

A prospective study of the association between endogenous hormones and depressive symptoms in postmenopausal women

Running Title: Postmenopausal hormone levels and depression

Joanne Ryan, MSc,^{1,2} Henry G. Burger, MD,³ Cassandra Szoeki, MBBS, PhD,¹ Philippe Lehert, PhD,⁴ Marie-Laure Ancelin, PhD,² and Lorraine Dennerstein, MBBS, PhD¹.

¹The University of Melbourne, Parkville, Victoria, Australia

²Inserm, U888, Montpellier, F-34093 France ; Univ Montpellier1, Montpellier, F-34000 France

³Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia.

⁴University of Mons, Belgium.

Funding/support: This study was supported by grants from the Victorian Health Promotion Foundation, the National Health and Medical Research Council of Australia, the Alzheimer's Association and the University of Melbourne. Prince Henry's Institute of Medical Research received grants from Organon Pty Ltd for the hormone assays.

Financial disclosure: Prof. Dennerstein reports Organon, Wyeth, Proctor & Gamble (research support) and Pfizer, Wyeth, Boehringer Ingelheim and Bayer Schering (expert panel and consulting fees).

Address for reprints and correspondence: Joanne Ryan, Department of Psychiatry, The University of Melbourne, Level 1 North, Royal Melbourne Hospital, Grattan Street, Parkville VIC, 3050, Australia. Tel: +61 383444520, Fax: +61 393474127. Email: jryan2@pgrad.unimelb.edu.au

ABSTRACT

Objective: Across a woman's lifetime, variations in hormone levels are known to influence mood and well-being. Whether absolute or changes in hormone levels over time are associated with depression among postmenopausal women remains unclear.

Design: The Melbourne Women's Midlife Health Project is a longitudinal population based study of women, who were followed through the menopause transition. This analysis is based on data collected from 138 postmenopausal women in years 11 and 13 of the study, who were assessed for the presence of depressive symptoms using the Centre for Epidemiological Studies Depression Scale. Logistic regression models were developed to determine whether absolute or changes in hormone levels were associated with depression.

Results: No significant associations were found between depressive symptoms and the absolute levels of sex-hormone binding globulin, testosterone, free androgen index, estradiol, free estradiol or FSH. On the other hand, women with a decline in total serum estradiol over the 2-year period had a more than 3-fold increased risk of depressive symptoms (OR: 3.5; 95% CI: 1.2-9.9). A large increase in FSH levels over this period was also associated with depressive symptoms (OR: 2.6; 95% CI: 1.0-6.7). These associations remained even after adjustment for initial depression score, as well as a range of potential confounding factors.

Conclusions: Changes in estradiol and to a lesser extent follicle-stimulating hormone levels are associated with an increased risk of depressive symptoms in postmenopausal women. These results further support a role for fluctuating rather than absolute hormone levels in later life depression.

Key words: Depression; Endogenous hormone levels; Estradiol; FSH; Postmenopause.

INTRODUCTION

Accumulating biological and epidemiological evidence supports a role for estrogens in depression. Estrogen receptors are present in the brain and estrogen has been shown to modulate neurotransmitter turn-over (see for review ¹) and stimulates serotonergic activity through the regulation of receptor number and function ², all of which strongly suggest that estrogen can influence mood and well-being. These findings are further supported by in vivo animal studies, where a sudden decrease in the brain's exposure to estrogen has been shown to disrupt neurosteroid signalling, leading to an increase in anxiety and depression ³.

The menopause transition is a period of marked hormone instability ⁴, with intense and irregular fluctuations in the levels of estrogen, which tend to decline up to the postmenopause. Research suggests that this period is associated with an increased risk for new onset depressive disorder ⁵ and a higher risk of depressive symptoms for at least some women ⁶⁻⁸. External stresses during the menopause transition also appear to have a more negative impact on women's mood ⁹. In addition, studies have shown that women who have undergone a surgical menopause and thus experience an abrupt drop in estrogen concentrations are also at a greater risk of depression ¹⁰⁻¹². Short-term randomised controlled trials have demonstrated the efficacy of estradiol in improving the mood of depressed perimenopausal women ^{13, 14}, and hormone therapy (HT) during the postmenopause may enhance the positive effects of antidepressants on depressed mood ¹⁵. On the other hand, the results of observational studies and randomised controlled trials (RCTs) have not all been so clear, and it is possible that certain women respond better to estrogen treatment than others ^{16, 17}.

Despite relatively strong evidence for a link between estrogens and depression, the results of studies which have looked at differences in serum estradiol levels between depressed and non-depressed women have been inconclusive ¹⁸⁻²¹. In addition, the majority of these studies have focused on hormone levels across the reproductive years or during the menopause transition. Whether there is an association between low estrogen concentrations and depression in postmenopausal women remains unclear, as does the involvement of other hormones in later life depression. Recent studies also suggest that rather than the absolute levels of plasma hormones, some women are particularly vulnerable to fluctuating hormone levels ^{22, 23}.

The objective of this study was therefore to determine whether absolute or changing levels of estradiol are associated with depression in a population-based sample of postmenopausal women. The influence of other hormones, namely follicle-stimulating hormone (FSH), testosterone, as well as sex-hormone binding globulin (SHBG), will also be examined.

METHOD

Study participants

This paper reports on post-hoc analysis of data collected from the Melbourne Women's Midlife Health Project (MWMHP), a population based study which began in 1991 and followed women through the menopause transition. The study protocol was approved by the University of Melbourne's Human Research Ethics Committee, and the procedures were followed in accordance with the ethical standards of the National Health and Medical Research Council. Information regarding the sampling procedure used for the MWMHP has been described in detail previously ²⁴ ²⁵. In brief, households were selected via random digital telephone dialling and women who were born in Australia, currently living in Melbourne and between 45 and 55 years of age were invited to participate. A total of 2001 women were recruited (response rate 71%) ²⁶ and they provided written informed consent. Subsequent interviews revealed that 51 of these women were actually outside the age limits for inclusion (39 were too young and 26 were older than 55 years), while a further 12 women refused to provide their date of birth and 4 women were not born in Australia. Of the remaining women, those eligible were invited to participate in the longitudinal study. Almost half of the women were not eligible for inclusion in this cohort because they had not menstruated in the

three prior months (n=442) and a number of women were using oral contraceptives (n=16) or HT (n=249). Another 427 women had undergone a hysterectomy and/or bilateral ovariectomy and 7 were excluded because they were unsure if they had undergone such an operation. Of the 779 women who met the specified eligibility criteria for the longitudinal cohort, 341 refused participation. The 438 women who agreed (response rate 56%) reported better self-rated health and were more likely to have a tertiary education, to exercise at least once a week, to have ever had a Papanicolaou smear and to have undergone dilatation and curettage, compared with the eligible women who refused to participate²⁷.

Assessment of depression

Depressive symptoms were assessed in years 11 and 13, using a shortened version of the 20-item Centre for Epidemiology Studies Depression Scale (CES-D)²⁸. This 10-item CES-D demonstrates good predictive accuracy when compared with the full length version and maintains high reliability^{29,30}. Women completed the short form of the CES-D individually by responding to a series of questions concerning their mood over the last week. The scores on all questions were summed to give a maximum of 30, with higher scores indicative of depressive symptoms. A cut-off of 10 was used to identify women with signs of clinical depression^{29,30}.

Serum hormone measurements

A fasting morning blood sample was taken from all postmenopausal women at the time of interview. Plasma blood samples were collected in EDTA sample tubes, which were then centrifuged and the plasma stored at -20°C within half an hour of collection. Samples were then transferred and stored for up to a year and a half at -80°C. All hormones were measured in year 11, however only the levels of estradiol and FSH were assessed in year 13. The double antibody radioimmunoassay (RIA) kit from Diagnostic Products Corporation (Los Angeles, USA) was used to measure total serum estradiol levels (E2). In a number of cases (39.1% of women in year 11 and 51.5% in year 13), the levels of E2 could not be determined as they were below the assay's sensitivity level. In this instance, the women were assigned the minimum detectable value of 20 pmol/l. The interassay coefficient of variation was 6.6% at 400 pmol/l and the between-assay coefficient of variation was 11% at 110 pmol/l and 13% at 470 pmol/l. Follicle-stimulating hormone (FSH) was measured using the TOSOH AIA1200 automated enzyme immunoassay (Abbott Laboratories), and total serum testosterone using an automated, chemiluminescent enzyme immunoassay (ACS180 from Chiron), which has a minimum detection of 0.3 nmol/L. The between-assay coefficient of variation at a testosterone level of 2 nmol/l was 12%. Sex hormone-binding globulin (SHBG) was measured by an automated chemiluminescent enzyme immunoassay using an Immulite Automated Analyser (Diagnostic Products Corporation, Los Angeles, USA). The coefficient of variation for SHBG was 7.9% at 82.4 nmol/l. As the level of bioavailable estradiol and testosterone were not directly measured, the free estradiol and androgen index (FAI) were calculated as the ratio of the measured estradiol or testosterone respectively, to the quantity of measured SHBG, multiplied by 100¹⁸. The change in E2 and FSH levels were determined by calculating the difference in measures over the 2-year follow-up period. Only the SHBG and FAI measures could be normalised using a log transformation, and the distribution of FSH levels on the original scale was approximately normal. The absolute values of the other hormones, as well as the change in E2 and FSH levels were treated uniquely as binary variables, with categories defined using the median value due to the relatively small sample size.

Other measures

As part of the questionnaires which were administered at each follow-up interview, information was obtained on socio-demographic and lifestyle characteristics, aging-related problems and overall health. The age of the women was recorded, as well as their level of education, their living status and current employment. They were questioned on their consumption of alcohol over the last week and whether they were current or past smokers. Body mass index (BMI: kg/m²) was calculated from height and weight measurements and women indicated their level of current physical activity.

Chronic health conditions were identified based on physical measurements and self-reporting. This included high cholesterol levels (total cholesterol, HDL, LDL), diabetes, hypertension, chronic asthma, heart disease, stomach or bowel ulcers, arthritis, rheumatism, cancer or migraine, or whether the women were taking medications for such conditions. A dichotomous variable was created to indicate whether a woman suffered from at least 3 of these health problems and they were also asked to evaluate their own health as better, worse or the same as their peers³¹.

Women indicated whether they were bothered by a list of 22 common symptoms in the last 2-weeks, which included experiencing dizzy spells, headaches, lack of energy, skin irritations and stiff joints. From this checklist, women who suffered from hot flushes and/or night sweats were also identified. The number and severity of hassles experienced in the last 2 weeks was identified using a shortened daily hassles scale³². Women's attitudes to menopause were assessed using an 8-item questionnaire in which the women noted their level of agreement to different statements about people's understanding of the menopause⁹.

Statistical analysis

Initial analysis compared the difference in year 11 characteristics according to depression status. Two-tailed chi-squared tests or t-tests were used to compare the categorical and continuous variables respectively, between depressed and non-depressed postmenopausal women in year 13. Hormones that were associated with depressive symptoms at the 20% significance level were analysed further in logistic regression models. The other characteristics which were associated with depressive symptoms were considered as potential confounding factors.

The odds ratios and 95% confidence intervals were initially calculated to determine the association between absolute or changes in hormone levels and depression status in year 13, while adjusting for year 11 depression scores (log CES-D). Multivariate logistic models were then generated with successive adjustment for *a priori* confounding factors: age and BMI, as well as alcohol consumption, the presence of hot flushes or night sweats and number of years since menopause. Previous work with the MWMHP has shown that estradiol, FSH and testosterone levels vary with age, and BMI is also associated with hormone levels^{27, 33, 34}. Hot flushes are significantly associated with declining estradiol levels and increasing FSH levels^{4, 35, 36}, and the levels of these hormones change depending on the number of years since menopause³⁷. In addition, hormone levels have also been shown to vary with alcohol intake³⁸. At the same time, these factors have all been linked to depression. Adjustment was also made for other covariates which were identified as potential confounders in the association between depressive symptoms and hormone levels from the t-tests and chi-squared analysis.

Secondary analysis was also undertaken to determine whether hormone levels were associated with new or incident cases of depression (CES-D \geq 10) in year 13. Rather than adjusting for year 11 CES-D score, this analysis excluded the 27 women with depressive symptoms in year 11.

SAS version 9.1 (SAS Institute, Inc., North Carolina) was used for the statistical analysis with a significance level of $p < 0.05$.

RESULTS

Study population

Of the women in the initial MWMHP longitudinal cohort, 291 participants remained after 13 years of follow-up (FU), giving a retention rate of 67% (Figure 1). The majority of women who were lost to FU had refused further participation in the study and the most common reason given was that they were "too busy with other things". In comparison to the women who dropped out of the study, those who remained until year 13 were better educated ($\chi^2=31.9$, $df=1$, $p < 0.001$), had less negative attitudes towards the menopause ($\chi^2=51.1$, $df=1$, $p < 0.001$), had better self-rated health ($\chi^2=31.9$, $df=1$, $p < 0.001$) and were more likely to undertake physical activities ($\chi^2=6.7$, $df=1$, $p=0.01$).

However they were also more likely to be either overweight or obese ($\chi^2=16.9$, $df=2$, $p=0.002$) and to drink larger quantities of alcohol ($\chi^2=18.5$, $df=1$, $p < 0.001$).

For the present analysis, women who had bled within the last 12 months and thus were not postmenopausal, were excluded ($n=19$)³⁹, as were current users of HT ($n=49$), women who were not assessed for depressive symptoms in year 11 or 13 ($n=21$) and those women who did not provide blood samples for hormone measurements in both years ($n=64$). This analysis is therefore based on a sub-sample of 138 postmenopausal women. In a number of respects these women were significantly different from the women who were selectively excluded from this analysis. Compared to the analysed sample, women not included in this analysis were more likely to have a lower educational level ($\chi^2=45.1$, $df=1$, $p<0.001$), to be current smokers ($\chi^2=8.1$, $df=1$, $p=0.005$), have poorer self-rated health ($\chi^2=29.1$, $df=1$, $p<0.001$) and more negative attitudes towards the menopause ($\chi^2=72.2$, $df=1$, $p<0.001$). The participants in this analysis however were more likely to consume larger quantities of alcohol ($\chi^2=16.7$, $df=1$, $p<0.001$), have no daily physical activity ($\chi^2=10.4$, $df=1$, $p=0.001$), to experience bothersome physical symptoms ($\chi^2=14.5$, $df=1$, $p<0.001$), and daily hassles ($\chi^2=8.3$, $df=1$, $p=0.004$) and to be either overweight or obese ($\chi^2=18.6$, $df=1$, $p<0.001$). There were no significant differences in depression status, hormone levels or in terms of the other variables, between women included in this analysis and those who were not.

Participant's characteristics

In year 11 of the study, the mean age of the women was 60.1, ranging from 55.9 to 66.8 years. Less than half of these women had a tertiary education, and there were few current smokers. The majority of women participated in regular physical activity and over a third claimed they were in good health. After the 2-year follow-up period, the prevalence of depression symptoms among these women was 25%. The year 11 socio-demographic, health and lifestyle characteristics of these women are summarised in Table 1. Women with depressive symptoms were significantly more likely to be younger or closer to the menopause, and were less likely to have participated in regular physical activity. They also differed in respect to other health-related factors such as their experience of hot flushes/night sweats or their number of daily hassles. These associations are in accordance with previous analysis based on the women recruited from the MWMHP¹².

The hormone levels of the women overall and according to their depression status are shown in Table 2. From year 11 to year 13, all women had an increase in FSH levels, with a median of increase of 8.3 IU/l. In year 11, 39.1% of women had the minimal detectable levels of estradiol and this increased to 51.5% at follow-up. Overall, 39.9% of the women had a decline in estradiol levels across the 2-year follow-up period, with a median change of 0pmol/l. In unadjusted analysis depressive symptoms were associated with higher levels of total estradiol in year 11, and there was a similar difference in free estradiol levels between depressed and non-depressed women, although this did not reach significance (Table 2). There were a higher percentage of depressed women who experienced a decline in estradiol levels over the 2-year period, and a large increase in FSH levels was significantly associated with depressive symptoms. There was no indication that the serum levels of either testosterone, FAI or SHBG were associated with depressive symptoms at follow-up ($p>0.20$) and thus they were not considered further in the analysis.

Hormone levels and depressive symptoms in year 13

Table 3 shows the results of the logistic regression analysis to determine whether levels of estradiol or FSH were associated with depressive symptoms over the 2-year follow-up. Initial analysis adjusting for only baseline depression score (model 1) suggested that a change in total estradiol or FSH levels was associated with the risk of depressive symptoms and there was also a trend for an association between depression and higher estradiol levels in year 11. Logistic models were then generated taking into account the covariates that *a priori* could potentially confound the association between depressive symptoms and hormone levels. Successive adjustment for age and BMI (model 2), and then with further inclusion of high alcohol consumption, number of years since menopause and hot flushes or night sweats (model 3), did not have a substantially effect on the results.

After this multivariate adjustment, a 2-year decline in estradiol levels remained the strongest risk factor for depressive symptoms. Women whose estradiol levels dropped between year 11 and 13,

had a 3.5-fold increased risk of having depressive symptoms in year 13. Likewise, changes in FSH levels also appeared to be associated with depression. The results suggest that women whose FSH levels increased by 9 IU/l, had more than 2-times the risk of depressive symptoms. The variables for the decline in estradiol, or the large increase in FSH were not associated with one another ($\chi^2=0.2$, d.f=1, $p=0.65$), and therefore, an association with one of these measures, did not necessarily imply an association with the other.

Using these models as a basis, adjustment was made for the other covariates which were found to be associated with depression status in year 11 at the 20% significance level (Table 1). After the addition of living alone, physical activity, more negative attitudes towards the menopause or a higher number of daily hassles, to model 3, a decline in estradiol or a large increase in FSH levels, were still significantly associated with depressive symptoms (data not shown). On the other hand, we did not find a significant association between depression and absolute levels of FSH or estradiol, in year 11 or 13.

The same hormone variables were examined in secondary analysis, to determine whether absolute or changing hormone levels predicted incident cases of depression, among women without depressive symptoms in year 11 (CES-D<10). Similar results were obtained to those described above (data not shown). Once again, even after multivariate analysis, a decrease in estradiol levels over the follow-up period was significantly associated with incident depression (adjusted OR=3.80, 95%CI: 1.15-12.5, $\chi^2=4.8$, df=1, $p=0.03$) and there was a trend for increased depression risk with a large increase in FSH levels (adjusted OR=3.21, 95%CI: 0.97-10.8, $\chi^2=3.6$, df=1, $p=0.06$).

DISCUSSION

The menopause transition is a period of marked hormone instability and the later phases of the transition are characterised by a steep increase in FSH levels, followed by a dramatic decline in estradiol levels⁴⁰. The levels of both of these hormones are then thought to stabilise as women enter the postmenopause⁴¹, but despite this, we have been able to demonstrate that hormone levels during the postmenopausal period may influence depression status. In particular, while we found no association between depressive symptoms and the absolute levels of estradiol or FSH, we determined that changes in the levels of these hormones were significantly associated with depressive symptoms. The risk of depressive symptoms increased over 3-fold with a decline in estradiol levels over the 2-year period and to a lesser extent with a large increase in FSH levels. These associations were independent of other factors that are linked to both depression and hormone levels. It has been suggested that the association between estradiol levels and depressed mood, at least for perimenopausal women, could be explained by the presence of vasomotor symptoms such as hot flushes. Indeed, studies with the data collected from the MWMHP, have shown that women with bothersome hot flushes were more likely to have higher negative mood scores and that hot flushes themselves were correlated with low estradiol and high FSH levels⁴². Confounding by hot flushes also explains associations between depression and estradiol that are only significant in unadjusted analysis⁴³. In our analysis however, even after adjustment for a range of variables, including age, body mass index, alcohol consumption, hot flushes or night sweats, as well as various socio-demographic, health and lifestyle factors, significant associations remained.

Absolute hormone levels and depressive symptoms

Contrary to our initial hypothesis and evidence in the literature which suggests that estradiol has a beneficial effect on mood, we did not find any association between absolute levels of either total or free estradiol and depressive symptoms. The majority of previous reports which have examined the relationship between serum hormone levels and depression have focused on the menopause transition. In general these studies have also failed to find an association between depression and either total plasma estradiol levels^{9, 18, 43-45}, bioavailable estradiol levels¹⁸ or levels of FSH^{9, 22, 45, 46}, although not consistently. Harlow and colleagues reported that women with a lifetime history of major depression had lower estradiol levels at both study enrolment and after follow-up²⁰, and positive associations between depressive symptoms and FSH have been found^{5, 20, 47}.

There are few studies which have examined differences in estradiol or FSH levels between depressed and non-depressed postmenopausal women. Of those that have, very small sample sizes may have limited the ability to detect significant effects in certain studies^{19, 46}. In a somewhat larger study of 265 volunteers aged 70 years and over, postmenopausal women with lower estradiol levels were more likely to have depressive symptoms, however only the results of unadjusted analysis were reported⁴⁸.

Changes in hormone levels are associated with depressive symptoms

Our finding that the risk of depressive symptoms was associated with changes in estradiol and FSH, rather than the absolute levels of these hormones, is supported by previous research^{5, 22, 23, 49}.

Freeman and colleagues found that the varying hormone milieu experienced by women during the menopause transition had an important negative effect on their mood²². In fact, they found that rapidly increasing FSH levels appeared to play a more central role, with decreasing estradiol levels associated with depressive symptoms in unadjusted analysis only. Other studies of perimenopausal women²⁰ however, as well as our study of the postmenopause, indicate that changes in estradiol levels were a stronger risk factor than variability in FSH. During the reproductive years, there is also evidence to suggest that changes, rather than the absolute levels of reproductive hormones such as estradiol, are more important in the etiology of depression and in determining a woman's vulnerability to developing depression⁵⁰. In addition, some have suggested that the actual rate of change of hormonal levels might also be relevant in predicting depression^{22, 51}. However the design of our study did not allow us to test this hypothesis. The negative consequences of a large change in hormone levels may help explain why surgical menopause is associated with an increased risk of depressive symptoms¹².

Previous analysis with data collected from the MWMHP, did not find a significant association between estradiol or FSH levels and mood⁴⁰. However this analysis made use of data collected during the first 13 years of the study, and thus examined changes in hormone levels across the entire menopause transition, without excluding women who using HT. In contrast, our current analysis focused solely on untreated postmenopausal women. In addition, as the CES-D was only introduced into the MWMHP in year 11, the previous analysis did not examine depressive symptoms as such, but instead used a measure of negative mood (negative affect scale).

Potential involvement of other steroid hormones

Testosterone has been shown to influence neurotransmitter activity⁵² and testosterone therapy can improve mood in premenopausal women with low testosterone levels⁵³. Treatment with testosterone either alone or in combination with estradiol, may also assist in the well-being of postmenopausal women⁵⁴. In this study however, we found that the levels of both total and bioavailable testosterone (FAI) were similar between women with depressive symptoms and those without. This finding appears relatively consistent in the literature, both in the case of perimenopausal^{22, 44-46, 55} and post-menopausal women^{18, 19, 46, 55, 56}. Likewise we did not find any association between depressive symptoms and the levels of SHBG, a circulating hormone-binding protein, which binds estrogens and androgens and transports them to their target tissues. However, in year 13 of the study, testosterone and SHBG were not measured and therefore a potential association with changes in the levels of these compounds cannot be ruled out.

It is also possible that other hormones which were not measured in our study could be associated with depressive symptoms in later life, such as DHEA and its sulphate metabolite DHEAS, which can reportedly enhance well-being⁵⁷. Lower DHEAS levels have been reported in depressed middle-aged and older women^{18, 45, 58}. On the other hand, some studies found no association between low levels of DHEA or DHEAS and depressive symptoms^{22, 44, 56} and treatment with these hormones might have no effect on mood or well-being⁵⁹. Unfortunately we have not yet analysed the levels of DHEAS in years 11 or 13 of this study. Earlier work on the MWMHP however, failed to find a significant association between DHEAS and mood⁶⁰.

Finally, the majority of studies which have looked at estrone^{18, 44-46, 48} or androstenedione^{18, 44, 55} have reported no association with depressive symptoms.

Limitations

A limitation of the present study is the relatively small sample size such that it is possible the negative findings result from a lack of power to detect statistical differences. In addition, as the hormones were only measured once in years 11 and 13, measurement error which results in bias towards the null hypothesis cannot be ruled out. However, despite these limitations, some significant associations were found. There are also limitations due to the floor effect with the hormone assays, in particular concerning testosterone and estradiol. Concentrations in the postmenopausal range for estradiol are around the limits of sensitivity for the assay used. More sensitive assays would allow better discrimination of differences in low hormone levels across women and consequently could allow better detection of associations with depressive symptoms⁶¹. The data collected concerning some of the covariates in this study were self-reported which may be subject to recall bias with depressed participants responding more negatively about their health. However, as these self-report measures concern the covariates, and since similar associations were seen in the unadjusted and adjusted analysis, it would appear that any bias did not have a substantial influence on our findings. Bias could also be introduced from the exclusion of women with missing data concerning some of the variables under analysis and those who were lost to follow-up. In particular, the women included in the current analysis had remained in the cohort for 13 years and thus bias may be caused by the loss of the women who had refused further participation in the study. Finally, although we attempted to control for a range of potential confounding factors that may explain the associations reported here, it is always possible that other unrecognized factors have influenced the results.

Strengths

Despite these limitations, this study has a number of strengths. The data used in this analysis come from a population-based prospective study of women recruited by random digit telephone dialling and therefore the results are relevant to postmenopausal women living in the community. Depressive symptoms were assessed using an instrument that has been validated in the general population and has been judged a reliable tool for detecting symptoms of depressed mood in older adults^{29, 30}. In addition, because detailed information was obtained on all of the participants in this study, we were able to adjust for a range of factors in the multivariate analysis, thus minimising any confounding.

Our findings suggest that changes in hormone levels rather than the absolute levels are associated with depressive symptoms in postmenopausal women. In particular, a decrease in estradiol levels and to a lesser extent a large increase in follicle-stimulating hormone levels, were associated with an increased risk of depressive symptoms over follow-up. This extends previous work with the MWMHP⁴⁰, which indicated the importance of considering varying rather than absolute hormone levels, but these findings warrant further investigation. Larger studies of postmenopausal women are needed, with more frequent measures of hormone levels over a longer period of time. This would allow a more accurate assessment of the true effect of these hormones on depression. Our results suggest a potential role for estrogen in later life depression and open-up the possibility that modulation of estradiol levels could be used as a therapeutic tool.

ACKNOWLEDGMENTS

We are indebted to the participants in the Melbourne Women's Midlife Health Project, who generously donated their time and participated in this research study. Joanne Ryan has Ph.D. scholarship from the University of Melbourne and Cassandra Szoeki is the recipient of a fellowship from the Royal Australian College of Physicians.

REFERENCES

1. Ancelin ML, Ritchie K. Lifelong endocrine fluctuations and related cognitive disorders. *Current Pharm Design* 2005;11(32):4229-52.
2. Andrade TG, Nakamuta JS, Avanzi V, Graeff FG. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT_{1A} receptors. *Behav Brain Res* 2005;163(1):18-25.
3. Maayan R, Strous RD, Abou-Kaoud M, Weizman A. The effect of 17beta estradiol withdrawal on the level of brain and peripheral neurosteroids in ovariectomized rats. *Neurosci Lett* 2005;384(1-2):156-61.
4. Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. *Recent Prog Horm Res* 2002;57:257-75.
5. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of hormones and menopausal status with depressed mood in women with no history of depression. *Arch Gen Psychiatry* 2006;63(4):375-82.
6. Bromberger JT, Matthews KA, Schott LL, et al. Depressive symptoms during the menopausal transition: The Study of Women's Health Across the Nation (SWAN). *J Affect Disord* 2007.
7. Maartens LW, Knottnerus JA, Pop VJ. Menopausal transition and increased depressive symptomatology: a community based prospective study. *Maturitas* 2002;42(3):195-200.
8. Schmidt PJ. Depression, the perimenopause, and estrogen therapy. *Ann NY Acad Sci* 2005;1052:27-40.
9. Dennerstein L, Lehert P, Burger H, Dudley E. Mood and the menopausal transition. *J Nerv Ment Dis* 1999;187(11):685-91.
10. McKinlay JB, McKinlay SM, Brambilla D. The relative contributions of endocrine changes and social circumstances to depression in mid-aged women. *J Health Soc Behav* 1987;28(4):345-63.
11. Porter M, Penney GC, Russell D, Russell E, Templeton A. A population based survey of women's experience of the menopause. *Br J Obstet Gynaecol* 1996;103(10):1025-8.
12. Dennerstein L, Guthrie JR, Clark M, Lehert P, Henderson VW. A population-based study of depressed mood in middle-aged, Australian-born women. *Menopause* 2004;11(5):563-8.
13. Schmidt PJ, Nieman L, Danaceau MA, et al. Estrogen replacement in perimenopause-related depression: a preliminary report. *Am J Obstet Gynecol* 2000;183(2):414-20.
14. Soares CN, Almeida OP, Joffe H, Cohen LS. Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch Gen Psychiatry* 2001;58(6):529-34.
15. Birkhauser M. Depression, menopause and estrogens: is there a correlation? *Maturitas* 2002;41(Suppl 1):S3-8.
16. Cohen LS, Soares CN, Poitras JR, Prouty J, Alexander AB, Shifren JL. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. *Am J Psychiatry* 2003;160(8):1519-22.
17. Zweifel JE, O'Brien WH. A meta-analysis of the effect of hormone replacement therapy upon depressed mood. *Psychoneuroendocrinology* 1997;22(3):189-212.
18. Barrett-Connor E, Von Muhlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo Study. *J Am Geriatr Soc* 1999;47(6):685-91.
19. Erdinler D, Bugay G, Ertan T, Eker E. Depression and sex hormones in elderly women. *Arch Gerontol Geriatr* 2004;39(3):239-44.
20. Harlow BL, Wise LA, Otto MW, Soares CN, Cohen LS. Depression and its influence on reproductive endocrine and menstrual cycle markers associated with perimenopause: the Harvard Study of Moods and Cycles. *Arch Gen Psychiatry* 2003;60(1):29-36.

21. Young EA, Midgley AR, Carlson NE, Brown MB. Alteration in the hypothalamic-pituitary-ovarian axis in depressed women. *Arch Gen Psychiatry* 2000;57(12):1157-62.
22. Freeman EW, Sammel MD, Liu L, Gracia CR, Nelson DB, Hollander L. Hormones and menopausal status as predictors of depression in women in transition to menopause. *Arch Gen Psychiatry* 2004;61(1):62-70.
23. Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev* 2005;4(2):141-94.
24. Dennerstein L, Smith AM, Morse C, et al. Menopausal symptoms in Australian women. *Med J Aust* 1993;159(4):232-6.
25. Smith AM, Dennerstein L, Morse CA, Hopper JL, Green A. Costs and benefits of the use of commercial market research approaches in large scale surveys. *Med J Aust* 1992;157(7):504.
26. Dennerstein L, Smith AM, Morse C. Psychological well-being, mid-life and the menopause. *Maturitas* 1994;20(1):1-11.
27. Burger HG, Dudley EC, Hopper JL, et al. The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. *J Clin Endocrinol Metab* 1995;80(12):3537-45.
28. Radloff L. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Measurement* 1977;1:385-401.
29. Andresen EM, Malmgren JA, Carter WB, Patrick DL. Screening for depression in well older adults: evaluation of a short form of the CES-D (Center for Epidemiologic Studies Depression Scale). *Am J Prev Med* 1994;10(2):77-84.
30. Furukawa T, Anraku K, Hiroe T, et al. Screening for depression among first-visit psychiatric patients: comparison of different scoring methods for the Center for Epidemiologic Studies Depression Scale using receiver operating characteristic analyses. *Psychiatry Clin Neurosci* 1997;51(2):71-8.
31. Smith AM, Shelley JM, Dennerstein L. Self-rated health: biological continuum or social discontinuity? *Soc Sci Med* 1994;39(1):77-83.
32. Kanner AD, Coyne JC, Schaefer C, Lazarus RS. Comparison of two modes of stress measurement: daily hassles and uplifts versus major life events. *J Behav Med* 1981;4(1):1-39.
33. Burger HG, Dudley EC, Cui J, Dennerstein L, Hopper JL. A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. *J Clin Endocrinol Metab* 2000;85(8):2832-8.
34. Santoro N, Torrens J, Crawford S, et al. Correlates of circulating androgens in mid-life women: the study of women's health across the nation. *J Clin Endocrinol Metab* 2005;90(8):4836-45.
35. Guthrie JR, Dennerstein L, Hopper JL, Burger HG. Hot flushes, menstrual status, and hormone levels in a population-based sample of midlife women. *Obstet Gynecol* 1996;88(3):437-42.
36. Dennerstein L, Lehert P, Guthrie JR, Burger HG. Modeling women's health during the menopausal transition: a longitudinal analysis. *Menopause* 2007;14(1):53-62.
37. Burger HG, Dudley EC, Hopper JL, et al. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. *J Clin Endocrinol Metab* 1999;84(11):4025-30.
38. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). *Cancer Causes Control* 2001;12(1):47-59.
39. Soules MR, Sherman S, Parrott E, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). *Fertil Steril* 2001;76(5):874-8.
40. Dennerstein L, Lehert P, Burger HG, Guthrie JR. New findings from non-linear longitudinal modelling of menopausal hormone changes. *Hum Reprod Update* 2007.

41. Burger HG, Hale GE, Robertson DM, Dennerstein L. A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. *Hum Reprod Update* 2007.
42. Guthrie JR, Dennerstein L, Taffe JR, Lehert P, Burger HG. Hot flushes during the menopause transition: a longitudinal study in Australian-born women. *Menopause* 2005;12(4):460-7.
43. Avis NE, Crawford S, Stellato R, Longcope C. Longitudinal study of hormone levels and depression among women transitioning through menopause. *Climacteric* 2001;4(3):243-9.
44. Gallicchio L, Schilling C, Miller SR, Zacur H, Flaws JA. Correlates of depressive symptoms among women undergoing the menopausal transition. *J Psychosom Res* 2007;63(3):263-8.
45. Schmidt PJ, Murphy JH, Haq N, Danaceau MA, St Clair L. Basal plasma hormone levels in depressed perimenopausal women. *Psychoneuroendocrinology* 2002;27(8):907-20.
46. Woods NF, Smith-Dijulio K, Percival DB, Tao EY, Taylor HJ, Mitchell ES. Symptoms during the menopausal transition and early postmenopause and their relation to endocrine levels over time: observations from the Seattle Midlife Women's Health Study. *J Womens Health (Larchmt)* 2007;16(5):667-77.
47. Huerta R, Mena A, Malacara JM, Diaz de Leon J. Symptoms at perimenopausal period: its association with attitudes toward sexuality, life-style, family function, and FSH levels. *Psychoneuroendocrinology* 1995;20(2):135-48.
48. Almeida OP, Lautenschlager N, Vasikaram S, Leedman P, Flicker L. Association between physiological serum concentration of estrogen and the mental health of community-dwelling postmenopausal women age 70 years and over. *Am J Geriatr Psychiatry* 2005;13(2):142-9.
49. Daly RC, Danaceau MA, Rubinow DR, Schmidt PJ. Concordant restoration of ovarian function and mood in perimenopausal depression. *Am J Psychiatry* 2003;160(10):1842-6.
50. Bao AM, Ji YF, Van Someren EJ, Hofman MA, Liu RY, Zhou JN. Diurnal rhythms of free estradiol and cortisol during the normal menstrual cycle in women with major depression. *Horm Behav* 2004;45(2):93-102.
51. Halbreich U. Gonadal hormones, reproductive age, and women with depression. *Arch Gen Psychiatry* 2000;57(12):1163-4.
52. Rubinow DR, Schmidt PJ. Androgens, brain, and behavior. *Am J Psychiatry* 1996;153(8):974-84.
53. Goldstat R, Briganti E, Tran J, Wolfe R, Davis SR. Transdermal testosterone therapy improves well-being, mood, and sexual function in premenopausal women. *Menopause* 2003;10(5):390-8.
54. Almeida OP. Sex playing with the mind. Effects of oestrogen and testosterone on mood and cognition. *Arq Neuropsiquiatr* 1999;57(3A):701-6.
55. Weber B, Lewicka S, Deuschle M, Colla M, Heuser I. Testosterone, androstenedione and dihydrotestosterone concentrations are elevated in female patients with major depression. *Psychoneuroendocrinology* 2000;25(8):765-71.
56. Bell RJ, Donath S, Davison SL, Davis SR. Endogenous androgen levels and well-being: differences between premenopausal and postmenopausal women. *Menopause* 2006;13(1):65-71.
57. Cawood EH, Bancroft J. Steroid hormones, the menopause, sexuality and well-being of women. *Psychol Med* 1996;26(5):925-36.
58. Yaffe K, Ettinger B, Pressman A, et al. Neuropsychiatric function and dehydroepiandrosterone sulfate in elderly women: a prospective study. *Biol Psychiatry* 1998;43(9):694-700.
59. Wolf OT, Neumann O, Hellhammer DH, et al. Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. *J Clin Endocrinol Metab* 1997;82(7):2363-7.

60. Dennerstein L, Randolph J, Taffe J, Dudley E, Burger H. Hormones, mood, sexuality, and the menopausal transition. *Fertil Steril* 2002;77 Suppl 4:S42-8.
61. Hogervorst E, Williams J, Combrinck M, David Smith A. Measuring serum oestradiol in women with Alzheimer's disease: the importance of the sensitivity of the assay method. *Eur J Endocrinol* 2003;148(1):67-72.

Figure Legend

Figure 1. Flow of participants from recruitment in the longitudinal cohort to analysis in this current study.

*Women who refused participation in year 11 but were part of the year 13 cohort.

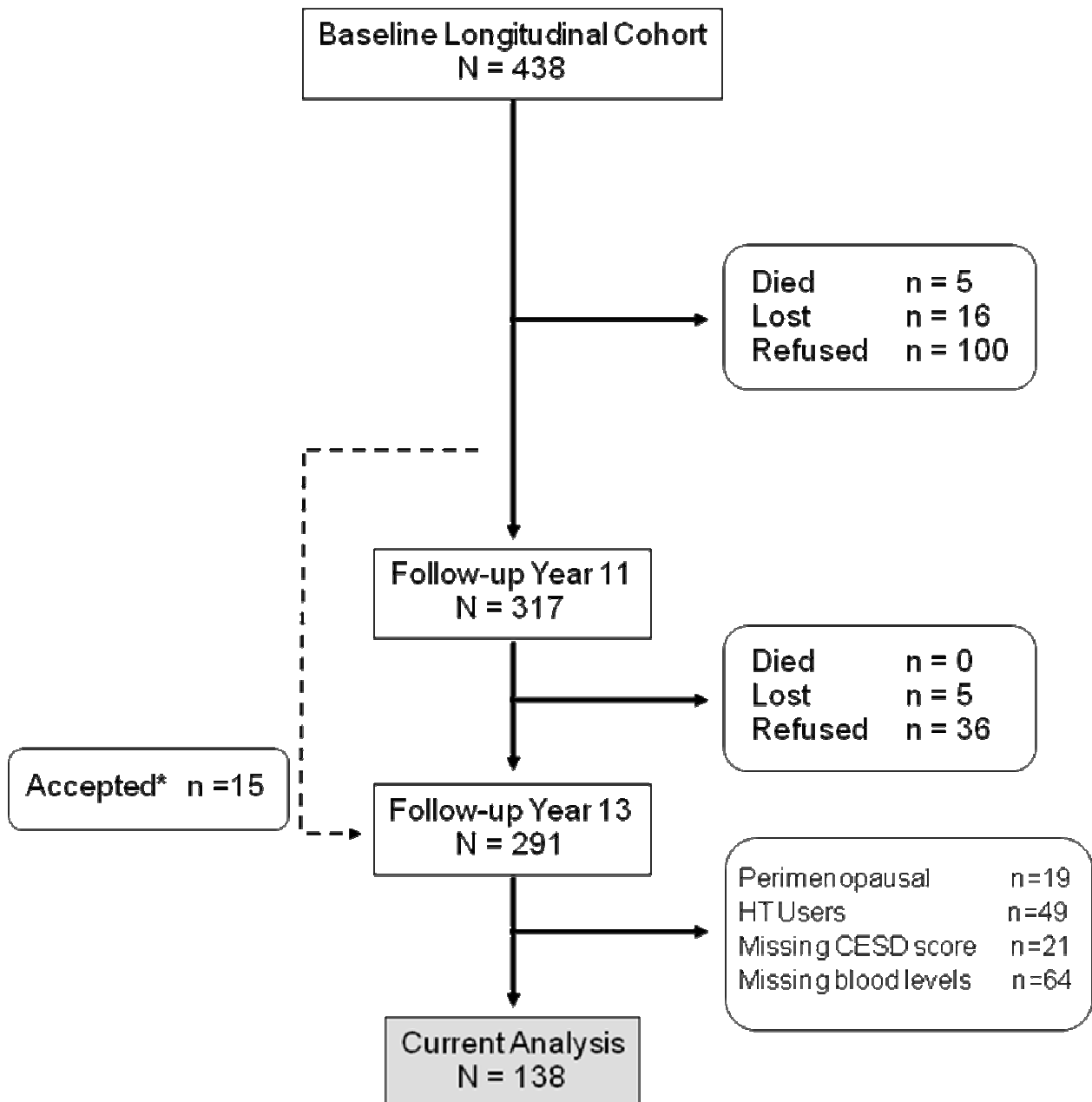


TABLE 1. Socio-demographic, health and lifestyle characteristics of the participants according to their depression status in year 13.

Characteristic	%		Test for difference	
	No Depression (n = 103)	Depression (n = 35)	Statistic (d.f.)	p
	Mean (sd)		t-value	
Age	60.3 (2.5)	59.2 (2.2)	2.4 (136)	0.02
Number of years since menopause	7.0 (2.3)	5.9 (2.5)	2.4 (136)	0.02
	Percentage		X²	
≥ 12 years of education	44.7	37.1	0.6 (1)	0.44
Living alone	19.4	31.4	2.2 (1)	0.14
No paid work	52.4	48.6	0.2 (1)	0.69
≥ 8 alcoholic drinks in the last week	22.3	14.3	1.1 (1)	0.31
Current smoker	3.9	5.7	0.2 (1)	0.65
No regular physical activity	10.7	25.7	4.8 (1)	0.03
Good self-rated health	38.8	34.3	0.2 (1)	0.63
A number of health problems ^a	22.3	17.1	0.4 (1)	0.52
Experiences hot flushes and/or night sweats	31.1	54.3	6.0 (1)	0.01
Experiences ≥ 6 bothersome physical symptoms	68.9	68.6	0.1 (1)	0.97
More negative attitudes towards menopause ^b	38.8	51.4	1.7 (1)	0.19
Higher number of daily hassles ^c	46.6	69.9	2.8 (1)	0.10
<i>Body Mass Index</i>				
< 25.0	33.0	40.0	7.6 (2)	0.02
Overweight (25 ≤ BMI < 30)	44.7	20.0		
Obese (BMI ≥ 30)	22.3	40.0		

^aIncludes having at least 3 of the following: high blood pressure, diabetes, heart disease, cancer, arthritis, regular migraines or other chronic conditions.

^bScoring less than or equal to 7 on the questionnaire concerning attitudes to aging, with lower scores indicating more negative attitudes (score range: -2 to 22).

^cScoring greater than or equal to 4 on the daily hassles scale, with higher scores indicating a higher number and/or severity of hassles.

TABLE 2. Serum hormone levels according to depression status in year 13.

Characteristic	Overall	Group	No		Test Statistic
			depression (n = 103)	Depression (n = 35)	
	Mean (sd)		Mean (sd)		p (t-value) 109 d.f
Sex-Hormone Binding Globulin(SHBG) ^a (nmol/l)	40.9 (1.6)		40.5 (1.6)	41.3 (1.4)	0.94 (-0.1)
Free Androgen Index (FAI) ^a	2.0 (1.7)		1.97 (1.2)	2.18 (1.9)	0.40 (-0.9)
Follicle-Stimulating Hormone (FSH) (IU/l)	70.8 (26.6)		71.0 (25.8)	70.2 (29.5)	0.88 (0.2)
	Median (IQR)^b		%		p (X²) 1 d.f.
SHBG (nmol/l)	42.1 (29.8; 55.4)	≥ 40	52.4	62.9	0.28 (1.2)
FAI	2.0 (1.4; 2.8)	≥ 2	50.5	51.4	0.92 (0.1)
Testosterone (nmol/l)	0.81 (0.7; 1.1)	≥ 0.8	49.5	57.1	0.61 (0.4)
Free Estradiol	31.7 (24.5; 40.9)	≥ 32	44.7	60.0	0.12 (2.5)
Total Estradiol (pmol/l)	23.7 (20.0; 32.6)	≥ 24	42.7	62.9	0.04 (4.3)
FSH (IU/l)	70.3 (52.3; 85.6)	≥ 70	54.4	42.9	0.24 (1.4)
Total Estradiol at Year 13 (pmol/l)	20.0 (20; 33.0)	> 20	44.7	60.0	0.12 (2.5)
FSH at Year 13 (IU/l)	80.2 (62.2; 95.4)	≥ 80	49.5	51.4	0.85 (0.1)
Decline in Estradiol over 2-yr follow-up (year 11 E2 level minus year 13 E2 level)	0 (-4.8; 5.2)	> 0	35.9	51.4	0.11 (2.6)
Large increase in FSH over 2-yr follow-up (year 13 FSH level minus year 11 FSH level)	8.3 (2.5; 16.9)	≥ 9	43.7	62.9	0.05 (3.8)

^aThe geometric mean is shown, given the log transformation of these measures.

^bInterquartile range (IQR).

TABLE 3. Adjusted Logistic Regression models for depression in year 13.

Hormone	Model 1 ^a		Model 2 ^b		Model 3 ^c	
	Odds ratio [95% CI]	p-value (χ^2 stat. ^o)	Odds ratio [95% CI]	p-value (χ^2 stat. ^o)	Odds ratio [95% CI]	p-value (χ^2 stat. ^o)
<u>Free E2</u>						
< 32	1					
≥ 32	1.48 [0.63-3.47]	0.37 (0.8)				
<u>Total E2</u>						
< 24 pmol/l	1 (referent)		1		1	
≥ 24 pmol/l	1.83 [0.78-4.34]	0.17 (1.9)	2.19 [0.85-5.67]	0.11 (2.6)	2.17 [0.80-5.91]	0.13 (2.3)
<u>Total E2 at year 13</u>						
< 20 pmol/l	1 (referent)					
≥ 20 pmol/l	1.06 [0.43-2.56]	0.91 (.01)				
<u>2-year decrease in E2</u>						
≤ 0 pmol/l	1 (referent)		1		1	
> 0 pmol/l	2.26 [0.95-5.37]	0.06 (3.4)	3.41 [1.24-9.36]	0.017 (5.7)	3.49 [1.23-9.95]	0.019 (5.5)
<u>FSH</u>						
< 70 IU/l	1 (referent)					
≥ 70 IU/l	0.78 [0.33-1.82]	0.56 (0.3)				
<u>FSH at year 13</u>						
< 80 IU/l	1 (referent)					
≥ 80 IU/l	1.39 [0.59-3.26]	0.45 (0.6)				
<u>2-year increase in FSH</u>						
< 9 IU/l	1 (referent)		1		1	
≥ 9 IU/l	2.70 [1.13-6.46]	0.03 (5.0)	2.62 [1.04-6.61]	0.04 (4.1)	2.57 [1.00-6.69]	0.05 (3.8)

^aModel 1 is adjusted for CES-D score (log) at baseline.

^bModel 2 is adjusted for CES-D score (log) at baseline, age and body mass index (<25, 25-30, ≥30).

^cModel 3 is adjusted for CES-D score (log) at baseline, age, body mass index (<25, 25-30, ≥30), high alcohol consumption (≥8 drinks in the last week), years since menopause and hot flushes or night sweats (presence vs absence).

^oTest statistic was compared to a χ^2 distribution with 1 degree of freedom.

