Onset of the climacteric phase by the mid-forties associated with impaired insulin sensitivity: a birth cohort study

Running title: Climacteric status and glucose at mid-40s.

Susanna M. Savukoski, MD[1,2], Eila TJ. Suvanto, MD[1,2], Juha P. Auvinen, MD[2,3,4], Paula RO. Pesonen, MSc[5], Sirkka M. Keinänen-Kiukaanniemi, MD[3], Katri S. Puukka, PhD[2,6,7], Tapani Ebeling, MD[2,8], and Maarit J. Niinimäki, MD[1,2]

[1]Department of Obstetrics and Gynaecology, PEDEGO Research Unit, Oulu University Hospital and University of Oulu, 90029 OYS, Finland [2]Medical Research Centre Oulu, Oulu University Hospital and University of Oulu, 90014 Oulu, Finland [3]Centre for Life Course Health Research, University of Oulu, 90014 Oulu, Finland [4]Oulunkaari Health Centre, Ii, Finland [5]Infrastructure for Population Studies, Faculty of Medicine, University of Oulu, 90014 Oulu, Finland [6]NordLab Oulu, Oulu University Hospital, 90029 Oulu, Finland [7]Department of Clinical Chemistry, University of Oulu, 90014 Oulu, Finland [8]Department of Internal Medicine, Oulu University Hospital and University of Oulu, 90029 OYS, Finland

Sources of funding: Juha Auvinen, Paula Pesonen, Sirkka Keinänen-Kiukaanniemi, Katri Puukka, Tapani Ebeling and Eila Suvanto received no financial support for the research, authorship, and/or publication of this article. Maarit Niinimäki received a research grant from the University of Oulu. Susanna Savukoski received a research grant from the Finnish Menopause Society, Juho Vainio Foundation and Finnish Medical Foundation.

Financial disclosures/conflicts of interest: None reported.

Corresponding author:

Susanna Savukoski (susanna.savukoski@oulu.fi). Address Kajaanintie 50, 90220 Oulu, Finland, phone +358408341559.

Abstract

Objective: To investigate whether early-onset menopausal transition associates with deteriorated glucose tolerance in women in their mid-forties.

Methods: A cross-sectional analysis of a cohort study including 2632 women of the Northern Finland Birth Cohort 1966. The participants were divided into two groups by their menstrual history and follicle stimulating hormone values at age 46: climacteric and preclimacteric women. Glucose and insulin parameters, as well as mathematical indices derived from them to evaluate insulin sensitivity, were compared between the groups. The results were adjusted for measured body mass index and smoking. The possible effect of hormone therapy was investigated in subanalyses excluding hormone therapy users.

Results: Climacteric women (n = 379) were more often current smokers at age 46 ($P = 0.008$), and their body mass indices increased more from 31 to 46 years ($P = 0.013$), compared to preclimacteric women (n = 2253). In a multivariable generalized linear model, being climacteric at age 46 was associated with several findings suggesting decreased insulin sensitivity: increase in glycated haemoglobin ($P < 0.001$), two-hour oral glucose tolerance test 30- and 60-minute insulin ($P = 0.040$ and 0.006, respectively) and area under the insulin curve ($P = 0.005$). Being climacteric also was associated with a decrease in the McAuley ($P = 0.024$) and Belfiore indices ($P = 0.027$) and glucose tolerance test 60-minute glucose ($P = 0.015$). In subanalyses excluding hormone therapy users (n = 94), the results did not change significantly.

Conclusions: Earlier onset of climacteric transition associates with impaired insulin sensitivity in middle-aged women.

Key words: insulin sensitivity / menopausal transition / age at menopause / oral glucose tolerance test / glycated haemoglobin

Introduction

The average age of menopause in Western countries is about 50–51 years [1,2], but according to a recent meta-analysis, about 12% of women face menopause by the age of 45 [3]. Women facing early menopause are at increased risk for cardiovascular diseases [4,5] and osteoporosis [6]. However, studies have reported conflicting results about whether age at menopausal transition influences the risk for diabetes [7–10]. The European Society of Human Reproduction and Embryology (ESHRE) guidelines do not recommend screening women with premature ovarian insufficiency (POI) for diabetes, as the evidence is insufficient [11].

Estrogen contributes to several processes which maintain normal glucose and insulin metabolism in the human body [12]. Physical changes during menopausal transition such as relative hyperandrogenism caused by decreasing estrogen and sex-hormone binding globulin and an increase in the amount of visceral fat may potentially impair insulin sensitivity [13–15]. However, studies have reported conflicting results about the effects of menopausal status on the risk for diabetes. A Chinese study reported that the risk for impaired glucose tolerance increases 6% for each year after menopause in women reaching menopause after the age of 49 and not taking hormone therapy (HT) [16]. However, a Japan Nurses' Health Study (JNHS) of 22,426 women, 40–59 years of age, suggested that menopausal state was not an independent risk factor for diabetes, as the results were adjusted for age, body mass index (BMI), use of HT, smoking, alcohol use and physical activity [9].

In our previous study, we reported that climacteric women at the age of 46 had a more unfavourable cardiovascular risk profile, compared with preclimacteric women at the same age: higher body fat percentage, higher total and low-density lipoprotein cholesterol and higher liver enzymes [17]. Based on the literature, we hypothesized that onset of the climacteric phase at a younger age might expose middle-aged women to impaired insulin sensitivity. In the present study, the objective was to investigate whether an earlier onset climacteric phase

associates with impaired glucose and insulin metabolism and prevalence of type 2 diabetes mellitus (T2DM). The study population consisted of more than 2600 women from Northern Finland Birth Cohort 1966 (NFBC1966). The cohort has been followed since the antenatal period with repeated clinical examinations and questionnaires. This study was based on a cross-sectional analysis of glucose metabolism at 31 and 46 years.

Methods

Study population

The NFBC1966 is a population sample that was recruited during pregnancy, based on 12,068 pregnant women living in the provinces of Oulu and Lapland, with their estimated date of delivery during 1966. Of the 12,231 children who were born, 12,058 were live births. The majority of the children were born in 1966, and they represented 96.3% of the births registered in the area during that time [18]. Data have been collected since the antenatal period, using questionnaires, clinical examinations, laboratory samples and imaging studies. The most recent follow-up studies were performed at the ages of 31 and 46 years. Both follow-up studies included a comprehensive questionnaire which was sent to every study participant who was alive and living in Finland. The 46-year questionnaire included questions about the study participants' menstrual history and current medications, including contraceptive and HT preparations. The study participants were also invited to submit to a clinical examination at both ages.

Ethical approval

The study design was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District (94/2011, 12/2003), and the principles of the Declaration of Helsinki and national guidelines have been rigorously followed. Permission to use the Care Register for Health Care was sought from the National Institute for Health and Welfare. A licence to use statistics on reimbursements for prescription medicines has been sought from the Social

Insurance Institution of Finland. Written, informed consent to use the cohort data collection and separately to use register data of them for scientific purposes was received from all study participants.

Group division

The female cohort participants who participated in the 46-year follow-up study were divided into climacteric (women who were either in late perimenopause or postmenopausal) and preclimacteric groups based on their follicle stimulating hormone (FSH) values and menstrual history. FSH values were determined using an immunochemiluminometric method (Advia Centaur XP; Siemens Healthcare Diagnostics Inc., Tarrytown, NY).

The criteria for being classified as climacteric were: 1) FSH value ≥ 25 IU/L and 2) amenorrhea ≥ 4 months (if the duration was known). The criteria for being classified as preclimacteric were: 1) FSH value < 25 IU/L and 2) still having regular/irregular menstrual cycles. As there are no general criteria for climacterium, these criteria were applied based on the ESHRE guidelines for POI and Stages of Reproductive Aging Workshop (STRAW) +10 staging system criteria for menopausal transition [11,19].

If the woman was hysterectomized or if she had a progestin-only treatment (peroral, capsule or intrauterine device), she was classified by FSH value only. Women currently using combined estrogen-progestin contraceptive pill or ring were excluded. Women using systemic HT were included automatically in the climacteric group. Women with discrepancies between FSH value and menstrual history were excluded from the study population, as were women who had discrepancies between medication reimbursements and self-reported use of HT.

Blood analyses

Clinical examinations at 31 and 46 years included blood samples. These samples were taken after an overnight fasting period, centrifuged immediately and stored at −20 °C and later at

−80 °C before analysis. The blood samples were analysed in NordLab Oulu (formerly Oulu University Hospital Laboratory), a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189).

Fasting glucose and insulin

Fasting plasma glucose (fP-gluc) and fasting serum insulin (fS-ins) levels at the age of 31 were determined by a glucose dehydrogenase method (Granutest 250; Diagnostica Merck, Darmstadt, Germany) and by radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden), respectively. Levels of fP-gluc and fS-ins at the age of 46 were analysed by using an enzymatic dehydrogenase method (Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, NY) and by a chemiluminometric immunoassay (Advia Centaur XP; Siemens Healthcare Diagnostics Inc., Tarrytown, NY), respectively.

Oral glucose tolerance test

A two-hour oral glucose tolerance test (OGTT) was performed in the 46-year follow-up study after an overnight (12 h) fasting period. The data on previously diagnosed diabetes and diabetes medications were based on self-reported diagnoses and medications noted in the questionnaire, hospital registers and medication registers from the Social Insurance Institution of Finland. Participants who were diagnosed with diabetes or who were currently using medication for diabetes were excluded from the OGTT.

At the beginning of the OGTT, capillary fingertip blood glucose was tested (Ascensia Contour; Bayer Diagnostics, Toronto, Canada). Participants with a result of 8.0 mmol/L or higher were excluded from the test, and only a fasting glucose sample was taken from them. The OGTT was performed by standardised protocol: the participants ingested a liquid containing 75g of glucose (GlucosePro; Comed, Tampere, Finland) within five minutes after the baseline glucose blood sample was taken. Serum insulin levels measured in mU/L and

plasma glucose levels measured in mmol/L were taken at baseline and at 30, 60 and 120 min after the 75g glucose intake (P-gluc^{0min,30min,60min,120min} and S-ins^{0min,30min,60min,120min}).

The study participants were classified by their OGTT results according to the World Health Organization (WHO) 1999 criteria: normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2DM [20]. Individuals with IFG and/or IGT were combined and categorized as having prediabetes.

Insulin sensitivity and beta-cell function indices

To investigate insulin sensitivity and beta-cell function, we calculated several mathematical indices. The indices used in this study were chosen based on the findings by earlier studies. Homeostatic model assessment (HOMA) method is widely used in clinical and epidemiological studies, to quantify insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) [21]. Matsuda–DeFronzo index [22], the Belfiore index and the area under the insulin curve (AUC-insulin) values have been reported to correlate well with euglycemic-hyperinsulinemic clamp in postmenopausal women [23,24]. The McAuley test has been reported to have high sensitivity and specificity to predict insulin sensitivity in euglycemic individuals [25,26]. AUC values for glucose and insulin were calculated using the trapezium method [27]. Formulas for other indices are shown in Table 1.

Glycated haemoglobin (HbA1c)

At the age of 46, the participants' concentrations of glycated and total haemoglobin were measured using the immunochemical assay method (Advia 1800; Siemens Healthcare Diagnostics Inc.; Tarrytown, NY) and the ratio was reported as a percentage of HbA1c.

Systemic estrogen users

Statistics for prescription medications were used to find study participants using systemic HT with estrogen. The study participants having systemic HT purchases (Anatomical Therapeutic Chemical [ATC] codes starting with G03C and G03F) during the one year prior to the 46-

year clinical examination who also reported the use of HT in clinical examination were classified as HT users. To evaluate the effect of HT on glucose metabolism, we did subanalyses excluding HT users.

Covariates

Earlier studies have shown that glucose metabolism is strongly affected by BMI [28] and smoking [29], so these were included in the adjusted model. Based on the data retrieved from the 31- and 46-year questionnaires, participants were defined as non-smokers, occasional/ex-smokers and current smokers at both ages. On clinical examination at age 31 and 46, height and weight were measured, and BMI was calculated. BMI values were divided into four classes: <25, 25–30, 30.01–35 and >35 kg/m^2.

Statistical analyses

The baseline characteristics have been presented with frequencies and proportions. Distributions of background variables in study groups were compared using Pearson's chi-square test. As a part of the members of the original cohort has been lost of follow-up, we also compared the background characteristics of women included into this study to those who fulfilled the questionnaire but did not attend to the clinical study.

The change in BMI from the age of 31 to 46 years was calculated and compared between the study groups with the Mann–Whitney *U* test. We performed a multivariable generalized linear model to investigate association between change in BMI from 31 to 46 years of age and climacteric status at age 46. In this model, change in BMI from 31 to 46 years of age was dependent and climacteric status at age 46 was independent variable. The model was adjusted with BMI and smoking at age 31. Glucose tolerance was compared between the study groups with Pearson's chi-square test.

Glucose and insulin variables and indices of insulin sensitivity and beta-cell function were first compared between the climacteric status groups with an independent sample *t*-test or

Mann–Whitney *U* test. To investigate association between glucose metabolism and climacteric status at age 46, multivariable generalized linear models have been executed, in which glucose and insulin metabolism outcomes were dependent variables and the climacteric status at age 46 was an independent variable. These models were adjusted with BMI and smoking at age 46. When investigating whether climacteric status at age 46 and glucose metabolism at age 31 were associated, we performed a multivariable generalized linear model in which glucose and insulin outcomes at age 31 were dependent variables and the climacteric status at age 46 was an independent variable, adjusting the models with BMI and smoking at age 31. On the grounds of Goodness of Fit tests, the interaction terms of the independent variables with *P*-value < 0.2 were included into the final models. For the models, the variables were normalised with logarithmic transformation.

For the analyses, a *P*-value < 0.05 was considered statistically significant. IBM SPSS Statistics for Windows, Version 24.0. (IBM, Armonk, NY) was used for analyses. Fig. 1 was created using CorelDRAW Graphics Suite 2019, Version 21.0.0.593 (Corel Corporation, Ottawa, Canada), and Fig. 2 and 3 were created with GraphPad Prism 8.0.1.244 (GraphPad Software, San Diego, California).

Results

Fig. 1 shows how the study groups were formed. The final study population consisted of 2632 women: 379 participants in the climacteric group (cases) and 2253 in the preclimacteric group (controls). Of the climacteric participants, 94 (24.8 %) were currently using HT. For 2/3 of climacteric women to who the time of last menstrual period was possible to determine, it was at the most two years [17]. According to the Care Register for Health Care, eight of the study participants were diagnosed with POI.

Background characteristics of the study population are shown in Table 2. The proportion of current smokers was higher in climacteric compared with preclimacteric women at the age of

46, whereas other baseline characteristics did not differ between the groups. Even though the distribution into BMI classes did not differ between the study groups, BMI values increased more from 31 to 46 years of age in the climacteric group (Fig. 2). Background characteristics at age 46 were available of 490 women who fulfilled the questionnaire but who did not attend the clinical study. As we compared these women to the women of our study population, BMI and education level did not differ between these groups, but women not attending the clinical study were more often current smokers (29.2 vs. 18.3 %, $P < 0.001$) and living without a partner (26.1 % vs. 21.9 %, $P = 0.041$).

In the multivariable generalized linear model with change in BMI from 31 to 46 years as dependent variable, adjusted with smoking and BMI at age 31, climacteric status at age 46 was not associated with BMI change ($\beta = 0.0354$, 95 % CI –0.060 to 0.767, $P = 0.094$).

Table 3 shows the fP-gluc, fS-ins and HbA1c values at the age of 46. In the multivariate generalized linear model, climacteric women had higher HbA1c levels. Unadjusted fP-gluc levels were higher in climacteric women, but there was no significant association between fP-gluc and climacteric status in the adjusted model. The fS-ins levels did not differ between the study groups and were not associated with climacteric status in the multivariate generalized linear model.

Insulin sensitivity and beta-cell function indices at age 46 are shown in Table 4. In the multivariate generalized linear model, climacteric women had higher AUC-insulin and lower Belfiore and McAuley indices. AUC-glucose, Matsuda–DeFronzo index, HOMA-IR and HOMA-β were not associated with climacteric status at age 46.

At age 31, fS-ins, fP-gluc levels and HOMA indices did not differ between the study groups divided by climacteric status at age 46 (Table 5).

The results of OGTT performed at the age of 46 are shown in Table 6. In the multivariate generalized linear model, being climacteric was associated with higher OGTT S-ins^{30min} and

S-ins^{60min} levels and lower P-gluc^{60min} levels. In the generalized linear model in which P-gluc^{60min} was dependent variable. the interaction term between BMI and climacteric status at age 46 was statistically significant (P = 0.023). As we performed this generalized linear model, adjusting with smoking, separately in different BMI classes, it seemed that P-gluc^{60min} levels were significantly lower only in climacteric women with BMI > 35 (β = -1.332, 95 % CI –2.513 to 10.152, P = 0.027). OGTT P-gluc^{30min} levels were significantly higher in climacteric women, but in the multivariate generalized linear model, these were not independently associated with climacteric status. The OGTT glucose and insulin curves are shown in Fig. 3.

As OGTT results were combined with previous diabetes diagnoses at age 46, 88.0% of climacteric and 88.0% of preclimacteric women had a normal glucose tolerance, 10.7% vs. 10.1% had prediabetes and 1.4% vs. 2.0% had diabetes, respectively. Distribution did not differ between the groups (P = 0.710).

We also compared all glucose and insulin variables at age 46 in subanalyses excluding HT users. In the subanalyses, there were no significant changes in adjusted results (data not shown).

Discussion

Our study findings suggest that onset of the climacteric phase by the mid-forties associates independently with deterioration of insulin sensitivity. However, differences in fasting and OGTT-derived glucose and insulin values were not clinically significant, and prevalence of abnormal glucose tolerance did not differ between the study groups. In our study population, women with an earlier onset climacteric phase also had other risk factors for adverse changes in glucose metabolism, as they gained more weight from 31 to 46 years of age, and they were more often current smokers at 46 years. The relationship between menopausal transition, BMI and smoking and glucose metabolism is multidimensional, as weight gain and smoking

are well-known risk factors for dysglycemia [30,31], and smoking also increases risk for earlier menopause [32,33], whereas menopausal transition alone does not independently seem to influence BMI [34,35].

In the case of insulin resistance, pancreatic beta-cells accelerate their insulin production, which leads to compensatory hyperinsulinemia [36]. In our study population, being climacteric at age 46 was independently associated with higher AUC-insulin. Higher insulin responses in the OGTT have been associated with an increased risk of developing T2DM, hypertension and coronary heart disease [37]. Being climacteric was associated with decreases in the McAuley and Belfiore indices, which indicate lower insulin sensitivity [25,38]. Earlier studies have reported that the McAuley index has high sensitivity and specificity to predict insulin sensitivity in euglycemic individuals [25,26], and the Belfiore index has been reported to have high correlation with hyperinsulinemic-euglycemic clamp test, which is considered to be the gold standard for measuring insulin sensitivity [38]. HOMA-IR, which did not differ between the climacteric and preclimacteric participants of our study, has been suggested to be a less sensitive and specific indicator of insulin sensitivity than the McAuley index [26]. HOMA- β values were not associated with climacteric status, suggesting that menopausal transition may not have a significant association with beta-cell function.

Very few studies have investigated insulin sensitivity in women facing early-onset menopausal transition. Ates et al [39] reported no difference in HOMA-IR between women with POI and healthy age-matched control women in their 30s. To the best of our knowledge, this is the first study to investigate insulin sensitivity in relation to menopausal status in women in their mid-forties. A few studies, however, have investigated insulin sensitivity in women facing menopausal transition in their 50s. A study by Toth et al [40] indicated that menopause does not significantly affect insulin sensitivity when measured with a hyperinsulinemic-euglycemic clamp test. A study by Mesch et al [41] reported that women in menopausal

transition and postmenopausal women had lower McAuley indices, compared to premenopausal women. However, this was analysed with analysis of variation (ANOVA), without taking into account the confounding factors.

In our study population, we found that women who were climacteric at age 46 had higher HbA1c levels. HbA1c is a biomarker representing average blood glucose levels for 2–3 months previous to the test [42]. To the best of our knowledge, there are no earlier studies investigating an association between menopausal status and HbA1c levels in women in their mid-forties, although a few earlier studies have suggested that postmenopausal status may associate with higher HbA1c levels in women aged 53 or older [43,44]. Higher HbA1 levels in non-diabetic adults have been associated with several adverse outcomes, such as increased risk for cardiovascular diseases [45,46], higher cancer morbidity and mortality [47] and higher overall mortality from all causes [46].

A somewhat contradictory result was seen in the multivariate generalized linear model of this study, as being climacteric seemed to associate with lower OGTT P-gluc^{60min} levels. However, as this analysis was performed separately in different BMI classes, the difference in OGTT P-gluc^{60min} levels between the study groups was significant only in women with BMI > 35. Hence, we suggest that this association may not be generalisable as a menopause-related change in glucose metabolism.

We did not find any differences in the prevalence of prediabetes or diabetes between the study groups at age 46, when combining the cases of OGTT-based undiagnosed diabetes and previously diagnosed cases of diabetes. As our exposed group also included perimenopausal women, we suggest that changes in glucose metabolism initiated because menopausal transition may take a longer time to develop to the level of prediabetes or diabetes. Earlier evidence on the effect of menopausal age on diabetes risk are controversial. Two European studies reported that younger menopausal age was an independent risk factor for T2DM in

women in their 60s [7,8], while the JNHS[9] or a follow-up study by Pandeya et al[10] including 126,721 women from white populations did not find an association between age at menopause and the incidence of diabetes. Leblanc et al[48] reported both early (<45 years) and late (>55 years) menopause increased the risk for T2DM in participants compared with those who underwent menopause from 45 to 55 years of age [48].

The mechanism of dysglycemia is multifactorial, and there are several risk factors that can be affected by lifestyle habits. Weight gain avoidance [49], not smoking [29], healthy diet [50] and moderate alcohol consumption [51] reduce the risk for T2DM. In a study by Mandrup et al[52], physical training decreased the OGTT insulin levels in postmenopausal women. There is evidence that HT may have favourable effect on glucose metabolism in postmenopausal women [15,53,54]. In a study by Margolis et al[55], fasting glucose and insulin, as well as HOMA-IR, were reduced after one year of estrogen-progestin treatment. Manson et al[56] reported lower rates of diabetes in HT users, but the decrease in risk disappeared during postintervention follow-up. As HT also carries some risk, it is not recommended for prevention of chronic diseases in postmenopausal women unless there are disturbing menopausal symptoms, except women with POI [57].

This study has several strengths. The data collection at 46 years included various glucose metabolism parameters, as well as two-hour OGTT glucose and insulin results. We also performed various dynamic tests for insulin sensitivity to investigate the aetiology of impaired glucose metabolism. We could connect glucose metabolism to climacteric status at the age of 46, as both FSH values and menstrual anamnesis were documented at the NFBC1966 46-year data collection, whereas most earlier studies have based menopausal status on self-reported menstrual anamnesis only. In addition, in our study, most of the exposed participants had faced climacterium quite recently, whereas several studies have investigated glucose metabolism in women far beyond menopause. Thus, our findings give

novel information of menopausal transition-related changes in glucose metabolism. Most earlier studies in this area have investigated menopausal status and glucose metabolism in women older than their 50s, while our study participants were several years below the average menopausal age. The most important covariates, BMI and smoking, were taken into account in the analyses, and these data were available for the majority of study participants. Information on the current use of diabetes medications, HT and the study participants' former diabetes diagnoses were retrieved from reliable sources, as a nationwide registry data was used.

A limitation of this study was that it was a cross-sectional evaluation of glucose metabolism in a cohort setting, and a further follow-up of the study participants' glucose metabolism was not yet available, as the upcoming follow-up study of NFBC1966 is still under preparation. Also, even though most of the study participants also took part in the 31-year follow-up study, there were fewer study participants in the 31-year analyses. Estimation of selection bias by comparing women dropping out at the age of 46 and those participating was unfortunately impossible. However, the overall response rate was 60.9%, which can be considered acceptable in this type of unselected population. The exact time of the last menstrual period was not reliably available for every study participant as many of them were using hormonal medications affecting the menstrual cycle. However, climacteric status was defined predominantly by using FSH measurements. In addition, there may be some other confounding factors, lifestyle related as well as genetic factors, which could not be taken into account in the analyses.

Conclusions:

Based on the study findings, we suggest that the onset of climacteric transition by the mid-40s associates with deteriorated insulin sensitivity in middle-aged women. The importance of healthy lifestyle habits should be especially emphasized in this population. The present study

offers novel information of glucose metabolism concerning women facing early-onset menopausal transition. As glucose metabolism evaluation of this study was cross-sectional, further follow-up studies would give additional information on causal relation between menopausal age and glucose metabolism, as well as whether early-onset menopausal transition accelerates adverse changes in insulin sensitivity compared to later onset climacteric phase.

Acknowledgments

This work was supported by the Northern Ostrobothnia Hospital District, Oulu University Hospital, the University of Oulu, the Finnish Menopause Society and the Finnish Medical Foundation. We thank the late professor Paula Rantakallio and the NFBC1966 participants in the 31-year and 46-year follow-up study and the Northern Finland Birth Cohort (NFBC) Project Centre.

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Table and figure legends

TABLE 1. *Formulas for insulin sensitivity and beta-cell function indices*
fP-gluc, fasting plasma glucose; fS-ins, fasting serum insulin; $gluc^{0min\text{-}120min}$, glucose values from two-hour oral glucose tolerance test; $S\text{-}ins^{0min\text{-}120min}$, insulin values from two-hour oral glucose tolerance test; AUC, area under the curve; fS-TG, fasting serum triglycerides.

FIG 1. Flow chart of the study population.

The study population consisted of female participants of the Northern Finland Birth Cohort 1966 (NFBC1966) who took part in all necessary parts of the 46-year follow-up study. FSH, follicle stimulating hormone; HT, hormone therapy

TABLE 2. *Background characteristics of the study population*

FIG 2: Change in BMI between 31 to 46 years of age.

TABLE 3. *Glucose and insulin metabolism variables in climacteric and preclimacteric women at age 46*

P[a], *P*-value from independent sample t-test ([c]) or from Mann-Whitney U test ([d]); IQR, interquartile range; β, slope of climacteric at age 46 in adjusted model; *P*[b], adjusted *P*-value in generalized linear model, in which glucose and insulin outcomes are dependent variables; fS-ins, fasting serum insulin; fP-gluc, fasting plasma glucose; HbA1c, glycated haemoglobin.
Independent variable: climacteric status at age 46
Adjusted variables: body mass index (BMI), smoking at age 46 and statistically significant interaction terms.
Interaction terms included in models: fS-ins: climacteric status*BMI
The logarithmic transformations were made for fP-gluc and fS-ins in the generalized linear models because of their skew distribution.
BMI: 4 classes: ≤ 25, 25.01-30, 30.01-35, >35
Smoking: 3 classes: non-smoker, former/occasional smoking, current smoker

TABLE 4. *Indices of insulin sensitivity and beta-cell function derived from fasting and OGTT measurements of glucose and insulin, in climacteric and preclimacteric women at age 46*

P[a], *P*-value from independent sample t-test ([c]) or from Mann-Whitney U test ([d]); IQR, interquartile range; β, slope of climacteric at age 46 in adjusted model; *P*[b], adjusted *P*-value in generalized linear model, in which indices of insulin sensitivity and beta-cell function are dependent variables; OGTT, oral glucose tolerance test; AUC, area under the curve; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment for beta-cell function
Independent variable: climacteric status at age 46
Adjusted variables: body mass index (BMI) at age 46, smoking at age 46 and statistically significant interaction terms.
Interaction terms included in models:
AUC-glucose: climacteric status*BMI
AUC-insulin, Belfiore index: BMI*smoking
HOMA-β: climacteric status*smoking
The logarithmic transformations were made for HOMA-β, HOMA-IR, AUC-insulin, Matsuda & DeFronzo for the generalized linear models because of their skew distribution.
BMI: 4 classes: ≤ 25, 25.01–30, 30.01–35, >35
Smoking: 3 classes: non-smoker, former/occasional smoking, current smoker

TABLE 5. *Glucose and insulin variables at 31 years, in groups divided by climacteric status at age 46*

P[a-]-value from Mann-Whitney U test; IQR, interquartile range; β, slope of climacteric at age 46 in adjusted model; *P*[b], adjusted *P*-value in generalized linear model, in which glucose and insulin outcomes at age 31 are dependent variables; fP-gluc, fasting plasma glucose; fS-ins, fasting serum insulin; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment for beta-cell function.
Independent variable: climacteric status at age 46
Adjusted variables: body mass index (BMI) at age 31, smoking at age 31 and statistically significant interaction terms. Interaction terms included in models:
fP-gluc, fS-ins, HOMA-IR: climacteric status*smoking
fS-ins, HOMA-IR: smoking*BMI
The logarithmic transformations were made for the variables in the multivariate generalized linear models because of their skew distribution.

BMI: 4 classes: ≤ 25, 25.01-30, 30.01-35, >35
Smoking: 3 classes: non-smoker, former/occasional smoking, current smoker

TABLE 6. *Oral glucose tolerance test (OGTT) results in climacteric and preclimacteric women at age 46*
P^a, *P*-value from independent sample t-test (^{c}) or from Mann-Whitney U test (^{d}); IQR, interquartile range; β, slope of climacteric in adjusted model; P^b, adjusted *P*-value in generalized linear model, in which OGTT outcomes are dependent variables.
Independent variable: climacteric status at age 46
Adjusted variables: body mass index (BMI) at age 46, smoking at age 46 and statistically significant interaction terms.
Interaction terms included in models:
OGTT P-gluc^{30min}, OGTT P-gluc^{60min}: climacteric status*BMI
OGTT P-gluc^{0min}, OGTT P-gluc^{60min}, OGTT P-gluc^{120min}, OGTT S-ins^{120min}: smoking*BMI

The logarithmic transformations were made for OGTT P-gluc^{0min}, OGTT S-ins^{0min}, OGTT S-ins^{30min}, OGTT S-ins^{60min} and OGTT S-ins^{120min} for the generalized linear models because of their skew distribution.
BMI: 4 classes: ≤ 25, 25.01–30, 30.01–35, >35
Smoking: 3 classes: non-smoker, former/occasional smoking, current smoker
Study participants with previous diabetes diagnosis/medication or who had OGTT P-gluc^{0min} ≥ 8.0 mmol/L were excluded from the test.

FIG 3. Glucose (A) and insulin (B) values in oral glucose tolerance test (OGTT).

Table1

TABLE 1. *Formulas for insulin sensitivity and beta-cell function indices*

Index	Formula	Reference for formula
HOMA-IR	fP-gluc x fS-ins / 22.5	Wallace *et al.*, 2004
HOMA- β	(20 x fS-ins)/ (fP-gluc – 3.5) x 100	Wallace *et al.*, 2004
Matsuda& DeFronzo	10 000/square foot of [(P-gluc^{0min} x S-ins^{0min}) x ((P-gluc^{0min} + P-gluc^{30min} + P-gluc^{60min} + P-gluc^{120min}) / 4) x ((S-ins^{0min} + S-ins^{30min} + S-ins^{60min} + S-ins^{120min}) / 4)]	Matsuda and DeFronzo, 1999
Belfiore index	2[/(AUC-insulin x AUC-glucose) +1]	Belfiore *et al.*, 1998
McAuley index	exp[2.63-0.28 ln (fS-ins)-0.31 ln (fS-TG)]	Mcauley *et al.*, 2001

Table2

TABLE 2. *Background characteristics of the study population*

	Climacteric *N* (%)	Preclimacteric *N* (%)	*P*-value
Body mass index at the age of 31			0.349
≤25	205 (74.3)	1086 (69.6)	
25.01–30	54 (19.6)	340 (21.8)	
30.01–35	10 (3.6)	88 (5.6)	
>35	7 (2.5)	48 (3.1)	
Body mass index at the age of 46			0.816
≤25	166 (45.7)	1048 (46.7)	
25.01–30	123 (33.9)	722 (32.2)	
30.01–35	51 (14.0)	307 (13.7)	
>35	23 (6.3)	168 (7.5)	
Smoking at the age of 31			0.226
Non-smoker	175 (49.4)	1102 (53.6)	
Former/occasional smoker	94 (26.6)	537 (26.1)	
Current smoker	85 (24.0)	418 (20.3)	
Smoking at the age of 46			0.008
Non-smoker	192 (51.9)	1303 (58.4)	
Former/occasional smoker	88 (24.1)	541 (24.2)	
Current smoker	88 (24.1)	387 (17.3)	
Marital status at age of 46			0.394
Unmarried	40 (10.7)	222 (9.9)	
Married/domestic partnership	298 (79.7)	1747 (77.8)	
Divorced	33 (8.8)	263 (11.7)	
Widow	3 (0.8)	13 (0.6)	
Education at age 46			
Basic	9 (2.4)	46 (2.0)	0.094
Secondary	231 (60.9)	1247 (55.3)	
Tertiary	139 (36.7)	960 (42.6)	

Table3

TABLE 3. *Glucose and insulin metabolism variables in climacteric and preclimacteric women at age 46*

Outcome		Mean ± SD/Median [IQR] (*N*)	*P*[a]	β (95% CI)	*P*[b]
fP-gluc(mmol/L)	Climacteric	5.30 [0.7] (353)			
	Preclimacteric	5.20 [0.6] (2221)	0.012[d]	0.010 (-0.002 to 0.023)	0.103
fS-ins (mU/L)	Climacteric	7.10 [5.2] (356)			
	Preclimacteric	7.20 [5.5] (2225)	0.958[d]	-0.201 (-0.411 to 0.009)	0.061
HbA1c (%)	Climacteric	5.5 ± 0.49 (361)			
	Preclimacteric	5.4 ± 0.47 (2249)	0.002[c]	0.091 (0.040-0.142)	<0.001

Table4

TABLE 4. *Indices of insulin sensitivity and beta-cell function derived from fasting and OGTT measurements of glucose and insulin, in climacteric women and preclimacteric women at age 46*

Outcome		Mean ± SD/Median [IQR] (*N*)	*P*[a]	β (95% CI)	*P*[b]
AUC-glucose (mmol*min/L)	Climacteric	763.5 [209.3] (308)			
	Preclimacteric	750.0 [214.5] (1886)	0.267[d]	-0.101 (-0.205 to 0.003)	0.057
AUC-insulin (mU*min)/L)	Climacteric	6238.5 [5397.0] (311)			
	Preclimacteric	5655.0 [4536.4] (1900)	0.003[d]	0.085 (0.025-0.145)	0.005
Matsuda-DeFronzo index	Climacteric	98.6 [74.3] (318)			
	Preclimacteric	102.1 [79.0] (1950)	0.163[d]	-0.036 (-0.096 to -0.023)	0.230
Belfiore index	Climacteric	0.7 ± 0.3 (307)			
	Preclimacteric	0.8 ± 0.3 (1879)	0.021[c]	-0.038 (-0.072 to -0.004)	0.027
McAuley index	Climacteric	8.1 ± 2.0 (355)			
	Preclimacteric	8.3 ± 2.0 (2224)	0.059[c]	-0.214 (-0.400 to -0.028)	0.024
HOMA-IR	Climacteric	1.8 [1.3] (351)			
	Preclimacteric	1.7 [1.4] (2211)	0.767[d]	0.017 (-0.042 to 0.076)	0.575
HOMA-β	Climacteric	78.3 [55.2] (351)			
	Preclimacteric	84.6 [54.6] (2210)	0.103[d]	0.030 (-0.075 to 0.134)	0.581

Table5

TABLE 5. *Glucose and insulin variables at 31 years, in groups divided by climacteric status at age 46*

Outcome		Median [IQR] (*N*)	*P*[a]	β (95% CI)	*P*[b]
fP-gluc	Climacteric	4.9 [0.5] (273)			
	Preclimacteric	4.9 [0.5] (1551)	0.907	-0.019 (-0.44 to 0.007)	0.146
fS-ins	Climacteric	7.3 [2.9] (273)			
	Preclimacteric	7.3 [3.1] (1550)	0.682	0.008 (-0.034 to 0.051)	0.695
HOMA-IR	Climacteric	1.6 [0.7] (270)			
	Preclimacteric	1.6 [0.8] (1545)	0.910	-0.092 (-0.189 to 0.06)	0.062
HOMA-β	Climacteric	111.8 [46.0] (270)			
	Preclimacteric	110.9 [56.9] (1544)	0.753	0.000 (-0.052 to 0.052)	0.998

Table6

TABLE 6. *Oral glucose tolerance test (OGTT) results in climacteric and preclimacteric women at age 46*

Outcome	Mean ±SD / Median [IQR] (*N*)		*P*[a]	β (95%CI)	*P*[b]
OGTT P-gluc^{0min} (mmol/L)	Climacteric Preclimacteric	5.3 [0.6] (319) 5.3 [0.6] (1954)	0.714[d]	-0.001 (-0.012 to 0.011)	0.918
OGTT P-gluc^{30min} (mmol/L)	Climacteric Preclimacteric	7.8 ± 1.6 (313) 7.6 ± 1.5 (1916)	0.034[c]	-0.389 (-1.154 to 0.377)	0.320
OGTT P-gluc^{60min} (mmol/L)	Climacteric Preclimacteric	6.9 ± 2.2 (313) 6.8 ± 2.2 (1905)	0.298[c]	-1.332 (-2.411 to -0.254)	0.015
OGTT P-gluc^{120min} (mmol/L)	Climacteric Preclimacteric	5.6 ± 1.4 (312) 5.7 ± 1.5 (1921)	0.254[c]	-0.111 (-0.282 to 0.060)	0.204
OGTT S-ins^{0min} (mU/L)	Climacteric Preclimacteric	7.3 [5.8] (319) 7.3 [5.9] (1954)	0.996[d]	-0.020 (-0.060 to 0.056)	0.933
OGTT S-ins^{30min} (mU/L)	Climacteric Preclimacteric	58.5 [45.8] (313) 54.7 [42.3] (1916)	0.048[d]	0.070 (0.003-0.137)	0.040
OGTT S-ins^{60min} (mU/L)	Climacteric Preclimacteric	60.3 [62.9] (313) 55.1 [49.2] (1913)	0.006[d]	0.101 (0.029-0.173)	0.006
OGTT S-ins^{120min} (mU/L)	Climacteric Preclimacteric	44.0 [39.0] (315) 41.8 [32.6] (1926)	0.113[d]	0.048 (-0.027 to 0.123)	0.210

Figure1

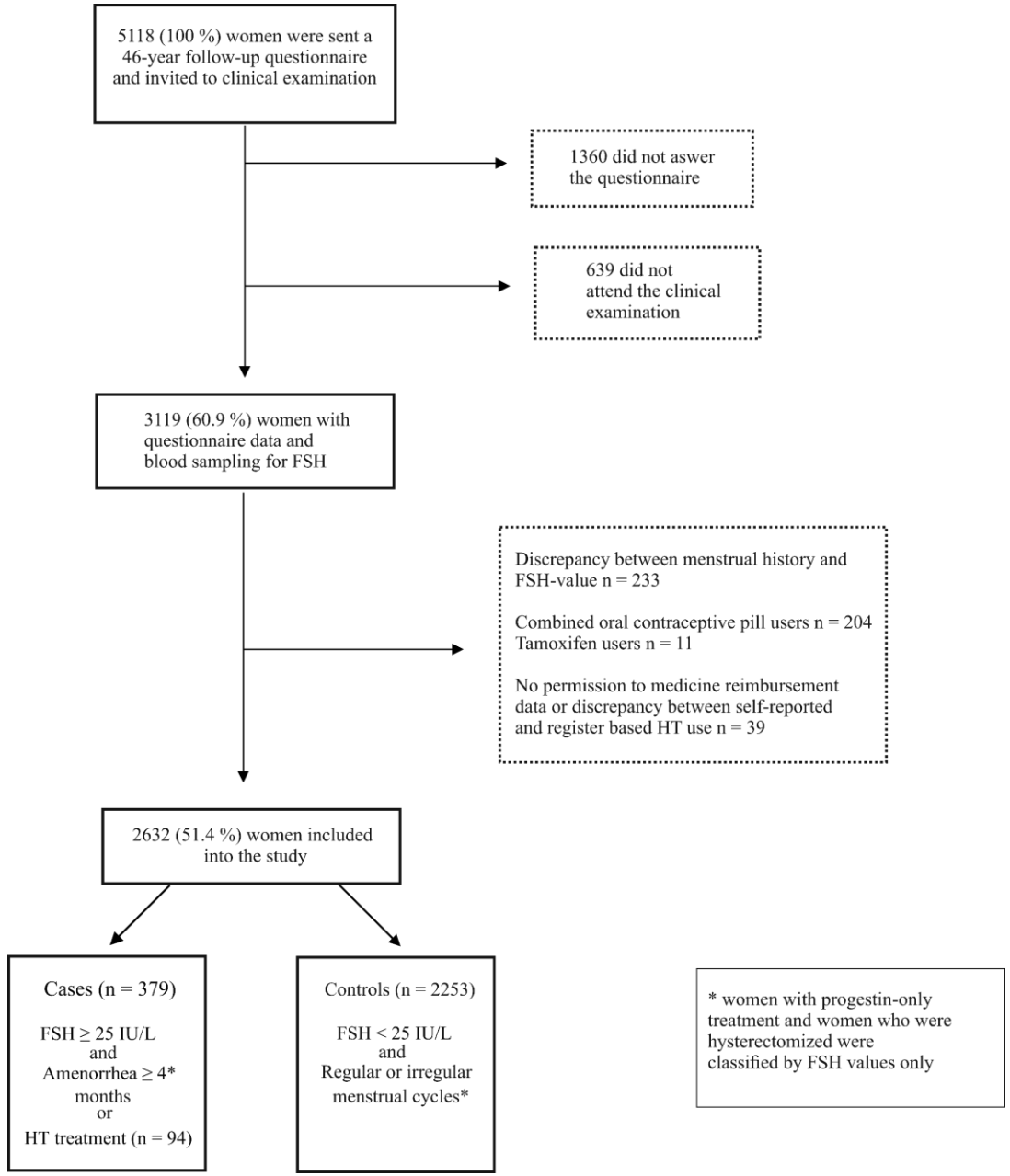

5118 (100 %) women were sent a 46-year follow-up questionnaire and invited to clinical examination
1360 did not aswer the questionnaire
639 did not attend the clinical examination
3119 (60.9 %) women with questionnaire data and blood sampling for FSH
Discrepancy between menstrual history and FSH-value n = 233
Combined oral contraceptive pill users n = 204
Tamoxifen users n = 11
No permission to medicine reimbursement data or discrepancy between self-reported and register based HT use n = 39
2632 (51.4 %) women included into the study
Cases (n = 379)
FSH ≥ 25 IU/L and Amenorrhea ≥ 4* months or HT treatment (n = 94)
Controls (n = 2253)
FSH < 25 IU/L and Regular or irregular menstrual cycles*
* women with progestin-only treatment and women who were hysterectomized were classified by FSH values only

Figure2

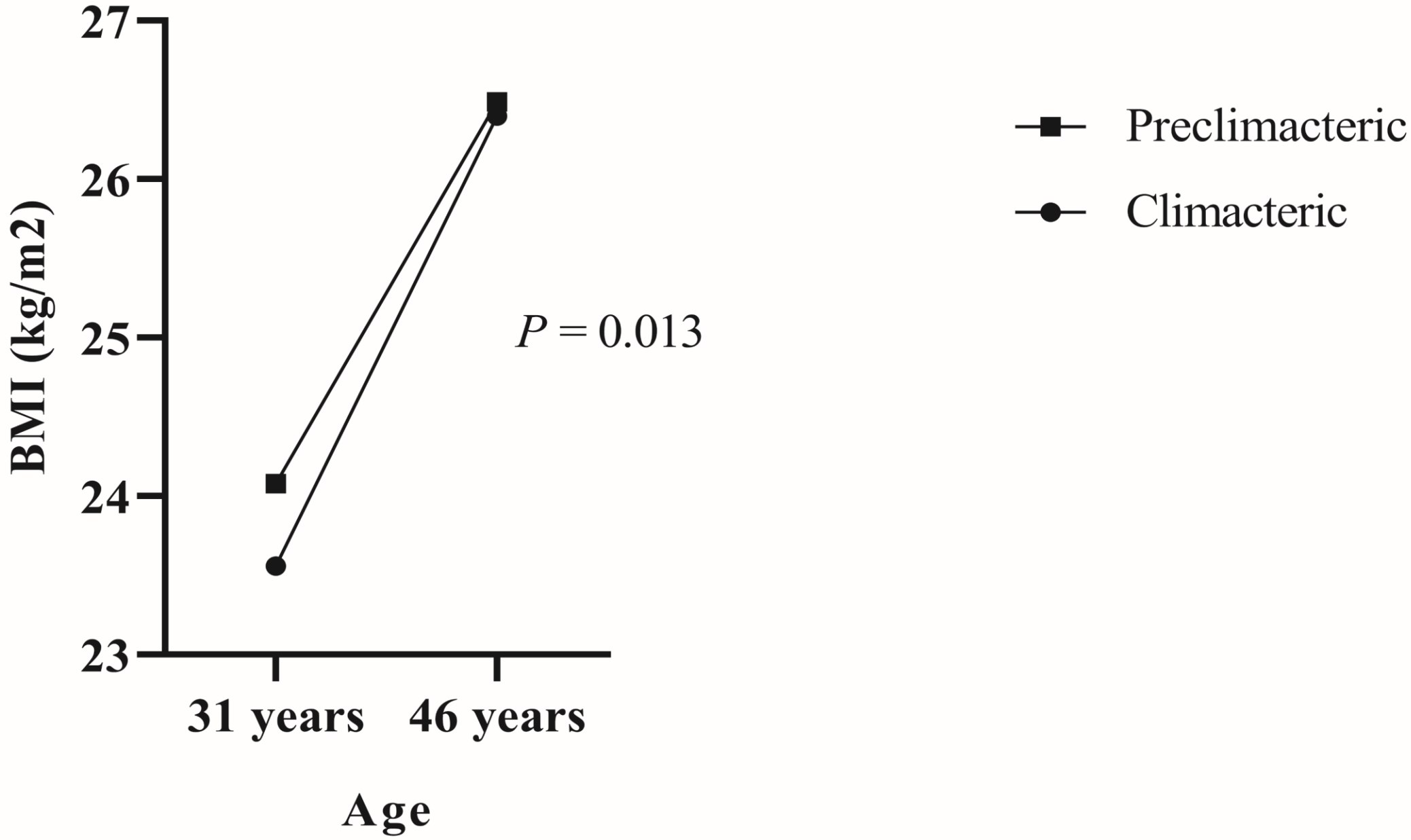
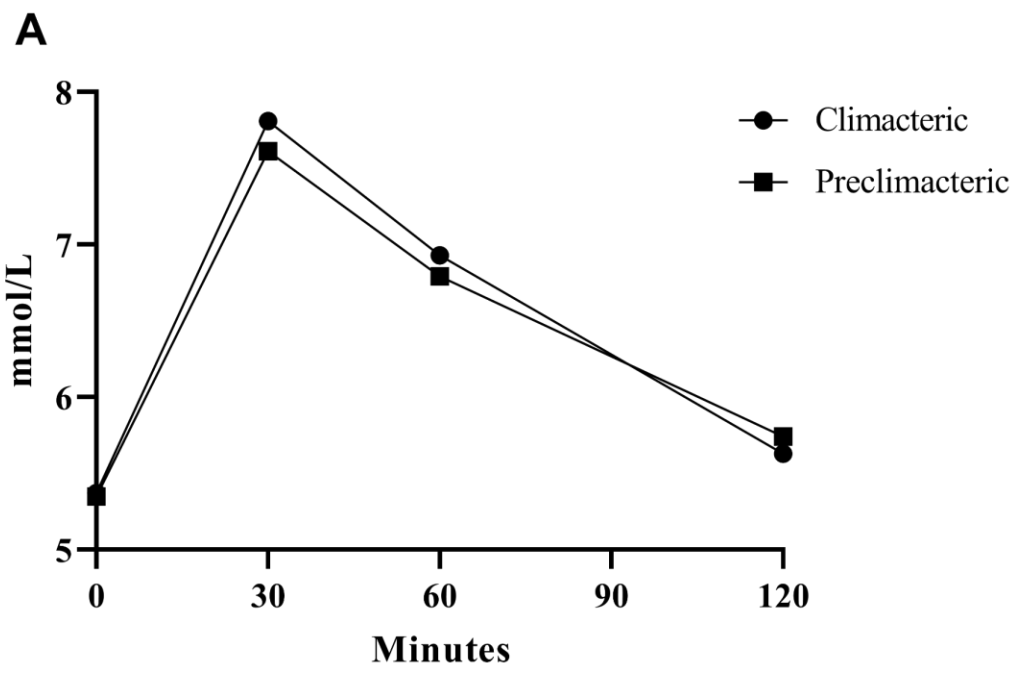

27
26
25
24
23
BMI (kg/m2)
31 years
46 years
Age
P = 0.013
Preclimacteric
Climacteric

Figure3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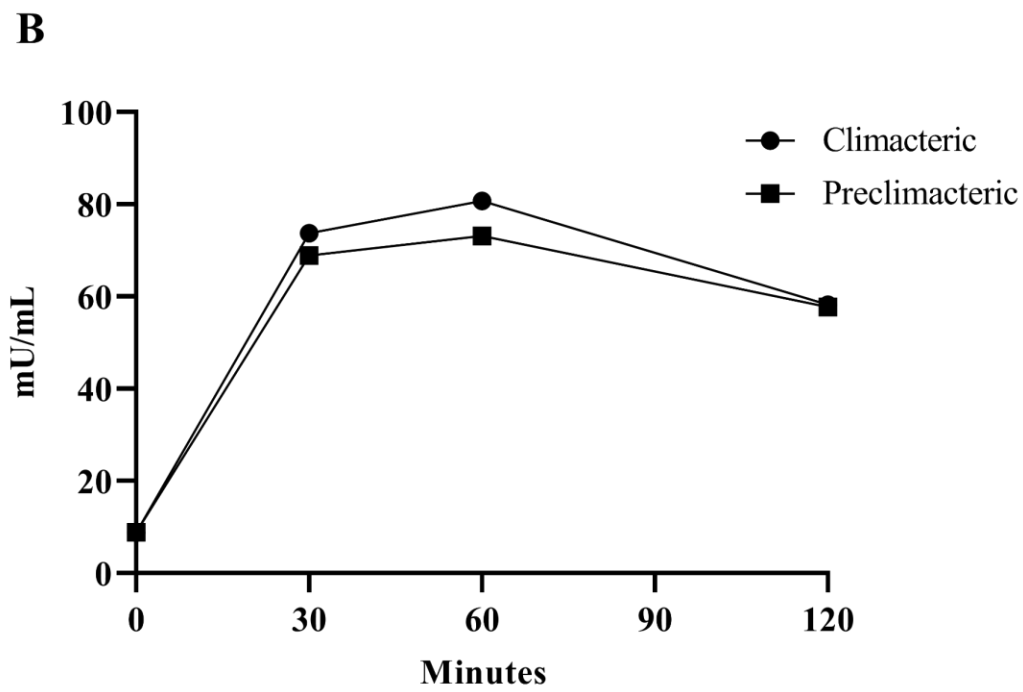
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