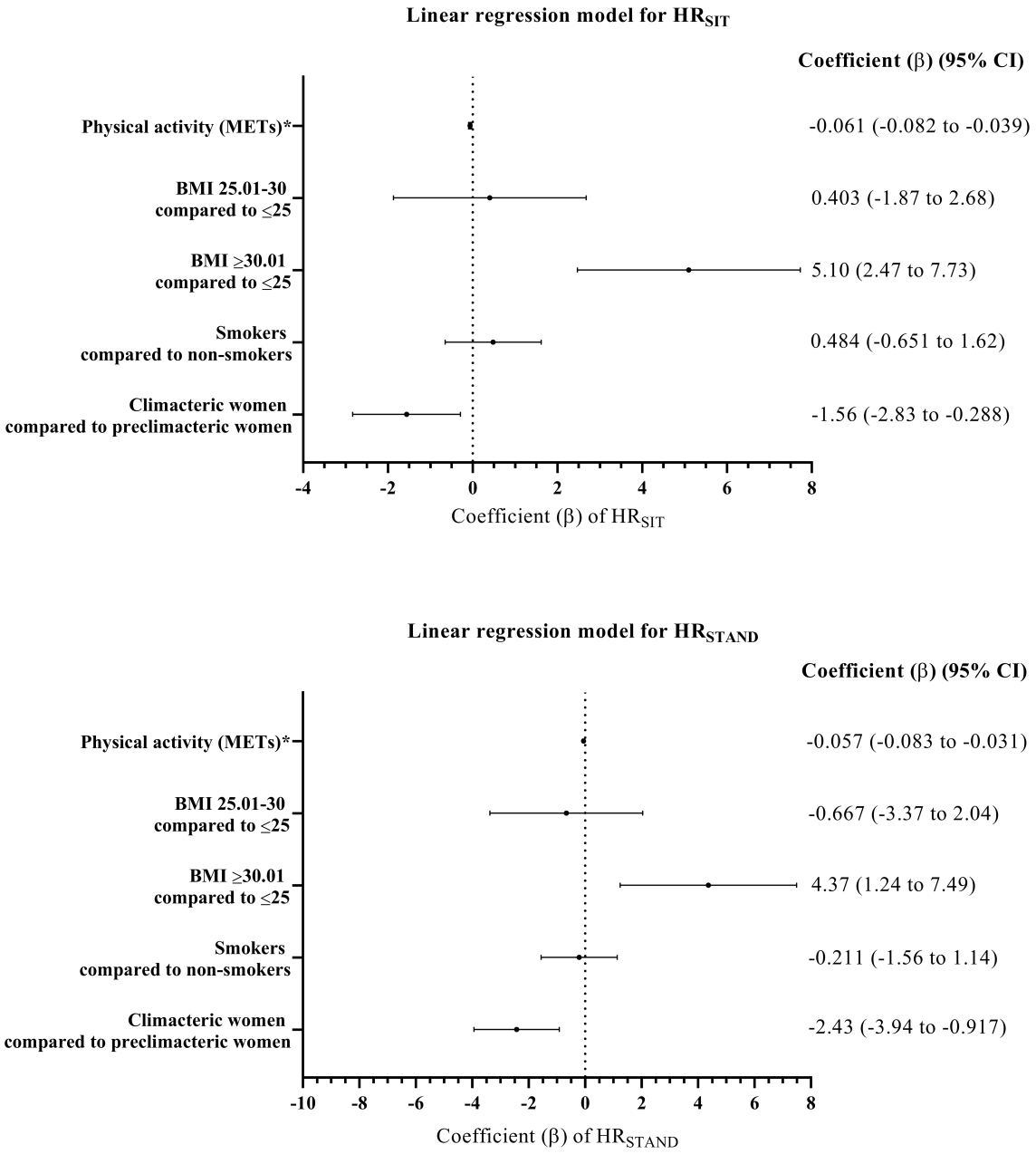

Figure 2. Mean HR in climacteric and preclimacteric women in seated and standing positions. In the multivariable linear regression models, HR measurements were the dependent variables, and climacteric status, smoking, BMI, and physical activity were the independent variables. Physical activity in MET (min/d) served as the continuous variable and is marked with *. Interactions included in the final model of HR_{SIT}: BMI*physical activity.

494 Interactions included in the final model of HR_{STAND}: BMI*physical activity. BMI, body mass

495 index; CI, confidence interval; HR, heart rate; MET, metabolic equivalent of task.

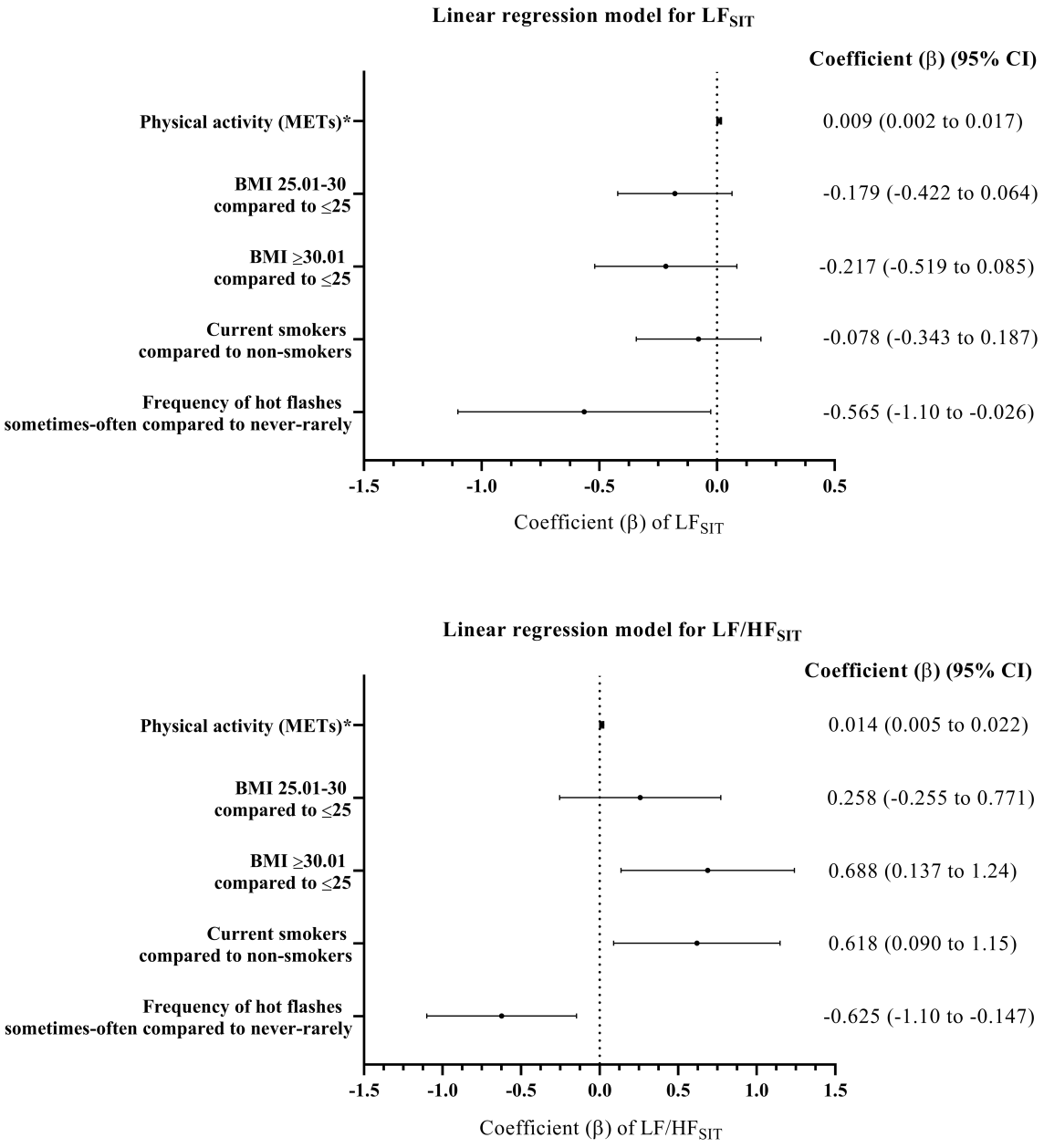

Figure 3. LF and LF/HF ratio in climacteric women in the seated position. In the multivariate linear regression models, LF and LF/HF measurements were the dependent variables, and frequency of hot flashes, smoking, BMI, and physical activity were the independent variables. Physical activity in MET (min/d) served as the continuous variable and is marked with *. Interactions included in the final model of LF_{SIT}: Hot flashes*physical activity. Interactions included in the final model of LF/HF_{SIT}: Hot flashes*physical activity, smoking*physical

activity, BMI*physical activity. BMI, body mass index; CI, confidence interval; HF, high frequency (0.15–0.4 Hz) power; LF, low frequency (0.04–0.15 Hz) power; MET, metabolic equivalent of task.